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PLA2G7/PAF-AH as a protective factor of *BRCA1* mutant breast cancer and ovarian cancer and negatively regulate the Wnt signaling pathway

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Contents

1. Abbreviations

ADP	adenosine diphosphate
AKT	protein kinase B
APC	adenomatous polyposis coli
AXIN	axin inhibitor
BRCA	breast cancer gene
BrDU	5-bromo-2-deoxyuridine
Cc	correlation coefficient
CI	confidence interval
CK1-alpha	casein kinase-1-α
DNA	deoxyribonucleic acid
ELISA	enzyme-linked immunosorbent assay
EOC	epithelial ovarian cancer
FBS	fetal bovine serum
FIGO	International Federation of Gynecology and Obstetrics
GAPDH	glycerinaldehyd-3-phosphat-dehydrogenase
GSK3β	glycogen synthase kinase-3β
ICC	immunocytochemistry
IHC	immunohistochemistry
IRScore	immunoreactive score
LEF	lymphatic enhancer factor
LRP5	lipoprotein receptor related protein 5
LRP6	lipoprotein receptor related protein 6
MMP	matrix metalloproteinase
mRNA	messenger ribonucleic acid
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
OS	overall survival
TCF	T cell factor
PAF	platelet activating factor
PBS	phosphate buffered saline

PI3K	phosphatidylinositol 3-kinase
PLA2G7/PAF-AH	platelet activating factor acetylhydrolase
PTAFR	platelet activating factor receptor
qPCR	quantitative polymerase chain reaction
Rac-1	ras-related C3 botulinum toxin substrate 1
Rho-A	ras homolog family member A
RIPA	radioimmunoprecipitation assay
ROC	receiver operating characteristic
RT	room temperature
siRNA	small interfering ribonucleic acid
Src/FAK	steroid receptor coactivator/focal adhesion kinase
STAT	signal transducer and activator of transcription
Tcf/Lef	transcription factor/ lymphoid enhancer-binding factor
TLE	transducin-like enhancer
WHO	world health organization

2 Introduction

2.1 Overview of the epidemiology and clinical features of breast and ovarian cancer

Breast cancer and ovarian cancer attract much attention in the research of female tumors due to their high incidence. With the continuous progress of research and clinical treatments in recent years, the survival rates of breast cancer and ovarian cancer have been improved. But they are still malignant tumors with a long incubation period, unobvious for the early-stage clinical symptoms, and the mortality rates are comparatively high. Studies have shown that 14 different types of cancers account for about 80% of all cancers[1]. These 14 high-incidence cancers include breast cancer and ovarian cancer.

2.1.1 Epidemiology and clinical features of breast cancer

Currently, cancer with the highest incidence among women is breast cancer. There are over one million breast cancer new cases worldwide every year. It is also the most common disease that causes women to die of cancer globally [2]. Generally, the long-term prognosis of advanced breast cancer is better than ovarian cancer [3]. However, the incidence rate is higher than ovarian cancer, and due to the limitations of screening methods, the survival rate of advanced breast cancer is still low[4]. The classic factors that guide oncologists to judge the prognosis of breast cancer are such as histological type, patient age, tumor size and tumor grade, axillary lymph node metastasis, etc. [5-8]. There are many new biomarkers have also been discovered in recent years. These molecules not only improve the ability to predict prognosis but also help to identify specific types of "high-risk" patients.

2.1.2 Epidemiology and clinical features of ovarian cancer

According to available statistics, ovarian cancer ranks in the top five diseases among European women leading to cancer deaths [9]. As the effective methods of early diagnosis at EOC are limited, the patient's relevant 5-year survival rate is less than 45% [10]. Some of the prognostic markers are widely used, such as FIGO (the disease stage at diagnosis), grading, high-volume ascites, older age, residual disease volume after surgery, and histological markers [11-13]. But, due to the difficulty and complexity of invasive tissue biopsy, there are rare non-invasive

prognostic biomarkers that are widely accepted.

2.1.3 The effect of gene mutation on breast and ovarian cancer, especially on HBOC

Including breast cancer and ovarian cancer, the vast majority cancers appear sporadic, while hereditary accounts for only about 5-10% of breast cancers and nearly 10% with ovarian cancers. These inherited breast and ovarian cancers with genetic characteristics are named HBOC [14-16]. About 60-80% of *BRCA* gene mutation carriers are at risk of suferring breast cancer, and at the ovarian cancer the risk is around 15-40%. *BRCA* gene mutations also increase the risk of other types of cancer [17-19]. Therefore, to identify high-risk groups for HBOC, screening the genes of *BRCA 1* and *BRCA 2* can be currently the most valid solutions for individuals. In clinical trials for healthy people with *BRCA* mutations, various preventive measures are proposed to reduce cancer risks. Such as regular intensive screenings, supply medical consultation and necessary *BRCA* screening for first-degree relatives of *BRCA* gene carriers, as well as breast reconstruction after prophylactic mastectomy, and/or oophorectomy or chemoprophylaxis for some individuals who are assessed with high-risk [20, 21].

2.2 *BRCA1* gene and its function

2.2.1 DNA repair and *BRCA1* related repair mechanisms

The first gene to be identified as a breast cancer susceptibility gene is *BRCA1*. Nearly 5-10% of overall breast cancer and 20-25% of hereditary background breast cancers are related to *BRCA1* gene [22, 23]. Previous studies have shown that *BRCA1* mutations will elevate risk to suffer from breast and ovarian cancer [24]. *BRCA1* gene on human chromosome 17q21 and the *BRCA2* gene on chromosome 13q12 are considered two important genes for both breast and ovarian cancer susceptibility [25, 26]. *BRCA1* and *BRCA2* encode proteins take part in the DNA double-strand (DBS) repair mechanisms [27-29].

The research has revealed that approximately 70,000 lesions happen to each human cell every day [30]. For both endogenous and exogenous DNA damage, approximately 75% of lesions are single-strand DNA (ssDNA) breaks, which are caused by oxidative damage during metabolism

or base hydrolysis. The ssDNA breaks can also be transformed into a more dangerous type, that is DNA double-strand breaks (DSBs). During evolution process, cells must obtain enzymes that repair DNA damage to restore the integrity of the genome [30, 31]. To maintain counteract DNA damages and genome integrity at high level, homologous recombination(HR) and non-homologous end-joining(NHEJ)are revealed for two principal pathways to repair DSBs [25, 32-34]. During homologous recombination (HR), the DSB is repaired by exchanging equivalent regions of DNA between homologous or sister chromosomes, whereas nonhomologous end-joining (NHEJ) relegates the ends without using a template[35]. A subset of DSBs are repaired during the S and G2 phases of cellcycle through HR [36]. In the HR repair process, the damaged DNA end is cut to expose a single-stranded overhang, allowing *BRCA1* (through the nucleation of Rad51 filaments) to search and capture this overhang by homology, and then complete the repair by performing templated synthesis. The DSB localization of the 53BP1 and *BRCA1* protein are negatively correlated, the level of *BRCA1* at double-strand break is significantly increased in 53BP1-deficient cells [37, 38]. NHEJ could be active in all cell stages but are usually dominant in G0 / G1 and G2[39]. *BRCA1* directly participates in HR, and more evidence supports the role of *BRCA1* also involved in the NHEJ repair system [40, 41]. Moreover, several studies have proved that BRCA1 interacts with enzymes capable of DNA structure and chromatin modification [42, 43].

2.2.2 Double-edged sword effect of *BRCA1* deficiency

BRCA1 is a vital protein which involved in DNA damage repairing process, for instance ubiquitination, DNA damage repair, transcriptional regulation, as well as the checkpoints of cell cycle [44-46]. Researches have shown the *BRCA1* deficiency leads to defects in repairment of DNA damage, aberrant centrosome replication, G2-M cell cycle checkpoint defects, increased apoptosis, genetic instability, growth retardation, and tumorigenesis [47-49]. An experiment in mouse model has proved that whole body knockout of the *BRCA1* gene (*BRCA1*-/-) leads to an increase in lethality on embryonic Day 7-8 [50]. In contrast, the exon 11 of *BRCA1* of a breast-specific deletion leads to breast tumor formation, combined with numerous genomic changes and cell lethality [51]. Thus, the deficient of *BRCA1* could be recognized as a double-edged sword. The massive tumor suppressor and tumorigenesis factors are dysregulated because of genome instability, which leads to tumorigenesis. However, the excessive damage of DNA could also trigger the lethal blockade by inducing apoptosis.

2.3 PAF-AH (Platelet-Activating Factor Acetylhydrolase) and its gene PLA2G7

2.3.1 PAF-AH and PLA2G7

Platelet-Activating Factor Acetylhydrolase (PAF-AH) is an enzyme involved in various biological effects that widely exist in many cells and tissues[52-54]. Currently, there are three kinds of PAF-AH the endocellular types I, II, and the plasma PAF-AH are revealed in mammals [55-57](Figure 1). The regulatory β subunit and the catalytic subunits alpha1 and alpha2 form the type I PAF-AH, which is announced to be a G protein-like complex. One allele of LIS1 gene disrupted can cause the lissencephaly [58-60]. Protein level in this locus is crucial, as no matter decreased and increased of LIS1 levels would affect brain development both in humans and in mice [60-63]. The protein primary structure of subunits in PAF-AH I exhibit low homology in plasma PAF-AH [64]. PAF-AH II is an enzyme usually distribute in cell. Its molecular weight is 40kDa, which contains 41% sequence identity with the plasma type [65]. And molecular weight of plasma-PAF-AH is 45kDa, with a monomeric polypeptide structure. In terms of function, these three homo-isomerases also show interesting intersections and differences. Biochemical analyses revealed that compared with PAF-AH II and plasma type, PAF-AH Ib proves higher specificity on glycerol phosphate for the sn-2 acetyl [65]. PAF-AH II and Plasma type preferentially hydrolyzing sn-2 butyryl and propionyl parts which are PAF analogues, while these phospholipids almost couldn`t be hydrolyzed by type Ib. Plasma and type II PAF-AH, both may take part in diverse pathological processes and be an important factor in oxidized phospholipids as a scavenger [55, 66]. Plasma PAF-AH usually closely combined to the HDL or LDL [57]. Although PAF-AH isoforms show low sequence homology, they also have the same functions in PAF catabolism and oxidative fragmentation of phospholipids [55, 67].

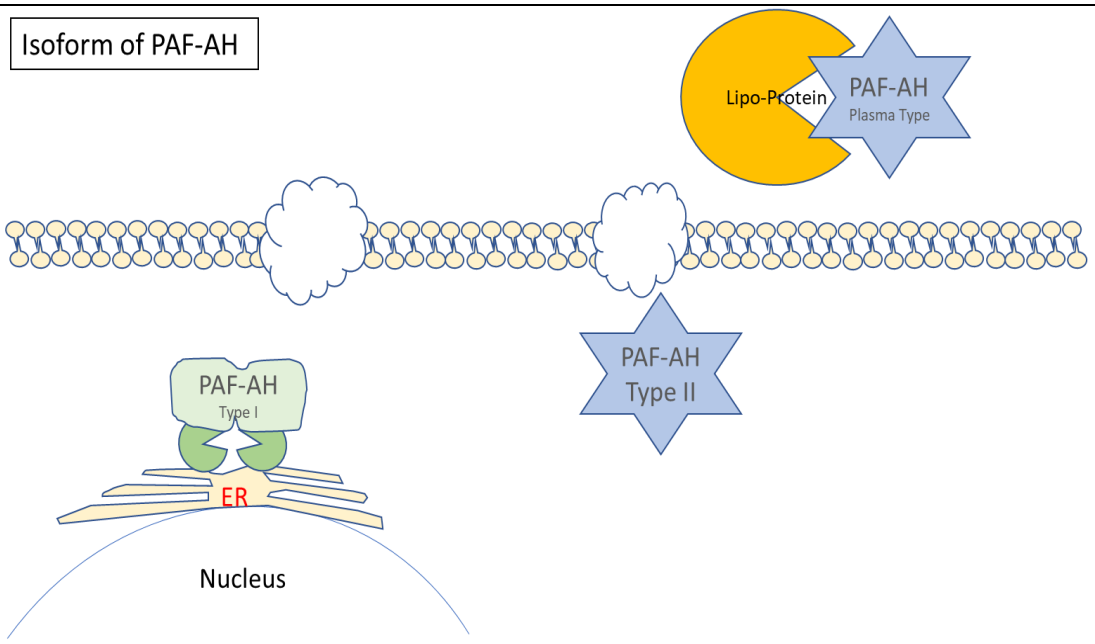


Figure 1: Three subtypes exist in mammals, the plasma PAF-AH and two intracellular types I and II. Plasma type also known as Lp-PLA2

It is reported 4% people from Japan have gene mutation in plasma PAF-AH and certain inflammatory-disorders associated with defective hereditary plasma PAF-AH [68, 69]. Some intracellular and extracellular enzymes also overlap with activity of PAF-AH. This also explains that vast majority of individuals can still maintain health when there are many defects caused by this mutation. These overlapping enzymes compensate for the serious clinical problems caused by the plasma PAF-AH deficiency.

Plasma PAF-AH which called Lp-PLA2 (Lipoprotein Associated Phospholipase A2) in the early days. It owns a motif called Gly-X-Ser-X-Gly which is found in some members of the α/β hydrolase superfamily, esterases, as well as lipases [70, 71]. The canonical α/β serine hydrolase folding is an important unit of human plasma PAF-AH, which contains catalytic triad with His351, Asp296, and Ser273 [72, 73]. The PLA2G7 gene contains 12 exons located on chromosome 6p12-21.1 [74].

2.3.2 Expression of plasma PAF-AH

Earlier in-vitro studies have revealed that hematopoietic cells and hepatocytes secret the plasma PAF-AH. During the process of differentiation from monocytes to macrophages,

activate-plasma PAF-AH is synthesized and secreted by macrophage cells [75, 76]. Upon the response for inflammatory stimuli, some blood cells (including mast cells, etc.) can secrete plasma PAF-AH as well. at the same time, mRNA plasma PAF-AH has been detected in thymus, tonsils, and placenta, which tissues with a large number of macrophages [74]. However, with the progress of research on plasma PAH-AH, it has gradually become a consensus that plasma PAF-AH is widely present in human tissues and cells. Studies have found that rat liver cells and human liver cancer cell lines/HepG2 can also secrete plasma PAF-AH [77]. And PAF-AH mRNA was expressed in the lung tissues, female tissues, Adipose& tissues, prostate, etc. (Summary found in: https://www.proteinatlas.org/ENSG00000146070-PLA2G7/tissue).

2.3.3 Regulation of PAF-AH/PLA2G7 activity

There were not many studies on PLA2G7 gene regulation. In the research on stem cells, Xiao et al. has revealed that Nrf3 (nuclear-factor erythroid 2-related factor 3) works as one of the upstream regulators of PLA2G7, which regulates gene expression through direct binding to the promoter regions [78].

The early studies have hypothesized that the binding with lipoproteins could regulate the PAF-AH level. Nearly 80% of plasma PAF-AH is combining with LDL, while the rest part is considered with HDL binding. Plasma PAF-AH can transfer between these two lipoproteins in vivo environment [79]. This also indicates that there may be a unique regulatory mechanism that interferes activity of plasma PAF-AH by changing the lipoproteins` structure and lipid composition [80, 81]. Another report showed that the site-directed mutagenesis of certain sites in plasma PAF-AH may affect its binding to the amino acid residues of LDL particles, such as Leu-116, Trp-115 and Tyr-205 [82].

2.3.4 Physiological function of PAF-AH/PLA2G7

Plasma PAF-AH was discovered in the early 1980s. Since the pro-inflammatory glycerophospholipid PAF can be hydrolyzed by it, PAF-AH is considered to have anti-inflammatory properties. In the following years, it was gradually proved that at position sn-2, PAF-AH hydrolyzes glycerophospholipids contains certain short and/or oxidized functionalities, and not for position sn-1's linkage preferentially [83]. The hydrolysis of PAF-AH

catalyzed substrates generates lysoPAF/lysophosphatidylcholine and oxidized fatty acids. And most of them are believed to have pro-oxidant activities and pro-inflammatory [83]. These studies have led to some disputed reviews about PAF-AH in disease and physiology [84-86]. PAF-AH degrades PAF by hydrolyzing and oxidized phospholipids with a specific acetate residue generated in inflammation and oxidant stress[56, 87-89]. PAF-like lipids are also effective antagonists of PAFR, and experiments have confirmed that PAF-AH also has a regulatory effect on PAFR antagonists[57, 86, 90].

2.3.5 Relationship between abnormal expression of PAF-AH/PLA2G7 and diseases

The abnormal activity has been confirmed of plasma PAF-AH within some certain diseases. Low activity of plasma PAF-AH has been observed in some disorders, including asthma [91, 92], juvenile rheumatoid arthritis [93], systemic lupus erythematosus [94], post-traumatic multiple organ failure[95], Crohn's diseases [96], sepsis[97] and acute myocardial infarction [98]. The mechanism underlying its occurrence and possible cumulative connection with PAF will be explained later in detail in the PAF section. On the contrary, a considerable number of diseases have also shown increased plasma PAF-AH activity, such as ischemic stroke[99], myocardial infarction[100], chronic cholestasis[101], essential hypertension [102], peripheral vascular disease [103], rheumatoid arthritis [104], diabetes mellitus [105]. In this present study, we aim to focused on the biological characteristics of plasma PAF-AH, hereafter called PAF-AH.

Gene polymorphisms also show different associations with diseases. A series of studies have revealed gene polymorphisms are related to function loss of PAF-AH, namely Gln281Arg mutation[106], Arg92His, Ile198Thr, Ala379Val[107, 108], and found that as Ile198Thr sited close to Tyr-205, and this may play an essential role for binding with LDL [82]. R92H polymorphism is also claimed to contribute to the risk increase of clinical atherosclerosis[109]. In contrast, the rs1051931 polymorphism of PLA2G7 has been reported as a predictor and protective factor of IVIG resistance in KD patients[110]. Similarly, V279F polymorphism in the PLA2G7 gene shows a protective effect on atherosclerosis[109].

The conclusions of the study indicate that increased methylation of the PLA2G7 gene promoter is a specific marker aging with gender-related, which enhances the coronary heart

disease (CHD) risk in women [111]. PLA2G7 methylation is also developing IAs risk in females or BAVMs risk of in males [112].

PAF-AH also exhibits the ability to degrade shortly oxidized and/or truncated chains of sn-2 in oxLDL type [86]. PAF-AH is considered to regulate anti-inflammatory effects by itself, because PAF and its analogs as well as truncated oxLDL may induce inflammation [113-115]. However, some studies have proved that PAF-AH also affects developing the inflammation. Studies on epidemiology have shown that enhanced PAF-AH is related to the increased CHD/stroke risks, mortality, and also describes the atherosclerotic effect of PAF-AH [116-118]. The expression of PLA2G7 in primary liver cancer is significantly higher compared with pre-tumor lesions [119]. Other related studies have proved that inhibiting PAF-AH has a positive therapeutic effect on diabetic retinopathy [120-122] and Alzheimer's disease[123]. Meanwhile, studies have discussed the role of PAF-AH express differentiation in various cancers. A retrospective study has revealed that PAF-AH may affect all aspects of tumor progression, including cell apoptosis, invasion, proliferation, angiogenesis, and metastasis. Research from Stafforini has showed that the anti-tumor and tumor-promoting effects of PAF-AH may coexist [124]. Comparing with the normal tissue, the PLA2G7 expression in certain tumor tissues is significantly higher. The expression of PLA2G7 in the plasma and tumor tissue of colon cancer patients is 50% higher compared with healthy individuals [125, 126]. Similar findings have also been observed in patients with liver metastases from colon cancer [127]. The expression of PLA2G7 in primary liver cancer is significantly higher compared with pre-tumor lesions[128]. The latest research on breast cancer and ovarian cancer has shown that PAF-AH works as the progressive factor in breast cancer, revealing that the high-level expression of PAF-AH/PLA2G7 may be related to more aggressive diseases with poor prognosis in TNBC [129]. On the contrary, our study also observed its high expression in *BRCA1* mutant breast cancer and ovarian cancer. It seems that the net effect of PAF-AH on tumors and tumor microenvironment (TME) is attributed to the combined effects of its products, secondary products, and substrates. Many studies have demonstrated different roles of PAF-AH in tumorigenesis through exogenous supplementation, overexpression, and non-specific inhibition under various experimental conditions. Therefore, it is difficult to obtain roughly consistent results throughout the study, especially considering the complex TME [124]. However, these data provide evidence on PAF-AH/PLA2G7 has participated in certain cancers pathological process.

2.4 PAF and its receptor PAFR

2.4.1 PAF as well as its production and metabolism

As the most critical hydrolysis substrate of PAF-AH, PAF(1-O-alkyl-2-acetyl-sn-glycero-3-phosphocholine) is one of the factors most closely related to the protective biological effects of PAF-AH.

As a potent mediator of proinflammatory phospholipid [130], many types of cells can synthesize and secrete PAF [131, 132]. Remodeling and de-novo synthesis are considered as two major ways to synthesize PAF. The remodeling pathway is considered to happen in the endoplasmic reticulum or nuclear membrane, Phospholipase A2 (PLA2) acts on lysophosphatidylcholine and arachidonic acid, and finally acetyl residues are transferred to LPC to produce PAF. The de novo synthesis pathway is another way of PAF synthesis, which aims to maintain the normal cell function with the required physiological level of PAF [133-135], while the remodeling pathway produces PAF as a result of inflammatory stimuli and involved in inflammation cascade [136]. If not combined with PAFR, PAF is rapidly degraded by the platelet activating factor acetylhydrolase (PAF-AH).

2.4.2 General function of PAF

PAF acts on many kinds of cells, the primary function of which is during physiological processes to mediate cell-cell interactions and cell function [136] , while this is also crucial in pathological processes[137]. PAF is involved in allergic diseases and inflammations, and tumors, and it is also related to reproductive, cardiovascular, nervous and immune systems. For tumorigenesis and tumor development, PAF has been recognized to play an essential part in thrombosis [138], and metastasis [139], and it is involved in angiogenesis, metastasis, anti-apoptosis, and oncogenic transformation [140-143]. Circulating cells and cancer cells secretes PAF to the tumor microenvironment, and mediates its effect through a particular G-protein-coupled receptor (PAFR) [144-148]. Highly expressed PAF has been detected in many cancer, including breast cancer [149, 150]. Especially, Zhang et. al have discovered that the PTAFR expression is increased in *BRCA1* mutant cell lines and tissues of *BRCA1* mutation carriers [151].

2.4.3 Expression and regulation of PAFR

PAFR is widely expressed on the surface of a variety of human cells, such as macrophages, endothelial cells, platelets as well as the white blood cells [152, 153]. PAFR is a seven-transmembrane G-protein-coupled receptor (GPCR) [154], similar to PAF and its homologous lipid molecules, PARF has a specific affinity with them [155-157]. After PAF binds to PAFR, it triggers various intracellular signaling cascades. Many studies have detected that PAFR is overexpressed in some tumors, such as the stomach cancer cell line JR-St [158], epidermoid carcinoma A431 cells [159], the endometrial cancer cell line HEC-1A [160], Kaposi's sarcoma cells [161], as well as breast cancer[149, 150]. Besides, the transgenic mice with overexpressed PAFR exhibit the melanocytic tumors and proliferative diseases [162]. Moreover, PAF has been confirmed to up-regulate the expression of PAFR in some types of cells, including rat epithelial cells [163] and human alveolar macrophages [164]. Therefore, PAFR also may provide the positive feedback loop for the effects of PAF[165]. Taken together, the relationship between PAF and PAFR is bi-directional and complex. On the one hand, PAF achieves its biological function by combining PAFR to trigger downstream signal transduction. On the other hand, PAF and PAF-like phospholipids also affect signal transmission by regulating the expression of PAFR, and even PAFR antagonists need to regulate PAFR by inhibiting exogenous PAF induction[166]. These findings prove that PAF/PAFR signaling pathway works a crucial part in tumorigenicity and tumor fate.

2.4.4 Phosphorylation cascade and PAF-PAFR signaling

Protein phosphorylation is a common regulatory pathway in organisms, playing an essential role in cell signal transduction. The serine phosphorylation functions through allosteric protein to activate the protein's activity. The tyrosine phosphorylation, in addition to allosterizing and activating the activity of the protein, has more critical functions to bind the protein to provide a structural gene, promoting its interactions with other proteins to compose the multi-protein complex. The formation of protein complexes further promotes protein phosphorylation. In the signal relay network, the phosphorylation of a signaling protein usually causes the downstream proteins to undergo phosphorylation sequentially, forming a phosphorylation cascade [167-169].

Aponte et al. have demonstrated that phospholipid mediated PAF can activate PAFR. The

activation induces the phosphorylation of various proteins and initiates multiple downstream signaling pathways, such as Paxillin, FAK, Src, and EGFR, leading to increased proliferation and invasiveness of ovarian cancer cells, especially through Src/FAK and downstream targets (such as PI3K) to promote the proliferation of cancer cells and induce MMP2/MMP9 to increase invasiveness [170].

2.5 β-catenin and Wnt/β-catenin signaling pathway

2.5.1 Wnt family and function

Many cell-signaling pathways, including the Wnt signaling pathway, participate and interfere with each other in developing tissues and organs of multicellular organisms to provide location information and induce cell fate regulation [171-174]. For example, Wnt genes, including TGF-β, FGF, and hedgehog and Notch protein, which are considered highly conserved regions in evolution, encoding a series of secreted protein growth factors [175]. The name Wnt (wingless-type MMTV integration site family) has derived from Wingless Drosophila melanogaster and its mouse homolog Int[176]. A total of 19 Wnt proteins have been confirmed in humans. These proteins play multiple roles in regulating cell death, polarity, migration, proliferation, and cell fate during development [176]. After completion of development, Wnt also plays a vital role in homeostasis. Many pieces of research have exhibited that aberrantly activate on Wnt pathway is related with various cancers [177-179].

2.5.2 The Wnt signaling pathway

The Wnt ligand performs signal transmission by combine with the receptor of frizzled family with seven-transmembrane structures and the participation of the co-receptors of LRP6 and LRP5. The structure consists of three-part, an exposed extracellular domain that can bind to Wnt, a frizzled protein receptor with a C-terminal tail in cell, and a seven-transmembrane region. According to the secondary structure prediction of the frizzled structure, a study has found that the frizzled protein is a member of the GPCR family [180]. At least three intracellular pathways can achieve Wnt signal transduction, such as the classic Wnt /β-catenin signaling pathway, the Wnt / Ca2 + pathway(non-classical), as well as the Wnt / PCP pathway [181-183](Figure 2). Now researches has validated that the Wnt/β-catenin pathway mainly regulates the determination of cell fate [184], the main effect of Wnt/Polarity pathway

regulates on cytoskeleton. However, the role of Wnt/Ca2+ pathway remains indistinct yet [181-183].

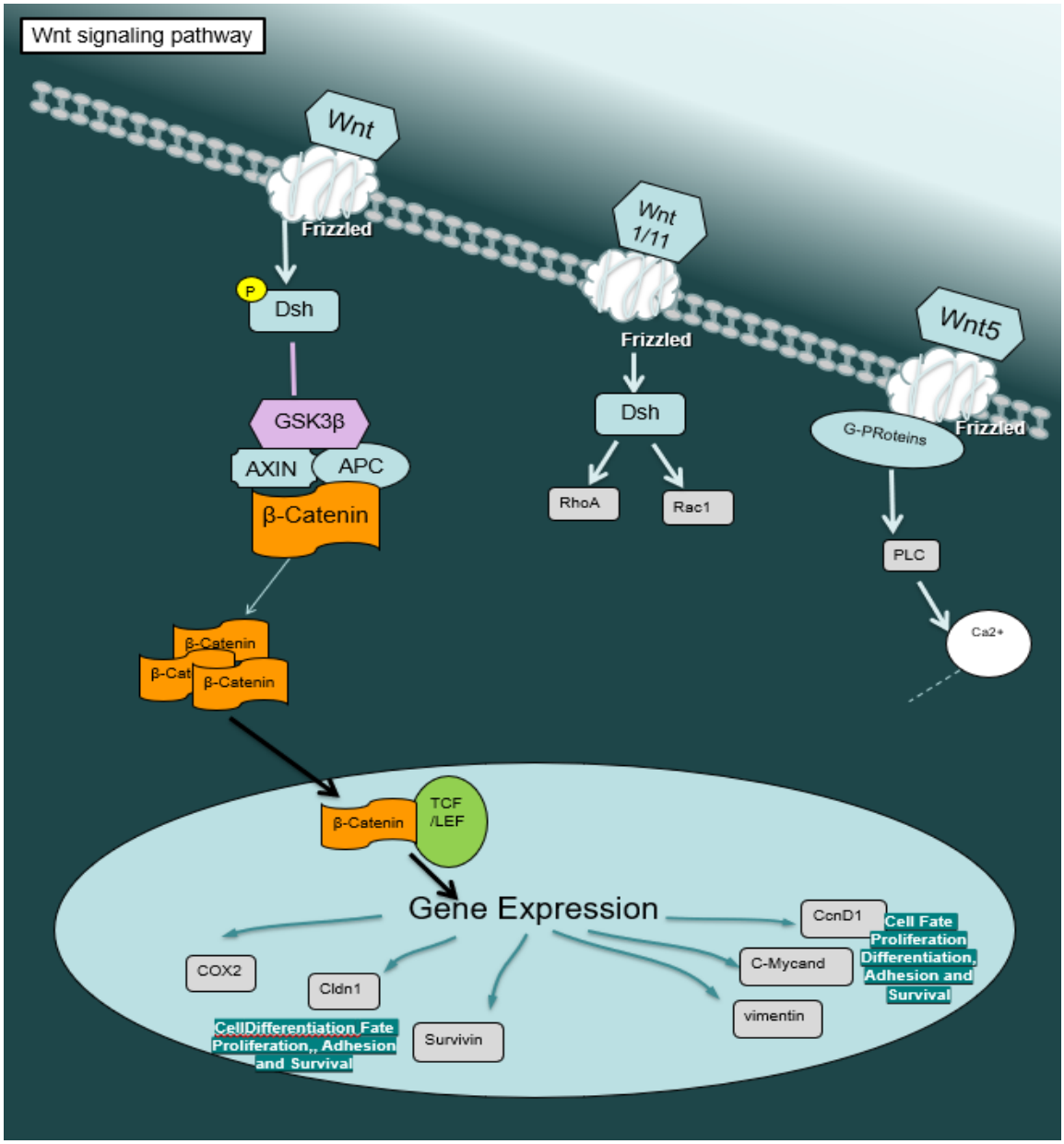


Figure 2: Three different intracellular Wnt signaling pathways

2.5.3 The canonical Wnt/β-catenin signaling pathway

The Wnt/β-catenin pathway keeps greatly conserved in evolution, which considered to be the most classic Wnt signal transduction pathway. The Wnt/β-catenin is able to activated by a series of Wnt proteins, including Wnt1, 3, 3a, 7a, 8 and it participates in transformation [185, 186]. Without Wnt signaling, β-catenin binds to cytoplasmic complex-related proteins, such as CK1-alpha, GSK3β, AXIN, and APC. This facilitates the β-catenin phosphorylate and interact

with β-TRCP (a protein contains β-transduction repeats) [187]. Finally, proteasome ubiquitinate and degrade β-catenin, which is also the main degradation pathway of β-catenin. Upon stimulation of Wnt signaling pathway, the Dsh (dehybridization) protein is activated due to the Wnt combines with the Wnt receptor Frizzled. Dsh1, 2, and 3 contain three highly conserved domains that are widely present in mammalian tissues, respectively are C-terminal DEP, a central PDZ domain, and an N-terminal DIX domain [188, 189]. The Disheveled protein activation enhances the GSK3β phosphorylation, which leads to the inhibition with the GSK3β function. Free and non-phosphorylated β-catenin gradually stores up in the cytoplasm and transfers into nucleus. Before Wnt signaling in the nucleus, LEF and TCF homologues binding to DNA in the promoter or enhancer region of the target gene with a specific sequence, and bind to Groucho and CTBP, and usually has the function of inhibiting gene expression [190]. The presence of the Wnt signaling increases the transportation of β-catenin to the nucleus and elevates it to binding on TCF/LEF (Lymph Enhancer Factor). The transcription is promoted, and multiple target genes are activated [191, 192]. The β-catenin can be delivered from the cadherin-bound pool and be useful for transmitting nuclear signaling [193]. The interaction of activated-catenin with SWI/SNF chromatin-remodeling complexes and CBP histone acetyltransferase, and binding of the BRG1 and Bcl9 to Pyg mediate transcriptional activation. β-catenin is competitively bound by Chibby and LEF1, and interacts with the C-terminus of β-catenin, causes inhibition for β-catenin-mediated transcriptional activation [194].

2.5.4 Target genes of β-catenin/TCF transcriptional regulation in tumors

Many genes have been confirmed to be the targets for the regulation of β-catenin/TCF transcription [191, 192]. These genes play the crucial part in the subsequent biological effects in tumors, including CcnD1 and c-Mycand, vimentin. Previous studies have confirmed that c-Myc and CcnD1 play a critical role in differentiation, proliferation, as well as cell growth in colon cancer. The c-Myc gene activation can induce the p53 expression, and eventually up-regulate p130/RB2 and p21WAF1, leading to growth arrest [195-197]. In human breast tumor cells, another target of β-catenin/TCF transactivation pathway is Vimentin, which take part in cell migration [198]. Wnt signaling is also capable by preventing cell apoptosis by up-regulating survivin, an anti-apoptotic protein, thereby enhancing cell viability [199, 200]. Wnt targets are several proteases including MMP26 and Matrilysin/MMP7 that can degrade the extracellular matrix[201], and cell adhesion molecules, including the NRCAM and CD44 [202, 203], which

can help tumor cells invade and metastasize. Cldn1 takes part in the β-catenin-TCF/LEF signaling pathway as well. The enhanced expression of Cldn1 could work as an essential part of the occurrence of colorectal tumors [204].

2.5.5 Breast and Ovarian Cancer & Wnt/β-catenin pathway

Recent studies have reported in invasive lobular breast carcinomas, the β-catenin complete loss from the cell membrane [205]. Besides, a β-catenin deficiency in membranous of invasive ductal carcinoma is associates with the canonical Wnt pathway activation and correlates with poorer outcomes in breast cancer patients [206, 207]. Meanwhile, in breast cancer PAF expression increases and it positively regulates the Wnt signaling pathway as a cofactor of the β-catenin transcription complex [208, 209].

Besides breast cancer, the Wnt/β-catenin inhibition has also been proved to enhance the anti-tumor immunity of ovarian cancer [210, 211], and reseach has exhibited that Wnt/β-catenin signal activation promotes cisplatin resistance in ovarian cancer[212].

2.6 Aim of the studies

At present, we haven`t gained enough in-depth knowledge of *BRCA1* mutant breast cancer and ovarian cancer. Especially, there are no precise and non-invasive biomarkers that predicted the disease risk and prognosis of *BRCA1* mutation carriers. We hypothesize that PAF-AH has differential expressions in *BRCA1* mutant breast cancer and ovarian cancer. And we predict PAF-AH may work as a non-invasive biomarker and indicate a better prognostic. The Wnt/β-catenin signaling works as a crucial biological process to maintain growth and stems during development. It also performs an essential part in tumorigenesis and tumor progression. Many studies have proved the role of PAF, the representative hydrolysis substrate of PAF-AH, is expressed increasingly in gynecological cancer and confirmed that PAF-PAFR is a poor prognostic factor. At the same time, a significant relationship between PAF-PAFR and Wnt/β-catenin signaling has also been revealed. Therefore, there is urgent to clarify in *BRCA1* mutant breast and ovarian cancer the significant role of PAF-AH.

The effect of PAF-AH in gynecological cancer remains little acknowledged. Most studies focus

on the mechanism of PAF-PAFR in tumor progression of breast and ovarian cancer. But the effect of PAF-AH on breast and ovarian cancer remains unclear. We aim to explore the protective impact with PAF-AH on *BRCA1* mutant breast and ovarian cancer, and clarified its mechanism as an important biomarker for better prognosis. This research is divided into three parts: 1) To clarify the significant relationship between PAF-AH and *BRCA1* breast and ovarian cancer. 2) To clarify the protective effect of PAF-AH in *BRCA1* breast and ovarian cancer. 3) To reveal the possible mechanisms of PAF-AH effect on Wnt/β-catenin. Based on ex-vivo and in-vitro studies, our purpose is to reveal the precise significance of PAF-AH for *BRCA1* mutant breast and ovarian cancer and in preparation role to find the potential therapeutic targets for the future.

3. Materials and Methods

3.1 Ethical Approval

All experiments were approved by the Ludwig-Maximilians University of Munich (LMU), Germany. The approval number of the ovarian cancer project is 227-09 and 19-972, and the approval number of the breast cancer project is 048-08. In this research, all tissue samples were collected from archival materials of the Obstetrics and Gynecology Department at the University of Ludwig Maximilian, which was initially used for pathological diagnosis and finished ahead of the present study. In the analysis, all patients` data kept completely anonymous and coded by the observers. The Ethics Committee approved the study of LMU. We ensure that the whole experimental procedures are strictly carried out and completed under the Helsinki Declaration standards (1975).

3.2 Patients and Specimens

In the breast cancer project, 110 tissue samples were analyzed from patients who had been through surgeries at the Obstetrics and Gynecology Department, LMU, 1987-2009 who diagnosed with breast cancer. The enrollment criteria were formulated as breast cancer, those only diagnosed with benign tumors or in situ cancer were ruled out from the cases, and no patient had been through adjuvant chemotherapy. Unfortunately, the follow-up data were missing. The fixing method were carried out by 4% buffered formalin, and the embedding on samples from tissues used paraffin for further immunohistochemical analysis. Gynecological pathologists assessed staging and grading for breast cancer cases. Detailed information of patients on the clinical features enrolled in this study included tumor grading, histology, and staging. The clinical-pathological variables are summarized in Table 2. 49.9 ± 13.2 years is Mean age of this cohort (41.8 ± 10.7 years of *BRCA1* associated cases; 53.6 ± 12.7 years of sporadic breast cancer). Survival analysis of breast cancer cohort was performed by using the online database https://kmplot.com/analysis/ with mRNA (gene chip).

Table 1: *Clinicopathologic characteristics of the breast cancer patients*

	Clinicopathological characteristics of patients		
		n (121)	*Percentage [%]*
Histology	Invasive ductal	80	66.1
	Invasive lobular	13	10.7
	Invasive medullary	12	9.9
	Invasively mucinous	1	0.8
	Unknown	15	11.8
Grading	1	4	3.3
	2	45	37.1
	3	60	49.6
	Unknown	12	9.9
Age	≥60	29	24.0
	<60	92	76.0
Primary tumor expansion	Tis	5	4.1
	T1a	3	2.5
	T1b	13	10.7
	T1c	22	21.5
	T2	43	36.2
	T3	12	9.9
	T4a	2	1.6
	T4b	2	1.6
	T4c	1	0.8
	T4d	6	5.0
	Tx	12	9.9
Nodal status	N0	38	31.4
	N1	34	28.1
	N2	9	7.4
	N3	7	5.8
	Nx	33	27.3
Distant Metastasis	M0	46	38.0
	M1	28	23.1
	Mx	47	53.5
BRCA1 mutation status	No BRCA1 mutation	28	23.1
	BRCA1 mutation	22	18.2
	Unknown	71	58.7

In ovarian cancer project, 156 tissue samples from patients who had been through EOC surgeries also at the Obstetrics and Gynecology Department from 1990-2002. The enrollment criteria were formulated as ovarian cancer, those only diagnosed with benign or borderline tumors were ruled out from the cases, and no patient had been through adjuvant chemotherapy. Munich Cancer Registry (Munich Tumor Center, Munich, Germany) applied the follow-up infomation. The method of fixing and embedding tissue samples is the same as above. The staging and grading information of EOC was assessed by gynecological pathologists. Detailed information about the clinical characteristics of patients enrolled in this study, including tumor grading, histology and staging, are listed in Table 2. The staging was performed according to FIGO classification from WHO (2014). There are 155 cases with primary tumor expansion data, 95 cases with lymph node invasion data, and 9 cases with distant metastasis data. There are thirteen patients who have residual disease after primary surgery.

Table 2: *Clinicopathologic characteristics of the ovarian cancer patients.*

Clinicopathological characteristics of patients			
		n	Percentage (%)
Histology	Serous	110	70.5
	Clear cell	12	7.7
	Endometrioid	21	13.5
	Mucinous	13	8.3
Primary tumor	TX	1	0.6
expansion	T1	40	25.6
	T2	18	11.5
	T3	97	62.3
Nodal status	pNX	61	39.1
	pN0	43	27.6
	pN1	52	33.3
Distant Metastasis	pMX	147	94.2
	pM0	3	1.9
	pM1	6	3.8
Grading Serous	Low	24	21.8
	High	80	72.7
Grading	G1	6	28.6
Endometrioid	G2	5	23.8
	G3	8	38.1
Grading Mucinous	G1	6	46.2
	G2	6	46.2
	G3	0	0
Grading Clear cell	G3	9	75
FIGO	I	35	22.4
	II	10	6.4
	III	103	66.0
	IV	3	1.9
Residual disease	Unknown	143	91.7
after primary surgery	Complete cytoreduction	11	7.1
	Incomplete cytoreduction	2	1.3
Age	≤ 60 years	83	53.2
	> 60 years	73	46.8

The *BRCA* mutation status of this EOC collective is unfortunately not available. Therefore, the *BRCA* mutation status was defined as unknown with a *BRCA* mutation probability of 10-20%. To investigate PAF-AH expression levels in *BRCA1* mutation carriers we stained additional tumor tissue of 107 patients with genetically confirmed *BRCA1* mutation (Table 3).

Table 3: *BRCA mutation status of analyzed patients.*

***BRCA* mutation status**	**n**	**Percentage (%)**
Mutation unknown	141	56.9
BRCA1 mutation	107	43.1
BRCA1	15	6
BRCA1+2	92	37

Furthermore, blood samples of EOC patients with/without *BRCA1* mutation were collected for this study, from 2019 to 2020. The characteristics of the patients with blood analysis are exhibited in Table 4.

Table 4: *Patient characteristics of the blood analysis.*

BRCA mutation status	n	Percentage (%)	Overall survival (months; median)	Progression free survival (months; median)
No mutation	17	73.9	25.0	17.0
BRCA1 mutation	6	26.1	34.5	28.0

3.2 Immunohistochemistry and Immunocytochemistry

After fixing and embedding tissue specimens were sliced into 3µm sections by our laboratory [213]. Xylol and 100% ethanol were used to dewaxing and washing the tumor slides. After incubation in methanol in 3% H_2O_2 for 20min, samples carried by slices were rehydrated by descending ethanol gradient. Then these slides were boiled in a sodium citrate buffer (pH = 6.0) mixed with 0.1 M citric-acid and 0.1 M sodium citrate. After cooking in the pressure boiler for 5min, slides were cooling and washing in PBS. Blocking solution (reagent 1, Zytochem-Plus HRP-Polymer-Kit (mouse/rabbit)) were used for preventing the non-specific binding,

After 30min, slides were incubated with anti-PAF-AH or anti-ß-Catenin antibodies at 4°C for 16h. On the second day, slides were washed with PBS and incubated at room temperature with the secondary antibodies/complexes (Reagent 3; Zytochem-Plus HRP Polymer-kit

(mouse/rabbit)). To visualize the staining chromogen-3, 3'-diaminobenzidine (DAB; Dako) was used for incubation for 10min. At last, slides with samples were counterstained with Hemalum and dehydrated one by one in ascending series of alcohol. The healthy placenta tissue sections used as the antibodies positive control, both negative and positive control were contained inside each experiment for the IHC staining to test antibody function and choose the adequate dilution of the antibody (Figure 3). Both positive and negative controls were performed in placenta and colon tissues to validate staining specificity. Slides with samples were analyzed under photomicroscope (Leitz, Wetzlar, Germany) by two independent observers. Multiplication of percentage range of positively stained cells and the optical staining intensity was used as semi-quantitative IRS score for the light microscopy analysis. The negative control shown as unstained and the positive signals were generating a brownish color.

Table 5: *Antibodies used in immunostainings.*

	Antibody	Dilution	Manufacturer
Breast cancer & Ovarian cancer	anti-PAF-AH (Cay-160603) Polyclonal rabbit IgG	1:200 (IHC) 1:50 (ICC)	MyBioSource, San Diego, CA, USA Cayman, Michigan, USA
Ovarian cancer	anti-GSK3β (phospho Y216) polyclonal rabbit IgG	1:1000 (IHC)	Abcam, Cambridge, UK
Breast cancer & Ovarian cancer	anti-β-catenin Roche-D178frzQ polyclonal rabbit IgG	1:300 (IHC) 1:200 (ICC)	Diagnostic BioSystems, Fremont, CA, USA

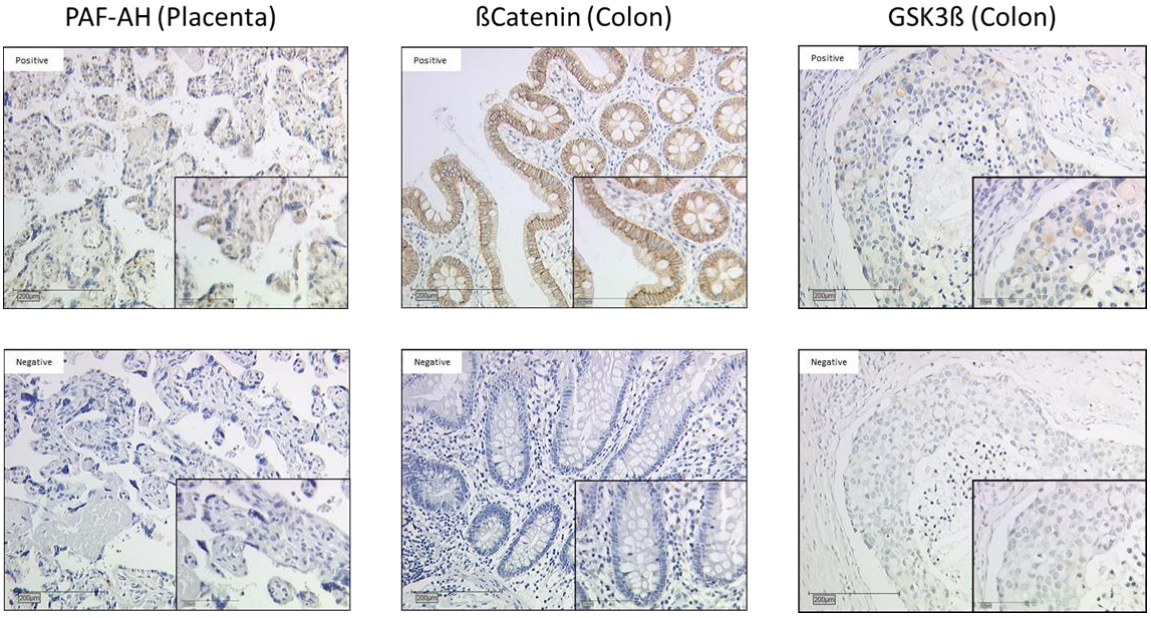


Figure 3. Positive and negative controls for each antibody.

HCC1937 and UWB1.289 cell lines were used in immunocytochemistry for each project. Cells were seeded on chamber slides (Millicell EZ SLIDE 8-well glass, Darmstadt, Germany) with a concentration of 5×10^3 cells/cm^2. Transfection was performed with adherent HCC1937 cells after 60h incubation. Untreated HCC1937 cells were served as control. After treatment, slides with cells were washed with 0.1 M PBS, ethanol 100%/methanol (1:1) were used to fixation for 15 min in room temperature and air-dried. Protein block (Dako, Glostrup, Denmark) was carried out in order to reduce non-specific background staining, for 25 min in RM. Thereafter, an anti-PAFAH 1:50 (Cayman, Polyclonal Cay-160603, 1:200 dilution, rabbit IgG, Michigan, USA) were carried out and ß-Catenin (ready to use, Roche, Ventana, Mouse IgG-1, Oregon, USA) directed against ß-Catenin for 16h at 4°C. The PBS were used with washing and slides incubated with a secondary antibody (Vector Laboratories) in RT. After 30 minutes, PBS was used with washing and then incubating with avidin-biotin-peroxidase complex (Vector Laboratories) at room temperature for 30 minutes. After developing the antigen-antibody complex, chromophore 3-amino-9-ethylcarbazole (AEC; Dako) and Mayer heme were carried out for counterstained. Finally, tap water was used to washing the slides and covered slides with Kaiser glycerin gelatin (Merck). Finally, the cell-loaded glass slide was washed with water, and the cover glass was sealed with Kaiser glycerin gelatin (Merck, Darmstadt, Germany).

3.3 Staining evaluation and statistical analysis

The IHC staining results were evaluated by two separate students in a double-blind process using the IRS[214]. The photomicroscope by Leitz (Wetzlar, Germany) was carried out to examine specimens and to observe specific PAFAH and ß-Catenin immunohistochemical staining reactions in the cells. A semi-quantitative immune response score (IR score, Remmele score[215]) was carried out for evaluating the intensity and distribution pattern of immunostaining. In order to obtain IR scoring results, the staining intensity was divided into four levels (0 = negative staining, 1 = weak, 2 = moderate, 3 = strong) and the percentage of positively stained cells was divided into the following five levels (0 = no staining, 1 = <10%, 2 = 11-50% , 3 = 51-80% , and 4 => 81%) then multiply these two results together. Nuclear and cytoplasmic staining of PAF-AH were completely carried out, as well as ß-Catenin was stained in nuclear and membrane in 110 tissue specimens, respectively. The median IRS-results from the three spots of each slide was calculated and used for further analyses.

With the help of SPSS 25.0 (v25, IBM) statistical analysis was accomplished successfully. Chi-square test was assessed for distribution of clinicopathological variables, and the IR score between different clinical and pathological subgroups were compared with the Mann-Whitney-U test [216]. Spearman analysis[217] was used to calculate the correlation between results of IHC staining. The log-rank testing was carried out for comparing survival-times and visualized in Kaplan-Meier plots. To confirm appropriate cut-off values ROC analysis, a widely accepted method for cut-off point selections, was accomplished. We determined Yudan index and defined it to be the maximum [218] to ensure that the best cut-off value maximizes the sensitivity and specificity sum. In order to obtain a multivariate analysis, a Cox regression model was implemented. When the P value is no larger than 0.05, it is recognized to be significant. The CT value of genes were get from qPCR, the formula $2^{-\Delta\Delta CT}$ was used for calculation of relative expression [219]. For data visualization and statistical analysis of in-vitro generated data Graph Pad Prism 7.03 (v7; San Diego) was used.

3.4 PAF-AH ELISA

To determine the PAF-AH concentration in serum samples an enzyme-linked immunosorbent assay (ELISA; R&D Systems, Minneapolis, MN, USA) was conducted abide by the instructions from company. The standard curve was created using a four-parameter logistic curve fit.

3.5 Cell lines

Breast cancer cell lines SK-BR-3(HER2+), HCC1937 (triple-negative, *BRCA1* mutant), MDA-MB-231 (triple-negative, *BRCA1* wildtype), BT-474(Luminal B), MCF-7(Luminal A), and a breast epithelial cell line MCF-10A (served as reference) were used in breast cancer project. The ovarian cancer cell lines SK-OV-3 (serous, *BRCA* WT), ES-2 (clear cell), UWB1.289 (serous, *BRCA1* negative), TOV-112D (endometrioid), and the ovarian epithelial cell line HOSEpiC (served as reference) were used in ovarian cancer project. All these cell lines were bought from the American Type Culture Collection (ATCC, Rockville, MD, USA). RPMI 1640 GlutaMAX medium (Gibco, Gibco, Paisley, UK) and 10% fetal bovine serum (FBS; Gibco, Paisley, UK) was used to maintaine the cell culturing with in incubator at 37°C under 5% CO_2 . MCF10A was thawed and maintained in culture with medium listed in Table 6.

Table 6: *Cell culture recipe for MCF-10A*

Component	Growth Medium[1]	Assay Medium[1] (Without EGF)
DMEM/F12 (Invitrogen #11330-032)	500.0 ml	500.0 ml
Horse Serum (Invitrogen#16050-122)	25.0 ml (5% final)	10.00 ml (2% final)
EGF (100 µg/ml stock)[2] (Peprotech, 1 mg)	100 µl (20ng/ml final)	--
Hydrocortisone (1mg/ml)[3] (Sigma #H-0888)	250 µl (0.5 mg/ml final)	250 µl (0.5 µg/ml final)
Cholera Toxin (1mg/ml stock)[4] (Sigma #C-8052)	50 µl (100 ng/ml final)	50µl (100 ng/ml final)
Insulin (10mg/ml stock)[5] (Sigma #I-1882)	500 µl (10µg/ml final)	500 µl (10µg/ml final)
Pen/Strep (100 x solution, Invitrogen #15070-063)	5.0 ml	5.0 ml

3.6 Real-time PCR

The mRNA Isolation was carried out according to instruction from the manufacturer by using the RNeasy Mini Kit (Qiagen). After isolation, the reverse transcription was carried out with the MMLV Reverse Transcriptase 1st-Strand cDNA Synthesis Kit (Epicentre, Madison), 1µg RNA was converted into the first-strand cDNA according to the protocols of the manufacturer. PCR was performed on all samples individually. The basal and after transfection mRNA expressions of PAFAH were quantified by qPCR using FastStart Essential DNA Probes Master and gene-specific primers (Roche). The $2^{-\Delta\Delta CT}$ method was carried out to quantify β-actin and GAPDH as housekeeping genes. After silencing, the expression of PAF-AH was compared with basal expression in HCC1937 cell line and UWB1.289 in each project, respectively. The primer sequences are available in the Table 7

3.7 siRNA knockdown

Lipofectamine RNAiMAX reagent (Invitrogen, Carlsbad) was used to transfect small interfering RNA (siRNA; 4 different sequences for PLA2G7; Qiagen Sciences, Maryland, USA) into UWB1.289 cells. A scrambled negative control siRNA (Qiagen, Hilden, Germany) was used as reference. UWB1.289 cells were seeded into 6-well plates and the transfection was carried out until the ratio of cell reached 60-70%. The cells were incubated with Opti-MEM Reduced Serum Medium (Thermo Fisher Scientific, Waltham, MA, USA) mixed with siRNA-PLA2G7 and Lipofectamine RNAiMAX. Breast cancer HCC1937 cells were harvested for further experiments after 60h transfected. For UWB1.289, after 36h cells were harvested and used for further experiments, in ovarian cancer project. Sequences of primers used in qPCR are listed in Table 7.

Table 7: *Sequences of primers used in qPCR to determine mRNA expression levels*.

PLA2G7	Forward: GGCTCTACCTTAGAACCCTGAAA Reverse: TTTTGCTCTTTGCCGTACCT
ACTB	Forward: TCCTCCCTGGAGAAGAGCTA Reverse: CGTGGATGCCACAGGACT
GAPDH	Forward: AGCCACATCGCTCAGACAC Reverse: GCCCAATACGACCAAATCC

3.8 Western blot

The western blot was performed according to formerly reported[220]. To sum up, all cells to be tested were lysed for 15min with 200 µL buffer at 4°C, containing 1:100 diluted RIPA buffer mixed in protease inhibitor (Sigma-Aldrich Co.). Lysates were detected with the Bradford assay [221] to identify the protein concentration. Protein extracts (65µg) were departed based on differences of molecular weight by using 12% SDS-PAGE and transferred on a polyvinylidene fluoride membrane (EMD Millipore, Billerica). To prevent the antibodies' non-specific binding the membrane was blocked in a well consisting of 1× casein solution (Vector Laboratories, Burlingame) for 1 hour. After casein saturation, the membrane was cut and incubated in diluted primary antibodies by shaking at 4°C gentlely overnight.

With breast cancer project, antibody against PAFAH (proteinTECH, Polyclonal 15526-1-AP,

1:200 dilution, rabbit IgG, Manchester, UK) and primary antibodies anti-GAPDH (GeneTex Co., Monoclonal GT-239, 1:1000 dilution, mouse IgG; Eching, Germany) were used respectively on each membrane. The result of GAPDH was served as a control. As for ovarian cancer project, primary antibodies a rabbit polyclonal antibody against PAF-AH (1:200 dilution; Cayman, Ann Arbor), a mouse monoclonal antibody against beta-actin antibody (1:1000 dilution; Sigma), and a mouse monoclonal antibody against GAPDH (1:1000 dilution; GeneTex Co., Eching, Germany) were used. GAPDH western blots served as controls.

Afterward, membranes were washed with 1:10 casein three times and then subjected to antibody anti-mouse/rabbit respectively with ABC-AmP reagent (VECTASTAIN ABC-AmP Kit for mouse/rabbit IgG; Vector Laboratories). The antibody complexes were visualized with BCIP/NBT substrates (Vectastain ABC-AmP Kit; Vector Laboratories). Bio-Rad Quantity One and Universal Hood II Software (Bio-Rad Laboratories Inc., Hercules) system were carried out for Western blotting detection and analysis. Western blots were performed for 9 times for every band.

3.9 Cell viability assay

Cell viability was measured by MTT assay. HCC1937 was used in breast cancer project and UWB1.289 was used in ovarian cancer project. For both assays cells were seeded on 96 well-plate with a concentration of 5000 cells/100µl. After incubated for 48h in 10%FCS in RPMI 1640 medium, transfection was carried out as described above. After transfection, cells were incubated with 20µl MTT solution (Sigma-Aldrich Co.) at a concentration of 5mg/ml for 2h. Aspirate all the solution, and use dimethyl sulfoxide (DMSO) 200µl to dissolve the precipitated formazan. Cell viability was measured with an Elx800 universal Microplate Reader at the wavelength of 595nm for OD value. Each round of experiment was validated for three times (n=3).

3.10 Proliferation assay

To measure the cell proliferation rate, HCC1937 was used in breast cancer project and UWB1.289 was used in ovarian cancer project. For both assays cells were seeded on 96 well-plate and transfection were carried out as described above. BrdU incorporation assay

(11647229001, Roche) was performed based on protocols. Absorbance value was quantified by a Elx800 universal Microplate Reader at 450 nm. Each experiment was performed for three times repeated for three wells(n=9).

3.11 Scratching assay

Breast cancer cell line HCC1937 was seeded on 24 well-plate (2×105 cells/ml). After 24h, the vertical line was drawn on the monolayer cells with a 100 µl pipet tip to make an artificial wound. Subsequently, the transfection was performed, and images of each scratch was taken at 0 h and 60 h after PLA2G7 gene knockdown. The cell migration was recorded with inverse phase contrast microscope with a camera (LEICA MC120 HD; Leica, Wetzlar, Germany). Microphotographs of wounded areas and areas recovered after the scratching were analyzed by Image J (https://imagej.nih.gov/ij/). The cell migration boundaries were defined as the difference of the area covered with cells at 60 h and 0 h.

In ovarian cancer project, UWB1.289 cells were seeded on 24 well-plate (2×10^5 cells/ml). The process for making scratch is the same as above. Subsequently, the transfection was performed, and boundaries of the scratches were taken at 0 h and 36 h after PLA2G7 gene knockdown. The calculation of scratch recovery is also the same as above. The cell migration area is defined as the difference of the area covered with cells at 36 h and 0 h.

4. Results

4.1 PAF-AH was only highly expressed in *BRCA1* mutant cases and implied a better OS, which correlated with Wnt signaling proteins.

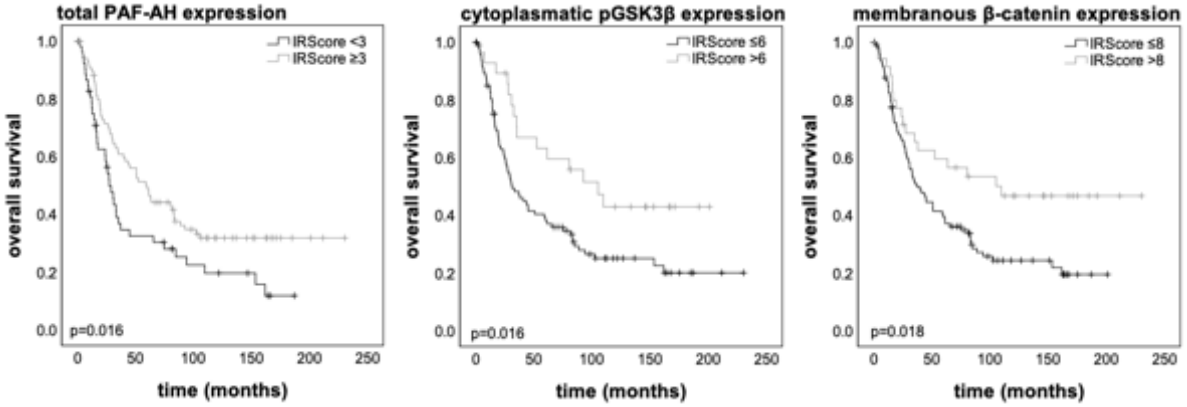
4.1.1 PAF-AH is an independent positive prognostic factor in EOC and correlates with the Wnt signaling's protein β-catenin and pGSK3β

To understand the role of PAF-AH in aberrant cell signaling and clinical context, the expression patterns of PAF-AH and the Wnt signaling proteins β-catenin and pGSK3β were investigated within a total 156 EOC specimens (Table 2) by IHC. For PAF-AH 86.52% of all successfully stained tissue samples were positive with a median (range) IRScore of 3 (0-12). 98.57% showed cytoplasmatic pGSK3β (Y216; median IRScore = 4 (0-12)) and all specimens showed membranous β-catenin (median IRScore = 8 (2-12)) expression. Strong positive correlations between nuclear/cytoplasmatic PAF-AH, cytoplasmatic pGSK3β and membranous β-catenin were found (Table 8).

***Table 8**: Correlations between PAF-AH and Wnt signaling proteins pGSK3β and β-catenin.*

	PAF-AH nucleus	**PAF-AH plasma**	**pGSK3β plasma**	**β-catenin membrane**
PAF-AH nucleus				
Cc	1	0.469	0.494	0.267
P	-	<0.001*	<0.001*	0.001*
N	141	141	135	140
PAF-AH plasma				
	0.469	1	0.448	0.291
	<0.001*	-	<0.001*	<0.001*
	141	141	135	140
pGSK3β plasma				
CC	0.494	0.448	1	0.224
P	<0.001*	<0.001*	-	0.008*
N	135	135	140	139
β-catenin membrane				
Cc	0.267	0.291	0.224	1
P	0.001*	<0.001*	0.008*	-
N	140	140	139	147

IRScores of PAF-AH (nucleus and cytoplasm), pGSK3β (plasma) and β-catenin (membrane) staining were associated with in between by performing Spearman's analysis. Asterisks were indicated for significant correlations (*: p≤0.01). n=number of patients, p=two-tailed significance, cc=correlation coefficient (Figure 4).



IRScore <3	IRScore ≤6	IRScore ≤8
n=53	n=96	n=101
median survival 27.4 months	median survival 31 months	median survival 36.9 months
IRScore ≥3	IRScore >6	IRScore >8
n=88	n=28	n=36
median survival 59.9 months	median survival 105.1 months	median survival 109.7 months

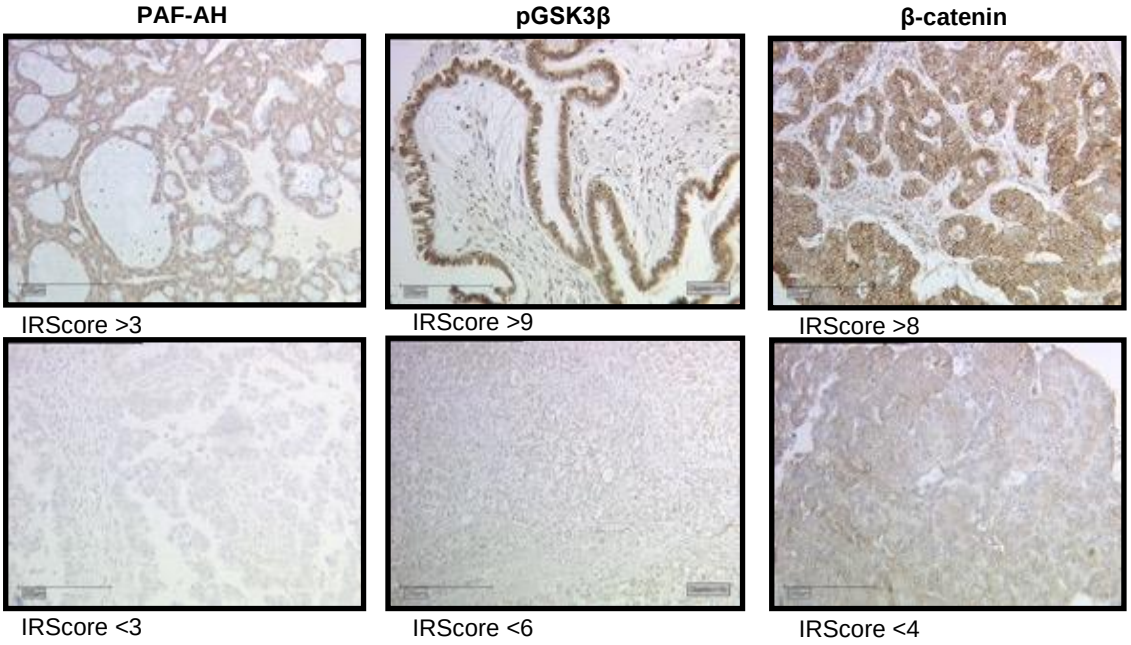


Figure 4: Univariate analyses and representative microphotographs of the immunostainings. The Kaplan Meier estimates (log-rank testing) show that high tumoral PAF-AH (IRScore ≥3; A) expression as well as high levels of cytoplasmatic pGSK3β (IRScore >6; B) and membranous β-catenin (IRScore >8; C) are associated with prolonged OS. The crosses (+) in graphs were marked as censoring events. Representative microphotographs of the immunostainings (10x magnification, scale bar = 200µm; D) show the difference between high expression (top) and low expression (bottom).

Combined survival analysis of the investigated proteins shows an even longer survival time (Figure 5). However, the subgroup with high expression levels of all factors (total PAF-AH, cytoplasmatic pGSK3β and membranous β-catenin) is quite small (n=11).

low expression of all proteins
n=128
median survival 35.2 months
high expression of all proteins
n=11
median survival 199.8 months

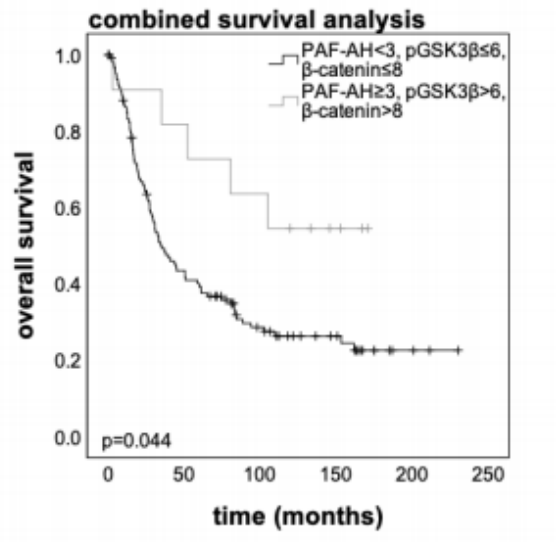


Figure 5: median OS 199.8 months vs. 35.2 months, p=0.044.

Age (>60 vs. ≤60 years, p=0.039), FIGO stage (III/IV vs. I/II, p=0.004), grading (high/G2-3 vs. low/G1, p=0.002), and tumoral PAF-AH expression (high vs. low, p=0.021) proved to be independent factors for prognosis of OS in the cohort. In contrast, cytoplasmic pGSK3β (p=0.645) and membranous β-catenin (p=0.745) were not the independent markers (Table 9). Due to insufficient data, the residual disease after primary surgery has been excluded for multivariate analysis.

Table 9: *Multivariate analysis confirmed independency of tumoral PAF-AH expression as positive prognostic factor for OS.*

Covariate	p	Hazard Ratio (95% CI)
Age >60 vs. ≤60	0.039*	1.637 (1.026-2.612)
FIGO III/IV vs. I/II	0.004*	2.585 (1.366-4.891)
Grading high/G2-3 vs. low/G1	0.002*	2.797 (1.436-5.449)
Total PAF-AH expression high (≥3) vs. low (<3)	0.021*	0.583 (0.369-0.921)
Cytoplasmatic pGSK3β (Y216) expression high (>6) vs. low (≤6)	0.645	0.877 (0.501-1.535)
Membranous β-catenin expression high (>8) vs. low (≤8)	0.745	0.736 (0.545-1.544)

Asterisks were indicated as the independent factors (*: p≤0.05). CI: confidence interval.

4.1.2 *BRCA1* mutant patients have higher PAF-AH levels in tumor tissue and in serum

PAF-AH expression was also detected by IHC in tumor tissue of *BRCA1* mutation carriers (n=107; Table 3). Interestingly, patients with *BRCA1* mutation or *BRCA1+2* mutations show significantly higher tumoral expression levels of PAF-AH (median IRScore=4) compared to patients with unknown *BRCA* mutation status (n=141; median IRScore=3), for which a mutation probability of 10-20% can be assumed (Figure 6)

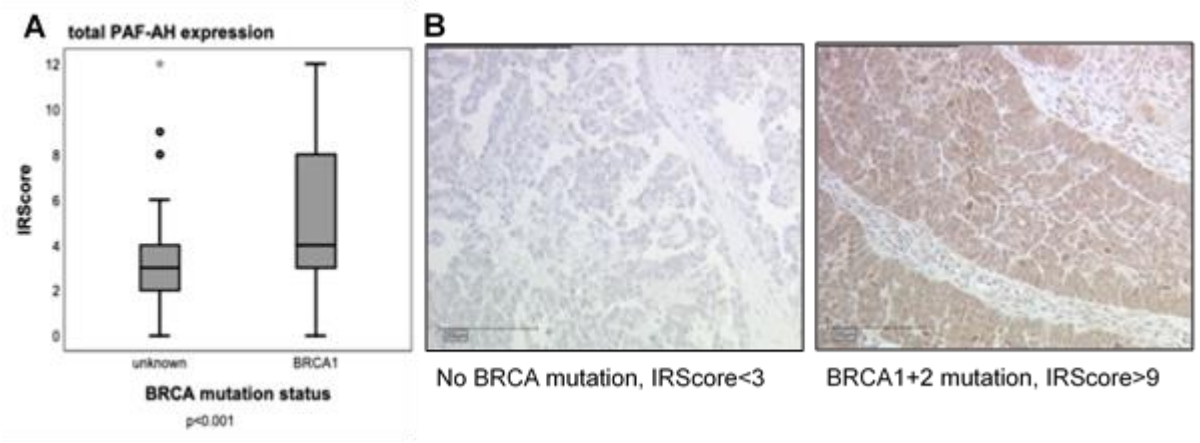


Figure 6: *BRCA1* mutation carriers show higher tumoral PAF-AH expression. PAF-AH expression is significantly higher in tumor tissue of *BRCA1* mutation carriers (median IRScore =4; p>0.001; A), when compared to patients with unknown *BRCA* mutation status (median IRScore =3) (Mann-Whitney-U-test). Representative microphotographs of PAF-AH immunostaining of patients with BRCA WT and *BRCA1+2* mutation are shown on the right (B) in 10x magnification (scale bar = 200µm).

Based on the results of IHC, the question arose, whether differences of PAF-AH expression between *BRCA* WT and BRCA mutation carriers can be detected in blood samples. In a preliminary analysis, PAF-AH serum concentrations of 6 *BRCA1* mutated and 17 *BRCA* WT EOC

patients were determined and collected before surgery(Table 3). Indeed, patients with genetically confirmed *BRCA1* mutation had significantly higher PAF-AH serum concentrations (median=264.56 ng/ml) than *BRCA* WT patients (median=176.35 ng/ml) (Figure 7).

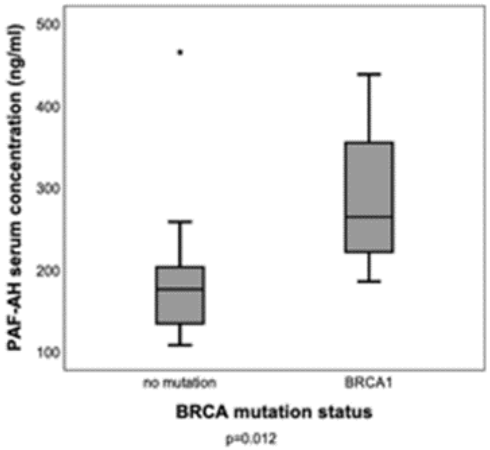


Figure 7: *BRCA1* mutation carriers show higher PAF-AH serum concentrations. PAF-AH serum concentrations were determined with ELISA. Higher level of PAF-AH was tested in serum samples of *BRCA1* mutation carriers (n=6), when compared to patients with *BRCA* WT (n=17) (p=0.012, Mann-Whitney-U-test).

4.1.3 PAF-AH is highly expressed in *BRCA1* mutant breast cancer tissue and is positively correlated with the β-catenin expression which implies a better prognosis

We used 121 slices for PAF-AH expression by IHC, and only 44 slices left were available for β-catenin detection. All slices were successfully stained. We observed 39 positive nuclear PAF-AH in 121 slices (32.2%), while 103 positive for cytoplasmic PAF-AH (85.1%). Median (range) IRS of PAF-AH in the nucleus and cytoplasm were respectively 0 (0, 9) and 3 (0, 12). 39 slices showed a positive membrane β-catenin in 44 slices (88.6%), and the median IRS was 6 (0, 12). Table 1 summarizes the clinical characteristics according to tumor grading, histology, and staging of the patients enrolled in the study. Unfortunately, the survival time and status of this collective were not available, so we did not do survival analysis within this collective. Survival analysis was performed by using the online database https://kmplot.com/analysis/(Figure 9 A, B) with mRNA (gene chip). The sensitivity of PAF-AH expression as a biomarker for the *BRCA1* mutation was utilized in the receiver operating characteristic (ROC) curve (Figure 9 C).

Both nuclear and total PAF-AH, as well as membrane ß-Catenin expressions, were visualized in box plot graphs (Figure 8). We observed that the *BRCA1* mutation group expressed significantly higher levels than the other two groups for nuclear PAF-AH ($p<0.001$), and membrane ß-Catenin showed a similar significance expression ($p<0.001$). Interestingly, patients with *BRCA1* mutation also showed significantly higher expression of total PAF-AH compared to patients with unknown *BRCA* mutation status and no *BRCA1* mutation. The mutation probability was assumed to be around 0.2-2.5% ($p <0.05$).

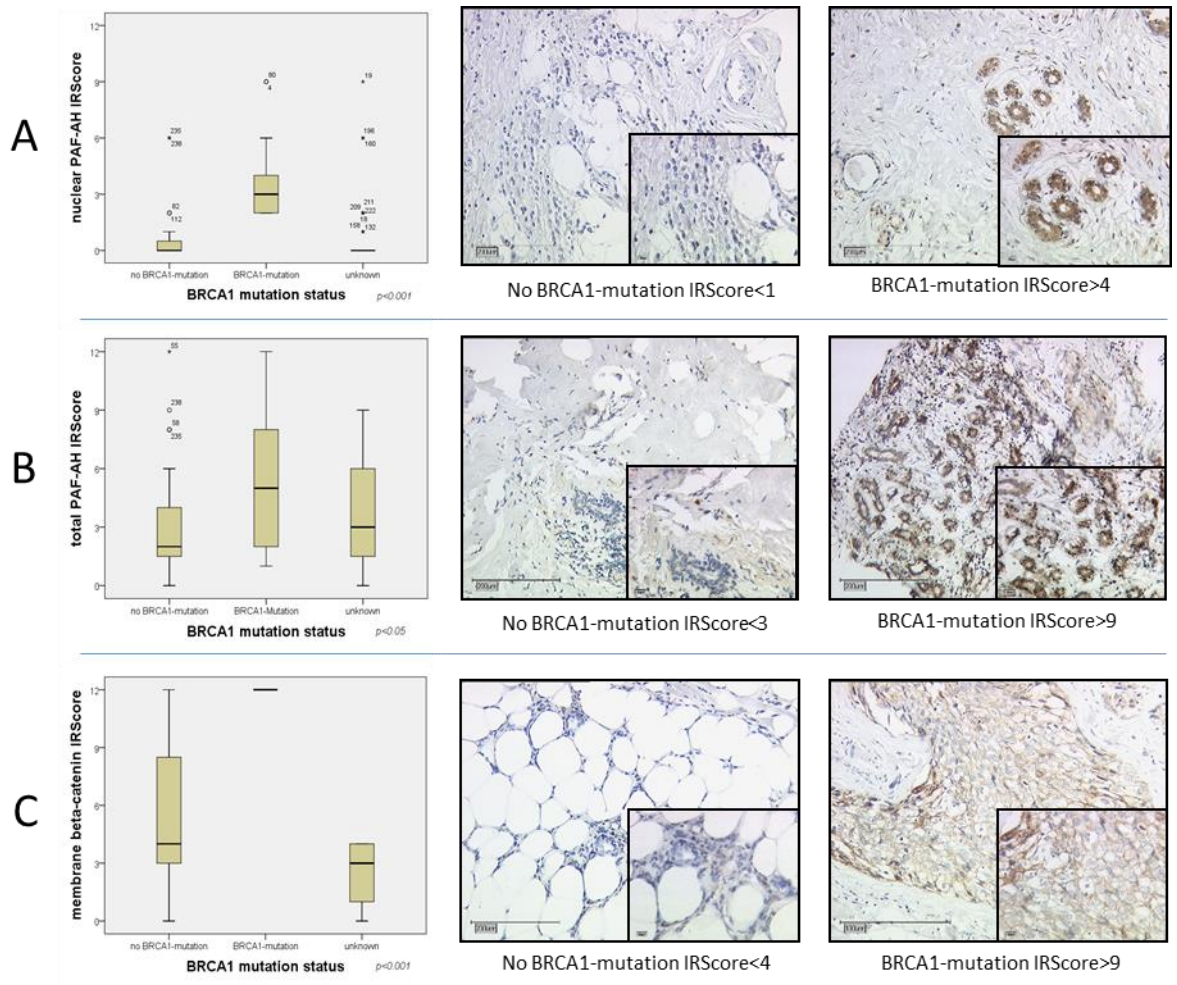


Figure 8. The figure in group A showed that nuclear PAF-AH in *BRCA1* mutation patients (n = 22; median IRScore = 3) significantly higher expressed than the other two groups ($p <0.001$). Within group B, the *BRCA1* mutation patients (median IRScore = 5) reflected a significant higher expression of total PAF-AH compared with unknown *BRCA* mutation status (n = 71; median IRScore = 3) and no *BRCA1* mutation (n = 28; median IRScore = 2) patients ($p <0.05$). The pictures in group C showed that the membrane ß-Catenin (n = 14; median IRScore = 12) in patients with *BRCA1* mutations was higher than no *BRCA1* mutation (n = 19; median IRScore = 4) and unknown *BRCA* mutation cohort (n = 11; median IRScore = 3) ($p <0.001$).

To better understand the correlation between PAF-AH and the crucial protein in Wnt signaling pathway, a correlation analysis was performed. The results showed a strong positive correlation between nuclear PAF-AH and membrane β-catenin($p<0.001$) (Table 10).

Table 10: *Correlation between PAF-AH and Wnt signaling protein β-catenin*

	PAF-AH nucleus	**PAF-AH plasma**	**β-catenin nucleus**	**β-catenin membrane**
PAF-AH nucleus				
Cc	1	0.225	-0.062	0.766
P	-	<0.05*	>0.05	<0.001*
N	121	121	44	44
PAF-AH plasma				
Cc	0.225	1	0.018	0.057
P	<0.05*	-	>0.05	>0.05
N	121	121	44	44
β-catenin nucleus				
CC	-0.062	0.018	1	-0.049
P	>0.05	>0.05	-	>0.05
N	44	44	44	44
β-catenin membrane				
Cc	0.766	0.057	-0.049	1
P	<0.001*	>0.05	>0.05	-
N	44	44	44	44

Table 10. IR-Scores of nucleus PAF-AH and membrane ß-catenin expressions were correlated to each other by Spearman's analysis. n = number of patients, p = two-tailed significance, cc = correlation coefficient.

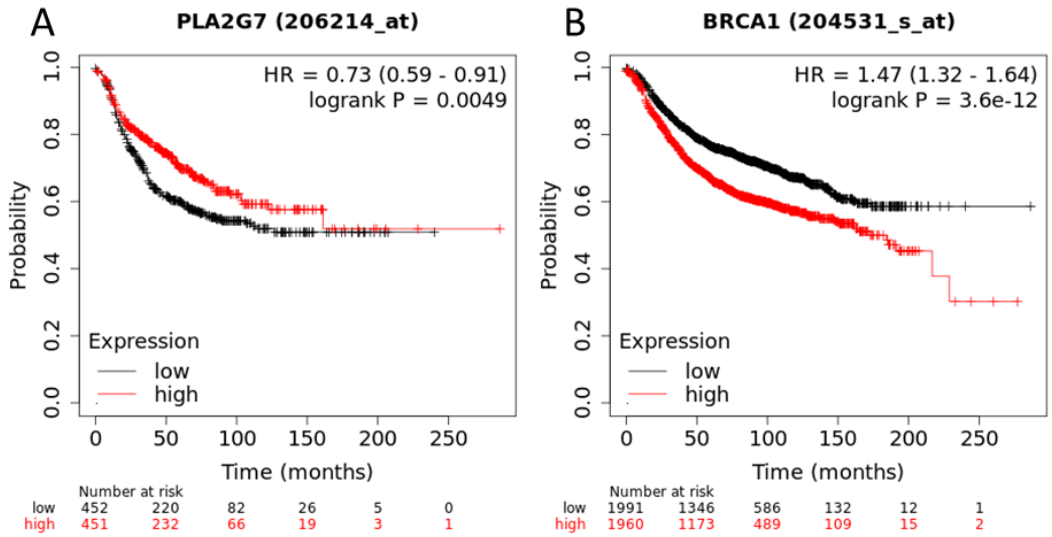


Figure 9. Survival analysis was performed by using the online database https://kmplot.com/analysis/(Figure 9 A, B) with mRNA (gene chip) detection results in breast cancer samples. A showed that the higher expression of PLA2G7 brings a relatively better prognosis in grade 3($p<0.01$), B showed *BRCA1* mutation patients (lower *BRCA1* expression) have a better OS compare with the non-*BRCA1* mutant group ($p<0.001$).

4.2 Only *BRCA1* mutated cell lines showed relevant expression of PLA2G7/PAF-AH

The basal mRNA (Figure 10A) and protein (Figure 10B) expression of PLA2G7/PAF-AH in five breast cancer cell lines were compared with the MCF10A cell line from breast epithelial. PLA2G7 in mRNA level ($p<0.01$) and PAF-AH in protein level ($p<0.001$) were observed with highly expressed in *BRCA1* mutant cell line HCC1937, compared to the other malignant and breast epithelial cell lines. The results of qPCR and Western blot were consistent and supported the conclusions from IHC.

The basal mRNA and protein expression of PLA2G7 in four ovarian cancer cell lines were compared with the cell line HOSEpiC from benign ovarian epithelial. PLA2G7 expression both on mRNA level ($p<0.05$; Figure 10C) and on protein level ($p<0.05$; Figure 10D) was significantly increased in *BRCA1* mutant ovarian cancer cell line UWB1.289 compared to HOSEpiC and other ovarian cancer cell lines. The results derived from qPCR and western blot are consistent with the results of IHC.

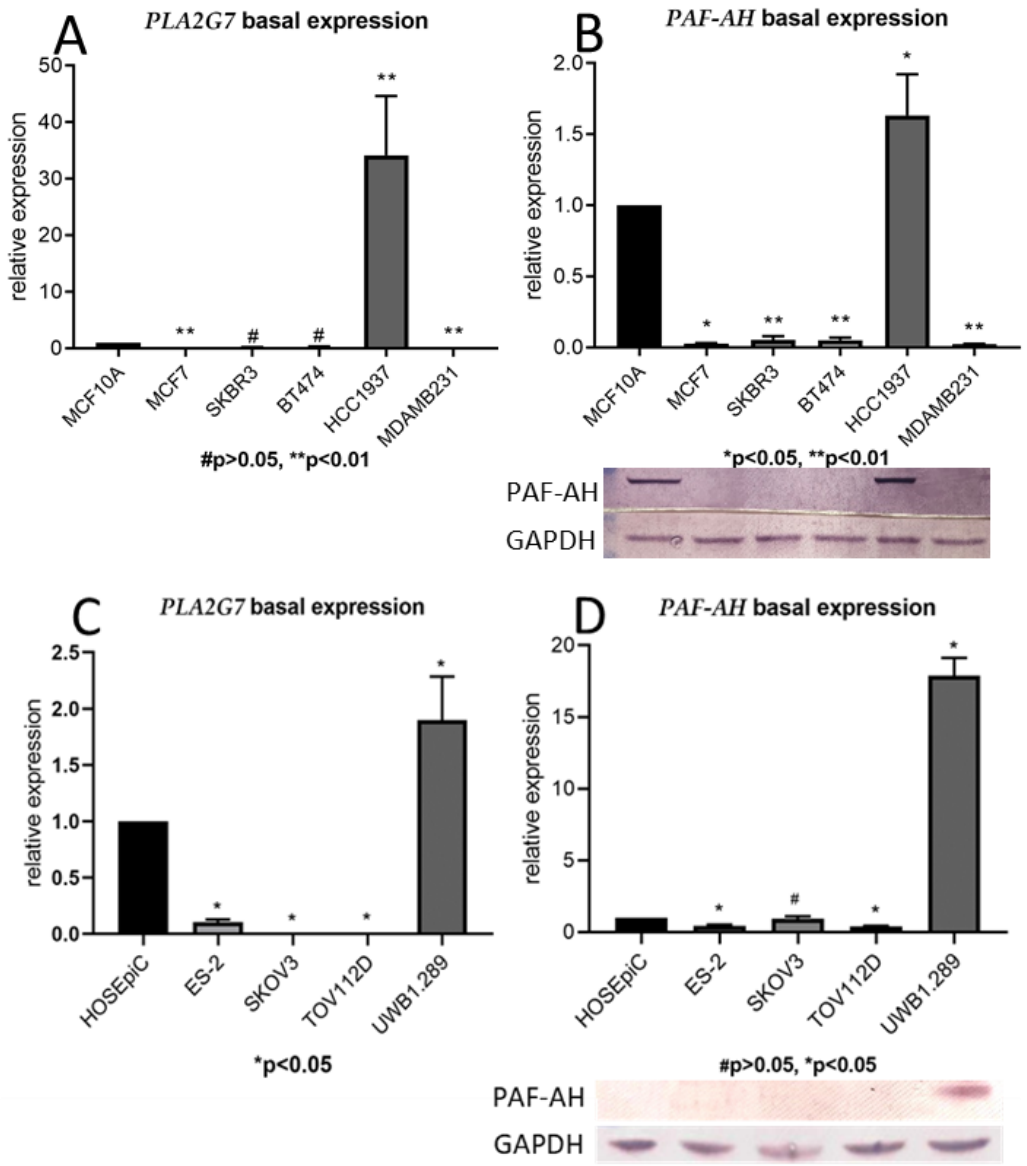


Figure 10. Expression of basal PLA2G7 (A) and PAF-AH (B) in five breast cancer cell lines were compared to the breast epithelial cell line MCF10A. Only the *BRCA1* mutant breast cancer cell line HCC1937 showed a relevant high expression of PLA2G7/PAF-AH. Only the *BRCA1* mutant ovarian cancer cell line UWB1.289 shows a relevant expression of PLA2G7/PAF-AH. Basal mRNA (qPCR; C) and protein (western blot analysis; D) expression of PLA2G7 in four ovarian cancer cell lines were compared with the benign ovarian cell line HOSEpiC.

4.3 PLA2G7 silencing was well established in *BRCA1* negative cell lines

To establishing the downregulation of PLA2G7 successfully, four different sequences of small interfering RNA-PLA2G7(S1-S4) were respectively transfected into HCC1937 and UWB1.289 with different time gradient, respectively. qPCR and western blot were conducted to quantify

the PLA2G7/PAF-AH expression. The best knockdown of PLA2G7 was established after an incubation time of 60h for HCC1937($p<0.01$; Figure 11A), and 36h for UWB1.289($p<0.01$; Figure 11C). Western blot analysis also significantly decreased expression in protein ($p<0.05$; Figure 11B, D), which concordant the downregulation in RNA level. Both were optimally downregulated in Sequence1.

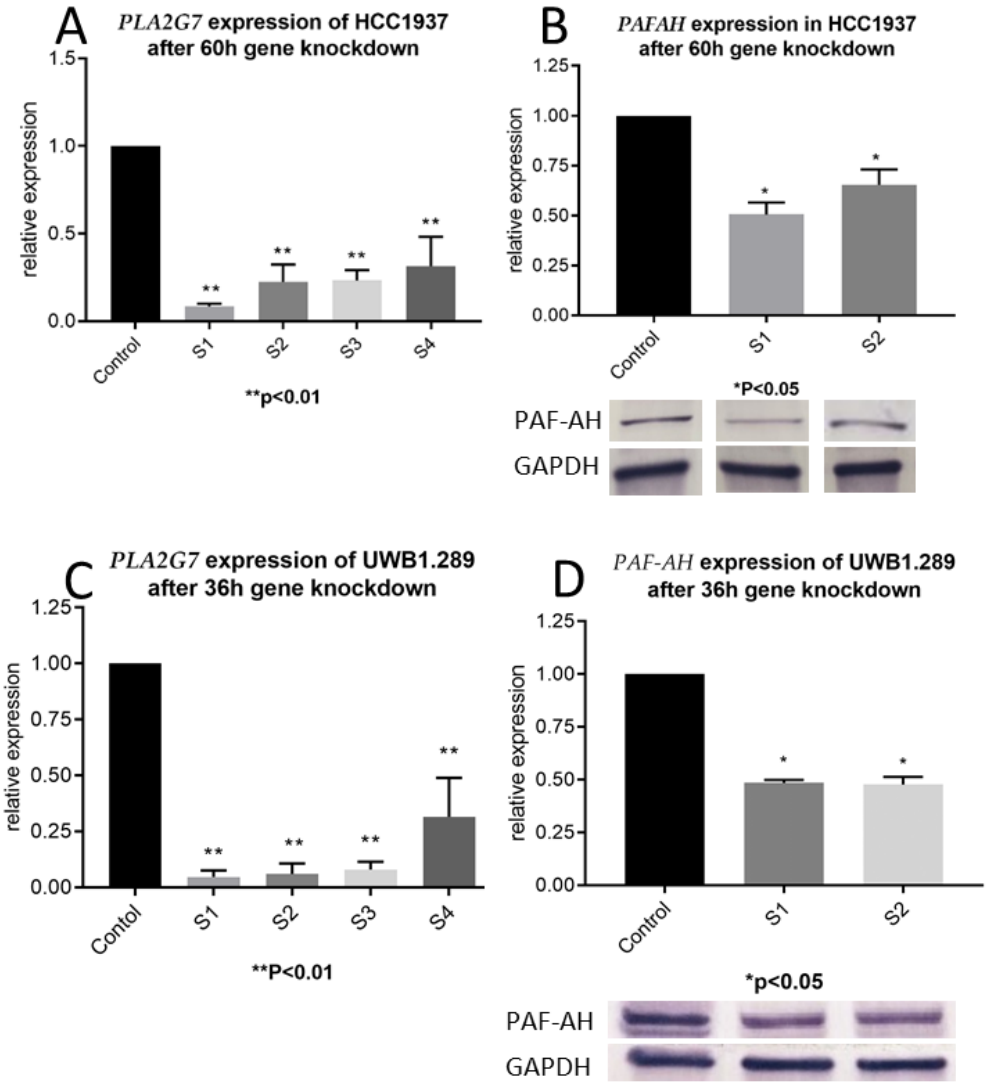


Figure 11: PLA2G7 expression after gene knockdown

4.4 PLA2G7 knockdown enhances viability, proliferation and motility of *BRCA1* negative cell lines

As degradation enzyme of PAF, we hypothesized that PAF-AH is a protective factor in ovarian cancer biology. Concordantly, the IHC results showed a positive association of PAF-AH expression with OS. To

characterize the cellular function of PAF-AH, viability, proliferation, and migration of UWB1.289 cells were investigated.

As shown in Figure 12A, the viability of HCC1937 increased after the PLA2G7 silencing. Furthermore, compare with the control group, HCC1937 transfected with siRNA-PLA2G7 also showed significantly higher absorbance (Figure 12B), which indicated ascend in the proliferation of breast cancer after gene silencing. Wound healing results exhibited after transfection cell migration ability was significantly activated (Figure 12C & D group). In summary, these results demonstrated that PLA2G7 silencing leads to cancer progression with the viability, proliferation, and migration in breast cancer. This conclusion was consistented with our hypothesis that PAF-AH as a protective factor in breast cancer, and concordantly with the results of our previous study on PAF-AH in ovarian cancer.

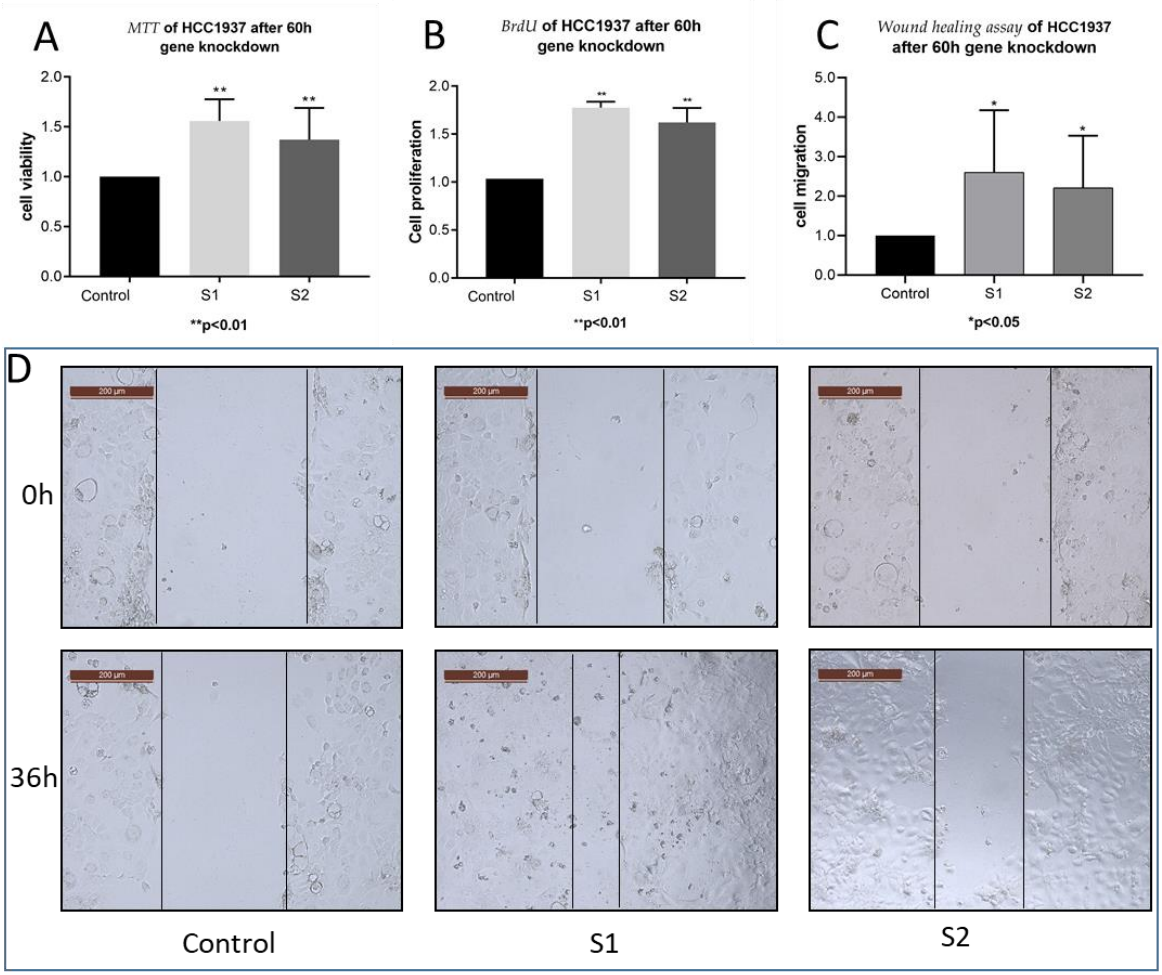


Figure 12. MTT result(A) showed after 60 hours of transfection by SiRNA-PLA2G7, the viability of HCC1937 significantly increased($p<0.01$). BrdU result(B) also exhibited gene knockdown groups were obviously ascending in proliferation compared to the control($p<0.01$). Wound healing assay (C & D group) result indicated the migration ability of PLA2G7 downregulated HCC1937 is significantly activated compared to the control group($p<0.05$).

Results of UWB1.289 cells under PLA2G7 knockdown with the siRNAs described above were compared with the results of an untreated control group (pseudo-knockdown with scrambled siRNA). As shown in Figure 13A, viability of UWB1.289 cells was increased by PLA2G7 silencing. Furthermore, PLA2G7 downregulated UWB1.289 cells exhibited significantly higher proliferation rates in comparison to the control group, which indicates that PLA2G7 knockdown induces the proliferation of EOC cells (Figure 13B). Results from the scratching assay show that after transfection of PLA2G7 siRNA the migration ability of UWB1.289 was significantly activated compared to the control group (Figure 13C). To sum up, the results indicate that PLA2G7 silencing causes cancer progression by an activation of viability, proliferation and migration.

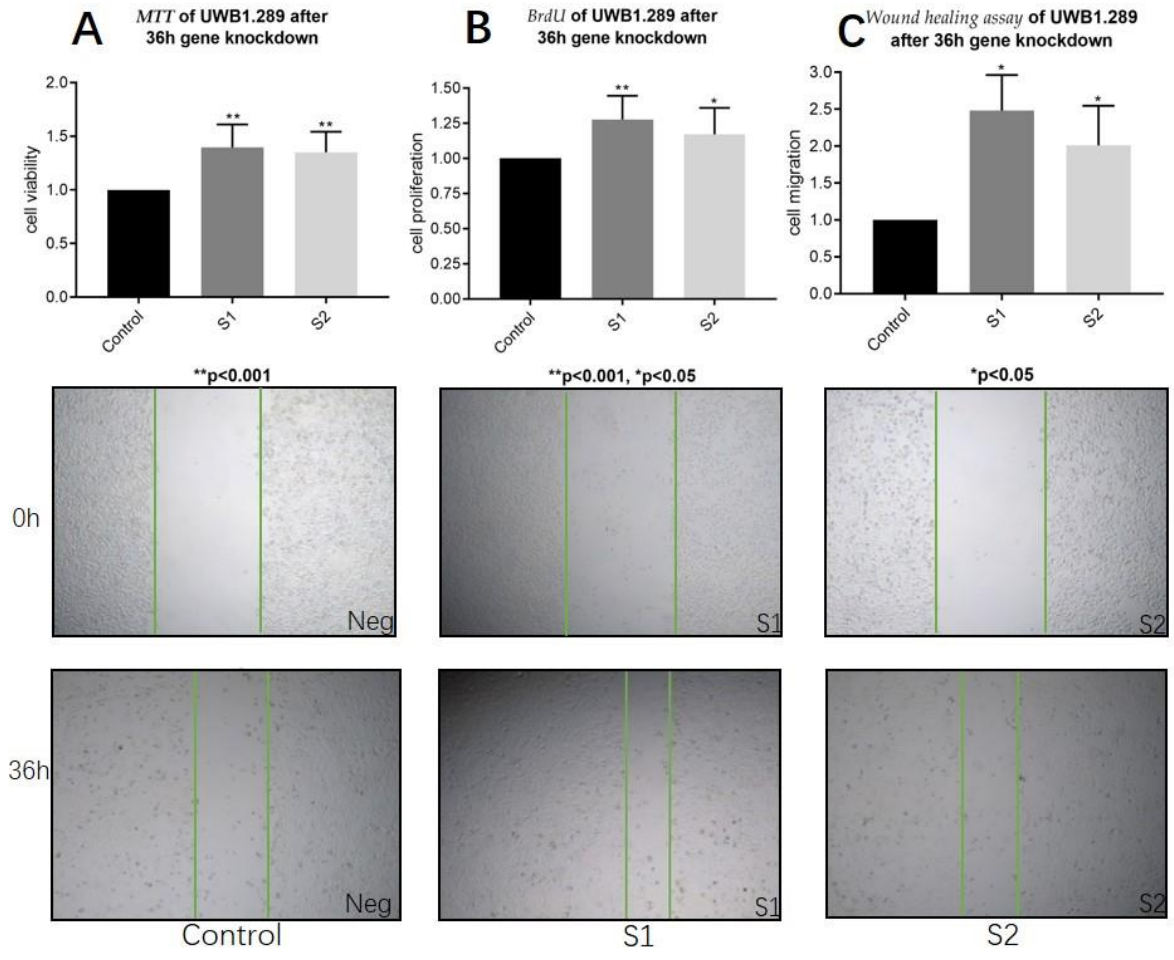


Figure 13: PLA2G7 silencing favors cancer progression by an activation of viability, proliferation and migration. MTT results show that after 36 hours siRNA (sequence S1 and S2) knockdown of PLA2G7 the viability of UWB1.289 increased significantly (A; $p<0.001$). DNA incorporation of BrdU was also significantly higher in the PLA2G7 downregulated group, indicating an increasing proliferation rate (B). The scratching assay proofed that the migration ability of PLA2G7 downregulated UWB1.289 cells is significantly activated compared to the control group (C; $p<0.05$).

4.4 The cellular distribution pattern of β-catenin changed by PLA2G7 knockdown from membrane to nucleus

After demonstrating the functional impact of PLA2G7 and its protein PAF-AH on cancer progression, we aimed to validate how PLA2G7 affects the Wnt/β-catenin signaling pathway. Based on significant correlation between PAF-AH and β-catenin found in IHC, a series of ICCs was carried out to prove an interplay of PAF-AH and β-catenin. HCC1937 cell line and UWB1.289 cell line were used respectively in each project. As expected, PAF-AH staining was downregulated after knockdown of PLA2G7 compared to the control with pseudo-knockdown (Figure 14). Interestingly, also the distribution of β-catenin changed by PLA2G7 knockdown. While membrane expression was weakened compared with the control group, the nuclear expression was enhanced.

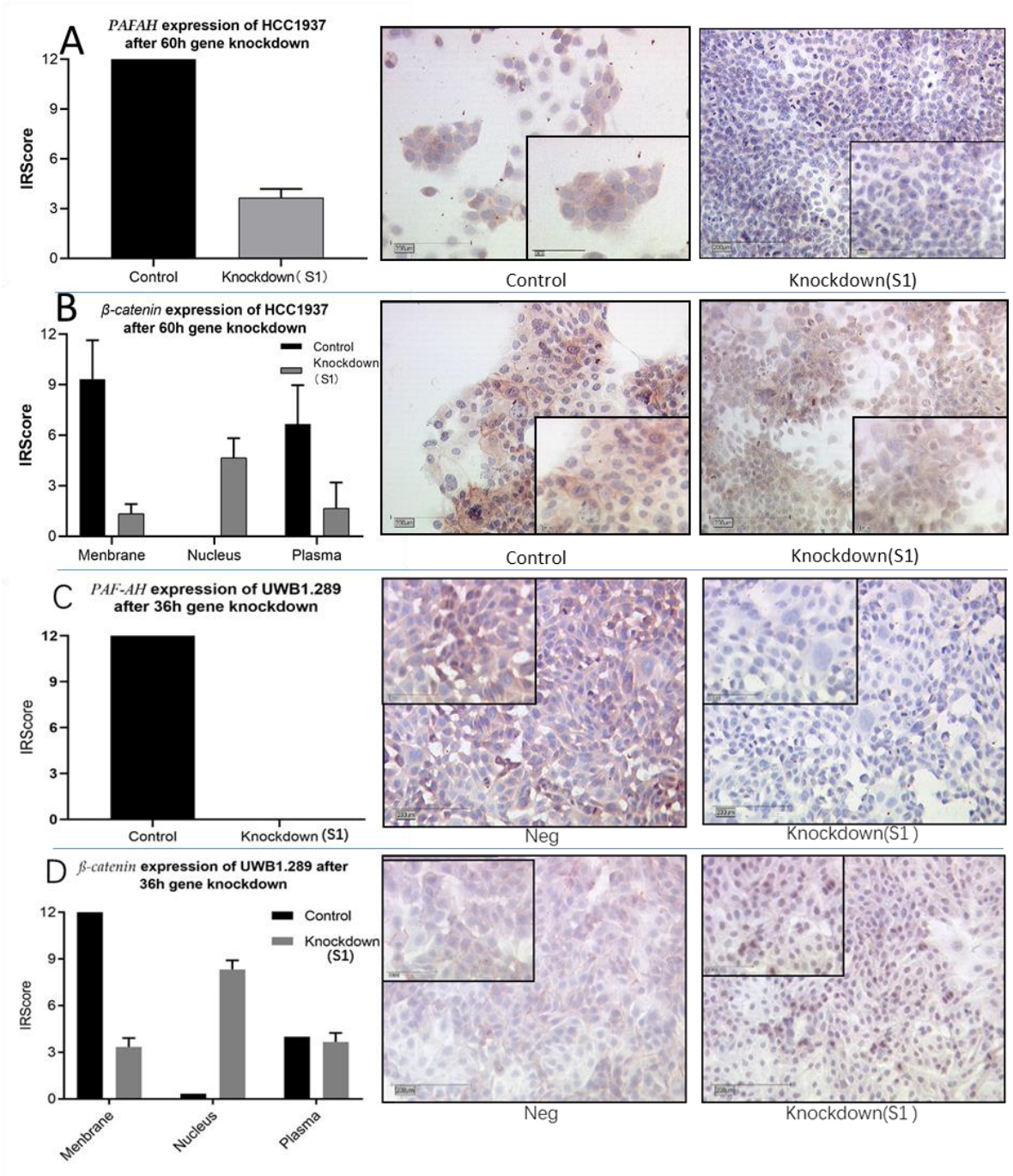


Figure 14: PAF-AH staining showed after 60 hours transfection with different siRNA-PLA2G7 in HCC1937, the expression of PAF-AH successfully suppressed (A). After the transfection of siRNA-PLA2G7, the expression of membrane ß-catenin was down regulated and nuclear expression increased (B). PLA2G7 silencing also caused a shift of β-catenin from membrane to nucleus in UWB1.289. ICC staining of PAF-AH and β-catenin changes after 36h silencing of PLA2G7 with siRNA (representative pictures are shown for sequence 1). The expression of PAF-AH was downregulated as expected (C). The distribution of β-catenin transferred from mainly in membrane to nucleus by PLA2G7 knockdown(D).

5 Discussion

5.1 The relationship between *BRCA1* mutation and high expression of PLA2G7/PAF-AH

During our experiments, we observed that the association between the high PLA2G7/PAF-AH expression and *BRCA1* mutation was consistent among different cancer entities. In the breast cancer project, we revealed a significant correlation between *BRCA1* mutation and high expression of PAF-AH ($p<0.05$), especially the high PAF-AH expression in the nucleus ($p<0.01$). Subsequently, five different breast cancer cell lines were tested to compare with a breast epithelial cell line using qPCR and Western blot analysis. The results were consistent with our findings in IHC that only *BRCA1* mutant cell line exhibited the high expression of PLA2G7/PAF-AH($p<0.01$).

In addition, in the ovarian cancer project, we also found a significant connection between *BRCA1* mutation cases and high PAF-AH expression ($p<0.001$). Moreover, the specimens with unknown *BRCA* status were compared with patients with mutations. Results showed that patients with *BRCA1* mutation or *BRCA1 + 2* mutations expressed a higher level of PAF-AH ($p<0.01$). Finally, the serum concentration of PAF-AH in blood samples of EOC patients was tested. The samples from patients with *BRCA1* mutations were compared with *BRCA* WT, and we found that *BRCA1* mutation carriers have had a significantly higher concentration of serum PAF-AH concentration($p=0.012$). Consistent results were also obtained with in-vitro experiments. *PLA2G7*/PAF-AH only showed significantly up-regulated in *BRCA1* mutant cell line compared with ovarian epithelial cells and other ovarian cancer cell lines ($p<0.05$).

So far, there is no direct evidence concerning the relationship between *BRCA1* and *PLA2G7*. Only one study has shown that *BRCA1*-defected mouse embryonic fibroblasts (MEF-*BRCA1*$^{\Delta/\Delta}$) express higher levels of *PLA2G7* compared with the wild-type counterparts (MEF-*BRCA1*$^{+/+}$) [222]. However, there is also some indirect evidence that *BRCA1* deficiency may increase the expression of PLA2G7 through a series of regulations. Gorrini et al. have reported that *BRCA1* regulates Nrf2-dependent antioxidant signaling by facilitating its stability and activation [223]. Besides, phospholipase A2 (also known as PLA2G7) is controlled by NRF2 in lipid catabolism due to its lipid properties[224-226]. In our study, we observed and confirmed

that a significant higher PLA2G7 / PAF-AH was only expressed in *BRCA1* mutant tissues and cells. Therefore, we concluded that PAF-AH could be used as a prognostic biomarker for *BRCA1* mutation carriers.

5.2 Highly expressed PAF-AH is associated with better prognosis

After the significant relationship between PAF-AH and *BRCA1* was confirmed, we also revealing that high PAF-AH expression was associated with a better prognosis in these two projects. As the information on survival time and status was missing in the breast cancer project, we did not perform the survival analysis. We explored the online database https://kmplot.com/analysis/mRNA (gene chip) for survival analysis. KM-plot showed that high expression of *PLA2G7* reflected a better prognosis in grade 3 patients ($p < 0.01$), while patients with *BRCA1* mutations (lower *BRCA1* expression) showed a better OS compared with non-*BRCA1* mutation carriers ($p < 0.001$). Subsequently, in-vitro experiments were carried out by using *BRCA1* mutant cells with knockdown of *PLA2G7*, and the results confirmed that depletion of *PLA2G7* enhanced viability ($p<0.01$), proliferation ($p< 0.01$), and motility ($p<0.05$) of HCC1937 cells compared with the control group (pseudo-knockdown with scrambled siRNA).

In the ovarian cancer project, we performed survival analysis based on survival time and status. The KM-plot showed that the high expression of PAF-AH (IRScore≥3; A) in tumors was significantly associated with the prolonged OS ($p<0.05$). Multivariate Cox regression model analysis confirmed in Age (>60 vs. ≤60 years, $p=0.039$), FIGO stage (III/IV vs. I/II, $p =0.004$), grading (high/G2-3 vs. low/G1, $p=0.002$), and tumoral PAF-AH expression (high vs. low, $p=0.021$) were independent factors for prognosis of OS in this cohort. Subsequently, the functional changes after PLA2G7 knockdown were also verified in MTT, BrdU, and scratch experiments. The results confirmed that viability ($p<0.001$), proliferation ($p<0.001$) and motility ($p<0.05$) of UWB1.289 cells were increased after PLA2G7 silencing.

We have explained the dual effects of PAF-AH on the disease from four perspectives before. According to our results, we exhibited the protective mechanism of PAF-AH on breast and ovarian cancer. And we summarized that, in addition to its connatural anti-inflammatory

effects, PAF-AH achieved its protective functions by interacting with the Wnt/β-catenin signaling pathway. We will comprehensively discuss this part together with the results of the next part.

5.3 Down-regulation of PAF-AH activates the Wnt/β-catenin signaling pathway

We also obtained and verified the negative regulation effect with PAF-AH on the Wnt/β-catenin signaling pathway in the two projects. In the ovarian cancer project, we selected the two representative proteins, β-catenin, and pGSK3β, of the canonical Wnt/β-catenin pathway in our research. According to the results of IHC, a strong positive correlation existed between nuclear/cytoplasmic PAF-AH, cytoplasmic pGSK3β ($p<0.001$), and membrane β-catenin ($p<0.001$). The high expression of the total PAF-AH ($p<0.05$), cytoplasmic pGSK3β($p<0.05$), and membrane β-catenin ($p<0.05$) were related to prolonged OS. Moreover, the combined survival analysis showed an even longer survival time ($p=0.044$). Besides, the multivariate Cox regression model results showed that Cytoplasmic pGSK3β ($p = 0.645$) and membranous β-catenin ($p = 0.745$) were not independent factors for prognosis of OS. Based on the correlation between PAF-AH and β-catenin found in IHC, a series of ICCs were carried out to prove the interplay between PAF-AH and β-catenin. As expected, compared with the control group, the staining of PAF-AH was reduced after knockdown of PLA2G7, while the membrane expression of β-catenin was reduced and the nuclear β-catenin was enhanced.

In the breast cancer project, we selected β-catenin to determine the relationship between the expression of PAF-AH and the classic Wnt/β-catenin pathway. Based on the results of IHC, a strong positive correlation existed between nuclear/cytoplasmic PAF-AH and membrane β-catenin. High expression of total PAF-AH protein and membrane β-catenin were related to prolonged OS. Accordingly, a series of ICCs were performed to verify the interaction between PAF-AH and β-catenin. Compared with the control group, the staining of PAF-AH was decreased after PLA2G7 knockdown, while the membrane expression of β-catenin was weakened, the nuclear expression of β-catenin was enhanced. These results were consistent with the findings in ovarian cancer.

In a study of platelet-activating factor receptor antagonists on the development of colon

cancer in rats, the IHC results of β-catenin have revealed that the differentiation of β-catenin distribution may reflect the malignant potential of the colon cancer crypts[227]. Another study on the invasion and metastasis of hepatocellular carcinoma has also shown that the cell distribution pattern of β-catenin (nuclear translocation) plays an active role in promoting metastasis [228]. These results are consistent with our findings. The high expression of membrane β-catenin implied a better prognosis compared with the high nuclear expression. We also observed that the benign cells (control cell lines) showed complete membrane expression of β-catenin. *BRCA1* mutation carriers with high expression of PAF-AH, which was associated a better prognosis, also showed high membrane expression of β-catenin.

5.4 PAF-AH affects Wnt/β-catenin signaling pathway through PAF(PAFR)

We summarized all the results and evidence from the literature in the two projects and put forward the following points to illustrate the possible mechanism of PAF-AH affected Wnt/β-catenin signaling pathway.

5.4.1 PAF-AH regulates Wnt/β-catenin signaling pathway by interfering with the GSK3β activity

PAF-AH regulates the PAF-PAFR signaling by hydrolysis, and the phosphorylation cascades caused by this signaling interfere with the activity of GSK3β. Studies have revealed that the GSK-3 activity is reduced after PAF treatment, and this down-regulation is related to PI3K/AKT activation [229, 230]. Cytoplasm GSK3β plays an essential effect in regulating β-catenin degradation, and both are key proteins in regulating the Wnt/β-catenin signaling pathway. When GSK3β is inactivated by phosphorylating at S9, the ubiquitination as well as proteasomal degradation of β-catenin fail. Moreover, β-catenin is accumulated and transferred into the nucleus[231], leading to the Wnt/β-catenin activation and its downstream gene expression. In this way, PAF-AH affected the Wnt/β-catenin activation (by affected the activities of GSK3β and β-catenin) by regulating the signaling of PAF-PAFR. (Figure 15)

5.4.2 PAF-PAFR regulates Wnt/β-catenin signaling pathway by the crosstalk with the nuclear translocation of STAT3

Lukashova et al. used unstimulated and PAF-stimulated MonoMac-1 cells for immunoprecipitation. The results show that PAF-PAFR signaling caused the tyrosine phosphorylation of the transcription factors of the STAT family, eventually leading to the phosphorylated STATs translocated to the nucleus. STAT3 has completed the phosphorylated and is transferred from the cytoplasm to the nucleus after PAF stimulation [232]. The nuclear translocation of STAT3 increased the transcription of β-catenin, thereby up-regulating β-catenin and further activating the Wnt pathway. Eventually, increased nuclear STAT3 resulted in the enhancement of survival, migration, and cell proliferation by crosstalk with Wnt/β-catenin signaling pathway [233, 234]. (Figure 15)

5.4.3 PAF acts on Wnt/β-catenin downstream genes

PAF acted on Wnt/β-catenin downstream genes by activated phosphorylation to promote cancer progression. Several studies confirmed that Src/FAK, FAK/STAT, and AKT are associated with cell carcinogenesis, tumor growth, angiogenesis, and metastasis [170, 222, 230, 235]. PAF-AH regulates the activation of the phosphorylation cascade by hydrolyzing PAF and modulating PAFR agonists[236]. This phosphorylation usually represents the early response to mitogenic induction, which activates a series of target genes downstream of Wnt/β-catenin signaling, including Src/FAK, FAK/STAT and AKT. Besides, the inactivation of GSK3β caused by AKT could activate Wnt/β-catenin signaling pathway and increase migration by causing Rac-1 activation and Rho-A inactivation [230, 237].(Figure 15)

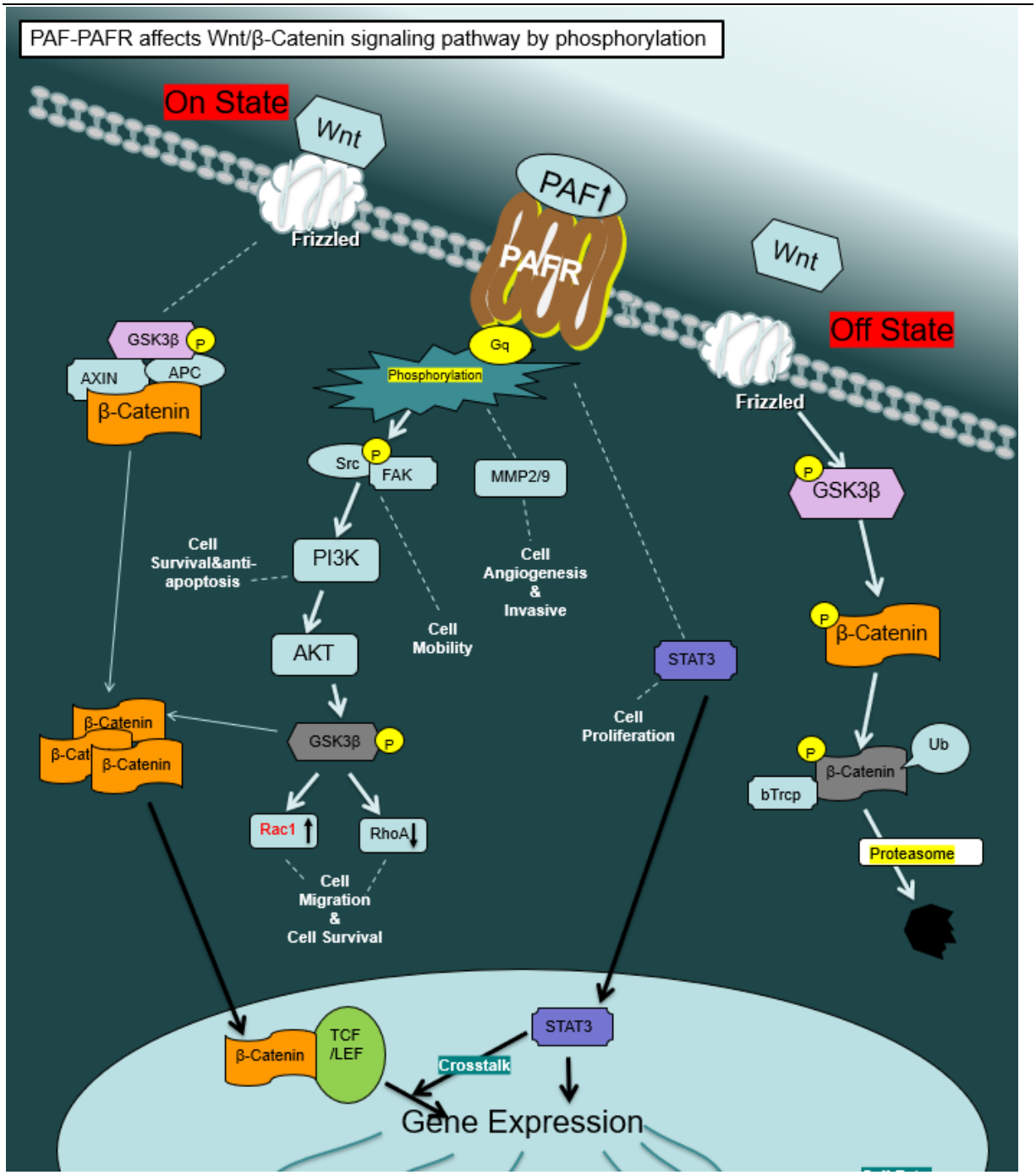


Figure 15: On state of Wnt/β-catenin signaling (on the left). Off state of Wnt/β-catenin signaling and degradation of β-catenin (on the right). PAF-PAFR signaling and cell functions changed after Phosphorylation activation(middle).

5.5 Other evidence that PAF-AH affects tumor progression

Interestingly, a study on KS cells has shown that the endogenous expression of adherens junctions (AJs) components (β-catenin, α-catenin, and VE-cadherin) are down-regulated in

PAF-stimulated cells. The destruction of adhesion of cadherin-catenin cell causes the separation of adjacent tumor cells, thereby increasing mobility and aggressiveness [238]. Meanwhile, PAF-initiated events of β-catenin up-regulate the phosphorylation pathway, causing β-catenin phosphorylation. The weakened connection between cadherin binding increases free β-catenin. The increase of free β-catenin can be degraded through the ubiquitin/proteasome pathway by GSK3β, and can also be transferred to the nucleus to activate the Wnt signaling pathway. However, this research did not detect the expression of nuclear β-catenin. The increase of free β-catenin and the degradation of β-catenin by ubiquitination are also contradictory factors.

Another impressive experimental result based on a mouse model has showed that the catalytic subunits of intracellular PAF-AH isoform IB can directly modulate the Wnt signaling pathway[239]. Although PAF-AH isoforms show low sequence homology, they also have the same functions in PAF catabolism and oxidative fragmentation of phospholipids [55, 67]. It means that in addition to affecting the Wnt signaling pathway through PAF, as the plasma isoform, the functional subunits of PAF-AH might also have similarities in regulating the Wnt signaling pathway.

5.6 Evidence that Wnt pathway may affect PAF-AH expression

The androgens regulate the release of PAF through Wnt/β-catenin-dependent pathways. The β-catenin silencing in PC-3 cells significantly reduces the expression of PAF induced by DHT, supporting the critical role of β-catenin in regulating PAF in PC-3 cells. These results indicate that β-catenin interacts with the androgen receptor signaling pathway to regulate PAF [240]. Zhang et al. announced that the expression of PTAFR is increased in *BRCA1* mutation cell lines and tissue specimens of *BRCA1* mutation carriers. Moreover, they have demonstrated that PAF/PTAFR-mediated malignant transition of non-malignant ovarian epithelial cells with *BRCA1* mutations induces proliferation and anti-apoptosis through FAK/STAT phosphorylation[151]. It also shows the complexity of the relationship between Wnt/β-catenin and PAF. Furthermore, the PAF-AH up-regulating may be correlated with the Wnt/β-catenin signaling and PAF expression.

However, the molecular interaction between PAF-AH and Wnt/β-catenin signaling pathway remains unclear which needs to be specified in future studies. The interplay between *BRCA1* and Wnt signaling pathway has been described in research by Wu et al. [231] . They have shown that epigenetic repression of *BRCA1* by Wnt effector Slug would lead to an inverse correlative association between Wnt signaling and *BRCA1* expression in basal-like breast cancer [231]. Moreover, a combination of oncogenic pathways for gastric cancer has also revealed significantly different pathways (such as SRC and β-catenin) and the close relationship between HDAC inhibition and *BRCA1*. They have predicted that the activation pattern of this pathway may imply a functional interaction between these pathways[241]. Based on the experimental results of ovarian cancer and breast cancer, we found that PAF-AH was highly expressed in *BRCA1* mutant cells and confirmed the relationship between PAF-AH and the canonical Wnt/β-catenin pathway. Nevertheless, we could not exclude the participation or crosstalk of other signaling pathways or regulatory factors, which led to the observed phenotype.

6 Summary

BRCA1 mutation carriers have a higher risk to suffer from cancer than wild-type *BRCA1*, especially those with HBOC (Hereditary Breast and Ovarian Cancer). However, among the breast and ovarian cancer patients, *BRCA1* mutation carriers had a better prognosis and a longer survival time. The mechanism underlying this contradiction remains unclear. Besides, there was no non-invasive biomarker to predict the incidence and prognosis of *BRCA1* carriers.

Previous researches have announced that the Wnt signaling pathway takes part in a crucial role in tumor progression and tumorigenesis. Investigations have also clarified the significance of the abnormally activated Wnt pathway for functional changes of cells. Studies have found that PAF works as an essential part in promoting the aberrant activation of the Wnt pathway and up-regulating the expressions of oncogenes downstream of Wnt signaling. However, the role of PAF-AH/*PLA2G7*, known as the PAF hydrolase, is still unclear. Interestingly, PAF-AH/*PLA2G7* is a protein/gene full of controversy and contradictions, and it has the functions of both suppressing and promoting cancer. Therefore, we investigated plasma PAF-AH/*PLA2G7* in our projects to prove its significance in *BRCA1* mutation carriers/patients by ex-vivo and in-vitro experiments.

We chose breast and ovarian cancer in our research, which are the most common tumor types associated with *BRCA1* mutations in the department of obstetrics and gynecology. First, immunohistochemistry was carried out to detect the PAF-AH/*PLA2G7* expression in the pathological specimens. The results indicated that a higher expression of PAF-AH was highly associated with the *BRCA1* mutation and associated with a better prognosis. The ELISA of serum samples also showed that *BRCA1* mutation carriers had a significantly higher expression of serum PAF-AH. Besides, in-vitro experiments were carried out in breast cancer and ovarian cancer cell lines for detections of PAF-AH and *PLA2G7*. Results confirmed that only *BRCA1* mutant cell lines had significantly higher PAF-AH/*PLA2G7* expression. Moreover, the *BRCA1* mutant (breast cancer and ovarian cancer) cell lines after knockdown of *PLA2G7* were used to test cell migration, cell proliferation, and cell viability. The results revealed that after the knockdown of *PLA2G7*, the viability, proliferation, and migration of cells were significantly enhanced. These experimental data in the breast cancer project and ovarian cancer project were highly consistent, further confirming our conclusion by IHC that PAF-AH/*PLA2G7* was a

protective factor for breast and ovarian cancer which associated with a better prognosis.

We further investigated how PAF-AH worked as a protective factor. We checked the correlations with representative proteins of the Wnt signaling pathway. In breast and ovarian cancer cell lines, we detected a correlation with β-catenin, and a correlation with GSK3β was detected in ovarian cancer. IHC results exhibited that the expressions of membrane β-catenin and cytoplasmic pGSK3β were highly correlated with a higher expression of PAF-AH, which were also significantly correlated with a better OS. In further experiments, we performed ICC to identify the high expression of membrane β-catenin in *BRCA1* mutant cell lines. As the expression of the β-catenin membrane disappeared, the nuclear expression of β-catenin was up-regulated after the knockdown of *PLA2G7*. The results were consistent in both breast cancer and ovarian cancer cell lines.

Taken together, these results confirmed our hypothesis that PAF-AH was a protective biomarker and associated with a better prognosis. It played a protective role by negatively regulating the activity of Wnt/β-catenin signaling pathway.

Finally, we recommend evaluating PAF-AH as a putative biomarker in *BRCA1* mutant breast and ovarian cancer in further studies.

7 Zusammenfassung

Frauen mit *BRCA1*-pathogenen Varianten haben ein höheres Krebsrisiko als *BRCA1*-Wildtyp, insbesondere HBOC-assoziierte Tumoren (erblicher Brust- und Eierstockkrebs). *BRCA1*-Träger haben jedoch statistisch gesehen eine bessere Prognose und eine längere Überlebenszeit als die durchschnittlichen Patienten, die an Brust- und / oder Eierstockkrebs leiden. Der Mechanismus dieses Widerspruchs bleibt unklar, und für *BRCA1*-Träger ist noch kein nicht-invasiver Biomarker zur Vorhersage der Inzidenz und Prognose verfügbar.

Der Wnt-Signalweg ist maßgeblich an der Tumorentstehung und Tumorprogression beteiligt, und der aktivierte Wnt-Signalweg ist an Veränderungen der Zellfunktion beteiligt. PAF spielt eine wesentliche Rolle bei der Förderung der pathologischen Aktivierung des Wnt-Signalwegs und der Aktivierung von Onkogenen downstream des Signalwegs. Die Rolle von PAF-AH / *PLA2G7*, bekannt als PAF-Hydrolase, bei der Pathogenese von Brust- und Eierstockkrebs ist noch unklar. Interessanterweise ist PAF-AH / *PLA2G7* ein Protein / Gen voller Kontroversen und Widersprüche, welches sowohl pro- als auch antionkogene Eigenschaften besitzt. Bis dato gab es keine Untersuchungen, die den Zusammenhang zwischen *BRCA1*-Mutation und PAF-AH / *PLA2G7* analysiert haben, um zu ergründen, ob die bessere Prognose von Brustkrebs- und Eierstockkrebspatientinnen mit der *BRCA1*-Mutation mit PAF-AH zusammenhängt. In unserem Projekt verwendeten wir sowohl Ex vivo- als auch In vitro-Experimente, um die Relevanz von PAF-AH / *PLA2G7* bei *BRCA1*-Mutationsträgern / Patienten zu untersuchen.

Zunächst wurden immunhistochemische Untersuchungen durchgeführt um die Expression von PAF-AH beim Mamma- und Ovarialkarzinom zu evaluieren. Die Ergebnisse zeigten, dass eine hohe PAF-AH-Expression mit der *BRCA1*-Mutation korrelierte und mit einer besseren Prognose verbunden war. Der ELISA-Test von Serumproben zeigte, dass Träger mit der *BRCA1*-Mutation einen signifikant höheren PAF-AH-Spiegel im Serum aufwiesen. Außerdem wurden In vitro-Experimente in Brustkrebs- und Eierstockkrebs-Zelllinien durchgeführt. Die Ergebnisse bestätigten, dass nur *BRCA1*-Mutantenzelllinien eine signifikant hohe PAF-AH / *PLA2G7*-Expression aufwiesen. Dann wurden die *BRCA1*-Mutanten-Zelllinien (Brustkrebs und Eierstockkrebs) nach siRNA-knockdown des *PLA2G7*-Gens auf Zellviabilität, Zellproliferation und Zellmigration untersucht. Die Ergebnisse zeigten, dass nach siRNA-knockdown von *PLA2G7* die Zellviabilität, die Zellproliferation und die Zellmigration signifikant verbessert

waren. Da sich diese Ergebnisse sowohl bei Brustkrebs- als auch Eierstockkrebszellen zeigten, erhärtete dies unsere immunhistochemischen Untersuchungen, dass es sich bei PAF-AH um einen positiven Prognosefaktor handelt.

Wir haben weiter untersucht, wie PAF-AH als Schutzfaktor wirkt. Dazu haben wir die Korrealtion mit Markern des Wnt-Signalwegs untersucht. In Brust- und Eierstockkrebs-Zelllinien wurde die Expression von β-catenin nachgewiesen, und die Expression von GSK3β wurde beim Eierstockkrebs gezeigt. Die IHC-Ergebnisse zeigten, dass die β-catenin- und cytoplasmatischen pGSK3β-Expression mit einer höheren PAF-AH-Expression korrelierten, was wiederum signifikant mit einem besseren OS assoziiert war. In weiteren Experimenten führten wir eine Immunzytochemie durch, um die β-catenin-Expression in mutierten *BRCA1*-Zelllinien nachzuweisen. Nach siRNA-knockdown von PLA2G7 kam es zu einer Verschiebung der zelluläre Expression von β-catenin von der Membran in den Kern. Diese Ergebnisse zeigten sich sowohl in Brustkrebs- als auch den Eierstockkrebs-Zelllinien.

Zusammenfassend bestätigten unsere Ergebnisse die Hypothese, dass PAF-AH ein protektiver Faktor ist und mit einer besseren Prognose assoziiert ist. PAF-AH führt zu einer Inaktivierung des Wnt / β-catenin-Signalwegs.

Weitere Studien müssen überprüfen, ob PAF-AH als Biomarker für Patientinnen mit *BRCA1*-mutiertem Brust- oder Eierstockkrebs eingesetzt werden kann.

8 References

1. In, T., *Facts & figures 2019: US cancer death rate has dropped 27% in 25 years.* 2019.
2. Ferlay, J., et al., *Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008.* Int J Cancer, 2010. **127**(12): p. 2893-917.
3. Chen, C., et al., *Clinical characteristics and survival outcomes of patients with both primary breast cancer and primary ovarian cancer.* Medicine (Baltimore), 2020. **99**(32): p. e21560.
4. Eccles, S.A., et al., *Critical research gaps and translational priorities for the successful prevention and treatment of breast cancer.* Breast Cancer Res, 2013. **15**(5): p. R92.
5. Armes, J.E., et al., *The histologic phenotypes of breast carcinoma occurring before age 40 years in women with and without BRCA1 or BRCA2 germline mutations: a population-based study.* Cancer, 1998. **83**(11): p. 2335-45.
6. Diab, S.G., et al., *Tumor characteristics and clinical outcome of tubular and mucinous breast carcinomas.* J Clin Oncol, 1999. **17**(5): p. 1442-8.
7. Livi, L., et al., *Tubular carcinoma of the breast: outcome and loco-regional recurrence in 307 patients.* Eur J Surg Oncol, 2005. **31**(1): p. 9-12.
8. Southey, M.C., et al., *Morphological predictors of BRCA1 germline mutations in young women with breast cancer.* Br J Cancer, 2011. **104**(6): p. 903-9.
9. Oberaigner, W., et al., *Survival for ovarian cancer in Europe: the across-country variation did not shrink in the past decade.* Acta Oncol, 2012. **51**(4): p. 441-53.
10. Siegel, R., D. Naishadham, and A. Jemal, *Cancer statistics, 2013.* CA Cancer J Clin, 2013. **63**(1): p. 11-30.
11. Aletti, G.D., et al., *Ovarian cancer surgical resectability: relative impact of disease, patient status, and surgeon.* Gynecol Oncol, 2006. **100**(1): p. 33-7.
12. Dembo, A.J., et al., *Prognostic factors in patients with stage I epithelial ovarian cancer.* Obstet Gynecol, 1990. **75**(2): p. 263-73.
13. Vergote, I., et al., *Prognostic importance of degree of differentiation and cyst rupture in stage I invasive epithelial ovarian carcinoma.* Lancet, 2001. **357**(9251): p. 176-82.
14. Ferlay, J., D.M. Parkin, and E. Steliarova-Foucher, *Estimates of cancer incidence and mortality in Europe in 2008.* Eur J Cancer, 2010. **46**(4): p. 765-81.
15. James, C.R., et al., *BRCA1, a potential predictive biomarker in the treatment of breast cancer.* Oncologist, 2007. **12**(2): p. 142-50.
16. Sharma Bhai, P., et al., *Next-Generation Sequencing Reveals a Nonsense Mutation (p.Arg364Ter) in MRE11A Gene in an Indian Patient with Familial Breast Cancer.* Breast Care (Basel), 2017. **12**(2): p. 114-116.
17. Kadouri, L., et al., *A novel BRCA-1 mutation in Arab kindred from east Jerusalem with breast and ovarian cancer.* BMC Cancer, 2007. **7**: p. 14.
18. Kauff, N.D., et al., *Risk-reducing salpingo-oophorectomy in women with a BRCA1 or BRCA2 mutation.* N Engl J Med, 2002. **346**(21): p. 1609-15.
19. Lynch, H.T., et al., *Hereditary breast cancer: part I. Diagnosing hereditary breast cancer syndromes.* Breast J, 2008. **14**(1): p. 3-13.
20. Koumpis, C., et al., *Prevalence of BRCA1 and BRCA2 mutations in unselected breast cancer patients from Greece.* Hered Cancer Clin Pract, 2011. **9**(1): p. 10.
21. Metcalfe, K.A., J.L. Semple, and S.A. Narod, *Time to reconsider subcutaneous mastectomy for breast-cancer prevention?* Lancet Oncol, 2005. **6**(6): p. 431-4.
22. Hall, M.J., et al., *BRCA1 and BRCA2 mutations in women of different ethnicities undergoing*

testing for hereditary breast-ovarian cancer. Cancer, 2009. **115**(10): p. 2222-33.
23. Novaković, S., et al., *Novel BRCA1 and BRCA2 pathogenic mutations in Slovene hereditary breast and ovarian cancer families.* Int J Oncol, 2012. **41**(5): p. 1619-27.
24. Kuchenbaecker, K.B., et al., *Risks of Breast, Ovarian, and Contralateral Breast Cancer for BRCA1 and BRCA2 Mutation Carriers.* Jama, 2017. **317**(23): p. 2402-2416.
25. Li, S. and Y. Wei, *Association of HMGB1, BRCA1 and P62 expression in ovarian cancer and chemotherapy sensitivity.* Oncol Lett, 2018. **15**(6): p. 9572-9576.
26. Miron, A., et al., *Testing for hereditary breast and ovarian cancer in the southeastern United States.* Ann Surg, 2000. **231**(5): p. 624-34.
27. Bracci, M., et al., *BRCA1 and BRCA2 Gene Expression: Diurnal Variability and Influence of Shift Work.* Cancers (Basel), 2019. **11**(8).
28. Laufer-Amorim, R., et al., *Comprehensive Genomic Profiling of Androgen-Receptor-Negative Canine Prostate Cancer.* Int J Mol Sci, 2019. **20**(7).
29. Murthy, P. and F. Muggia, *Women's cancers: how the discovery of BRCA genes is driving current concepts of cancer biology and therapeutics.* Ecancermedicalscience, 2019. **13**: p. 904.
30. Lindahl, T. and D.E. Barnes, *Repair of endogenous DNA damage.* Cold Spring Harb Symp Quant Biol, 2000. **65**: p. 127-33.
31. Friedberg, E.C., *A brief history of the DNA repair field.* Cell Res, 2008. **18**(1): p. 3-7.
32. Wang, L., et al., *p62-mediated Selective autophagy endows virus-transformed cells with insusceptibility to DNA damage under oxidative stress.* PLoS Pathog, 2019. **15**(4): p. e1007541.
33. Yaneva, M., et al., *Non-homologous end joining, but not homologous recombination, enables survival for cells exposed to a histone deacetylase inhibitor.* Nucleic Acids Res, 2005. **33**(16): p. 5320-30.
34. Han, L., et al., *Mouse Rad1 deletion enhances susceptibility for skin tumor development.* Mol Cancer, 2010. **9**: p. 67.
35. Haber, J.E., *Deciphering the DNA Damage Response.* Cell, 2015. **162**(6): p. 1183-5.
36. Rothkamm, K., et al., *Pathways of DNA double-strand break repair during the mammalian cell cycle.* Mol Cell Biol, 2003. **23**(16): p. 5706-15.
37. Escribano-Díaz, C., et al., *A cell cycle-dependent regulatory circuit composed of 53BP1-RIF1 and BRCA1-CtIP controls DNA repair pathway choice.* Mol Cell, 2013. **49**(5): p. 872-83.
38. Tang, J., et al., *Acetylation limits 53BP1 association with damaged chromatin to promote homologous recombination.* Nat Struct Mol Biol, 2013. **20**(3): p. 317-25.
39. Lieber, M.R., et al., *Mechanism and regulation of human non-homologous DNA end-joining.* Nat Rev Mol Cell Biol, 2003. **4**(9): p. 712-20.
40. Lavin, M.F., *ATM and the Mre11 complex combine to recognize and signal DNA double-strand breaks.* Oncogene, 2007. **26**(56): p. 7749-58.
41. Rupnik, A., N.F. Lowndes, and M. Grenon, *MRN and the race to the break.* Chromosoma, 2010. **119**(2): p. 115-35.
42. Bochar, D.A., et al., *BRCA1 is associated with a human SWI/SNF-related complex: linking chromatin remodeling to breast cancer.* Cell, 2000. **102**(2): p. 257-65.
43. Cantor, S.B., et al., *BACH1, a novel helicase-like protein, interacts directly with BRCA1 and contributes to its DNA repair function.* Cell, 2001. **105**(1): p. 149-60.
44. Deng, C.X. and F. Scott, *Role of the tumor suppressor gene BRCA1 in genetic stability and mammary gland tumor formation.* Oncogene, 2000. **19**(8): p. 1059-64.
45. Gallardo-Rincón, D., et al., *Clinical Evaluation of BRCA1/2 Mutation in Mexican Ovarian*

Cancer Patients. Transl Oncol, 2020. **13**(2): p. 212-220.
46. Welcsh, P.L., et al., *BRCA1 transcriptionally regulates genes involved in breast tumorigenesis.* Proc Natl Acad Sci U S A, 2002. **99**(11): p. 7560-5.
47. Deng, C.X., *BRCA1: cell cycle checkpoint, genetic instability, DNA damage response and cancer evolution.* Nucleic Acids Res, 2006. **34**(5): p. 1416-26.
48. Lahusen, T.J., et al., *BRCA1 function in the intra-S checkpoint is activated by acetylation via a pCAF/SIRT1 axis.* Oncogene, 2018. **37**(17): p. 2343-2350.
49. Xu, X., et al., *BRCA1 represses DNA replication initiation through antagonizing estrogen signaling and maintains genome stability in parallel with WEE1-MCM2 signaling during pregnancy.* Hum Mol Genet, 2019. **28**(5): p. 842-857.
50. Hakem, R., et al., *The tumor suppressor gene BRCA1 is required for embryonic cellular proliferation in the mouse.* Cell, 1996. **85**(7): p. 1009-23.
51. Xu, X., et al., *Conditional mutation of BRCA1 in mammary epithelial cells results in blunted ductal morphogenesis and tumour formation.* Nat Genet, 1999. **22**(1): p. 37-43.
52. Foulks, J.M., et al., *PAF-acetylhydrolase expressed during megakaryocyte differentiation inactivates PAF-like lipids.* Blood, 2009. **113**(26): p. 6699-706.
53. Sharma, J., et al., *Activation of group VI phospholipase A2 isoforms in cardiac endothelial cells.* Am J Physiol Cell Physiol, 2011. **300**(4): p. C872-9.
54. Yang, J., et al., *Serum activity of platelet-activating factor acetylhydrolase is a potential clinical marker for leptospirosis pulmonary hemorrhage.* PLoS One, 2009. **4**(1): p. e4181.
55. Karasawa, K., et al., *Plasma platelet activating factor-acetylhydrolase (PAF-AH).* Prog Lipid Res, 2003. **42**(2): p. 93-114.
56. Stafforini, D.M., *Biology of platelet-activating factor acetylhydrolase (PAF-AH, lipoprotein associated phospholipase A2).* Cardiovasc Drugs Ther, 2009. **23**(1): p. 73-83.
57. Stafforini, D.M., *Plasma PAF-AH (PLA2G7): Biochemical Properties, Association with LDLs and HDLs, and Regulation of Expression.* Enzymes, 2015. **38**: p. 71-93.
58. Lo Nigro, C., et al., *Point mutations and an intragenic deletion in LIS1, the lissencephaly causative gene in isolated lissencephaly sequence and Miller-Dieker syndrome.* Hum Mol Genet, 1997. **6**(2): p. 157-64.
59. Pilz, D.T., et al., *LIS1 and XLIS (DCX) mutations cause most classical lissencephaly, but different patterns of malformation.* Hum Mol Genet, 1998. **7**(13): p. 2029-37.
60. Reiner, O., et al., *Isolation of a Miller-Dieker lissencephaly gene containing G protein beta-subunit-like repeats.* Nature, 1993. **364**(6439): p. 717-21.
61. Bi, W., et al., *Increased LIS1 expression affects human and mouse brain development.* Nat Genet, 2009. **41**(2): p. 168-77.
62. Cahana, A., et al., *Targeted mutagenesis of Lis1 disrupts cortical development and LIS1 homodimerization.* Proc Natl Acad Sci U S A, 2001. **98**(11): p. 6429-34.
63. Hirotsune, S., et al., *Graded reduction of Pafah1b1 (Lis1) activity results in neuronal migration defects and early embryonic lethality.* Nat Genet, 1998. **19**(4): p. 333-9.
64. Hattori, K., et al., *cDNA cloning and expression of intracellular platelet-activating factor (PAF) acetylhydrolase II. Its homology with plasma PAF acetylhydrolase.* J Biol Chem, 1996. **271**(51): p. 33032-8.
65. Hattori, K., et al., *Purification and characterization of platelet-activating factor acetylhydrolase II from bovine liver cytosol.* J Biol Chem, 1995. **270**(38): p. 22308-13.
66. Arai, H., et al., *Platelet-activating factor acetylhydrolase (PAF-AH).* J Biochem, 2002. **131**(5): p. 635-40.
67. Arai, H., *Platelet-activating factor acetylhydrolase.* Prostaglandins Other Lipid Mediat, 2002.

68-69: p. 83-94.

68. Unno, N., et al., *Association of a G994 -->T missense mutation in the plasma platelet-activating factor acetylhydrolase gene with risk of abdominal aortic aneurysm in Japanese.* Ann Surg, 2002. **235**(2): p. 297-302.
69. Miwa, M., et al., *Characterization of serum platelet-activating factor (PAF) acetylhydrolase. Correlation between deficiency of serum PAF acetylhydrolase and respiratory symptoms in asthmatic children.* J Clin Invest, 1988. **82**(6): p. 1983-91.
70. Tjoelker, L.W., et al., *Plasma platelet-activating factor acetylhydrolase is a secreted phospholipase A2 with a catalytic triad.* J Biol Chem, 1995. **270**(43): p. 25481-7.
71. Tjoelker, L.W., et al., *Anti-inflammatory properties of a platelet-activating factor acetylhydrolase.* Nature, 1995. **374**(6522): p. 549-53.
72. Samanta, U. and B.J. Bahnson, *Crystal structure of human plasma platelet-activating factor acetylhydrolase: structural implication to lipoprotein binding and catalysis.* J Biol Chem, 2008. **283**(46): p. 31617-24.
73. Tyurin, V.A., et al., *Specificity of lipoprotein-associated phospholipase A(2) toward oxidized phosphatidylserines: liquid chromatography-electrospray ionization mass spectrometry characterization of products and computer modeling of interactions.* Biochemistry, 2012. **51**(48): p. 9736-50.
74. Stafforini, D.M., et al., *Platelet-activating factor acetylhydrolase deficiency. A missense mutation near the active site of an anti-inflammatory phospholipase.* J Clin Invest, 1996. **97**(12): p. 2784-91.
75. Elstad, M.R., et al., *Platelet-activating factor acetylhydrolase increases during macrophage differentiation. A novel mechanism that regulates accumulation of platelet-activating factor.* J Biol Chem, 1989. **264**(15): p. 8467-70.
76. Narahara, H., R.A. Frenkel, and J.M. Johnston, *Secretion of platelet-activating factor acetylhydrolase following phorbol ester-stimulated differentiation of HL-60 cells.* Arch Biochem Biophys, 1993. **301**(2): p. 275-81.
77. Satoh, K., et al., *Platelet-activating factor (PAF) stimulates the production of PAF acetylhydrolase by the human hepatoma cell line, HepG2.* J Clin Invest, 1991. **87**(2): p. 476-81.
78. Xiao, Q., et al., *Nrf3-Pla2g7 interaction plays an essential role in smooth muscle differentiation from stem cells.* Arterioscler Thromb Vasc Biol, 2012. **32**(3): p. 730-44.
79. Stafforini, D.M., et al., *Human plasma platelet-activating factor acetylhydrolase. Association with lipoprotein particles and role in the degradation of platelet-activating factor.* J Biol Chem, 1987. **262**(9): p. 4215-22.
80. Ostermann, G., et al., *The contribution of individual lipoproteins to the degradation of platelet-activating factor in human serum.* Haemostasis, 1989. **19**(3): p. 160-8.
81. Stafforini, D.M., et al., *Lipoproteins alter the catalytic behavior of the platelet-activating factor acetylhydrolase in human plasma.* Proc Natl Acad Sci U S A, 1989. **86**(7): p. 2393-7.
82. Stafforini, D.M., et al., *Molecular basis of the interaction between plasma platelet-activating factor acetylhydrolase and low density lipoprotein.* J Biol Chem, 1999. **274**(11): p. 7018-24.
83. Rosenson, R.S. and D.M. Stafforini, *Modulation of oxidative stress, inflammation, and atherosclerosis by lipoprotein-associated phospholipase A2.* J Lipid Res, 2012. **53**(9): p. 1767-82.
84. Chen, C.H., *Platelet-activating factor acetylhydrolase: is it good or bad for you?* Curr Opin Lipidol, 2004. **15**(3): p. 337-41.
85. Karabina, S.A. and E. Ninio, *Plasma PAF-acetylhydrolase: an unfulfilled promise?* Biochim

Biophys Acta, 2006. **1761**(11): p. 1351-8.
86. Marathe, G.K., et al., *To hydrolyze or not to hydrolyze: the dilemma of platelet-activating factor acetylhydrolase.* J Lipid Res, 2014. **55**(9): p. 1847-54.
87. Castro Faria Neto, H.C., et al., *Regulating inflammation through the anti-inflammatory enzyme platelet-activating factor-acetylhydrolase.* Mem Inst Oswaldo Cruz, 2005. **100 Suppl 1**: p. 83-91.
88. Karasawa, K., *Clinical aspects of plasma platelet-activating factor-acetylhydrolase.* Biochim Biophys Acta, 2006. **1761**(11): p. 1359-72.
89. Prescott, S.M., et al., *Platelet-activating factor and related lipid mediators.* Annu Rev Biochem, 2000. **69**: p. 419-45.
90. Foulks, J.M., et al., *PAF-acetylhydrolase expressed during megakaryocyte differentiation inactivates PAF-like lipids.* Blood, The Journal of the American Society of Hematology, 2009. **113**(26): p. 6699-6706.
91. Triggiani, M., et al., *Characterization of platelet-activating factor acetylhydrolase in human bronchoalveolar lavage.* Am J Respir Crit Care Med, 1997. **156**(1): p. 94-100.
92. Tsukioka, K., et al., *Increased plasma level of platelet-activating factor (PAF) and decreased serum PAF acetylhydrolase (PAFAH) activity in adults with bronchial asthma.* J Investig Allergol Clin Immunol, 1996. **6**(1): p. 22-9.
93. Tetta, C., et al., *Release of platelet-activating factor in systemic lupus erythematosus.* Int Arch Allergy Appl Immunol, 1990. **91**(3): p. 244-56.
94. Tselepis, A.D., et al., *Association of the inflammatory state in active juvenile rheumatoid arthritis with hypo-high-density lipoproteinemia and reduced lipoprotein-associated platelet-activating factor acetylhydrolase activity.* Arthritis Rheum, 1999. **42**(2): p. 373-83.
95. Partrick, D.A., et al., *Reduced PAF-acetylhydrolase activity is associated with postinjury multiple organ failure.* Shock, 1997. **7**(3): p. 170-4.
96. Serebruany, V.L., et al., *Depressed plasma platelet-activating factor acetylhydrolase in patients presenting with acute myocardial infarction.* Cardiology, 1998. **90**(2): p. 127-30.
97. Graham, R.M., et al., *Plasma degradation of platelet-activating factor in severely ill patients with clinical sepsis.* Crit Care Med, 1994. **22**(2): p. 204-12.
98. Kald, B., et al., *Platelet-activating factor acetylhydrolase activity in intestinal mucosa and plasma of patients with Crohn's disease.* Digestion, 1996. **57**(6): p. 472-7.
99. Satoh, K., et al., *Platelet-activating factor acetylhydrolase in plasma lipoproteins from patients with ischemic stroke.* Stroke, 1992. **23**(8): p. 1090-2.
100. Ostermann, G., et al., *The degradation of platelet-activating factor in serum and its discriminative value in atherosclerotic patients.* Thromb Res, 1988. **52**(6): p. 529-40.
101. Meade, C.J., et al., *Serum PAF acetylhydrolase and chronic cholestasis.* Lancet, 1991. **338**(8773): p. 1016-7.
102. Hofmann, B., et al., *Enhanced degradation of platelet-activating factor in serum from diabetic patients.* Haemostasis, 1989. **19**(3): p. 180-4.
103. Dulioust, A., et al., *Paf-acether acetylhydrolase activity is increased in patients with rheumatic diseases.* Scand J Rheumatol, 1992. **21**(4): p. 161-4.
104. Satoh, K., et al., *Increased activity of the platelet-activating factor acetylhydrolase in plasma low density lipoprotein from patients with essential hypertension.* Prostaglandins, 1989. **37**(6): p. 673-82.
105. Ostermann, G., et al., *Plasma from atherosclerotic patients exerts an increased degradation of platelet-activating factor.* Thromb Res, 1987. **47**(3): p. 279-85.
106. Yamada, Y. and M. Yokota, *Loss of activity of plasma platelet-activating factor*

acetylhydrolase due to a novel Gln281-->Arg mutation. Biochem Biophys Res Commun, 1997. **236**(3): p. 772-5.

107. Bell, R., et al., *Systematic screening of the LDL-PLA2 gene for polymorphic variants and case-control analysis in schizophrenia.* Biochem Biophys Res Commun, 1997. **241**(3): p. 630-5.
108. Kruse, S., et al., *The Ile198Thr and Ala379Val variants of plasmatic PAF-acetylhydrolase impair catalytical activities and are associated with atopy and asthma.* Am J Hum Genet, 2000. **66**(5): p. 1522-30.
109. Santoso, A., et al., *Associations between four types of single-nucleotide polymorphisms in PLA2G7 gene and clinical atherosclerosis: a meta-analysis.* Am J Cardiovasc Dis, 2017. **7**(6): p. 122-133.
110. Gu, X., et al., *The rs1051931 G>A Polymorphism in the PLA2G7 Gene Confers Resistance to Immunoglobulin Therapy in Kawasaki Disease in a Southern Chinese Population.* Front Pediatr, 2020. **8**: p. 338.
111. Jiang, D., et al., *Elevated PLA2G7 gene promoter methylation as a gender-specific marker of aging increases the risk of coronary heart disease in females.* PLoS One, 2013. **8**(3): p. e59752.
112. Liu, Y., et al., *Methylation of Phospholipase A2 Group VII Gene Is Associated with Brain Arteriovenous Malformations in Han Chinese Populations.* J Mol Neurosci, 2020. **70**(7): p. 1056-1063.
113. Hartley, A., D. Haskard, and R. Khamis, *Oxidized LDL and anti-oxidized LDL antibodies in atherosclerosis - Novel insights and future directions in diagnosis and therapy<sup/>.* Trends Cardiovasc Med, 2019. **29**(1): p. 22-26.
114. Que, X., et al., *Oxidized phospholipids are proinflammatory and proatherogenic in hypercholesterolaemic mice.* Nature, 2018. **558**(7709): p. 301-306.
115. Serbulea, V., D. DeWeese, and N. Leitinger, *The effect of oxidized phospholipids on phenotypic polarization and function of macrophages.* Free Radic Biol Med, 2017. **111**: p. 156-168.
116. Thompson, A., et al., *Lipoprotein-associated phospholipase A(2) and risk of coronary disease, stroke, and mortality: collaborative analysis of 32 prospective studies.* Lancet, 2010. **375**(9725): p. 1536-44.
117. Zalewski, A. and C. Macphee, *Role of lipoprotein-associated phospholipase A2 in atherosclerosis: biology, epidemiology, and possible therapeutic target.* Arterioscler Thromb Vasc Biol, 2005. **25**(5): p. 923-31.
118. Zhang, H., et al., *Amelioration of atherosclerosis in apolipoprotein E-deficient mice by combined RNA interference of lipoprotein-associated phospholipase A2 and YKL-40.* PLoS One, 2018. **13**(8): p. e0202797.
119. Lu, J., et al., *Dual roles of endogenous platelet-activating factor acetylhydrolase in a murine model of necrotizing enterocolitis.* Pediatr Res, 2010. **68**(3): p. 225-30.
120. Moschos, M.M., et al., *"Association between platelet activating factor acetylhydrolase and diabetic retinopathy: Does inflammation affect the retinal status?".* Prostaglandins Other Lipid Mediat, 2016. **122**: p. 69-72.
121. Siddiqui, M.K., et al., *Lp-PLA(2) activity is associated with increased risk of diabetic retinopathy: a longitudinal disease progression study.* Diabetologia, 2018. **61**(6): p. 1344-1353.
122. Staurenghi, G., et al., *Darapladib, a lipoprotein-associated phospholipase A2 inhibitor, in diabetic macular edema: a 3-month placebo-controlled study.* Ophthalmology, 2015. **122**(5): p. 990-6.

123. Doody, R.S., et al., *Lipoprotein-associated phospholipase A2, homocysteine, and Alzheimer's disease.* Alzheimers Dement (Amst), 2015. **1**(4): p. 464-71.
124. Stafforini, D.M., *Diverse Functions of Plasma PAF-AH in Tumorigenesis.* Enzymes, 2015. **38**: p. 157-79.
125. Denizot, Y., et al., *Tissue concentrations of platelet-activating factor in colorectal carcinoma: inverse relationships with Dukes' stage of patients.* Oncogene, 2003. **22**(46): p. 7222-4.
126. Denizot, Y., et al., *Elevated plasma phospholipase A2 and platelet-activating factor acetylhydrolase activity in colorectal cancer.* Mediators Inflamm, 2004. **13**(1): p. 53-4.
127. Denizot, Y., et al., *Platelet-activating factor and liver metastasis of colorectal cancer.* Int J Cancer, 2005. **113**(3): p. 503-5.
128. Cervantes-Anaya, N., et al., *Downregulation of sorting nexin 10 is associated with overexpression of miR-30d during liver cancer progression in rats.* Tumour Biol, 2017. **39**(4): p. 1010428317695932.
129. Lehtinen, L., et al., *PLA2G7 associates with hormone receptor negativity in clinical breast cancer samples and regulates epithelial-mesenchymal transition in cultured breast cancer cells.* J Pathol Clin Res, 2017. **3**(2): p. 123-138.
130. Tsoupras, A., R. Lordan, and I. Zabetakis, *Inflammation, not Cholesterol, Is a Cause of Chronic Disease.* Nutrients, 2018. **10**(5).
131. Bazan, N.G., *Synaptic lipid signaling: significance of polyunsaturated fatty acids and platelet-activating factor.* J Lipid Res, 2003. **44**(12): p. 2221-33.
132. Snyder, F., *Platelet-activating factor and related acetylated lipids as potent biologically active cellular mediators.* Am J Physiol, 1990. **259**(5 Pt 1): p. C697-708.
133. Wang, H., et al., *Platelet-activating factor (PAF) up-regulates plasma and tissue PAF-acetylhydrolase activity in the rat: effect of cycloheximide.* Pediatr Res, 1997. **42**(5): p. 597-603.
134. Snyder, F., *Metabolic processing of PAF.* Clin Rev Allergy, 1994. **12**(4): p. 309-27.
135. Snyder, F., *The ether lipid trail: a historical perspective.* Biochim Biophys Acta, 1999. **1436**(3): p. 265-78.
136. Massey, C.V., et al., *Molecular and cellular actions of platelet-activating factor in rat heart cells.* J Clin Invest, 1991. **88**(6): p. 2106-16.
137. Prescott, S.M., et al., *Platelet-activating factor and related lipid mediators.* Annual review of biochemistry, 2000. **69**(1): p. 419-445.
138. Kelesidis, T., et al., *The Role of Platelet-Activating Factor in Chronic Inflammation, Immune Activation, and Comorbidities Associated with HIV Infection.* AIDS Rev, 2015. **17**(4): p. 191-201.
139. Kim, K.J., et al., *Platelet-activating factor enhances tumour metastasis via the reactive oxygen species-dependent protein kinase casein kinase 2-mediated nuclear factor-κB activation.* Immunology, 2014. **143**(1): p. 21-32.
140. Ferreira, M.A., et al., *Tumor growth, angiogenesis and inflammation in mice lacking receptors for platelet activating factor (PAF).* Life Sci, 2007. **81**(3): p. 210-7.
141. Garcia, C.C., et al., *Platelet-activating factor receptor plays a role in lung injury and death caused by Influenza A in mice.* PLoS Pathog, 2010. **6**(11): p. e1001171.
142. Kume, K. and T. Shimizu, *Platelet-activating factor (PAF) induces growth stimulation, inhibition, and suppression of oncogenic transformation in NRK cells overexpressing the PAF receptor.* J Biol Chem, 1997. **272**(36): p. 22898-904.
143. Melnikova, V.O., et al., *Crosstalk between protease-activated receptor 1 and platelet-activating factor receptor regulates melanoma cell adhesion molecule (MCAM/MUC18)*

expression and melanoma metastasis. J Biol Chem, 2009. **284**(42): p. 28845-55.
144. Bito, H., et al., *Cloning, expression and tissue distribution of rat platelet-activating-factor-receptor cDNA.* Eur J Biochem, 1994. **221**(1): p. 211-8.
145. Bito, H., et al., *Molecular cloning of a novel type of somatostatin receptor and platelet-activating factor receptor cDNAs from rat.* Ann N Y Acad Sci, 1993. **707**: p. 480-1.
146. Frost, B.L., T. Jilling, and M.S. Caplan, *The importance of pro-inflammatory signaling in neonatal necrotizing enterocolitis.* Semin Perinatol, 2008. **32**(2): p. 100-6.
147. Kravchenko, V.V., et al., *Platelet-activating factor induces NF-kappa B activation through a G protein-coupled pathway.* J Biol Chem, 1995. **270**(25): p. 14928-34.
148. Zimmerman, G.A., et al., *Brief review: molecular mechanisms of neutrophil binding to endothelium involving platelet-activating factor and cytokines.* J Lipid Mediat, 1990. **2 Suppl**: p. S31-43.
149. Anandi, V.L., et al., *Platelet-activating factor promotes motility in breast cancer cells and disrupts non-transformed breast acinar structures.* Oncol Rep, 2016. **35**(1): p. 179-88.
150. Bussolati, B., et al., *PAF produced by human breast cancer cells promotes migration and proliferation of tumor cells and neo-angiogenesis.* Am J Pathol, 2000. **157**(5): p. 1713-25.
151. Zhang, L., et al., *Activated networking of platelet activating factor receptor and FAK/STAT1 induces malignant potential in BRCA1-mutant at-risk ovarian epithelium.* Reprod Biol Endocrinol, 2010. **8**: p. 74.
152. Salajegheh, A., *Angiogenesis in health, disease and malignancy*. 2016: Springer.
153. Stafforini, D.M., et al., *Platelet-activating factor, a pleiotrophic mediator of physiological and pathological processes.* Crit Rev Clin Lab Sci, 2003. **40**(6): p. 643-72.
154. Sugimoto, T., et al., *Molecular cloning and characterization of the platelet-activating factor receptor gene expressed in the human heart.* Biochem Biophys Res Commun, 1992. **189**(2): p. 617-24.
155. Honda, Z., S. Ishii, and T. Shimizu, *Platelet-activating factor receptor.* J Biochem, 2002. **131**(6): p. 773-9.
156. Li, W. and T.M. McIntyre, *Platelet-activating factor receptor affects food intake and body weight.* Genes Dis, 2015. **2**(3): p. 255-260.
157. Prescott, S.M., G.A. Zimmerman, and T.M. McIntyre, *Platelet-activating factor.* J Biol Chem, 1990. **265**(29): p. 17381-4.
158. Bussolino, F., et al., *Platelet activating factor produced in vitro by Kaposi's sarcoma cells induces and sustains in vivo angiogenesis.* J Clin Invest, 1995. **96**(2): p. 940-52.
159. Maggi, M., et al., *Platelet-activating factor mediates an autocrine proliferative loop in the endometrial adenocarcinoma cell line HEC-1A.* Cancer Res, 1994. **54**(17): p. 4777-84.
160. Dent, P., et al., *Radiation-induced release of transforming growth factor alpha activates the epidermal growth factor receptor and mitogen-activated protein kinase pathway in carcinoma cells, leading to increased proliferation and protection from radiation-induced cell death.* Mol Biol Cell, 1999. **10**(8): p. 2493-506.
161. Mutoh, H., et al., *Platelet-activating factor (PAF) positively auto-regulates the expression of human PAF receptor transcript 1 (leukocyte-type) through NF-kappa B.* Biochem Biophys Res Commun, 1994. **205**(2): p. 1137-42.
162. Ishii, S. and T. Shimizu, *Platelet-activating factor (PAF) receptor and genetically engineered PAF receptor mutant mice.* Prog Lipid Res, 2000. **39**(1): p. 41-82.
163. Shimizu, T., H. Mutoh, and S. Kato, *Platelet-activating factor receptor*, in *Platelet-Activating Factor and Related Lipid Mediators 2*. 1996, Springer. p. 79-84.
164. Wang, H., et al., *Platelet-activating factor receptor mRNA is localized in eosinophils and*

epithelial cells in rat small intestine: regulation by dexamethasone and gut flora. Immunology, 1999. **97**(3): p. 447-54.
165. Mutoh, H., et al., *Platelet-activating factor (PAF) positively auto-regulates the expression of human PAF receptor transcript 1 (leukocyte-type) through NF-κB.* Biochemical and biophysical research communications, 1994. **205**(2): p. 1137-1142.
166. Garrido, D., et al., *Characterization of the phospholipid platelet-activating factor as a mediator of inflammation in chickens.* Frontiers in veterinary science, 2017. **4**: p. 226.
167. Dombradi, V., J. Krieglstein, and S. Klumpp, *Regulating the regulators. Conference on protein phosphorylation and protein phosphatases.* EMBO Rep, 2002. **3**(2): p. 120-4.
168. Thomas, G., et al., *The ins and outs of protein phosphorylation. Workshop report: control of signaling by protein phosphorylation.* EMBO Rep, 2000. **1**(1): p. 11-5.
169. Li, Y., et al., *Co-occurring protein phosphorylation are functionally associated.* PLoS Comput Biol, 2017. **13**(5): p. e1005502.
170. Aponte, M., et al., *Activation of platelet-activating factor receptor and pleiotropic effects on tyrosine phospho-EGFR/Src/FAK/paxillin in ovarian cancer.* Cancer Res, 2008. **68**(14): p. 5839-48.
171. Davis, L.A. and N.I. Zur Nieden, *Mesodermal fate decisions of a stem cell: the Wnt switch.* Cell Mol Life Sci, 2008. **65**(17): p. 2658-74.
172. Sedgwick, A.E. and C. D'Souza-Schorey, *Wnt Signaling in Cell Motility and Invasion: Drawing Parallels between Development and Cancer.* Cancers (Basel), 2016. **8**(9).
173. So, J., et al., *Wnt/β-catenin signaling cell-autonomously converts non-hepatic endodermal cells to a liver fate.* Biol Open, 2013. **2**(1): p. 30-6.
174. Yang, Y., *Wnt signaling in development and disease.* Cell Biosci, 2012. **2**(1): p. 14.
175. Pelullo, M., et al., *Wnt, Notch, and TGF-β Pathways Impinge on Hedgehog Signaling Complexity: An Open Window on Cancer.* Front Genet, 2019. **10**: p. 711.
176. Loh, K.M., R. van Amerongen, and R. Nusse, *Generating Cellular Diversity and Spatial Form: Wnt Signaling and the Evolution of Multicellular Animals.* Dev Cell, 2016. **38**(6): p. 643-55.
177. Khramtsov, A.I., et al., *Wnt/beta-catenin pathway activation is enriched in basal-like breast cancers and predicts poor outcome.* Am J Pathol, 2010. **176**(6): p. 2911-20.
178. López-Knowles, E., et al., *Cytoplasmic localization of beta-catenin is a marker of poor outcome in breast cancer patients.* Cancer Epidemiol Biomarkers Prev, 2010. **19**(1): p. 301-9.
179. Rask, K., et al., *Wnt-signalling pathway in ovarian epithelial tumours: increased expression of beta-catenin and GSK3beta.* Br J Cancer, 2003. **89**(7): p. 1298-304.
180. Niehrs, C., *The complex world of WNT receptor signalling.* Nat Rev Mol Cell Biol, 2012. **13**(12): p. 767-79.
181. Huang, H. and X. He, *Wnt/beta-catenin signaling: new (and old) players and new insights.* Curr Opin Cell Biol, 2008. **20**(2): p. 119-25.
182. Karner, C., K.A. Wharton, Jr., and T.J. Carroll, *Planar cell polarity and vertebrate organogenesis.* Semin Cell Dev Biol, 2006. **17**(2): p. 194-203.
183. Kohn, A.D. and R.T. Moon, *Wnt and calcium signaling: beta-catenin-independent pathways.* Cell Calcium, 2005. **38**(3-4): p. 439-46.
184. Clevers, H., *Wnt/beta-catenin signaling in development and disease.* Cell, 2006. **127**(3): p. 469-80.
185. Arnsdorf, E.J., P. Tummala, and C.R. Jacobs, *Non-canonical Wnt signaling and N-cadherin related beta-catenin signaling play a role in mechanically induced osteogenic cell fate.* PLoS One, 2009. **4**(4): p. e5388.

186. Castelo-Branco, G., et al., *Differential regulation of midbrain dopaminergic neuron development by Wnt-1, Wnt-3a, and Wnt-5a.* Proc Natl Acad Sci U S A, 2003. **100**(22): p. 12747-52.
187. Li, X., et al., *Protein phosphatase 2A and its B56 regulatory subunit inhibit Wnt signaling in Xenopus.* Embo j, 2001. **20**(15): p. 4122-31.
188. Markowitz, S.D. and M.M. Bertagnolli, *Molecular origins of cancer: Molecular basis of colorectal cancer.* N Engl J Med, 2009. **361**(25): p. 2449-60.
189. Reya, T. and H. Clevers, *Wnt signalling in stem cells and cancer.* Nature, 2005. **434**(7035): p. 843-50.
190. Barolo, S., *Transgenic Wnt/TCF pathway reporters: all you need is Lef?* Oncogene, 2006. **25**(57): p. 7505-11.
191. Henderson, B.R. and F. Fagotto, *The ins and outs of APC and beta-catenin nuclear transport.* EMBO Rep, 2002. **3**(9): p. 834-9.
192. Mosimann, C., G. Hausmann, and K. Basler, *Beta-catenin hits chromatin: regulation of Wnt target gene activation.* Nat Rev Mol Cell Biol, 2009. **10**(4): p. 276-86.
193. Giles, R.H., J.H. van Es, and H. Clevers, *Caught up in a Wnt storm: Wnt signaling in cancer.* Biochim Biophys Acta, 2003. **1653**(1): p. 1-24.
194. Liu, X., et al., *Transcription factor achaete-scute homologue 2 initiates follicular T-helper-cell development.* Nature, 2014. **507**(7493): p. 513-8.
195. He, T.C., et al., *Identification of c-MYC as a target of the APC pathway.* Science, 1998. **281**(5382): p. 1509-12.
196. Klaus, A. and W. Birchmeier, *Wnt signalling and its impact on development and cancer.* Nat Rev Cancer, 2008. **8**(5): p. 387-98.
197. Lin, C.M., et al., *Apigenin-induced lysosomal degradation of β-catenin in Wnt/β-catenin signaling.* Sci Rep, 2017. **7**(1): p. 372.
198. Gilles, C., et al., *Transactivation of vimentin by beta-catenin in human breast cancer cells.* Cancer Res, 2003. **63**(10): p. 2658-64.
199. Ambrosini, G., C. Adida, and D.C. Altieri, *A novel anti-apoptosis gene, survivin, expressed in cancer and lymphoma.* Nature medicine, 1997. **3**(8): p. 917-921.
200. Muzio, L.L., et al., *Expression of the apoptosis inhibitor survivin in aggressive squamous cell carcinoma.* Experimental and molecular pathology, 2001. **70**(3): p. 249-254.
201. Wu, B., S.P. Crampton, and C.C. Hughes, *Wnt signaling induces matrix metalloproteinase expression and regulates T cell transmigration.* Immunity, 2007. **26**(2): p. 227-39.
202. Conacci-Sorrell, M.E., et al., *Nr-CAM is a target gene of the beta-catenin/LEF-1 pathway in melanoma and colon cancer and its expression enhances motility and confers tumorigenesis.* Genes Dev, 2002. **16**(16): p. 2058-72.
203. Schmitt, M., et al., *CD44 functions in Wnt signaling by regulating LRP6 localization and activation.* Cell Death Differ, 2015. **22**(4): p. 677-89.
204. Miwa, N., et al., *Involvement of claudin-1 in the beta-catenin/Tcf signaling pathway and its frequent upregulation in human colorectal cancers.* Oncol Res, 2001. **12**(11-12): p. 469-76.
205. Bànkfalvi, A., et al., *Immunophenotypic and prognostic analysis of E-cadherin and beta-catenin expression during breast carcinogenesis and tumour progression: a comparative study with CD44.* Histopathology, 1999. **34**(1): p. 25-34.
206. Lin, S.Y., et al., *Beta-catenin, a novel prognostic marker for breast cancer: its roles in cyclin D1 expression and cancer progression.* Proc Natl Acad Sci U S A, 2000. **97**(8): p. 4262-6.
207. Wong, S.C., et al., *Expression of frizzled-related protein and Wnt-signalling molecules in invasive human breast tumours.* J Pathol, 2002. **196**(2): p. 145-53.

208. Jung, H.Y., et al., *PAF and EZH2 induce Wnt/β-catenin signaling hyperactivation.* Mol Cell, 2013. **52**(2): p. 193-205.
209. Wang, X., et al., *PAF-Wnt signaling-induced cell plasticity is required for maintenance of breast cancer cell stemness.* Nat Commun, 2016. **7**: p. 10633.
210. Doo, D.W., et al., *Inhibition of the Wnt/β-catenin pathway enhances antitumor immunity in ovarian cancer.* Ther Adv Med Oncol, 2020. **12**: p. 1758835920913798.
211. Marchion, D.C., et al., *Gene expression data reveal common pathways that characterize the unifocal nature of ovarian cancer.* Am J Obstet Gynecol, 2013. **209**(6): p. 576.e1-576.e16.
212. Huang, L., et al., *Role of Wnt/β-catenin, Wnt/c-Jun N-terminal kinase and Wnt/Ca(2+) pathways in cisplatin-induced chemoresistance in ovarian cancer.* Exp Ther Med, 2016. **12**(6): p. 3851-3858.
213. Scholz, C., et al., *Glycodelin A is a prognostic marker to predict poor outcome in advanced stage ovarian cancer patients.* BMC Res Notes, 2012. **5**: p. 551.
214. Remmele, W. and H.E. Stegner, *[Recommendation for uniform definition of an immunoreactive score (IRS) for immunohistochemical estrogen receptor detection (ER-ICA) in breast cancer tissue].* Pathologe, 1987. **8**(3): p. 138-40.
215. Remmele, W., et al., *Comparative histological, histochemical, immunohistochemical and biochemical studies on oestrogen receptors, lectin receptors, and Barr bodies in human breast cancer.* Virchows Archiv A, 1986. **409**(2): p. 127-147.
216. Whitney, J., *Testing for differences with the nonparametric Mann-Whitney U test.* J Wound Ostomy Continence Nurs, 1997. **24**(1): p. 12.
217. *Spearman's rank correlation coefficient.* Bmj, 2018. **362**: p. k4131.
218. Youden, W.J., *Index for rating diagnostic tests.* Cancer, 1950. **3**(1): p. 32-5.
219. Thomsen, R., et al., *Analysis of qPCR data by converting exponentially related Ct values into linearly related X0 values.* J Bioinform Comput Biol, 2010. **8**(5): p. 885-900.
220. Tremmel, E., et al., *Thyronamine regulation of TAAR1 expression in breast cancer cells and investigation of its influence on viability and migration.* Breast Cancer (Dove Med Press), 2019. **11**: p. 87-97.
221. Campion, E.M., S.T. Loughran, and D. Walls, *Protein Quantitation and Analysis of Purity.* Methods Mol Biol, 2017. **1485**: p. 225-255.
222. Hong, R., et al., *Preventing BRCA1/ZBRK1 repressor complex binding to the GOT2 promoter results in accelerated aspartate biosynthesis and promotion of cell proliferation.* Mol Oncol, 2019. **13**(4): p. 959-977.
223. Gorrini, C., et al., *BRCA1 interacts with Nrf2 to regulate antioxidant signaling and cell survival.* J Exp Med, 2013. **210**(8): p. 1529-44.
224. Dodson, M., et al., *Modulating NRF2 in Disease: Timing Is Everything.* Annu Rev Pharmacol Toxicol, 2019. **59**: p. 555-575.
225. Paek, J., et al., *Mitochondrial SKN-1/Nrf mediates a conserved starvation response.* Cell Metab, 2012. **16**(4): p. 526-37.
226. Garg, S., et al., *Interrelationship between nuclear factor-erythroid-2-related factor 2, NADPH quinone oxidoreductase and lipoprotein-associated phospholipase A2 expression in young patients of metabolic syndrome.* International Journal of Diabetes in Developing Countries, 2018. **39**: p. 74-81.
227. Furihata, T., et al., *Evaluation of the malignant potential of aberrant crypt foci by immunohistochemical staining for β-catenin in inflammation-induced rat colon carcinogenesis.* International journal of molecular medicine, 2002. **9**(4): p. 353-358.
228. Yu, Y., et al., *Electrochemical Detection and Distribution Analysis of β-Catenin for the*

Evaluation of Invasion and Metastasis in Hepatocellular Carcinoma. Anal Chem, 2016. **88**(7): p. 3879-84.

229. Hamel-Côté, G., et al., *Regulation of platelet-activating factor-induced interleukin-8 expression by protein tyrosine phosphatase 1B.* Cell Communication and Signaling, 2019. **17**(1): p. 21.
230. Nandy, D., et al., *Role of AKT-glycogen synthase kinase axis in monocyte activation in human beings with and without type 2 diabetes.* J Cell Mol Med, 2010. **14**(6b): p. 1396-407.
231. Wu, Z.Q., et al., *Canonical Wnt signaling regulates Slug activity and links epithelial-mesenchymal transition with epigenetic Breast Cancer 1, Early Onset (BRCA1) repression.* Proc Natl Acad Sci U S A, 2012. **109**(41): p. 16654-9.
232. Lukashova, V., et al., *G-protein-independent Activation of Tyk2 by the Platelet-activating Factor Receptor.* Journal of Biological Chemistry, 2001. **276**(26): p. 24113-24121.
233. Armanious, H., et al., *STAT3 upregulates the protein expression and transcriptional activity of β-catenin in breast cancer.* International journal of clinical and experimental pathology, 2010. **3**(7): p. 654.
234. Fragoso, M.A., et al., *The Wnt/β-catenin pathway cross-talks with STAT3 signaling to regulate survival of retinal pigment epithelium cells.* PLoS One, 2012. **7**(10): p. e46892.
235. Penna, C., et al., *Post-ischaemic activation of kinases in the pre-conditioning-like cardioprotective effect of the platelet-activating factor.* Acta Physiol (Oxf), 2009. **197**(3): p. 175-85.
236. Stafforini, D.M., *Plasma PAF-AH (PLA2G7): Biochemical Properties, Association with LDLs and HDLs, and Regulation of Expression.* The Enzymes, 2015. **38**: p. 71-93.
237. Dufourcq, P., et al., *Regulation of endothelial cell cytoskeletal reorganization by a secreted frizzled-related protein-1 and frizzled 4- and frizzled 7-dependent pathway: role in neovessel formation.* Am J Pathol, 2008. **172**(1): p. 37-49.
238. Boccellino, M., et al., *Platelet-activating factor regulates cadherin-catenin adhesion system expression and beta-catenin phosphorylation during Kaposi's sarcoma cell motility.* Am J Pathol, 2005. **166**(5): p. 1515-22.
239. Livnat, I., et al., *PAF-AH Catalytic Subunits Modulate the Wnt Pathway in Developing GABAergic Neurons.* Front Cell Neurosci, 2010. **4**.
240. Xu, B., et al., *Effects of platelet-activating factor and its differential regulation by androgens and steroid hormones in prostate cancers.* British Journal of Cancer, 2013. **109**(5): p. 1279-1286.
241. Ooi, C.H., et al., *Oncogenic pathway combinations predict clinical prognosis in gastric cancer.* PLoS Genet, 2009. **5**(10): p. e1000676.

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