Aus der Medizinischen Klinik und Poliklinik IV
der Ludwig-Maximilians-Universität München
Direktor: Prof. Dr. med. Martin Reincke

Der Einfluss von Ki67-index, Tumorlokalisation, Alter und Mismatch-Reparatur-Status auf das Überleben von Patienten mit Kolorektalkarzinom

Dissertation

Zum Erwerb des Doktorgrades der Merdizin
an der medizinischen Fakultät
der Ludwig-Maximilians-Universität zu München

vorgelegt von

Pan Li

aus

Shan Dong Provinz, China

2017

Mit genehmigung der Medizinischen Fakultät
der Universität München

Berichterstatter:	Prof. Dr. Dr. Fuat S. Oduncu, MA, EMB, MBA
Mitberichterstatter:	PD Dr. med. habil. Dr. med. univ. Jens Neumann
Dekan:	Prof. Dr. med. Dent. Reinhard Hickel
Tag der mündlichen Prüfung:	07.12.2017

Erklärung

Hier mit erkläre ich, dass die Arbeit meine ursprüngliche Arbeit ist. Die Arbeit und Ergebnisse in der Arbeit wurden eigenständig durchgeführt. Ein Teil der Daten zum Kolorektalkarzinom wurde in Zusammenarbeit mit Dr. Zhitao Xiao erarbeitet (Sun Yat-sen Universität Krebs Zentrum, Guangzhou, China). Herr Qingjian Ou hat mit technischer Unterstützung beigetragen (Sun Yat-sen Universität Krebs Zentrum, Guangzhou, China), Dr. Braciak unterstütze die Veröffentlichungen redaktionell (Klinikum der Universität München, Medizinische Klinik und Poliklinik IV).

Alle Ergebnisse wurden in den folgenden Beiträgen veröffentlicht oder akzeptiert :

1. Pan Li, Zhitao Xiao, Todd A. Braciak, Qingjian Ou, Gong Chen, Fuat S. Oduncu. Verbindung zwischen Ki67-Index und klinikopathologischen Merkmalen bei Kolorektalkarzinome. Oncol Res Treat 2016; 39:696-702.
2. Pan Li, Zhitao Xiao, Todd A. Braciak, Qingjian Ou, Gong Chen, Fuat S. Oduncu. Auswirkungen von Alter und Mismatch-Reparatur-Status auf das Überleben bei Kolorektalkrebs. Cancer Medicine, 2017; 6(5):975-981.
3. Pan Li, Zhitao Xiao, Todd A. Braciak, Qingjian Ou, Gong Chen, Fuat S. Oduncu. Ein Zusammenhang zum Überleben wird durch die Kombination der Faktoren des Mismatch-Reparaturstatus, der Lage des Tumors und des Alters bei Ausbruch bei Kolorektal-Patienten gesehen. Plos One 2017, 10:0172799 .

Die vorliegende Doktorarbeit begann im April 2016 mit der Betreuung von Prof. Dr. Dr. Fuat Oduncu an der Medizinischen Klinik und Poliklinik IV, Klinikum der Universität München.

München, 11.12.2017,

Doktorantin: Pan Li Unterschrift:

Supervisor: Prof. Fuat S. Oduncu Unterschrift:

Declaration

I hereby declare that the thesis is my original work. The work and results presented in the thesis were performed independently. Part of the data in colorectal cancer was collected in cooperation with Dr. Zhitao Xiao (Sun Yat-sen University Cancer Center, Guangzhou, China). Mr. Qingjian Ou provided with technical support (Sun Yat-sen University Cancer Center, Guangzhou, China), Dr. Braciak helped to revise the published manuscripts (Klinikum der Universität München, Medizinische Klinik und Poliklinik IV).

All three papers were published or accepted:

1. Pan Li, Zhitao Xiao, Todd A. Braciak, Qingjian Ou, Gong Chen, Fuat S. Oduncu. Association between Ki67 Index and Clinicopathological Features in Colorectal Cancer. Oncol Res Treat 2016; 39:696-702.
2. Pan Li, Zhitao Xiao, Todd A. Braciak, Qingjian Ou, Gong Chen, Fuat S. Oduncu. Impact of age and mismatch repair status on survival in colorectal cancer. Cancer Medicine, 2017; 6(5):975-981.
3. Pan Li, Zhitao Xiao, Todd A. Braciak, Qingjian Ou, Gong Chen, Fuat S. Oduncu. A relationship to survival is seen by combining the factors of mismatch repair status, tumor location and age of onset of colorectal cancer patients. Plos One 2017, 10:0172799 .

All the data presented in the thesis will not be used in any other thesis for scientific degree application. The work for the thesis began in April 2016 with the supervision by Prof. Dr. Dr. Fuat Oduncu at the Medizinische Klinik und Polikinik IV, Klinikum der Universität München.

München, 11.12.2017,

Doctor student: Pan Li Signature:

Supervisor: Prof. Fuat S. Oduncu Signature:

Introduction

Colorectal cancer accounts worldwide for about 1 million new cases per year and is the third most common cancer in men and the second most common in women. Although recent advances in treatment have improved the prognosis, CRCs remain to present 8 % of all cancers [1-3]. Research has shown that the most CRCs arise in colorectal polyps before developing into malignant disease. Therefore, resecting the adenomatous polyps by colonoscopy can help to prevent CRCs [4]. The incidence of CRC has been found to be associated with increasing age. CRCs can occur in young people, but around 90 % of CRCs are diagnosed after 40 years of age and incidences continue to increase even more rapidly after this age. In addition, older patients with CRC have unique treatment challenges [5]. Although there is a way to completely prevent CRCs taking advantage of screening methods, investigating the biomarkers and clinical factors of CRC that can lead to optimize diagnosis and treatment regimens are essential.

A common feature of all cancers is the imbalance of the proliferative activity and cell death. Ki67 is nucleus antigen. Ki67 antibody recognized the corresponding antigen from G1 to M-phase of the cell proliferation, but is absent from resting cells (G0). Therefore, Ki67 is an excellent marker to determine the growth fraction of tumor cells, and likewise it could probably reflect clinical process [6]. Many studies have shown a predictive function of Ki67 in breast cancer and neuroendocrine tumors [7–9]. The quantification of Ki67 expression as Ki67 labeling index (Ki67-Li) was demonstrated significantly higher in liver metastases than primary tumor of CRC [10]. Ki67 staining of core biopsies of lymph node were significantly associated with poor prognosis of CRC, the nuclear staining of Ki67 demonstrated decreased levels in rectal cancer with chemotherapy with cetuximab [11, 12, 14]. Higher Ki67-Li was observed as a biomarker to detect renal cell carcinoma (RCC) with high risk for metastases [13]. Ki67 was found to be highly expressed in the proliferative basal half of the crypt and was significantly elevated in ulcerative colitis (UC) colons compared to non-UC control samples [15, 16]. In CRCs, the analysis of Ki67 has shown different patterns connected with patients' age, pathological tumor differentiation, and lymph node metastasis [17-20]. However, not all studies are in agreement with the associations between Ki67-Li and clinicopathological information for CRCs. We set out to investigate whether assessment of Ki67 could also help to define the prognosis for patients with CRC more accurately.

The notion that age can be a significant prognostic factor in CRC remains controversial. Some studies have shown poorer prognosis for younger patients with CRC [31-34]. Contradictory, other authors have demonstrated that younger patients with CRC have better prognoses compared with elderly ones [35, 36]. Furthermore, little is known about the biological mechanisms responsible for the rise in CRC with aging. Few studies have explored that the molecular profiles of CRC patients have been shown to differ between various age groups of CRC [21-25]. Whether patients within these various age groups have a different biological behavior remains controversial, for example, if some biomarkers are expressed in specific age groups.

The Evaluation of Genomic Applications in Practice of Prevention (EGAPP) recommended screening deficient mismatch repair (MMR) status for all CRC patients in 2009 [26]. MMR proteins are nuclear enzymes which correct mismatched nucleotides and insertion-deletion loops (IDLs) during DNA replication [27]. It was indicated that Stage II CRC patients with dMMR have a better outcome [28]. Some current studies have shown that CRCs with dMMR were more prevalent in younger patients [29, 30]. Despite these early findings about MMR status, the association of OS for age of disease onset and MMR status in CRC patients is still not clear.

In this current study, we sought to find the association of MMR status based on age and disease outcomes in advantage of the recommendations of the EGAPP to test MMR status [26]. We performed systematic IHC screening for MLH1, MSH2, MSH6 and PMS2 proteins in a large number of Chinese CRC patients assessed in our institute since 2011.

Another clinical factor that we also wanted to explore is the location of the primary CRCs, which might be useful as a prognostic factor. Different CRC locations are derived from various embryonic tissues. The right colon, which includes cecum, ascending colon, and proximal two thirds of the transverse colon, is derived from the embryonic midgut. The left colon includes distal one third of the transverse colon, descending colon, sigmoid colon, and rectum, which is derived from the embryonic hindgut [37]. Some recent studies have indicated that the clinical characteristics and biological factors for CRC differ across these different locations [38-41]. One study has indicated that the primary tumor location could serve as prognostic factor in metastatic CRC [42]. Some studies have shown a correlation between patients' gender and tumor location [43, 44]. Tumor location and age of onset are two important clinical characteristics for CRC patients. To date, only few studies have evaluated the correlations between these two factors to the prognosis of CRC. It has been previously reported that early-onset right colon cancers were a subset for most Lynch Syndrome cases; this group of patients were with earlier stages of disease at diagnosis and had better prognosis [45]. In another study, Bax/Bcl-2 ratio showed decreased levels in CRC patients older than 50 and in colon compared to rectal cancer [46].

Currently, the associations between biological, clinical features and tumor locations have been made. Mucinous histology, wild type p53, high microsatellite instability and BRAF mutation tend to be found in right colon [47-49]. In contrast, left colon cancers are frequently found to be with chromosomal instability [48]. Rectal cancers tend to be more frequently associated with lung metastases compared with colon cancers [50]. While approximately 50 % of the CRC occur in rectal and sigmoid area, during the past decades it found a shift towards right colon. However, the impact of this change in tumor location to CRC prognosis has not yet been completely analyzed. The age of tumor onset is also considered as an important clinical factor in regard to overall survival of CRCs. Many studies have demonstrated that the incidence of CRC increases significantly with advancing age. However, the overall viewpoint that age is a significant prognostic factor in CRC has been controversial. Various studies have reported poorer prognosis among young patients with CRC [51-53].

CRCs with dMMR tend to be located in proximal colon and have poor differentiation with increased numbers of lymph nodemetastases compared with CRCs with pMMR [54]. Previous studies have shown that right colon cancers are associated with worse prognosis than left colon and rectal cancers [55]. This finding would appear to be contrary with observations that right colon cancers are more

likely to be dMMR [56]. To date, only few studies have looked for a relationship between MMR status and prognosis of CRC by tumor locations [57-59].

Increasing studies have shown that the biological profiles of CRCs may differ across the tumor location [60-62], which suggests that the mechanism of CRC development can differ across the tumor location [63, 64]. While right colon and left colon cancers are frequently studied in combination with each other, and in combination with those arising in the rectum, only a few studies have looked at the combination of tumor location and stage. In one earlier study, it was reported that tumor location was a strong predictor of high T, N and stages of disease [65]. In yet another study looking at disease staging and tumor location Stage II right colon cancers were shown to exhibit higher microsatellite instability indicating that tumor location may affect differential responses to cancer therapy [66]. It was reported that patients with right side colon cancer were older, more often females and had a poorer prognosis than patients with left side colon cancer [67]. Currently, only few studies have evaluated the correlation between age of tumor onset and tumor location with regard to the overall survival of CRCs [68, 69].

To date, there has been no large-scale analysis to investigate the combined impact of Ki67, MMR status, tumor location and age of disease onset with regard to the overall survival of CRC. Therefore, we analyzed the association of CRC overall survival, expression of Ki67, MMR status and the age of onset and tumor location using a large data following the recommendation of EGAPP.

Einleitung

Weltweit treten etwa 1 Million neue Fälle von Kolorektalkarzinomen pro Jahr auf und es handelt sich um die dritthäufigste Krebsart bei Männern und die zweithäufigste bei Frauen. Obwohl neueste Fortschritte in der Behandlung die Prognose verbessert haben, machen CRCs immer noch 8 % aller Krebserkrankungen aus [1-3]. Die Forschung hat gezeigt, dass die meisten CRCs in kolorektalen Polypen entstehen, bevor sie sich zu malignen Erkrankungen entwickeln. Daher kann die Resektion der adenomatösen Polypen durch Koloskopie helfen, um CRCs zu verhindern [4]. Die Inzidenz von CRC wurde mit zunehmendem Alter assoziiert. CRCs können bei jungen Leuten auftreten, aber etwa 90 % der CRCs werden nach dem 40. Lebensjahr diagnostiziert und die Inzidenz steigt nach diesem Alter noch schneller an. Darüber hinaus gibt es für ältere Patienten mit CRC einzigartige Behandlungsmethoden [5]. Es ist zwar möglich mithilfe von verbesserten Screeningmethoden CRC vollständig zu verhindern. Die Biomarker und klinischen Faktoren von CRC zu untersuchen, die zur Optimierung von Diagnose- und Behandlungsschemata führen können, ist unerlässlich.

Ein gemeinsames Merkmal aller Krebsarten ist das Ungleichgewicht der proliferativen Aktivität und des Zelltods. Ki67 ist ein Antigen, das sich im Kern befindet. Der monoklonale Antikörper von Ki67 erkannte das Antigen von der G1 bis zur M-Phase der Zellproliferation, fehlt aber bei ruhenden Zellen (G0). Daher ist Ki67 ein hervorragender Marker, um den Wachstumsanteil der Tumorzellen zu bestimmen, und ebenso könnte er vermutlich den klinischen Prozess widerspiegeln [6]. Viele Studien haben eine prädiktive Funktion von Ki67 bei Brustkarzinomen und neuroendokrinen Tumoren gezeigt [7-9]. Die Quantifizierung der Ki67-Expression als Ki67-Markierungsindex (Ki67-Li) wurde bei Lebermetastasen von CRC signifikant höher nachgewiesen [10]. Die Ki67-Färbung der Kernbiopsien des Lymphknotens war signifikant mit einer schlechten Prognose von CRC assoziiert, die Kernfärbung von Ki67 zeigte eine verminderte Konzentration an Rektalkarzinomen mit Chemotherapie mit Cetuximab [11, 12, 14]. Höheres Ki67-Li wurde als Biomarker beobachtet, um das Nierenzellkarzinom (RCC) mit hohem Risiko für Metastasen zu erkennen [13]. Ki67 wurde in der proliferativen basalen Hälfte der Krypta stark exprimiert und war im Vergleich zu Nicht-UC-Kontrollproben signifikant erhöht [15, 16]. In CRCs hat die Analyse von Ki67 verschiedene Muster in Verbindung mit Patientenalter, pathologischer Tumordifferenzierung und Lymphknotenmetastase gezeigt [17-20]. Allerdings stimmen nicht alle Studien mit den Assoziationen zwischen Ki67-Li und klinikopathologischen Informationen für CRCs überein. Wir wollten untersuchen, ob die Beurteilung von Ki67 auch dazu beitragen könnte, die Prognose für Patienten mit CRC genauer zu definieren.

Die Vorstellung, dass das Alter ein signifikanter prognostischer Faktor in CRC sein kann, bleibt umstritten. Einige Studien haben eine schlechtere Prognose für jüngere Patienten mit CRC gezeigt [31-34]. Im Widerspruch dazu haben andere Autoren gezeigt, dass jüngere Patienten mit CRC bessere Prognosen im Vergleich zu älteren haben [35, 36]. Darüber hinaus ist wenig über die biologischen Mechanismen, die für den Anstieg der CRC mit dem Alter verantwortlich sind, bekannt. Nur wenige Studien haben untersucht, ob sich die molekularen Profile von Patienten mit CRC bei verschiedenen Altersgruppen unterscheiden [21-25]. Ob die Patienten innerhalb dieser verschiedenen Altersgruppen ein anderes biologisches Verhalten haben, bleibt umstritten, z.B. ob manche Biomarker in bestimmten Altersgruppen auftreten.

Die Evaluation von genomischen Anwendungen in der Praxis der Prävention (EGAPP) hat das Screening von defizienten Mismatch-Reparatur (MMR) Status für alle CRC Patienten im Jahr 2009 empfohlen [26]. MMR-Proteine sind Nukleus-Enzyme, die bei der DNA-Replikation nicht übereinstimmende Nukleotide und Insertions-Deletions-Loops (IDLs) korrigieren [27]. Es wurde darauf hingewiesen, dass die Stage II CRC Patienten mit dMMR ein besseres Ergebnis haben [28]. Einige aktuelle Studien haben gezeigt, dass dMMR bei jüngeren Patienten mit CRC häufiger waren [29, 30]. Trotz dieser frühen Erkenntnisse über den MMR-Status ist der Zusammenhang von OS mit dem Alter bei Krankheitsbeginn und dem MMR-Status bei CRC-Patienten noch nicht klar.

In unsere Studie haben wir versucht, den Zusammenhang mit dem MMR-Status auf der Grundlage von Alters- und Krankheitsresultaten nach den Empfehlungen des EGAPP zu finden, um den MMR-Status zu testen [26]. Wir haben systematische IHC-Screening für MLH1-, MSH2-, MSH6- und PMS2-Gene an einer großen Anzahl von chinesischen CRC-Patienten in unserem Institut seit 2011 durchgeführt und bewertet.

Ein weiterer klinischer Faktor, den wir auch erforschen wollten, ist die Lokalisation der primären CRCs, die als prognostischer Faktor nützlich sein könnte. Verschiedene CRC-Lokalisationen werden aus verschiedenen embryonalen Geweben entgenommen. Der rechte Kolon, der Cecum, den aufsteigenden Kolon und proximal zwei Drittel des transversalen Kolon enthält, wird aus dem embryonalen Middarm abgeleitet. Der linke Kolon schließt ein distales Drittel des transversalen Kolon ein, den absteigenden Kolon, Sigmoiddarm und Rektum, das aus dem embryonalen Enddarm stammt [37]. Einige neuere Studien haben gezeigt, dass sich die klinischen Merkmale und biologischen Faktoren für CRC an diesen verschiedenen Standorten unterscheiden [38-41]. Eine Studie hat gezeigt, dass die primäre Tumorlokalisation als prognostischer Faktor im metastatischen CRC dienen könnte [42]. Einige Studien haben eine Korrelation zwischen dem Geschlecht und dem Tumor der Patienten gezeigt [43, 44]. Tumorlokalisation und Alter bei Krankheitsbeginn sind zwei wichtige klinische Merkmale für CRC-Patienten. Bisher haben nur wenige Studien die Korrelationen zwischen diesen beiden Faktoren für die Prognose von CRC ausgewertet. Es wurde bereits berichtet, dass jüngere Patienten mit rechtem Kolonkarzinom eine Untermenge für die meisten Lynch-Syndrom-Fälle waren, diese Gruppe von Patienten waren in frühren Stadien der Krankheit bei der Diagnose und hatten eine bessere Prognose [45]. In einer anderen Studie zeigte das Bax / Bcl-2-Verhältnis ein verringertes Niveau bei CRC-Patienten, die älter als 50 waren, sowie bei Kolonkarzinomen im Vergleich zu Rektumkarzinomen [46].

Derzeit hat man die Zusammenhänge zwischen Biomarkern, klinischen Merkmalen und Tumorlokalisation untersucht. Muzinöse Histologie, Wildtyp p53, hohe Mikrosatelliteninstabilität und BRAF-Mutation neigen dazu, im rechten Kolon aufzutreten [47-49]. Im Gegensatz dazu sind linke Kolonkarzinome häufig mit chromosomaler Instabilität zu finden [48]. Rektale Karzinome treten häufiger zusammen mit Lungenmetastasen als mit Kolonkarzinomen auf [50]. Während etwa 50% des CRC im rektalen und sigmoiden Bereich auftreten, fand in den vergangenen Jahrzehnten eine Verschiebung zum rechten Dickdarm statt. Allerdings ist die Auswirkung dieser Veränderung der Tumorposition auf die CRC-Prognose noch nicht vollständig analysiert worden. Das Alter bei Tumorbeginn wird auch als wichtiger klinischer Faktor für das Gesamtüberleben von CRCs angesehen.

Viele Studien haben gezeigt, dass die Inzidenz von CRC mit zunehmendem Alter deutlich zunimmt. Allerdings ist die allgemeine Sichtweise, dass das Alter ein signifikanter prognostischer Faktor in CRC, umstritten. Verschiedene Studien haben über eine schlechtere Prognose bei jungen Patienten mit CRC berichtet [51-53].

CRCs mit dMMR neigen dazu, sich im proximalen Kolon zu befinden und haben eine schlechte Differenzierung mit einer erhöhten Anzahl von Lymphknotenmetastasen im Vergleich zu CRCs mit pMMR [54]. Frühere Studien haben gezeigt, dass die rechte Kolonkarzinome mit einer schlechteren Prognose assoziiert sind als linke Kolon- und Rektalkarzinome [55]. Diese Feststellung scheint den Beobachtungen zu widersprechen, dass mehr rechte Kolonkarzinome mit dMMR auftreten [56]. Bisher haben nur wenige Studien einen Zusammenhang zwischen dem MMR-Status und der Prognose von CRC in Bezug auf Tumorlokalisation gesucht [57-59].

Immer mehr Studien haben gezeigt, dass sich die biologischen Profile von CRCs durch die Tumorlokalisation unterscheiden können [60-62], was darauf hindeutet, dass sich der Mechanismus der CRC-Entwicklung durch die Tumorlokalisation unterscheiden kann [63, 64]. Während rechte und linke Kolonkarzinome häufig in Kombination miteinander untersucht werden und in Kombination mit denen, die im Rektum entstehen, haben nur wenige Studien die Kombination von Tumorlokalisation und Stadium untersucht. In einer früheren Studie wurde berichtet, dass die Tumorposition ein starker Prädiktor für hohe T, N und Stadien der Erkrankung war [65]. In einer weiteren Studie, die sich auf die Stadien und die Tumorlokalisation stützt, wurde gezeigt, dass die Dickdarmkrebserkrankungen eine höhere Mikrosatelliteninstabilität aufweisen, was darauf hinweist, dass die Tumorposition unterschiedliche Auswirkungen bei der Krebstherapie haben kann [66]. Es wurde berichtet, dass Patienten mit rechten Kolonkarzinomen älter waren, häufiger Frauen waren und eine schlechtere Prognose als Patienten mit linken Kolonkarzinomen haten [67]. Derzeit haben nur wenige Studien die Korrelation zwischen dem Alter bei Krankheitbeginn und der Tumorposition hinsichtlich des Gesamtüberlebens von CRCs untersucht [68, 69].

Bisher gab es keine groß angelegte Analyse, um die kombinierten Auswirkungen von Ki67, MMR-Status, Tumorort und Alter bei Krankheitsbeginn im Hinblick auf das Gesamtüberleben von CRC zu untersuchen. Daher analysierten wir den Zusammenhang von CRC mit Gesamtüberleben, Ausdruck von Ki67, MMR-Status und Alter bei Krankheitsbeginn, Tumorlokalisation mit großen Daten nach der Empfehlung von EGAPP.

Reference

1. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A: Global cancer statistics, 2012. CA Cancer J Clin 2015; 65: 87–108.
2. Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM: Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int J Cancer 2010; 127: 2893– 2917.
3. Sung JJ, Lau JY, Goh KL, Leung WK (2005) Increasing incidence of colorectal cancer in Asia: implications for screening. Lancet Oncol 6: 871-876.
4. Vogelstein B, Fearon ER, Hamilton SR, Kern SE, Preisinger AC, et al. (1988) Genetic alterations during colorectal-tumor development. N Engl J Med 319: 525-532.
5. Imperiale TF, Juluri R, Sherer EA, Glowinski EA, Johnson CS, Morelli MS. A risk index for advanced neoplasia on the second surveillance colonoscopy in patients with previous adenomatous polyps. GastrointestEndosc. 2014-09-01 2014;80(3):471-478.
6. Scholzen T, Gerdes J: The Ki-67 protein: from the known and the unknown. J Cell Physiol 2000; 182: 311–322.
7. Fujimori Y, Fujimori T, Imura J, Sugai T, Yao T, Wada R, Ajioka Y, Ohkura Y: An assessment of the diagnostic criteria for sessile serrated adenoma/polyps: SSA/Ps using image processing software analysis for Ki67 immunohistochemistry. DiagnPathol 2012; 7: 59.
8. Pathmanathan N, Balleine RL: Ki67 and proliferation in breast cancer. J ClinPathol 2013; 66: 512–516.
9. Gerdes J, Schwab U, Lemke H, Stein H: Production of a mouse monoclonal antibody reactive with a human nuclear antigen associated with cell proliferation. Int J Cancer 1983; 31: 13–20.
10. Krause P, Flikweert H, Monin M, Seif AHA, Helms G, Cantanhede G, Ghadimi BM, Koenig S: Increased growth of colorectal liver metastasis following partial hepatectomy. ClinExp Metastasis 2013; 30: 681–693.
11. Zhang Z, Wang M, Zhou L, Feng X, Cheng J, Yu Y, Gong Y, Zhu Y, Li C, Tian L, Huang Q: Increased HMGB1 and cleaved caspase-3 stimulate the proliferation of tumor cells and are correlated with the poor prognosis in colorectal cancer. J ExpClin Cancer Res 2015; 34: 51.
12. Jakob C, Liersch T, Meyer W, Becker H, Baretton GB, Aust DE: Predictive value of Ki67 and p53 in locally advanced rectal cancer: correlation with thymidylate synthase and histopathological tumor regression after neoadjuvant 5-FU-based chemoradiotherapy. World J Gastroenterol 2008; 14: 1060–1066.
13. Weber T, Meinhardt M, Zastrow S, Wienke A, Fuessel S, Wirth MP: Immunohistochemical analysis of prognostic protein markers for primary localized clear cell renal cell carcinoma. Cancer Invest 2013; 31: 51–59.
14. Debucquoy A, Haustermans K, Daemen A, Aydin S, Libbrecht L, Gevaert O, De Moor B, Tejpar S, McBride WH, Penninckx F, Scalliet P, Stroh C, Vlassak S, Sempoux C, Machiels JP: Molecular response to cetuximab and efficacy of preoperative cetuximab-based chemoradiation in rectal cancer. J ClinOncol 2009; 27: 2751– 2757.
15. Dame MK, Jiang Y, Appelman HD, Copley KD, McClintock SD, Aslam MN, Attili D, Elmunzer BJ, Brenner DE, Varani J, Turgeon DK: Human colonic crypts in culture: segregation of immunochemical markers in normal versus adenoma-derived. Lab Invest 2014; 94: 222–234.
16. Burum-Auensen E, Deangelis PM, Schjolberg AR, Roislien J, Andersen SN, Clausen OP: Spindle proteins Aurora a and BUB1B, but not Mad2, are aberrantly expressed in dysplastic mucosa of patients with longstanding ulcerative colitis. J ClinPathol 2007; 60: 1403– 1408.
17. Gurzu S, Jung J, Mezei T, Pavai Z: The correlation between the immunostains for p53 and Ki67 with bcl-2 expression and classical prognostic factors in colorectal carcinomas. Rom J MorpholEmbryol 2007; 48: 95– 99.
18. Stromar IK, Jakic-Razumovic J: The value of immunohistochemical determination of topoisomerase IIalpha and Ki67 as markers of cell proliferation and malignant transformation in colonic mucosa. ApplImmunohistochemMolMorphol 2014; 22: 524–529.
19. Jiang SM, Wang RB, Yu JM, Zhu KL, Mu DB, Xu ZF: (Correlation of VEGF and Ki67 expression with sensitivity to neoadjuvant chemoradiation in rectal adenocarcinoma). ZhonghuaZhong Liu ZaZhi 2008; 30: 602–605.
20. Vernillo R, Lorenzi B, Banducci T, Minacci C, Vindigni C, Lucenti FA, Lorenzi M: Immunohistochemical expression of p53 and Ki67 in

colorectal adenomas and prediction of malignancy and development of new polyps. Int J Biol Markers 2008; 23: 89–95.

21. Fearon ER, Vogelstein B. A genetic model for colorectal tumorigenesis. Cell. 1990-06-01 1990;61(5):759-767.
22. Khodapasand E, Jafarzadeh N, Farrokhi F, Kamalidehghan B, Houshmand M. Is Bax/Bcl-2 ratio considered as a prognostic marker with age and tumor location in colorectal cancer? Iran Biomed J. 2015-01-20 2015;19(2):69-75.
23. Boardman LA, Litzelman K, Seo S, et al. The association of telomere length with colorectal cancer differs by the age of cancer onset. ClinTranslGastroenterol. 2014-01-20 2014;5:e52.
24. Baars JE, Kuipers EJ, van Haastert M, Nicolai JJ, Poen AC, van der Woude CJ. Age at diagnosis of inflammatory bowel disease influences early development of colorectal cancer in inflammatory bowel disease patients: a nationwide, long-term survey. J Gastroenterol. 2012-12-01 2012;47(12):1308-1322.
25. O'Connell JB, Maggard MA, Liu JH, Etzioni DA, Livingston EH, Ko CY. Do young colon cancer patients have worse outcomes? World J Surg. 2004-06-01 2004;28(6):558-562.
26. Recommendations from the EGAPP Working Group: genetic testing strategies in newly diagnosed individuals with colorectal cancer aimed at reducing morbidity and mortality from Lynch syndrome in relatives. GENET MED. 2009 2009-01-01;11(1):35-41.
27. Kolodner RD, Marsischky GT. Eukaryotic DNA mismatch repair. CurrOpin Genet Dev. 1999-02-01 1999;9(1):89-96.
28. Boland CR, Goel A. Microsatellite instability in colorectal cancer. Gastroenterology. 2010-06-01 2010;138(6):2073-2087.
29. Limburg PJ, Harmsen WS, Chen HH, et al. Prevalence of alterations in DNA mismatch repair genes in patients with young-onset colorectal cancer. ClinGastroenterolHepatol. 2011-06-01 2011;9(6):497-502.
30. Schofield L, Watson N, Grieu F, et al. Population-based detection of Lynch syndrome in young colorectal cancer patients using microsatellite instability as the initial test. Int J Cancer. 2009-03-01 2009;124(5):1097-1102.
31. Lieu CH, Renfro LA, de Gramont A, et al. Association of age with survival in patients with metastatic colorectal cancer: analysis from the ARCAD Clinical Trials Program. J ClinOncol. 2014-09-20 2014;32(27):2975-2984.
32. Cusack JC, Giacco GG, Cleary K, et al. Survival factors in 186 patients younger than 40 years old with colorectal adenocarcinoma. J Am Coll Surg. 1996-08-01 1996;183(2):105-112.
33. Schellerer VS, Merkel S, Schumann SC, et al. Despite aggressive histopathology survival is not impaired in young patients with colorectal cancer : CRC in patients under 50 years of age. Int J Colorectal Dis. 2012-01-01 2012;27(1):71-79.
34. McMillan DC, McArdle CS. The impact of young age on cancer-specific and non-cancer-related survival after surgery for colorectal cancer: 10-year follow-up. Br J Cancer. 2009-08-18 2009;101(4):557-560.
35. Li Q, Cai G, Li D, Wang Y, Zhuo C, Cai S. Better long-term survival in young patients with non-metastatic colorectal cancer after surgery, an analysis of 69,835 patients in SEER database. PLoS One. 2014-01-20 2014;9(4):e93756.
36. Schellerer VS, Merkel S, Schumann SC, et al. Despite aggressive histopathology survival is not impaired in young patients with colorectal cancer : CRC in patients under 50 years of age. Int J Colorectal Dis. 2012-01-01 2012;27(1):71-79.
37. Glebov OK, Rodriguez LM, Nakahara K, Jenkins J, Cliatt J, Humbyrd CJ. (2003) Distinguishing right from left colon by the pattern of gene expression. Cancer Epidemiol Biomarkers Prev 12: 755–762. PMID: 12917207
38. Han NY, Kim MJ, Park BJ, Sung DJ (2014) Location of rectal cancer as determined using rectal magnetic resonance imaging, and its relationship with pulmonary metastasis. Turk J Gastroenterol 25: 661–668. doi: 10.5152/tjg.2014.5616 PMID: 25599778
39. Papagiorgis PC, Zizi AE, Tseleni S, Oikonomakis IN, Nikiteas NI (2012) The pattern of epidermal growth factor receptor variation with disease progression and aggressiveness in colorectal cancer depends on tumor location. Oncol Lett 3: 1129–1135. doi: 10.3892/ol.2012.621 PMID: 22783405
40. Boisen MK, Johansen JS, Dehlendorff C, Larsen JS, Osterlind K, Hansen J. (2013) Primary tumor location and bevacizumab effectiveness in patients with metastatic colorectal cancer. Ann Oncol 24: 2554– 2559. doi: 10.1093/annonc/mdt253 PMID: 23864097
41. Andrieu N, Launoy G, Guillois R, Ory-Paoletti C, Gignoux M (2004) Estimation of the familial relative risk of cancer by site from a French population based family study on colorectal cancer (CCREF study). Gut 53: 1322–1328. doi: 10.1136/gut.2003.036376 PMID: 15306593

42. Loupakis F, Yang D, Yau L, Feng S, Cremolini C, Zhang W, et al. (2015) Primary tumor location as a prognostic factor in metastatic colorectal cancer. J Natl Cancer Inst 107.

43. Matanoski G, Tao XG, Almon L, Adade AA, Davies-Cole JO (2006) Demographics and tumor characteristics of colorectal cancers in the United States, 1998–2001. Cancer 107: 1112–1120. doi: 10.1002/ cncr.22008 PMID: 16838314

44. Nawa T, Kato J, Kawamoto H, Okada H, Yamamoto H, Kohno H, et al. (2008) Differences between right- and left-sided colon cancer in patient characteristics, cancer morphology and histology. Journal of Gastroenterology and Hepatology 23: 418–423. doi: 10.1111/j.1440-1746.2007.04923.x PMID: 17532785

45. Perea J, Cano JM, Rueda D, Garcia JL, Inglada L, Osorio I. (2015) Classifying early-onset colorectal cancer according to tumor location: new potential subcategories to explore. Am J Cancer Res 5: 2308– 2313. PMID: 26328262

46. Khodapasand E, Jafarzadeh N, Farrokhi F, Kamalidehghan B, Houshmand M (2015) Is Bax/Bcl-2 ratio considered as a prognostic marker with age and tumor location in colorectal cancer? Iran Biomed J 19: 69–75. doi: 10.6091/ibj.1366.2015 PMID: 25864810

47. Tran B, Kopetz S, Tie J, Gibbs P, Jiang ZQ, Lieu CH, et al. (2011) Impact of BRAF mutation and microsatellite instability on the pattern of metastatic spread and prognosis in metastatic colorectal cancer. Cancer 117: 4623–4632. doi: 10.1002/cncr.26086 PMID: 21456008

48. Iacopetta B (2002) Are there two sides to colorectal cancer? Int J Cancer 101: 403–408. doi: 10.1002/ ijc.10635 PMID: 12216066

49. Fukatsu H, Kato J, Nasu JI, Kawamoto H, Okada H, Yamamoto H, et al. (2007) Clinical characteristics of synchronous colorectal cancer are different according to tumour location. Dig Liver Dis 39: 40–46. doi: 10.1016/j.dld.2006.07.015 PMID: 16996329

50. Tan KK, Lopes GLJ, Sim R (2009) How uncommon are isolated lung metastases in colorectal cancer? A review from database of 754 patients over 4 years. J GastrointestSurg 13: 642–648. doi: 10.1007/ s11605-008-0757-7 PMID: 19082673

51. Cusack JC, Giacco GG, Cleary K, Davidson BS, Izzo F, Skibber J, et al. (1996) Survival factors in 186 patients younger than 40 years old with colorectal adenocarcinoma. J Am CollSurg 183: 105–112. PMID: 8696540

52. Li M, Li JY, Zhao AL, Gu J (2011) Do young patients with colorectal cancer have a poorer prognosis than old patients? J Surg Res 167: 231–236. doi: 10.1016/j.jss.2010.10.040 PMID: 21316708

53. Schellerer VS, Merkel S, Schumann SC, Schlabrakowski A, Fortsch T, Schildberg C, et al. (2012) Despite aggressive histopathology survival is not impaired in young patients with colorectal cancer: CRC in patients under 50 years of age. Int J Colorectal Dis 27: 71–79. doi: 10.1007/s00384-011-12918 PMID: 21881876

54. Jass JR, Do KA, Simms LA, Iino H, Wynter C, Pillay SP, et al. (1998) Morphology of sporadic colorectal cancer with DNA replication errors. Gut 42: 673–679. PMID: 9659163

55. Meguid RA, Slidell MB, Wolfgang CL, Chang DC, Ahuja N (2008) Is there a difference in survival between right- versus left-sided colon cancers? Ann SurgOncol 15: 2388–2394. doi: 10.1245/s10434008-0015-y PMID: 18622647

56. Minoo P, Zlobec I, Peterson M, Terracciano L, Lugli A (2010) Characterization of rectal, proximal and distal colon cancers based on clinicopathological, molecular and protein profiles. Int J Oncol 37: 707– 718. PMID: 20664940

57. Sinicrope FA, Rego RL, Halling KC, Foster N, Sargent DJ, La Plant B, et al. (2006) Prognostic impact of microsatellite instability and DNA ploidy in human colon carcinoma patients. Gastroenterology 131: 729–737. doi: 10.1053/j.gastro.2006.06.005 PMID: 16952542

58. Sanchez JA, Krumroy L, Plummer S, Aung P, Merkulova A, Skacel M, et al. (2009) Genetic and epigenetic classifications define clinical phenotypes and determine patient outcomes in colorectal cancer. Br J Surg 96: 1196–1204. doi: 10.1002/bjs.6683 PMID: 19787768

59. Breivik J, Lothe RA, Meling GI, Rognum TO, Borresen-Dale AL, Gaudernack G. (1997) Different genetic pathways to proximal and distal colorectal cancer influenced by sex-related factors. Int J Cancer 74: 664–669. PMID: 9421366

60. Yamauchi M, Morikawa T, Kuchiba A, Imamura Y, Qian ZR, et al. (2012) Assessment of colorectal cancer molecular features along bowel subsites challenges the conception of distinct dichotomy of proximal versus distal colorectum. Gut 61: 847-854.

61. Kim GP, Colangelo LH, Wieand HS, Paik S, Kirsch IR, et al. (2007) Prognostic and predictive roles of high-degree microsatellite instability in colon cancer: a National Cancer Institute-National Surgical Adjuvant Breast and Bowel Project Collaborative Study. J

ClinOncol 25: 767-772.

62. Minoo P, Zlobec I, Peterson M, Terracciano L, Lugli A (2010) Characterization of rectal, proximal and distal colon cancers based on clinicopathological, molecular and protein profiles. Int J Oncol 37: 707-718.
63. Iacopetta B (2002) Are there two sides to colorectal cancer? Int J Cancer 101: 403-408.
64. Li FY, Lai MD (2009) Colorectal cancer, one entity or three. J Zhejiang Univ Sci B 10: 219-229.
65. Hemminki K, Santi I, Weires M, Thomsen H, Sundquist J, et al. (2010) Tumor location and patient characteristics of colon and rectal adenocarcinomas in relation to survival and TNM classes. BMC Cancer 10: 688.
66. Phipps AI, Lindor NM, Jenkins MA, Baron JA, Win AK, et al. (2013) Colon and rectal cancer survival by tumor location and microsatellite instability: the Colon Cancer Family Registry. Dis Colon Rectum 56: 937-944.
67. Hansen IO, Jess P (2012) Possible better long-term survival in left versus right-sided colon cancer - a systematic review. Dan Med J 59: A4444.
68. Lieu CH, Renfro LA, de Gramont A, et al. Association of age with survival in patients with metastatic colorectal cancer: analysis from the ARCAD Clinical Trials Program. J ClinOncol. 2014-09-20 2014;32(27):2975-2984.
69. Cusack JC, Giacco GG, Cleary K, et al. Survival factors in 186 patients younger than 40 years old with colorectal adenocarcinoma. J Am Coll Surg. 1996-08-01 1996;183(2):105-112.

Table of Contents

Part 1 ..... **696-702**

Abstract 696

Introduction 696

Patients and Methods 697

Patients 697

Immunohistochemical Staining for Ki67 697

Statistical Analysis 697

Results 698

Discussion 698

Conclusion 701

Disclosure Statement 701

References 701

Part 2 ..... **1-10**

Certificate of acception 1

Abstract 2

Introduction 3

Materials and Methods 3

Patients 3

Treatment and Follow-up 4

Immunohistochemistry 4

Statistical Analysis 5

Results 5

Discussion 7

Conflict of Interest Statement 9

References 9

Part 3 ..... **1-11**

Abstract 1/11

Introduction 2/11

Methods 3/11
Patients 3/11
Treatment and Follow-up 3/11
Immunohistochemistry 4/11
Statistical Analysis 4/11
Results 4/11
Discussion 6/11
Autor Contributions 9/11
References 9/11
Part 4 ..... **I-IV**
Summary I
Acknowledgement III
Curriculum Vita IV

Oncology Research and Treatment

Original Article

Oncol Res Treat 2016;39:696–702
DOI: 10.1159/000450623

Received: July 21, 2016
Accepted: August 30, 2016
Published online: October 13, 2016

Association between Ki67 Index and Clinicopathological Features in Colorectal Cancer

Pan Li[a] Zhi-Tao Xiao[b] Todd A. Braciak[a] Qing-Jan Ou[b] Gong Chen[b] Fuat S. Oduncu[a]

[a]Department of Hematology and Oncology, Medizinische Klinik und Poliklinik IV, Klinikum der Universität München, Munich, Germany; [b]Department of Colorectal Surgery, State Key Laboratory of Oncology in South China, Sun Yat-sen University Cancer Center, Guangzhou, China

Keywords
Colorectal cancer · Ki67 · Prognosis · Biomarker

Summary
Background: Conflicting results have been reported about the association between the Ki67 labeling index (Ki67-Li) and clinical outcome in patients with colorectal cancer (CRC). **Patients and Methods:** Ki67 expression was assessed by immunohistochemistry (IHC) in 2,233 consecutive CRC cases. **Results:** We determined 992 cases to have a low and 1,241 cases to have a high Ki67-Li (representing an approximately 44–56% breakdown in distribution between low versus high patients designated by phenotype). Stage III patients with a high Ki67-Li had higher 3-year disease-free survival (DFS) and overall survival (OS) than those with a low Ki67-Li (DFS 70 vs. 61%; p = 0.02 and OS 75 vs. 64%; p = 0.008). We also found significantly improved 3-year progression-free survival (PFS) for stage IV patients in the high versus the low Ki67-Li group (PFS 14 vs. 10%; p = 0.02). Yet, we found no statistical differences in prognosis for stage I and II patients and in OS for stage IV patients between high versus low Ki67-Li (p > 0.05). **Conclusion:** Our results suggest that high Ki67-Li can be an independent prognostic biomarker to aid the assessment of patient outcomes in both stage III and IV CRC.

Introduction

Colorectal cancer (CRC) is the third most common cancer in men and the second most common cancer in women worldwide [1]. Data collected in 2008 showed that worldwide there were over 1.23 million new cases, and over 608,000 patients died from CRC that year [2]. Several factors influence the prognosis, including clinical, histopathological, and biological factors related to the stage of the CRC tumor. Therefore, investigations into the molecular mechanisms of CRC that can lead to novel biomarkers to optimize diagnosis and/or treatment regimens are essential.

Ki67 expression has been shown to be strictly associated with cell proliferation. During interphase, the Ki67 antigen can be exclusively detected within the cell nucleus, whereas in mitosis most of the protein relocates to the chromosomal surface. Ki67 protein is present during all active phases of the cell cycle (G1, S, G2, and mitosis), but is absent from resting cells (G0). Because of these qualities, Ki67 is an excellent marker to determine the growth fraction of a given cell population, and likewise it could mark growing tumor cells [3].

Ki67 expression analysis is already used to estimate the prognosis of breast cancer and neuroendocrine tumors due to its expression pattern restricted to phases of the cell cycle in differentiating cells [4–6]. It was reported that liver metastases of CRC demonstrated a significantly higher Ki67 labeling index (Ki67-Li) [7]. Ki67 expression was found to be significantly higher in tumor tissues than in peritumoral tissues, and Ki67 levels were significantly associated with lymph node metastasis and poor prognosis of CRC [8]. However, some studies have shown that Ki67 has no correlation with patient survival [9, 10]. We set out to investigate whether assessment of Ki67 could also help to more accurately define the prognosis for patients with CRC.

Correspondence can also be sent to: Dr. Gong Chen, Department of Colorectal Surgery, State Key Laboratory of Oncology in South China, Sun Yat-sen University Cancer Center, East Dongfeng Road 651, 510060 Guangzhou, China, e-mail: chengong@sysucc.org.cn

KARGER

Fax +49 761 4 52 07 14
Information@Karger.com
www.karger.com

Accessible online at:
www.karger.com/ort

Prof. Dr. Dr. Fuat S. Oduncu
Department of Hematology and Oncology, Medizinische Klinik und Poliklinik IV
Klinikum der Universität München
Ziemssenstr. 1, 80336 Munich, Germany
fuat.oduncu@med.uni-muenchen.de

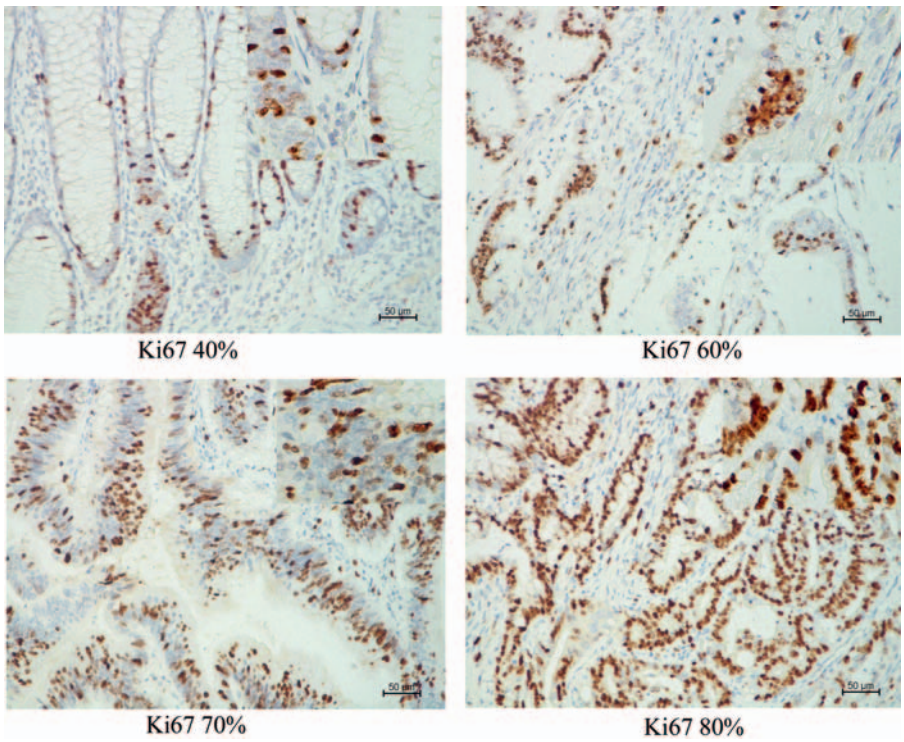

Fig. 1. Examples of immunostaining for Ki67.

Patients and Methods

Patients

The ethics committee of the Sun Yat-sen University Cancer Center approved this study, and informed consent was obtained from all patients at the beginning of the study. A total of 4,500 histologically confirmed CRC patients were recruited and 2,233 tumor tissues were obtained after operation for analysis from the Sun Yat-sen University Cancer Center between May 2011 and May 2016. All patients were of Chinese origin. The clinical and family histories of each patient were reviewed. Finally, 2,233 cases were selected for analysis following the outlined strict exclusion criteria: age less than 18 years and older than 85 years, severe complication, multiprimary cancer, synchronous and metachronous CRC, family history (first-degree and second-degree relatives with any cancer), familial adenomatous polyposis, and death not from tumor-related causes. The primary tumor site was categorized as proximal colon if the tumor was located above the splenic flexure or distal colon if it was located at or below the splenic flexure and rectum. Stage I (T1–2 N0) and stage II (T3–4 N0) CRC patients without high-risk clinical features (e.g. T4 stage, bowel perforation or clinical bowel obstruction, inadequate lymph node sampling, poorly differentiated history) were treated with radical surgery or endoscopic removal of the tumor alone. Stage II (T3–4N0) CRC patients with high-risk clinical features received chemotherapy comprising capecitabine; folinic acid+5-fluorouracil (5-FU)+oxaliplatin (mFOLFOX); and capecitabine+oxaliplatin (XELOX). Stage III (TxN1–2) patients received radical surgery and 12 cycles of adjuvant mFOLFOX/XELOX within a 6-month period. All stage IV (T×N×M1) patients with local tumor complications or resectable metastases received palliative surgery or radical surgery. The first-line treatment for stage IV CRC was the mFOLFOX/FOLFIRI (folinic acid+5-FU+irinotecan) regimen. 89 patients with rectal cancer also received neo-chemoradiotherapy. Responses were evaluated in accordance with the RECIST guidelines. After surgery, tumor recurrence was detected by physical examination, serum carcinoembryonic antigen (CEA) assay, and abdominal and thoracic imaging; patients were monitored every 3–6 months for the first 3 years, every 6 months for the following 2 years, and then annually for patients surviving beyond 5 years. The duration of follow-up was defined as the time between surgery and disease recurrence, death, or last hospital contact (scheduled follow-up or telephone contact). The cutoff date for this analysis was May 2016. The median follow-up time for living patients in this study was 4.3 years.

Immunohistochemical Staining for Ki67

All diagnosed CRC patients, independent of clinical criteria, were prospectively tested using IHC for the expression of MMR proteins on the second day after operation. Blocks of formalin-fixed, paraffin-embedded adenocarcinoma tissue comprising an area of normal colorectal mucosa adjacent to the tumor were selected in each case. Staining for Ki67 tissue expression was performed using the primary anti-Ki67 antibody (1:50 clone MIB-1; Dako, Tokyo, Japan). The Ki67-Li was calculated for each sample as the percentage of positively stained tumor cells among all counted tumor cells. Immunostained sections were evaluated by 2 pathologists blinded to the patients' clinical characteristics. Discordant cases were reviewed by a third pathologist to reach a consensus. Ki67 had a moderate to intense reaction in most cases, both in well-differentiated adenocarcinomas and in moderately or poorly differentiated tumors. Representative examples of immunostaining are shown in figure 1. The mean percentage of positively stained cells for all cases was 47%. In our study, a percentage of < 50% positively stained tumor cells among all counted tumor cells was considered a low Ki67-Li, and a percentage of ≥ 50% was considered a high Ki67-Li, which was in concordance with previous studies [11].

Statistical Analysis

Data are described as frequencies given in percentage values. The differences in distribution between the variables examined were assessed with the X^2 or the Fisher's exact test. The primary end point used in the analysis was disease-free survival (DFS) defined as the time from the date of surgery to the first event (local or distant disease recurrence), or progression-free survival (PFS) calculated as the time from the start of surgery to clinical or radiological progression. Patients who were alive and relapse-free at the last contact were censored at the time of last follow-up. Overall survival (OS) was defined as the time elapsed from the date of surgery until tumor-induced death. Surviving patients were censored at the time of last follow-up. Median follow-up and 95% confidence intervals (CI) were calculated using the reverse Kaplan-Meier method. The survival curve was estimated using the Kaplan-Meier method and compared using the log-rank test. Univariate and multivariate Cox proportional

hazards models were used to explore associations between Ki67-Li, location, age, stage, differentiation grade, and gender. The score and likelihood ratio test p values were used to test the statistical significance of each covariate in the univariate and multivariate Cox models, respectively. All statistical tests were two-sided, and p values ≤ 0.05 were considered statistically significant. Statistical analyses were performed using SPSS software (IBM Corp., Armonk, NY, USA).

Results

Of the 2,233 CRC patients evaluated, 992 were found to have a low Ki67-Li with an overall prevalence of 44.4%. 1,241 were found to have high Ki67 expression with a prevalence of 55.6%. This selected patient population included 1,316 males and 917 females of which 32.8 and 22.8%, respectively, were found to present with a high Ki67-Li while the male versus female distribution of low Ki67-Li was 26.2 and 18.3%, respectively. Detailed clinicopathological information for the patients included in this study is shown in table 1. In addition, we found that a high Ki67-Li was more likely to be noticed in the right (59.6%) or left (60.3%) colon versus the rectum (49.9%) ($p < 0.001$), and in older (40–59 years, 56.3%; 60–85 years, 56.9%) versus younger (20–39 years, 46.3%; $p = 0.028$) patients. Gender, cancer stage, T (tumor) stage, N (lymph node) stage, M (metastasis) stage, and pathological differentiation showed no statistical differences in univariate Cox analysis ($p > 0.05$) for Ki67 expression. All parameters were dichotomy variables. Among the variables analyzed in the multivariate Cox model, age (hazard ratio (HR) 2.29, 95% CI 2.23–2.33, $p = 0.025$) and location (HR 2.36, 95% CI 2.31–2.38, $p = 0.020$) were significantly associated with DFS. Age (HR 2.15, 95% CI 2.09–2.18, $p = 0.022$) and location (HR 2.40, 95% CI 2.36–2.43, $p = 0.018$) also had a statistically significant correlation with OS.

Assessment of Ki67-Li as Prognostic Marker

Patients with stage III CRC and a high Ki67-Li had a statistically significant improvement in DFS (70%) and OS (75%) compared to patients with low Ki67-Li tumors (DFS 61%; HR 0.72, 95% CI 0.55–0.95, $p = 0.02$ and OS 64%; HR 0.68, 95% CI 0.51–0.91, $p = 0.008$). We also found significant improvement in the 3-year PFS for stage IV patients in the high Ki67-Li group (PFS 14%) versus the low Ki67-Li group (PFS 10%) (HR 1.23, 95% CI 1.04–1.57, $p = 0.02$). However, OS showed no statistical difference in stage IV patients with high Ki67-Li tumors (OS 19%) versus low Ki67-Li tumors (OS 18%) (HR 1.22, 95% CI 0.99–1.51, $p = 0.06$). From the analysis of stage I patients, we found no differences in the 3-year DFS or OS between high Ki67-Li patients versus those with a low Ki67-Li (DFS 94 vs. 93%; HR 0.84, 95% CI 0.35–2.03, $p = 0.70$ and OS 95 vs. 93%; HR 0.69, 95% CI 0.27–1.74, $p = 0.43$). Likewise, no difference was found in the 3-year DFS and OS of high versus low Ki67-Li stage II patients (DFS 83 vs. 78%; HR 0.77, 95% CI 0.57–1.05, $p = 0.09$ and OS 85 vs. 81%; HR 0.82, 95% CI 0.59–1.13, $p = 0.22$). The survival plots for Ki67-Li are shown in figure 2.

Discussion

Here, we evaluated the expression of Ki67 in CRC tissues and whether assessment of the Ki67-Li could be a valuable prognostic tool in this disease. We found that a high Ki67-Li appeared to be an independent and good prognostic biomarker for survival in stage III and IV CRC patients while it provided no evidence for a prognostic effect in stage I or II CRC patients. The reasons for the discrepancies between the effect of Ki67 expression in the various CRC stages are not clear. However, the differences may account for the many roles of Ki67 in cell growth and regulation.

It has been previously shown that the rate of tumor cell proliferation can be assessed by detecting the fraction of cells with nuclei expressing Ki67 [6]. The Ki67 antigen binds to the nuclei of proliferating cells throughout the cell cycle, except during the G0 and early G1 phases, marking cellular expansion [12]. Monitoring of the Ki67 protein has already been used as a clinical predictor for the prognosis of breast cancer and neuroendocrine tumors due to its expression pattern restricted to growth phases of the cell cycle found in differentiating cells [4, 5]. Nuclear staining of Ki67 was also shown to be significantly associated with disease-specific survival in a multivariate model of clear cell renal cell carcinoma and provided the ability to predict disease outcome [13]. It has also been reported that neoadjuvant chemoradiotherapy with cetuximab decreased the levels of Ki67-Li in rectal cancer [14]. With regard to colon tissue, Ki67 was found to be highly expressed in the proliferative basal half of the crypt [15]. Also, it has been shown that Ki67 was significantly elevated in ulcerative colitis (UC) colons compared to non-UC control samples, indicating that proliferation is enhanced in UC colonic mucosa [16]. With regard to CRC itself, it has been reported that Ki67 expression correlates with patient age, pathological tumor differentiation, and lymph node involvement [17]. In addition, a strong correlation between Ki67 expression has also been seen in all levels of epithelial dysplasia and tumor grades [18]. In another study, Ki67-Li was found to significantly correlate with lymph node metastasis, but not tumor size, age, or gender, in rectal adenocarcinomas [19]. It has also been shown that Ki67 expression is not related to atypia [20].

From our large dataset of Chinese CRC patients studied here, we found that high Ki67-Li correlated with old age and better survival for stage III or IV colon cancers, but not with gender, tumor stage, or pathological differentiation. Overall, the results of our attempt to find an association between Ki67-Li and prognosis for the general population of patients with CRC were conflicting. Inconsistent effects of Ki67 expression with regard to patient outcomes also appear to have been evidenced in other studies. A previous study using Kaplan-Meier survival analysis showed that survival was significantly shorter for CRC patients with higher expression of Ki67 versus lower [21, 22]. Yet, lower TNM and Dukes stage and higher Ki67 expression and presence of Ki67 hot spot areas in histopathological samples in rectal carcinoma were associated with better survival rates [23]. High Ki67 expression was also found to correlate well with response of chemoradiation therapy in locally advanced rectal cancers [24]. In contrast to this finding, another

Table 1. Clinicopathological characteristics of the patients

Characteristic	Ki67-Li, n (%)		p value
	low (< 50%) (992/44.4)	high (≥ 50%) (1,241/55.6)	
Sex			0.496
Male	584 (26.2)	732 (32.8)	
Female	408 (18.3)	509 (22.8)	
Age, years			0.028
20–39	101 (4.5)	87 (3.9)	
40–59	448 (20.1)	576 (25.8)	
60–85	443 (19.8)	587 (25.9)	
Pathology			0.270
G1	38 (1.7)	66 (3.0)	
G2	896 (40.1)	1,106 (49.5)	
G3	11 (0.5)	7 (0.3)	
Mucinous	42 (1.9)	53 (2.4)	
Signet-ring	5 (0.2)	9 (0.4)	
Cancer stage			0.165
I	134 (6.0)	197 (8.8)	
IIA	281 (12.6)	329 (14.7)	
IIB	102 (4.6)	123 (5.5)	
IIC	19 (0.9)	23 (1.0)	
IIIA	21 (0.9)	34(1.5)	
IIIB	186 (8.3)	266 (11.9)	
IIIC	47 (2.1)	45 (2.0)	
IVA	124 (5.6)	141 (6.3)	
IVB	78(3.5)	83 (3.7)	
T stage			0.197
T1	35 (1.6)	60 (2.7)	
T2	127 (5.7)	177 (7.9)	
T3	519 (23.3)	664 (29.7)	
T4a	251 (11.2)	277 (12.4)	
T4b	59 (2.6)	63 (2.8)	
N stage			0.143
N0	619 (27.7)	749 (33.6)	
N1	268 (12.0)	344 (15.4)	
N2	105 (4.7)	147 (6.6)	
M stage			0.411
M0	790 (35.4)	1,014 (45.5)	
M1	131 (5.9)	149 (6.7)	
M2	70 (3.1)	75 (3.4)	
Location			< 0.001
Right colon	211 (9.4)	310 (13.9)	
Left colon	294 (13.2)	446 (20.0)	
Rectum	487 (21.8)	485 (21.7)	
Metastasis			0.011
Yes	366 (16.4)	393 (17.6)	
No	626 (28.0)	848 (38.0)	
Alive			0.005
Yes	655 (29.3)	889 (39.8)	
No	337 (15.1)	352 (15.8)	

study showed that patients with high tumor regression actually had significantly lower Ki67 expression within their tumors than non-responders [25]. Finally, well-differentiated adenocarcinomas from liver cancers were found to have a high number of low Ki67-Li vessels that might account for differences in tumor growth [26].

Many of these earlier studies on the effects of Ki67 expression utilized only a small number of cases in their analysis. A more recent study in CRC that analyzed 1,653 cases reported that high Ki67 expression is an independent prognostic marker for better survival [27]. The results of this earlier study are in slight contrast to those of the current study. In our study, we also found a high Ki67-Li to be an good independent prognostic biomarker but only for stage III or IV CRC patients. We found no prognostic effect for Ki67 indexing in stage I or II CRC patients.

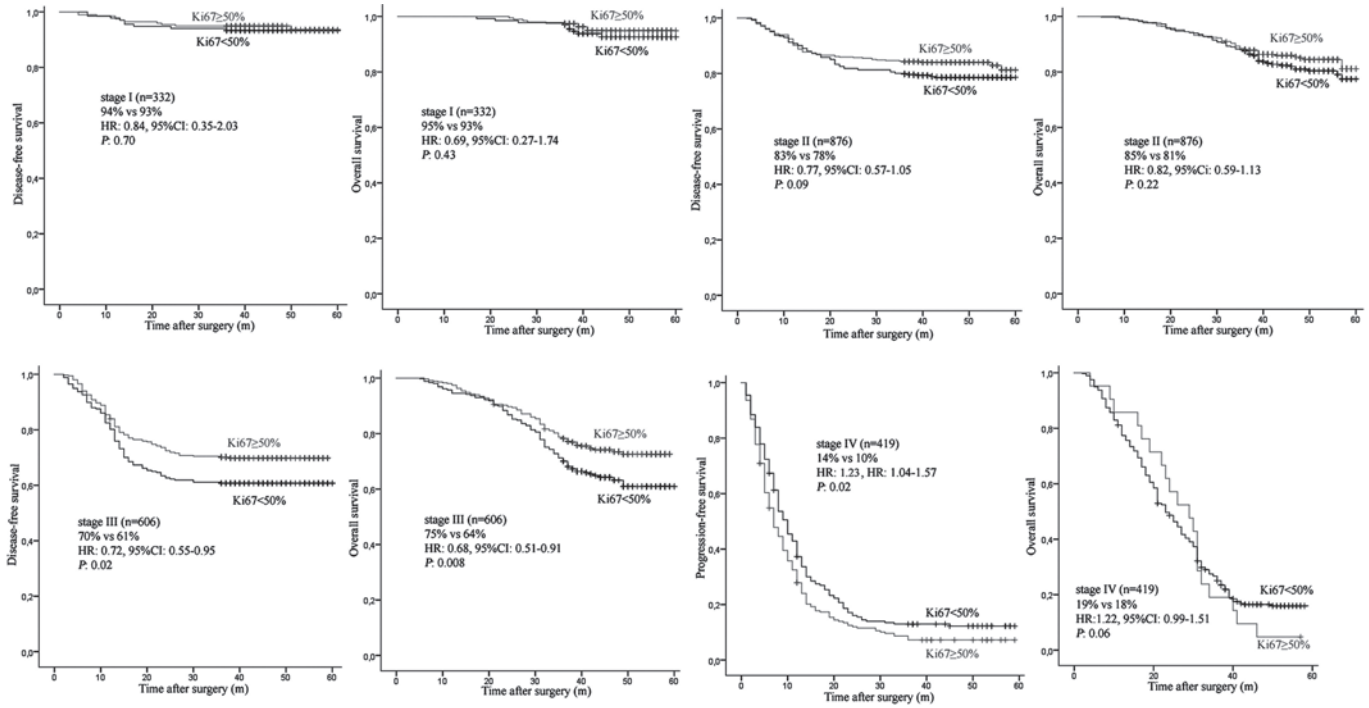

Fig. 2. Disease-free survival (DFS) and overall survival (OS) according to Ki67 expression in colorectal carcinoma (CRC) stage I–IV.

One of the criteria that might account for the above differences is the classification of Ki67 indexing. The previous study stratified the patient groups into 3 categories of low, moderate, and high whereas we used only 2 designations of either high or low. Only 17% of the previous study's 1,653 patients were considered to have a high Ki67-Li. Second, our study was conducted in only 1 hospital in China, and this population may not be comparable to the previous study's patient population nor does it necessarily reflect the entire population of China. Finally, CRC is a disease induced by multiple biological, genetic, and environmental factors that could account for differences in tumor response, and each of these elements should be examined in future studies.

Conclusion

Our data demonstrate that patients with a high Ki67-Li have a statistically significant reduction in their rates of tumor recurrence as well as better survival rates compared to patients with a low Ki67-Li for stage III CRC. While no statistically significant differences were found for patients with stage I and II tumors and a high Ki67-Li, for stage IV CRC patients Ki67-Li was an effective prognostic factor predicting PFS but not OS. Finally, we demonstrated a close correlation between high Ki67-Li and old age and colon cancer. This study provides further population-specific evidence for Ki67 expression in CRC and use of Ki67-Li monitoring for patient management.

Disclosure Statement

The authors have declared no conflicts of interest.

References

1 Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A: Global cancer statistics, 2012. CA Cancer J Clin 2015;65:87–108.
2 Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM: Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int J Cancer 2010;127:2893–2917.
3 Scholzen T, Gerdes J: The Ki-67 protein: from the known and the unknown. J Cell Physiol 2000;182:311–322.
4 Fujimori Y, Fujimori T, Imura J, Sugai T, Yao T, Wada R, Ajioka Y, Ohkura Y: An assessment of the diagnostic criteria for sessile serrated adenoma/polyps: SSA/Ps using image processing software analysis for Ki67 immunohistochemistry. Diagn Pathol 2012;7:59.
5 Pathmanathan N, Balleine RL: Ki67 and proliferation in breast cancer. J Clin Pathol 2013;66:512–516.
6 Gerdes J, Schwab U, Lemke H, Stein H: Production of a mouse monoclonal antibody reactive with a human nuclear antigen associated with cell proliferation. Int J Cancer 1983;31:13–20.
7 Krause P, Flikweert H, Monin M, Seif AHA, Helms G, Cantanhede G, Ghadimi BM, Koenig S: Increased growth of colorectal liver metastasis following partial hepatectomy. Clin Exp Metastasis 2013;30:681–693.
8 Zhang Z, Wang M, Zhou L, Feng X, Cheng J, Yu Y, Gong Y, Zhu Y, Li C, Tian L, Huang Q: Increased HMGB1 and cleaved caspase-3 stimulate the proliferation of tumor cells and are correlated with the poor prognosis in colorectal cancer. J Exp Clin Cancer Res 2015;34:51.

9 Gurzu S, Szentirmay Z, Jung I: Molecular classification of colorectal cancer: a dream that can become a reality. Rom J Morphol Embryol 2013;54:241–245.
10 Debucquoy A, Goethals L, Libbrecht L, Perneel C, Geboes K, Ectors N, McBride WH, Haustermans K: Molecular and clinico-pathological markers in rectal cancer: a tissue micro-array study. Int J Colorectal Dis 2009;24:129–138.
11 Wang H, Wen J, Wang H, Guo Q, Shi S, Shi Q, Zhou X, Liu Q, Lu G, Wang J: Loss of expression of EphB1 protein in serous carcinoma of ovary associated with metastasis and poor survival. Int J Clin Exp Pathol 2014;7:313–321.
12 Jakob C, Liersch T, Meyer W, Becker H, Baretton GB, Aust DE: Predictive value of Ki67 and p53 in locally advanced rectal cancer: correlation with thymidylate synthase and histopathological tumor regression after neoadjuvant 5-FU-based chemoradiotherapy. World J Gastroenterol 2008;14:1060–1066.
13 Weber T, Meinhardt M, Zastrow S, Wienke A, Fuessel S, Wirth MP: Immunohistochemical analysis of prognostic protein markers for primary localized clear cell renal cell carcinoma. Cancer Invest 2013;31:51–59.
14 Debucquoy A, Haustermans K, Daemen A, Aydin S, Libbrecht L, Gevaert O, De Moor B, Tejpar S, McBride WH, Penninckx F, Scalliet P, Stroh C, Vlassak S, Sempoux C, Machiels JP: Molecular response to cetuximab and efficacy of preoperative cetuximab-based chemoradiation in rectal cancer. J Clin Oncol 2009;27:2751–2757.
15 Dame MK, Jiang Y, Appelman HD, Copley KD, McClintock SD, Aslam MN, Attili D, Elmunzer BJ, Brenner DE, Varani J, Turgeon DK: Human colonic crypts in culture: segregation of immunochemical markers in normal versus adenoma-derived. Lab Invest 2014;94:222–234.
16 Burum-Auensen E, Deangelis PM, Schjolberg AR, Roislien J, Andersen SN, Clausen OP: Spindle proteins Aurora a and BUB1B, but not Mad2, are aberrantly expressed in dysplastic mucosa of patients with longstanding ulcerative colitis. J Clin Pathol 2007;60:1403–1408.
17 Gurzu S, Jung J, Mezei T, Pavai Z: The correlation between the immunostains for p53 and Ki67 with bcl-2 expression and classical prognostic factors in colorectal carcinomas. Rom J Morphol Embryol 2007;48:95–99.
18 Stromar IK, Jakic-Razumovic J: The value of immunohistochemical determination of topoisomerase IIalpha and Ki67 as markers of cell proliferation and malignant transformation in colonic mucosa. Appl Immunohistochem Mol Morphol 2014;22:524–529.
19 Jiang SM, Wang RB, Yu JM, Zhu KL, Mu DB, Xu ZF: (Correlation of VEGF and Ki67 expression with sensitivity to neoadjuvant chemoradiation in rectal adenocarcinoma). Zhonghua Zhong Liu Za Zhi 2008;30:602–605.
20 Vernillo R, Lorenzi B, Banducci T, Minacci C, Vindigni C, Lucenti FA, Lorenzi M: Immunohistochemical expression of p53 and Ki67 in colorectal adenomas and prediction of malignancy and development of new polyps. Int J Biol Markers 2008;23:89–95.
21 Lin MX, Wen ZF, Feng ZY, He D: (Expression and significance of Bmi-1 and Ki67 in colorectal carcinoma tissues). Ai Zheng 2008;27:1321–1326.
22 Ma YL, Peng JY, Zhang P, Liu WJ, Huang L, Qin HL: Immunohistochemical analysis revealed CD34 and Ki67 protein expression as significant prognostic factors in colorectal cancer. Med Oncol 2010;27:304–309.
23 Salminen E, Palmu S, Vahlberg T, Roberts PJ, Soderstrom KO: Increased proliferation activity measured by immunoreactive Ki67 is associated with survival improvement in rectal/recto sigmoid cancer. World J Gastroenterol 2005;11:3245–3249.
24 Kikuchi M, Mikami T, Sato T, Tokuyama W, Araki K, Watanabe M, Saigenji K, Okayasu I: High Ki67, Bax, and thymidylate synthase expression well correlates with response to chemoradiation therapy in locally advanced rectal cancers: proposal of a logistic model for prediction. Br J Cancer 2009;101:116–123.
25 Jakob C, Liersch T, Meyer W, Becker H, Baretton GB, Aust DE: Predictive value of Ki67 and p53 in locally advanced rectal cancer: correlation with thymidylate synthase and histopathological tumor regression after neoadjuvant 5-FU-based chemoradiotherapy. World J Gastroenterol 2008;14:1060–1066.
26 Ceausu RA, Cimpean AM, Gaje P, Gurzu S, Jung I, Raica M: CD105/Ki67 double immunostaining expression in liver metastasis from colon carcinoma. Rom J Morphol Embryol 2011;52:613–616.
27 Melling N, Kowitz CM, Simon R, Bokemeyer C, Terracciano L, Sauter G, Izbicki JR, Marx AH: High Ki67 expression is an independent good prognostic marker in colorectal cancer. J Clin Pathol 2016;69:209–214.

Cancer Medicine

Open Access

ORIGINAL RESEARCH

Impact of age and mismatch repair status on survival in colorectal cancer

Pan Li[1], Zhi-Tao Xiao[2], Todd A. Braciak[1], Qing-Jian Ou[2], Gong Chen[2] & Fuat S. Oduncu[1]

[1]Department of hematology and oncology, Medizinische Klinik und Poliklinik IV, Ludwig Maximilians University, Munich, Germany
[2]Department of Colorectal Surgery, State Key Laboratory of Oncology in South China, Sun Yat-sen University Cancer Center, Guangzhou, China

Keywords
Age, biomarker, colorectal cancer, MMR status, prognosis

Correspondence
Fuat S. Oduncu, Department of hematology and oncology, Medizinische Klinik und Poliklinik IV, Ludwig Maximilians University, Ziemssenstr. 1, D-80336 Munich, Germany.
Tel: +49-(0)-89-4400-52205;
Fax: +49-(0)-89-4400-54412;
E-mail: fuat.oduncu@med.uni-muenchen.de
and
Gong Chen, Department of Colorectal Surgery, State Key Laboratory of Oncology in South China, Sun Yat-sen University Cancer Center, East Dongfeng Road 651, D-510060 Guangzhou, China.
Tel: +86-13500030531;
Fax: +86-(0)-20-87343584;
E-mail: chengong@sysucc.org.cn

Funding information
No funding information provided.

Received: 19 September 2016; Revised: 5 December 2016; Accepted: 14 December 2016

Cancer Medicine **2017; 6(5):975–981**

doi: 10.1002/cam4.1007

Abstract

Previous studies have suggested that deficiencies in mismatch repair genes (dMMR) often occur in patients with colorectal cancer (CRC) and contribute to disease etiology. Here, we looked for a correlation of MMR status to disease outcomes from a large number of Chinese CRC patients stratified by the age of onset of disease. A total of 2233 CRC patients were analyzed and tissue biopsies of surgically removed tumors scored for MMR gene status. The patient distribution after classification consisted of 188 younger aged patients (20–39 years of age), 1024 middle aged patients (40–59 years of age), and 1020 older aged patients (60–85 years of age). In this analysis, the expression of four MMR genes was assessed by immunohistochemistry (IHC). We found that the young group of CRC patients with dMMR had higher overall survival (OS) than the young group of patients with proficient MMR (pMMR) (77% vs. 56%, $P = 0.03$). Middle-aged patients with dMMR also had higher OS than middle-aged group patients with pMMR (78% vs. 68%, $P = 0.012$). However, we found no statistical difference in OS between dMMR and pMMR status in the older group of patients (75% vs. 71%, $P = 0.224$). Finally, the middle- and older-aged group set of patients had higher OS than the young group of patients (69% vs. 71% vs. 59%, $P = 0.008$). These data demonstrated that the age of disease onset can be an important factor to help evaluate the prognosis of CRC when combined with the analysis of MMR status within tumor biopsied tissue.

Introduction

The development of colorectal cancer (CRC) can be a slow process, with early stages of disease often presenting with no clinical symptoms to the patient. It has been indicated that most CRCs are adenocarcinomas arising from noncancerous adenomatous polyps [1,2]. Like many other types of cancer, CRC has been found to have an associated higher incidence with increasing age. The risk of CRC has a marked increase in occurrence after reaching 40 years of age and incidence continues to increase even more rapidly after this age. Overall, the risk of CRC doubles with each succeeding decade of age, and continues to rise exponentially in incidence with age [3].

Little is known about the precise biochemical mechanisms responsible for the rise in CRC with aging. Many possible causes for this increase in CRC incidence have been suggested. One model of CRC progression proposed by Vogelstein et al. indicates that malignancy arises as a result of accumulation of mutations in tumor suppressor genes and oncogenes [4]. Indeed, the molecular profiles of CRC patients have been shown to differ between various age groups of patients examined [5–7]. Whether patients within these various age groups have a different

biological behavior including MMR activity remains controversial.

MMR genes correct mismatched nucleotides and insertion–deletion loops (IDLs) in DNA caused by polymerase errors, chemical modifications, and recombination between heterologous DNA sequences [8]. It was previously demonstrated that stage II CRC patients with dMMR have a better prognosis and it was indicated that some patients may actually be harmed by 5-FU treatment [9]. More recent studies have found that CRC tumors with dMMR were more prevalent in younger patients [10,11]. Despite these early findings about MMR status, there still has been no conclusive study to prove an association with overall survival (OS) for age of disease onset and MMR status in CRC patients.

In this study, we sought to find if difference in MMR status based on age could be useful to identify groups of patients that would have better disease outcomes. Following the recommendations of the EGAPP [12], we performed systematic immunohistochemistry (IHC) screening of CRC tumor tissues looking for microsatellite instability of patients. Here, we had access to a large number of Chinese CRC patients who were operated on to remove their primary tumors and whose tissues were assessed in our institute since 2011. Our findings indicate that the combined analysis of age of onset and MMR status could provide some prognostic information about these CRC patient outcomes.

Materials and Methods

Patients

The ethics committee of Sun Yat-sen University Cancer Center approved this study and informed consents for all patients were obtained at the beginning of the study. A total of 4500 patients with histologically confirmed CRC tumors were recruited from the Sun Yat-sen University cancer center between May 2011 and May 2016 for this study. Clinical and familial histories for each of these patients were reviewed. From these recruited patients, 2233 cases were finally selected for analysis after applying strict exclusion criteria that included: age less than 18 years and older than 85 years, severe complication, multiprimary cancer, synchronous and metachronous CRC, family history (first-degree and second-degree relatives had any kind of cancer), familial adenomatous polyposis, death not due to tumor-related reason, and incomplete follow-up record were not included for study. The primary tumor location site was categorized as right colon if the tumor was located above the splenic flexure, and left colon if it was located at or below the splenic flexure or as from the rectum. The median follow-up on surviving patients was 4.3 years.

Treatment and follow-up

Stage I (T1–2 N0) and stage II (T3–4 N0) CRC patients without high-risk clinical features (e.g., T4 stage, bowel perforation or clinical bowel obstruction, inadequate lymph node sampling, poorly differentiated histology) were treated with radical surgery or endoscopic removal of the tumor alone. Stage II (T3–4 N0) CRC patients with high-risk clinical features were recommended to follow the capecitabine+oxaliplatin (XELODA) treatment regimen. Stage III (Tx N1–2) patients were designated to receive radical surgery and 12 cycles of adjuvant mFOLFOX/XELOX regimen treatment within a 6-month period. All stage IV (Tx Nx M1) patients received palliative surgery or radical surgery. The first-line treatment for stage IV CRC was mFOLFOX/FOLFIRI (folinic acid+5-FU+irinotecan) chemotherapy regimen. Patient clinical responses were evaluated in accordance with the RECIST guidelines. After surgery, tumor recurrence was determined by physical examination, serum carcinoembryonic antigen (CEA) assay, or abdominal and thoracic imaging taken every 3–6 months for the following 3 years after initial therapy, then every 6 months for the next 2 years, and finally followed by annual checkup. In addition, Sun Yat-sen University cancer center has an independent follow-up department. The colleagues call the patients or the family members regularly and register the survival status and health condition. The cutoff date of analysis for inclusion in this study was May 2016.

Immunohistochemistry

Blocks of formalin-fixed, paraffin-embedded CRC adenocarcinoma tissue comprising an area of normal colorectal mucosa adjacent to the tumor were selected in each case. Cases with complete nuclear loss of MMR expression in invasive tumor cells but with retained expression in inflammatory cells and/or adjacent normal tissue as positive controls were considered MMR deficient. Staining was performed using the following primary antibodies: mouse anti-human mutL homolog 1 (MLH1) (dilution 1:150, clone OTI1C1, zhongshan jiqiao, Beijing), rabbit anti-human mutS homolog2 (MSH2) (dilution 1:100, clone ZA0622, zhongshan jiqiao, Beijing, mouse anti-human mutS homolog 6 (MSH6) (dilution 1:150, clone OTI5D1, zhongshan jiqiao, Beijing), and mouse anti-human postmeiotic segregation increased 2 (PMS2) (dilution 1:150, clone OTI2G5, zhongshan jiqiao, Beijing). Whole-tissue sections were analyzed independently by two pathologists and were blinded to any of the patients' clinical characteristics. Any discordant cases were reviewed by a supplementary pathologist in order to reach a consensus on the tumor characterization. Illustrative

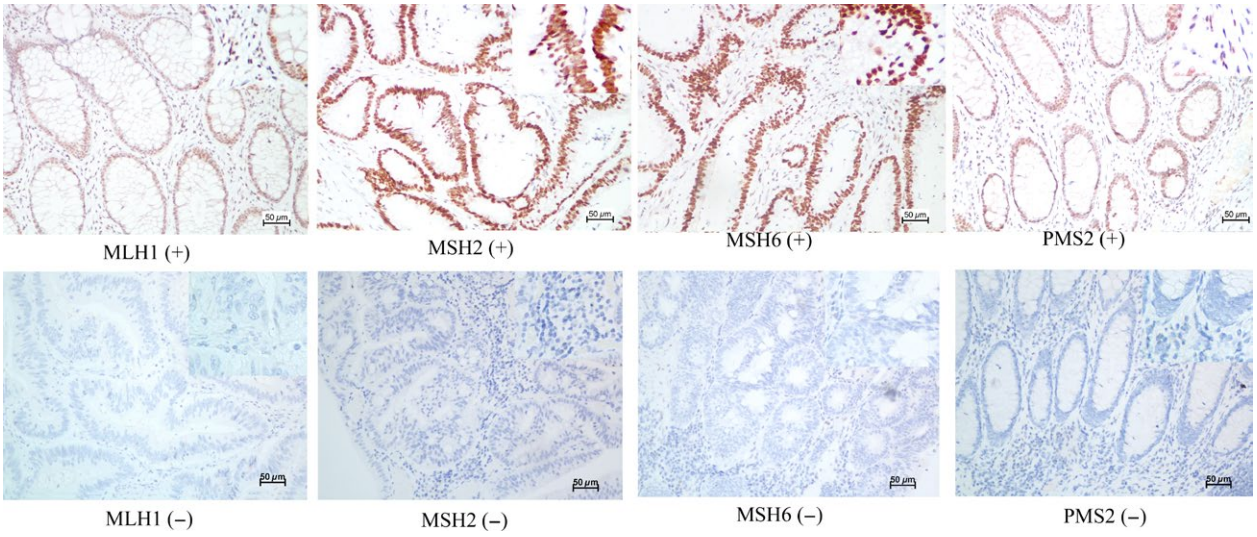

Figure 1. Illustrative immunostainings. Positive (upper panel) and negative (lower panel) for MLH1, MSH2, MSH6, and PMS2.

immunostainings of recovered CRC tumor sections are shown in Figure 1.

Statistical analysis

Patient data are described in frequencies (percentages) of cases with the given phenotype. Differences in distributions between the variables examined were assessed by the χ^2 or the Fisher's exact test. The primary end point of the study was OS, defined as the time elapsed from the date of surgery until the tumor-induced death of the patient. Surviving patients were censored on the last follow-up date. Median follow-up and the 95% CI were calculated using the reverse Kaplan–Meier method. The survival curve was estimated with the Kaplan–Meier method and compared using the log-rank test. The score and likelihood ratio test *P*-values were used to test the statistical significance of each covariate in the univariate and multivariable Cox models, respectively. All statistical tests were two-sided, and *P*-values less than or equal to 0.05 were considered statistically significant. Statistical analyses were performed using SPSS software.

Results

A total of 2001 (89.6%) of all CRC specimens examined showed retained expression in tumor cells for MLH1, MSH2, MSH6, and PMS2 proteins. In comparison, loss of expression in at least one of the four MMR genes was found to occur in 232 of 2233 patients analyzed representing a dMMR status for 10.4% of the CRC patients examined. The patients analyzed included 188 patients (8.4%) classified as young (20–39 years old), 1024 (45.9%) as middle-aged patients (40–59 years old), and 1020 (45.7%) as older-aged patients (60–85 years old). Their median age at diagnosis was 58 years (ranged 18–85 years). The patient demographics and tumor characteristics by age are listed in Table 1.

In addition, we found that 55.3% of the younger patient group were female. With regard to dMMR status, we found that tumors with dMMR status tended to be noticed in higher incidence in younger patients (13.8%) compared to middle-age (12.2%) and older patients (7.9%) ($P < 0.001$). In univariate analysis, the MMR status (HR: 1.56, 95% CI: 1.18–2.06, $P = 0.002$) and tumor stage (HR: 0.19, 95% CI: 0.16–0.23, $P < 0.001$) showed statistical significance. However, among the variables analyzed in the multivariate Cox model, only tumor stage (HR: 0.20, 95% CI: 0.17–0.24, $P < 0.001$) was shown to be significantly associated with OS regarding the age of disease onset. We found that gender (HR: 0.96, 95% CI: 0.82–1.11, $P = 0.561$), tumor location (HR: 0.92, 95% CI: 0.83–1.01, $P = 0.064$), and pathological differentiation (HR: 0.97, 95% CI: 0.84–1.12, $P = 0.700$) showed no statistical differences by univariate Cox analysis ($P > 0.05$) regarding the age of disease onset.

Our most important finding in this study was that we could find an association for dMMR status and age of disease onset to OS for our Chinese CRC patients examined. As part of this analysis, we found that age alone had associations with OS. Here, middle- and older-aged patients had higher OS than younger-aged grouped patients (69% vs. 71% vs. 59%, HR: 1.07, 95% confidence interval (CI): 0.91–1.25, $P = 0.008$). In our results, the middle- (69%) and older-aged (71%) patients have similar OS with no significant difference. However, when the middle- and

Table 1. Clinicopathological characteristics of the patients.

Characteristics	Ages (*n*/%)			*P*-value
	20–39 years (188/8.4)	40–59 years (1024/45.9)	60–85 years (1021/45.7)	
Gender				<0.001
Male	84 (3.8)	599 (26.8)	633 (28.3)	
Female	104 (4.7)	425 (19.0)	388 (17.4)	
Location				0.228
Right colon	52 (2.3)	243 (10.9)	226 (10.1)	
Left colon	59 (2.6)	323 (14.5)	358 (16.0)	
Rectum	77 (3.4)	458 (20.5)	437 (19.6)	
Pathology				0.095
G1	18 (0.8)	48 (2.1)	38 (1.7)	
G2	152 (6.8)	919 (41.2)	931 (41.7)	
G3	0 (0.0)	5 (0.2)	13 (0.6)	
Mucinous	13 (0.6)	46 (2.1)	36 (1.6)	
Signet-ring	5 (0.2)	6 (0.3)	3 (0.1)	
Stage				<0.001
I	13 (0.6)	129 (5.8)	189 (8.5)	
IIA	57 (2.6)	273 (12.2)	280 (12.5)	
IIB	13 (0.6)	119 (5.3)	93 (4.2)	
IIC	3 (0.1)	17 (0.8)	22 (1.0)	
IIIA	6 (0.3)	25 (1.1)	24 (1.1)	
IIIB	30 (1.3)	209 (9.4)	213 (9.5)	
IIIC	14 (0.6)	47 (2.1)	31 (1.4)	
IVA	28 (1.3)	123 (5.5)	114 (5.1)	
IVB	24 (1.1)	82 (3.7)	55 (2.5)	
MMR status				<0.001
dMMR	26 (1.2)	125 (5.6)	81 (3.6)	
pMMR	162 (7.3)	899 (40.3)	940 (42.1)	
Alive				0.001
Yes	111 (5.0)	707 (31.7)	726 (32.5)	
No	77 (3.4)	317 (14.2)	295 (13.2)	

older-aged patients are compared with younger-aged patients (59%), statistical significance is shown. When age was analyzed in conjunction with MMR status, we found that younger CRC patients with dMMR had higher OS than young patients with proficient MMR (pMMR) (77% vs. 56%, HR: 0.42, 95% CI: 0.19–1.02, $P = 0.03$). Likewise, we found that middle-aged patients with dMMR also had higher OS than middle-aged patients with pMMR (78% vs. 68%, HR: 0.63, 95% CI: 0.43–0.92, $P = 0.012$). This association of age with dMMR did not hold for older-age group patients. Here, we found no statistical difference in OS between dMMR and pMMR status for older-aged CRC patients (75% vs. 71%, HR: 0.76, 95% CI: 0.48–1.20, $P = 0.224$). Patient survival plots with regard to tumor location and MMR status are shown in Figure 2.

Discussion

Multiple risk factors contribute to the capacity of an individual to develop CRC. Disturbingly, younger-aged patients are more likely to be diagnosed with later stage disease when their cancers are discovered [13]. However, it is unclear whether this reflects differing biology between young versus older patients or simply that a low rate of CRC screening is performed at this young age. In our present Chinese population-based CRC study, we looked to see if age and MMR status had an impact on the survival of these CRC patients.

It was previously reported that younger patients have a higher prevalence of mucinous or signet-ring types of carcinoma [14,15]. Yet here, in our study, we found that pathological differentiation showed no statistical difference to OS ($P > 0.05$). Another study demonstrating a linkage to age and CRC demonstrated that the age at diagnosis of inflammatory bowel disease was an indicator of early development of CRC in inflammatory bowel disease patients [7].

In younger patients, CRC tend to present more commonly as stage III or IV disease, which may reflect differing biology in younger-aged CRC patients, but could

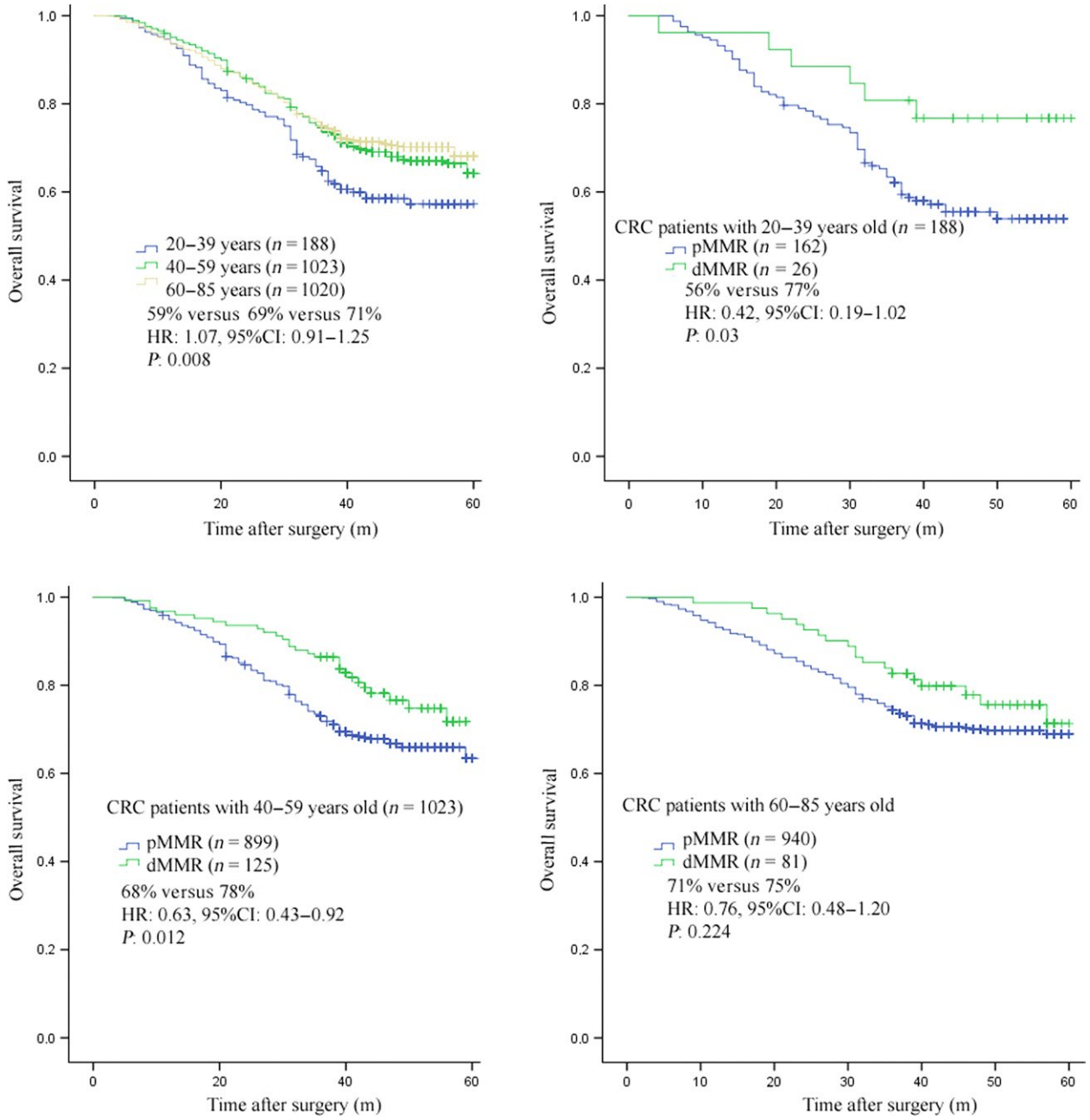

Figure 2. Overall survival (OS) according to MMR status and age in colorectal carcinoma (CRC) stage I-IV.

also be reflective of later diagnosis because of the rarity of this condition in that age group, and/or less surveillance in general of cancers in this age group [13]. In concordance with this previous study, here, we also found that younger patients were more likely to present as stage IIIB or IVB disease.

The evidence is increasing to indicate that the molecular profiles of CRC cells can differ in various age groups of patients and that this will influence the given patient's disease outcome and response to therapy [5–7]. It was reported that CRC patients above the age of 50 showed decreased Bax/Bcl-2 ratios that might differentially control tumor cell apoptosis between the various age groups of patients [5]. Also, it has been shown that peripheral blood leukocyte (PBL) telomere length varies according to the age of CRC onset, perhaps impacting immune cell functions differentially [6]. With regard to MMR status, a more recent set of studies found that dMMR was more

Table 2. Univariate analysis of overall survival.

		95% CI		
Variable	HR	Lower	Upper	*P*-value
Tumor stage	0.19	0.16	0.23	<0.001
Gender	0.96	0.82	1.11	0.561
Tumor location	0.92	0.83	1.01	0.064
Pathology	0.97	0.84	1.12	0.700
MMR status	1.56	1.18	2.06	0.002

prevalent in younger CRC patients [10,11]. Likewise, we found that tumors with a dMMR status tended to be more prevalent in the younger group of patients (13.8%) versus the middle- (12.2%) or older-aged patients (7.9%) ($P < 0.001$).

MMR corrects mismatched nucleotides and insertion–deletion loops (IDLs) in DNA caused by polymerase errors, chemical modifications, and recombination between heterologous DNA sequences [8]. It was demonstrated that stage II patients with dMMR have a better prognosis and may actually be harmed by 5-FU treatment [9]. In our study, young CRC patients with dMMR had higher OS than young patients with pMMR ($P = 0.03$). We also found that middle-aged patients with dMMR also had higher OS than middle-aged patients with pMMR ($P = 0.012$). However, we found no statistical difference in OS between dMMR and pMMR status in older-aged patients ($P = 0.224$). While not yet known, these differences in disease response may be based upon the molecular profile or epigenetics among these various aged patients.

Overall, the notion that age can be a significant prognostic factor in CRC has been somewhat controversial. Various studies have reported poorer prognosis for younger patients with CRC [16–19]. Yet, other authors have demonstrated that younger patients with CRC surgically treated appeared to have a higher cancer-specific survival rate than elderly ones [13,19,20]. In our study here, we found that middle- and older-aged patients had higher OS than young patients ($P = 0.008$), indicating that again there is some link to age and CRC patient disease response.

In conclusion, the observation that cancer-related mortality did not decrease with increasing age exemplified that, although elderly patients have a shorter life expectancy based on their age and preexistent conditions, they did still benefit from cancer treatment. The combination of age and MMR was a significant predictor of overall survival in our study, reflecting the importance of optimizing patient beyond treatment. The potential efficacy of age-tailored and molecular profiled interventions as components of personalized care clearly need to be investigated further in future studies.

Conflict of Interest

The authors declare that they have no competing interests.

References

1. Siegel, R. L., K. D. Miller, and A. Jemal. 2015. Cancer statistics, 2015. CA Cancer J. Clin. 65:5–29.
2. Edwards, B. K., E. Ward, B. A. Kohler, C. Eheman, A. G. Zauber, R. N. Anderson, et al. 2010. 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. Cancer 116:544–573.
3. Imperiale, T. F., R. Juluri, E. A. Sherer, E. A. Glowinski, C. S. Johnson, and M. S. Morelli. 2014. A risk index for advanced neoplasia on the second surveillance colonoscopy in patients with previous adenomatous polyps. Gastrointest. Endosc. 80:471–478.
4. Fearon, E. R., and B. Vogelstein. 1990. A genetic model for colorectal tumorigenesis. Cell 61:759–767.
5. Khodapasand, E., N. Jafarzadeh, F. Farrokhi, B. Kamalidehghan, and M. Houshmand. 2015. Is Bax/Bcl-2 ratio considered as a prognostic marker with age and tumor location in colorectal cancer? Iran. Biomed. J. 19:69–75.
6. Boardman, L. A., K. Litzelman, S. Seo, R. A. Johnson, R. J. Vanderboom, G. W. Kimmel, et al. 2014. The association of telomere length with colorectal cancer differs by the age of cancer onset. Clin. Transl. Gastroenterol. 5:e52.
7. Baars, J. E., E. J. Kuipers, M. van Haastert, J. J. Nicolai, A. C. Poen, and C. J. van der Woude. 2012. Age at diagnosis of inflammatory bowel disease influences early development of colorectal cancer in inflammatory bowel disease patients: a nationwide, long-term survey. J. Gastroenterol. 47:1308–1322.
8. Kolodner, R. D., and G. T. Marsischky. 1999. Eukaryotic DNA mismatch repair. Curr. Opin. Genet. Dev. 9:89–96.
9. Boland, C. R., and A. Goel. 2010. Microsatellite instability in colorectal cancer. Gastroenterology 138:2073–2087.
10. Limburg, P. J., W. S. Harmsen, H. H. Chen, S. Gallinger, R. W. Haile, J. A. Baron, et al. 2011. Prevalence of alterations in DNA mismatch repair genes in patients with young-onset colorectal cancer. Clin. Gastroenterol. Hepatol. 9:497–502.
11. Schofield, L., N. Watson, F. Grieu, W. Q. Li, N. Zeps, J. Harvey, et al. 2009. Population-based detection of Lynch syndrome in young colorectal cancer patients using microsatellite instability as the initial test. Int. J. Cancer 124:1097–1102.

12. Recommendations from the EGAPP Working Group. 2009. genetic testing strategies in newly diagnosed individuals with colorectal cancer aimed at reducing morbidity and mortality from Lynch syndrome in relatives. Genet. Med. 11:35–41.
13. O'Connell, J. B., M. A. Maggard, J. H. Liu, D. A. Etzioni, E. H. Livingston, and C. Y. Ko. 2004. Do young colon cancer patients have worse outcomes? World J. Surg. 28:558–562.
14. Ganapathi, S., D. Kumar, N. Katsoulas, D. Melville, S. Hodgson, C. Finlayson, et al. 2011. Colorectal cancer in the young: trends, characteristics and outcome. Int. J. Colorectal Dis. 26:927–934.
15. Neufeld, D., B. Shpitz, N. Bugaev, M. Grankin, J. Bernheim, E. Klein, et al. 2009. Young-age onset of colorectal cancer in Israel. Tech. Coloproctol. 13:201–204.
16. Lieu, C. H., L. A. Renfro, A. de Gramont, J. P. Meyers, T. S. Maughan, M. T. Seymour, et al. 2014. Association of age with survival in patients with metastatic colorectal cancer: analysis from the ARCAD Clinical Trials Program. J. Clin. Oncol. 32:2975–2984.
17. Cusack, J. C., G. G. Giacco, K. Cleary, B. S. Davidson, F. Izzo, J. Skibber, et al. 1996. Survival factors in 186 patients younger than 40 years old with colorectal adenocarcinoma. J. Am. Coll. Surg. 183:105–112.
18. Schellerer, V. S., S. Merkel, S. C. Schumann, A. Schlabrakowski, T. Foertsch, C. Schildberg, et al. 2012. Despite aggressive histopathology survival is not impaired in young patients with colorectal cancer: CRC in patients under 50 years of age. Int. J. Colorectal Dis. 27:71–79.
19. McMillan, D. C., and C. S. McArdle. 2009. The impact of young age on cancer-specific and non-cancer-related survival after surgery for colorectal cancer: 10-year follow-up. Br. J. Cancer 101:557–560.
20. Li, Q., G. Cai, D. Li, Y. Wang, C. Zhuo, and S. Cai. 2014. Better long-term survival in young patients with non-metastatic colorectal cancer after surgery, an analysis of 69,835 patients in SEER database. PLoS ONE 9:e93756.

PLOS ONE

RESEARCH ARTICLE

A relationship to survival is seen by combining the factors of mismatch repair status, tumor location and age of onset in colorectal cancer patients

Pan Li[1], Zhitao Xiao[2], Todd A. Braciak[1], Qingjian Ou[2], Gong Chen[2]*, Fuat S. Oduncu[1]*

1 Department of Hematology and Oncology, Medizinische Klinik und Poliklinik IV, Ludwig Maximilians University, Munich, Germany, **2** Department of Colorectal Surgery, State Key Laboratory of Oncology in South China, Sun Yat-Sen University Cancer Center, Guangzhou, China

* chengong@sysucc.org.cn (GC); fuat.oduncu@med.uni-muenchen.de (FSO)

Citation: Li P, Xiao Z, Braciak TA, Ou Q, Chen G, Oduncu FS (2017) A relationship to survival is seen by combining the factors of mismatch repair status, tumor location and age of onset in colorectal cancer patients. PLoS ONE 12(3): e0172799. doi:10.1371/journal.pone.0172799

Editor: William B. Coleman, University of North Carolina at Chapel Hill School of Medicine, UNITED STATES

Received: September 5, 2016

Accepted: January 16, 2017

Published: March 2, 2017

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Funding: The authors received no specific funding for this work.

Competing interests: The authors have declared that no competing interests exist.

Abstract

Background

The progression of colorectal cancer (CRC) may differ depending on the location of the tumor and the age of onset of the disease. Previous studies also suggested that the molecular basis of CRC varies with tumor location, which could affect the clinical management of patients. Therefore, we performed survival analysis looking at different age groups and mismatch repair status (MMR) of CRC patients according to primary tumor location in an attempt to identify subgroups of CRC that might help in the prognosis of disease.

Methods

A group of 2233 patients operated on to remove their CRC tumors were analyzed (521 with right colon cancer, 740 with left colon cancer and 972 with rectal cancer). The expression of four MMR genes was assessed by immunohistochemistry (IHC), independent of clinical criteria. From the data collected, a predictive model for overall survival (OS) could be constructed for some associations of tumor location and age of onset using Kaplan-Meier, logistic and Cox regression analysis.

Results

When tumor location was considered as the lone factor, we found no statistical difference in overall survival (OS) between right cancer (68%), left cancer (67%) or rectal cancer tumor locations (71%) (HR: 1.17, 95%CI (confidence interval): 0.97–1.43, $P = 0.057$). When age of onset was considered, middle age (40–59 years) and older (60–85 years) patients were found to have higher OS than younger onset cancer (20–39 years) patients (69% vs 71% vs 59%, HR: 1.07, 95% confidence interval (CI): 0.91–1.25, $P = 0.008$). When both age of onset and tumor location were considered in combination as disease factors, we found that the subgroup of patients with left colon cancer from middle age (69%) and older (67%) aged patients had higher OS than younger (54%) patients (HR: 0.89, 95%CI: 0.68–1.16, $P =$

0.048). However in patients with right colon cancers, we found no statistical difference is OS between younger, middle age or older grouped patients (60% vs 71% vs 67%, HR: 0.84, 95% CI: 0.61–1.16, $P = 0.194$). With regard to rectal located cancers, we found that younger (62%) and middle age (68) patients had lower OS than older (77%) patients (HR:1.46, 95% CI: 1.13–1.88, $P = 0.004$). The rates of deficient MMR (dMMR) was 10.4%. We found no statistical difference in OS stratified by tumor locations. However, right colon cancer patients with dMMR (86%) had higher OS than those with proficient MMR (pMMR) (63%) (HR: 3.01, 95% CI: 1.82–4.97, $P<0.001$). Left colon cancer patients with dMMR (76%) also had higher OS than those with pMMR (66%) (HR: 1.67, 95% CI: 0.95–2.92, $P = 0.01$). Oppositely, rectal cancer patients with dMMR (60%) had lower OS than those pMMR (68%) (HR: 0.77, 95% CI: 0.51–1.17, $P = 0.04$).

Conclusions

These data demonstrate that primary tumor location can be an important factor when considered along with age of onset for the prognosis of CRC. Primary tumor location is also an important factor to evaluate the predictive effect of MMR status for the prognosis of CRC.

Introduction

It is estimated that colorectal cancer causes over 600,000 deaths worldwide annually, which makes this disease the fourth most common cause of cancer-related death[1,2]. Tumor location is now being considered to be an important factor for pathogenesis of CRC. Currently, noteworthy correlations between certain biological, clinical features and the anatomic subsites of the tumor have been made. Right colon cancers are more likely to be characterized by mucinous histology, high microsatellite instability, wild type p53 and BRAF mutation[3,4,5]. In contrast, left colon cancers are frequently found to be infiltrating, constricting lesions, with a phenotype that involves chromosomal instability[4]. While rectal located cancers are known to be more strongly associated with the presence of isolated pulmonary metastases than colon cancer [6]. While approximately 50% of the CRC occur in rectosigmoid area, a shift in location towards the proximal colon during the past few decades has been noted. The impact of this shift in CRC tumor location with regard to prognosis has not yet clearly been defined.

Another factor that has been considered that might have prognostic impact is the age of disease onset. It is known that the incidence of CRC increases markedly with advancing age. However, the overall notion that age is a significant prognostic factor in CRC has been controversial. Various studies have reported poorer prognosis among young patients with CRC[7]. While other reports have demonstrated that younger patients with CRC surgically treated to remove primary tumors appeared to have a higher specific survival rate than elderly patients [8,9].

CRCs with dMMR have distinct clinical and pathological features that commonly include proximal colon predominance, poor differentiation and increased numbers of tumor-infiltrating lynphonotes compared with CRCs with pMMR[10]. Most prior studies have suggested that right colon cancers are associated with higher mortality than left colon and rectally located cancers[11]. This finding would appear to be inconsistent with observations that right colon cancers are more likely to be dMMR[12]. To date, only a limited number of prospective studies have looked for a relationship between MMR status and prognosis of CRC by subsite across

the colorectum[13,14]. Although many of these previous studies had large study populations, many case groups became small after stratification by MMR status and tumor site were used for analysis. While the dMMR phenotype has been reported to be up to 10-fold higher in frequency as seen in sporadic proximal compared to left tumors[15], there remains a need to better understand the epidemiologic and clinical profile of MMR status between right and left colon or rectally located cancers. Currently, only a limited number of prospective studies have evaluated the relation between tumor location and age of onset with regard to prognosis of CRC [16] [17].

To date, there has been no systematical analysis looking at the combined influence of MMR status, location and age of disease onset with regard to the prognosis of CRC. Therefore, we analyzed the relationship of CRC overall survival, MMR status and the age of onset stratified by tumor location using a large number of Chinese CRC patients whose disease outcomes were established.

Methods

Patients

The ethics committee of Sun Yat-Sen University Cancer Center approved this study and the written informed consents for all patients were obtained at the beginning of the study. A total of 4500 histologically confirmed patients with CRC were recruited after operation from Sun Yat-Sen University cancer center between May 2011 and May 2016. All patients were of Chinese origin. The clinical and family history for each of these patients was reviewed. Finally, 2233 cases were selected for analysis after application of the following strict exclusion criteria: patients age less than 18 years and older than 85 years, severe complication, multi-primary cancer, synchronous and metachronous CRC, family history (first-degree and second-degree relatives had any kind of cancer), familial adenomatous polyposis, death not due to tumor-related reason were not included in the study. The primary tumor location was categorized as right colon if the tumor was located above the splenic flexure or left colon if it was located at or below the splenic flexure and rectum. The median follow-up on living patients was 4.3 years.

Treatment and follow-up

Stage I (T1–2 N0) and stage II (T3–4 N0) CRC patients without high-risk clinical features (e.g. T4 stage, bowel perforation or clinical bowel obstruction, inadequate lymph node sampling, poorly differentiated histology) were treated with radical surgery or endoscopic removal of the tumor alone. Stage II (T3–4 N0) CRC patients with high-risk clinical features were recommended to follow the XELODA/mFOLFOX/XELOX treatment regimen. Stage III (Tx N1–2) patients were scheduled to receive radical surgery and 12 cycles of adjuvant mFOLFOX/XELOX regimen treatment within a 6-month period. All stage IV (Tx Nx M1) patients received either palliative surgery or radical surgery. The first-line treatment for Stage IV CRC was the mFOLFOX/FOLFIRI chemotherapy regimen. Eighty-nine patients with rectal cancer also received neo-chemoradiotherapy. Responses were evaluated in accordance with the RECIST guidelines. After surgery, tumor recurrence was monitored by physical examination, serum carcinoembryonic antigen (CEA) assay, and abdominal and thoracic imaging taken every 3–6 months for the first 3 years following initial therapy, then every 6 months for the following 2 years, and finally from an annual check up. The duration of the patient follow-up was defined as the time between surgery and disease recurrence, death or last hospital contact (scheduled follow-up or telephone contact). The cutoff date for this analysis was May 2016.

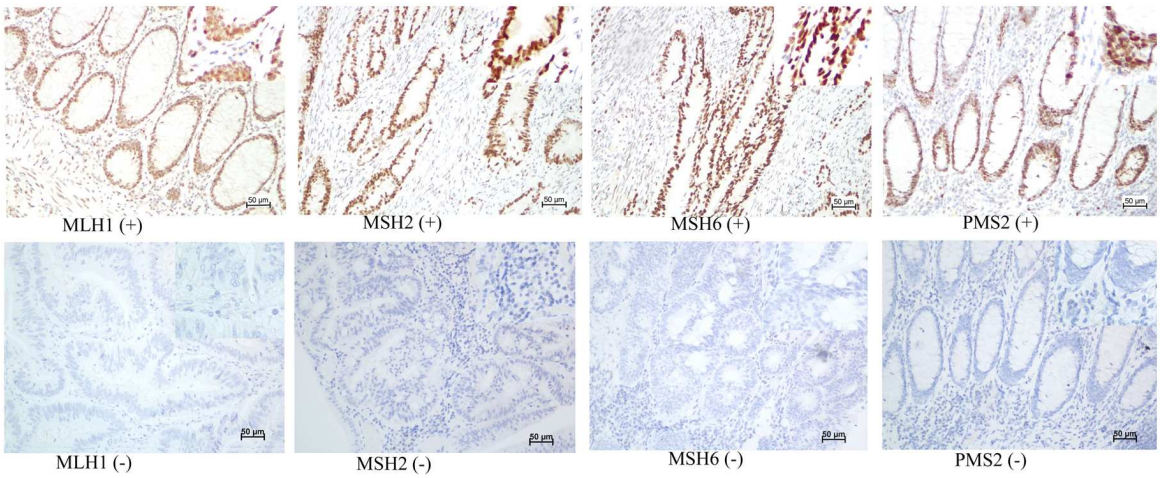

Fig 1. Illustrative immunostainings. Positive (upper panel) and negative (lower panel) for MLH1, MSH2, MSH6 and PMS2.

doi:10.1371/journal.pone.0172799.g001

Immunohistochemistry

Blocks of formalin-fixed, paraffin-embedded adenocarcinoma tissue comprising an area of normal colorectal mucosa adjacent to the tumor were selected in each case. Cases with complete nuclear loss of expression in invasive tumor cells with retained expression in inflammatory cells and/or adjacent normal tissue as positive controls were considered MMR deficient. Staining was performed using the following primary antibodies: mouse anti-human mutL homolog 1 (MLH1) (dilution 1:150, clone OTI1C1, zhongshan jiqiao, Beijing), rabbit anti-human mutS homolog2 (MSH2) (dilution 1:100, clone ZA0622, zhongshan jiqiao, Beijing, mouse anti-human mutS homolog 6 (MSH6) (dilution 1:150, clone OTI5D1, zhongshan jiqiao, Beijing), and mouse anti-human postmeiotic segregation increased 2 (PMS2) (dilution 1:150, clone OTI2G5, zhongshan jiqiao, Beijing). Whole tissue sections were read separately by two pathologists blinded to the patients' clinical characteristics. Discordant cases were reviewed by a supplementary pathologist to reach a consensus. Illustrative immunostainings are shown in Fig 1.

Statistic analysis

Patient data were described as frequencies (percentages) in our analysis. Differences in distributions between the variables examined were assessed with the X^2 or the Fisher's exact test. The primary end point for this study was OS, defined as the time elapsed from the date of surgery until tumor-induced death. Surviving patients were censored on the last follow-up date. Median follow-up and the 95% CI were calculated using the reverse Kaplan–Meier method. The survival curve was estimated with the Kaplan–Meier method and compared using the log-rank test. Univariate and multivariable Cox proportional hazards models were used to explore the association of age, location, stage, differentiation grade and gender. The score and likelihood ratio test *P* values were used to test the statistical significance of each covariate in the univariate and multivariable Cox models, respectively. All statistical tests were two-sided, and *P* values less than or equal to 0.05 were considered statistically significant. Statistical analyses were performed using SPSS software.

Results

The examined patient population included 521 (23.3%) right colon cancers, 740 (33.1%) left colon cancers and 972 (43.5%) rectal located cancers. A total of 2001 (89.6%) CRC specimens

Table 1. Clinicopathological characteristics of patients.

Characteristic	Locations (n/%)			*P* value
	Right colon (521/23.3)	Left colon (740/33.1)	Rectum (972/43.5)	
Gender				0.164
Male	298 (13.3)	457 (20.5)	561 (25.1)	
Female	223 (10.0)	283 (12.7)	411 (18.4)	
Age				0.286
20–39 years	52 (2.3)	59 (2.6)	77 (3.4)	
40–59 years	243 (10.9)	323 (14.5)	458 (20.5)	
60–85 years	226 (10.1)	358 (16.0)	437 (19.6)	
Pathology				<0.001
G1	40 (1.8)	27 (1.2)	37 (1.7)	
G2	426 (19.1)	675 (30.2)	901 (40.3)	
G3	5 (0.2)	4 (0.2)	9 (0.4)	
Mucinous	45 (2.0)	27 (1.2)	23 (1.0)	
Signet-ring	5 (0.2)	7 (0.3)	2 (0.1)	
Stage				<0.001
I	27 (1.2)	74 (3.3)	230 (10.3)	
IIA	195 (8.7)	230 (10.3)	185 (8.3)	
IIB	23 (1.0)	36 (1.6)	166 (7.4)	
IIC	14 (0.6)	14 (0.6)	14 (0.6)	
IIIA	7 (0.3)	16 (0.7)	32 (1.4)	
IIIB	119 (5.3)	165 (7.4)	168 (7.5)	
IIIC	25 (1.1)	33 (1.5)	34 (1.5)	
IVA	63 (2.8)	98 (4.4)	104 (4.7)	
IVB	48 (2.1)	74 (3.3)	39 (1.7)	
MMR status				
dMMR	117 (5.2)	55 (2.5)	60 (2.7)	<0.001
pMMR	404 (18.1)	685 (30.7)	912 (40.8)	
Alive				0.057
Yes	355 (15.9)	494 (22.1)	695 (31.1)	
No	166 (7.4)	246 (11.0)	277 (12.4)	

doi:10.1371/journal.pone.0172799.t001

showed retained expression of MLH1, MSH2, MSH6 and PMS2 in tumor cells. In comparison, loss of expression in at least one of the four MMR genes occurred in only 232 of 2233 patients analyzed (10.4%). The results for demographics and tumor characteristics by location are listed in Table 1.

In general, we found that colon cancers (77.1%) were more likely to be mucinous or signet-ring phenotype tumors than rectal cancers (22.9%) ($P<0.001$). The OS between the four stages of CRC showed significant differences (stage I: 94%, stage II: 83%, stage III: 70% and stage IV: 18%, HR 0.93, 95%CI: 0.65–2.38, $P<0.001$). With regard to tumor location alone, we found no statistical difference in OS between right (68%), left (67%) or rectal located cancers (71%) (HR: 1.17, 95%CI: 0.97–1.43, $P = 0.057$).

When age of disease onset was considered as a prognostic factor alone, we found that middle age (40–59 years) and older (60–85 years) patients had a statistically significant higher OS than younger (20–39 years) patients (69% vs 71% vs 59%, HR: 1.07, 95% CI: 0.91–1.25, $P = 0.008$). Additional stratification in patient prognosis profiling could be evidenced when age of disease onset was considered along with tumor location. When these factors were analyzed together, we found that left colon located cancers for both middle age (69%) and older

(67%) patients had a statistically significant higher OS than that for younger (54%) patients (HR: 0.89, 95%CI: 0.68–1.16, $P = 0.048$). However this effect was not seen for right colon located cancers, in this case we found no statistical difference in OS between younger, middle age or older patients (60% vs 71% vs 67%, HR: 0.84, 95% CI: 0.61–1.16, $P = 0.194$). An effect was seen in rectal located cancers when age and tumor location were considered. Here, a statistically significant difference in survival was found for both younger (62%) and middle aged (68%) patients that had a lower OS than older (77%) patients (HR: 1.46, 95%CI: 1.13–1.88, $P = 0.004$).

When dMMR status was considered with location, right colon cancer patients with dMMR (86%) had higher OS than those patients with pMMR (63%) (HR: 3.01, 95% CI: 1.82–4.97, $P<0.001$). Likewise, left colon cancer patients with dMMR (76%) also had higher OS than those with pMMR (66%) (HR: 1.67, 95% CI: 0.95–2.92, $P = 0.01$). Oppositely, we found rectal cancer patients with dMMR (60%) had lower OS than those with pMMR (68%) (HR: 0.77, 95% CI: 0.51–1.17, $P = 0.04$).

Among the variables analyzed in the multivariate Cox model, stage (HR 3.86, 95%CI: 3.69–4.01, $P = 0.001$), pathological differentiation (HR 2.14, 95%CI: 2.07–2.19, $P<0.001$) and MMR status (HR 1.23, 95%CI: 1.19–1.26, $P<0.001$) were significantly associated with tumor location. However, gender (HR 1.39, 95%CI: 1.35–1.42, $P = 0.164$) and the age of patients (HR 2.38, 95%CI: 2.33–2.42, $P = 0.286$) showed no statistical significance with tumor location. The survival plots of tumor location and age are shown in Fig 2.

Discussion

Colorectal cancer has reported high morbidity and mortality rates worldwide, and according to the GLOBOCAN report from 2008, an estimated 1.2 million people suffered from CRC, accounting for approximately 10% of all cancer patients[18].

Various risk factors for CRC have been identified, and these form the basis for screening recommendations to be provided for patients. CRC, like other types of cancer, demonstrates an increasing incidence with age. In this current study, middle aged and older classified patients accounted for 45.9 and 45.7 percent of the CRC patient population studied with younger patients only accounting for the remaining 8.4% of the CRC cases. This patient distribution for CRC we found in our study was concordant with the patient breakdown seen in previous study[19].

The notion that age is a significant prognostic factor in CRC has previously been suggested but this conclusion has remained somewhat controversial. Previous studies have reported poorer prognosis among younger patients with CRC[7]. While others have reported that younger patients with CRC among those surgically treated appeared to have a higher specific survival rate versus more elderly patients [8,9]. In our report here on Chinese CRC patients, we found middle and older aged patients had higher OS than younger patients ($P = 0.008$). Our observation that cancer related mortality did not decrease with increasing age after those reaching 50 years old exemplifies the idea that, although elderly patients have a shorter life expectancy based on their age, still benefited from cancer treatment.

Another factor that we wanted to test that might be useful as a prognostic factor in CRC is the location of the primary CRC tumor. Different locations within the colon are derived from different embryonic tissues. The right colon (cecum, ascending colon, and proximal two thirds of the transverse colon) is derived from the embryonic midgut, whereas the left colon (distal one third of the transverse colon, descending colon, sigmoid colon, and rectum) is derived from the embryonic hindgut[20]. It is likely that these different origins of colon tissue could contribute to CRC disease pathology by independent processes. Indeed, there is already

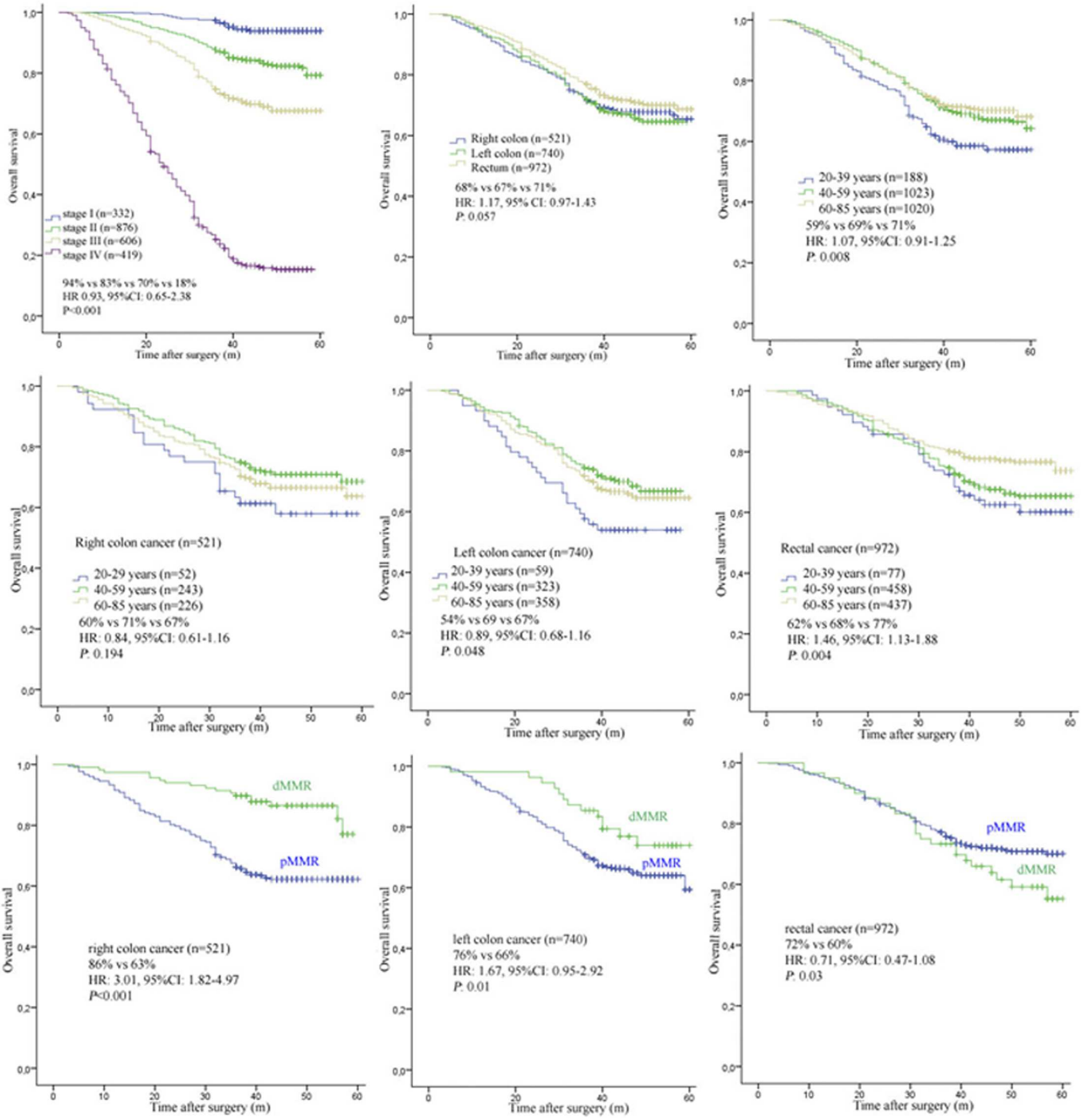

Fig 2. OS according to the MMR status, tumor location and age.

doi:10.1371/journal.pone.0172799.g002

evidence to indicate that the clinical characteristics and molecular profiles of gene and protein expression for CRC differ across these different tissue sites[21,22,23,24]. One previous study has already shown that the primary tumor location could serve as prognostic factor in metastatic CRC[25]. Other studies reported about some correlation between patients' sex and

tumor location [26,27]. However, in this study we found no statistical association between sex and tumor location. In this study, we found no statistical difference in OS between right cancer, left cancer and rectal cancer ($P = 0.057$). It is not yet clear why we did not find a similar result when analyzing tumor location alone independent of age of disease onset. Yet, when tumor location was considered along with age of onset, we could find statistically significant associations for tumor location to disease survival.

Tumor location and age of onset are two basic clinical characteristics of CRC. To date, only a limited number of prospective studies have evaluated the relation between tumor location and age of onset to the prognosis of CRC. It has been previously reported that right-sided early-onset colon cancers were a subset in which most Lynch Syndrome cases could be found, with earlier stages of disease at diagnosis having better prognosis[16]. In another study, the Bax/Bcl-2 ratio was statistically correlated for CRC against age and tumor location. Here, patients older than 50 showed decreased levels of Bax/Bcl-2 ratio. Moreover, with regard to tumor location, the Bax/Bcl-2 ratio was found to be significantly lower in colon compared to rectal cancer[17]. However, one potential caveat to these findings is that both studies only focused on a select age group of cancer patients.

In our current study, we found two possibly important associations for tumor location and age of disease onset to OS of CRC patients. First in left colon located cancers, middle (69%) and older aged (67%) patients were found to have higher OS than younger (54%) patients ($P = 0.048$). Second in rectal located cancers, we found the younger (62%) and middle aged (68) patients had lower OS than older (77%) patients ($P = 0.004$). According to the well-accepted model of CRC progression by Vogelstein, malignancy arises with aging as a result of accumulation of mutations in tumor suppressor genes and oncogenes [28,29]. Because we have noticed differences in OS with regard to tumor location and not necessarily due to age, our results suggest that another factor in disease could be from accumulation effect of mutations that differ in various tissue locations. Recently, it was reported that human intestinal microbiota and bacterial metabolites contributed to the aetiology of CRC[30]. It is likely that possible mechanisms are involved and gaining understanding of these processes will aid in the prognosis of CRC patients.

From our analysis we were able to find that more frequent occurrences of mucinous, high-grade and signet-ring phenotype in right colon cancers (9.6%) compared with left colon cancer (4.6%) and rectal cancer (2.6%). It is of note that right lesions have more frequently been reported to be related to dMMR status tumors. One explanation for this finding is that the right and left colon arise from different embryological sources [31].

In clinical practice, the tumor's MMR status is increasingly being used to guide clinical management. Stage II patients with dMMR have a better prognosis and may actually be harmed by 5-FU treatment[32]. We found that patients with right tumors had no prognostic difference compared with patients who had left colon and rectal tumors, which contradicted to the previous findings of others[33,34]. Yet in our current study, we did find that dMMR status could show benefit as a prognostic biomarker for right and left colon cancer. As well, dMMR gave indications for bad prognosis of rectal cancers. Overall we found that the separate evaluation of tumor location appears to have some predictive and prognostic value when combined with MMR status for colon and rectal cancers.

Conclusions

We have found that the combination of age and tumor location can be a significant predictor of overall survival in CRC. This finding solidifies the notion that despite the morbidity and mortality associated with any CRC diagnosis, baseline patient characteristics might still

provide predictive information as to a given patient's outcome and also can help the clinician to predict the clinical course and response of chemotherapy. Our findings also confirm the importance of location of primary tumor and indicate that tumors resected from different locations of the colorectum have different dMMR status that can work as prognostic biomarkers in colorectal cancer.

The increasing trend in the CRC mortality indicate that improved primary and secondary prevention measures are particularly still needed and could be further aided by better prognostic methods being made available.

Author Contributions

Conceptualization: PL.

Data curation: PL ZTX.

Formal analysis: PL ZTX.

Investigation: PL ZTX.

Methodology: PL.

Project administration: GC FSO.

Resources: QJO.

Software: PL ZTX.

Supervision: GC FSO.

Validation: GC FSO.

Visualization: GC FSO.

Writing – original draft: PL.

Writing – review & editing: TAB.

References

1. Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. (2010) Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int J Cancer 127: 2893–2917. doi: 10.1002/ijc.25516 PMID: 21351269
2. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. (2011) Global cancer statistics. CA Cancer J Clin 61: 69–90. doi: 10.3322/caac.20107 PMID: 21296855
3. Tran B, Kopetz S, Tie J, Gibbs P, Jiang ZQ, Lieu CH, et al. (2011) Impact of BRAF mutation and microsatellite instability on the pattern of metastatic spread and prognosis in metastatic colorectal cancer. Cancer 117: 4623–4632. doi: 10.1002/cncr.26086 PMID: 21456008
4. Iacopetta B (2002) Are there two sides to colorectal cancer? Int J Cancer 101: 403–408. doi: 10.1002/ijc.10635 PMID: 12216066
5. Fukatsu H, Kato J, Nasu JI, Kawamoto H, Okada H, Yamamoto H, et al. (2007) Clinical characteristics of synchronous colorectal cancer are different according to tumour location. Dig Liver Dis 39: 40–46. doi: 10.1016/j.dld.2006.07.015 PMID: 16996329
6. Tan KK, Lopes GLJ, Sim R (2009) How uncommon are isolated lung metastases in colorectal cancer? A review from database of 754 patients over 4 years. J Gastrointest Surg 13: 642–648. doi: 10.1007/s11605-008-0757-7 PMID: 19082673
7. Cusack JC, Giacco GG, Cleary K, Davidson BS, Izzo F, Skibber J, et al. (1996) Survival factors in 186 patients younger than 40 years old with colorectal adenocarcinoma. J Am Coll Surg 183: 105–112. PMID: 8696540
8. Li M, Li JY, Zhao AL, Gu J (2011) Do young patients with colorectal cancer have a poorer prognosis than old patients? J Surg Res 167: 231–236. doi: 10.1016/j.jss.2010.10.040 PMID: 21316708

9. Schellerer VS, Merkel S, Schumann SC, Schlabrakowski A, Fortsch T, Schildberg C, et al. (2012) Despite aggressive histopathology survival is not impaired in young patients with colorectal cancer: CRC in patients under 50 years of age. Int J Colorectal Dis 27: 71–79. doi: 10.1007/s00384-011-1291-8 PMID: 21881876
10. Jass JR, Do KA, Simms LA, Iino H, Wynter C, Pillay SP, et al. (1998) Morphology of sporadic colorectal cancer with DNA replication errors. Gut 42: 673–679. PMID: 9659163
11. Meguid RA, Slidell MB, Wolfgang CL, Chang DC, Ahuja N (2008) Is there a difference in survival between right- versus left-sided colon cancers? Ann Surg Oncol 15: 2388–2394. doi: 10.1245/s10434-008-0015-y PMID: 18622647
12. Minoo P, Zlobec I, Peterson M, Terracciano L, Lugli A (2010) Characterization of rectal, proximal and distal colon cancers based on clinicopathological, molecular and protein profiles. Int J Oncol 37: 707–718. PMID: 20664940
13. Sinicrope FA, Rego RL, Halling KC, Foster N, Sargent DJ, La Plant B, et al. (2006) Prognostic impact of microsatellite instability and DNA ploidy in human colon carcinoma patients. Gastroenterology 131: 729–737. doi: 10.1053/j.gastro.2006.06.005 PMID: 16952542
14. Sanchez JA, Krumroy L, Plummer S, Aung P, Merkulova A, Skacel M, et al. (2009) Genetic and epigenetic classifications define clinical phenotypes and determine patient outcomes in colorectal cancer. Br J Surg 96: 1196–1204. doi: 10.1002/bjs.6683 PMID: 19787768
15. Breivik J, Lothe RA, Meling GI, Rognum TO, Borresen-Dale AL, Gaudernack G. (1997) Different genetic pathways to proximal and distal colorectal cancer influenced by sex-related factors. Int J Cancer 74: 664–669. PMID: 9421366
16. Perea J, Cano JM, Rueda D, Garcia JL, Inglada L, Osorio I. (2015) Classifying early-onset colorectal cancer according to tumor location: new potential subcategories to explore. Am J Cancer Res 5: 2308–2313. PMID: 26328262
17. Khodapasand E, Jafarzadeh N, Farrokhi F, Kamalidehghan B, Houshmand M (2015) Is Bax/Bcl-2 ratio considered as a prognostic marker with age and tumor location in colorectal cancer? Iran Biomed J 19: 69–75. doi: 10.6091/ibj.1366.2015 PMID: 25864810
18. Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. (2010) Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int J Cancer 127: 2893–2917. doi: 10.1002/ijc.25516 PMID: 21351269
19. O'Connell JB, Maggard MA, Livingston EH, Yo CK (2004) Colorectal cancer in the young. Am J Surg 187: 343–348. doi: 10.1016/j.amjsurg.2003.12.020 PMID: 15006562
20. Glebov OK, Rodriguez LM, Nakahara K, Jenkins J, Cliatt J, Humbyrd CJ. (2003) Distinguishing right from left colon by the pattern of gene expression. Cancer Epidemiol Biomarkers Prev 12: 755–762. PMID: 12917207
21. Han NY, Kim MJ, Park BJ, Sung DJ (2014) Location of rectal cancer as determined using rectal magnetic resonance imaging, and its relationship with pulmonary metastasis. Turk J Gastroenterol 25: 661–668. doi: 10.5152/tjg.2014.5616 PMID: 25599778
22. Papagiorgis PC, Zizi AE, Tseleni S, Oikonomakis IN, Nikiteas NI (2012) The pattern of epidermal growth factor receptor variation with disease progression and aggressiveness in colorectal cancer depends on tumor location. Oncol Lett 3: 1129–1135. doi: 10.3892/ol.2012.621 PMID: 22783405
23. Boisen MK, Johansen JS, Dehlendorff C, Larsen JS, Osterlind K, Hansen J. (2013) Primary tumor location and bevacizumab effectiveness in patients with metastatic colorectal cancer. Ann Oncol 24: 2554–2559. doi: 10.1093/annonc/mdt253 PMID: 23864097
24. Andrieu N, Launoy G, Guillois R, Ory-Paoletti C, Gignoux M (2004) Estimation of the familial relative risk of cancer by site from a French population based family study on colorectal cancer (CCREF study). Gut 53: 1322–1328. doi: 10.1136/gut.2003.036376 PMID: 15306593
25. Loupakis F, Yang D, Yau L, Feng S, Cremolini C, Zhang W, et al. (2015) Primary tumor location as a prognostic factor in metastatic colorectal cancer. J Natl Cancer Inst 107.
26. Matanoski G, Tao XG, Almon L, Adade AA, Davies-Cole JO (2006) Demographics and tumor characteristics of colorectal cancers in the United States, 1998–2001. Cancer 107: 1112–1120. doi: 10.1002/cncr.22008 PMID: 16838314
27. Nawa T, Kato J, Kawamoto H, Okada H, Yamamoto H, Kohno H, et al. (2008) Differences between right- and left-sided colon cancer in patient characteristics, cancer morphology and histology. Journal of Gastroenterology and Hepatology 23: 418–423. doi: 10.1111/j.1440-1746.2007.04923.x PMID: 17532785
28. Fearon ER, Vogelstein B (1990) A genetic model for colorectal tumorigenesis. Cell 61: 759–767. PMID: 2188735

29. Vogelstein B, Fearon ER, Hamilton SR, Kern SE, Preisinger AC, Leppert M, et al. (1988) Genetic alterations during colorectal-tumor development. N Engl J Med 319: 525–532. doi: 10.1056/NEJM198809013190901 PMID: 2841597

30. Louis P, Hold GL, Flint HJ (2014) The gut microbiota, bacterial metabolites and colorectal cancer. Nat Rev Miscrobiol 10:661–72.

31. Iacopetta B (2002) Are there two sides to colorectal cancer? Int J Cancer 101: 403–408. doi: 10.1002/ijc.10635 PMID: 12216066

32. Boland CR, Goel A (2010) Microsatellite instability in colorectal cancer. Gastroenterology 138: 2073–2087. doi: 10.1053/j.gastro.2009.12.064 PMID: 20420947

33. Rees M, Tekkis PP, Welsh FK, O'Rourke T, John TG (2008) Evaluation of long-term survival after hepatic resection for metastatic colorectal cancer: a multifactorial model of 929 patients. Ann Surg 247: 125–135. doi: 10.1097/SLA.0b013e31815aa2c2 PMID: 18156932

34. O'Connell JB, Maggard MA, Ko CY (2004) Colon cancer survival rates with the new American Joint Committee on Cancer sixth edition staging. J Natl Cancer Inst 96: 1420–1425. doi: 10.1093/jnci/djh275 PMID: 15467030

Summary

In our large data, we accessed 2,233 colorectal cancer patients to analyze the Ki67 expression, MMR status, age of onset and tumor locations regarding overall survival of the CRCs. In the first part, we focused on the possibility of Ki67 as a prognostic biomarker for CRC. Our study has shown that stage III CRC patients with a high Ki67 expression had an improved overall survival compared to patients with a low Ki67 expression. However, for stage I and II CRCs we found no statistical difference between patients with high Ki67-Li and patients with low Ki67-Li. Remarkably, it is shown that stage IV CRC patients with low Ki67-Li had improved progression free survival (PFS). However, this benefit was not found in the overall survival (OS) for the same stage patients. In previous studies, the prognostic effect of Ki67-Li was shown in breast cancer and renal cell cancer. Our study provided further large-data evidence for Ki67 expression as a biomarker for CRCs management.

In the second part, we analyzed the association between age of tumor onset and MMR status in regard to CRC prognosis. In general, young patients had a decreased OS rate compared with middle-age and elderly patients. When stratified by MMR status, the young patients with dMMR status had better OS than young patients with pMMR status. The situation was similar for middle-age patients. However, the prognostic impact of MMR showed no statistical difference for elderly patients. This result demonstrated that the prognostic influence of MMR status may shift during different ages of disease onset.

In the third part, we analyze the relationship of age and tumor location and the association between MMR status and tumor location. In general, young patients with CRC had the worst prognosis compared with middle-age and elderly patients. However, the elderly rectal cancer patients had better prognosis compared with young and middle-age patients. When MMR status was considered, the colon cancer patients with dMMR had better prognosis than the patients with pMMR. However, concerning rectal cancer patients, the patients with pMMR had better prognosis than the patients with dMMR.

At present, the treatment of CRC is in a period of stagnation. More research is required combining the clinical factors and biological mechanisms to find further solutions.

Zusammenfassung

In unseren großen Datenbank haben wir auf 2.233 Patienten mit Kolorektalkarzinomen zugegriffen, um die Ki67-Expression, den MMR-Status, das Alter bei Krankheitsbeginn und die Tumorlokalisation in Bezug auf das Gesamtüberleben der CRCs zu analysieren. Im ersten Teil untersuchten wir die Möglichkeit von Ki67 als prognostischen Biomarker für CRC. Unsere Studie hat ergeben, dass Stadium III CRC Patienten mit einer hohen Ki67-Expression ein verbessertes Gesamtüberleben im Vergleich zu Patienten mit einer niedrigen Ki67-Expression hatten. Allerdings fanden wir für die Stadium I und II CRCs keinen statistischen Unterschied zwischen Patienten mit hohem Ki67-Li und Patienten mit niedrigem Ki67-Li. Überraschenderweise zeigte sich, dass Stadium IV CRC Patienten mit niedrigem Ki67-Li ein verbessertes progressionsfreies Überleben (PFS) hatten. Allerdings wurde dieser Benefit nicht im Gesamtüberleben (OS) für die Patienten im gleichen Stadium gefunden. In früheren Studien wurde die prognostische Wirkung von Ki67-Li bei Brustkarzinomen und Nierenzellkarzinomen gezeigt. Unsere Studie lieferte weitere Beisweise aus einer großen Datenbank für Ki67 als Biomarker für CRCs Management.

Im zweiten Teil analysierten wir den Zusammenhang zwischen dem Alter bei Krankheitsbeginn und dem MMR-Status in Bezug auf die CRC-Prognose. Im Allgemeinen hatten junge Patienten eine verminderte OS-Rate im Vergleich zu Patienten mittleren Alters und älteren Patienten. Wenn sie durch den MMR-Status geschichtet wurden, hatten die jungen Patienten mit dMMR-Status eine bessere Prognose als junge Patienten mit pMMR-Status. Die Situation war ähnlich für Patienten mittleren Alters. Allerdings zeigte die prognostische Wirkung von MMR keinen statistischen Unterschied bei älteren Patienten. Dieses Ergebnis zeigte, dass sich der prognostische Einfluss des MMR-Status je nach Alter bei Ausbruch der Erkrankung verschieben kann.

Im dritten Teil analysieren wir das Verhältnis von Alter und Tumorlokalisation und den Zusammenhang zwischen MMR-Status und Tumorlokalisation. Im Allgemeinen hatten junge Patienten mit CRC eine schlechte Prognose im Vergleich zu Patienten mittleren Alters und älteren Patienten. Allerdings hatten die älteren Patienten mit Rektalkarzinomen bessere Prognose im Vergleich zu jungen und Patienten mittleren Alters. Als der MMR-Status in Betracht gezogen wurde, hatten bei den Patienten mit Kolonkarzinomen diejenigen mit dMMR eine bessere Prognose als diejenigen mit pMMR. Bei den rektalen Krebspatienten hatten diejenigen mit pMMR jedoch eine bessere Prognose als diejenigen mit dMMR.

Gegenwärtig befindet sich die Behandlung von CRC in einer Zeit der Stagnation. Wir müssen mehr Forschungen machen, die die klinischen Faktoren und biologischen Mechanismen kombinieren, um weitere Behandlungsmöglichkeiten zu finden.

Acknowledgement

I would appreciate the MD study in LMU, which I will keep as one of the most important experience in my life.

First of all, I would like to express my great gratitude to my supervisor, Prof. Dr. Dr. Fuat Oduncu. He provided me with great working environment and always had patience for all scientifical problems.

Foremost, I would like to express my deeply gratitude to my advisor in China, Prof. Gong Chen. He provided full support to finish this cooperation.

My sincere thanks also go to Dr. Zhitao Xiao, who helpt me to collect data during his free time.

In particularly, I am sincerely grateful to Mr. Qingjian Ou, who gave me great encouragement and provided all necessary techinical supports.

I want to give especial thanks to Dr. Todd Braciak, who helped to improve my English expression and revise the manuscripts for me.

Sincerely thank all the colleagues in hamotology and oncology department. They gave me warm support for my daily work.

Last but important, I would thank my family for the endless love and encouragement.