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Platelets, Microenvironment and Hepatocellular Carcinoma

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Abstract

Hepatocellular carcinoma (HCC) is the commonest type of liver cancer and has a high mortality rate. Currently treatment options are limited and new therapies are urgently needed. Platelets are enucleated small cells, derived from mature megakaryocytes and besides their role in thrombosis; they actively take part in carcinogenesis and metastasis. Platelet number in the blood is associated with disease progression, overall survival and HCC subgrouping. Both thrombocytosis and thrombocytopenia are associated with HCC phenotype and size, related with other factors like cirrhosis background. Platelet counts and also platelet-to-lymphocyte ratio (PLR) and neutrophil-to-lymphocyte ratio (NLR) are considered in decision making in management. Since platelets also take up nucleotides and cytokines from tumor cells, isolating and studying platelets might provide valuable information for understanding tumor cells and may help to develop personalized treatment. Anticoagulants and antiplatelet agents are commonly used potential cancer therapeutics, which are also being studied for HCC treatment. Thus, platelets are one aspect of a complex microenvironmental milieu, the affects the biology of HCC and other tumors.

Keywords: Platelets; Hepatocellular carcinoma; Thrombocytosis; Thrombocytopenia; Microenvironment

Abbreviations

ADP: Adenosine Diphosphate; ALKP: Alkaline Phosphatase; AspECT: Aspirin and Esomeprazole Chemoprevention in Barrett's Metaplasia; ASPREE: Aspirin in Reducing Events in the Elderly; CAPP3: Cancer Prevention Project; CAFs: Cancer-Associated Fibroblastic cells; CTLs: Cytotoxic T lymphocytes; DCP: Des-γ-Carboxyprothrombin; EGF: Epidermal Growth Factor; EMT: Epithelial-to-Mesenchymal Transition; ECM: Extracellular Matrix; FGF: Fibroblast Growth Factor; HBV: Hepatitis B Virus; HCV: Hepatitis C Virus; HCC: Hepatocellular Carcinoma; HGF: Hepatocyte Growth Factor; IGF-1: Insulin-like Growth Factor-1; IFN-γ: Interferon- γ; MHC-1: Major-Histocompability Complex-1; MMPs: Matrix Metalloproteinases; NK: Natural Killer; NLR: Neutrophil-to-Lymphocyte Ratio; NPCs: Non-hepatocyte Cells; PI3K: Phosphatidyl-Inositol-3 Kinase; PDGF: Platelet-Derived Growth Factor; PLR: Platelet-to-Lymphocyte Ratio; PVT: Portal Vein Thrombosis; PARs: Protease-Activated Receptors; EPIC: The Evaluation of 7E3 for the Prevention of Ischaemic Complications; TXA2: Thromboxane A2; JNK: c-Jun NH2-terminal Kinase; TF: Tissue Factor; TGF-β: Transforming Growth Factor-β; TCIPA: Tumor Cell Induced Platelet Aggregation; ukCAP: United Kingdom Colorectal Adenoma Prevention; VEGF: Vascular Endothelial Growth Factor; AFP: α-Fetoprotein; GGTP: γ-Glutamyl Transpeptidase

Introduction

Liver cancer is the fifth most frequent cancer globally, ranks second among the causes of death from all cancer and hepatocellular carcinoma (HCC) is the most common type of cancer of the liver [1,2]. The increasing risk of HCC is attributed to chronic hepatitis C infection and subsequent cirrhosis, fatty liver disease and alcohol-related cirrhosis [3]. A vast majority of HCC develops with fibrosis and in the background of cirrhosis [4]. Surveillance of cirrhotic patients is a critical approach to detect early-stage HCC which is generally asymptomatic, and so many patients tend to be diagnosed at intermediate or late-stage of tumor [5]. There are no treatment options to reverse the development of advanced HCC, and patients with untreated advanced tumors have an overall survival rate of 7 months, whereas with the use of the only FDA approved systemic therapy, multikinase inhibitor Sorafenib, overall survival rate increased up to 10 months [6]. The need to find new markers in early-stage diagnosis of HCC and generate new approaches for therapies underlies the importance of better understanding tumor formation. In this review we focus on the importance of tumor microenvironment and stress the microenvironmental role of platelets in HCC.

HCC microenvironment

Tumor microenvironment, which has well appreciated importance for the development of tumor cells, is a complex system, that includes stromal, endothelial and immune cells, cytokines and growth factors, proteolytic enzymes like matrix metalloproteinases (MMPs), extracellular matrix (ECM) proteins and microvesicles and platelets [7,8]. As an important provider of the other microenvironment elements, stromal cells have had great attention, since new approaches and knowledge are available to enrich our insights into their role in tumor formation and metastasis.

Stromal cells play pivotal roles during liver fibrosis, tumorigenesis and metastasis. Hepatocytes are the main parenchymal liver cells and are responsible for the vital metabolic activities of the liver. Although molecular changes in hepatocytes are considered to be the main driver in liver tumorigenesis, the role of changes in non-hepatocellular cells has also been appreciated recently. Non-hepatocyte cells (NPCs) consist of hepatic stellate cells and fibroblasts taking part in keeping

the structural liver integrity and ECM production; epithelial and endothelial cells lining the bile ducts and the blood vessels, respectively; progenitor cells giving rise to both hepatic and non-hepatic cells; blood cells, that include platelets responsible for coagulation; immune cells, mainly Kupffer cells-the resident macrophages- in normal physiology and other immune cells whose presence varies in HCC, such as lymphocytes-mainly effector T-cells, tumor-associated macrophages, myeloid-derived suppressor cells, neutrophils and finally cancer-associated fibroblasts [6,7,9]. Many studies reveal genetic alterations in stromal cells providing a suitable niche for both tumor initiation and homing of metastatic cells [10].

Stromal tumor cells have been reviewed in three classes by Hanahan and Coussens based on their contribution to the hallmarks of cancer [11]. They grouped stromal cells as Angiogenic Vascular Cells, endothelial cells and pericytes; Cancer-Associated Fibroblastic Cells (CAFs), α-SMA+ myofibroblasts, mesenchymal stem cells, activated local fibroblasts and activated adipocytes; and as the third group Infiltrating Immune Cells, T and B cells, macrophages, monocytes, neutrophils, mast cells and platelets. Expression and secretion of mitogenic signals by stromal cells such as epidermal growth factor (EGF), fibroblast growth factor (FGF), hepatocyte growth factor (HGF) and insulin-like growth factor-1 (IGF-1) have been highlighted under many hallmark capabilities along with ECM modifying proteolytic enzymes. In the context of this review, platelets among the HCC stromal cells will further be considered regarding their implication in HCC.

Roles of platelets in HCC microenvironment: Platelets (also known as thrombocytes) are enucleated small cells, separated from mature megakaryocytes by cytoplasmic fragmentation and join the blood circulation, with concentrations ranging from 150-350 × 109/l [12]. The first and most well-known role of platelets is in thrombosis (coagulation) and their microscopic recognition dates back to 1882 [13].

In case of injury and vascular damage, the process of thrombosis is initiated. It involves platelet activation, adhesion to the injured blood vessel wall, aggregation and secretion of platelet granules which may contain many secretory factors such as IGF-1, EGF, HGF, FGF, vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF) etc. [14,15]. Since these secretory molecules are elevated also in tumor microenvironment, the contributing role of platelets to tumor formation and metastasis is not surprising. Platelets have been associated with tumor growth and metastasis for decades [16-20]. The effect of platelets and platelet secreted molecules has been investigated in multiple studies of many types of cancer since [21].

Thrombocytosis refers to elevated platelet number in blood, which is typically found in case of an infection. However, there is also a strong correlation between thrombocytosis and clinical tumor biology, found in various types of cancers including gastric cancer, lung cancer, ovarian cancer and HCC [22-25]. The correlation exists strongly in solid tumors, and a poor prognosis has been found when associated with thrombocytosis, and frequency of thrombocytosis also increased with advanced tumor stages [26-28]. It was shown by Cravioto-Villanueva et al. that patients with rectal cancer who had preoperative low platelet counts (<350000/mm^3) have a 5-year survival rate with a ratio of 81% comparing to patients with high preoperative platelet counts (>350000 mm^3), who had a survival rate of only 25 months [28].

In HCC, a restrospective study with 1154 HCC patients has found a significant association with thrombocytosis (platelet count >400000 mm^3) and larger tumor volume, high α-fetoprotein (AFP) levels, as well as poor survival and lower incidence of receiving therapy [29]. They recorded a drop in platelet count after tumor resection among patients with thrombocytosis and an elevation in platelet count after recurrence. Initial studies have also reported thrombocytosis with hepatoblastoma observed in children and suggested platelet count as a strong diagnostic marker [30,31]. A more recent cohort study in Taiwan considered platelet count as a more reliable marker as an extrahepatic metastasis predictor in early stage HCC [32].

In contrast to thrombocytosis, thrombocytopenia refers to low platelet count and is associated with HCC developing on a cirrhotic background liver. It is mostly associated with liver fibrosis, a decrease in platelet counts were observed with increased pathological fibrosis scores with HCC patients in hepatitis C virus (HCV) background [33]. Thrombocytopenia is considered as a predictive and prognostic factor in HCC (Figure 1) along with AFP, and has therefore been suggested for risk evaluation in screening [34]. In a recent systematic review, low level preoperative platelet count (<100000/mm^3) was found to be significantly related to worse overall survival and recurrence-free survival [35], likely as a reflection of liver fibrosis. Overall, platelet counts have been, suggested to be considered for decision making in treatment regimen [36,37].

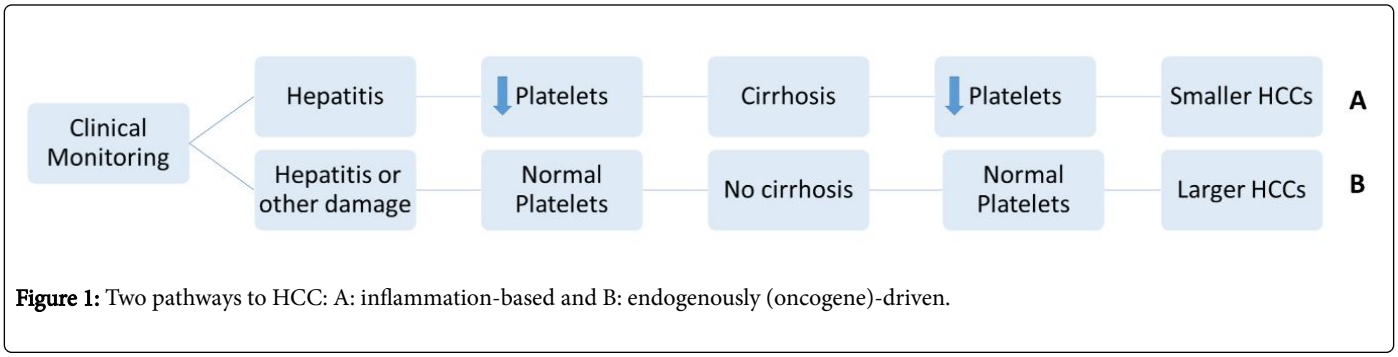

Figure 1: Two pathways to HCC: A: inflammation-based and B: endogenously (oncogene)-driven.

Considering HCC as a heterogeneous group of diseases, it has been suggested that patients with different clinical profiles might have different disease patterns and should be considered differently for treatment and prognosis [38,39]. Studies involving clinical parameters of patients with small or large HCCs have shown that along with other factors such as AFP, tumor size is correlated with platelet counts [40].

HCCs associated with thrombocytosis are often in non-cirrhotic liver and diagnosed in association with larger tumors; whereas HCCs associated with thrombocytopenia are associated with small tumor size

(<3 cm), lower blood albumin and worse liver function and a fibrotic background [38,41-43]. Thus, biological features such as AFP levels and portal vein thrombosis (PVT) are important categorizing factors along with platelet number. High platelet number associates with higher AFP levels, increased γ-glutamyl transpeptidase (GGTP), higher alkaline phosphatase (ALKP), low billirubin levels and with low prothrombin time [44]. Prospective study data analysis evaluated clinical parameters and patient survival, and showed that although blood platelet number, AFP and GGTP levels correlate with increasing tumor size, platelet number and AFP were not found to correlate with higher risk of death. Whereas GGTP, billirubin levels, PVT, tumor size and number were found to be associated with poor survival [40]. Another study subgrouping HCC according to tumor size has identified that large tumor size correlated with PVT positivity, high platelet/AFP ratio, chronic alcohol consumption and poor survival, whereas small tumor size was correlated with PVT negativity and low platelet/AFP ratio, therefore suggest that the relationship of micro-environmental factors and macro-environmental factors affecting HCC should be evaluated for HCC subgrouping [45].

A relationship between platelet counts and HCC metastasis was also established; Morimoto and colleagues demonstrated a significant correlation between extrahepatic metastasis of HCC and high platelet counts, large number of tumors, high serum des-γ-carboxyprothrombin (DCP) and Child-Pugh Class A [46]. Interestingly, studies involving HCC patients with extrahepatic metastasis compared to non-metastasis, have revealed a correlation between metastasis and increasing tumor size, more tumor multifocality, higher PVT, higher blood AFP, higher DCP, elevated ALKP and lower cirrhosis [47]. Metastasis correlated with lower survival was only observed in patients with small tumors because patients with larger tumors had poor survival regardless of presence or absence of metastasis [47]. Metastases are much commoner in HCC without cirrhosis and thus with normal platelet levels, rather than in cirrhotic, thrombocytopenic HCC [39,45,46].

Besides the platelet number, the ratios with other stromal immune cells like neutrophils and lymphocytes also have prognostic value. Li et al. have suggested that platelet-to-lymphocyte ratio (PLR) but not the platelet count itself is an important prognosis factor for the overall survival of patients with advanced stage HCC [48], patients with lower PLR had better 3-month survival rates and suggested to be better candidates in consideration of targeted drug therapies. Another study associated PLR and neutrophil-to-lymphocyte ratio (NLR) with recurrence rates after liver transplantation [49] and suggested PLR as a good predictor for tumor recurrence after liver transplantation and NLR as a good indicator of 5-year patient survival. Moreover, Xue et al. have related platelet count, pretreatment PLR and post-treatment PLR with metastatic potential of HCC cells and regarded these ratios as independent risk factors for HCC metastasis [50]. They also confirmed the link between increased platelet counts with poor survival and worse progression.

The prognostic values of NLR and PLR were also evaluated; higher NLR and PLR were found to associate with tumor size greater than 3 cm [51]. Yet another follow-up study suggest that the combination of post-operative NLR-PLR is a better tool than considering NLR or PLR alone as a predictive factor, in regard to recurrence and overall survival in patients with hepatitis B virus (HBV)-related HCC after liver resection [52]. It was suggested that for overcoming recurrence, post-operative systemic inflammatory state should take the main focus instead of pre-operative systemic inflammatory state in HCC patients who received liver resection (51). Thus, platelet count, NLR and PLR may be taken into consideration in managing patients with HCC.

Mechanisms for tumor cell effects on platelets: The relationship between platelets and cancer cells is bidirectional (Figure 2), since tumor cells stimulate platelet formation and aggregation, whereas platelets stimulate the growth of tumor cells and promote their metastasis through activation and secretion of several molecules. Platelet activation by tumor cells involves molecules including Tissue Factor (TF), adenosine diphosphate (ADP), Thrombin, Thromgouboxane A2 (TXA2) and MMPs (mainly MMP-2 and MMP-14) are directly secreted or indirectly stimulated by tumor cells and this mechanism is referred to tumor cell induced platelet aggregation (TCIPA) [53]. TF is the main activator of coagulation and microparticles carrying active TF were found to be secreted from tumor cells [54]. TF binding to FVIIa creates a cascade of events resulting in conversion of prothrombin to thrombin, fibrin formation and finally platelet activation [55]. ADP is normally stored in dense-granules of platelets, released upon activation and binds to the G-protein coupled receptors P2Y1 and P2Y21 which are located on the platelets themselves [12]. ADP is found to be expressed and secreted by tumor cells and responsible for platelet aggregation in cancer [12,56].

Thrombin serine protease has an important modifying role in several steps of coagulation and is known to activate G-protein coupled protease-activated receptors (PARs) [57]. PARs are also expressed in cancer cells including HCC cells, thrombin activated PARs (PAR-1 and PAR-4) cause invasion and migration [58-60]. Xue et al. suggested thrombin as an independent prognostic factor for HCC and have shown correlations between thrombin level and metastatic potential, tumor relapse and poor prognosis [61]. Secreted TXA2 is a soluble agonist of platelets and was found to be secreted by tumor cells which in turn bind to its receptor and initiate a downstream signaling that results in platelet activation and recruitment of additional platelets [62]. MMP-1, MMP-2 and MMP-9 were shown to be secreted by tumor cells and activate TCIPA [12]. Also Stone et al. have shown that interleukin-6 secreted from the tumor cells and thrombopoietin produced in the liver are linked to thrombocytosis and silencing of these molecules reverses elevation in platelet number in mice.

Besides these well-known mechanisms, an emerging topic concerns the "education of platelets by tumor cells". Recent studies show that platelets take up pro-angiogenic cytokines, proteins and RNA which are secreted by tumor cells. Klement et al. have shown that platelets selectively take up angiogenic regulators like VEGF, PDGF and FGF2, in contrast to other plasma proteins like albumin [63]. Similarly Battinelli et al. have observed a differential release of VEGF when platelets are exposed to breast cancer cell line MCF-7 [64]. In luminar breast cancer cells, Kuznetsov et al. have shown that platelets absorb breast tumor derived cytokines and travel to other tissues to deliver these factors, hence enable distant metastasis and recurrence [65]. More recently, tumor-derived RNA was found to be taken up by the platelets. In an initial study, differential expression of 200 platelet genes of metastatic lung cancer patients and controls demonstrated a mechanism of altered platelet function in the presence of metastasis [66]. Nilsson et al. have shown that tumor cells secrete membrane vesicles which transfer tumor-derived RNA into the platelets and also by studying platelets isolated from glioma and prostate cancer patients; they have shown that platelets carry a specific RNA signature of the tumor [67]. Best et al. have used similar approach to identify the different molecular tumor subtypes and locations of primary tumor types, including hepatobilary tumors, with high accuracy using RNA-

Seq data of 228 patients [68]. These findings strongly suggest platelets as a valuable tool for cancer diagnostics.

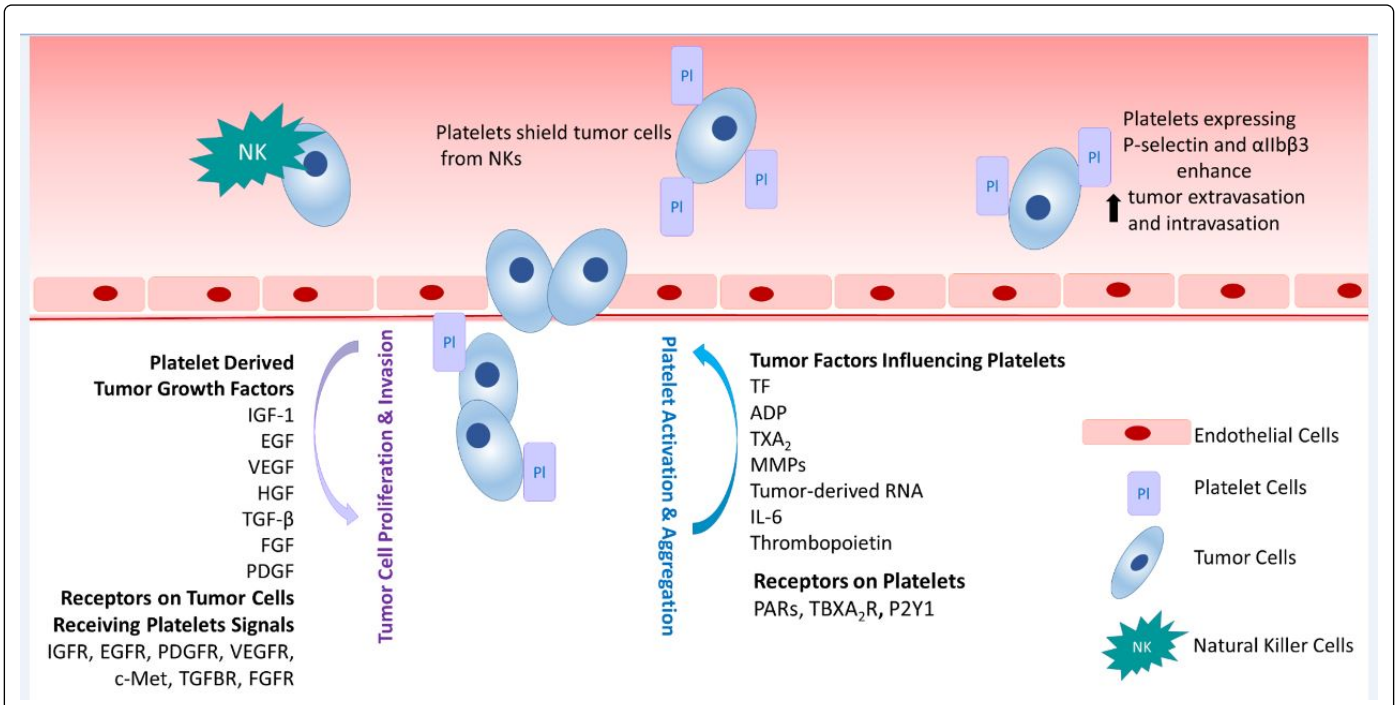

Figure 2: Relationships between platelets and tumor growth and metastasis. Platelet cells secrete many growth factors which promote tumor cell proliferation and invasion. They also promote tumor cell intravasation and extravasation. In addition they shield tumor cells in blood from natural killer cells. Tumor cells, in turn, secrete factors that promote platelet activation and aggregation.

Mechanisms for platelet effects on tumor growth and metastasis: As tumor cells activate platelets, so activated platelets in turn contribute to several steps of carcinogenesis. The most well appreciated contribution of platelets is the secretion of granules containing (1) growth factors (IGF-1, EGF, VEGF, HGF, transforming growth factor-β (TGF-β), FGF, PDGF etc.) (2) coagulation factor (prothrombin, fibrinogen, factor V, and factor VIII) (3) pro-angiogenic and anti-angiogenic factors (angiopoietin-1, angiostatin etc.) (4) MMPs and Tissue inhibitor of Metalloproteinases (TIMPs) (MMP-1, MMP-2, MMP-3, MMP-9, MT1-MMP, MMP-14, TIMP-1 and TIMP-2), (5) proinflammatory mediators (C-X-C motif chemokines, such as CXCL4, CXCL7 and CXCL12) and (6) immunologic molecules (C1 inhibitor and IgG) [12,21,69,70]. By releasing these factors, platelets initiate various signaling cascades that result in proliferation and survival of tumor cells (Figure 2). Recent data identifying the effects of platelet extracts on HCC cell lines have shown that platelets and platelet derived factors increase cell proliferation, invasion and migration whereas decrease apoptosis and medium AFP levels, through JNK signaling [69]. In colon and breast cancer cells, epithelial-to-mesenchymal transition (EMT) was demonstrated to be activated by platelet-derived TGF-β, also a direct contact between platelets and tumor cells were shown to activate NF-κB and TGF-β/Smad pathways, promoting more invasive and metastatic cells [71]. Another study with ovarian cancer cells also pointed out the importance of platelet-derived TGF-β in proliferative capacity of tumor cells [72]. Secretory platelet granules with cytokines also trigger angiogenesis, these pro-angiogenic effects are generally attributed to VEGF, PDGF, TGF-β, IGF-1 and endostatin [73]. These cytokines might be produced directly in the platelets or taken up from the tumor cells. Nevertheless, since tumor cells grow without blood circulation up to 1-2 mm3, pro-angiogenic factor stimulation is necessary for tumor cells to grow further which is also provided by the platelets [74]. Platelets help the tumor cells to adhere to the blood vessel wall through expressions of P-selectin (CD62P) and αIIbβ3 and enhance both intravasation and extravasation, also in lung and liver cancers endothelial-derived P-selectin was found to be just as important as platelet-derived P-selectin [75]. Metastasis capacity was shown to diminish drastically when P-selectin is inhibited and P-selectin-mediated melanoma cell rolling and metastasis formation was found to be attenuated *in vivo* [76]. Once the tumor cells enter the bloodstream, tumor cell-platelet aggregates form and this union gives tumor cells a survival advantage in the circulation since there is a high shear stress [77]. The tumor cell-platelet alliance in the blood also helps the tumor cells to evade immune surveillance, mainly from natural killer (NK) cells, was shown by Nieswandt and colleagues both *in vitro* as well as *in vivo* [78]. Major-histocompability complex-1 (MHC-1) on platelets was shown to be transferred to tumor cells to escape from NK cells and thereby impairing cytotoxicity and IFN-γ production by NK cells [79]. Another important step in metastasis, the homing, is also encouraged by platelets. Platelets secrete the chemokines CXCL5 and CXCL7 when they are in contact with tumors, this result in granulocyte recruitment and formation of "early metastatic niches" through CXCR2 receptor therefore increases metastatic seeding and progression [80].

In the case of HCC, HBV induced HCC has been shown to associate with CD8 T cell response to control acute HBV infection, but an inadequate response might result in hepatocyte damage which might eventually lead to cirrhosis [81]. Platelet reduction was found to be

linked to reduced virus-specific cytotoxic T lymphocytes (CTLs) in the liver of mice with acute viral hepatitis and concluded that platelet activation contributes to HCC formation through CD8 T cell accumulation [82].

The effects of platelets in tumor biology additionally relate to drug resistance in HCC therapy. Doxorubicin and the multikinase inhibitors Sorafenib (Nexavar) and Regorafenib (Stivarga) are HCC therapies. Several in-vitro studies on platelets and the use of these drugs in HCC cells have shown that platelets modulate cancer-suppressive actions of the inhibitors mainly through antagonizing effects of growth factors EGF and IGF-1 [83-86].

Role of platelet inhibitors in HCC prevention and therapy: Anticoagulants and antiplatelet agents have been commonly used to inhibit tumor growth and metastasis for many cancer types including colon cancer, esophageal cancer, liver cancer etc. [87]. Aspirin is one of the most studied to target the platelets and large clinical trials were initiated to assess the use to reduce cancer risk, these trials include aspirin and esomeprazole chemoprevention in Barrett's metaplasia (AspECT), united kingdom colorectal adenoma prevention (ukCAP), Cancer Prevention Project (CAPP3) and aspirin in reducing events in the elderly (ASPREE) [12,87]. Although there are also studies which claim no preventive effect of aspirin on cancer [88,89], data analysis of several trials demonstrated aspirin treatment lowers cancer incidence and distant metastasis for several cancer types including HCC [90]. The important study of Sitia et al. on aspirin affecting HCC, examined the long-term treatment of aspirin which blocks TXA2 production and clopidogrel which blocks the P2Y12 ADP receptor to inhibit platelet activation decreased intrahepatic accumulation of HBV triggered CD8 T cells and other inflammatory cells. These agents reduced HBV-based hepatocellular injury and proliferation, prevented HCC development and increased overall mouse survival [91,92]. Besides aspirin, other drugs targeting platelet activation were studied; Prasugrel and Ticagrelor, which inhibit P2Y12 ADP and thrombin receptor PAR-1 inhibitor Vorapaxar were studied in-vivo to detect their effect on hepatocellular adenoma and carcinoma [88,93]. Abciximab is among the most well-known antibodies targeting platelets, The Evaluation of 7E3 for the Prevention of Ischaemic Complications (EPIC) trial was initiated to test its effects on patients at high risk undergoing percutaneous revascularization procedures, later due to its role in platelet aggretation, and it was used also for cancer epidemiology studies [94]. It is the monoclonal antibody targeting αIIbβ3-Integrin (GP IIb/IIIa) and was found to induce apoptosis of MCF-7 breast cancer cells. Earlier studies have shown that it blocks the release of VEGF from platelets and metastasis *in vivo* [95,96].

Heparin is an activator of platelets and can cause a slight decrease in platelet count in the beginning of treatment which is then followed by platelet activation [97]. Studies on mechanisms of heparin on platelet activation have shown that heparin requires the binding to GPIIb-IIIa (integrin αIIbβ3) to trigger platelet aggregation [98]. Gao et al. have also demonstrated the binding of heparin to GPIIb-IIIa results in activation of phosphatidyl-inositol-3 kinase (PI3K) [99]. There is debate of using heparin for cancer patients to inhibit thromboembolic events, since heparin activates platelets and also interacts with tumor cell signaling. Studies show that heparin effects on HCC cells are context dependent. Thus, Ozen et al. have found that in the presence of HGF, heparin represses HGF-induced c-Met signaling, whereas in our recent unpublished data we have observed that in the absence of HGF, heparin activates c-Met signaling in an HGF-independent manner [100]. The role of heparin on platelet interaction with tumor cells and its modulating role in their signaling is being further investigated. Our microarray data of heparin induced HCC cell line SK-HEP-1 has revealed upregulation of molecules which might be responsible from platelet functions including: Collagen Type VI, EGR-1, Interleukin 8, CXCL-2. Collagen Type VI is known to be responsible for platelet adhesion and aggregation through its collagenous domain in vascular regions with low shear stress [101]. Collagen type VI was found to induce alpha-granule secretion and up-regulation of cell surface glycoprotein IIb/IIIa along with Collagen Types I, III, V and also Collagen Type VI was shown to be more effective in alpha-granule secretion from platelets than Types I and III [102]. EGR-1 is another upregulated gene whose decreased activity in the presence of heparin and HGF, resulted in supressions of MMP-2, MMP-2 and MT1-MP [100]. Whereas in our recent unpublished studies we have seen that in the absence of HGF, heparin increases EGR-1 mediated MMP-2 and MMP-9 activities. Since it is known that MMP-2 has an important role in platelet activation and aggregation, heparin induced EGR-1 might target platelet activation through MMP-2 [103-105]. IL-8 (also known as CXCL8) is well known for its iflammatory role, presence in tumor microenvironment, secretion by activated platelets and as a ligand for CXCR2 [106]. CXCL2 belongs to the CXC chemokines and is produced by many cells including tumor cells, CXCL-2 can also bind to the receptor CXCR2 [107]. CXCR2 has a role in platelet-granulocyte signaling that promotes early metastatic niches and metastasis [81]. Therefore heparin induced secretion of CXCL8 and CXCL2 from HCC tumor cells might be activating CXCR2 and promote platelet regulated metastasis.

As proteomics tools are more readily available to enrich our understanding of platelets, studies on platelet proteome, phosphoproteome, kinome and interactome would provide an insight into defining new drug targets in HCC. Such effort is already being made for other diseases [108]. PlateletWeb is a great tool to study systems biological analysis and integrated networks of platelets and might provide great contribution for platelet mediated cancer studies [109].

Conclusion

Along with tumor internal factors, microenvironment has important roles in tumor formation, extravasation, intravasation, homing (metastasis formation) and drug resistance. Platelets and platelet-derived factors are among the targets in cancer therapy owing to their significant roles.

Low platelets (thrombocytosis) are closely correlated with increasing degree of cirrhosis, and have thus been suggested to be a cirrhosis-surrogate (33). Thus, monitoring of platelet counts in patients with hepatitis (a cirrhosis precursor) is used to monitor for the development of increasing liver fibrosis and thus of cirrhosis. Platelet levels are also a well-described risk factor for developing HCC in patients with cirrhosis. Thus, monitoring of platelet counts in patients with known cirrhosis is used to assess for any suspicion of HCC development.

Platelet count changes are not themselves an HCC tumor marker, but merely an indicator of risk for HCC development in patients with cirrhosis. However, it is becoming increasingly clear that in patients who have an HCC diagnosis, the level of platelets-normal or low- is likely a reflection of 2 HCC phenotypes (25,38,40,41) with different natural history. Thus:

- Hepatitis > decreasing platelets > cirrhosis > decreasing platelets > smaller HCCs
- Hepatitis or other damage > normal platelets > Low or no cirrhosis > larger HCCs

In pathway B, platelet factors appear to contribute to increasing HCC size

Platelet counts, NLR and PLR have useful prognostic value in HCC and can be considered in treatment planning. In addition, they can be included in screening programs, can also be used in the identification of tumor subtyping to predict disease prognosis. Actions modulating platelet activity and number can increase the effects of medical therapies. Potential clinical agents for doing this include aspirin and the newer non aspirin drugs such as clopidogrel, prasugrel and ticagrelor, as well as platelet antibodies.

Conflict of Interest

The authors have no conflicts of interest to declare.

References

1. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, et al. (2015) Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer 136: E359-386.
2. Venook AP, Papandreou C, Furuse J, de Guevara LL (2010) The incidence and epidemiology of hepatocellular carcinoma: a global and regional perspective. Oncologist 15 Suppl 4: 5-13.
3. El-Serag HB (2011) Hepatocellular carcinoma. N Engl J Med 365: 1118-1127.
4. Fattovich G, Stroffolini T, Zagni I, Donato F (2004) Hepatocellular carcinoma in cirrhosis: incidence and risk factors. Gastroenterology 127: S35-50.
5. Villanueva A, Llovet JM (2011) Targeted therapies for hepatocellular carcinoma. Gastroenterology 140: 1410-1426.
6. Prieto J, Melero I, Sangro B (2015) Immunological landscape and immunotherapy of hepatocellular carcinoma. Nat Rev Gastroenterol Hepatol 12: 681-700.
7. Tu T, Budzinska MA, Maczurek AE, Cheng R, Di Bartolomeo A, et al. (2014) Novel aspects of the liver microenvironment in hepatocellular carcinoma pathogenesis and development. Int J Mol Sci 15: 9422-9458.
8. Thompson AI, Conroy KP, Henderson NC (2015) Hepatic stellate cells: central modulators of hepatic carcinogenesis. BMC Gastroenterol 15: 63.
9. Hernandez-Gea V, Toffanin S, Friedman SL, Llovet JM (2013) Role of the microenvironment in the pathogenesis and treatment of hepatocellular carcinoma. Gastroenterology 144: 512-527.
10. Han KQ, He XQ, Ma MY, Guo XD, Zhang XM, et al. (2015) Inflammatory microenvironment and expression of chemokines in hepatocellular carcinoma. World J Gastroenterol 21: 4864-4874.
11. Hanahan D, Coussens LM (2012) Accessories to the crime: functions of cells recruited to the tumor microenvironment. Cancer Cell 21: 309-322.
12. Mezouar S, Frère C, Darbousset R, Mege D, Crescence L, et al. (2016) Role of platelets in cancer and cancer-associated thrombosis: Experimental and clinical evidences. Thromb Res 139: 65-76.
13. Ribatti D, Crivellato E (2007) Giulio Bizzozero and the discovery of platelets. Leuk Res 31: 1339-1341.
14. Gay LJ, Felding-Habermann B (2011) Contribution of platelets to tumour metastasis. Nat Rev Cancer 11: 123-134.
15. Kurokawa T, Zheng YW, Ohkohchi N (2016) Novel functions of platelets in the liver. J Gastroenterol Hepatol 31: 745-751.
16. Gasic GJ, Gasic TB, Stewart CC (1968) Antimetastatic effects associated with platelet reduction. Proc Natl Acad Sci USA 61: 46-52.
17. Iakovleva MP (1955) Thrombocytes in the blood in cancer and other diseases of the stomach. Vopr Onkol 1: 63-71.
18. Pankratova NG (1957) Thrombocytosis and the thrombocyte formula in the diagnosis of lung cancer and suppurative diseases. Vestn Khir Im I I Grek 78: 32-39.
19. Refvem O (1960) Thrombocythemia as an independent phenomenon in cancer. Nord Med 64: 950-953.
20. Prizhivoit GN (1964) Megakaryocyte system and thrombocyte reaction on the growth of malignant neoplasms. Acta Unio Int Contra Cancrum 20: 1568-1571.
21. Goubran HA, Stakiw J, Radosevic M, Burnouf T (2014) Platelets effects on tumor growth. Semin Oncol 41: 359-369.
22. Ikeda M, Furukawa H, Imamura H, Shimizu J, Ishida H, et al. (2002) Poor prognosis associated with thrombocytosis in patients with gastric cancer. Ann Surg Oncol 9: 287-291.
23. Maráz A, Furák J, Varga Z, Kahán Z, Tiszlavicz L, et al. (2013) Thrombocytosis has a negative prognostic value in lung cancer. Anticancer Res 33: 1725-1729.
24. SÅ‚abuszewska-JÃ‚Å WA, Dmoch-Gajzlerska E, Kozakiewicz B, Jakiel G (2015) The prognostic significance of thrombocytosis in ovarian cancer. Ann Agric Environ Med 22: 731-735.
25. Carr BI, Guerra V (2013) Thrombocytosis and hepatocellular carcinoma. Dig Dis Sci 58: 1790-1796.
26. Stone RL, Nick AM, McNeish IA, Balkwill F, Han HD, et al. (2012) Paraneoplastic thrombocytosis in ovarian cancer. N Engl J Med 366: 610-618.
27. Chadha AS, Kocak-Uzel E, Das P, Minsky BD, Delclos ME, et al. (2015) Paraneoplastic thrombocytosis independently predicts poor prognosis in patients with locally advanced pancreatic cancer. Acta Oncol 54: 971-978.
28. Cravioto-Villanueva A, Luna-Perez P, Gutierrez-de la Barrera M, Martinez-Gómez H, Maffuz A, et al. (2012) Thrombocytosis as a predictor of distant recurrence in patients with rectal cancer. Arch Med Res 43: 305-311.
29. Hwang SJ, Luo JC, Li CP, Chu CW, Wu JC, et al. (2004) Thrombocytosis: a paraneoplastic syndrome in patients with hepatocellular carcinoma. World J Gastroenterol 10: 2472-2477.
30. Nickerson HJ, Silberman TL, McDonald TP (1980) Hepatoblastoma, thrombocytosis, and increased thrombopoietin. Cancer 45: 315-317.
31. Shafford EA, Pritchard J (1993) Extreme thrombocytosis as a diagnostic clue to hepatoblastoma. Arch Dis Child 69: 171.
32. Lee CH, Lin YJ, Lin CC, Yen CL, Shen CH, et al. (2015) Pretreatment platelet count early predicts extrahepatic metastasis of human hepatoma. Liver Int 35: 2327-2336.
33. Lu SN, Wang JH, Liu SL, Hung CH, Chen CH, et al. (2006) Thrombocytopenia as a surrogate for cirrhosis and a marker for the identification of patients at high-risk for hepatocellular carcinoma. Cancer 107: 2212-2222.
34. Huang YC, Huang CF, Chang KC, Hung SF, Wang JH, et al. (2011) Community-based screening for hepatocellular carcinoma in elderly residents in a hepatitis B- and C-endemic area. J Gastroenterol Hepatol 26: 129-134.
35. Pang Q, Qu K, Zhang JY, Song SD, Liu SS, et al. (2015) The prognostic value of platelet count in patients with hepatocellular carcinoma: A systematic review and meta-analysis. Medicine (Baltimore) 94: e1431.
36. Venkat R, Hannallah JR, Krouse RS, Maegawa FB (2016) Preoperative thrombocytopenia and outcomes of hepatectomy for hepatocellular carcinoma. J Surg Res 201: 498-505.
37. Nakayama H, Takayama T (2015) Management before hepatectomy for hepatocellular carcinoma with cirrhosis. World J Hepatol 7: 2292-2302.
38. Carr BI, Guerra V, Pancoska P (2012) Thrombocytopenia in relation to tumor size in patients with hepatocellular carcinoma. Oncology 83: 339-345.
39. Giuliante F, De Rose AM, Guerra V, Ardito F, Nuzzo G, et al. (2013) Clinical characteristics and survival of European patients with resectable large hepatocellular carcinomas. J Gastrointest Cancer 44: 329-335.

40. Carr BI, Guerra V, Giannini EG, Farinati F, Ciccarese F, et al. (2014) Significance of platelet and AFP levels and liver function parameters for HCC size and survival. Int J Biol Markers 29: e215-223.
41. Carr BI, Lin CY, Lu SN (2014) Platelet-related phenotypic patterns in hepatocellular carcinoma patients. Semin Oncol 41: 415-421.
42. Carr BI, Guerra V (2013) Thrombocytosis and hepatocellular carcinoma. Dig Dis Sci 58: 1790-1796.
43. Carr BI, Guerra V, De Giorgio M, Fagiuoli S, Pancoska P (2012) Small hepatocellular carcinomas and thrombocytopenia. Oncology 83: 331-338.
44. Carr BI, Guerra V (2013) Hepatocellular carcinoma size: platelets, γ-glutamyl transpeptidase, and alkaline phosphatase. Oncology 85: 153-159.
45. Pancoska P, Carr BI (2014) Macro- and micro-environmental factors in clinical hepatocellular cancer. Semin Oncol 41: 185-194.
46. Morimoto Y, Nouso K, Wada N, Takeuchi Y, Kinugasa H, et al. (2014) Involvement of platelets in extrahepatic metastasis of hepatocellular carcinoma. Hepatol Res 44: E353-359.
47. Carr BI, Guerra V (2016) Hepatocellular carcinoma extrahepatic metastasis in relation to tumor size and alkaline phosphatase levels. Oncology 90: 136-142.
48. Li X, Chen ZH, Xing YF, Wang TT, Wu DH, et al. (2015) Platelet-to-lymphocyte ratio acts as a prognostic factor for patients with advanced hepatocellular carcinoma. Tumour Biol 36: 2263-2269.
49. Lai Q, Castro SE, Rico Juri JM, Pinheiro RS, Lerut J (2014) Neutrophil and platelet-to-lymphocyte ratio as new predictors of dropout and recurrence after liver transplantation for hepatocellular cancer. Transpl Int 27: 32-41.
50. Xue TC, Ge NL, Xu X, Le F, Zhang BH et al. (2016) High platelet counts increase metastatic risk in huge hepatocellular carcinoma undergoing transarterial chemoembolization. Hepatol Res .
51. Wang Q, Blank S, Fiel MI, Kadri H, Luan W, et al. (2015) The severity of liver fibrosis influences the prognostic value of inflammation-based scores in hepatitis B-associated hepatocellular carcinoma. Ann Surg Oncol 22 Suppl 3: S1125-1132.
52. Li C, Wen TF, Yan LN, Li B, Wang WT, et al. (2015) Postoperative neutrophil-to-lymphocyte ratio plus platelet-to-lymphocyte ratio predicts the outcomes of hepatocellular carcinoma. J Surg Res 198: 73-79.
53. Jurasz P, Alonso-Escolano D, Radomski MW (2004) Platelet-cancer interactions: mechanisms and pharmacology of tumour cell-induced platelet aggregation. Br J Pharmacol 143: 819-826.
54. Thomas GM, Panicot-Dubois L, Lacroix R, Dignat-George F, Lombardo D, et al. (2009) Cancer cell-derived microparticles bearing P-selectin glycoprotein ligand 1 accelerate thrombus formation in vivo. J Exp Med 206: 1913-1927.
55. Ruf W, Yokota N, Schaffner F (2010) Tissue factor in cancer progression and angiogenesis. Thromb Res 125 Suppl 2: S36-38.
56. Bastida E, Ordinas A, Jamieson GA (1981) Differing platelet aggregating effects by two tumor cell lines: absence of role for platelet-derived ADP. Am J Hematol 11: 367-378.
57. Snyder KM, Kessler CM (2008) The pivotal role of thrombin in cancer biology and tumorigenesis. Semin Thromb Hemost 34: 734-741.
58. Kaufmann R, Rahn S, Pollrich K, Hertel J, Dittmar Y, et al. (2007) Thrombin-mediated hepatocellular carcinoma cell migration: cooperative action via proteinase-activated receptors 1 and 4. J Cell Physiol 211: 699-707.
59. Kaufmann R, Henklein P, Henklein P, Settmacher U (2009) Green tea polyphenol epigallocatechin-3-gallate inhibits thrombin-induced hepatocellular carcinoma cell invasion and p42/p44-MAPKinase activation. Oncol Rep 21: 1261-1267.
60. Mußbach F, Henklein P, Westermann M, Settmacher U, Böhmer FD, et al. (2015) Proteinase-activated receptor 1- and 4-promoted migration of Hep3B hepatocellular carcinoma cells depends on ROS formation and RTK transactivation. J Cancer Res Clin Oncol 141: 813-825.
61. Xue YH, Zhang XF, Dong QZ, Sun J, Dai C, et al. (2010) Thrombin is a therapeutic target for metastatic osteopontin-positive hepatocellular carcinoma. Hepatology 52: 2012-2022.
62. Stegner D, Dütting S, Nieswandt B (2014) Mechanistic explanation for platelet contribution to cancer metastasis. Thromb Res 133 Suppl 2: S149-157.
63. Klement GL, Yip TT, Cassiola F, Kikuchi L, Cervi D, et al. (2009) Platelets actively sequester angiogenesis regulators. Blood 113: 2835-2842.
64. Battinelli EM, Markens BA, Italiano JE Jr. (2011) Release of angiogenesis regulatory proteins from platelet alpha granules: modulation of physiologic and pathologic angiogenesis. Blood 118: 1359-1369.
65. Kuznetsov HS, Marsh T, Markens BA, Castaño Z, Greene-Colozzi A, et al. (2012) Identification of luminal breast cancers that establish a tumor-supportive macroenvironment defined by proangiogenic platelets and bone marrow-derived cells. Cancer Discov 2: 1150-1165.
66. Calverley DC, Phang TL, Choudhury QG, Gao B, Oton AB, et al. (2010) Significant downregulation of platelet gene expression in metastatic lung cancer. Clin Transl Sci 3: 227-232.
67. Nilsson RJ, Balaj L, Hulleman E, van Rijn S, Pegtel DM, et al. (2011) Blood platelets contain tumor-derived RNA biomarkers. Blood 118: 3680-3683.
68. Best MG, Sol N, Kooi I, Tannous J, Westerman BA, et al. (2015) RNA-Seq of tumor-educated platelets enables blood-based pan-cancer, multiclass, and molecular pathway cancer diagnostics. Cancer Cell 28: 666-676.
69. Carr BI, Cavallini A, D'Alessandro R, Refolo MG, Lippolis C, et al. (2014) Platelet extracts induce growth, migration and invasion in human hepatocellular carcinoma in vitro. BMC Cancer 14: 43.
70. Menter DG, Tucker SC, Kopetz S, Sood AK, Crissman JD, et al. (2014) Platelets and cancer: a casual or causal relationship: revisited. Cancer Metastasis Rev 33: 231-269.
71. Labelle M, Begum S, Hynes RO (2011) Direct signaling between platelets and cancer cells induces an epithelial-mesenchymal-like transition and promotes metastasis. Cancer Cell 20: 576-590.
72. Cho MS, Bottsford-Miller J, Vasquez HG, Stone R, Zand B, et al. (2012) Platelets increase the proliferation of ovarian cancer cells. Blood 120: 4869-4872.
73. Sabrkhany S, Griffioen AW, Oude Egbrink MG (2011) The role of blood platelets in tumor angiogenesis. Biochim Biophys Acta 1815: 189-196.
74. Nishida N, Yano H, Nishida T, Kamura T, Kojiro M (2006) Angiogenesis in cancer. Vasc Health Risk Manag 2: 213-219.
75. Coupland LA, Chong BH, Parish CR (2012) Platelets and P-selectin control tumor cell metastasis in an organ-specific manner and independently of NK cells. Cancer Res 72: 4662-4671.
76. Ludwig RJ, Boehme B, Podda M, Henschler R, Jager E, et al. (2004) Endothelial P-selectin as a target of heparin action in experimental melanoma lung metastasis. Cancer Res 64: 2743-2750.
77. Egan K, Cooke N, Kenny D (2014) Living in shear: platelets protect cancer cells from shear induced damage. Clin Exp Metastasis 31: 697-704.
78. Nieswandt B, Hafner M, Echtenacher B, Männel DN (1999) Lysis of tumor cells by natural killer cells in mice is impeded by platelets. Cancer Res 59: 1295-1300.
79. Placke T, Örgel M, Schaller M, Jung G, Rammensee HG, et al. (2012) Platelet-derived MHC class I confers a pseudonormal phenotype to cancer cells that subverts the antitumor reactivity of natural killer immune cells. Cancer Res 72: 440-448.
80. Labelle M, Begum S, Hynes RO (2014) Platelets guide the formation of early metastatic niches. Proc Natl Acad Sci USA 111: E3053-3061.
81. Maini MK, Schurich A (2012) Platelets harness the immune response to drive liver cancer. Proc Natl Acad Sci USA 109: 12840-12841.
82. Iannacone M, Sitia G, Isogawa M, Marchese P, Castro MG, et al. (2005) Platelets mediate cytotoxic T lymphocyte-induced liver damage. Nat Med 11: 1167-1169.
83. D'Alessandro R, Refolo MG, Lippolis C, Giannuzzi G, Carella N, et al. (2014) Antagonism of sorafenib and regorafenib actions by platelet factors in hepatocellular carcinoma cell lines. BMC Cancer 14: 351.

84. Refolo MG, D'Alessandro R, Lippolis C, Messa C, Carella N, et al. (2014) Modulation of doxorubicin mediated growth inhibition of hepatocellular carcinoma cells by platelet lysates. Anticancer Agents Med Chem 14: 1154-1160.

85. D'Alessandro R, Messa C, Refolo MG, Carr BI (2015) Modulation of sensitivity and resistance to multikinase inhibitors by microenvironmental platelet factors in HCC. Expert Opin Pharmacother 16: 2773-2780.

86. Lippolis C, Refolo MG, D'Alessandro R, Carella N, Messa C et al (2015) Resistance to multikinase inhibitor actions mediated by insulin like growth factor-1. Journal of Experimental & Clinical Cancer Research 34

87. Serebruany VL, Cherepanov V, Cabrera-Fuentes HA, Kim MH (2015) Solid cancers after antiplatelet therapy: Confirmations, controversies, and challenges. Thromb Haemost 114: 1104-1112.

88. Zhang SM, Cook NR, Manson JE, Lee IM, Buring JE (2008) Low-dose aspirin and breast cancer risk: results by tumour characteristics from a randomised trial. Br J Cancer 98: 989-991.

89. Cook NR, Lee IM, Gaziano JM, Gordon D, Ridker PM, et al. (2005) Low-dose aspirin in the primary prevention of cancer: The Women's Health Study: a randomized controlled trial. JAMA 294: 47-55.

90. Algra AM, Rothwell PM (2012) Effects of regular aspirin on long-term cancer incidence and metastasis: a systematic comparison of evidence from observational studies versus randomised trials. Lancet Oncol 13: 518-527.

91. Sitia G, Aiolfi R, Di Lucia P, Mainetti M, Fiocchi A, et al. (2012) Antiplatelet therapy prevents hepatocellular carcinoma and improves survival in a mouse model of chronic hepatitis B. Proc Natl Acad Sci USA 109: E2165-2172.

92. Sitia G, Iannacone M, Guidotti LG (2013) Anti-platelet therapy in the prevention of hepatitis B virus-associated hepatocellular carcinoma. J Hepatol 59: 1135-1138.

93. Gebremeskel S, LeVatte T, Liwski RS, Johnston B, Bezuhly M (2015) The reversible P2Y12 inhibitor ticagrelor inhibits metastasis and improves survival in mouse models of cancer. Int J Cancer 136: 234-240.

94. Califf RM, Lincoff AM, Tcheng JE, Topol EJ (1995) An overview of the results of the EPIC trial. Eur Heart J 16 Suppl L: 43-49.

95. Kononczuk J, Surazynski A, Czyzewska U, Prokop I, Tomczyk M, et al. (2015) αIIbβ3-Integrin ligands: Abciximab and Eptifibatide as proapoptotic factors in MCF-7 human breast cancer cells. Curr Drug Targets 16: 1429-1437.

96. Amirkhosravi A, Amaya M, Siddiqui F, Biggerstaff JP, Meyer TV, et al. (1999) Blockade of GpIIb/IIIa inhibits the release of vascular endothelial growth factor (VEGF) from tumor cell-activated platelets and experimental metastasis. Platelets 10: 285-292.

97. Greinacher A (2011) Platelet activation by heparin. Blood 117: 4686-4687.

98. Chong BH, Ismail F (1989) The mechanism of heparin-induced platelet aggregation. Eur J Haematol 43: 245-251.

99. Gao C, Boylan B, Fang J, Wilcox DA, Newman DK, et al. (2011) Heparin promotes platelet responsiveness by potentiating αIIbβ3-mediated outside-in signaling. Blood 117: 4946-4952.

100. Ozen E, Gozukizil A, Erdal E, Uren A, Bottaro DP, et al. (2012) Heparin inhibits hepatocyte growth factor induced motility and invasion of hepatocellular carcinoma cells through early growth response protein 1. PLoS One 7: e42717.

101. Ross JM, McIntire LV, Moake JL, Rand JH (1995) Platelet adhesion and aggregation on human type VI collagen surfaces under physiological flow conditions. Blood 85: 1826-1835.

102. Alberio L, Dale GL (1998) Flow cytometric analysis of platelet activation by different collagen types present in the vessel wall. Br J Haematol 102: 1212-1218.

103. Knezevic I, Leisner TM, Lam SC (1996) Direct binding of the platelet integrin alphaIIbbeta3 (GPIIb-IIIa) to talin. Evidence that interaction is mediated through the cytoplasmic domains of both αIIb and β3. J Biol Chem 271: 16416-16421.

104. Soslau G, Mason C, Lynch S, Benjamin J, Ashak D, et al. (2014) Intracellular matrix metalloproteinase-2 (MMP-2) regulates human platelet activation via hydrolysis of talin. Thromb Haemost 111: 140-153.

105. Choi WS, Jeon OH, Kim HH, Kim DS (2008) MMP-2 regulates human platelet activation by interacting with integrin αIIbβ3. J Thromb Haemost 6: 517-523.

106. Waugh DJ, Wilson C (2008) The interleukin-8 pathway in cancer. Clin Cancer Res 14: 6735-6741.

107. Baggiolini M, Dewald B, Moser B (1997) Human chemokines: an update. Annu Rev Immunol 15: 675-705.

108. García Á (2016) Platelet clinical proteomics: Facts, challenges and future perspectives. Proteomics Clin Appl .

109. Boyanova D, Nilla S, Birschmann I, Dandekar T, Dittrich M (2012) Platelet web: A systems biologic analysis of signaling networks in human platelets. Blood 119: e22-e34.