MULTIDISCIPLINARY MANAGEMENT OF COLORECTAL CANCER: SURGICAL AND IMMUNOLOGICAL ASPECTS

DISSERTATION

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"Die naturwissenschaftliche Frage ist die logische Hypothese, welche von einem bekannten Gesetz durch Analogie und Induktion weiterschreitet; die Antwort darauf giebt das Experiment, welches in der Frage selbst vorgeschrieben liegt."

Rudolf Virchow, 1849

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I. Summary

Colorectal carcinoma is one of the global leading causes of tumor-associated death. The management of colorectal cancer currently involves surgical and non-surgical interventions. For patients with locally restricted tumor stages, appropriate surgical approach to remove the primary lesion is currently the mainstay of curative treatments. For hepatic, pulmonary or peritoneal metastatic colorectal cancer, systemic therapy represents the backbone of treatment and surgical resection in selected patients with resectable metastases (pulmonary, hepatic, peritoneal) may also lead to improved survival. Additionally, recent advances in genomic technologies, immunology, and cell biology have led to novel understandings about the modulations of the microenvironment in all stages of colorectal carcinoma.

The main objective of this dissertation was on the one hand to prove the effect of laparoscopic approach for colorectal carcinoma management. On the other hand, from an experimental perspective, it is to reveal that the inflammatory microenvironment might be associated with tumorigenesis in parasite infestation. Additionally, because bone marrow-derived mesenchymal stem cells (MSC) are important components that could mediate interactions between the inflammatory microenvironment and cancer cells, this study also demonstrated the molecular mechanisms associated with irradiation and cellular interactions of tumor cells and MSC cells.

The first manuscript investigated surgical strategies for primary colorectal cancer (Ref.1) and revealed that improved oncological control was provided with complete mesocolic excision (CME) for colonic cancer, comparable to precise local lymph node dissection

particularly in the technically advanced surgical procedure of laparoscopic left hemicolectomy.

Nevertheless, surgical intervention in elderly patients are still associated with elevated postoperative morbidity and mortality as well as dysfunction. Thus, in the second paper, it was investigated that colorectal cancer treatment in patients with elevated perioperative risk caused more surgical complications. Additionally, it was demonstrated that laparoscopic (minimally invasive) surgery was superior in oncologic outcome and survival, especially for those with renal or respiratory dysfunction as well as stage III carcinoma (Ref.2)

To introduce the aspect of inflammation on cancer development, the third paper shows a clinical case collection of schistosomal colorectal carcinoma and its prognostic factors and clinicopathological characteristics. Immune reactions against schistosomal eggs in the colorectal mucosa lead to inflammatory disease, and the repetitive inflammatory reaction is to be relevant for carcinogenesis. Correlation of clinical parameters showed, that patients with rectosigmoid schistosomiasis presented an elevated cancer antigen 125 (CA-125) level and the majority of these patients were at the earlier stage of carcinoma). (Ref.3)

Additionally, radiotherapy not only induces cytoreduction of colorectal cancer but also remodels the tumor microenvironment by promoting tumor-associated tropism reaction of peripheral BM-MSC circulation. However, the function of irradiation mediated recruited BM-MSCs and local MSCs were poorly defined, they do play a crucial role in immune microenvironment modulation (Ref.4). Indeed, the role of bone marrow-derived

mesenchymal stem cells remained controversial in tumor development even without radiation intervention: Previously, it was reported that BM-MSC modulates the immune response of lymphocytes, resulting in immune tolerance which might induce local recurrence or distant metastasis. In contrast, BM-MSC is capable to release diverse cytokines/chemokines and possess cytotoxicity effect against the tumor. In the fourth paper, it was shown that bone marrow-derived mesenchymal stem cells could potentially enhance the anticancer activity of colorectal cancer radiotherapy.

This cumulative dissertation is based on 4 articles which were published in peer-review scientific journals in the timeframe from 2015 to 2018 (JCR and http://www.scimagojr.com):

Ref.1-**Feng, H**., Zhao, X., Zhang, Z., Han, D., Mao, Z., Lu, A., & Thasler, W. E. (2016). Laparoscopic Complete Mesocolic Excision for Stage II/III Left-Sided Colon Cancers: A Prospective Study and Comparison with D3 Lymph Node Dissection. *Journal of Laparoendoscopic & Advanced Surgical Techniques*, 26(8), 606-613. (***impact factor: 1.322#, Quartiles: Surgery, Q2***)

Ref.2- **Feng H**, Schiergens TS, Mao ZH, Zhao J, Shen X, Lu AG, Thasler WE. Long-term outcomes and propensity score matching analysis: rectal cancer resection for patients with elevated preoperative risk. *Oncotarget*. 2017 Apr 11;8(15):25679. (***impact factor: 5.168*, Quartiles: Oncology, Q1***)

Ref.3**- Feng, H**., Lu, A., Zhao, X., Han, D., Zhao, J., Shi, L., Schiergens TS, Lee SM, Zhang WP, ... & Thasler, W. E. (2015). Comparison of non-schistosomal rectosigmoid cancer and schistosomal rectosigmoid cancer. *World Journal of Gastroenterology*, 21(23), 7225-7232. (***impact factor: 3.411#, Quartiles: gastroenterology and hepatology, Q2***)

Ref.4- **Feng, H**., Zhao, J., Schiergens TS, Wang, P., Ou, B., Rami Al-Sayegh, Li, M., Lu, A., Yin, S., & Thasler, W. E. (2018) Bone marrow-derived mesenchymal stromal cells promote colorectal cancer cell death under low-dose irradiation. *British Journal of Cancer*. 2018 Jan 2; Epub. (***impact factor: 5.922#, Quartiles: Medicine, Q2***)

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The present dissertation evaluates the management of CRC in four different aspects:

- surgical technique of lymph node dissection (Ref.1),
- the oncological outcome of minimally invasive surgery (Ref.2),
- the influence of chronic inflammation in carcinogenesis (Ref.3),
- the modulation of the immune microenvironment by MSCs (Ref.4),

In addition to the published results, the experimental work during the last four years explored the mechanisms of immune modulation in colorectal cancer liver metastases. Currently, the experiments investigate the function of CXCR6+iNKT cells in metastatic colorectal cancers and the modulation of the microenvironment by the crosstalk of Kupffer cells and hepatic resident invariant nature killer T cells in liver metastases. (see outlook)

II. LIST OF FIGURES AND TABLES

III. LIST OF ABBREVIATIONS AND ACRONYMS

2D	Two-dimensional
3D	Three-dimensional
5-FU	5-Fluorouracil
ACS	American Chemical Society
ALPPS	Associating Liver Partition with Portal Vein Ligation for Staged Hepatectomy
AKT	Ak strain transforming (RAC-alpha serine/threonine-protein kinase)
BDCA-3	blood dendritic cells antigen
BM-MSC	bone marrow mesenchymal stromal cell
CA-125	cancer antigen 125
CAR	chimeric antigen receptor
CCL	CC chemokine ligands
CCR	CC chemokine receptors
CRC	colorectal cancer
CRLM	colorectal cancer liver metastases
Cr-POSSUM	Colorectal cancer-Physiological and Operative Severity Score for the enUmeration of Mortality and Morbidity
CT	computed tomography
CTu	the core of the tumor
CTLA4	cytotoxic T-lymphocyte-associated antigen 4
CME	complete mesocolic excision
CXCL	CXC chemokine ligands
CXCR	CXC chemokine receptors
D3	D3 lymph node station
DC	dendritic cell
EGFR	Epidermal growth factor receptor
ELISA	Enzyme-Linked Immunosorbent Assay
EMT	epithelial-mesenchymal transition
ERK	extracellular signal-regulated kinase (ERK
FDA	Food and Drug Administration
FLR	future liver remnant
IFN-γ	Interferon-gamma
IL-2	Interleukin 2
IMRT	intensity-modulated radiation therapy
IM	tumor invasive margin
iNKT	invariant NKT
KC	Kupffer cell
LODDS	log odds of positive lymph nodes
MSCs	mesenchymal stem cells
NK cell	Natural Killer cell
NKT	natural killer T
NL	adjacent normal liver tissue
PD-1	programmed cell death protein 1
pDC	plasmacytoid DC

PI3K	phosphatidylinositol 3-kinase
PVE	portal vein embolization
RFA	radiofrequency ablation
RILD	radiation-induced liver disease
SRBT	stereotactic body radiation therapy
SRA	superior rectal artery
TLR	Toll-like receptor
TME	total mesorectal excision
TNF-α	tumor necrosis factor alpha
UV-C	ultraviolet C
VEGF	Vascular endothelial growth factor
WHO	world health organization
X-ray	X-radiation

1. Introduction

Over the past two decades, the incidence of colorectal cancer declined by about 2-4% per year in both genders, with the pace accelerating in more recent decade from 2004 to 2013, which might be attributed to alterations in risk factors and screening programs (Edwards et al, 2010), especially colonoscopy screening and the removal of precancerous polyps (Siegel et al, 2012). However, colorectal carcinoma is still the third leading cause of death according to the most recently released data from the American Cancer Society (ACS, Edwards, et al, 2017). Furthermore, the incidence increases each year in younger adults (< 50 years old) (SEER et al, 2012). Globally, colorectal carcinoma is also listed as the third most frequent carcinoma diagnosed in both genders (Jemal et al, 2011), especially in European countries, Australia, East Asia, and North America according to the World Health Organization (WHO) GLOBOCAN database (http://globocan.iarc.fr/Pages/Map.aspx).

1.1 Management of CRC from a surgical perspective

Concerning primary colorectal cancer, surgery is currently the most appropriate choice of curative treatment (Young et al, 2014). The current management is a complete mesocolic excision (CME) procedure for colonic carcinoma or the total mesorectal excision (TME) approach of the rectum to remove the tumor as well as the lymphatic drainage basin of the affected colonic segment or rectum, including the major vascular pedicle. However, CME and TME are associated with an elevated risk of postoperative morbidity and mortality (Beets et al, 2016). In patients with comorbidities and advanced age, the mortality rate is as high as 10-15%, the overall morbidity rate increased from 19%

to 26% (Antoniou et al, 2015). The increasing awareness of postoperative morbidity, as well as the influence of quality of life after colorectal cancer surgery, induced an increasing interest in surgical approach selection among conventional open surgery, laparoscopic surgery, robotic surgery, and transanal endoscopic surgery with the aim to be as radical as possible concerning the tumor but less invasive concerning the patient

Especially the implementation of minimally invasive surgical techniques, the comparative study of conventional and laparoscopic surgical approach for colorectal carcinoma has been conducted for decades.

Concerning the laparoscopic approach, constant effort (e.g. Natural-orifice transluminal endoscopic surgery (NOTES)) has been paid to minimize the surgical trauma and reduce surgery associated morbidity. Meanwhile, numerous studies focused on perioperative management such as postoperative pain, mortality within 30 days, and length of hospital stay. According to these studies, laparoscopy showed favorable outcomes. Additionally, there was no difference in oncological outcomes when comparing conventional open surgery to laparoscopic surgery. Furthermore, elderly patients do profit from laparoscopic surgery even in their oncologic outcome.

For localized primary colorectal cancer (Stage I and II), the 5-year overall survival rate is around 90%. However, those patients only account for approximately 2/5 of all colorectal cancer patients (Siegel et al, 2017). 1/5 to 1/4 of patients were stage IV carcinoma when diagnosed, 15–25% of patients were with synchronous liver metastasis (Nordlinger et al, 2009, Lykoudis et al, 2014, Adam et al, 2015), 25% of patients showed metachronous liver metastases.

Even for CRLM, liver resection is still the most effective treatment to offer long-term disease-free survival (Jones et al, 2017). However, the 5-year overall survival rate declined rapidly to 14% (Siegel et al, 2017) and only a minority of 20% of patients are suitable for surgery (Vatandoust et al. 2015, Garden et al. 2006) and 30% of these patients will develop recurrence (Jones et al, 2012). It is quite a challenge when colorectal cancer has already spread to distant sites to form metastasis, which made multimodal management of CRC necessary.

1.2 Comprehensive Management of CRC and CRLM

Adjuvant or neoadjuvant chemotherapy regimens are usually recommended for patients with lymph node metastases. However, plenty of patients with metastasis are not suitable for surgical therapeutic strategies due to morbidities, organ dysfunction, or non-hepatic disease progression. (Garden et al., 2006; Alberts et al., 2005). Alternative treatment options for tumor control e.g. locally ablative regimens prolonged survival. Treatment modalities currently being used include:

1) chemotherapy seem to be the optimal option for improving symptoms and extending life expectancy (**Table 1).** In addition, the use of chemotherapy in a neoadjuvant setting allows increasing patients with CRLM eligible for resection by 3-20 % (Misiakos et al, 2011; García et al,2015). However, the efficacy was still modest, and these therapies were also associated with significant toxicities. Inspiringly, to date, multidisciplinary research aims to prolong survival, as well as to increase the percentage of patients previously considered unresectable to be significantly down-staged and eligible for hepatic resection.

2) Targeted therapy, utilizing monoclonal antibodies such as cetuximab, bevacizumab, and panitumumab, has led to improved oncological outcomes. The development of molecular targeted therapies accelerated with the understanding of the molecular pathways that mediate cancer cell proliferation, invasion, and angiopoiesis. Prolongation of patient survival and improvement in the quality of life has been associated with the use of these therapeutic strategies. (García et al,2015)

3) Ablative techniques, such as radiofrequency ablation (RFA) (Cirocchi et al. 2012), irreversible electroporation (Fruhling et al.2017), use heating probes to destroy tumors within the liver. The 5-year survival rates of CRLM after RFA range from 20.0% to 48.5%. The local progression rate is 8.8%~ 40.0%.

4) Techniques to improve resectability include portal vein embolization (PVE) (Wicherts et al. 2010), Associating Liver Partition with Portal Vein Ligation for Staged Hepatectomy (ALPPS) (Vennarecci et al. 2014), and two-stage hepatectomy. PVE before major/extended hepatectomy could increase Future Liver Remnant (FLR), to convert unresectable to resectable tumors. Though infrequently being used, two-stage hepatectomy is a vital and curative surgical approach for highly selected CRLM patients with bilobar multinodular hepatic metastasis.

5) Irradiation, intensity-modulated radiation therapy (IMRT, Krishnan et al., 2006) and Stereotactic Body Radiation Therapy (SBRT) are approaches that can reduce the damage of radiation-induced liver disease (RILD) for hepatic metastasis(Lawrence et al., 1992; Cheng et al., 2002).

6) Cancer immunotherapy: Food and Drug Administration (FDA) approval of anti-programmed cell death protein 1 (anti-PD-1), anti-PD-L1, and of anti-Cytotoxic T-lymphocyte-associated antigen 4 (anti-CTLA4) for the treatment of metastatic cancer has engendered novel awareness among surgeons and physicians of anticancer effect of checkpoint inhibitor (Sharma et al. 2015, Pardoll et al. 2012). Additionally, remarkable efforts of the pharmaceuticals above were shown in urinary cancer, gynecological cancer, and Hodgkin's lymphoma, even after therapeutic failure of conventional therapies. Despite clinic success in a variety of malignant tumors, evidence in colorectal cancer is lacking (Jacobs et al, 2015). Regarding the complicated tumor microenvironment of colorectal cancer, it is essential to investigate the immune mechanism and its modulation in the management of colorectal cancer.

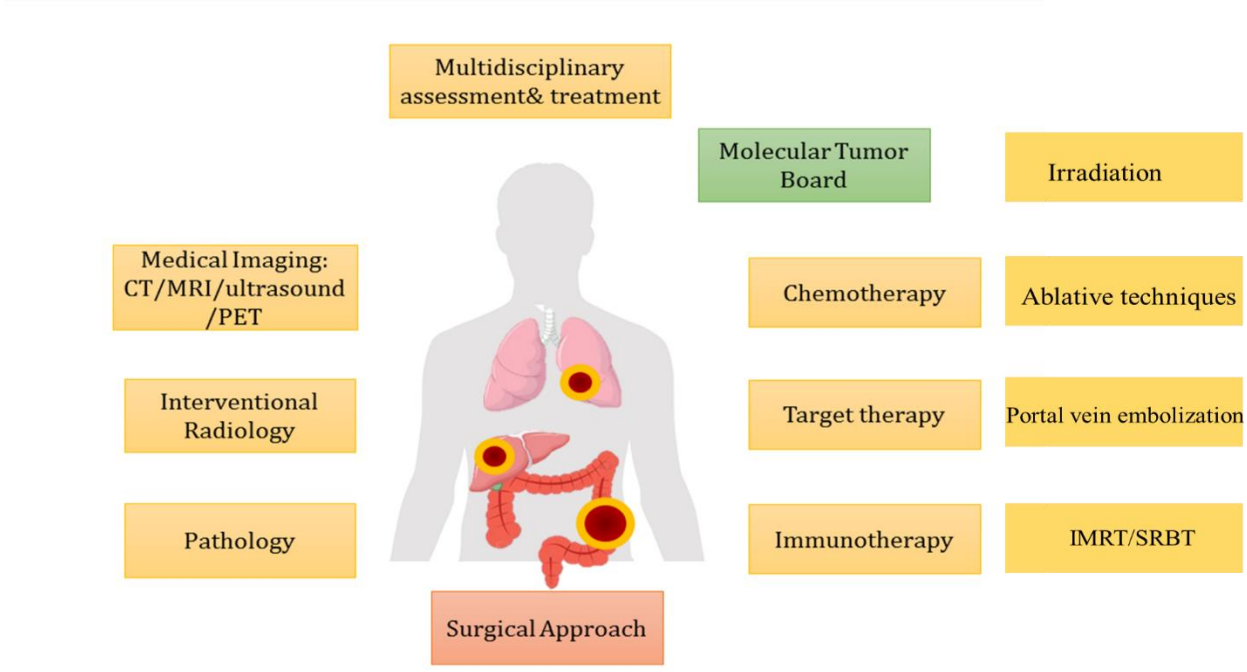

Figure 1: Multimodal management of colorectal cancer.

Table 1: Overview of non-surgical treatment options in colorectal cancer

Chemotherapy	Target therapy	Immunotherapy
5-Fluorouracil (5-FU)	Small-molecule drugs (such as VEGF Receptor 2 inhibitor)	Monoclonal antibodies
capecitabine (Xeloda)	Serine/threonine kinase inhibitors	Adoptive cell therapy (such as CAR T-Cell Therapies)
irinotecan (Camptosar)	Monoclonal antibodies Regorafenib (Stivarga)	Toll-like receptor (TLR) agonists
Leucovorin (folinic acid)	Cetuximab target EGFR	Cancer vaccines
oxaliplatin (Eloxatin)	Vectibix (panitumumab) target EGFR Bevacizumab targets VEGF	Interleukin-2 (IL-2) & tumor-infiltrating lymphocytes
Aflibercept Tipiracil	ramucirumab (Cyramza) aflibercept (Zaltrap)	Immune checkpoint inhibitors (such as PD-1/PD-L1 inhibitors and CTLA-4 inhibitors)

1.3 Tumor microenvironment and immune modulation in CRC

Among the multimodal management of colorectal cancer treatment, immunotherapy per se, (not only immune checkpoint inhibitors but also CAR-T and monoclonal antibody-based therapy) is hopefully changing the therapeutic option for patients. Recently, the American FDA has approved immunotherapy mostly in the second line setting for multiple stage IV cancer after progression on chemotherapy. Nevertheless, disease progression has been reported after the treatment of immunotherapy in non-small cell lung cancer, as well as head& neck malignancy (Saâda-Bouzid E et al. 2017). In fact, the tumor microenvironment is the vital area of the crosstalk among cancer cells, stromal cells, and the immune system via cytokines and chemokines. In response to certain

chemokines, various immune cell subsets and mesenchymal stem cells (MSCs) migrate into the microenvironment, differentiate, and regulate tumor immune responses in a spatiotemporal manner. It is suspected that MSCs can regulate both, adaptive as well as innate immunity and exert immune modulatory activity, which confer tremendous potential for clinic application. MSCs are able to secrete alterative cytokines and chemokines to possess anti-tumor properties (Pommey et al, Galipeau et al, 2006; Hendijani et al, Javanmard et al, Liotta et al, 2015). However, from the other side, MSC could also construct a suppressive immune microenvironment, resulting in immune tolerance and the promotion of cancer cell proliferation, which increases the risk of tumor relapse (Houthuijzen et al, 2012; Chen et al, 2015). Furthermore, the secretion of chemokines and cytokines, the interaction of MSCs and tumor cells under radiation could complete and enhance the efficacy of the radiotherapy.

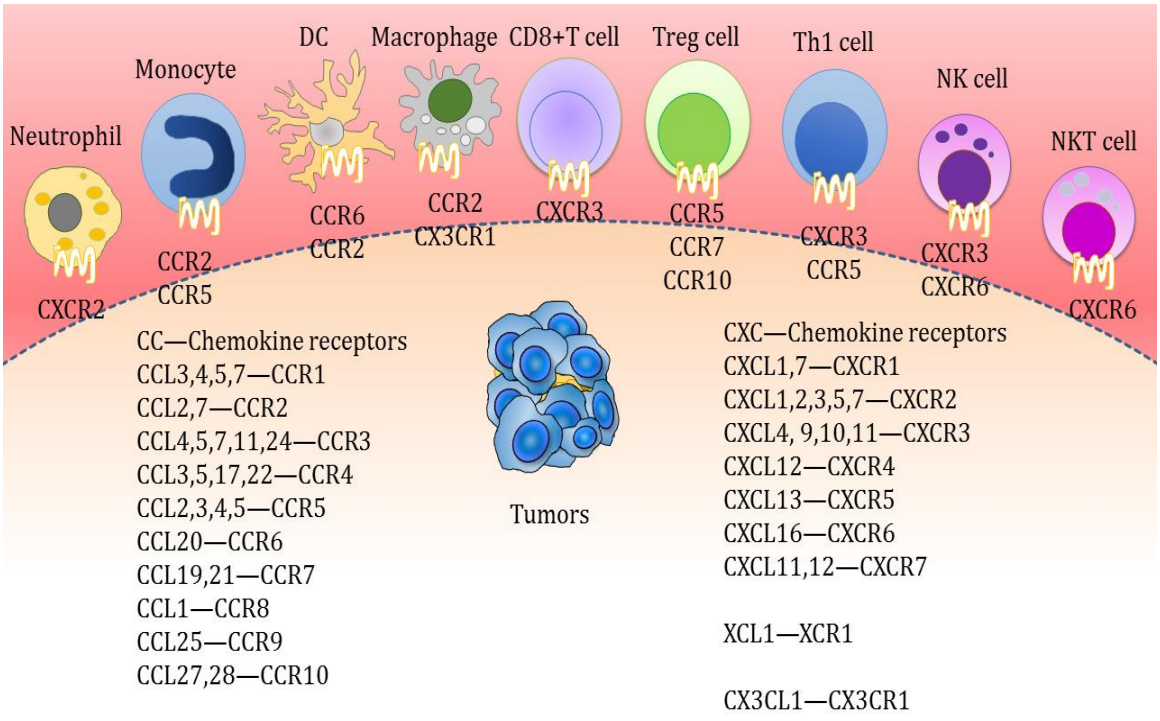

Figure 2: chemokine receptors and ligand pairings

2. Objectives

The translational research presented in this dissertation aimed to investigate the management of colorectal cancer from clinical and experimental aspects.

From a surgical perspective: It has been stated by several randomized clinical trials that the oncological outcomes and long-term survival rates were equivalents between the open and laparoscopic approach to treat rectal carcinoma. In addition, shorter hospital stay, rapid recovery, less hemorrhage, and reduced complications rates were achieved by a laparoscopic approach. However, surgery is related to substantial morbidity and mortality for patients with co-morbidities and elder patients. The first session of the dissertation aimed to analyze the outcome of patients with rectal cancer and elevated operative risk, either after conventional or laparoscopic approach.

From the experimental perspective: the aim of this part was to evaluate the interaction of inflammatory microenvironment and tumorigenesis as a therapeutic target. BM-MSC are important components which are able to mediate interactions between the inflammatory microenvironment and cancer cells. This study also demonstrated the tumor modulatory function of MSCs under radiation. Considering pluripotency and cancer tropism reaction of mesenchymal stromal cells, our study supposed that mesenchymal stromal cells could either sensitize or obtund the radiotherapy effect in colorectal carcinoma.

3. Summary of results

Paper I: Management of CRC from the perspective of surgical technique

Feng, H., Zhao, X., Zhang, Z., Han, D., Mao, Z., Lu, A., & Thasler, W. E. (2016). Laparoscopic Complete Mesocolic Excision for Stage II/III Left-Sided Colon Cancers: A Prospective Study and Comparison with D3 Lymph Node Dissection. *Journal of Laparoendoscopic & Advanced Surgical Techniques*, 26(8), 606-613. (***impact factor: 1.322[#], Quartiles: Surgery, Q2***)

Comparative study was performed among laparoscopic D3 lymphadenectomy and complete mesocolic excision (CME) to analyze the resemblance and distinction among different approaches for stage II and stage III colonic carcinoma left hemicolectomy.

1. The quality of lymph node dissection was comparable. The mean mesenteric area of the specimen was significant different ($P < .0001$), with 5730mm^2 in a superior rectal artery (SRA) preserving D3 lymph node dissection, 8145 mm^2 in non-preserving approach, and 8745 mm^2 in CME group.

2. Despite the quantity of lymphadenectomy measured by the lymph node count was higher in CME specimens, log odds of positive lymph nodes (LODDS) or positive nodes or lymph node ratio (LNR) were similar among the groups.

3. The difference among perioperative morbidity measured by the recovery time of bowel function was insignificant. Although operative time in D3 lymphadenectomy was longer, the variance was insignificant. Concerning the anastomotic leakage ($p = 0.34$) and vascular comorbidity ($p = 0.64$), the differences were insignificant either.

Paper II: Management of CRC from the perspective of comorbidity

> **Feng H**, Schiergens TS, Mao ZH, Zhao J, Shen X, Lu AG, Thasler WE. Long-term outcomes and propensity score matching analysis: rectal cancer resection for patients with elevated preoperative risk. *Oncotarget*. 2017 Apr 11;8(15):25679. (***impact factor: 5.168*, Quartiles: Oncology, Q1***)

This paper discussed how personalized risk assessment has significant implications for colorectal cancer surgical practice and management. Total mesorectal excision (TME) for rectal carcinoma is relevant to comorbidity and mortality, especially in patients experiencing comorbidities as well as elder patients. This study investigated the prognosis in these patients who went through conventional or laparoscopic rectal resection. The retrospective study recruited 132 high operative risk patients after propensity score matching from 2007 to 2011 with the elevated operative risk patient defining as Cr-POSSUM > 5% plus other risk factors.

1. The overall complication rate: conventional approach (71 %) > laparoscopic rectal resection (41%, p=0.0005).

2. Conventional open surgery was positively relevant to advanced Clavien-Dindo grading (p=0.02).

3. There was a positive correlation (p=0.01) between Clavien grading and Cr-POSSUM;

4. Patients with stage III-IV colorectal cancer and patients with preoperative respiratory disease or renal dysfunction experienced better overall survival rate through the laparoscopic approach (p< 0.0001, p=0.03, p=0.049).

Locoregional recurrence rate was similar between laparoscopic (6%) and conventional (8%) surgical approaches. No significant difference could be detected between patients underwent various approaches in stage I~II (p = 0.13, HR 0.5565, 95%CI 0.26-1.19).

However, the overall survival rate was elevated significantly in the laparoscopic surgery group with stage III-IV carcinoma (p<0.0001). These results demonstrated the advantages of the laparoscopic approach for elevated operative risk patients with rectal carcinoma

Paper III:

> **Feng, H**., Lu, A., Zhao, X., Han, D., Zhao, J., Shi, L., Schiergens TS, Lee SM, Zhang WP, ... & Thasler, W. E. (2015). Comparison of non-schistosomal rectosigmoid cancer and schistosomal rectosigmoid cancer. *World Journal of Gastroenterology*, 21(23), 7225-7232. (***impact factor: 3.411#, Quartiles: gastroenterology and hepatology, Q2***)

The retrospective study recruited 26 rectosigmoid carcinoma patients with colonic schistosomiasis diagnosed via endoscopy and pathological evaluations from 2009 to 2013. The clinicopathological features were compared for patients with non-schistosomal rectosigmoid carcinoma and schistosomal rectosigmoid carcinoma.

1. Patients with rectosigmoid schistosomiasis expressed a significantly elevated level of biomarker CA-125 and the majority presented with an earlier stage of cancer(p=0.003).

2. Different morphological characteristics of endoscopic finding----60% presented as fungating mass polyps, 30% were congestive and ulcerative polyps, cauliflower-like masses and annular masses were presented as 30% and 10%, respectively.

3. Thickened walls of the bowls, as well as linear and tram-track calcifications, could be detected by computed tomography (CT) scans.

Paper IV:

Feng, H., Zhao, J., Schiergens TS, Wang, P., Ou, B., Rami Al-Sayegh, Li, M., Lu, A., Yin, S., & Thasler, W. E. (2018) Bone marrow-derived mesenchymal stromal cells promote colorectal cancer cell death under low-dose irradiation. *British Journal of Cancer*. 2018 Jan 2; Epub. (***impact factor: 5.922*, Quartiles: Medicine, Q2***)

This paper discussed if the application of combined therapy of hBM-MSCs and radiotherapy has implications for colorectal cancer treatment. In the present study, 3D culture modules, co-cultivation modules, and colorectal tumor organoids were established. The flow cytometry and enzyme-linked immunosorbent array were applied to analyze the cytokines and chemokine secretion in mesenchymal stromal cells under low-dose radiation. X-ray was used to modulate the irradiating microenvironment.

1. *In vitro*, BM-MSCs could induce the epithelial-mesenchymal transitions (EMT) progression of colorectal cancer cells;

2. Low-dose ionizing radiation-induced BM-MSCs to release TNF-α and IFN-γ and present anticancer effect in 2D and 3D co-cultivation system.

3. Certain cytokine (TNF-α, IFN-γ) secreted by BM-MSCs under irradiation lead to impaired proliferation and apoptosis of colorectal cancer cells in 3D and co-culture system.

4. Irradiation of the co-cultivation system leads to caspase3 cleavage of the cancer cells. Additionally, it attenuated PI3K/AKT and ERK phosphorylation in colorectal cancer cells.

Taken together, potentially, BM-MSCs enhanced the effect of radiation associated cytotoxicity against colorectal carcinoma. Additionally, CRC patients might benefit from the conjunction of BM-MSC with radiotherapy.

4. References

Adam R, De Gramont A, Figueras J, et al. Managing synchronous liver metastases from colorectal cancer: a multidisciplinary international consensus[J]. *Cancer Treatment Reviews*, 2015, 41(9): 729-741.

Antoniou, S. A., Antoniou, G. A., Koch, O. O., Pointner, R., & Granderath, F. A. (2015). Laparoscopic colorectal surgery confers lower mortality in the elderly: a systematic review and meta-analysis of 66,483 patients. *Surgical endoscopy*, 29(2), 322.

Beets, G. L., Figueiredo, N. F., & Beets-Tan, R. G. (2017). Management of rectal cancer without radical resection. *Annual review of medicine*, 68, 169-182.

Cirocchi R, Trastulli S, Boselli C, et al. Radiofrequency ablation in the treatment of liver metastases from colorectal cancer[J]. *Cochrane Database of Systematic Reviews*, 2012, 6(6).

Edwards B K, Ward E, Kohler B A, et al. Annual Report to the Nation on the Status of Cancer, 1975-2006, Featuring Colorectal Cancer Trends and Impact of Interventions (Risk Factors, Screening, and Treatment) to Reduce Future Rates[J]. *Cancer*, 2010, 116(3): 544-573.

Fairfax, K. C., Everts, B., Smith, A. M., & Pearce, E. J. (2013). Regulation of the development of the hepatic B cell compartment during Schistosoma mansoni infection. *The Journal of Immunology*, 191(8), 4202-4210.

Fruhling, P., Nilsson, A., Duraj, F., Haglund, U., & Noren, A. (2017). Single-center nonrandomized clinical trial to assess the safety and efficacy of irreversible electroporation (IRE) ablation of liver tumors in humans: Short to mid-term results. *Ejso*, 43(4), 751-757.

García-Alfonso, P., Ferrer, A., Gil, S., Dueñas, R., Pérez, M. T., Molina, R., Capdevila, J., Safont, M. J., Castañón, C., Cano, J. M., … Lara, R. (2015). Neoadjuvant and conversion treatment of patients with colorectal liver metastasis: the potential role of bevacizumab and other antiangiogenic agents. *Targeted oncology*, 10(4), 453-65.

Garden O J, Rees M, Poston G J, et al. Guidelines for resection of colorectal cancer liver metastases[J]. *Gut,* 2006: 1-8.

Guillou PJ, Quirke P, Thorpe H, Walker J, Jayne DG, Smith AM, Heath RM, Brown JM, Group MRC CLASICC Trial Group (2005) Short-term endpoints of conventional versus laparoscopic-assisted surgery in patients with colorectal cancer (MRC CLASICC trial): multicentre, randomised controlled trial. *Lancet* 365:1718–1726

Jacobs, J., Smits, E., Lardon, F., Pauwels, P., & Deschoolmeester, V. (2015). Immune checkpoint modulation in colorectal cancer: What's new and what to expect. *Journal of immunology research*, *2015*.

Jemal, A., Bray, F., Center, M. M., Ferlay, J., Ward, E., & Forman, D. (2011). Global cancer statistics. *CA: a cancer journal for clinicians*, 61(2), 69-90.

Jones, R. P., & Poston, G. J. (2017). Resection of liver metastases in colorectal cancer in the era of expanding systemic therapy. *Annual review of medicine*, 68, 183-196.

Jones, R. P., Jackson, R., Dunne, D. F. J., Malik, H. Z., Fenwick, S. W., Poston, G. J., & Ghaneh, P. (2012). Systematic review and meta‐analysis of follow‐up after hepatectomy for colorectal liver metastases. *British Journal of Surgery*, 99(4), 477-486.

Lykoudis, P. M., O'reilly, D., Nastos, K., & Fusai, G. (2014). Systematic review of surgical management of synchronous colorectal liver metastases. *British Journal of Surgery*, 101(6), 605-612.

Misiakos, E. P., Karidis, N. P., & Kouraklis, G. (2011). Current treatment for colorectal liver metastases. *World J Gastroenterol*, 17(36), 4067-4075.

Nagarsheth, N., Wicha, M. S., & Zou, W. (2017). Chemokines in the cancer microenvironment and their relevance in cancer immunotherapy. *Nature Reviews Immunology*.

Nordlinger, B., Van Cutsem, E., Gruenberger, T., et al. (2009). Combination of surgery and chemotherapy and the role of targeted agents in the treatment of patients with colorectal liver metastases: recommendations from an expert panel. *Annals of Oncology*, 20(6), 985-992.

Pardoll, D. M. (2012). The blockade of immune checkpoints in cancer immunotherapy. *Nature Reviews Cancer*, 12(4), 252-264.

Saâda-Bouzid, E., Defaucheux, C., Karabajakian, A., Palomar Coloma, V., Servois, V., Paoletti, X., ... & Peyrade, F. (2017). Hyperprogression during anti-PD-1/PD-L1 therapy in patients with recurrent and/or metastatic head and neck squamous cell carcinoma. *Annals of Oncology*.

Sharma, P., & Allison, J. P. (2015). The future of immune checkpoint therapy. *Science,* 348(6230), 56-61.

Siegel, R. L., Ward, E. M., & Jemal, A. (2012). Trends in colorectal cancer incidence rates in the United States by tumor location and stage, 1992–2008. *Cancer Epidemiology and Prevention Biomarkers*, 21(3), 411-416.

Siegel, R. L., Miller, K. D., & Jemal, A. (2017). Cancer statistics, 2017. *CA: a cancer journal for clinicians*, 67(1), 7-30.

Smith, P., Fallon, R. E., Mangan, N. E., Walsh, C. M., Saraiva, M., Sayers, J. R., et al. (2005). Schistosoma mansoni secretes a chemokine binding protein with anti-inflammatory activity. *Journal of Experimental Medicine*, 202(10), 1319-1325.

Society, A.C., 2017. Cancer facts and figures 2017.

Surveillance, Epidemiology, and End Results (SEER) Program. SEER*Stat Database: Incidence-SEER 9 Regs Research Data with Delay-Adjustment, Malignant Only, Nov. 2015 Sub (1975-2013) <Katrina/Rita Population Adjustment>-Linked To County Attributes-Total US, 1969-2014 Counties. Bethesda, MD: National Cancer Institute, Division of Cancer Control and Population Sciences, Surveillance Research Program, Surveillance Systems Branch; 2016.

Vatandoust, S., Price, T. J., & Karapetis, C. S. (2015). Colorectal cancer: Metastases to a single organ. *World journal of gastroenterology*, 21(41), 11767.

Vennarecci, G., Laurenzi, A., Sandri, G. B., Rizzi, E. B., Cristofaro, M., et al. (2014). The ALPPS procedure for hepatocellular carcinoma. *Ejso*, 40(8), 982-988.

Wicherts, D. A., de Haas, R. J., Andreani, P., Sotirov, D., Salloum, C., et al. (2010). Impact of portal vein embolization on long‐term survival of patients with primarily unresectable colorectal liver metastases. *British Journal of Surgery*, 97(2), 240-250.

Young, P. E., Womeldorph, C. M., Johnson, E. K., Maykel, J. A., Brucher, B., et al. (2014). Early detection of colorectal cancer recurrence in patients undergoing surgery with curative intent: current status and challenges. *J Cancer*, 5(4), 262-271.

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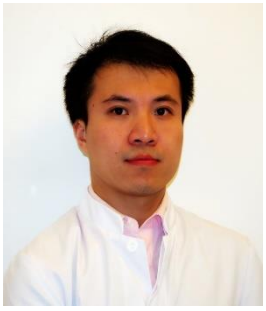
6. Curriculum vitae



Hao Feng Sept.10, 1984 Chinese Married

Academic Background

- Please check the printed version

Publications

Original Articles:

Feng H, Tong H, Yan J, He M, Chen W, Wang J. Genomic Features and Clinical Characteristics of Adolescents and Young Adults With Cholangiocarcinoma. *Front Oncol*. 2020 Jan 14;9:1439. doi: 10.3389/fonc.2019.01439. PMID: 32010606.

Yan, J., **Feng H***(Co-first),* Wang, H., Yuan, F., Yang, C., Liang, X., ... & Wang, J. Hepatic artery classification based on three-dimensional CT.. *British Journal of Surgery.2020 Feb*, doi:10.1002/bjs.11458. PMID: 32057096

Feng H, Jing-kun Zhao, Tobias Schiergens, puxiongzhi Wang, Baochi Ou, Rami Al-Sayegh, Minglun Li, Aiguo LU, Shuai Yin, and Wolfgang Thasler Bone marrow-derived mesenchymal stem cells promote colorectal cancer cell death under low dose irradiation. British Journal of Cancer (2018), 1–13
doi: 10.1038/bjc.2017.415

Zhao J, Ou B, Han D, Wang P, Zong Y, Zhu C, Liu D, Zheng M, Sun J, **Feng H** (*Corresponding*), Lu A. Tumor-derived CXCL5 promotes human colorectal cancer metastasis through activation of the ERK/Elk-1/Snail and AKT/GSK3β/β-catenin pathways. *Mol Cancer***.** 2017 Mar 29;16(1):70.
doi: 10.1186/s12943-017-0629-4. PMID: 28356111

Ou B, Zhao J, Guan S, Wangpu X, Zhu C, Zong Y, Ma J, Sun J, Zheng M, **Feng H** *(Corresponding),* Lu A. Plk2 promotes tumor growth and inhibits apoptosis by targeting Fbxw7/Cyclin E in colorectal cancer. *Cancer Lett* 2016 Oct 1;380(2):457-66.
doi: 10.1016/j.canlet.2016.07.004. PMID: 27423313

Feng H, Schiergens TS, Mao ZH, Zhao J, Shen X, Lu AG, Thasler WE. Long-term outcomes and propensity score matching analysis: rectal cancer resection for patients with elevated preoperative risk. *Oncotarget.* 2016 Dec 9.
doi: 10.18632/oncotarget.13827. PMID: 27974672

Dong W, Lu A, Zhao J, Yin S, Ou B, **Feng H** *(Corresponding)*, An efficient and simple co-culture method for isolating primary human hepatic cells: Potential application for tumor microenvironment research. *Oncol Rep***.** 2016 Oct;36(4):2126-34.
doi: 10.3892/or.2016.4979. PMID: 27498714

Jingkun Zhao, Baochi Ou, **Hao Feng**, Puxiongzhi Wang, Shuai Yin, Congcong Zhu, Shenjie Wang, Chun Chen, Minhua Zheng, Yaping Zong, Jing Sun, Aiguo Lu. Overexpression of CXCR2 predicts poor prognosis in patients with colorectal cancer. *Oncotarget*
doi: 10.18632/oncotarget.16086

Ou B, Zhao J, Guan S, **Feng H**, Wangpu X, Zhu C, Zong Y, Ma J, Sun J, Shen X, Zheng M, Lu A.CCR4 promotes metastasis via ERK/NF-κB/MMP13 pathway and acts downstream of TNF-α in colorectal cancer. *Oncotarget*. 2016 Jul 26;7(30):47637-47649.
doi: 10.18632/oncotarget.10256. PMID: 27356745

Feng H, Zhao XW, Zhang Z, Han DP, Mao ZH, Lu AG, Thasler WE. Laparoscopic Complete Mesocolic Excision for Stage II/III Left-Sided Colon Cancers: A Prospective Study and Comparison with D3 Lymph Node Dissection. *J Laparoendosc Adv Surg Tech A*. 2016 Aug;26(8):606-13.
doi: 10.1089/lap.2016.0120. PMID: 27183112

Schiergens TS, Lüning J, Renz BW, Thomas M, Pratschke S, **Feng H,** Lee SM, Engel J, Rentsch M, Guba M, Werner J, Thasler WE. Liver Resection for Non-colorectal Non-neuroendocrine Metastases: Where Do We Stand Today Compared to Colorectal Cancer? *J Gastrointest Surg***.** 2016 Jun;20(6):1163-72.
doi: 10.1007/s11605-016-3115-1. PMID: 26921025

Ma J, Zhao J, Lu J, Wang P, **Feng H**, Zong Y, Ou B, Zheng M, Lu A. Cadherin-12 enhances proliferation in colorectal cancer cells and increases progression by promoting EMT. *Tumour Biol***.** 2016 Jul;37(7):9077-88.
doi: 10.1007/s13277-015-4555-z. PMID: 26762412

Wang P, Zhao J, Yang X, Guan S, **Feng H**, Han D, Lu J, Ou B, Jin R, Sun J, Zong Y, Feng B, Ma J, Lu A, Zheng M. PFDN1, an indicator for colorectal cancer prognosis, enhances tumor cell proliferation and motility through cytoskeletal reorganization. *Med Oncol*. 2015 Dec;32(12):264.
doi: 10.1007/s12032-015-0710-z. PMID: 26553318

Schiergens TS, Dörsch M, Mittermeier L, Brand K, Küchenhoff H, Lee SM, **Feng H**, Jauch KW, Werner J, Thasler WE. Thirty-day mortality leads to underestimation of postoperative death after liver resection: A novel method to define the acute postoperative period. *Surgery***.** 2015 Dec;158(6):1530-7.
doi: 10.1016/j.surg.2015.07.019. PMID: 26298028

Feng H, Lu AG, Zhao XW, Han DP, Zhao JK, Shi L, Schiergens TS, Lee SM, Zhang WP, Thasler WE. Comparison of non-schistosomal rectosigmoid cancer and schistosomal rectosigmoid cancer. *World J Gastroenterol*. 2015 Jun 21;21(23):7225-32.
doi: 10.3748/wjg.v21.i23.7225. PMID: 26109809

Lu J, Ma J, Cai W, Wangpu X**, Feng H**, Zhao J, Guan S, Zong Y, Lu A. CC motif chemokine ligand 19 suppressed colorectal cancer in vivo accompanied by an increase in IL-12 and IFN-γ. *Biomed Pharmacother***.** 2015 Feb;69:374-9.
doi: 10.1016/j.biopha.2014.12.032. PMID: 25661385

Lu AG, Zhao XW, Mao ZH, Han DP, Zhao JK, Wang P, Zhang Z, Zong YP, Thasler W, **Feng H**

(*Corresponding*), Challenge or opportunity: outcomes of laparoscopic resection for rectal cancer in patients with high operative risk. *J Laparoendosc Adv Surg Tech A.* 2014 Nov;24(11):756-61. doi: 10.1089/lap.2014.0163. PMID: 25376002

Lu J, Zhao J, **Feng H**, Wang P, Zhang Z, Zong Y, Ma J, Zheng M, Lu A. Antitumor efficacy of CC motif chemokine ligand 19 in colorectal cancer. *Dig Dis Sci.* 2014 Sep;59(9):2153-62. doi: 10.1007/s10620-014-3138-y. PMID: 24705643

Zhao J, Li P, **Feng H,** Wang P, Zong Y, Ma J, Zhang Z, Chen X, Zheng M, Zhu Z, Lu A. Cadherin-12 contributes to tumorigenicity in colorectal cancer by promoting migration, invasion, adhesion and angiogenesis. *J Transl Med.* 2013 Nov 15;11:288. doi: 10.1186/1479-5876-11-288.PMID: 24237488

Long-term outcome of laparoscopic-assisted right-hemicolectomy with D3 lymphadenectomy versus open surgery for colon carcinoma. Han DP, Lu AG, **Feng H**, Wang PX, Cao QF, Zong YP, Feng B, Zheng MH. *Surg Today.* 2014 May;44(5):868-74. doi: 10.1007/s00595-013-0697-z. PMID: 23989942

7. Appendix:

Paper I
Paper II
Paper III
Paper IV

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Laparoscopic Complete Mesocolic Excision for Stage II/III Left-Sided Colon Cancers: A Prospective Study and Comparison with D3 Lymph Node Dissection

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Abstract

Background: To investigate the similarities and differences of laparoscopic complete mesocolic excision (CME) to a colon resection with a D3 lymphadenectomy for the stage II/III left-sided colon carcinoma.
Methods: Patients between July 2011 and August 2014 were randomized into D3 and CME groups. Mesenteric area, log odds of positive lymph nodes (LODDS), and other operative parameters were collected and assessed.
Results: The average specimen sizes were $5730 \pm 828\,mm^2$ in superior rectal artery (SRA)-preserving D3, $8145 \pm 1022\,mm^2$ in SRA-nonpreserving D3, and $8745 \pm 1039\,mm^2$ in the CME group; the differences were significant ($P < .0001$). The number of lymph nodes collected from CME specimens was larger, but the CME specimens did not contain an elevated value of LODDS or positive nodes or lymph node ratio (LNR). There were also no significant differences between recovery times of bowel function. Although it took more operation time in D3 approach, especially in SRA-preserving D3 operation, the difference was not significant. Concerning the leakage rate ($P = .34$) and vessel-related complications ($P = .64$), there were no significant differences either.
Conclusions: Both standard D3 resection and CME could achieve a high quality of mesocolic plane grade for stage II/III colon cancer. The LODDS and LNR were comparable, and those were not relevant to mesenteric size.

Introduction

During the last decades, from the first laparoscopic hemicolectomy performed by Jacob in 1993[1] and the D3 excision in 1996 to the concept of complete mesocolic excision (CME), which was raised in 2009,[2] the radical dissection rate of colonic cancer has been improved to pursue a clearer and adequate surgical margin, lower local recurrence rate, and better long-term postoperative outcome.[3,4]

Based on the anatomy and embryology, CME has already been the standard procedure for colon cancer excision, which ensures the complete removal of the mesocolon by ''high ligation of central vascular,'' ''cutting off main vessel,'' and ''central lymph node dissection.'' However, D3 excision has also achieved more than 15 years of satisfactory clinical outcomes in Asia, especially in Japan, China, and South Korea.[5] D3 excision underlined the dissection of lymph nodes that were located at the root of the central vessels.[6,7] This study is to investigate the similarities and differences of laparoscopic CME to a colon resection with a D3 lymphadenectomy for the stage II/III left-sided colon carcinoma.

Materials and Methods

Patient selection

A prospective trial was performed in the Shanghai Minimally Invasive Surgery Center between July 2011 and August 2014. Forty-one cases with colorectal cancer went through laparoscopic left-sided colon resection and were randomly divided into two groups: the D3 and CME group. Primary outcome (lymph node ratio [LNR]) and secondary outcomes (size of mesentery specimen, positive lymph nodes, leakage, etc.) were compared between the two groups.

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The inclusion criteria were patients with descending colon, sigmoid-descending colon junction identified by preoperative histopathological biopsy, computed tomography (CT) scan, MRI, and endoscopy findings; with preoperative tumor stage II or III according to the AJCC 7th, emergency resections were excluded, so that 38 patients were included in this study. Video and photographs of the operation and the resected samples were assessed by three independent professional observers to evaluate the quality of the approaches.

Informed consent was obtained from all patients. The primary outcome of this study was the LNR, which ranges from 0.2 to 0.4; a previous study demonstrated that the values were well spread across 0.2–0.4, with a mean of 0.28 and standard deviation (SD) of 0.27 and 20 patients per arm. An independent *t*-test would provide 80% power to detect a difference between groups at two-sided α of 0.05. Assuming a dropout rate of 20%, we thus aimed at enrolling 20 subjects in each arm.

Eligible patients were randomly allocated to CME or D3 colorectal surgery. To obtain a balanced distribution of different surgical procedures in the two groups, randomization lists were generated by a computer program, assuming that all patients had the same probability of undergoing CME or D3 surgery. Randomization by individual random numbers was also performed. Assignments were made by means of sealed sequenced masked envelopes that were opened, before the induction of anesthesia, by a nurse unaware of the trial design.

Ethics statement

Protocol approval for all research performed was obtained from the Medical Ethics Committee of Shanghai Ruijin Hospital according to the Declaration of Helsinki.

Operation procedure

Laparoscopic CME in left hemicolectomy. The dissection started at the left colic artery (LCA), and the root of inferior mesenteric artery (IMA) was then ligated with Ham-o-Locks, preserving the preaortic fascia (PAF)[8,9] and the superior hypogastric plexus (SHP)[10] (Fig. 1A, B). Dissection was performed until the pancreas was detected after an incision of the

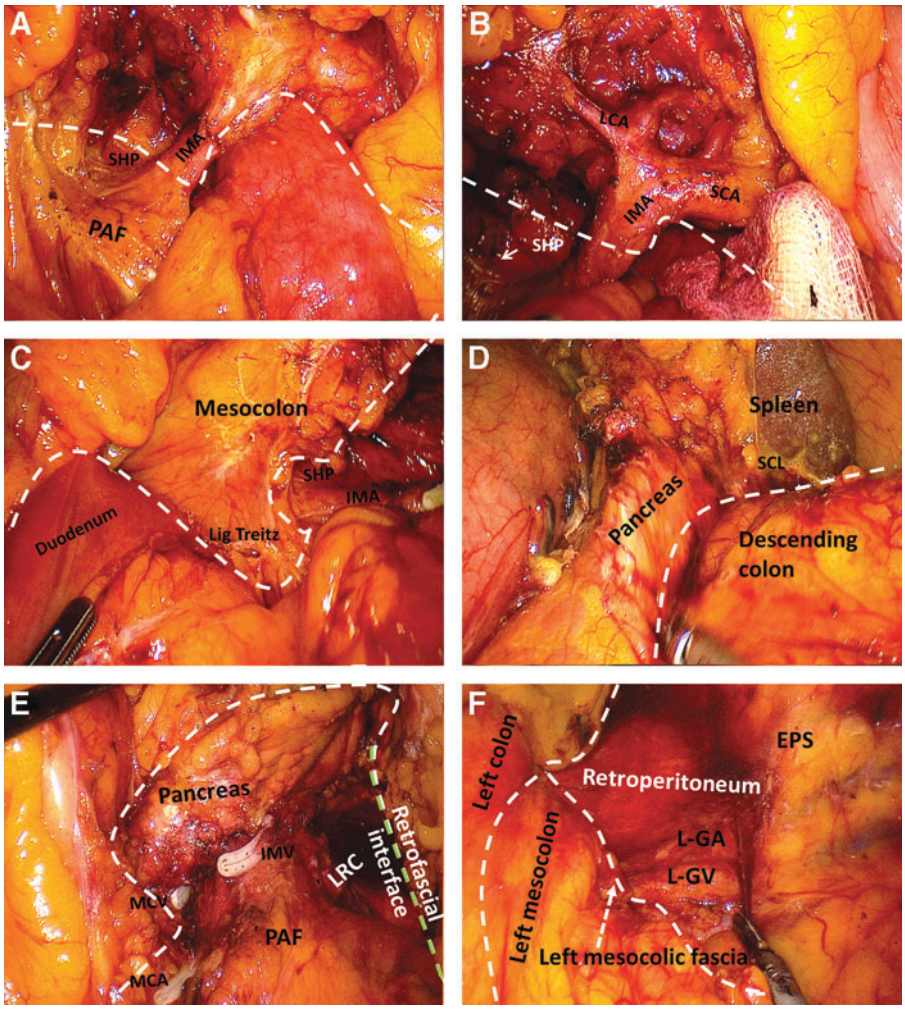

FIG. 1. Laparoscopic CME in left hemicolectomy. **(A,B)** the dissection and ligation of IMA; **(C,D,E)**, IMV exposure ligation; **(F)**, left lateral peritoneal reflection mobilization. CME, complete mesocolic excision; EPS, extraperitoneal space; IMA, inferior mesenteric artery; IMV, inferior mesenteric vein; LCA, left colic artery; L-GA, left gonadal artery; L-GV, left gonadal vein; Lig Treitz, Treitz ligament; LRC, left retrocolic space; MCA, middle colic artery; MCV, middle colic vein; PAF, preaortic fascia; SCA, sigmoid artery; SCL, splenocolic ligament; SHP, superior hypogastric plexus.

Treitz ligament (Lig Treitz) (Fig. 1C). After separating the right gastrocolic ligament (RGL) and splenocolic ligament (SCL) (Fig. 1D), the origin of the inferior mesenteric vein (IMV) was ligated after dissecting the inferior edge of the pancreas (Fig. 1E). Lymph nodes surrounding the mid-colic artery (MCA) and its left branch were dissected, and the vessel was ligated. The dissection of the splenic flexure area was laterally followed by dissection of the left lateral peritoneal reflection, colofascial and mesofascial plane mobilization, and dissection of the peritoneal reflection at the base of mesosigmoid to the extraperitoneal space (EPS) (Fig. 1F). The Toldt's fascia was dissected from the iliac ridge to the splenic flexure to dissect the phrenicocolic ligament (PCL) and sustentaculum lienis, which acted as a brassiere to the lower pole of the spleen.

Laparoscopic D3 lymph node dissection. The harvested lymph nodes were mapped according to the Japanese guideline. According to this guideline, lymph nodes over the root of IMA were defined as N3 lymph nodes,[11] and therefore, the D3 lymph node dissection was performed as follows[12]: for the cancers of descending colon, and for those tumors whose feeding vessels proximal to sigmoid colon were the first branch of sigmoid colon artery (SCA), the root of IMA was exposed with an ultrasonic surgical device. The LCA was then exposed, clipped, and cut, however, the superior rectal artery (SRA) was preserved—SRA-preserving D3. In the other way (SRA-nonpreserving D3), the central vessel IMA should be ligated at the root.[13] After the lymph node (LN) dissection, the mesentery of the sigmoid and descending colon was mobilized posteriorly in a medial to lateral approach, maintaining the layer on the left ureter and gonadal vessels[14,15] (Fig. 2).

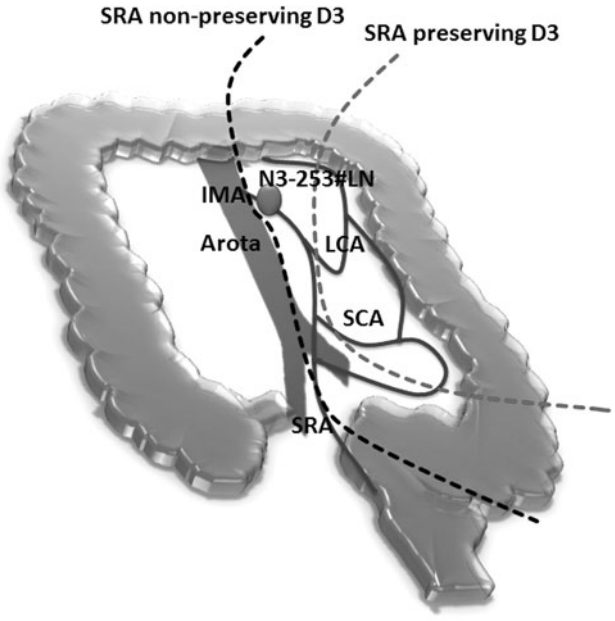

FIG. 2. Schema of laparoscopic D3 left hemicolectomy. The gray line shows the cutting strategy for SRA preservation and the black line shows the cutting strategy of the D3 dissection without SRA preservation. LCA, left colic artery; SCA, sigmoid artery; SRA, superior rectal artery.

Calculation of mesenteric size

Previous studies measured the length of specimens, or used softwares to measure the macroscopic area. Instead, this study calculated the area of left mesocolon with a formula. Important specimen parameters were included to reduce measurement errors induced by the inconsistency of proportional scale (Supplementary Fig. S1; Supplementary Data are available online at www.liebertpub.com/lap).

Assessments of the operation

The plane of dissection was evaluated by a histopathologist on the basis of the presence and extent of any identifiable mesocolic defect. The grading system referred to the system reported by West et al.,[16] including dissection in the *mesocolic plane* (intact mesocolon), *intramesocolic plane* (significant mesocolic defects that do not expose the muscularis propria), or *muscularis propria plane* (significant and extensive defects that expose areas of muscularis propria).

Log odds of positive lymph nodes

First, the fresh specimens were examined by the surgeons, the lymph nodes were carefully mapped according to their location, and then, whether there were more lymph nodes that remained was double checked by the pathology department. The LNR was defined as the number of positive lymph nodes divided by the number of examined lymph nodes (NELN). Log odds of positive lymph nodes (LODDS) were classified according to log (number of positive nodes + 0.5)/(number of total examined nodes-positive nodes + 0.5) values as follows: LODDS0 (≤ -1.36), LODDS1 (-1.36 to -0.53), and LODDS2 (≥ -0.53).[17]

Statistical methods

Logistic regression was performed to analyze the correlation of mesentery sizes and LNR. A paired Student's *t*-test and ANOVA were also used. Analyses were performed with Stat View 5.0 for Windows (SAS Institute, Inc., Cary, NC). The χ^2 test or Fisher's exact test was applied to analyze the categorical variables, as appropriate. A probability (*P)* value of <.05 was regarded as statistically significant.

Results

There was no significant difference in age ($P = .35$), gender ($P = .57$), BMI ($P = .54$), tumor location ($P = .20$), *p*tumor stage ($P = .74$), intraoperative blood loss ($P = .46$), and splenic bleeding ($P = .65$) among these groups.

Assessment of plane grades

Eighteen cases were considered as *mesocolic plane* grade in SRA-preserving D3 group, 19 cases were considered as *mesocolic plane* grade in the SRA-nonpreserving D3 group. In CME group, *mesocolic plane* grade and *intramesocolic plane* grade were 19 and 1 cases; the differences between the two groups were insignificant.

Mesentery size

The average specimen sizes were $5730 \pm 828\,mm^2$ (SRA-preserving D3), $8145 \pm 1022\,mm^2$ (SRA-nonpreserving D3),

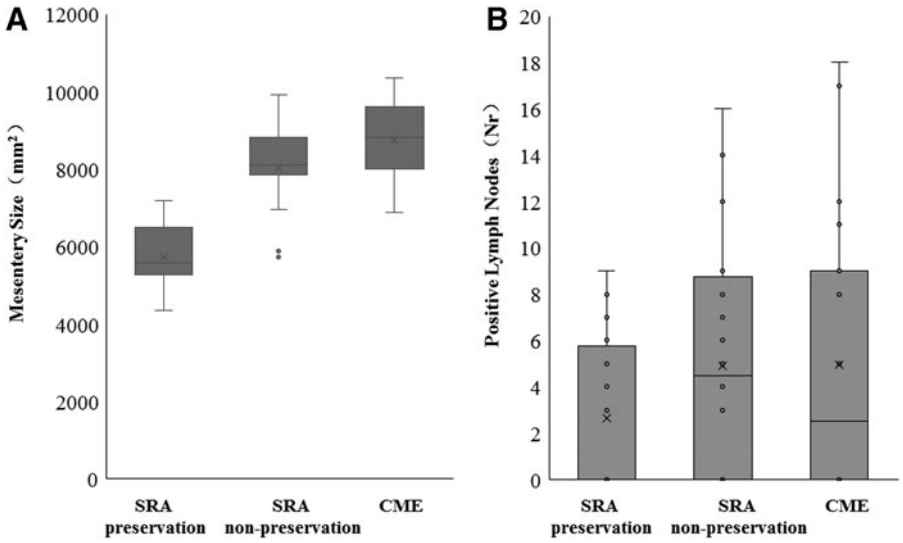

FIG. 3. The correlation among area of mesentery **(A)**, positive lymph node **(B)**, and surgical procedures.

and $8745 \pm 1039\,mm^2$ (CME group), respectively. The differences between SRA-preserving and SRA-nonpreserving groups were significant ($P < .0001$), so were the differences among the three groups ($P < .0001$). It was because that the incisal margin should be ~ 10 cm longitudinally in D3 excision, following the so-called 10 cm rule, the length was measured by laparoscopic forceps during the operation. However, there was no significant difference between the SRA-nonpreserving group and CME group ($P = .0538$) (Figs. 3 and 4).

Positive lymph node, LODDS, and LNR

More lymph nodes were harvested in SRA-nonpreserving D3 and CME specimens compared to SRA-preserving D3 specimens ($P1 = .004$, $P = .05$), but not between the former two groups ($P = .67$) (Table 1 and Figs. 3 and 4). The LNRs were 24%, 31%, and 25% in those groups and did not show a significant difference ($P = .75$, $P1 = .57$, $P2 = .49$). Furthermore, the regression analysis showed that the mesentery size was related to total LN, but not to the LNR. Nine in 31 cases of stage III patients had the positive center lymph nodes. Interestingly, we also found unilateral double ureters in one of the patients (Fig. 5C).

Other articles suggested that LODDS provide more valuable information than LNR independently of the NELN. In the present study, there was no significant differences in LODDS among each group ($P = .80$). And notably, LODDS and LNR curves have a similar trend.

In this study, the distance between proximal margin/distal margin and central vessel was measured soon after the fresh specimens were collected, so were the Δa and sample length.

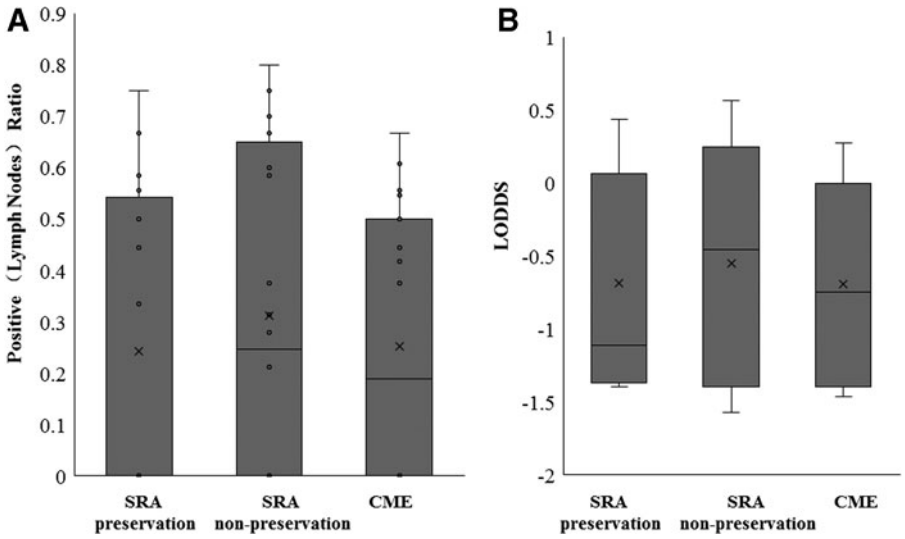

FIG. 4. The correlation among positive lymph node ratio **(A)**, LODDS **(B)**, and surgical procedures. LODDS, log odds of positive lymph nodes.

Table 1. Characteristics of Patients and Specimens

	SRA-preserving D3 (n = *20*)	*SRA-nonpreserving D3* (n = *20*)	*CME* (n = *20*)	P
Age	58.1	66.8	61.8	$P = .35$
Gender (male/female)	3/8	7/6	7/7	$P = .57$
BMI (kg/m^2)	25.8	26.2	26.9	$P = .54$
Tumor location				
Descending colon	13 (1)	9	12	
Splenic flexure	8 (1)	6	5	$P = .20$
Sigmoid-descending colon junction	0	5	3	
pTumor stage				
II	11	8	10	$P = .74$
III	9	12	10	
Area of mesentery (mm^2)	5730 ± 828	8145 ± 1022	8745 ± 1039	$P < .0001$, $P1 < .0001$, $P2 = .0538$
Sample length (cm)	24.6	28.2	31.2	$P = .0001$, $P1 = .0003$, $P2 = .99$
Minimum radius (mm)	110.8	119.8	118.4	$P = .11$, $P1 = .08$, $P2 = .64$
Intraoperative blood loss (mL)	78.3	90.8	92.1	$P = .18$, $P1 = .11$, $P2 = .85$
Splenic bleeding	1	3	2	$P = .65$, $P1 = .38$, $P2 = .57$
Positive LN	2.65 ± 3.3	4.90 ± 5.1	4.95 ± 6.0	$P = .52$, $P1 = .24$, $P2 = .87$
Total number of LN	10.2 ± 2.0	14.8 ± 3.5	15.8 ± 7.4	$P = .05$, $P1 = .004$, $P2 = .67$
LNR	0.24 ± 0.3	0.31 ± 0.3	0.25 ± 0.3	$P = .75$, $P1 = .57$, $P2 = .49$
LODDS				
LODDS0	5	7	8	$P = .80$
LODDS1	6	2	2	$P1 = .56$, $P2 = .57$
LODDS2	9	11	10	
PM, ED (mm)	105.5, 46.1	110.69, 47.69	111.14, 47.36	
SinΔa	0.79	0.92	0.94	$P < .0001$, $P1 < .0001$, $P2 = .08$

*P*1, *P*-value between SRA-preserving and SRA-nonpreserving D3; *P2*, *P*-value between SRA-nonpreserving D3 and CME; *P*, *P*-value among the three groups.

CME, complete mesocolic excision; ED, excision edge; LN, lymph node; LRC, left retrocolic space; LNR, lymph node ratio; LODDS, log odds of positive lymph nodes; PM, proximal margin mesocolon; SRA, superior rectal artery.

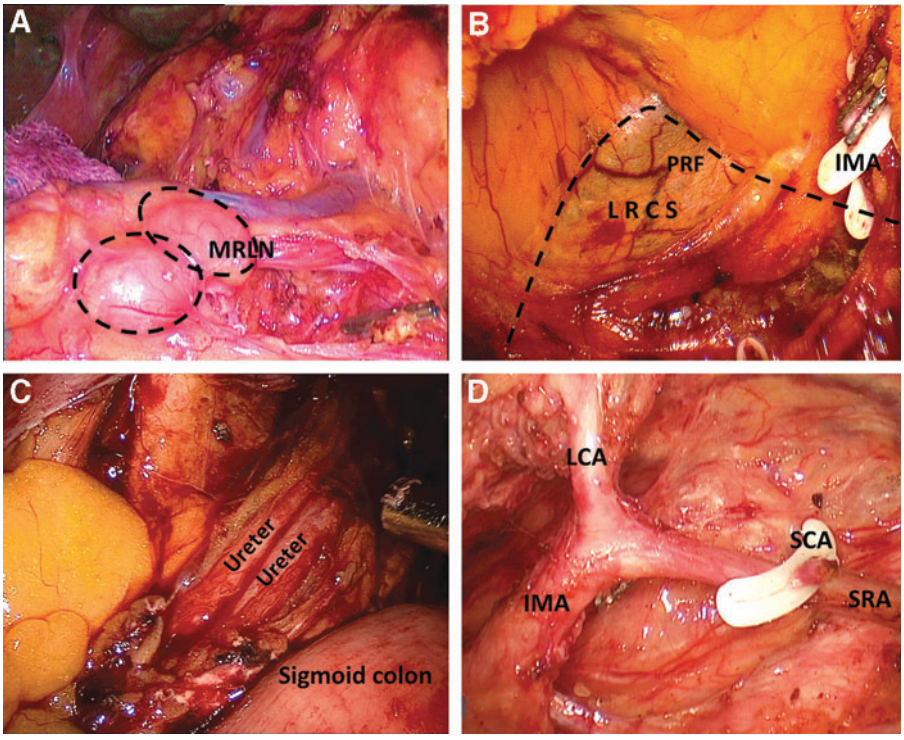

FIG. 5. **(A)** lymph node metastasis at the root of vessel; **(B)**, accurate exposure of PRF and LRC; **(C)**, ureter variation; **(D)**, variation of branch vessels of sigmoid and LCA. IMV, the inferior mesenteric vein; LCA, left colic artery; LRCS, left retrocolic space; MRLN, mesenteric root lymph nodes; PRF, prerenal fascia; SCA, sigmoid artery; SRA, superior rectal artery.

Table 2. Short-Term Postoperative Outcomes

	SRA-preserving D3 (n = *20*)	*SRA-nonpreserving D3* (n = *20*)	*CME* (n = *20*)	P
Operation time	124.6	119.8	119.0	.67
Bowel function recovery	2.7	2.7	2.9	.81
Liquid diet	1.67	1.69	1.64	.98
Postoperative analgesia	2	2	4	.56
Leakage	0	1	2	.34
Vessel-related complication	0	1	1	.64

CME, complete mesocolic excision; SRA, superior rectal artery.

There were significant differences among the three groups concerning the sinα ($P < .0001$), but not between the SRA-preserving group and CME group. Other data are shown in Table 1.

Postoperative short-term outcomes

There was no significant difference in bowel function recovery time, liquid diet time, and postoperative analgesia ($P = .56$) among these three groups (Table 2). It took more operating time in D3 operation (SRA-preserving D3, 124.6 minutes, SRA-nonpreserving D3, 119.8 minutes, CME 119 minutes, $P = .67$), especially in SRA-preserving D3 operation, owing to the separation and dissection for SCA and SRA, but the difference was not significant. We evaluated the postoperative complications, respectively: one patient got leakage 6 days after operation in the D3 group and one patient got leakage 5 days after operation in the CME group ($P = .34$). Concerning the vessel-associated complications, there was no significant difference ($P = .64$).

Discussion

CME and D3, unified and complementary

In 2006, the Japanese Society for Cancer of Colon and Rectum published the 7th edition guideline; the guideline defined again the lymph node grouping and recommended the same range for D3 dissection. A radical D3 lymph node dissection of colon cancer should be performed to remove the paracolic lymph nodes (N1), intermediate lymph nodes (N2), and central lymph nodes (N3).[18] In 2010, the Chinese Ministry of Health issued the Chinese standard for colorectal cancer treatment,[19] which recommended that lymph node dissection for advanced colon cancers without distant metastasis should cover the same three groups. For left-sided colon cancers, D3 dissection specially clears the lymph nodes located along the root of IMA (between the origin of the artery and that of the LCA). The advantage of D3 excision could be (1) to avoid excess mobilization of the transverse colon for anastomosis and (2) to preserve the normal sigmoid colon in view of minimally invasive surgery. What is the difference between the SRA-nonpreserving D3 and CME? Theoretically, they should be equivalent procedures with the principles of both procedures being the same. Rates for intact mesocolic plane were both high as were lymph node yield, but both were significantly greater in the CME specimens; however, lymph node positivity rates were equivalent. It was postulated that the differences between these specimens were likely related to the technique adopted by each country. The Japanese D3 has previously shown that positive lymph node spread rarely occurs beyond 10 cm from the tumor and as such, D3 rarely resects more than 10 cm from the tumor. The CME specimens in contrast were significantly longer, and hence, the resulting larger mesocolic surface area and lymph node counts. Survival and local recurrence data are similarly impressive.

Both CME and D3 approach advocate careful dissection along embryologic planes, resulting in a marked improvement in the oncologic quality of the specimen without increasing the postoperative complications or mortality rates.[20] Compared to SRA-nonpreserving D3, the CME technique usually includes removal of the next vascular arcade beyond the 10 cm margin to ensure that there was no remaining regional paracolic nodes; it probably also accounts for the marked increased resected area of mesentery in CME specimens.[21] In our previous study, which included 31 cases with left-sided colon cancer that underwent laparoscopic CME between September 2010 and December 2012, the complications were observed in 4 cases (12.9%), the total number of lymph nodes removed was 13.9 ± 5.0, including 4.7 ± 2.6 paracolic lymph nodes, 5.4 ± 3.7 intermediate lymph nodes, and 5.4 ± 3.7 nodes at the mesocolic root.[22]

Center vessel ligation (CVL) and infarct layer dissection are the key points of CME technology, the main vessel ties are always performed at the root. The advantages of CVL are showed in several studies, while controversial conclusions also exist, which suggested no demonstrable benefit. Considering the harvested lymph nodes, several lines of evidence suggested that lymph node metastasis is a stochastic, rather than a stepwise, phenomenon,[23] therefore, it is not surprising that some clinical researches failed to identify a survival benefit from extensive lymphadenectomy compared to standard resection, which suggested that a therapeutic benefit of extensive lymphadenectomy in colon cancer may not be convincing. That might also explain the similarly impressive reported outcomes of left-sided colonic tumors in both CME and D3 excision. This study showed that mesentery size was not relevant to LNR or positive LN. As reported before, significant longitudinal spread is rare, about 0% for left-sided tumor; therefore, it was not surprising that the CME specimens did not contain an increased number of positive nodes. In contrast, Le Voyer et al. suggested that the number of total lymph nodes was relevant to prognosis of colorectal cancer although some of which were negative.[24] Especially for patients with pN1 tumors, survival improved as the number of collected lymph nodes increased. An absolute 23% improvement (90% vs. 67%) in a 5-year survival was achieved if more than 40 lymph nodes were identified, compared with those patients who had 10 or fewer nodes.[24]

Some strategies for laparoscopic CME in left hemicolectomy

It is necessary to remove the perivascular sheath by sharp dissection for better central vessel root exposure, especially for patients with lymph node metastasis (Fig. 5A). Second, following the accurate surgical planes and spaces is also quite important, especially the mesofascial plane,[25] retrofascial interface, PAF, and LRC (Fig. 5B). Third, there are various vascular alterations. For instance, there are 6 kinds of variations of the branch vessels of sigmoid and LCA,[26] the routes of ureters also have kinds of variations (Fig. 5C,D).

Splenic flexure mobilization. Around the splenic flexure, Toldt's fascia spreads to the dorsal side of pancreatic tail, and so, if the surgeon separates splenic flexure followed by Toldt's fascia, this might naturally enter the dorsal side of pancreas, which might damage the vessels and pancreatic structure. There are two approaches to separate the splenic flexure, one is lateral approach of splenic flexure mobilization (SFM-L), which is to separate the Toldt's fascia then upwards to splenic flexure, the other; is to open gastrocolic ligament first and gradually divide the left side toward splenic flexure, which is also named anterior approach of splenic flexure mobilization (SFM-A). In detail, in SFM-L, the surgeon would stand at the right side of patients, after mobilization of the omental bursa from the lateral side (close to the tail of the pancreas) was performed. In SFM-A approach, the right-sided patient position was changed to an anti-Trendelenburg position with the surgeon standing between the patient's legs. The camera should also be moved from the supraumbilical to the trocar in the middle abdomen to achieve sufficient distance to the splenic flexure. However, the latter might be easier to distinguish the pancreatic tail with visual control, so as not to damage the structure. It was suggested that there was a significantly higher rate of intraoperative complications in the SFM-L group compared to the SFM-A group. It is also reported that a shorter operative time can be taken during the medial to lateral approach (Supplementary Fig. S2).

Recognize PAF, retrofascial interface, and the mesofascial plane. There were prerenal fascia posterolateral to the left mesocolon, PAF median to the left mesocolon, parietal layer of the pelvic fascia posterolateral to the mesorectum, which continued at different sites. Peritoneum resection was performed along the aorta to the top, the PAF is exposed under the peritoneal connective tissue, which extends upward till the dorsal side of the duodenum and contains autonomic nerve fibers inside.

Recently, Coffey et al. found that the peritoneal occurs wherever the mesenteric organ is opposed to the retroperitoneum, so that to gain access to the mesofascial plane, a peritonotomy of the peritoneal reflection in this location is required. When this is lengthened, and the interface placed on stretch, it might be possible to separate sharply the components of the mesofascia interface without disrupting the integrity of either.[27,28]

It also showed the contiguity among the mesorectal fascia, the mesosigmoidal fascia, and the left mesocolic fascia.[29] Thus, these represent different regions of the same fascial entity. In keeping with this, the mesorectal fascia, Gerota's fascia, and the anterior pararenal fascia are different regions of the same entity.[30]

Preserve the SRA or not?

Both D3 and CME could get a high rate of *mesocolic plane* grade for stage II and stage III patients,[31] and CME extends longitudinal mesocolon resection. In this study, a long-term follow-up and larger sample quantity would be essential. D3 excision is flexible in choosing whether to preserve SRA or not, which was determined by the clinical preoperative staging as well as intraoperative staging. SRA preservation may reduce the anastomotic tension compared to the SRA-nonpreserving D3 approach, so as to reduce the leakage rate[32]; however, there were not enough evidences to show whether it promotes early bowel function recovery. In contrast, it was also reported that SRA preservation could be performed without compromising the quality of lymph node dissection and relapse-free survival, but no advantage of SRA-preserving approach was demonstrated.[33] In addition, the LCA might be absent in 12% patients,[34] and so, CT reconstruction is recommended for the D3 approach.

In conclusion, both D3 and CME can achieve high quality of *mesocolic plane* grade for stage II and stage III patients. The LODDS and LNR were comparable and without significant correlation with the mesenteric area.

Disclosure Statement

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References

1. Jacobs M, Verdeja JC, Goldstein HS, et al. Minimally invasive colon resection (laparoscopic colectomy). Surg Laparosc Endosc 1991;1:144–150.
2. Hohenberger W, Weber K, Matzel K, et al. Standardized surgery for colonic cancer: Complete mesocolic excision and central ligation technical notes and outcome. Colorectal Dis 2008;11:354–364.
3. Clinical Outcomes of Surgical Therapy Study Group. A comparison of laparoscopically assisted and open colectomy for colon cancer. N Engl J Med 2004;350:2050–2059.
4. Bertelsen CA, Bols B, Ingeholm P, Jansen JE, Neuenschwander AU, Vilandt J. Can the quality of colonic surgery be improved by standardization of surgical technique with complete mesocolic excision? Colorectal Dis 2011;13: 1123–1129.
5. Han DP, Lu AG, Feng H, Wang PX, Cao QF, Zong YP, Feng B, Zheng MH. Long-term outcome of laparoscopic assisted right-hemicolectomy with D3 lymphadenectomy versus open surgery for colon carcinoma. Surg Today 2014;44:868–874.
6. Japanese Society for Cancer of the Colon and Rectum. Japanese Classification of Colorectal Carcinoma 2nd English edition. Tokyo: Kanehara & Co., Ltd; 2009.
7. Higuchi T, Sugihara K. Complete mesocolic excision with central vascular ligation as standardised surgical technique for colonic cancer: A Japanese multicentre study. Dis Colon Rectum 2010;53:646.

8. van Schaik J, van Baalen JM, Visser MJ, et al. Nerve preserving aortoiliac reconstruction surgery: Anatomical study and surgical approach. J Vasc Surg 2001;33:983–989.
9. Sato T, Hashimoto M. Morphological analysis of the fascial lamination of the trunk. Bull Tokyo Med Dent Univ 1984; 31:21–32.
10. Zhang C, Ding Zi-hai, Li Guo-xin, et al. Anatomical observations of pelvic autonomic nerves concerning with total mesorectal excision. Chin J Clin Anat 2006;24: 60–64.
11. Kobayashi M, Okamoto K, Namikawa T, et al. Laparoscopic lymph node dissection around the inferior mesenteric artery for cancer in the lower sigmoid colon and rectum. Surg Endosc 2006;20:563–569.
12. Watanabe T, Itabashi M, Shimada Y, et al. Japanese Society for Cancer of the Colon and Rectum (JSCCR) guidelines 2010 for the treatment of colorectal cancer. Int J Clin Oncol 2012;17:1–29.
13. Sekimoto M, Takemasa I, Mizushima T, et al. Laparoscopic lymph node dissection around the inferior mesenteric artery with preservation of the left colic artery. Surg Endosc 2011;25:861–866.
14. Milsom JW, Boehm B, Decanini C, Fazio VW. Laparoscopic oncologic proctosigmoidectomy with low colorectal anastomosis in a cadaver model. Surg Endosc 1994;8: 1117–1123.
15. Milsom JW, Boehm B, Nakajima K. Laparoscopic Colorectal Surgery. New York: Springer-Verlag, 1996.
16. West NP, Hohenberger W, Weber K, Perrakis A, Finan PJ, Quirke P. Complete mesocolic excision with central vascular ligation produces an oncologically superior specimen compared with standard surgery for carcinoma of the colon. J Clin Oncol 2010;28:272–278.
17. Arslan NC, Sokmen S, Canda AE, Terzi C, Sarioglu S. The prognostic impact of log odds of positive lymph nodes in colon cancer. Colorectal Dis 2014;16:O386–O392.
18. Japanese Research Society for Cancer of the Colon and Rectum. General Rules for Clinical and Pathological Studies on Cancer of Colon, Rectum and Anus, 7th edn. Tokyo: Kanehara & Co. Ltd., 2006.
19. Wang JP. Chinese standard for the diagnosis and treatment of colorectal cancer. Chi J Gastrointest Surg 2010; 14:1–4.
20. Shimada Y, Hamaguchi T, Moriya Y, et al. Randomized phase III study of adjuvant chemotherapy with oral uracil and tegafur plus leucovorin versus intravenous fluorouracil and levofolinate in patients (pts) with stage III colon cancer (CC): Final results of Japan Clinical Oncology Group study (JCOG0205). J Clin Oncol 2012;30. (Suppl; abstract 3524)
21. Japanese Society for Cancer of the Colon and Rectum. Japanese Classification of Colorectal Carcinoma. Tokyo: Kanehara & Co. Ltd., 2009.
22. Zong YP, Han DP, Lu AG, Feng H, et al. Laparoscopic complete mesocolic excision (CME) for left-sided colon cancer: Analysis of feasibility and technical tips. Chin J Pract Surg 2014;34:85–89.
23. Willaert W, Mareel M, Van De Putte D, Van Nieuwenhove Y, Pattyn P, Ceelen W. Lymphatic spread, nodal count and the extent of lymphadenectomy in cancer of the colon. Cancer Treat Rev 2014;40:405–413.
24. Le Voyer TE, Sigurdson ER, Hanlon AL, Mayer RJ, Macdonald JS, Catalano PJ, Haller DG. Colon cancer survival is associated with increasing number of lymph nodes analyzed: A secondary survey of intergroup trial INT-0089. J Clin Oncol 2003;21:2912–2919.
25. Culligan K, Remzi FH, Soop M, Coffey JC. Review of nomenclature in colonic surgery—Proposal of a standardised nomenclature based on mesocolic anatomy. Surgeon 2013;11:1–5.
26. Sinkeet S, Mwachaka P, Muthoka J, Saidi H . Branching pattern of inferior mesenteric artery in a Black African population: A dissection study. ISRN Anat 2012;2013: 962904.
27. Coffey JC, Sehgal R, Culligan K, Dunne C, McGrath D, Lawes N, Walsh D. Terminology and nomenclature in colonic surgery: Universal application of a rule-based approach derived from updates on mesenteric anatomy. Tech Coloproctol 2014;18:789–794.
28. Coffey JC, Culligan K, Walsh LG, Sehgal R, Dunne C, McGrath D, Walsh D, Moore M, Staunton M, Scanlon T, Dewhurst C, Kenny B, O'Riordan C, O'Brien JM, Quondamatteo F, Dockery P. An appraisal of the computed axial tomographic appearance of the human mesentery based on mesenteric contiguity from the duodenojejunal flexure to the mesorectal level. Eur Radiol 2015. [Epub ahead of print]
29. Kontovounisios C, Kinross J, Tan E, et al. Complete mesocolic excision in colorectal cancer: A systematic review. Colorectal Dis 2015;17:7–16.
30. Coffey JC, Dillon M, Sehgal R, Dockery P, Quondamatteo F, Walsh D, Walsh L. Mesenteric-based surgery exploits gastrointestinal, peritoneal, mesenteric and fascial continuity from duodenojejunal flexure to the anorectal junction—A review. Dig Surg 2015;32:291–300.
31. Kobayashi H, West NP, Takahashi K, Perrakis A, Weber K, Hohenberger W, Quirke P, Sugihara K. Quality of surgery for stage III colon cancer: Comparison between England, Germany, and Japan. Ann Surg Oncol 2014;21 Suppl 3: 398–404.
32. Rosenblum JD, Boyle CM, Schwartz LB. The mesenteric circulation: Anatomy and physiology. Surg Clin N Am 1997;77:289–306.
33. Wakahara T, Toyokawa A, Ashitani H, Tsuchida S, Hasegawa Y. Comparison of laparoscopic sigmoidectomy with and without preservation of the superior rectal artery: A single-institution retrospective study. Asian J Endosc Surg 2015;8:29–33.
34. Kornblith PL, Boley SJ, Whitehouse BS. Anatomy of the splanchnic circulation. Surg Clin N Am 1992;72:1–30.

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Clinical Research Paper

Long-term outcomes and propensity score matching analysis: rectal cancer resection for patients with elevated preoperative risk

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ABSTRACT

BACKGROUND: It is still controversial about the treatment strategy for rectal cancer patients with elevated operative risk and elder rectal cancer patients.

METHODS: This study presented a retrospective single center experience in rectal cancer proctectomy for high operative risk patients. High operative risk patient was defined as Cr-POSSUM > 5% combined with associated risk factors. 220 in 1477 consecutive patients met the inclusion criteria.

RESULTS: 132 patients were selected (66:66) after propensity score matching. The total complication rate between conventional open rectal resection (71 %) and laparoscopic surgery (41%) was significantly different (p = 0.0005). There is a significantly positive correlation between open surgery and advanced Dindo Classification (p = 0.02). Cr-POSSUM is positively correlated with Dindo Classification (p = 0.01). There was no significant difference in survival rate among stage I~II, different age groups or different Cr-POSSUM score sub-groups. However, stage III-IV tumor patients in laparoscopic group experienced improved overall survival rate. (p < 0.0001). For patients with preoperative pulmonary or renal disease, patients in laparoscopic group also had better long term prognosis (p = 0.03, p = 0.049).

CONCLUSIONS: The results demonstrate the potential advantages of laparoscopic rectal cancer resection for high operative risk patients, especially for the patients with preoperative respiratory or renal disease and stage III cancer.

INTRODUCTION

Rectal cancer is associated with substantial morbidity and mortality, especially in elder patients and those with co-morbidities. Outcome after these surgeries depends both on modifiable factors, such as perioperative medical care, and on physiological tolerance of surgical trauma. Over the last two decades, we have seen a continuous improvement of the quality of laparoscopic surgery in rectal cancer, especially in specialized centers with longstanding experience and high annual volumes. Several studies that compared laparoscopic and conventional open resection for rectal cancer show no difference with respect to local recurrence or overall and disease-free survival after 3, 5 [1, 2] even 10 years [3], respectively. More recently, long-term data including the MRC CR07 [4], MRC CLASICC trial, Comparison of Open *versus* laparoscopic surgery for mid or low Rectal cancer After Neoadjuvant chemoradiotherapy (COREAN) trial [5], the Colorectal cancer laparoscopic or Open Resection (COLOR II) trial [6] have released long-term survival rates. Though some of the randomized control trials have included patients with elevated preoperative risk (American Society of Anesthesiologists classification 3 and 4), these patients were generally recruited to clinical trials less often than younger patients and therefore

are under-represented in publications about cancer treatment [7]. Because of this heterogeneous, can these recommendations from major studies, such as laparoscopic rectal operations are safe and sound, be extrapolated to the fragile subset of patients with more comorbidity or do they need to be modified? The aim of this study is to analysis the survival and outcomes in patients with rectal cancer associated with high operative risk in conventional open rectal resection group (OpS) and laparoscopic rectal resection group (LaPS).

MATERIALS AND METHODS

Patients

This study included all 1477 consecutive patients undergoing radical surgical resection for rectal cancer in a tertiary referral teaching hospital - Shanghai Ruijin Hospital between September 2007 and Nov 2011. 220 patients were considered with high operative risk. Patients were admitted to Gastrointestinal Surgical Centre or Minimally Invasive Surgical Centre. Both centers belong to Department of General Surgery. The operative conditions, anesthesia management as well as perioperative management were at the same level. Both surgical teams had the same operative quality of rectal cancer. Emergency protectomy was excluded.

Diagnoses and tumor stage

The diagnoses were made preoperatively and then confirmed by postoperative pathology. The tumor node metastasis (TNM) staging of colon and rectal cancer system (American Joint Committee on Cancer Manual, 7th edition) was used. The criteria for neoadjuvant radiochemotherapy were patients with rectal cancer of the lower and middle third of the rectum and suspected T3 or T4 tumors and patients with pathological lymph nodes as demonstrated by CT or MRI-scan.

Surgical procedures and quality control

Patient demographics were extracted routinely by trained registrars from the hospital records. Patients were assigned preoperatively to the laparoscopic or open approach based on clinical criteria and imaging, including chest radiograph, abdominal computed tomography, and colonoscopy etc. Patients' preference had also been considered. Conversion cases were deemed necessary remained in the laparoscopic surgery group for all outcomes by intention-to-treat analysis. The preoperative preparation and the techniques of the procedures were described previously. With our experience from open total mesorectal excision, laparoscopic surgery was performed according to the same oncologic principles [8, 9]. Briefly, laparoscopic surgery was done with five trocars, the rectum was mobilized with monopolar cautery or an ultrasonic scalpel, dissecting between the visceral and parietal pelvic fascia without injuring the hypogastric nerves. Laparoscopic and open procedures were performed by four senior surgeons with their specialist team from the division of Gastrointestinal Surgery or division of Minimally Invasive Surgery in Ruijin Hospital. In the LapS group, surgery was performed by a systemic team of surgeons with abundant experience and expertise in conventional colorectal surgery and laparoscopic skills. In the OpS group, another fixed group of experienced surgeons specializing in colorectal surgery executed the surgery. [9]

Statistical methods

Analyses were performed with Stat View 5.0 for Windows (SAS Institute Inc., Cary, NC, USA). The X^2 test or Fisher's exact test was applied to analyze the categorical variables. The results were subjected to a nonparametric Mann-Whitney U test. A Student's *t*-test was also used to analyze the intragroup differences. The Kaplan-Meier method was used to analyze the overall survival of patients; the log-rank test was used to compare patient survival between groups. Cox-regression model was used for multivariate analysis. Logistic regression was used to analyze the correlation of Cr-POSSUM and Dindo-Demartines-Clavien Classification. $P < 0.05$ was regarded as statistically significant.

Propensity score matching

Propensity score matching was applied to reduce the effect of treatment selection bias and potential confounding effect, thereby creating a quasi-randomized experiment. This matching is done using a generalized SAS macro that matches Ops to LapS at a 1:1 ratio, using an algorithm to maximize the number of propensity score match. Patients were selected based on this score calculating for baseline characteristics; that is age, gender, tumor size, tumor location, tumor stage, Cr-possum value and radiochemotherapy at baseline in patients.

Risk evaluation

Patients with a predicted Colorectal Physiologic and Operative Severity Score for the enumeration of Mortality and Morbidity (Cr-POSSUM) ≥5% OR criteria below [10] were managed as 'high operative risk':

1. Aged > 60 years

PLUS undergoing re-do surgery

Table 1: The patient demographics and histopathological tumor assessment

Clinical or pathologic feature	OpS (*n* = 66)	LapS (*n* = 66)	*P*-value	95%CI	
Sex ratio (Male: Female)	45:22	46:21	0.85	-0.1794-0.1491	
Age (years)	69±11.2	68±12.1	0.59	-2.984-5.196	
Body mass index (kg/m2)	28.1	27.9	0.44		
pTumor stage (AJCC)					
I	18	17			
IIA	17	10			
IIIB	6	12			
IIIA	3	5	0.85	-0.2982-0.3588	
IIIB	10	12			
IIIC	4	7			
IV	8	3			
Lymph node metastasis					
N0	42	40			
N1	15	17	0.81		
N≥2	9	9			
Tumor size (diameter, cm)	3.60±1.58	3.57±0.84	0.92	-0.3812-0.4239	
Tumor location from anal verge (cm)	6.18±1.94	6.36±2.06			
Low-rectal (0~5cm)	23	35	0.54	-0.5259-0.9978	
Mid-rectal (6~10cm)	40	26			
Upper-rectal (>10cm)	3	5			
Type of surgery					
APR	44	49			
LAR	18	15	0.28		
Others	4	2			
Chemo-and/or radiotherapy	23	26	0.61	-0.2204-0.1294	
Stoma formed					
No	23	21			
Ileostomy	20	29	0.58		
Colostomy	23	16			
Resection margin R0	65	65			
R1	1	1	--		
Total mesorectal excision					
Complete	46	38			
Nearly complete	11	17	0.52		
Unknown	3	4			
Incomplete	6	7			

OR have acute or chronic renal impairment (sCr > 130 μmol/L)

OR have diabetes mellitus

OR are strongly suspected clinically to have any significant risk factor for the cardiac or respiratory disease. (e. g. chronic obstructive pulmonary disease, history of ischemic heart disease, congestive heart failure, arrhythmias, angina pectoris, or cardiac risk index > 12 etc.)

2. have shock of any cause, any age group.

Cr-POSSUM scores were calculated for each patient retrospectively from their medical records. The calculating software is freely available on the internet (http://www.riskprediction.org.uk/index-cr.php, Risk Prediction in Surgery)

RESULTS

There was no significant difference between each group concerning the age (69±11.2 *vs* 68±12.1 years

Table 2: Preoperative risk, postoperative complications and other outcomes

Clinical or pathologic feature	OpS (*n*=66)	LapS (*n*=66)	*P*-value
Preoperative risk			
Cr-possum Score			
~ 10 percent	29	36	
10 ~ 20 percent	24	15	0.65
20~ percent	13	15	
Undergoing re-do surgery	3	2	
Acute or chronic renal impairment	18	12	0.30
Diabetes	13	8	0.34
Cardiac disease	33	21	0.051
Respiratory disease	33	22	0.08
Cerebrovascular disease	2	1	-
Dindo-Demartines-Clavien Classification			
Dindo 1	33	41	
Dindo 2	24	9	0.92
Dindo 3	7	14	
Dindo 4	2	2	
Surgical complications			
Anastomotic leakage	5	6	-
Prolong ileus	1	1	-
Intra-abdominal abscess	2	1	-
Urological complication	6	5	-
(transurethrale catheter-related problem, urinary tract infection/retension, ureter leakage)			
Perineal wound complication	9	2	**0.03**
(wound dehiscence, wound infections, wound necrosis, abscess or delayed wound healing)			
perforation	1	1	-
Gastrointestinal haemorrhage	2	3	-
Rectal stump abscess	4	1	0.37
DVT	0	1	-
General complications			
Cardiac complication	4	3	-
Respiratory complication	10	2	**0.03**
Neurological symptoms	1	0	-
Renal complication	3	0	0.24
Ascites	0	1	-
Return to normal bowel function	5.5	4.0	
30-day mortality	1	0	-

Cr-POSSUM=Colorectal Physiologic and Operative Severity Score for the enumeration of Mortality and Morbidity. DVT, deep vein thrombosis

old, p = 0.5907). The Body mass index (BMI) were 28.1kg/m^2 and 27.9kg/m^2 (p = 0.437). The tumor size was 3.60±1.58cm and 3.57±0.84cm, respectively (p = 0.916), and located in 6.18cm and 6.36cm from the anal verge. The tumor stage, postoperative radiochemotherapy, circumferential resection margin (< 2mm) positivity (LapS 1of 66 [2%] *vs* OpS 1 of 66 [2%]), distal margin, macroscopic completeness of the resection (incomplete rate: LapS 9% *vs* OpS 10%), locoregional recurrence rate (LapS 4of 66 [6%] *vs* OpS 5 of 66 [8%]) did not differ between laparoscopic and open surgery groups.

Operative risk

The distribution of ages and Cr-POSSUM were showed in Table 1. 37 patients (56%) in OpS group were with a Cr-POSSUM score ≥10%, 13 patients (20% in total) of which were with a Cr-POSSUM score ≥20%; while in LapS group, the amount of patients with score above 10% and 20% were 30 patients (45%) and 15 patients (22%), respectively. Concerning the 4 patients whose scores were below 5% in OpS group, three patients were older than 50 years old with pulmonary dysfunction, one patient was 59 years old undergoing re-do surgery. In LapS group, three in five patients were beyond 50 years old combining with pulmonary dysfunction; one was with chronic renal impairment; one patient experienced re-do surgery. In total, there were 3 and 2 patients in each group underwent re-do surgery, eighteen and twelve patients suffered from acute or chronic renal impairment, thirteen and eight patients were suffering from diabetes mellitus in OpS and LapS group, respectively. 50%, 50% patients in OpS group and 32%, 33% patients were suffering from Cardiac and respiratory disease, respectively. 3% patients in the open surgery group have cerebrovascular disease. Generally speaking, there was no significant difference between the two groups in preoperative risk.

Postoperative complications and outcomes

The postoperative complications included surgical complications as well as general complications. Surgical complications contain anastomotic leakage [11], ileus, intra-abdominal abscess, urological or perineal wound complications, fistula, hemorrhage and deep vein thrombosis (Table 2). And there were no significant differences between two groups except that laparoscopic group has a significant lower wound complication rate (2 *vs* 9). General complications include cardiac, respiratory, neurological and renal complications, Ascites etc. Cardiac complications happened in 4 and 3 patients respectively in OpS and LapS groups, containing postoperative heart failure, arrhythmia, angina and ischemic heart diseases, while, the number of patients in the laparoscopic group with respiratory complications was significantly lower (p = 0.03). Notably, the total complication rate between conventional open rectal resection (71 %) and laparoscopic surgery (41%) showed a significant difference (p = 0.0005). 2 cases (3%) in the LapS group were converted to open surgery in the present study.

The correlation analysis of Cr-POSSUM and dindo-demartines-clavien classification

There is no significant difference between laparoscopic surgery and conventional surgical procedure in the distribution of Dindo-Demartines-Clavien Classification (p = 0.92). There is a significant

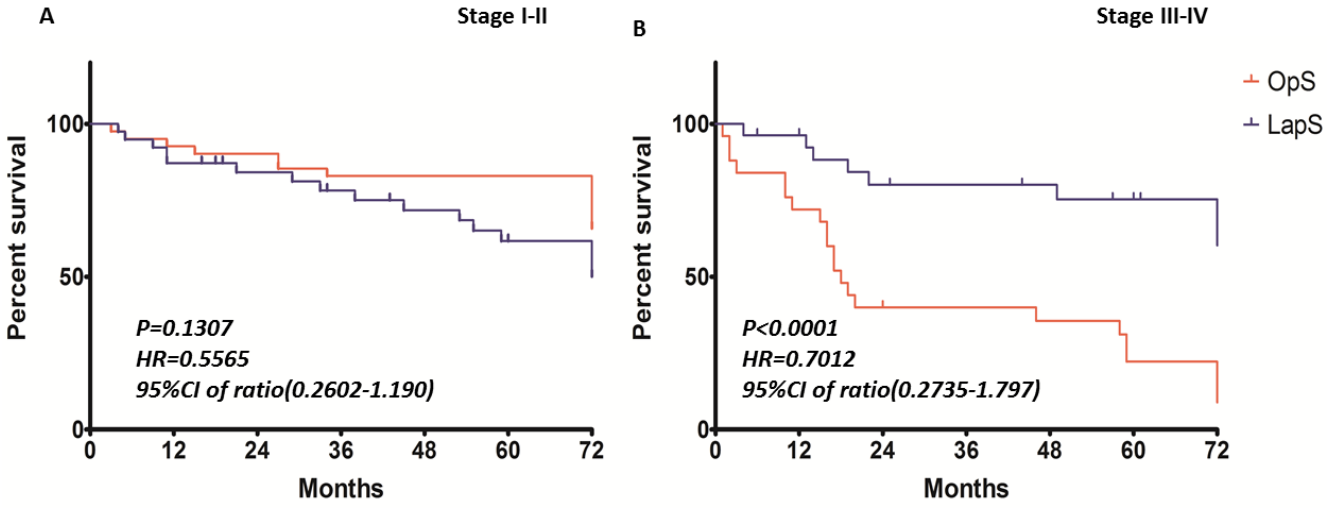

Figure 1: 5-year overall survival rates of Different Tumor Stages. After Log-rank analysis, no difference could be found between patients undergoing laparoscopic and open rectal resection in stage I~II (p = 0.13, HR 0.5565, 95%CI 0.26-1.19, Figure1A), whereas the overall survival rate was statistically significantly higher in LapS group with stage III-IV tumor (p < 0.0001, HR 0.70, 95%CI 0.27-1.79, Figure1B)

positive correlation between open surgery and the Dindo-Demartines-Clavien Classification (Estimate = 0.7495, p= 0.02, 95%CI 1.102~4.062). In addition, Cr-POSSUM is positively correlated with Dindo-Demartines-Clavien Classification (Estimate = 0.0458, p = 0.01, 95%CI 1.010~1.085).

5-year overall survival, disease-free survival and disease-specific survival rates of different tumor stages and Cr-POSSUM score sub-groups

The median follow-up is 49.5 months. Using Log-rank analysis, no difference could be found between patients undergoing laparoscopic and open rectal resection in stage I~II (p = *0.13, HR 0.5565, 95%CI 0.26-1.19*), whereas the overall survival rate was statistically significantly higher in LapS group with stage III-IV tumor (p < 0.0001, HR 0.70, 95%CI 0.27-1.79) Figure 1. We further used Cox regression to analyze the 132 patients; it also showed patients undergoing laparoscopic rectal resection had a better overall survival rate.

The 5- year overall survival curves of patients in different Cr-POSSUM score sub-groups are shown in Figure 2D, 2E, 2F. The actuarial survivals of the laparoscopic and open groups with Cr-POSSUM valuing 10~20% was without significantly different (p = 0.12, HR 2.02, 95%CI 0.83-4.90), so was for patients with Cr-POSSUM below 10% (p = 0.46) or above 20% (p = 0.64). The 5-year disease-free survival and disease specific survival are showed in Table 3.

Overall survival, disease-free survival and disease-specific survival rates of patients with preoperative cardiac, renal or respiratory diseases

The overall survival rates of patients with cardiovascular, pulmonary and renal diseases are shown in Figure 2A, 2B, 2C. In patients with the preoperative pulmonary disease, the 5-year overall survival rates of all stages and every different stage in these two groups were significantly different (p = 0.03 [OS], p = 0.02 [DFS]), while in patients with cardiovascular disease, the 5-year overall survivals were not significantly different (p = 0.9). For patients with the preoperative renal disease, the 5-year overall survival rates benefit from laparoscopic surgery with a significant difference. (p = 0.049), however, the disease-specific survival was not significantly different.

Furthermore, although people older than 75 years account for only 5~10% of the overall population in developed countries and some developing countries, 35~45% of patients with rectal cancer are in this age group. This proportion may increase in the future because

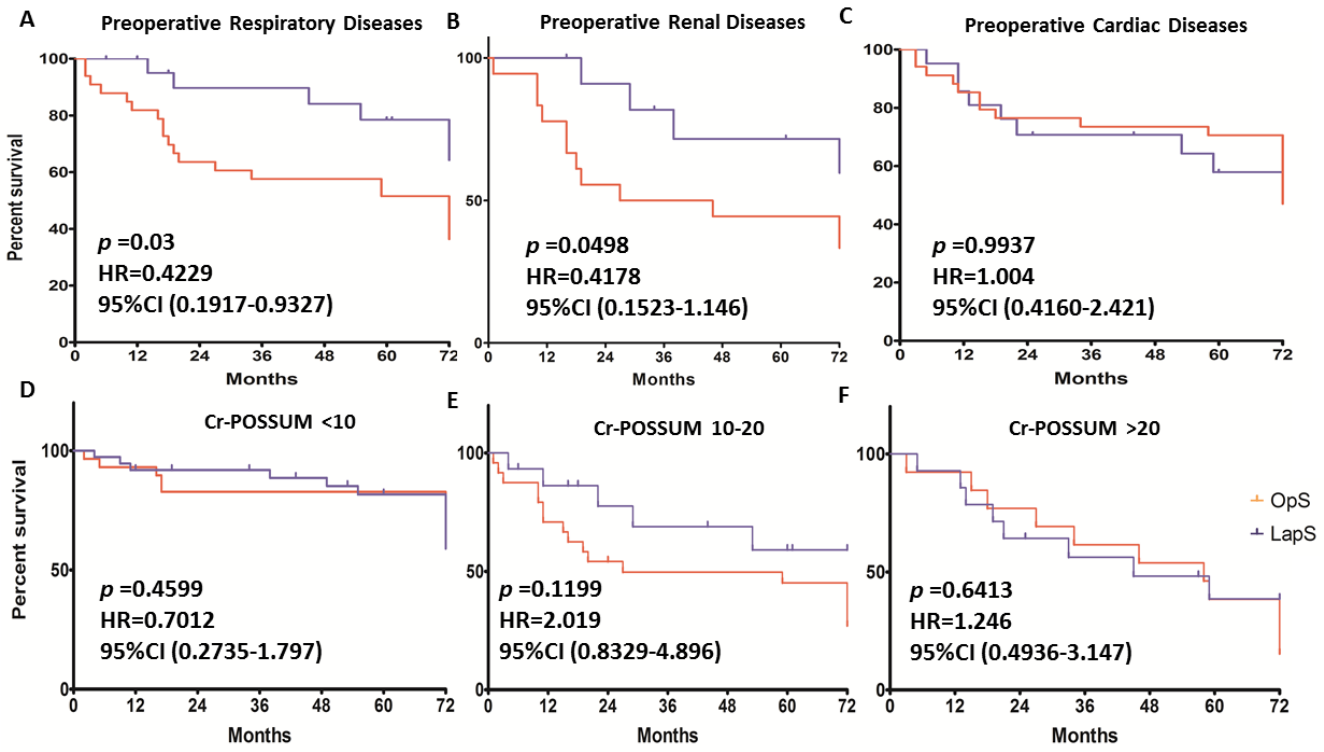

Figure 2: Overall survival rates of patients with preoperative diseases or patients in different Cr-POSSUM score sub-groups. A.-C., the overall survival rates of patients with cardiovascular, pulmonary and renal diseases. **D.- F.**, The 5- year survival curves of patients in different Cr-POSSUM score sub-groups.

Table 3: Disease free survival, disease specific survival and overall survival

Subgroup			5-year survival	HR(95%CI)	*p*
Stage I~II	Disease free survival	LapS	76.7%	0.58(0.28~1.19)	0.14
		OpS	89.1%		
	Disease specific survival	LapS	74.1%	0.63(0.29~1.36)	0.24
		OpS	88.6%		
Stage III~IV	Disease free survival	LapS	60%	5.14(2.27~11.68)	<0.0001
		OpS	38.4%		
	Disease specific survival	LapS	62.1%	5.57(2.42~12.81)	<0.0001
		OpS	38.4%		
Preoperative respiratory disease	Disease free survival	LapS	82.5%	0.40(0.18~0.87)	0.02
		OpS	64.6%		
	Disease specific survival	LapS	86.4%	0.45(.020~1.04)	0.047
		OpS	73.3%		
Preoperative renal disease	Disease free survival	LapS	80.9%	0.41(0.15~1.12)	0.049
		OpS	59.9%		
	Disease specific survival	LapS	81.9%	0.38(0.13~1.15)	0.06
		OpS	61.6%		
Preoperative cardiac disease	Disease free survival	LapS	71.9%	0.98(0.41~2.37)	0.98
		OpS	75.6%		
	Disease specific survival	LapS	75.2%	0.81(0.32~2.09)	0.68
		OpS	76.9%		
Cr-POSSUM<10	Disease free survival	LapS	84.2%	0.68(0.27~1.70)	0.40
		OpS	89.1%		
	Disease specific survival	LapS	84.2%	2.34(1.08~5.07)	0.43
		OpS	82.9%		
Cr-POSSUM 10~20	Disease free survival	LapS	73.8%	1.39(0.48~4.01)	0.54
		OpS	66.5%		
	Disease specific survival	LapS	73.8%	1.28(0.43~3.78)	0.66
		OpS	68.8%		
Cr-POSSUM >20	Disease free survival	LapS	63.6%	1.44(0.54~3.82)	0.46
		OpS	61.1%		
	Disease specific survival	LapS	72.5%	2.49(0.81~7.64)	0.11
		OpS	57.6%		
>75	Disease free survival	LapS	64.4%	0.89(0.42~1.89)	0.76
		OpS	60.7%		
	Disease specific survival	LapS	69.4%	0.66(0.29~1.42)	0.32
		OpS	59.1%		
<75	Disease free survival	LapS	62.9%	0.79(0.36~1.72)	0.55
		OpS	60.8%		
	Disease specific survival	LapS	62.9%	0.79(0.36~1.72)	0.55
		OpS	60.8%		

of demographics of an aging population, and increases in life expectancy [12]. Thus, we separated the patients into two sub-groups (~75, > 75) by age (LapS 23 of 66 [35%] > 75y, OpS 26 of 66 [39%] > 75y). The overall survival rate (Figure 3), disease-free survival rate, disease specific survival rate and the complication rate (not show) did not differ significantly in each group.

DISCUSSION

Recently, the continual innovations of surgical approach are a major step towards the idea of personalized medicine, we should notice that it is still controversial about the treatment strategy for elderly patients with rectal tumor and those with elevated operative risk. Especially for patients with elevated operative risk, patients are most vulnerable when their pre-existing comorbidities make them susceptible to perioperative risk [13, 14, 15].

The COREAN trial demonstrated similar disease-free survival (Lap79.2% *vs* Open 72.5%) and overall survival rates (Lap 91.7% *vs* Open 90.4%). The 3-year disease-free survival rate (Lap74.8% *vs* Open 70.8%) and overall survival rates were similar between both approaches in COLOR II trial as well. More recently, American College of Surgeons Oncology Group [ACOSOG] Z6051 trial [16] and Australasian Laparoscopic Cancer of the Rectum Randomized Clinical Trial [AlaCaRT] [17] investigated the non-inferiority of minimally invasive compared with open pelvic dissection for rectal cancer patients. The results suggest that a laparoscopic resection may not be oncologically justified in many patients requiring protectomy for rectal cancer. However, it was also reported that the follow-up studies to the ACOSOG Z6051 and ALaCaRT trials may show that long-term oncologic outcome are not compromised by a laparoscopic approach and slightly favorable outcomes might be seen as demonstrated by the COREAN and COLOR II trials. Other randomized trials and systematic reviews have also reported that laparoscopic and open proctectomy have similar oncological outcomes [18]. However, little solid evidence exists in support of laparoscopic or open proctectomy for patients with high operative risk, although some literature showed that perioperative morbidity did not differ between two groups (Table 4).

It is well accepted that laparoscopic approach is equivalent in the treatment of rectal cancer and shows advantages of shorter hospitalization and faster recovery, lower blood loss and lower complications rates [19], especially in patients with low rectal cancer [20, 21, 22].

Pulmonary comorbidities have been considered as an independent predictor of poor outcome in patients undergoing colectomy and appear to be enhanced in patients with chronic renal diseases. Chronic kidney diseases require dialysis is also a known surgical risk factor that in bowel resection increases the risk of death nearly 6-fold and doubles the complication rate. Therefore, some literature suggests laparoscopic surgery is not attempted for these patients considering their body habitus or longer operative time or creation of pneumoperitoneum which may be potentially associated with adverse pathophysiological changes, including hypercapnia, reduced venous return. However, in this study, patients with preoperative respiratory diseases and

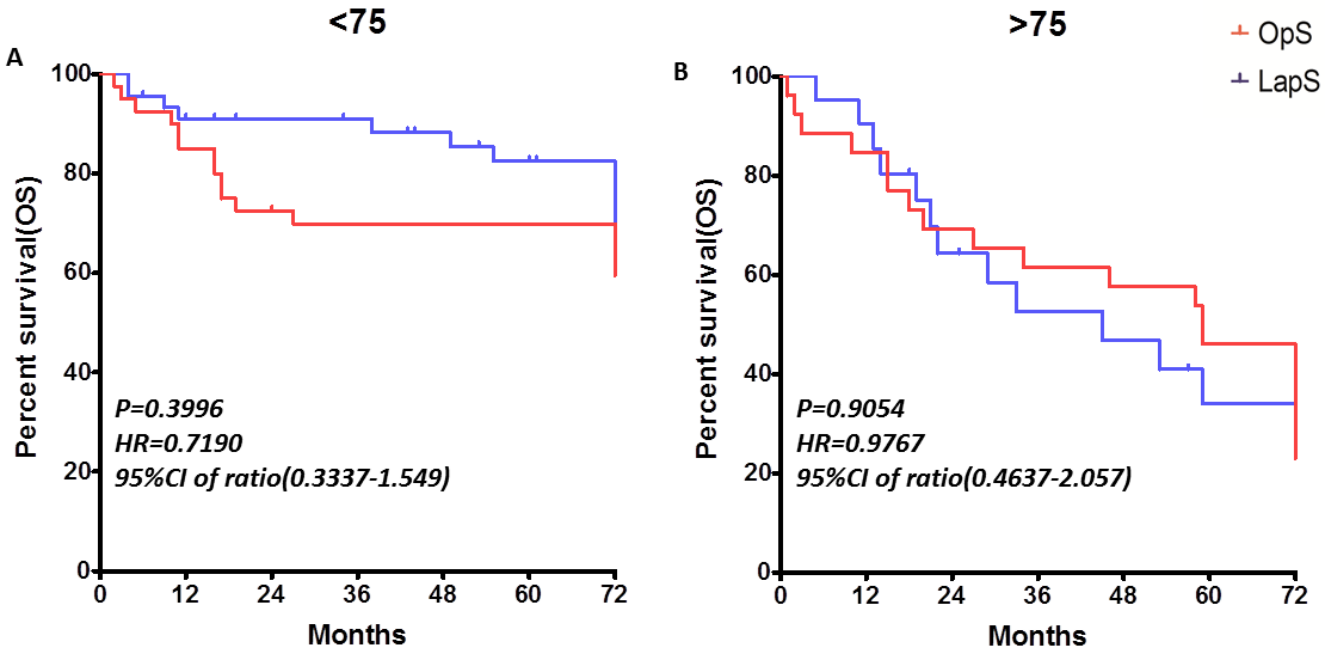

Figure 3: The overall survival rates in age sub-groups (~75, > 75).

Table 4: Recent comparative series in advanced rectal cancer

Reference	Year	Lap: Open	Follow up	Stage	Survival	*P* value
Park *et al*	2009	170:374	36m (2-75)	1-3	3-year DFS lap 77.5%	0.29
					Open 82.6%	
Laurent *et al*	2009	238:233	52m(1-151)	1-3	5-year DFS lap 82%	NS
					Open 79%	
				3	5-year DFS lap ~69%	NS
					Open ~69%	
				3	5-year OS lap ~72%	0.02
					Open ~52%	
Law *et al*	2009	111:310	34m	3	5-year OS lap 56.6%	0.33
					Open 50%	
Li *et al*	2011	113:123	74.8m	3	5-year OS lap 66.7%	0.85
					Open 70.3%	
				1-3	5-year OS lap 77.9%	0.91
					Open 78.9%	
Liang *et al*	2011	69:174	Until 3 year	1-3	3-year OS	NS
Baik *et al*	2011	54:108	Until 5 year	3	5-year OS lap 91.7%	0.30
					Open 77.2%	
				3	5-year DFS lap ~58.8%	0.63
					Open ~51.5%	
Law *et al*	2012	814:1197	40.3m	3	5-year OS lap ~58%	0.18
					Open ~48%	
Park*et al*	2013	404:404	Until 3 years	1-3	5-year OS lap 82.1%	0.44
					Open 81.3%	
				3	5-year OS lap ~70%	0.26
					Open ~73%	
				3	5-year DFS lap ~69%	0.18
					Open ~59%	
Asoglu *et al*	2013	513:0	31m(7-64)	3	5-year OS lap ~70%	-
Good *et al*[30]	2013	130:0	40m	3	5-year OS lap 75.6%	-
				4	5-year OS lap 53.8%	-
Ng SS *et al*	2014	136:142	Until 10 years	1-3	10-year OS lap ~58%	
					Open ~48%	
				3	10-year RR lap 25.8%	0.08
					Open 43.2%	
Reibetanz *et al*[29]	2014	170:170	48m vs 46m	1-3	3-year OS	NS
Bonjer et al (COLORII)	2015	699:345	Until 3 year	1-3	3-year OS lap 86.7% Open 83.6% DFS lap 74.8% Open 70.8% RR lap 5% Open 5%	NS NS NS
Jeong et al (COREAN)	2015	170:170	Until 3 year	1-4	3-year OS lap 91.7% Open 90.4% DFS lap 79.2% Open 72.2%	NS NS

OS, overall survival; DFS, disease free survival; RR, recurrence rate

renal diseases benefit from laparoscopic surgery, which was consistent with previous reports. The reasons might be lower pain rate, less complication rate in laparoscopic surgery, and also it might be attributable to the enhanced post-operative recovery of lung function in laparoscopic group [23, 24]. Besides, a lung-protective PEEP during pneumoperitoneum might be also valuable for preventing intratidal recruitment/derecruitment [25].

Presently, better preoperative risk assessment should be introduced, objective and accurate evaluation of risk should become routine procedures, those would be helpful to predict and avoid postoperative complications by selecting the appropriate surgical approach. Cr-POSSUM model is a promising specialized tool for monitoring surgical outcomes in colorectal cancer surgery, which might be more accurate than P-POSSUM score [26, 27] in pre-operative use. In present research, patients suffering stage III/IV tumor with a laparoscopic surgery (60%) had primarily a significantly better outcome than patients undergoing open surgery (38.4%), as compared with DFS rates of 64.9% after laparoscopic surgery and 52.0% after open surgery among patients with stage III disease in the COREAN study. There was no significant difference in different Cr-POSSUM subgroups. Other study findings showed that elder patients might benefit most from improved short-term postoperative outcomes following the laparoscopic surgery [28]. Our research did not indicate significant improvements in the overall survival in different age group. The comparable survival rates were reported in series of literature. But the present study showed superior survival in laparoscopic resection, especially in stage III/IV cancers. We reviewed recent researchers: in 2010, the UK MRC CLASICC trial demonstrated that the 5-year overall survival rate (OSR) was 60.3% for laparoscopic rectal resection *versus* 52.9% for open surgery. Feliciotti´s group [29] (62.5%*vs* 60.6%), Ng *et al* [30] (63.9 %*vs* 55%), Law´s group [31] (71.1%*vs* 59.3%), Jayne *et al* [32] (60.3%*vs* 52.9%) and Baik *et al* [33] (90.8% *vs* 88.5%) all presented a better 5-year OSR for laparoscopic rectal resection, though the differences were not significant. Recently, it was reported that laparoscopic resection is associated with more favorable 5-year OS in stage II and III cancer [34, 35]. These results were not influenced by postoperative chemotherapy, which was given similarly after both approaches, especially for stage III cancer. The lower complication rate associated with laparoscopic resection might contribute to the better OS, this reason is more pronounced in the patients with high preoperative risk [36, 37, 38, 39]. Given the increased mortality and morbidity, all efforts should be made to medically optimize these patients preoperatively. One of the limitations of this study is the sample number, though the estimated power was 0.8 ($\alpha = 5\%$). For an instant, only a few patients with diabetes or cerebrovascular diseases were involved in the analyses which still need to be further improved under larger sample amount. Although a randomized controlled trial should be conducted to confirm the findings of the present study, the authors believe that the present study is of value in proposing the future studies.

Author contributions

Lu AG, Thasler WE revised, partly designed and finally approved the article to be published. Feng H, Lu AG, designed the research and performed the follow-up study; Feng H, performed the clinical research with Mao ZH; Feng H, Zhao JK, and Schiergens T analyzed the data and wrote the manuscript.

CONFLICT OF INTEREST

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REFERENCES

1. Kim JG, Heo YJ, Son GM, Lee YS, Lee IK, Suh YJ, Cho HM, Chun CS. Impact of laparoscopic surgery on the long-term outcomes for patients with rectal cancer. ANZ J Surg. 2009; 79:817–23.
2. Lujan J, Valero G, Hernandez Q, Sanchez A, Frutos MD, Parrilla P. Randomized clinical trial comparing laparoscopic and open surgery in patients with rectal cancer. Br J Surg. 2009; 96:982–89.
3. Agha A, Benseler V, Hornung M, Gerken M, Iesalnieks I, Fürst A, Anthuber M, Jauch KW, Schlitt HJ. Long-term oncologic outcome after laparoscopic surgery for rectal cancer. Surg Endosc. 2014; 28:1119–25.
4. Quirke P, Steele R, Monson J, Grieve R, Khanna S, Couture J, O'Callaghan C, Myint AS, Bessell E, Thompson LC, Parmar M, Stephens RJ, Sebag-Montefiore D, and MRC CR07/NCIC-CTG CO16 Trial Investigators, and NCRI Colorectal Cancer Study Group. Effect of the plane of surgery achieved on local recurrence in patients with operable rectal cancer: a prospective study using data from the MRC CR07 and NCIC-CTG CO16 randomised clinical trial. Lancet. 2009; 373:821–28.
5. Jeong SY, Park JW, Nam BH, Kim S, Kang SB, Lim SB, Choi HS, Kim DW, Chang HJ, Kim DY, Jung KH, Kim TY, Kang GH, et al. Open versus laparoscopic surgery for mid-rectal or low-rectal cancer after neoadjuvant chemoradiotherapy (COREAN trial): survival outcomes of an open-label, non-inferiority, randomised controlled trial. Lancet Oncol. 2014; 15:767–74.
6. Bonjer HJ, Deijen CL, Abis GA, Cuesta MA, van der Pas MH, de Lange-de Klerk ES, Lacy AM, Bemelman WA, Andersson J, Angenete E, Rosenberg J, Fuerst A, Haglind

E, and COLOR II Study Group. A randomized trial of laparoscopic versus open surgery for rectal cancer. N Engl J Med. 2015; 372:1324–32.

7. Townsley CA, Selby R, Siu LL. Systematic review of barriers to the recruitment of older patients with cancer onto clinical trials. J Clin Oncol. 2005; 23:3112–24.
8. Feng B, Zhu QL, Xia Y, Lu AG, Wang ML, Li JW, Hu WG, Zang L, Mao ZH, Dong F, Ma JJ, Zheng MH. Direct and indirect costs and long-term survival of laparoscopic anterior resection for rectal cancer. Med Sci Monit. 2010; 16:PH97–102.
9. Zheng MH, Feng B, Hu CY, Lu AG, Wang ML, Li JW, Hu WG, Zang L, Mao ZH, Dong TT, Dong F, Cai W, Ma JJ, et al. Long-term outcome of laparoscopic total mesorectal excision for middle and low rectal cancer. Minim Invasive Ther Allied Technol. 2010; 19:329–39.
10. The Royal College of Surgeons of England and Department of Health. Report on the Periop-erative Care of the Higher Risk General Surgical Patient 2011.The Higher Risk General Surgical Patient Towards Improved Care for a Forgotten Group. 2011 1-30:12-13
11. Jannasch O, Klinge T, Otto R, Chiapponi C, Udelnow A, Lippert H, Bruns CJ, Mroczkowski P. Risk factors, short and long term outcome of anastomotic leaks in rectal cancer. Oncotarget. 2015; 6:36884–93. doi: 10.18632/oncotarget.5170.
12. Manceau G, Karoui M, Werner A, Mortensen NJ, Hannoun L. Comparative outcomes of rectal cancer surgery between elderly and non-elderly patients: a systematic review. Lancet Oncol. 2012; 13:e525–36.
13. Roviello F, Marrelli D, De Stefano A, Messano A, Pinto E, Carli A. Complications after surgery for gastric cancer in patients aged 80 years and over. Jpn J Clin Oncol. 1998; 28:116–22.
14. Sklow B, Read T, Birnbaum E, Fry R, Fleshman J. Age and type of procedure influence the choice of patients for laparoscopic colectomy. Surg Endosc. 2003; 17:923–29.
15. Scheidbach H, Schneider C, Hügel O, Yildirim C, Lippert H, Köckerling F. Laparoscopic surgery in the old patient: do indications and outcomes differ? Langenbecks Arch Surg. 2005; 390:328–32.
16. Fleshman J, Branda M, Sargent DJ, Boller AM, George V, Abbas M, Peters WR Jr, Maun D, Chang G, Herline A, Fichera A, Mutch M, Wexner S, et al. Effect of laparoscopic-assisted resection vs open resection of stage II or III rectal cancer on pathologic outcomes: the ACOSOG Z6051 randomized clinical trial. JAMA. 2015; 314:1346–55.
17. Stevenson AR, Solomon MJ, Lumley JW, Hewett P, Clouston AD, Gebski VJ, Davies L, Wilson K, Hague W, Simes J, and ALaCaRT Investigators. ALaCaRT Investigators. Effect of laparoscopic-assisted resection vs open resection on pathological outcomes in rectal cancer: the ALaCaRT randomized clinical trial. JAMA. 2015; 314:1356–63.
18. Vennix S, Pelzers L, Bouvy N, Beets GL, Pierie JP, Wiggers T, Breukink S. Laparoscopic versus open total mesorectal excision for rectal cancer. Cochrane Database Syst Rev. 2014; 4:CD005200.
19. Marks JH, Kawun UB, Hamdan W, Marks G. Redefining contraindications to laparoscopic colorectal resection for high-risk patients. Surg Endosc. 2008; 22:1899–904.
20. Ströhlein MA, Grützner KU, Jauch KW, Heiss MM. Comparison of laparoscopic vs. open access surgery in patients with rectal cancer: a prospective analysis. Dis Colon Rectum. 2008; 51:385–91.
21. Mohamed ZK, Law WL. Outcome of tumor-specific mesorectal excision for rectal cancer: the impact of laparoscopic resection. World J Surg. 2014; 38:2168–74.
22. Salihoglu Z, Baca B, Koksal S, Hakki Hamzaoglu I, Karahasanoglu T, Avci S, Ozben V. Analysis of laparoscopic colorectal surgery in high-risk patients. Surg Laparosc Endosc Percutan Tech. 2009; 19:397–400.
23. Stage JG, Schulze S, Møller P, Overgaard H, Andersen M, Rebsdorf-Pedersen VB, Nielsen HJ. Prospective randomized study of laparoscopic versus open colonic resection for adenocarcinoma. Br J Surg. 1997; 84:391–96.
24. Asoglu O, Balik E, Kunduz E, Yamaner S, Akyuz A, Gulluoglu M, Kapran Y, Bugra D. Laparoscopic surgery for rectal cancer: outcomes in 513 patients. World J Surg. 2013; 37:883–92.
25. Wirth S, Biesemann A, Spaeth J, Schumann S. Pneumoperitoneum deteriorates intratidal respiratory system mechanics: an observational study in lung-healthy patients. Surg Endosc. 2017; 31:753-760.
26. Horzic M, Kopljar M, Cupurdija K, Bielen DV, Vergles D, Lackovic Z. Comparison of P-POSSUM and Cr-POSSUM scores in patients undergoing colorectal cancer resection. Arch Surg. 2007; 142:1043–48.
27. Bromage SJ, Cunliffe WJ. Validation of the CR-POSSUM risk-adjusted scoring system for major colorectal cancer surgery in a single center. Dis Colon Rectum. 2007; 50:192–96.
28. Allardyce RA, Bagshaw PF, Frampton CM, Frizelle FA, Hewett PJ, Rieger NA, Smith JS, Solomon MJ, Stevenson AR, and Australasian Laparoscopic Colon Cancer Study Group. Australasian Laparoscopic Colon Cancer Study shows that elderly patients may benefit from lower postoperative complication rates following laparoscopic versus open resection. Br J Surg. 2010; 97:86–91.
29. Feliciotti F, Guerrieri M, Paganini AM, De Sanctis A, Campagnacci R, Perretta S, D'Ambrosio G, Lezoche E. Long-term results of laparoscopic versus open resections for rectal cancer for 124 unselected patients. Surg Endosc. 2003; 17:1530–35.
30. Ng SS, Leung KL, Lee JF, Yiu RY, Li JC, Teoh AY, Leung WW. Laparoscopic-assisted versus open abdominoperineal resection for low rectal cancer: a prospective randomized

trial. Ann Surg Oncol. 2008; 15:2418–25. Erratum

31. Law WL, Poon JT, Fan JK, Lo SH. Comparison of outcome of open and laparoscopic resection for stage II and stage III rectal cancer. Ann Surg Oncol. 2009; 16:1488–93.
32. Jayne DG, Thorpe HC, Copeland J, Quirke P, Brown JM, Guillou PJ. Five-year follow-up of the Medical Research Council CLASICC trial of laparoscopically assisted versus open surgery for colorectal cancer. Br J Surg. 2010; 97:1638–45.
33. Baik SH, Gincherman M, Mutch MG, Birnbaum EH, Fleshman JW. Laparoscopic vs open resection for patients with rectal cancer: comparison of perioperative outcomes and long-term survival. Dis Colon Rectum. 2011; 54:6–14.
34. Law WL, Poon JT, Fan JK, Lo OS. Survival following laparoscopic versus open resection for colorectal cancer. Int J Colorectal Dis. 2012; 27:1077–85.
35. Laurent C, Leblanc F, Wütrich P, Scheffler M, Rullier E. Laparoscopic versus open surgery for rectal cancer: long-term oncologic results. Ann Surg. 2009; 250:54–61.
36. Park IJ, Choi GS, Lim KH, Kang BM, Jun SH. Laparoscopic resection of extraperitoneal rectal cancer: a comparative analysis with open resection. Surg Endosc. 2009; 23:1818–24.
37. Reibetanz J, Germer CT. Oncological long-term results after open versus laparoscopic surgery for rectal cancer. [Article in German] Chirurg. 2014; 85:1113–14.
38. Good DW, O'Riordan JM, Moran D, Keane FB, Eguare E, O'Riordain DS, Neary PC. Laparoscopic surgery for rectal cancer: a single-centre experience of 120 cases. Int J Colorectal Dis. 2011; 26:1309–15.
39. Schwenk W, Böhm B, Witt C, Junghans T, Gründel K, Müller JM. Pulmonary function following laparoscopic or conventional colorectal resection: a randomized controlled evaluation. Arch Surg. 1999; 134:6–12.

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ORIGINAL ARTICLE

Retrospective Study

Comparison of non-schistosomal rectosigmoid cancer and schistosomal rectosigmoid cancer

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Abstract

AIM: To compare the clinicopathological features of patients with non-schistosomal rectosigmoid cancer and schistosomal rectosigmoid cancer.

METHODS: All the patients with rectosigmoid carcinoma who underwent laparoscopic radical surgical resection in the Shanghai Minimally Invasive Surgical Center at Ruijin Hospital affiliated to Shanghai Jiao-Tong University between October 2009 and October 2013 were included in this study. Twenty-six cases of colonic schistosomiasis diagnosed through colonoscopy and pathological examinations were collected. Symptoms, endoscopic findings and clinicopathological characteristics were evaluated retrospectively.

RESULTS: There were no significant differences between patients with and without schistosomiasis in gender, age, CEA, CA19-9, preoperative biopsy findings or postoperative pathology. Patients with rectosigmoid schistosomiasis had a significantly higher CA-125 level and a larger proportion of these patients were at an early tumor stage (P = 0.003). Various morphological characteristics of schistosomiasis combined with rectosigmoid cancer could be found by colonoscopic examination: 46% were fungating mass polyps, 23% were congestive and ulcerative polyps, 23% were cauliflower-like masses, 8% were annular masses. Only 27% of the patients were diagnosed with rectal carcinoma preoperatively after the biopsy. Computed tomography (CT) scans showed thickened intestinal walls combined with linear and tram-track calcifications in 26 patients.

CONCLUSION: Rectosigmoid carcinoma combined with schistosomiasis is associated with higher CA-125 values and early tumor stages. CA-125 and CT scans have a reasonable sensitivity for the accurate diagnosis.

Key words: Schistosomiasis; Rectosigmoid cancer; Colonoscopy; Biomarker; Diagnosis

Core tip: The association between schistosomiasis and colorectal malignancy has long been suggested in the literature. This study aimed to improve our understanding of the relationship between *Schistosoma japonicum*-related enteropathy and rectosigmoid carcinoma, with a particular focus on laboratory examination, endoscopic findings and clinicopathological characteristics of rectosigmoid schistosomiasis.

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INTRODUCTION

Human schistosomiasis is a prevalent parasitic disease caused by trematode flukes of the genus Schistosoma, of which *Schistosoma mansoni, Schistosoma japonicum* (*S. japonicum*) *and Schistosoma haematobium* are the three major species. By conservative estimates, at least 230 million people worldwide are infected with *Schistosoma* spp, and it is important to acknowledge that schistosomiasis is now becoming a cause for concern in Europe, especially in southern Europe, because of climate change as well as infected travelers who return from endemic areas[1]. A number of epidemiological data has suggested a close etiological relationship between colorectal cancer and schistosomiasis, especially *S. japonica*[2,3]. The microenvironmental changes and inflammation may form a causal link between schistosome chronic infection and colorectal carcinogenesis[4,5]. However, as the symptoms of colonic schistosomiasis are nonspecific and may mimic other gastrointestinal problems, this condition could be under diagnosed[6], and there is little relevant clinical data in the medical literature, mostly limited to case reports[7-9]. On the other hand, in some schistosoma-endemic areas, colonic schistosomiasis can be correctly diagnosed while colorectal cancer may be missed, especially when CEA or CA19-9 levels are within the normal range.

Detailed knowledge about schistosomiasis is necessary to improve the accuracy of clinical diagnosis. At present, colorectal neoplasia associated with schistosoma has only been reported on few occasions.

This research was conducted retrospectively, based on the recent data of schistosomal rectosigmoid cancer, including surgical findings and clinicopathological characteristics, to find a sensitive biomarker that might improve the accuracy of clinical diagnosis, and discuss the probable etiological role of chronic schistosomal infestation in rectosigmoid cancer.

MATERIALS AND METHODS

Patient selection and diagnoses

In this study, retrospective analysis was conducted for 26 consecutive cases in patients diagnosed with rectosigmoid carcinoma combined with colonic schistosomiasis between 01-10-2009 and 01-11-2013, who underwent surgical resection at the Shanghai Minimally Invasive Surgical Center of Ruijin Hospital, which is affiliated to Shanghai Jiao-Tong University. Those patients were admitted to hospital because of liquid or pasty diarrhea, abdominal pain, pain on colon palpation or hematochezia. Colonoscopies were performed in the outpatient service of our department 1-14 d before hospitalization. Two to three biopsies were obtained and sent to the department of pathology. CT scans, magnetic resonance imaging and laboratory examinations were performed in the outpatient service or on the first day of hospitalization. Patients who were diagnosed with rectosigmoid carcinoma in the same time period without schistosomiasis were selected as a control group. After surgical resection, all specimens were reviewed histopathologically, and the pathological TNM stages were determined according to the classification established by the American Joint Committee on Cancer (AJCC, 7th edition). The gold standard for diagnosis of schistosomiasis depends on finding ova by microscopy in the colon, rectum or stool.

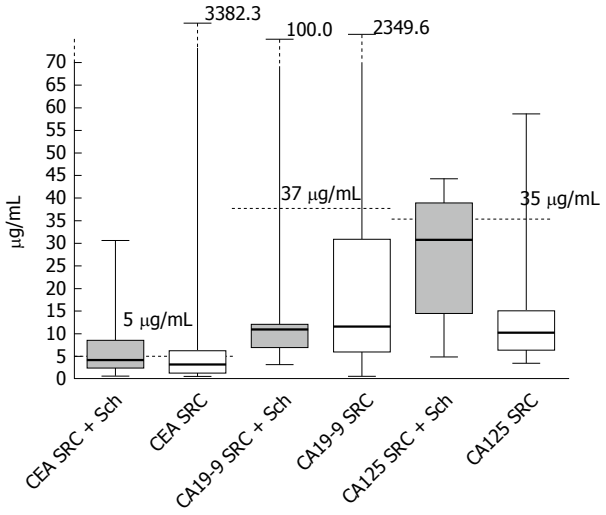

Figure 1 Quantities of CEA, CA19-9 and CA125 in the peripheral blood of patients who had rectosigmoid carcinoma with or without schistosomiasis. Dotted lines define the normal values. RSC: Rectosigmoid carcinoma; Sch: Schistosomiasis.

Examination and data collection

Abdominal ultrasonography, laboratory profiles, urine and stool tests were acquired after being admitted to hospital. Data on clinicopathological characteristics and treatments were collected routinely from the hospital records by trained registrars.

Statistical analysis

Analyses were performed using Stat View 5.0 for Windows (SAS Institute Inc., Cary, NC, United States). The χ^2 test or Fisher's exact test were applied to analyze the categorical variables. The results were subjected to a nonparametric Mann-Whitney *U* test. A Student's *t*-test was also used to analyze the intragroup differences. $P < 0.05$ was regarded as statistically significant. The statistical methods of this study were reviewed by Yi-Fei Zhang from the Institute for Stroke and Dementia Research Hospital of the University of Munich.

RESULTS

Clinical characteristics

In this study, 26 patients were diagnosed with sigmoid or rectal carcinoma combined with rectosigmoid schistosomiasis. Of these patients, 69.2% were male, while 31% out of the patients were female. 12 patients (46%) had elevated CEA values (> 5 μg/mL) and 47% patients (7/15) had abnormal CA-125 values (> 35 U/mL). The distribution of these biomarkers is shown in Figure 1. It worth noting that only 1 patient (9%, 1/11) in this series had an abnormal CA19-9 value (> 37.0 U/mL). In addition, there were no significant differences in gender, age, CEA value, CA19-9 value or findings from preoperative biopsy when these two groups were compared, based on characteristics and colonoscopic findings. Instead, patients with rectosigmoid schistosomiasis had significantly higher CA-125 values than those without ($P = 0.0001$) (Table 1). 85% of the patients who were diagnosed with sigmoid or rectal carcinoma combined with rectosigmoid schistosomiasis were at early tumor stages (stage Ⅰ or stage Ⅱ), compared to 47% of patients without schistosomiasis ($P = 0.003$).

Table 1 Patient characteristics and colonoscopic findings *n* (%) or mean ± SD

	With schistosomiasis (*n* = 26)	Without schistosomiasis (*n* = 34)	*P* value
Gender male/female	18/8	22/12	
Age (yr)	60.7 ± 10.6	63 ± 8.7	0.6100
CEA	4.2 ± 8.8	82.9 ± 428.1	0.3800
CA19-9	10.9 ± 306.5	35.1 ± 65.1	0.1300
CA-125	27.4 ± 3.3	12.7 ± 10.0	0.0001
Preoperative biopsy			
Carcinoma	7 (26.9)	16 (47.1)	
Hyperplastic polyps	5 (19.2)	8 (23.5)	
Villous adenoma	3 (11.5)	1 (2.9)	
Tubular adenoma	3 (11.5)	1 (2.9)	0.3400
Others	8 (30.7)	8 (23.5)	
Morphology			
Congestive, Ulcerative	6 (23.1)	13 (38.2)	
Fungating mass	12 (46.2)	8 (23.5)	0.1600
Cauliflower-like mass	6 (23.1)	6 (17.6)	
Annular	2 (7.6)	7 (20.6)	
Tumor stage			
Ⅰ	16 (61.5)	6 (17.6)	
Ⅱ	6 (23.1)	10 (29.4)	0.0030
Ⅲ	4 (15.4)	17 (50)	
Ⅳ	0	1 (2.9)	
Differentiation			
Well	16 (61.5)	15 (44.1)	
Moderate	7 (26.9)	16 (47.1)	0.4000
Poor	3 (11.5)	3 (8.8)	
Postoperative pathology			
Adenocarcinoma	16 (61.5)	30 (88.2)	
Signet-ring cell carcinoma	7 (7.7)	0	0.1300
Mucinous adenocarcinoma	3 (30.8)	4 (11.8)	

Endoscopic examination

Various morphological characteristics of schistosomiasis combined with rectosigmoid cancer were found in colonoscopic examinations (Figure 2). The fungating mass polyp was the major morphological type, being present in around 46% of all 26 patients. For the remaining patients, six (23%) had congestive and ulcerative polyps, six (23%) had cauliflower-like masses and two (8%) were annular type. Preoperative rectosigmoid biopsy provides an efficient but insensitive way of visualizing eggs, especially for those with low worm burdens. In this study, only seven patients (27%) were diagnosed with rectosigmoid carcinoma preoperatively; 19% of the biopsies showed hyperplastic polyps, and 8% and 23% revealed intraepithelial neoplastic changes (Table 1).

CT presentation

Abdominal CT enhanced dynamic scans (CTA) demon-

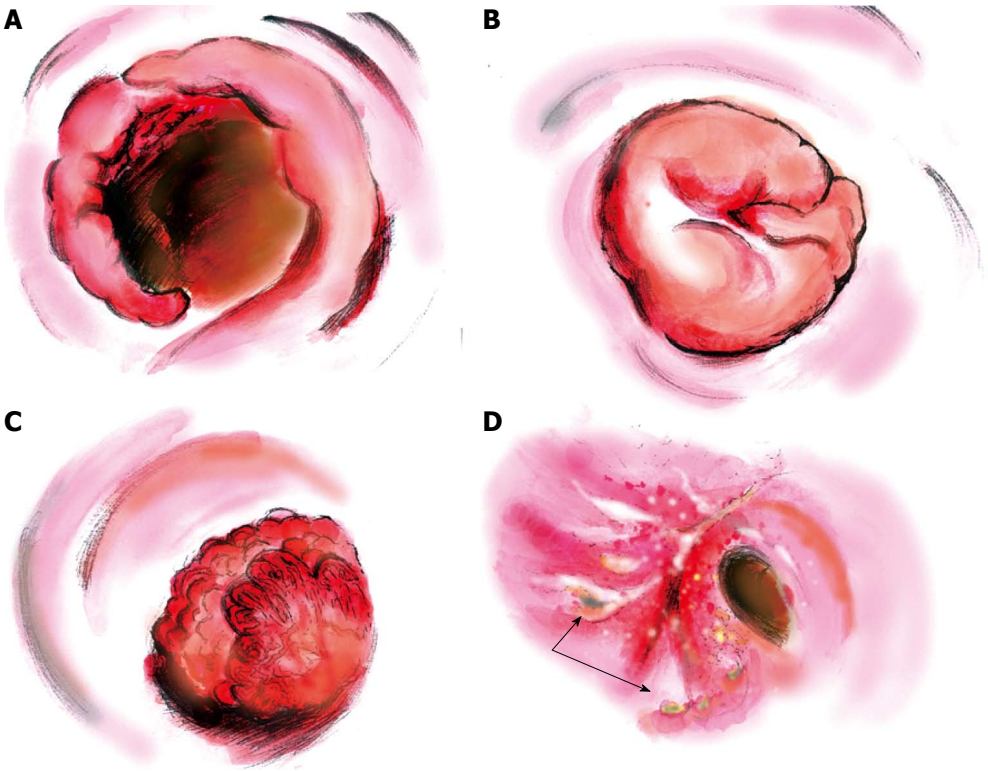

Figure 2 Endoscopic findings showing different morphological characteristics of schistosomiasis combined with rectal cancer. A: Annular; B: Fungating mass; C: Cauliflower-like mass; D: Congestive, ulcerative (black arrow).

strated evenly thickened intestinal walls combined with linear and tram-track calcifications in 26 patients, with (8%) or without (92%) perirectal fatty infiltration, the rectal lumen were locally narrowed in the primary lesions. Calcified ova could be found in 22 patients (85%). No significant lymphadenopathy was demonstrated. For those who had intestinal stenosis and for whom colonoscopic examinations were not recommended, virtual colonoscopy and virtual dissection were used to assess the condition and situation (Figure 3B-D).

Laparoscopic surgical finding and pathology characters

Irregular thickening of the intestinal wall was found in 25 patients (96%) during operations or postoperative sample assessments. Most of the patients were at Stage Ⅰ (62%), and 23% and 15% were at Stage Ⅱ and Stage Ⅲ, respectively. In 18 patients (69%), schistosomal ova were only found in the submucosal layer; in 19% the ova had infiltrated muscularis propria, and serosal infiltrations were found in 12% of the patients. In 8% of the patients, schistosomal ova could be found infiltrating into the surrounding lymph nodes postoperatively. In 21 patients (81%), schistosomal ova could be found inside the tumor, while ova from the remaining 19% of patients were found in the adjacent tissues (Figure 4). Considering the pathological profiles, the largest percentage had well differentiated tumors and adenocarcinoma observed in postoperative pathological examination. The information on signet-ring cell carcinomas (8%) and mucinous adenocarcinomas (31%) were also included in the present study (Table 2).

DISCUSSION

Schistosomal rectal cancer: better or worse prognosis?

Although schistosomiasis has been controlled in endemic regions[10] in the tropics and subtropics, previous *S. japonicum* infection might lead to complications, such as chronic intestinal schistosomiasis and hepatosplenic schistosomiasis, and this condition is significantly associated with both liver cancer and colorectal cancer[11]. Schistosome infection may have a negative effect on the prognosis of colorectal cancer[12]; it has been reported that the five-year survival rate was 45.6% out of 430 cases complicated with schistosomiasis, which was significantly lower than in those without schistosomiasis (50.9% out of 2717)[13]. In the present study, schistosomal rectosigmoid cancer seemed to be related to early tumor stage, possibly because schistosomiasis-related intestinal damages are mainly granuloma and fibrosis resulting from schistosomal ova deposition, especially in the large intestine[14-16]. Continuous epithelial proliferation adjacent to a chronic schistosomal ulcer and polyp formation, which lead to more obvious symptoms, might encourage the patients to seek a medical examination earlier. Wang *et al*[17] analyzed 30 patients with schistosomal rectal cancer and showed that schistosomiasis (P = 0.026)

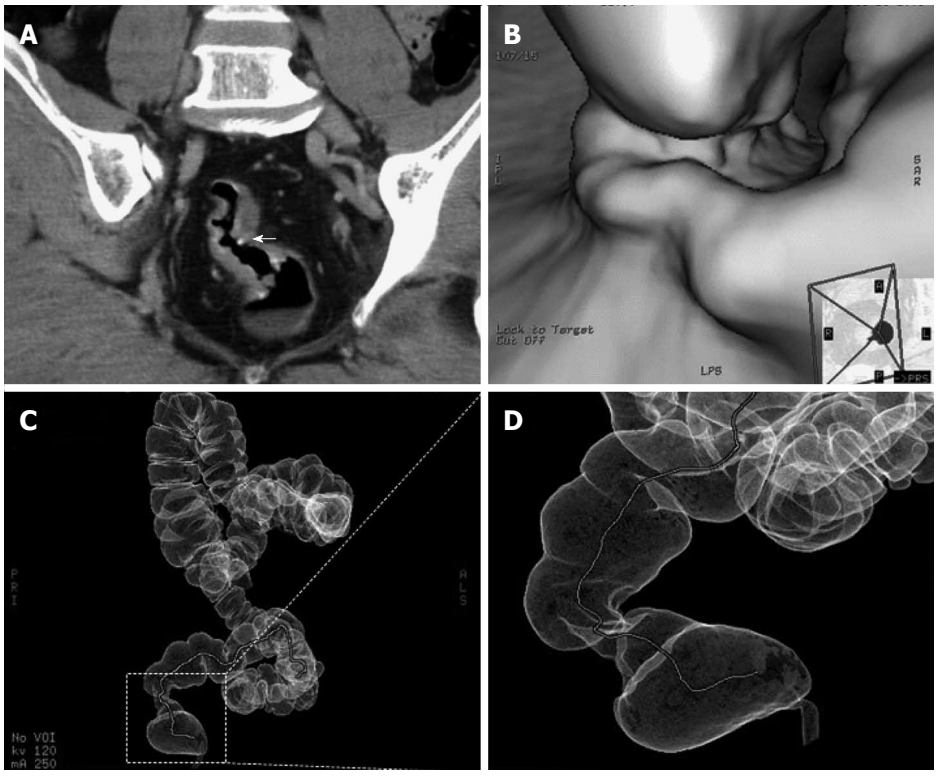

Figure 3 Computed tomography presentation. A: Computed tomography (CT) scan showing curvilinear calcification in the rectosigmoid colon and calcified, conglomerate nodules (arrow) protruding from the wall of the rectosigmoid colon; B: Lobulated polypus in the rectum; C, D: CTVC enables three-dimensional view of walls of the colon as a result of reconstruction of multislice CT images. The colorectal stenosis is showed in the area surrounding by dotted lines. CTVC: CT virtual colonoscopy.

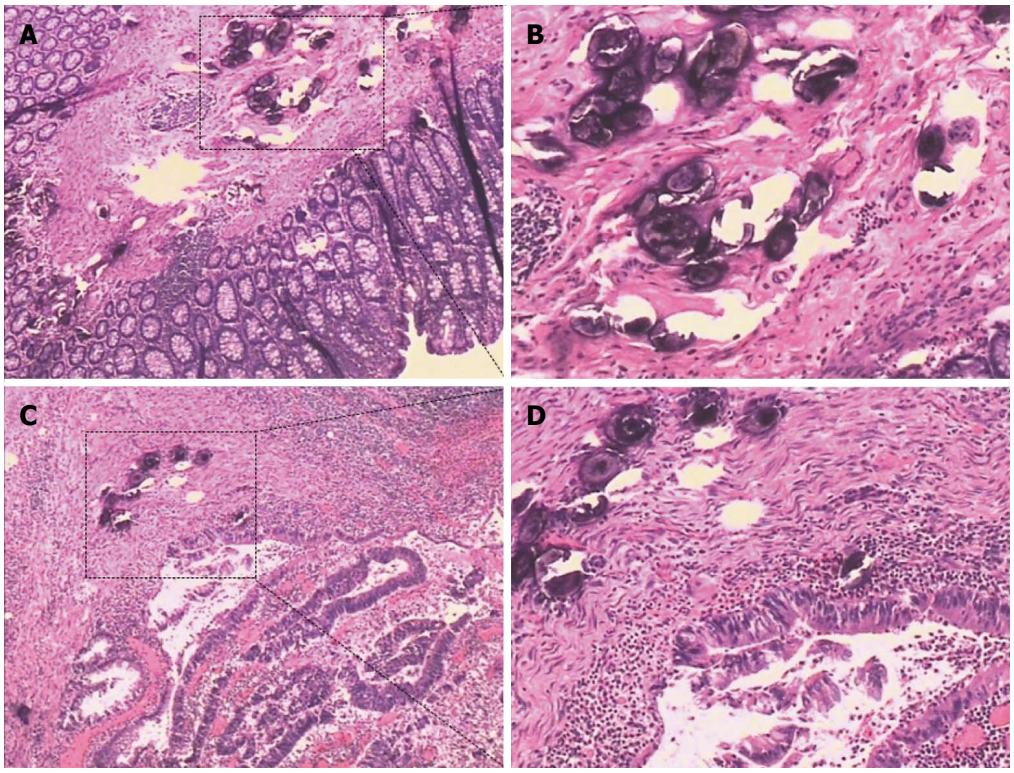

Figure 4 Pathological features of schistosomiasis-associated rectal adenocarcinoma. A, B: Schistosomiasis ova in tumor adjacent tissues. C, D: Schistosomiasis ova in tumor tissues.

Table 2 Laparoscopic surgery findings and pathological characteristics *n* (%)

	Male, *n*	Female, *n*	Total
Schistosomal ova position			
Submucosa infiltration	12	6	18 (69.2)
Muscularis propria	4	1	5 (19.2)
Serosal infiltration	2	1	3 (11.5)
Infiltration in the sLNs	1	1	2
Intra-tumor tissue	14	7	21 (80.8)
Para-tumor tissue	4	1	5 (19.2)
Calcification ova (CT)	15	7	22 (84.6)
Irregular thickening of the intestinal wall	17	8	25 (96.2)
Rough serosal surface	2	1	3 (11.5)

CT: Computed tomography; sLNs: Surrounding lymph nodes.

was statistically significantly correlated with overall survival (OS). Schistosomiasis was an independent prognostic factor for worse DFS and OS in multivariate analysis[17].

Sensitivity of biomarker examinations

In cases of intestinal cancer associated with schistosomiasis, the location of the cancer was predominately the rectum[18], followed by the sigmoid colon, and then the other parts of the colon, while small intestinal cancer with a relatively lower distribution of *S. japonicum* eggs is quite rare[4,19]. Fan *et al*[20] analyzed 285 pathological specimens with colorectal cancer from surgical operations in an endemic area for schistosomiasis and found that cancer in the rectum and sigmoid colon accounted for 44% and 27% in the 220 cases of cancer combined with schistosomiasis, respectively. In those patients without schistosomiasis, the comparative figure was 23% and 18%, respectively, with a significant difference[20]. Peripheral blood tumor marker IL-2, TNF-2 and CEA might be elevated in those patients[21]. Yu *et al*[22] divided schistosomal egg induced polyps into three types: fibrous type, mixed type and epithelial proliferative type; CEA and PNA receptors were present in 18/20 (90%) and 6/18 of epithelial proliferative type, respectively. In the present study, 46% and 47% patients had elevated CEA or CA-125, whilst few patients had abnormal CA19-9. On the other hand, after statistical analysis, schistosomal rectosigmoid carcinoma was only associated with a higher CA-125 level.

Is colonoscopy specific?

Colonoscopy provides valuable information for the diagnosis of colonic schistosomiasis[23]. Liu *et al*[3] systematically described morphological types of schistosomal colorectal cancer (endophytic/ulcerative, exophytic/fungating, annular, giant polyp and IIc), and found that the ulcerative type were the most common cases. However, in this study, which focused on rectosigmoid cancers, fungating masses seemed form the majority of the cases. The endoscopic findings of schistosomal rectosigmoid cancer were non-specific.

Considering the diagnoses of carcinoma, only 27% of the patients were diagnosed with rectal carcinomas preoperatively, 19% of the biopsies showed hyperplastic polyps, and 8% and 23% revealed low or high grade intraepithelial neoplastic changes, respectively. Considering the diagnosis of colonic schistosomiasis: if schistosoma ova are not observed in biopsies, the near-normal crypts with excess mucus and diffuse or focal infiltration of eosinophilic granulocytes may be highly suggestive of colonic schistosomiasis[24]. Therefore, multisite biopsies are recommended to improve the accuracy of diagnosis. Ye *et al*[25] analyzed clinical and endoscopic manifestations for 96 patients, and found that epidemiological investigations and colonoscopic examinations combined with multi-block and multi-site biopsies may improve the rate of correct diagnosis of intestinal schistosomiasis.

Recent technological advances have significantly enhanced the role of imaging in the detection, characterization, and management of infectious diseases involving the large intestine. Lee *et al*[26] reported that CT demonstrated calcifications resembling tram tracks in the sigmoid colon and postulated that the tram-track appearance is noted only in the distal large intestine because this portion of the colon has a thicker, muscular layer than the proximal colon. Irregular thickening of the intestinal wall, soft tissue masses, multiple *S. japonicum* ova calcifications inside the tumor with obscured margins and multiple intestinal masses in some patients, are important CT features of CRC with schistosomiasis. Zhang *et al*[27] compared the CT presentation and pathological characteristics and found that the intestinal wall was irregularly thickened in 95% of the patients, with soft tissue masses in 5% patients. Linear, spotty and small patchy calcifications were seen in 104 (80%) patients, with 96 out of 130 patients having ill-defined margins[27]. In support of this view, in our study, CT scan and CT virtual colonography have a reasonable sensitivity and specificity for detecting these lesions. CT allowed the visualization of evenly thickened intestinal wall combined with linear and tram-track calcifications in all 26 patients.

Recent studies have also thrown some light on the molecular events associated with schistosomal colorectal cancer. Ruan *et al*[28] found that the expressions of vascular growth factors including PD-ECGF and VEGF are higher in the colorectal carcinoma patients with schistosomiasis than in those without. Zalata *et al*[29] found that signet ring cell carcinoma and mucinous adenocarcinoma both exhibited intense c-myc expression compared with non-mucinous carcinoma ($P = 0.001$). When adjusting for *S. mansoni* infection, 58% of schistosomal colorectal cancer cases were Bcl-2 positive compared with only 33% of non-schistosomal colorectal cancers ($P = 0.046$). They also suggested that the genotoxic agents produced

endogenously through the course of *Schistosomiasis mansoni* infection may play a role in CRC- *Schistosoma mansoni* pathogenesis through the dysregulation of apoptosis by the alteration of the expression pattern of Bcl-2 protein[29].

A recent study showed that the prognosis of patients with schistosomal rectal cancer is worse than those with non-schistosomal rectal cancer; therefore, a diagnosis of schistosomiasis might be necessary[17]. In the present research, CA-125 levels and CT scans have a sufficient sensitivity to diagnose rectosigmoid carcinoma combined with schistosomiasis.

COMMENTS

Background

Colorectal cancer coexisting with schistosomiasis is a typical schistosomiasis-related intestinal damage, especially in the sigmoid colon and rectum.

Research frontiers

Epidemiological data have suggested that a close relationship exists between colorectal cancer and schistosomiasis, especially when infected with *Schistosoma japonicum*. However, there have been little available data regarding the role of *Schistosoma japonicum* in rectosigmoid carcinoma.

Innovations and breakthroughs

The authors compared the clinicopathological features of patients with non-schistosomal rectosigmoid cancer or schistosomal rectosigmoid cancer, and analyzed the laboratory examinations, endoscopic findings and clinicopathological characteristics between those two groups. There were no significant differences in CEA values, CA19-9 values or findings from preoperative biopsies. However, patients with rectosigmoid schistosomiasis had significantly higher CA-125 values.

Applications

According to this study, rectosigmoid carcinoma combined with schistosomiasis might be associated with higher CA-125 values and early stages; CA-125 and computed tomography (CT) scans have a sufficient sensitivity for accurate diagnosis.

Terminology

Endoscopy and CT scans contribute to the diagnosis of schistosomal rectosigmoid carcinoma, although they are nonspecific. A correct diagnosis of schistosomal rectosigmoid carcinoma can be established by endoscopy as well as CT scans in combination with its clinicopathological characteristics and laboratory tests (CA-125).

Peer-review

The manuscript written by Feng *et al* retrospectively evaluated the endoscopic findings and clinicopathological characteristics of schistosomal rectosigmoid cancer. The findings are important and provide novel information for the management of patients with rectosigmoid carcinoma, as well as schistosomiasis. However, there are some concerns that need to be addressed.

REFERENCES

1 **Colley DG**, Bustinduy AL, Secor WE, King CH. Human schistosomiasis. *Lancet* 2014; **383**: 2253-2264 [PMID: 24698483 DOI: 10.1016/S0140-6736(13)61949-2]

2 **Chen MG**. Assessment of morbidity due to Schistosoma japonicum infection in China. *Infect Dis Poverty* 2014; **3**: 6 [PMID: 24529186 DOI: 10.1186/2049-9957-3-6]

3 **Liu W**, Zeng HZ, Wang QM, Yi H, Mou Y, Wu CC, Hu B, Tang CW. Schistosomiasis combined with colorectal carcinoma diagnosed based on endoscopic findings and clinicopathological characteristics: a report on 32 cases. *Asian Pac J Cancer Prev* 2013; **14**: 4839-4842 [PMID: 24083755 DOI: 10.7314/APJCP.2013.14.8.4839]

4 **Steinmann P**, Keiser J, Bos R, Tanner M, Utzinger J. Schistosomiasis and water resources development: systematic review, meta-analysis, and estimates of people at risk. *Lancet Infect Dis* 2006; **6**: 411-425 [PMID: 16790382 DOI: 10.1016/S1473-3099(06)70521-7]

5 **Konishi T**, Watanabe T, Shibahara J, Nagawa H. Surveillance colonoscopy should be conducted in patients with colorectal Shistosomiasis even after successful treatment of the disease. *Int J Immunopathol Pharmacol* 2006; **19**: 245-246 [PMID: 16569365]

6 **Mohamed AR**, al Karawi M, Yasawy MI. Schistosomal colonic disease. *Gut* 1990; **31**: 439-442 [PMID: 2110925 DOI: 10.1136/gut.31.4.439]

7 **Botes SN**, Ibirogba SB, McCallum AD, Kahn D. Schistosoma prevalence in appendicitis. *World J Surg* 2015; **39**: 1080-1083 [PMID: 25609120 DOI: 10.1007/s00268-015-2954-3]

8 **Li WC**, Pan ZG, Sun YH. Sigmoid colonic carcinoma associated with deposited ova of Schistosoma japonicum: a case report. *World J Gastroenterol* 2006; **12**: 6077-6079 [PMID: 17009414 DOI: 10.3748/wjg.v12.i37.6077]

9 **Issa I**, Osman M, Aftimos G. Schistosomiasis manifesting as a colon polyp: a case report. *J Med Case Rep* 2014; **8**: 331 [PMID: 25296942 DOI: 10.1186/1752-1947-8-331]

10 **Gryseels B**, Polman K, Clerinx J, Kestens L. Human schistosomiasis. *Lancet* 2006; **368**: 1106-1118 [PMID: 16997665 DOI: 10.1016/S0140-6736(06)69440-3]

11 **Gray DJ**, Ross AG, Li YS, McManus DP. Diagnosis and management of schistosomiasis. *BMJ* 2011; **342**: d2651 [PMID: 21586478 DOI: 10.1136/bmj.d2651]

12 **Chen MC**, Chang PY, Chuang CY, Chen YJ, Wang FP, Tang YC, Chou SC. Colorectal cancer and schistosomiasis. *Lancet* 1981; **1**: 971-973 [PMID: 6112388]

13 Schistosomiasis and its prognostic significance in patients with colorectal cancer. National Cooperative Group on Pathology and Prognosis of Colorectal Cancer. *Zhonghua Zhongliu Zazhi* 1986; **8**: 149-151 [PMID: 3021419]

14 **Madbouly KM**, Senagore AJ, Mukerjee A, Hussien AM, Shehata MA, Navine P, Delaney CP, Fazio VW. Colorectal cancer in a population with endemic Schistosoma mansoni: is this an at-risk population? *Int J Colorectal Dis* 2007; **22**: 175-181 [PMID: 16786317 DOI: 10.1007/s00384-006-0144-3]

15 **Singh KP**, Gerard HC, Hudson AP, Boros DL. Differential expression of collagen, MMP, TIMP and fibrogenic-cytokine genes in the granulomatous colon of Schistosoma mansoni-infected mice. *Ann Trop Med Parasitol* 2006; **100**: 611-620 [PMID: 16989687 DOI: 10.1179/136485906X118530]

16 **Chen Y**, Boros DL. Polarization of the immune response to the single immunodominant epitope of p38, a major Schistosoma mansoni egg antigen, generates Th1- or Th2-type cytokines and granulomas. *Infect Immun* 1999; **67**: 4570-4577 [PMID: 10456902]

17 **Wang M**, Zhang YC, Yang XY, Wang ZQ. Prognostic analysis of schistosomal rectal cancer. *Asian Pac J Cancer Prev* 2014; **15**: 9271-9275 [PMID: 25422211 DOI: 10.7314/APJCP.2014.15.21.9271]

18 Schistosomes, liver flukes and Helicobacter pylori. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Lyon, 7-14 June 1994. *IARC Monogr Eval Carcinog Risks Hum* 1994; **61**: 1-241 [PMID: 7715068]

19 **Hao WG**, Wang XM, Yi WQ, Gao YP. [Analysis of lesions in 498 schistosomiasis patients]. *Zhongguo Xuexichongbing Fangzhi Zazhi* 2013; **25**: 428, 432 [PMID: 24358761]

20 **Fan XL**, Cheng HJ. Discussion on the relationship between schistosomiasis and colorectal cancer – histopathological analysis of 285 cases. *Zhongguo Xuexichongbing Fangzhi Zazhi* 1992; **3**: 62

21 **Hamed MA**, Ahmed SA, Khaled HM. Efficiency of diagnostic biomarkers among colonic schistosomiasis Egyptian patients. *Mem Inst Oswaldo Cruz* 2011; **106**: 322-329 [PMID: 21655820 DOI: 10.1590/S0074-02762011000300011]

22 **Yu XR**, Chen PH, Xu JY, Xiao S, Shan ZJ, Zhu SJ. Histological classification of schistosomal egg induced polyps of colon and their clinical significance. An analysis of 272 cases. *Chin Med J* (Engl) 1991; **104**: 64-70 [PMID: 1908760]

23 **Cao J**, Liu WJ, Xu XY, Zou XP. Endoscopic findings and clinicopathologic characteristics of colonic schistosomiasis: a

report of 46 cases. *World J Gastroenterol* 2010; **16**: 723-727 [PMID: 20135720 DOI: 10.3748/wjg.v16.i6.723]
24 **Yosry A**. Schistosomiasis and neoplasia. *Contrib Microbiol* 2006; **13**: 81-100 [PMID: 16627960 DOI: 10.1159/000092967]
25 **Ye C**, Tan S, Jiang L, Li M, Sun P, Shen L, Luo H. Endoscopic characteristics and causes of misdiagnosis of intestinal schistosomiasis. *Mol Med Rep* 2013; **8**: 1089-1093 [PMID: 23969514 DOI: 10.3892/mmr.2013.1648]
26 **Lee RC**, Chiang JH, Chou YH, Rubesin SE, Wu HP, Jeng WC, Hsu CC, Tiu CM, Chang T. Intestinal schistosomiasis japonica: CT-pathologic correlation. *Radiology* 1994; **193**: 539-542 [PMID: 7972775 DOI: 10.1148/radiology.193.2.7972775]
27 **Zhang W**, Wang PJ, Shen X, Wang GL, Zhao XH, Seema SF, Zheng SQ, Li MH. CT presentations of colorectal cancer with chronic schistosomiasis: A comparative study with pathological findings. *Eur J Radiol* 2012; **81**: e835-e843 [PMID: 22658847 DOI: 10.1016/j.ejrad.2012.02.020]
28 **Ruan SL**, Wang B, Lu QM, Dong LR, Cao CX, Xu SL, Shen WY. [Expression of vascular growth factors in intestinal tissues in colorectal carcinoma patients with schistosomiasis japonica]. *Zhongguo Xuexichongbing Fangzhi Zazhi* 2013; **25**: 250-254 [PMID: 24024441]
29 **Zalata KR**, Nasif WA, Ming SC, Lotfy M, Nada NA, El-Hak NG, Leech SH. p53, Bcl-2 and C-Myc expressions in colorectal carcinoma associated with schistosomiasis in Egypt. *Cell Oncol* 2005; **27**: 245-253 [PMID: 16308474]

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Bone marrow-derived mesenchymal stromal cells promote colorectal cancer cell death under low-dose irradiation

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Background: Radiotherapy remains one of the cornerstones to improve the outcome of colorectal cancer (CRC) patients. Radiotherapy of the CRC not only help to destroy cancer cells but also remodel the tumour microenvironment by enhancing tumour-specific tropism of bone marrow-derived mesenchymal stromal cell (BM-MSC) from the peripheral circulation. However, the role of local MSCs and recruited BM-MSC under radiation were not well defined. Indeed, the functions of BM-MSC without irradiation intervention remained controversial in tumour progression: BM-MSC was previously shown to modulate the immune function of major immune cells, resulting in an impaired immunological sensitivity and to induce an increased risk of tumour recurrence. In contrast, it could also secrete various cytokines and possess anticancer effect.

Methods: Three co-cultivation modules, 3D culture modules, and cancer organoids were established. The induction of cytokines secretion in hBM-MSCs after irradiation was analysed by ELISA array and flow cytometry. AutoMac separator was used to separate hBM-MSC and CRC automatically. Cells from the co-cultured group and the control group were then irradiated by UV-C lamp and X-ray. Proliferation assay and viability assay were performed.

Results: In this study, we show that BM-MSCs can induce the EMT progression of CRC cells *in vitro*. When irradiated with low doses of ultraviolet radiation and X-rays, BM-MSCs show an anti-tumour effect by secreting certain cytokine (TNF-α, IFN-γ) that lead to the inhibition of proliferation and induction of apoptosis of CRC cells. This was further verified in a 3D culture model of a CRC cell *in vitro*. Furthermore, irradiation on the co-culture system induced the cleavage of caspase3, and attenuated the phosphorylation of phosphatidylinositol 3-kinase (PI3K)/AKT and extracellular signal-regulated kinase in cancer cells. The signal pathways above might contribute to the cancer cell death.

Conclusions: Taken together, we show that BM-MSC can potentially promote the effect of radiotherapy in CRC.

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Colorectal cancer (CRC) remains one of the most common cancers worldwide, with ~746 000 and 614 000 new diagnosed cases per year in men and women, respectively (Schreuders *et al*, 2015). Despite rapid advances in multiple therapy strategies of cancer, the efficacy of current treatment strategies is still far from expected.

Mesenchymal stromal cells (MSCs) are a heterogeneous group of progenitor cells that are important for tissue regeneration. Cancer is considered as 'wounds that never heal' and thus MSCs, in response to chemokine, are continuously recruited and integrated into the tumour microenvironment. MSCs within tumour microenvironment could exert both pro-apoptotic and pro-survival effects on tumours and modulate the immune functions by altering the cytokine secretion profile of antigen presenting cells, T cells, and natural killer cells. It has been proven that MSCs could secrete various cytokines and possess anticancer effects (Pommey and Galipeau, 2006; Hendijani and Javanmard, 2015; Liotta *et al*, 2015). However, on the other hand, evidence was provided that MSC could also induce an inflammatory and immune suppression microenvironment, resulting in an impaired immunological sensitivity and the promotion of tumour growth, which gives rise to an increased risk of tumour recurrence (Houthuijzen *et al*, 2012; Chen *et al*, 2015).

In radiation oncology, classical viewpoints insist ionising radiation works by penetrating and damaging the DNA of cancerous tissue, which leads to cellular death. However, the complex interaction between the cancer cell and stromal cells, especially the function of MSCs under radiation, are not very much investigated. Owing to the complexity of interactions of different cell types within the tumour microenvironment, we speculated that the components of tumour microenvironment could also affect the systemic anti-cancer effect of radiotherapy. At present, researchers used heterogeneous culture of marrow stromal cells and claim they are MSCs. Actually, MSCs contain several sub-populations: stromal cells, progenitor cells, fibroblasts, and stem cells. In the present study, the authors used MSCs containing a subpopulation of stem cells (Galderisi and Giordano, 2014). Considering multipotency and tumour tropism of MSCs, we further supposed that MSCs could either sensitive or blunt the radiotherapy effect in CRC. Growing evidence has shown that low doses of radiation also have profound effects on cellular functions. Concerning stem cells, owing to their longer lifespan, they could sustain more rounds of lower doses of radiation, which may severely affect cellular function but not cellular physiology (Fazel *et al*, 2009). In addition, low doses of radiation could have already induced a reduction in cycling MSCs and an increase in apoptotic cells, and these proportions did not grow progressively as the doses increased (Alessio *et al*, 2015).

To verify this hypothesis, we investigated the radiotherapy sensitivity of CRC cell and bone marrow-derived mesenchymal stromal cell (BM-MSC) under radiation *in vitro*. Our finding suggests that: (1) MSCs can induce the mesenchymal phenotype of CRC *in vitro*; (2) BM-MSCs under low-dose radiation show an anti-tumour effect in 2D and 3D co-cultivation models by secreting certain cytokine (TNF-α, IFN-γ). (3) Irradiation on the co-culture system induced the cleavage of caspase3, and attenuated the phosphorylation of phosphatidylinositol 3-kinase (PI3K)/AKT and extracellular signal-regulated kinase (ERK) in cancer cells. PI3K-Akt signalling pathway is a signal transduction pathway that promotes survival and growth in response to extracellular signals. The suppression of this signalling pathway might lead to cancer cell death. Therefore, the combination of administration of MSC with radiotherapy might improve the outcome of CRC patients. This study provides clues for an improved therapy alternative to sensitise radiotherapy in CRC patients.

MATERIALS AND METHODS

Cell lines. Human CRC cell lines (HT-29, SW1116, and SW620) were obtained from Shanghai Digestive Surgery Key Lab, which was purchased and generated from American Type Culture Collection (Manassas, VA, USA). CRC cells were cultured in RPMI 1640 medium with GlutaMAX (GIBCO) supplemented with 10% fetal bovine serum (FBS) and penicillin–streptomycin (100 U ml^{-1} penicillin,100 µg ml^{-1} streptomycin). Cells were maintained at 37 °C in a 5% CO_2 incubator (Thermo Fisher Scientific, Darmstadt, Germany) according to standard protocols. Pancreatic cancer BxPC3 and DanG cell lines were cultured in GIBCO Dulbecco's Modified Eagle Medium (DMEM, Thermo Fisher Scientific). The medium was replaced routinely every 2–3 days. When 70–80% cell confluence has reached, the cells were sub-cultured. Poietics Normal Human Bone Marrow-Derived Mesenchymal stromal cells were complimentary from Group of Dr med Tobias Schiergens, which were purchased from Lonza (Walkersville, MD, USA) (Mayer–Wagner *et al*, 2011). MSC was cultured in StemMACS MSC Expansion Media (130-091-680, Miltenyi Biotec, Bergisch Gladbach, Germany) supplemented with human StemMACS MSC Expansion Media Kit XF (Miltenyi Biotec), 10% FBS (VWR Life Science, Visalia, CA, USA) and penicillin–streptomycin. Fibroblast was isolated from cancer specimens of CRC patients. The project was approved by the Research Ethics Boards of Ruijin Hospital affiliated to Shanghai Jiao Tong University and the informed consent was signed by the patients.

Cell counting. The growth medium was discarded from the culture dish (Corning, Inc., Christiansburg, VA, USA) and the remaining adherent cells were collected by trypsinisation. Cells were then counted with the CASY TT Cell Counter (Roche Diagnostics, Indianapolis, IN, USA). Viable cells were discriminated from dead/apoptotic cells by trypan blue exclusion.

UV and ionising irradiation. Cells were seeded at a density of 2.5×10^3 cells cm^{-2} in 12-well plates. On the next day, Cells were irradiated with a 254 nm UV-C lamp (UVP Inc., Upland, CA, USA) at a dose of 10 J m^{-2}, which was measured with a UVX radiometer. Irradiated cells were allowed to grow for 48 ~ 72 h without changing the medium, according to a previously published protocol, the dose has also been indicated in this publication (Lu *et al*, 2012). Supernatant and the adherent cells growing on the dish were collected, respectively. Viable adherent cells were counted. For X-rays irradiation, cells were irradiated with an RS225 X-Ray irradiator (200 kV, 10 mA, 1 Gy per 66 s) with the total dose of 10 Gy. After irradiation, cells were returned to the incubator and incubated for 24–48 h. The dose of the ultraviolet radiation and ionising radiation used in this manuscript has been indicated in other publications (Brozyna *et al*, 2007; Gullo *et al*, 2008; Deacon *et al*, 2008; Sato *et al*, 2014; Fujita *et al*, 2015). The optimal condition has also been confirmed by our preliminary experiment.

The co-culture model. To investigate the interaction of CRC cells with MSCs, we used three different co-culture models: the wedge-gap dish (Figure 1D), μ–Slide 2 × 9 well (ibidi, Munich, Germany, Figure 2F), and ibidi culture insert μ–dish (ibidi, Supplementary Figure 1C). For 2 × 9 well μ–Slide, 50 µl CRC cells were seeded in the centre minor well and 50 μl MSCs in each surrounding well at a density of 2.5×10^3 cells cm^{-2}. After cell attachment, 500 µl RPMI1640 complete growth medium was added to the whole growth area, allowing the cells in different nine wells to share common growth medium under irradiation. For wedge-gap dishes (Figure 1D), 2 ml suspended CRC cells and 4 ml suspended MSCs were seeded in the inner dish and outer dish, respectively (MSC was seeded 12 h later after the attachment of CRC cells), with the

density of 2.5×10^3 cells cm^{-2}. Similarly, 15 ml RPMI1640 complete growth medium was added into dishes after cell attachment, allowing inner and outer cells to share growth medium. Cells were then treated with irradiation for 24–48 h.

Wound-healing assay of MSC and CRC cell. Wound-healing assay of 'two different cell line' was performed to investigate the interactions of different cell types. For this purpose, MSCs and CRC cells (2.5×10^3 cells cm^{-2}) were seeded into each well in ibidi's μ-Dish$^{35\,mm,high}$ (ibidi), respectively. (Supplementary Figure 1B). The insert was removed after cell attachment and 3 ml RPMI 1640 complete growth medium was added to the culture dish. For the control group, both wells in the insert were seeded with the same cell line (either CRC cells or MSCs). Subsequently, the co-culture system was treated with irradiation of 10 J cm^{-2} for 96 h, with irradiation untreated cells as the control group.

Cell proliferation assay and colony formation assay. For cell proliferation assay (Figure 3A), 4×10^4 SW1116 or HT29 cells were seeded with or without (the control group) 1×10^4 MSCs in 12-well plate. The cells were then treated with 10 J cm^{-2} irradiation for 1 h per 6 h. Total cells in each well were counted at 12 h, 36 h, and 72 h, respectively.

Similarly, in Figure 3C and D, CRC cells and MSCs was mixed and seeded in ultra-low attachment 24-well plates with a different ratio (CRC: MSCs, 25:25, 50:25, 100:25, 200:25). The same number of CRCs seeded without MSCs in the plate was used as the control group. Different amounts of CRC cells (50, 75, 125, and 225), which were seeded in the plate, respectively, was used as the blank control. Cells were then treated with 10 J cm^{-2} irradiation for 1 h per 8 h, lasting for 8 days. Cell colonies were counted on the 9th day. Independent experiments were repeated at least three times.

In Figure 3E, 6×10^6 CRC cells were mixed with or without 10^6 MSC cells and then seeded in the co-culture system (Supplementary Figure 1B). Cells were treated with 10 J cm^{-2} irradiation for 1 h per 8 h. Total cells in each well were counted at 24 h, 48 h, 72 h, 96 h, and 120 h, respectively.

CFSE cell proliferation assay. CRC cells were collected and pelleted from the co-culture model 2 (Supplementary Figure B) from the experiment group and the control group, respectively. After washed with PBS twice, CRC cells were re-suspended and incubated with CellTrace CFSE (1:1000 dilution) staining solution for 20 min in dark. Cells were pelleted again and resuspended in fresh pre-warmed complete culture medium. The results were analysed by flow cytometry. The experiments were repeated three times.

ELISA. Culture medium from the co-culture system after irradiation was collected at 6, 12, and 24 h, respectively. Cytokines concentration was determined by sandwich ELISA using a Human Th1/Th2/Th17 Cytokines Multi-Analyte ELISArray Kit (Qiagen, Hilden, Germany), following the manufacturer's instructions.

Immunoblot analysis. Cells were homogenised and lysed in RIPA buffer supplemented with proteinase inhibitor. An equal amount of proteins (25 μg) were loaded and run on 12% SDS-PAGE gel and transferred onto PVDF membranes following electrophoresis. After the incubation with 5% milk in TBS/T for 1 h, the membrane was incubated with the primary antibodies at 4 °C overnight. The primary antibodies used in this experiment were: anti-total AKT, anti-total Erk, anti-Phospho-AKT, anti-Phospho Erk1/2, anti-procaspase 3, anti-Caspase3, anti-PI3K, anti-beta-actin, anti-GFAP, anti-vimentin, anti-desmin, anti-alpha-smooth muscle actin, and anti-Phospho Stat3 (Cell signaling, Danvers, MA, USA). GAPDH (Cell Signaling) was used as the loading control.

Immunofluorescence. Cells cultured in eight-well chamber slides (Falcon, BD, Germany) and culture-inserts (ibidi) were washed twice with cold PBS, fixed with 4% para-formaldehyde for 15 min, permeabilised with 0.1 % Triton X-100 for 5 min, blocked with 5 % BSA, incubated with indicated primary antibodies: anti-GFAP and anti-desmin (Sigma, Darmstadt, Germany), anti-Lgr5 (Abcam, Cambridge, UK), anti-α-SMA, and anti-Vimentin (R&D, Minneapolis, MN, USA), APC-anti-CD271 (Miltenyi Biotech, Auburn, CA, USA) at 4 °C overnight and followed by anti-rabbit Alexa fluor 488 secondary antibody and anti-mouse Alexa Fluor 568-conjugated secondary antibody (Life technology, Darmstadt, Germany). The cells were then stained with anti-fade DAPI (Life Technology) for nuclear staining, and the images were acquired with an Olympus Axion microscope (Olympus, Tokyo, Japan).

Flow cytometry. PE-CD133, FITC-CD44 (Biolegend, San Diego, CA, USA), LGR5, APC-CD271 antibodies were used for flow cytometry. Take CD133 for example, the expression of CD133 antigen on hybrids and parental CRC cells were performed by flow cytometry. Cells were stained with PE-conjugated monoclonal anti-human CD133 (Becton Dickinson, San Jose, CA, USA). Isotype control IgG-PE, served as a control. After stained 30 min, samples were analysed by flow cytometry (FACS Calibur, BD Biosciences, San Jose, CA, USA) and data were analysed using CellQuest software and BD FACSDiva6.0 software (BD Biosciences). Intracellular staining flow cytometry followed the standard protocol provided by BD. CFSE (Biolegend, San Diego, CA, USA) and 7-AAD/Annexin V kit (eBioscience, Thermo Fisher Scientific, Darmstadt, Germany) were used to perform proliferation assay and cell apoptosis assay.

Selected isolation of MSCs from the co-culture system. Cell separation from co-culture system was performed using the CD271+ MicroBeads isolation kit (Miltenyi Biotech) as recommended by the manufacturer. Separation occurs in a MACS Column, which induces a high-gradient magnetic field (~0.6 Tesla) when placed in an AutoMACS Separator (Miltenyi Biotech). After the automatic sorting, CD271+ MSCs and CRC cells were separated in different falcon tubes for further analysis.

Organoid culture. Fresh CRC tissue samples were cut into small pieces using a scalpel, washed with ice-cold PBS containing antibiotic 3~5 times, and subsequently digested with 0.05% trypsin, 0.02% EDTA (Thermo Fisher Scientific, Waltham, MA, USA) for 12 min at 37 °C with shaking every 15 min. The remaining fragments were additionally treated with Collagenase NB 4G (SERVA Electrophoresis GmbH, Heidelberg, Germany) at 37 °C for 20 min. The pellet was re-suspended in 24 ml 40% Percoll PLUS/Percoll, placed in 50-ml polystyrene conical centrifuge tube (BD Biosciences) and overlaid with 9 ml 70% Percoll solution. Centrifuge immediately at 2500 rpm (Eppendorf 5810R centrifuge) for 20 min (brake off), at room temperature. The cell fraction was carefully and gently collected above the interphase band (above 1.065 g ml^{-1}) by using a sterile Pasteur pipet, then pelleted at 1500 rpm (Eppendorf, Hamburg, Germany) for 7 min at 4 °C. The cell pellet was suspended with Matrigel (growth factor reduced; BD Biosciences) and dispensed into 48-well culture plates (25 ml matrigel per well), which have also cover with single layer of MSC. The basal culture medium for human intestinal organoids was prepared as recently described (Fujii *et al*, 2016).

Analysis of publicly available data sets. To analyse CD271 mRNA expression in colorectal adenocarcinoma, we obtained the data from TCGA, by using www.cbioportal.org. Specifically, on the home page of the website, select 'Query', then, select 'Colorectal Adenocarcinoma (TCGA, Provisional)', enter CD271 (NGFR) gene in the 'enter gene set', download data from Plots and Survival data, click 'mRNA expression *Z*-score (all genes)' from Select Genomic Profiles, the NGFR mRNA *Z*-scores of 382 cases will appear. To

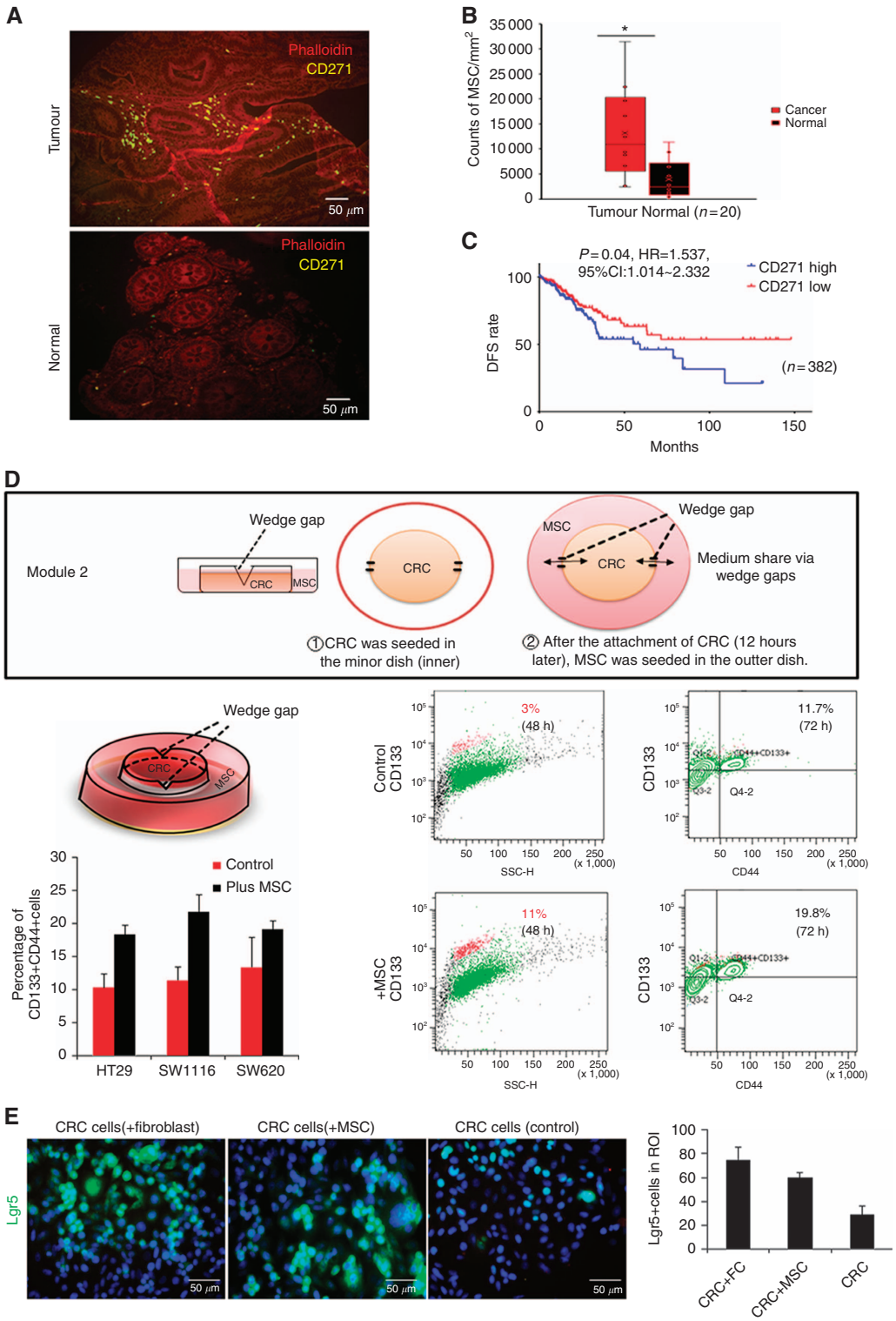
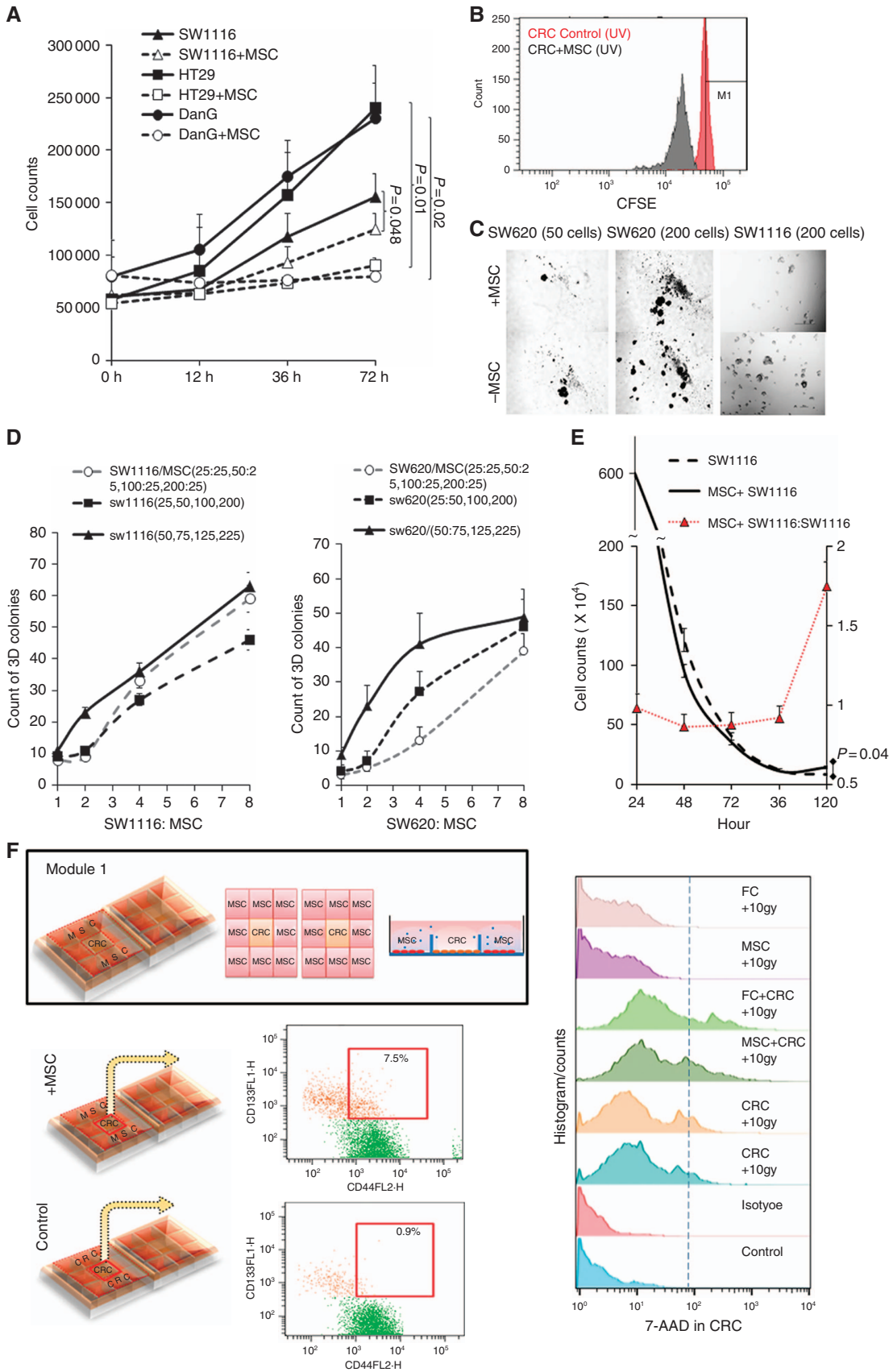


Figure 1. Co-cultivation with MSCs promoted colorectal cancer cells (CRC) to obtain stemness *in vitro*. (**A**) Human colorectal cancer tissues and adjacent normal tissues were stained with the anti-CD271 antibody (MSCs marker, yellow). MSCs could be found surrounding the tumour lesions (200x). (**B**) Counts of CD271 + MSCs of the slides were calculated respectively using ImageJ. MSCs were enriched in tumour site (Red box) compared with normal tissues (black box). (**C**) Disease-free survival (DFS) rate was analysed basing on TCGA data set. Patients were separated into CD271 mRNA *z*-score high and low groups. (**D**) Co-cultivation model for MSCs and CRC. The 3.5 cm and 10 cm dish share medium through two wedge gaps when the medium is enough to reach the level of the wedge gap. If the medium did not reach the wedge gap level, cells in two dishes could grow independently. FACS analysis of CD133 + CRC stem cell-like cells, CD133 + CD44 + stem cell-like cells before and after co-cultivation with MSCs. Three cell lines HT29, SW1116, and SW620 were taken into consideration. (**E**) Immunofluorescence staining and quantification showed the proportion of LGR5 + CRC stem cell-like cells increased after co-cultured with fibroblast or mesenchymal stromal cells compared with control group. *$P<0.05$.

analyse the effect of NGFR expression on prognostic of CRC patients, we generated Kaplan–Meier survival curve of CRC patients with low or high expression of NGFR by using PRISM.

Statistical analysis. All continuous values were expressed as mean ± s.d. and all experiments were repeated three times. The results were subjected to a nonparametric Mann–Whitney *U*-test.



A paired Student's *t*-test, unpaired *t*-test, two-way ANOVA were also used to analyse the intragroup and intergroup differences. All statistical analyses were done using GraphPad Prism7 (GraphPad Software Inc., La Jolla, CA, USA) and Stat View 5.0 for Windows (SAS Institute Inc., Cary, NC, USA). Student's *t*-test was also used to test differences in cell viability assays. A *P*-value <0.05 was considered statistically significant.

RESULTS

The proportion of CRC stem cell-like cells increased when co-cultured with MSCs. CD271 is a biomarker for mesenchymal stem/stromal cells and follicular dendritic cells in the colorectal tumour site. In CRC specimens, the CD271 + MSC density (counts per mm^3, $n=20$) was significantly higher ($P=0.037$, Figure 1A and B) than in adjacent normal tissues (5 ~ 10 cm from the proximal tumour margin). Basing on TCGA publicly available data sets, it was found that high expression of NGFR (CD271) mRNA in the tumour tissues (mRNA *z*-score > -0.3115) was related to worse disease-free survival (DFS) (Figures 1C, $P=0.04$, HR = 1.537, 95% CI: 1.014 ~ 2.332, $n=382$). This might suggest that high density of CD271 + MSC is relevant with a poor DFS rate of the patients. In *in vitro* experiment, CRC displayed the morphological characteristics of epithelial–mesenchymal transition after co-cultured with BM-MSCs for 72 h (Supplementary Figure 1A). To further identify whether MSC-CRC cell-cell adhesion was important for this alteration, three different co-culture models were established. After 72 h co-cultivation in ibidi μ-Dish (module 1, Figure 2F) and wedge-gap dish (module 2, Figure 1D), flow cytometry showed significantly elevated proportion of CD133 + cells (48 h, 11 ± 3.7 *vs* 3 ± 1.9%, $P<0.05$), CD133 + CD44 + cells (Figure 1D, 72 h. 19.74 ± 0.7426 *vs* 11.73 ± 0.9979, $P<0.0001$, 95% CI 5.374 ~ 10.65,) and Lgr5 + cells (Figure 1E, 72 h, CRC + fibroblast, 75 ± 10.8 *vs* CRC + MSC, 60 ± 3.8 *vs* MSC, 29 ± 7) in cancer cells from co-cultivation groups.

Cancer cells underwent epithelial-mesenchymal transition and MSC differentiated into mature cancer-associated fibroblasts (CAF) in the co-culture model. In the MSC-CRC wound-healing assay, MSCs showed greater mobility than CRC cells (Supplementary Figure 1B). Besides, MSCs exhibited a series of morphological changes, including elongated phenotype, reduced adhesion, and increased migration, which were normally observed in the differentiation process of MSCs to CAFs (Direkze *et al*, 2004). Immunofluorescence analysis of MSCs which were co-cultured with CRC cells for 48 h revealed upregulated expression of α-SMA, whereas downregulated expression of desmin, suggesting the differentiation of MSCs to fibroblasts (Supplementary Figure 1C (a–f) D) (Wang *et al*, 2004). Meanwhile, immunofluorescence staining of CRC cells revealed up-regulated vimentin, fibronectin, snail as well as GFAP protein expression and downregulated E-cadherin expression, indicating the progression of epithelial–mesenchymal transition (Supplementary Figure 1A, Figure1C, G-I).

CRC cells showed more attenuated proliferation and viability in the co-culture system than in the non-co-culture system under irradiation. Though it was found that high density of CD271 + MSC is relevant with a poor DFS rate of the CRC patients, however, radiotherapy was not taken into consideration. What is the function of MSCs when under radiation?

CRC cells (4×10^4 cells) and MSCs (1×10^4 cells) were seeded and cultured in module 2 (module 2, Figure 1D), with CRC cells (4×10^4 cells) cultured alone as the control group. Cells were then irradiated with 10 J cm^{-2} irradiation for 1 h per 8 h, lasting for 72 h. Viable CRC cells and MSCs in each group were collected and counted every 12 h, respectively. Proliferation assays were performed in CRC cells. CRC cells from co-culture group showed a significantly attenuated proliferation capability (Figure 2A and B).

In the 3D colony formation assay, different numbers of CRC cells (25, 50, 100, and 200 cells) were seeded in ultra-low attachment plates as the blank control group, additional 25 MSCs were seed to each well to set up the co-culture group, making the final CRC: MSC ratio 1:1, 2:1, 4:1, and 8:1. The negative control group was established by using additional 25 CRC cells instead of 25 MSCs. Cells were then treated with 10 J cm^{-2} irradiation for 1 h per 8 h, lasting for 8 days. Cell colonies were counted on the 9th day (Figure 2C). The negative control group exhibited a significantly enhanced colony formation capability ($p_{sw620}=0.035$, $p_{sw1116}=0.027$, $p_{HT29}=0.043$), especially with CRC:MSC ratio within 2:1 ~4:1 (Figure 2D). Based on these results, we suggest that the MSCs impaired the proliferation and colony formation capability of CRC cells under irradiation. Next, we use module 2, which can include more cells to verify the finding.

Specifically, 6×10^6 CRC cells and 1×10^6 MSC cells were seeded in the inner well and outer well of the co-culture system, respectively (module 2). The control group was established by seeding only the same amount of CRC cells in the inner well. Cells were treated with 10J cm^{-2} irradiation for 1 h every 8 h. Cell counts in each well were calculated at 24 h, 48 h, 72 h, 96 h, and 120 h, respectively. The result is consistent with former results. Especially within 96 h, CRC cell counts declined more rapidly in the co-culture group than CRC cells alone (Figure 2E). Intriguingly, the number of viable CRC cells from the co-culture system stayed stable after 96 h. However, the number of C xRC cells in the control group was still decreasing (Figure 2E, Supplementary Figure 1E). Accordingly, this could be owing to that the MSC-induced part of cancer cells to maintain/gain stemness at the early

Figure 2. Co-culture system showed attenuate proliferation and viability under UV irradiation. (**A**) This is a representative figure of proliferation experiment. In total, 4×10^4 SW1116, HT29 or DanG cells were seeded with or without (control group) 1×10^4 MSCs in 12-well plate. In total, 10 J cm^{-2} irradiation was performed for 1 h and last for 6 h and total cell numbers in each well were counted at 12 h, 36 h, and 72 h respectively. (**B**) CSFE assay of control and co-cultivation group. (**C**, **D**) 25, 50, 100, or 200 SW1116, SW620 cells were seeded in ultra-low attachment 24-well plates, respectively (control), or following by additional 25 bone marrow-derived mesenchymal stromal cells seeding in each well. Twenty-five more SW1116 cells were also seeded instead of 25 MSCs as blank control. After 10 J cm^{-2} irradiation 1 h per 8 h for 8 days, cell colonies were counted on the 9th day. Independent experiments were repeated 2 ~ 3 times. Mean value were represented. (**E**) 6×10^6 SW1116 cells were seeded in co-culture system (Supplementary Figure 2B), with or without 10^6 MSC cells seeding in the inserted well. 10 J cm^{-2} irradiation was performed for 1 h and last for 8 h and total cell numbers in each well were counted at 24 h, 48 h, 72 h, 96 h, and 120 h, respectively. The colorectal cancer cells decreased more rapidly in the co-culture group than control group (SW1116 only) at the beginning (0 ~ 96 h) after irradiation, however, turned to be slower and stayed stable after 96 h. (**F**) Co-culture model for MSCs and CRC. The μ–Slide 2×9 well harbours two arrays of 3×3 square fields where cells can be cultivated independently within the small square or share the same growth medium within the total 3×3 square fields. After co-cultivation for 48 h, flow cytometry showed more CD133 + CD44 + colorectal cancer stem cell-like cells than the control group. However, there were also more 7-AAD + dead cells compared with the control group. *P < 0.05.

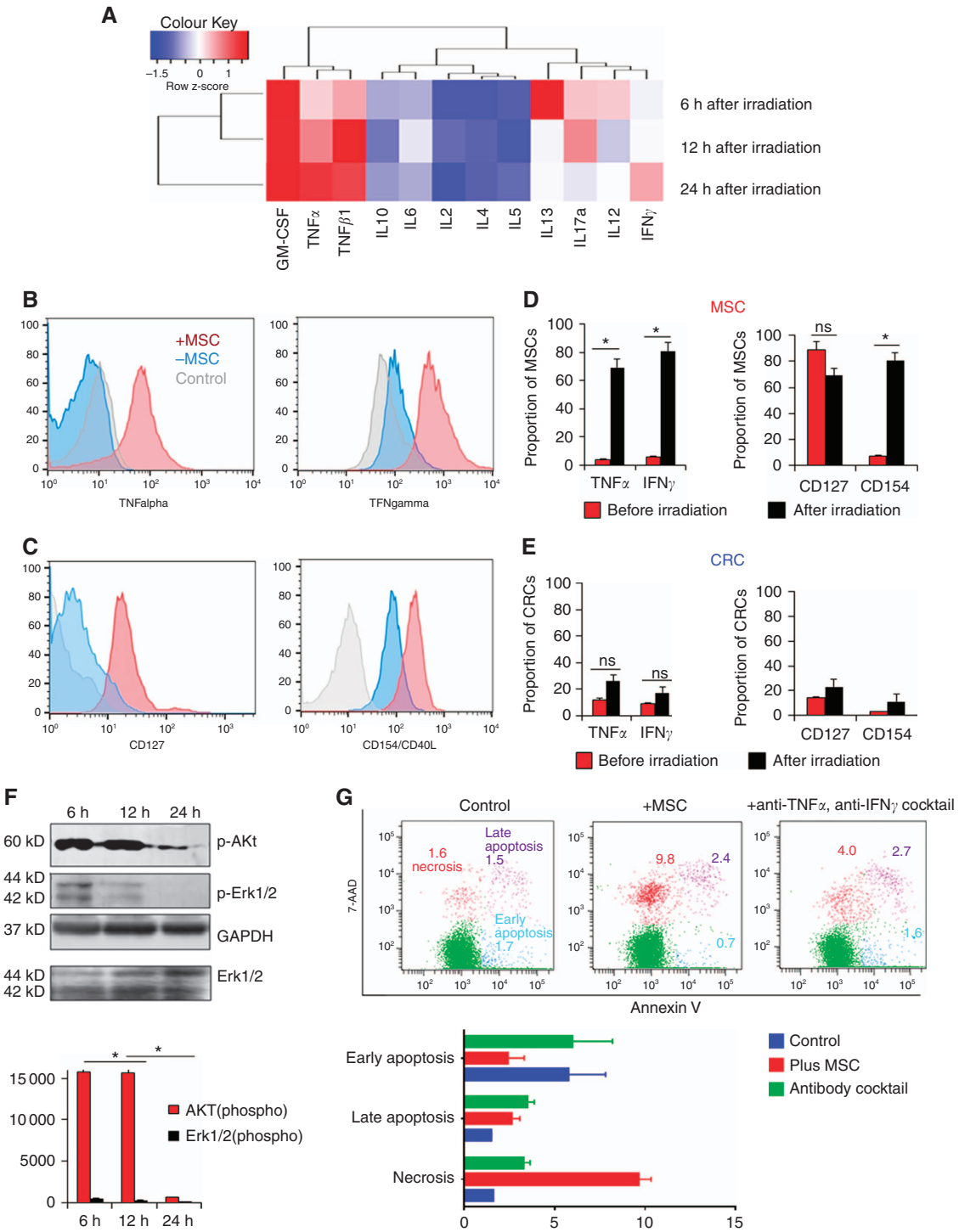


Figure 3. Alteration of cytokine levels and protein expressions in MSCs or CRC cells from the co-cultivation system. (**A**) Supernatant from MSCs and CRC cells co-cultivation model were collected at 6 h, 12 h, and 24 h after irradiation. ELISA array was performed, R studio was used for calculating and establishing the heat map. (**B**–**E**) To identify the origin of cytokines, MSCs, and CRCs were collected from the irradiated co-culture system, respectively. Elevated TNFa, IFN-gamma, and CD154 (CD40L) were detected by flow cytometry in MSCs rather than CRC cells. Proportions of cells are shown in column; (**D**): expression of cytokines in MSC from co-cultivated model before and after irradiation; (**E**): cytokines expression of CRC cells in co-cultivated model before and after irradiation; (**F**): PI3K/AKT signal pathway protein p-Akt and p-Erk1/2 from colorectal cancer cells were significantly suppressed in the coculture system after 6 h, 12 h, and 24 h UV irradiation; (**G**): after 10 Gy ionising irradiation and 24 h incubation, colorectal cancer cell line showed significantly increased necrosis rate, in the coculture model tested by 7-AAD/Annexin V cell apoptosis assay. *$P<0.05$.

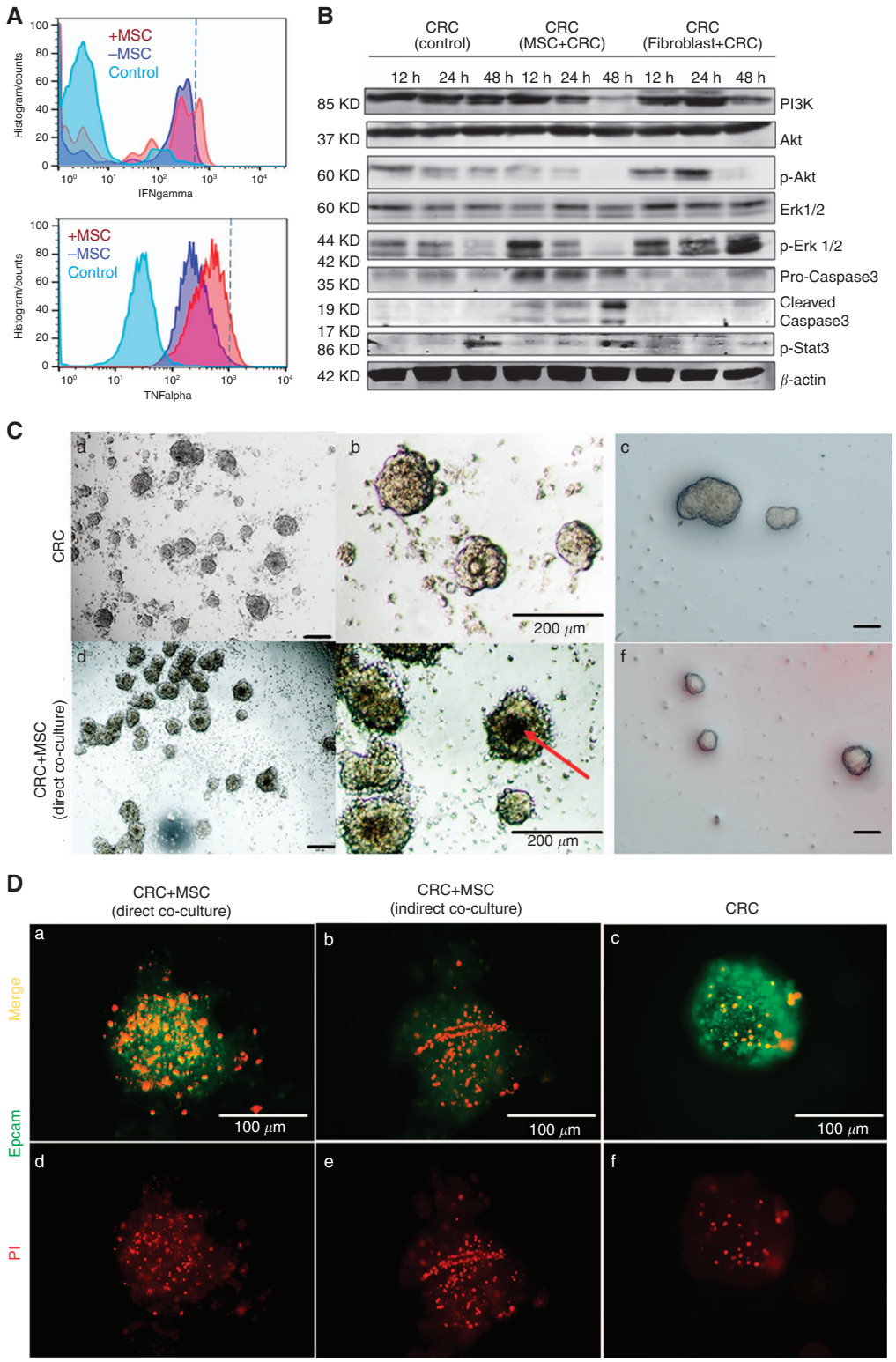


Figure 4. Co-cultivation showed increased cell death and suppression of AKT signal pathway of colorectal cancer cells under ionising irradiation. (**A**) Ionising irradiation could induce TNFα and IFN5 secretion by MSC in the co-cultivated system. (**B**) Colorectal cancer cell ERK and AKT signalling pathways were suppressed in the co-cultivated system, meanwhile, cleaved caspase 3, and p-Stat3 in CRC cells were activated in CRC + MSC co-culture group. (**C**) The same number of 3D spheroids (CRC cells and CRC cells + MSCs) were transferred into an ultra-low attachment plate and treated with 10 J cm^{-2} irradiation for 1 h in every 6 h. Dark cores (red arrow), which were reported to be dead cells, could be observed in the co-culture group. Tumour organoids were co-cultivated with or without MSCs, the volumes of tumour organoids turned to be smaller in the cocultivation group (f, a single layer of MSC was seeded below the Matrigel layer) compared with CRC without MSCs feeding after irradiation. (**D**) To further confirm the cytotoxicity effect of MSC under irradiation, PI staining was performed on two co-culture models (direct CRC cells-MSCs contact and indirect co-cultivation), as well as colorectal cancer spheroids. the co-cultivation group, both direct co-cultivation and indirect co-cultivation showed more dead cells under irradiation even in 3D culture condition.

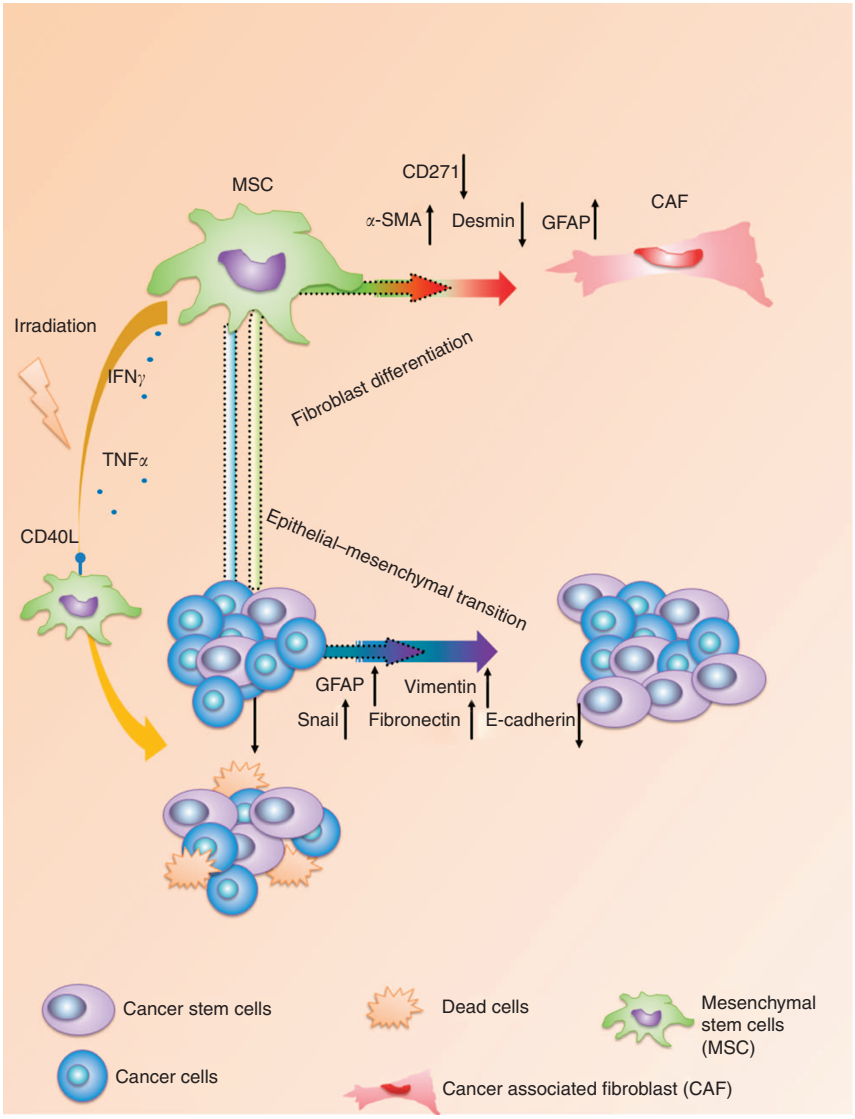


Figure 5. Schematic representation of colorectal cancer cells apoptosis induced by irradiation treated bone marrow-derived mesenchymal stromal cells.

phase of irradiation, thereafter those cancer stem cells-like cells could be resistant to irradiation. Subsequently, the hypothesis was further approved by stem cell staining (CRC + MSC *vs* CRC Control: 7.533 ± 0.48 *vs* $0.95 \pm 0.23\%$, $P < 0.0001$, 95% CI, $-7.703 \sim -5.453$) and 7-AAD live/dead cell staining via flow cytometry (Figure 2F).

MSCs secrete IFNγ, TNFα when co-cultured with CRC cell under irradiation. To investigate the reason behind the finding, we supposed that cytokine alteration induced by MSCs might affect the CRC cells. To verify the hypotheses, cells grown in the co-culture system was treated with 10 J cm^{-2} irradiation for 1 h in every 6 h. The supernatant was collected afterward at 6 h, 12 h, and 24 h, respectively. ELISA array was performed with the supernatant and the result was presented in Figure 3A. It reveals that the supernatant from the co-culture system contained increased concentration of GM-CSF, which was reported by others. Besides, elevated TGF-β1, IFNγ, and TNFα were also detected. In contrast, IL13 decreased significantly after the ultraviolet radiation (UV) irradiation. To investigate the cell origin of TNFα or IFNγ, flow cytometer analysis was performed. We found that it is MSC rather than CRC cell that is responsible for the secretion of TNFα, IFNγ, and expression of CD154 (also known as CD40L), which can induce cytotoxicity against CRC (Figure 3B-E).

Phosphorylated AKT (p-Akt) and phosphorylated Erk1/2 were suppressed in CRC cells from the co-cultured system. Next, we investigate the change of signal pathways, which are involved in the cell proliferation by western blot. Specifically, the result revealed that p-Akt and p-Erk1/2, which are important protein in PI3K/AKT signal pathway, were significantly suppressed in CRC cells when co-cultured with MSCs under UV irradiation conditions (Figure 3F). Taken together, suppression of p-Akt and Erk1/2 in CRC cells, when incubated with MSC under irradiation, contributes to the arrest of the cell proliferation and cell death.

CRC cells showed increased apoptosis and suppressed AKT signal pathway in the co-culture system under ionising irradiation. Subsequently, we investigate the cell apoptosis and AKT signal pathway in CRC cells in the co-culture system. Specifically, CRC cells and MSCs were co-cultured and irradiated under same conditions as described before. Cell death and cell apoptosis assay showed significantly increased cell death of CRC cells, especially necrosis of cells in co-culture group. For instance, SW620 isolated from the co-culture module 2 display significantly

higher proportion of necrotic cells (9.7 ± 1.2 *vs* 1.6 ± 0.1%) and late apoptotic cells (2.6 ± 0.8 *vs* 1.5 ± 0.05%) after irradiation ($P = 0.0012$). Despite the heterogeneity among different cancer cell lines, total cell death and necrosis rate of CRC cells were increased significantly in the co-culture group (Figure 3G). In consistent with the result of UV irradiation, ionising irradiation could also induce TNFα and IFNγ secretion by MSC in the co-culture system (TNFα: 13.6 *vs* 1.2%, $P < 0.05$; IFNγ 22.1 *vs* 4.6%, $P < 0.01$, Figure 4A). When the co-culture system was treated with TNFα and IFNγ neutralising antibodies, CRC cells displayed attenuated cell death rate ($P = 0.03$, Figure 3G). Moreover, ERK and AKT signalling pathways were suppressed while apoptosis pathway was activated in CRC cells. Interestingly, p-Stat3 was also activated in CRC cells, which might be the result of feedback regulation to rescue CRC cells from death (Figure 4B).

CRC cells showed increased apoptosis in the 3D co-culture system under ionising irradiation. Afterward, the cytotoxicity of MSC under ionising irradiation was performed in the 3D co-culture system. CRC cells were co-cultivated with or without MSC in the hanging-drop plates to form 3D spheroids (direct co-cultured). The same number of spheroids were then transferred into a 96-well ultra-low attachment plate and treated with 10J cm^{-2} irradiation for 1 h in every 6 h. Dark cores, which were reported to be dead cells, could be observed in the co-culture group (Figure 4C). Tumour organoids derived from three patients were also sub-cultured with or without MSC (see Method), the volumes of tumour organoids turned to be smaller in the co-cultivation group (Figure 4F) after irradiation. To further confirm the cytotoxicity effect of MSC under irradiation, immunofluorescence staining was performed on two co-culture models (direct and indirect), as well as CRC spheroids. In consistent with our hypothesis, the co-cultivation group showed more dead cells under irradiation even in 3D condition (Figure 4D).

DISCUSSION

Radiation therapy could render tumour cells visible to the immune system of patients. In addition to the direct effects of radiation, the ensuing immune response orchestrates the expression of inflammatory mediators and cytokines, which act on local milieu and neighbouring tumour cells (Sologuren *et al*, 2014). Although MSCs are not originally identified as immune cells, MSCs possesse various immune regulatory capacities and are pivotal components in the tumour microenvironment, being able to home and infiltrate into the tumour stroma. Up to date, results of previous studies are controversial about its biological interaction with CRC cells, particularly in terms of promotion versus inhibition of tumour progress (Guan and Chen, 2013).

In normal tissues, MSCs were observed to be radiation-protective through its well-known ability of regenerative functions after ionising radiation (Gao *et al*, 2012; Li *et al*, 2013; Nicolay *et al*, 2015; Wilson *et al*, 2015). It was proposed that MSC may also be protective in a similar way for CRC cells after radiation. Thereafter, some research groups found that after the treatment of MSC-conditioned medium, breast cancer cells could exhibit elevated proliferation capability as well as radiation-resistance, owing to the high levels of insulin-like growth factor-1 in the medium (Yang *et al*, 2014). Meanwhile, evidence was also provided that MSC could induce both inflammatory and immune suppressive micro-environment (Eterno *et al*, 2014), indicating that the cytokines, e.g. G-CSF, IL1a, and TNFα etc., were tightly regulated by environmental challenges (Zhukareva *et al*, 2010).

In contrast, most recent investigations demonstrated that MSCs were able to inhibit tumour growth by apoptosis induction (Han *et al*, 2014). Moreover, it seems that MSCs, in combination with irradiation, were able to enhance the systematic anti-tumour effect of ionising radiation and thus synergistically increase the efficiency of radiotherapy (de Araújo Farias *et al*, 2015). One of the reasons might be that radiation-induced acute damage to MSCs and a perpetual cascade of cytokines triggered the recruitment of immune cells (Wirsdörfer and Jendrossek, 2016). Intriguingly, lower doses of irradiation could selectively stimulate the proliferation of MSCs but not tumour cells *in vitro* via the activation of MAPK/ERK signalling pathway (Liang *et al*, 2011). In addition, MSCs could also possess superior antioxidant ROS-scavenging capacity and more active DNA double-strand break repair systems to facilitate their radioresistance (Chen *et al*, 2006). In the present study, we demonstrated that, under a low dose of radiation, MSCs might improve the anticancer responses by releasing various cytokines such as IFNγ and TNFα and expressing upregulated CD154, and attenuated the proliferative activity and viability of CRC cells, producing a potent cytotoxic synergistic effect on CRC cells. Besides, other groups have found that MSC can express iNOS, secrete IL-12, IL-2, and other foreign gene-encoded cytokines to inhibit tumour progress or the proliferation of other cells (Nakamura *et al*, 2004; Xiang *et al*, 2009; Gao *et al*, 2010; Zinöcker and Vaage, 2012; Jeong *et al*, 2015).

Cell death was just one aspect of cancer biology. Another vital aspect was the invasion of the cells, which is directly related to metastasis. To evaluate whether X-ray irradiation lead to increased metastases, the transwell invasion assay was performed. Though the CRC and MSC co-culture group showed stronger invasive capability, there was no difference between CRC + MSC group and CRC group when cultivated under irradiation.

Furthermore, recent data also suggested that MSCs exhibited potentials for inhibiting tumour proliferation or spread through cell cycling arresting and cell death-related signalling pathways activation (Chang *et al*, 2015). For example, Khakoo *et al* reported that reducing tumour cell growth could be observed *ex vivo* when treated with human bone marrow MSC by Akt activity inhibition of tumour cells (Khakoo *et al*, 2006). In the present study, irradiation on the co-culture system induced the cleavage of caspase3, and attenuated the PI3K/ AKT as well as ERK in cancer cells. Similarly, it was reported that MSC associated anticancer effect was mainly by suppressing PI3K/Akt signalling pathway and subsequently increasing the protein level of cell death factors (Ma *et al*, 2012). Specifically, combination of MSCs and radiations resulted in the cleavages of caspase 9/3, increased phosphorylation of JNK and decreased phosphorylation of PI3K/AKT and ERK in cancer cells. Suppression of PI3K/Akt signalling cascades could lead to the blockade of both cell cycle progression and cell growth during CRC development. Han *et al* (2014) reported that JNK inhibition reversed the apoptotic ability of MSCs to cleave caspase 9/3 in prostate cancer cells, indicating that the JNK pathway might also be activated when PI3K was suppressed. Aikin *et al* (2004) claimed that suppression of PI3K was the reason of increased JNK phosphorylation and cell death. Many mechanisms might be involved in the suppression of PI3K/Akt signalling pathway, e.g., cytokines (IGF-1, TNFα *et al*) (Liu *et al*, 2017), PTEN over-expression, and miRNA. Concerning the cytokines, though it was reported that TNFα could inhibit the apoptosis of several cancer cells by inducing the phosphorylation of Akt at both Ser473 and Thr308, the switch of promoting or inhibiting apoptosis effect was mainly dependent on the concentration of TNFα (Sandra *et al*, 2002). Other mechanisms included ROS-induced PI3K/Akt signalling pathway activation and radiation-associated bystander effect of MSCs on CRCs. Radiation-induced bystander effect is to descript the phenomenon that cells which exposed to irradiation could communicate their DNA damage response condition to bystander cells, which have not been directly irradiated. Cytokines induced ROS-production (Schaue *et al*, 2012), Activation of NF-çB signalling pathway, and NF-çB induced enzymatic systems like

iNOS were suggested to be the molecular underpinnings of this bystander effect (Han *et al*, 2003).

In co-culture experiments without UV irradiation, we found that MSCs prompted the epithelial-mesenchymal-transition and CRC cell stemness. Similarly, Xue JG *et al* reported that co-culture of gastric cancer cells and umbilical cord MSCs increased the expression of stemness factors and EMT markers, such as N-cadherin, Vimentin, α-SMA, and fibroblast activation protein, in gastric cancer cells (Direkze *et al*, 2004; Xue *et al*, 2015) Though MSC was important in promoting an inflammatory and immune suppression microenvironment, the function of MSCs was also interfered by the tumour microenvironment. In this study, we also observed an elevated expression of TGF-β, particularly at the early phase of post-irradiation, in the supernatant of the co-culture model. We verified that elevated expressions of TGF-β could promote the differentiation of MSCs to CAFs (Wang *et al*, 2004; Quante *et al*, 2011). Similarly, other groups have reported that TGF-β can stimulate hypomethylation of MSCs and induce gene expression profiles alterations towards myofibroblast signature-expressing biomarkers. However, the limitation of this study is lack of *in vivo* perspective. Concerning preclinical situation, the study from de Araújo Farias *et al* might give a clue. They reported that *in vitro* MSCs are a source of anti-tumour cytokines after a low dose of irradiation, MSCs decreased the proliferative activity of tumour cells, producing a potent cytotoxic synergistic effect on tumour cells (Direkze *et al*, 2004; de Araújo Farias *et al*, 2015; Xue *et al*, 2015).

It was recently demonstrated that radiations might induce senescence of MSCs which affect the functions. Consequently, senescent cells could block the proliferation of cancer cells and induced apoptosis (Özcan *et al*, 2015, 2016; Alessio *et al*, 2017). Thus, we performed the acid *β*-galactosidase assay. Cells were irradiated with the total dose of 10 Gy. We found the proportion of senescent MSCs was increasing after the irradiation ($P = 0.037$) (Supplementary Figure 2A). However, when we incubated the cancer cells with the conditional medium from control or senescent MSC, there was no significant effect on CRC cells by acid *β*-galactosidase staining. Secretomes and exosomes had an important role in anti-tumour activities. However, in the present study, the effect was not significant. It might be owing to the effect becoming impaired when secretomes were collected from senescent cells previously in contact with cancer cells as reported before (Özcan *et al*, 2015; Alessio *et al*, 2017).

Concerning the limitations of this study, the *in-vivo* experiment needs to be further performed to verify not only the therapeutic achievement but also bystander effects of MSCs administration in CRC patients. In addition, concerning the similarities and differences between of UV-C and X-ray in the present study, for UV radiation, the main damages are on the same DNA strand. However, X-rays induced both single and double strand breaks. Another difference is that the X-rays are more penetrating, which is also why they are used in radiation oncology. However, the similarities could be found even at the beginning of the signalling response cascade. Both these damages allow the phosphorylation of histone variant 2AX and form characteristic 'repair foci'. UV and ionising irradiation were used together in lots of studies (Aszterbaum *et al*, 1999). Because both UV and ionising radiation (IR) produce oxidised bases DNA damage, they could also elicit complex cellular responses involving several similar signalling pathways, for example, NF-çB signalling pathway, MAPK signalling pathway, PI3K/AKT signalling pathway, and ROS-associated signalling pathways (Rieger and Chu, 2004).

Because MSC differentiated into CAF during the cocultivation with tumour cells, it is important to examine the BM-MSCs as a function of time: before and after this differentiation occurs. It was reported that the CAF associated genes such as SDF-1, CCL2, MMP9, and PDGFRB were upregulated in the 30-day tumour conditional media exposed MSC. Most of the top 25 upregulated genes were involved in glycoprotein and binding process and in cellular metabolism. (Mishra *et al*, 2008). Those CAF-like cells could stimulate tumour survival and proliferation, angiogenesis, and metastatic spread in xenograft models (Guilloton *et al*, 2012).

Concerning the clinical setting, the time period might change the treatment strategy. In brief, it took more or less 5–6 weeks. However, the functional consequence of MSC plus radiation is controversial. The detailed properties of radiation-surviving endogenous MSCs are not well documented in human and animal studies. It is generally agreed that the physiologic properties of surviving MSCs after a life-threatening dose of radiation are more likely to differ significantly from those before radiation exposure, despite their having an active DNA damage responding pathway. Even the anti-fibrotic or pro-fibrotic effect is still not certain, from one hand, MSC could stimulate the cell proliferation of fibroblast and modulate relevant soluble mediators and proteinases after external radiation (Haubner *et al*, 2015), on the other hand, the post-irradiation injection of MSC could induce host secretion of HGF and PGE2 to control the activation of fibroblasts. To summary, the effects differ a lot owing to the various models and dose setting. Thus, to establish a standard, more clinically relevant model to evaluate the effect of MSC as well as radiation is essential. The present study is just offering evidence from one perspective.

Taken together, this study provided a possibility to enhance the anti-tumour effect of radiotherapy by utilising MSCs treatment. (Figure 5). When irradiated with low-dose irradiation, BM-MSCs show an anti-tumour effect by secreting cytokines like TNF-α, IFN-γ that lead to the inhibition of proliferation and induction of apoptosis of CRC cells. In addition, suppression of PI3K/AKT signal pathway proteins p-Akt and Erk1/2 in CRC cells also contributed to the arrest of cancer cell proliferation and cell death under irradiation when incubated with MSC.

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CONFLICT OF INTEREST

The authors declare no conflict of interest

REFERENCES

Aikin R, Maysinger D, Rosenberg L (2004) Cross-talk between phosphatidylinositol 3-kinase/AKT and c-jun NH2-terminal kinase mediates survival of isolated human islets. *Endocrinology* **145**(10): pp 4522–4531.

Alessio N, Esposito G, Galano G, De Rosa R, Anello P, Peluso G, Tabocchini MA, Galderisi U (2017) Irradiation of mesenchymal stromal cells with low and high doses of alpha particles induces senescence and/or apoptosis. *J Cell Biochem* **118**(9): 2993–3002.

Alessio N, Capasso S, Di Bernardo G, Cappabianca S, Casale F, Calarco A, Cipollaro M, Peluso G, Galderisi U (2017) Mesenchymal stromal cells having inactivated RB1 survive following low irradiation and accumulate damaged DNA: Hints for side effects following radiotherapy. *Cell Cycle* **16**(3): 251–258.

Alessio N, Del Gaudio S, Capasso S, Di Bernardo G, Cappabianca S, Cipollaro M, Peluso G, Galderisi U (2015) Low dose radiation induced senescence of human mesenchymal stromal cells and impaired the autophagy process. *Oncotarget* **6**(10): pp8155.

Aszterbaum M, Epstein J, Oro A, Douglas V, LeBoit PE, Scott MP, Epstein EH (1999) Ultraviolet and ionizing radiation enhance the growth of BCCs and trichoblastomas in patched heterozygous knockout mice. *Nat Med* **5**(11): 1285–1291.

Brozyna A, Zbytek B, Granese J, Carlson JA, Ross J, Slominski A (2007) Mechanism of UV-related carcinogenesis and its contribution to nevi/melanoma. *Expert Rev Dermatol* **2**(4): pp451–469.

Chang PY, Qu YQ, Wang J, Dong LH (2015) The potential of mesenchymal stem cells in the management of radiation enteropathy. *Cell Death Dis* **6**(8): ppe1840.

Chen F, Zhuang X, Lin L, Yu P, Wang Y, Shi Y, Hu G, Sun Y (2015) New horizons in tumor microenvironment biology: challenges and opportunities. *BMC Med* **13**(1): pp45.

Chen MF, Lin CT, Chen WC, Yang CT, Chen CC, Liao SK, Liu JM, Lu CH, Lee KD (2006) The sensitivity of human mesenchymal stem cells to ionizing radiation. *Int J Radiat Oncol Biol Phys* **66**(1): pp244–253.

Deacon DH, Hogan KT, Swanson EM, Chianese-Bullock KA, Denlinger CE, Czarkowski AR, Schrecengost RS, Patterson JW, Teague MW, Slingluff CL (2008) The use of gamma-irradiation and ultraviolet-irradiation in the preparation of human melanoma cells for use in autologous whole-cell vaccines. *BMC Cancer* **8**(1): pp360.

de Araújo Farias V, O'Valle F, Lerma BA, de Almodóvar CR, López-Peñalver JJ, Nieto A, Santos A, Fernández BI, Guerra-Librero A, Ruiz-Ruiz MC, Guirado D (2015) Human mesenchymal stem cells enhance the systemic effects of radiotherapy. *Oncotarget* **6**(31): pp31164.

Direkze NC, Hodivala-Dilke K, Jeffery R, Hunt T, Poulsom R, Oukrif D, Alison MR, Wright NA (2004) Bone marrow contribution to tumor-associated myofibroblasts and fibroblasts. *Cancer Res* **64**(23): pp8492–8495.

Eterno V, Zambelli A, Pavesi L, Villani L, Zanini V, Petrolo G, Manera S, Tuscano A, Amato A (2014) Adipose-derived Mesenchymal Stem Cells (ASCs) may favour breast cancer recurrence via HGF/c-Met signaling. *Oncotarget* **5**(3): pp613.

Fazel R, Krumholz HM, Wang Y, Ross JS, Chen J, Ting HH, Shah ND, Nasir K, Einstein AJ, Nallamothu BK (2009) Exposure to low-dose ionizing radiation from medical imaging procedures. *N Engl J Med* **361**(9): pp849–857.

Fujii M, Shimokawa M, Date S, Takano A, Matano M, Nanki K, Ohta Y, Toshimitsu K, Nakazato Y, Kawasaki K, Uraoka T (2016) A colorectal tumor organoid library demonstrates progressive loss of niche factor requirements during tumorigenesis. *Cell Stem Cell* **18**(6): pp827–838.

Fujita M, Yamada S, Imai T (2015) Irradiation induces diverse changes in invasive potential in cancer lines. *Semin Cancer Biol* **35**: 45–52.

Galderisi U, Giordano A (2014) The gap between the physiological and therapeutic roles of mesenchymal stem cells. *Med Res Rev* **34**(5): pp1100–1126.

Gao P, Ding Q, Wu Z, Jiang H, Fang Z (2010) Therapeutic potential of human mesenchymal stem cells producing IL-12 in a mouse xenograft model of renal cell carcinoma. *Cancer Lett* **290**(2): pp157–166.

Gao Z, Zhang Q, Han Y, Cheng X, Lu Y, Fan L, Wu Z (2012) Mesenchymal stromal cell-conditioned medium prevents radiation-induced small intestine injury in mice. *Cytotherapy* **14**(3): pp267–273.

Guan J, Chen J (2013) Mesenchymal stem cells in the tumor microenvironment. *Biomed Rep* **1**(4): pp517–521.

Guilloton F, Caron G, Ménard C, Pangault C, Amé-Thomas P, Dulong J, De Vos J, Rossille D, Henry C, Lamy T, Fouquet O (2012) Mesenchymal stromal cells orchestrate follicular lymphoma cell niche through the CCL2-dependent recruitment and polarization of monocytes. *Blood* **119**(11): pp2556–2567.

Gullo CA, Hwang WY, Poh CK, Au M, Cow G, Teoh G (2008) Use of ultraviolet-light irradiated multiple myeloma cells as immunogens to generate tumor-specific cytolytic T lymphocytes. *J Immune Based Ther Vaccines* **6**(1): 2.

Han D, Antunes F, Canali R, Rettori D, Cadenas E (2003) Voltage-dependent anion channels control the release of the superoxide anion from mitochondria to cytosol. *J Biol Chem* **278**(8): pp5557–5563.

Han I, Yun M, Kim EO, Kim B, Jung MH, Kim SH (2014) Umbilical cord tissue-derived mesenchymal stem cells induce apoptosis in PC-3 prostate cancer cells through activation of JNK and downregulation of PI3K/AKT signaling. *Stem Cell Res Ther* **5**(2): pp54.

Haubner F, Muschter D, Pohl F, Schreml S, Prantl L, Gassner HG (2015) A co-culture model of fibroblasts and adipose tissue-derived stem cells reveals new insights into impaired wound healing after radiotherapy. *Int J Mol Sci* **16**(11): pp25947–25958.

Hendijani F, Javanmard SH (2015) Dual protective and cytotoxic benefits of mesenchymal stem cell therapy in combination with chemotherapy/radiotherapy for cancer patients. *Crit Rev Eukaryot Gene Expr* **25**(3): 203–207.

Houthuijzen JM, Daenen LGM, Roodhart JML, Voest EE (2012) The role of mesenchymal stem cells in anti-cancer drug resistance and tumour progression. *Br J Cancer* **106**(12): pp1901.

Jeong KY, Lee EJ, Kim SJ, Yang SH, Sung YC, Seong J (2015) Irradiation–induced localization of IL–12–expressing mesenchymal stem cells to enhance the curative effect in murine metastatic hepatoma. *Int J Cancer* **137**(3): pp721–730.

Khakoo AY, Pati S, Anderson SA, Reid W, Elshal MF, Rovira II, Nguyen AT, Malide D, Combs CA, Hall G, Zhang J (2006) Human mesenchymal stem cells exert potent antitumorigenic effects in a model of Kaposi's sarcoma. *J Exp Med* **203**(5): pp1235–1247.

Li Y, Xu J, Mao L, Liu Y, Gao R, Zheng Z, Chen W, Le A, Shi S, Wang S (2013) Allogeneic mesenchymal stem cell therapy for bisphosphonate-related jaw osteonecrosis in Swine. *Stem Cells Dev* **22**(14): pp2047–2056.

Liang X, So YH, Cui J, ZHAO XU X, Y CAI L, LI W (2011) The low-dose ionizing radiation stimulates cell proliferation via activation of the MAPK/ERK pathway in rat cultured mesenchymal stem cells. *J Radiat Res* **52**(3): pp380–386.

Liotta F, Querci V, Mannelli G, Santarlasci V, Maggi L, Capone M, Rossi MC, Mazzoni A, Cosmi L, Romagnani S, Maggi E (2015) Mesenchymal stem cells are enriched in head neck squamous cell carcinoma, correlates with tumour size and inhibit T-cell proliferation. *Br J Cancer* **112**(4): pp745.

Liu Q, Guan JZ, Sun Y, Le Z, Zhang P, Yu D, Liu Y (2017) Insulin-like growth factor 1 receptor-mediated cell survival in hypoxia depends on the promotion of autophagy via suppression of the PI3K/Akt/mTOR signaling pathway. *Mol Med Rep* **15**(4): pp2136–2142.

Lu AG, Feng H, Pu-Xiong-Zhi Wang DP, Han XHC, Zheng MH (2012) Emerging roles of the ribonucleotide reductase M2 in colorectal cancer and ultraviolet-induced DNA damage repair. *World J Gastroenterol* **18**(34): pp4704.

Ma Y, Hao X, Zhang S, Zhang J (2012) The in vitro and in vivo effects of human umbilical cord mesenchymal stem cells on the growth of breast cancer cells. *Breast Cancer Res Treat* **133**(2): pp473–485.

Mayer–Wagner S, Passberger A, Sievers B, Aigner J, Summer B, Schiergens TS, Jansson V, Müller PE (2011) Effects of low frequency electromagnetic fields on the chondrogenic differentiation of human mesenchymal stem cells. *Bioelectromagnetics* **32**(4): pp283–290.

Mishra PJ, Mishra PJ, Humeniuk R, Medina DJ, Alexe G, Mesirov JP, Ganesan S, Glod JW, Banerjee D (2008) Carcinoma-associated fibroblast–like differentiation of human mesenchymal stem cells. *Cancer Res* **68**(11): pp4331–4339.

Nakamura K, Ito Y, Kawano Y, Kurozumi K, Kobune M, Tsuda H, Bizen A, Honmou O, Niitsu Y, Hamada H (2004) Antitumor effect of genetically engineered mesenchymal stem cells in a rat glioma model. *Gene Ther* **11**(14): pp1155.

Nicolay NH, Perez RL, Saffrich R, Huber PE (2015) Radio-resistant mesenchymal stem cells: mechanisms of resistance and potential implications for the clinic. *Oncotarget* **6**(23): pp19366.

Özcan S, Alessio N, Acar MB, Toprak G, Gönen ZB, Peluso G, Galderisi U (2015) Myeloma cells can corrupt senescent mesenchymal stromal cells and impair their anti-tumor activity. *Oncotarget* **6**(37): pp39482.

Özcan S, Alessio N, Acar MB, Mert E, Omerli F, Peluso G, Galderisi U (2016) Unbiased analysis of senescence associated secretory phenotype (SASP) to identify common components following different genotoxic stresses. *Aging* **8**(7): pp1316.

Pommey S, Galipeau J (2006) The use of mesenchymal stromal cells in oncology and cell therapy. *Bull Cancer* **93**(9): pp901–907.

Quante M, Tu SP, Tomita H, Gonda T, Wang SS, Takashi S, Baik GH, Shibata W, DiPrete B, Betz KS, Friedman R (2011) Bone marrow-derived myofibroblasts contribute to the mesenchymal stem cell niche and promote tumor growth. *Cancer Cell* **19**(2): pp257–272.

Rieger KE, Chu G (2004) Portrait of transcriptional responses to ultraviolet and ionizing radiation in human cells. *Nucleic Acids Res* **32**(16): pp4786–4803.

Sandra F, Matsuki NA, Takeuchi H, Ikebe T, Kanematsu T, Ohishi M, Hirata M (2002) TNF inhibited the apoptosis by activation of Akt serine/

threonine kinase in the human head and neck squamous cell carcinoma. *Cell Signal* **14**(9): pp771–778.
Sato K, Imai T, Okayasu R, Shimokawa T (2014) Heterochromatin domain number correlates with X-ray and carbon-ion radiation resistance in cancer cells. *Radiat Res* **182**(4): pp408–419.
Schaue D, Kachikwu EL, McBride WH (2012) Cytokines in radiobiological responses: a review. *Radiat Res* **178**(6): pp505–523.
Schreuders EH, Ruco A, Rabeneck L, Schoen RE, Sung JJ, Young GP, Kuipers EJ (2015) Colorectal cancer screening: a global overview of existing programmes. *Gut* **64**(10): pp1637–1649.
Sologuren I, Rodríguez-Gallego C, Lara PC (2014) Immune effects of high dose radiation treatment: implications of ionizing radiation on the development of bystander and abscopal effects. *Transl Cancer Res* **3**(1): pp18–31.
Wang D, Park JS, Chu JS, Krakowski A, Luo K, Chen DJ, Li S (2004) Proteomic profiling of bone marrow mesenchymal stem cells upon transforming growth factor β1 stimulation. *J Biol Chem* **279**(42): pp43725–43734.
Wilson JG, Liu KD, Zhuo H, Caballero L, McMillan M, Fang X, Cosgrove K, Vojnik R, Calfee CS, Lee JW, Rogers AJ (2015) Mesenchymal stem (stromal) cells for treatment of ARDS: a phase 1 clinical trial. *Lancet Respir Med* **3**(1): pp24–32.
Wirsdörfer F, Jendrossek V (2016) The role of lymphocytes in radiotherapy-induced adverse late effects in the lung. *Front Immunol* **7**: 591.
Xiang J, Tang J, Song C, Yang Z, Hirst DG, Zheng QJ, Li G (2009) Mesenchymal stem cells as a gene therapy carrier for treatment of fibrosarcoma. *Cytotherapy* **11**(5): pp516–526.
Xue J, Zhu Y, Sun Z, Ji R, Zhang X, Xu W, Yuan X, Zhang B, Yan Y, Yin L, Xu H (2015) Tumourigenic hybrids between mesenchymal stem cells and gastric cancer cells enhanced cancer proliferation, migration and stemness. *BMC Cancer* **15**(1): pp793.
Yang HY, Qu RM, Lin XS, Liu TX, Sun QQ, Yang C, Li XH, Lu W, Hu XF, Dai JX, Yuan L (2014) IGF-1 from adipose-derived mesenchymal stem cells promotes radioresistance of breast cancer cells. *Asian Pac J Cancer Prev* **15**(23): pp10115–10119.
Zhukareva V, Obrocka M, Houle JD, Fischer I, Neuhuber B (2010) Secretion profile of human bone marrow stromal cells: donor variability and response to inflammatory stimuli. *Cytokine* **50**(3): pp317–321.
Zinöcker S, Vaage JT (2012) Rat mesenchymal stromal cells inhibit T cell proliferation but not cytokine production through inducible nitric oxide synthase. *Front Immunol* **3.**

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Current work

Hepatic resident iNKT cells recruitment and immune microenvironment modulation in colorectal liver metastasis

Aim: The aim of this ongoing study is to investigate the modulatory effect of the hepatic immune microenvironment by the crosstalk of hepatic resident iNKT cells and Kupffer cell as well as dendritic cells in hepatic metastases of colorectal cancer.

Methods: Various of sub-classification of iNKT cells, Kupffer cell as well as dendritic cells located in the core of tumor, cancer invasive margin, as well as adjacent normal hepatic tissue were detected and analyzed by BD flow cytometry (LSR Fortessa™), immunofluorescence, and colorectal malignancy tissue microarray(n=138). *Ex vivo* 3D co-cultivation simulation and Quansys Multiplex assay were also performed.

Interim results: In metastatic status, CXCR6 positive iNKT cells had a positive correlation with long term outcome. Additionally, iNKT cells, especially type 2 iNKT cells (NKT2) were enriched at the invasive margin. Interestingly, despite less iNKT cells presenting at the core of tumor, the proportion of iNKT cells out of lymphocytes elevated to appropriate five folds and expressed enhanced IFNγ, IL9, and FasL compared to normal hepatic tissue and invasive margin. This implied the vital role of iNKT cells in immunological surveillance. Both dendritic cells and Kupffer cells had the capability to promote iNKT cells activation, the proportion of BDCA-3+DCs dramatically increased from normal tissue to the core of the tumor. Though BDCA-3+DCs expressing an enhanced level of CXCL16 comparing to other subtypes, the competency of CD1d mediated iNKT activation was inferior to plasmacytoid DC. Plasmacytoid DC expressed

with significantly higher α-GalCer: CD1d complex formation in ex vivo assays, implying the vital role of pDC in NKT activation. In the present study, Kupffer cells also showed the potential to awaken iNKT cells. Intriguingly, iNKT released Th2 cytokines when activated by Kupffer cells. On contrast, Th1 biased immune response might be detected when NKT cells were activated by dendritic cells. Furthermore, KC subtypes also differed from location to location. Additionally, transmembrane CD1d expression, intra/tmCXCL16 expression, and Th1/2/17 biased immune responses of Kupffer cells and dendritic cells were related to lipid metabolism preferences of these cells.

"The task of science is to stake out the limits of the knowable, and to center consciousness within them."

1849, R. Virchow, *Der Mensch*