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Review Article

Multifaceted role of cancer educated platelets in survival of cancer cells

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ABSTRACT

Platelets, the derivatives of megakaryocytes, pose dynamic biological functions such as homeostasis and wound healing. The mechanisms involved in these processes are utilized by cancerous cells for proliferation and metastasis. Platelets through their activation establish an aggregate termed as Tumor cell induced platelet aggregation (TCIPA) that aids in establishing a niche for the primary tumor at secondary site while recruiting granulocytes and monocytes. The study of these close interactions between the tumor and the platelets can be exploited as biomarkers in liquid biopsy for early cancer detection, thereby increasing the life expectancy of cancer patients.

1. Introduction

Platelets originate from large progenitor cells often known as megakaryocytes and are abundantly found in blood (150–400 × 10^9/L) [1]. Platelets pose dynamic biological functions including hemostasis, arterial thrombosis, immunity, inflammation, vessel remodeling and angiogenesis [2,3]. In this review, a novel role of platelets in liquid biopsy has been depicted in association with oncogenesis. This study can prove to be valuable as it can help in diagnosis of early cancer development.

2. Platelets role in tumor microenvironment

The complete mechanism of action of platelets on tumor is still a mystery, yet there are evidences that suggest an interplay between platelets and tumor formation [4]. In previous studies, it is suggested that platelets get activated upon recruitment to tumor microenvironment followed by secretion of certain mitogenic and angiogenic proteins from platelets that accelerate tumor proliferation [5,6]. Based on the literature, a generalized model depicting the interaction of platelets with primary tumor has been shown in Fig. 1.

Furthermore, tumors are also known to release certain angiogenic and mitogenic factors promoting tumor metastasis [7]. These released angiogenic factors initiate angiogenesis in the tumor microenvironment. Tumor and platelet interaction, helping in tumor progression, is also mediated by interaction of TLR4 present on platelets and HMGB1 released by tumor cells [8]. A dying tumor releases HMGB1 to bind itself to platelets via TLR4 which mediates clustering by adhesion causing increase in tumor progression [9]. Therefore, it is suggested that tumor cell survival and dissemination is dependent upon platelet activation.

3. Platelets role in intravasation of tumor

Platelets facilitate tumor cell metastasis via interaction with selectins and glycoproteins [5,10–13]. They induce EMT and degradation of ECM, thereby increasing the permeability of blood vessels surrounding the tumor [13–15].

Platelets have also been seen secreting Lysophosphatidic acid also known as LPA. In plasma an interaction between the autotaxin and integrin released by platelets increases autotaxin activity; hence, converting more of plasma Lysophosphatidylcholine (LPC) into Lysophosphatidic acid (LPA). LPA is a lipid possessing certain signaling properties of growth factors [16,17]. LPA apart from playing roles in cellular proliferation and survival also aids in metastasis of multiple types of cancers by assisting in reversal of differentiation and tumor invasion by means of multiple cascades of G protein coupled receptors (LPA1–6) [18]. Out of these 6 receptors, the blockage of LPA1 and LPA2 is sufficient to inhibit tumor invasion as these play an important part in CTC development [19,20]. Upregulation of LPA was observed in gynecologic, breast, ovarian, thyroid, colon, prostate and melanoma cancers [21,22,23].

LPA enhances the activity of MMP7, MMP9 and MMP2 in tumor cells [24–29]. MMPs are endopeptidases that play an important role in

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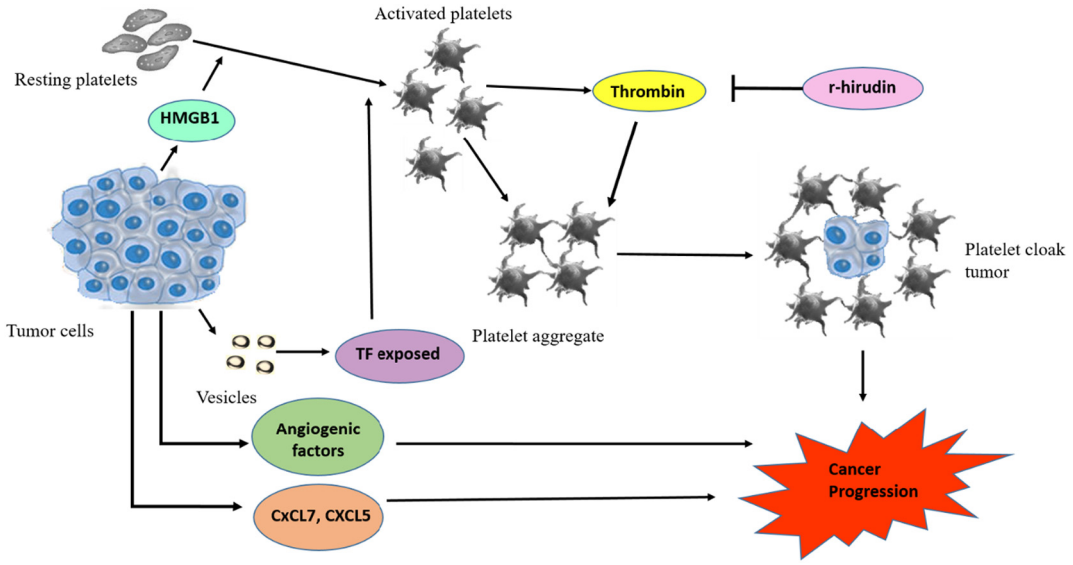

Fig. 1. Tumor dependent Platelet activation: Tumor cells induce platelet activation via release of certain factors that induce aggregation that lead to cancer progression.

tumor progression by degrading as well as remodulating the ECM (Extracellular matrix) [30]; thus, allowing the detachment of tumor from its place of origin and enter into blood circulation to get metastasized [28].

EMT in tumor cells is also initiated by TGF-β and PDGF released by platelets upon activation [31–35]. Effect of TGF-β and PGE2, released by platelets, on induction of EMT, remodeling of ECM and metastasis is backed up by studies of many researchers [15,33]. Another study showed localization of platelets on leading edge of tumor as well as the expression of Snail1 which is an EMT associated transcription factor [14]. Some other studies also reveal that tissue factor expression on tumor cells induce activation of platelets [36,37]. Platelets also get activated by the tumor cells by ADP, thrombin and via ligation of TLR-4 [5,29,38]. These activated platelets later release ATP, serotonin and histamine. After a tumor cell has undergone EMT the step that follows it is invasion.

To summarize we can say increased production of MMPs promote degradation of ECM [28,29]. LPA released by platelets further enhances the activity of MMPs [39]; hence, promoting the entry of tumor cell in to the bloodstream [40,41].

Despite of progress in determining tumor platelet interactions, no direct evidence is available indicating platelet affecting intravasation of tumor cell [5]. Further experimentation is required to identify the mechanisms underlying this key step during metastasis. The above procedure is summarized in Fig. 2.

4. Platelets role in tumor cell induced platelet aggregation (TCIPA) formation

After entering blood vessels, tumor tends to translocate itself towards distant body parts [42]. The ability of tumor to metastasize to secondary sites depend upon its ability to activate platelets. Platelets in blood vessels get activated either when they come in direct contact with the tumor or when tumor interacts with the agonistic mediators released by platelets, eventually triggering an aggregation of platelets called tumor cell induced platelet aggregation (TCIPA) [43]. Certain agonist mediators involve thrombin, tumor associated proteinases, TxA2 and ADP etc. [44,45,38]. The ability of tumor to form aggregate and form a heterogeneous emboli has also been observed within the microvasculature of murine models [46]. Moreover, previous studies also reveal that patients suffering from cancer has increased number of activated platelets in blood [47,48,49] and this activation of platelets leading to aggregation has been found correlating with metastatic potential of cancer both in in-vivo and in-vitro studies [4].

In platelets one of the two basic pathways are involved in their activation leading ultimately to TCIPA formation as show in Fig. 2. One of that pathways is prompted by CLEC-2 and GPVI which includes a cascade of tyrosine phosphorylation downstream of the ITAM associated with the receptor or hemITAM respectively. This leads to complete activation of platelets [50]. The second pathway involves TXA2, thrombin and ADP (soluble agonists) which upon interaction with G protein coupled receptors initiate specific downstream signaling cascade and help in activation of platelets.

CLEC2 plays a role in platelet activation in vivo by interacting with an unknown ligand [51]. The only known ligand for CLEC2 is a transmembrane protein podoplanin [52], not present in blood vessels. But expression of podoplanin is seen in several tumors [53] and by blocking podoplanin a decrease in formation of TCIPA was seen in vitro [54] and decrease in metastasis was observed in vivo [54–56]. So it can be concluded that hampering CLEC2-podoplanin contact can be a promising strategy for metastasis prevention. Although it should be noted that this interaction is required for separating lymph and blood vessels during development of an embryo [57,58] and an adult mice [59,60].

As far as GPVI is concerned it is the collagen receptor which is particular for platelets and this receptor is known to cause strong cellular activations ultimately promoting TCIPA. This was further proved by clinical studies as its absence or deficiency ultimately decreased experimental metastasis by 50% while had no effect on primary tumor growth [61]. This might be of interest clinically as interfering GPVI minimally affected platelet's function of hemostasis [62].

According to the activation induced by second described pathway, ADP and TXA2 secreted by tumor cells help in recruitment of platelets to initiate platelet aggregation; thus, helping them to survive in blood circulation from shear stress [63,64,5,65]. Two ADP receptors which are G protein coupled and help in aggregate formation are expressed by platelets and are known as $P2Y_1$ and $P2Y_{12}$ [66]. Although tumor cell secrete ADP [67–69] but a reduction in metastasis was observed in the absence of $P2Y_{12}$ [67]. Furthermore, inhibitors of ADP decreased the potential of coaggregate (tumor and platelets) formation [70]. In addition to ADP and TXA2, thrombin also plays a part in platelet aggregation via activation. It has been reported in studies that expression of platelet tissue factor, involved in production of thrombin from zymogen prothrombin, has been detected in different types of tumors

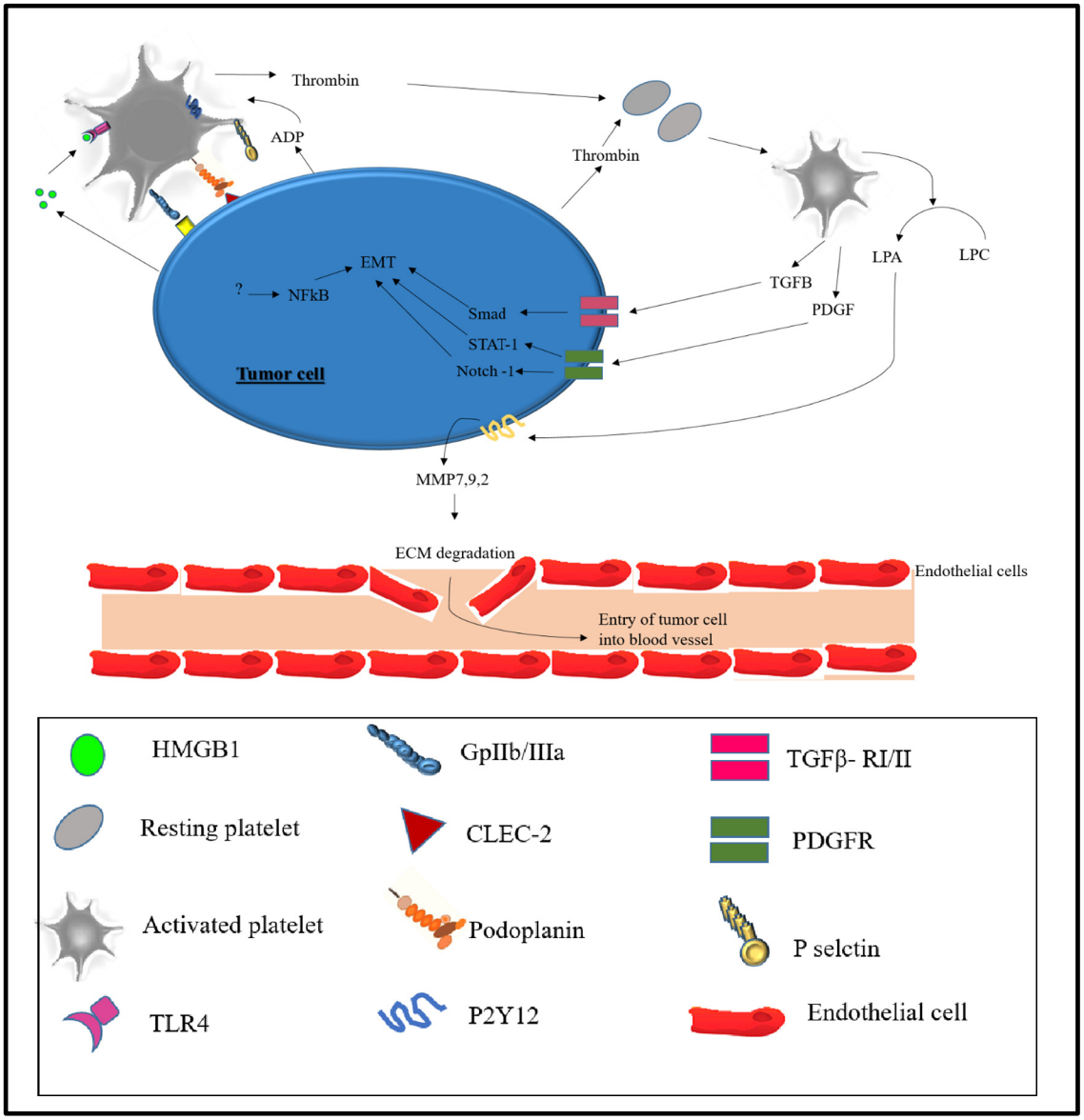

Fig. 2. Platelets favor metastasis: Release of ADP and Thrombin causes activation of platelets that interact with tumor cell to start a cascade of new events.

[71–73]. Increased expression of thrombin acts as an important agonist for platelet activation and coagulation by cleaving Protease-activated receptor 4 (PAR4) and PAR1 present on human platelets. Moreover, Inhibition of thrombin by hirudin in a cancer cell line inhibited TCIPA [74]. The deficiency of PAR-4 made the platelets of mice to be insensitive to the effect caused by thrombin and this helped in protecting them from experimentally induced metastasis just like as if deficiency of platelets could have done [75]. It is important to note here that PAR-4 heterozygosity helps moderately in hemostasis [76] and it also aids in reduction of experimentally induced metastasis. So, a reduction of signaling by thrombin is enough for decreasing metastasis. Mice also stayed protected from this induced metastasis if they lacked Gαq which is a subunit of G protein and is important downstream of PAR-4 and other receptors.

Integrins present on the surface of cancer cells are also seen playing their part in TCIPA as they assist in adhesion of cell to cell. These integrins are hence involved in interaction of tumor with the platelets via binding of GPIIb/IIIa from platelets primarily with integrin αvβ3 expressed on tumor cells [5]. Cell adhesion ability of tumor cell was seen to be increased with enhanced expression of GPIIb/IIIa receptor of platelets in presence of fibrinogen in ovary cells of Chinese hamster. Thus, Inhibition of both αvβ3 or GPIIb/IIIa by the use of antibodies can inhibit or decrease platelets and melanoma cell adhesion ultimately reducing metastatic potential [77]. It has also been observed that inhibition of αvβ3 by monoclonal antibody and reduction of platelet number decreased pulmonary metastasis [77]. Moreover, inhibition of GPIIb/IIIa also reduced aggregation and adhesion potential of tumor cells [78]. According to reports GPIIb/IIa activation is not only required for TCIPA but it also is important for secretion of pro angiogenic factors from α granules of platelets further assisting in tumor proliferation and metastasis [79,80]. Although it is not known that how much important is a specific platelet activator for metastasis while some of the data suggests that it depends on the type of tumor cell and the mechanism through which it activates the platelet [81]. Thus, it can be concluded that contact of αvβ3 with GPIIb/IIIa mediate platelet activation. After platelets get activated a change in confirmation of integrin occur i.e. inside out activation, enabling them to interact with their ligands with high affinity to form aggregates [82] eventually helping in TCIPA and further in tumor dissemination by adhering tumor with endothelium due to their ability of mediating cell to cell interactions.

Platelet aggregation to form TCIPA is also initiated by upregulation of surface receptors of platelets i.e. P-selectin which upon binding to

mucin type glycoprotein mediates tumor-platelet interactions [70,83]. This interaction not only allows for the migration of tumor as well as enhances its adhesion to vascular endothelium [84]. The mice that are deficient in P-selectin show attenuated tumor growth which indicates the importance of these adhesion molecules for tumor metastasis [83].

The role of GPIb-IX-V complex in tumor metastasis and formation of TCIPA is controversial as the earlier experiments indicated pro-metastatic role of GPIbα [74,85], whereas there were others who reported no effects on in vitro blockage of GPIb. The subunit of GPIb- IX-V complex i.e. GPIbα, used for binding of ligand, when binds to vWF, it decelerates platelets and adhere them to the site of injury. GPIbα has also been seen binding with P-selectin of either the activated platelets or endothelial cells which then mediates the leukocyte and platelet interactions by interacting with Mac-1. GPIbα inhibition in vivo by monoclonal antibody (Fab-fragment) caused an increase in metastasis of melanoma which indicates role of GPIbα to be inhibitory. However, on the other hand a sharp contrast was observed in a study in which mice lacking GPIbα showed reduced metastasis and it was independent of the fact if the mice lack only the GPIbα extracellular domain or GPIb-IX-V entirely [86]. To date no acceptable explanation has been provided for this but another report also indicated reduced metastasis in mice deficient in vWF [87]. As GPIb-vWF interaction are an essential requirement for adhesion of platelets; therefore, in the deficiency of vWF, the aggregates of platelets and tumor (TCIPA) might shift themselves to smaller blood vessels in place of larger ones because they will provide the aggregate with lesser shear force. In mice, deficient in P selectin, no effect of GPIb blockage was observed on promotion of metastasis [88]. This observation came out as a surprise for scientists because metastasis was promoted during lack of vWF regardless of absence of P- selectin only from endothelial cells and not from the platelets. This further caused a flaw in the formation of body of Weibel-Palade in mice who were deficient in vWF. According to some experiments the blockage or complete absence of P-selectin was found to be favorable for experimental metastasis [83], whereas the P-Selectin from endothelial cells and platelets was found to be involved in tumor dissemination [89].

In addition, if normal platelet number exists but their activity is somehow reduced this could also reduce production of TCIPA leading to emboli formation [90,73,91]. So, here we can conclude that Thrombin, ADP, Tumor associated proteinases and TXA2 released by tumor or direct interaction of tumor cell with platelets cause platelet activation [44] [45]. Platelets after getting activated get attached to the CTC via highly expressed P- selectin and GPIIb-IIIa-fibrinogen Bridge [92] forming TCIPA.

It is important to note here that the reduction in number of platelets or thrombocytopenia due to anti-platelet sera [93,94] or by a defect in platelet production [89,75] decreased metastasis. Interestingly, count of platelet number that are 30% of that of the control levels were enough to promote metastasis [89]; hence, indicating that just like in hemostasis and thrombosis [95] only very large decrease in amount of platelets resulted in an impaired overall response by platelets in vivo.

5. Platelets role in evasion of tumor from immune surveillance

Hematogenous metastasis is supported by TCIPA as it entangles the emboli in the microvasculature and the aggregate formed also then helps in escaping from immune surveillance. Tumor cells circulating in blood also known as CTC (circulating tumor cells) have very low half-life due to the challenges faced by them given by the immune system in the form of rapid clearance as well as the mechanical destruction by shear forces of blood [5,65]. The mechanism through which a tumor cell induces platelet aggregation could be different for different cancer cell types. Since less than 0.1% of the cancer cells found in blood survive [96] and form TCIPA; therefore, it is important for them to confer the theme of survival advantage. The molecular mechanism involved in overall survival of CTC by platelets is yet to be understood. According to one of the presented hypothesis, envelopment of tumor by activated platelets provide it with a shield against immune system as attack by NK cells is dependent on physical interaction with the CTC [97,94,98].

This envelopment of NK cells can be explained as activated platelets through their GPIIb/IIIa receptors bind with fibrinogen to make fibrin clot. This clot entangles the tumor in the mesh created by platelets and fibrin network to form a heteroaggregate. All of this procedure occurs within the blood vessels [5,63]. This heterogeneous clot formed now shields the tumor from the attack of immune system cells i.e. lysis by NK cells [99,98]. Although NK cells are most important for antitumor activity [100,101,97] but a direct interaction with the tumor is required for this activity. Mice which were deficient in NK cells showed more susceptibility towards metastasis [102,103,104] and low activity by NK cells in peripheral blood showed increased link to the risk of developing cancer in certain epidemiological studies [105].

Other studies proved that platelet depletion in the absence of NK cells either does not affect or moderately affects metastasis. Depletion of fibrin cross linker FXIII or fibrinogen decreased the occurrence of metastasis. It is important to mention here that in mice, deficient in NK cells, decreased number of fibrinogens did not enhanced metastasis [98]. Moreover, according to a study platelets not only shield tumor cell from attack of NK cells but also protects it by downregulating the activity of NK cells [94]. This might be the result of decreased expression of NKG2D on NK cells. Evidence also exists that proves the condition of thrombocytopenia increases the CTC lysing ability of NK cells both in vivo as well as in vitro [94].

Activated platelets also release TGF-β upon interaction with tumor cell. Release of TGF-β causes a reduction in expression of activating receptors on the surface of NK cells [106]. Moreover, by the release of thrombin, tumor cell associated tissue factor and resulting deposition of fibrin further promotes the evasion of tumor from the immune surveillance ultimately promoting metastasis [98]. These all interactions of tumor with activated platelets help to initiate the cascade of coagulation and promote tumor dissemination.

Platelets when activated are capable of transferring the MHC complex to the circulating tumor cells. This allows the tumor to act as host cell; thus, helping them to mimic as normal body cells and escape the immune surveillance. TCIPA also hampers dendritic cell maturation by the release of platelet granules containing VEGF [107,108,109,110]. Efficient TCIPA can evade immune surveillance, and, thus, can promote metastasis [4]. Moreover, Pro-thrombotic environment helps in the promotion of metastasis. Antiplatelet agents decrease [4] metastatic potential of tumor cell as a result of insufficient shielding from immune cells by platelets. Tumor cell also sometimes mimic platelets to escape from immune attack by expressing receptors and adhesion molecules present on surface of platelets [111]. The above stated mechanisms are described in Fig. 3.

6. Platelets role in protection of tumor cell from shear stress

This potential of platelets to be pro-metastatic attributes to the fact that they enhance the adhesion properties of tumor and protects it physically from shear forces of vessels [5,63,98,112,61,113].

GPIIb/IIIa integrin found on platelets interacts with αvβ integrin found on tumor cell to promote the cohesion and adhering of tumor cell with the walls of blood vessels, and, thus, providing protection to the tumor from the shear forces of blood flow [5]. This can be further proved as application of inhibitors of GPIIb/IIIa decreased the colonization potential of tumor [78]. Thus, we can say that the interaction between these two integrins after the activation of platelets help in migration, adhesion and ultimately survival of tumor cell. [5,63,77].

7. Platelets role in tumor extravasation

Tumor cells while roaming may get stuck due to their large size and get extravasated [114]. Microparticles derived from platelets and

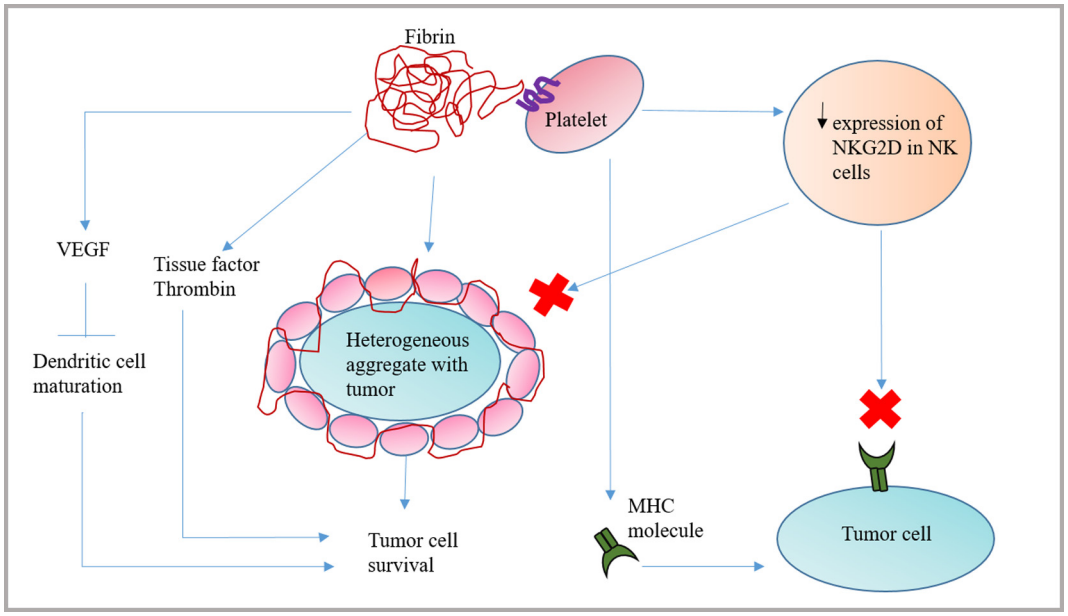

Fig. 3. Evasion of tumor cells for immune system: Platelets decrease the expression of NKG2D in NK cells and bind to Fibrin and tumor cell to form a heterogeneous aggregate to evade from the immune system.

platelets itself coaggregate with tumor to promote metastasis via helping in arresting of the tumor cell in blood vessel and ultimately extravasation [4,5,29,115,63,33]. Role played by platelets in extravasation is explored by various researchers. [63,113]. Platelets are known to release certain cytokines and growth factors present in α-granules of platelets which not only help in metastasis of tumor but also in addition of creating a protective and supportive environment helps in attachment of tumor cells with vascular endothelium ultimately promoting extravasation.

CTC after escaping through all stress and reaching a distant point, starts to localize in that organ. For that to occur CTC has to come out of the blood vessel i.e. extravasation so, it anchors itself to endothelial cells and then break in through the extracellular matrix (ECM). The process of extravasation involves the interaction between the adhesion receptors present on endothelial cells and CTC and this interaction is thought to be regulated by platelets. EMT and MET play a pivotal role in cancer invasiveness and metastasis [116]. Circulating tumor cells are often known to possess properties like the cells which are going through Epithelial–Mesenchymal Transition (EMT) [117]. Circulating tumor cells (CTC) of breast and prostate cancer when studied were seen co-expressing Vimentin, Cytokeratin (CK), E-cadherin and N-cadherin [118]. If these signaling molecules were inhibited using their specific inhibitors as Snail, Zeb and Twist relapse of metastasis was prevented [119]. Although the mechanism that helps CTC to uphold the state of EMT is yet to be explained. Platelets, however, not only are seen protecting CTC from immune system but also help in maintaining the state of EMT [33].

According to certain evidences this process involves not only the interaction of CTC with platelets but also with the leukocytes which collectively induce the expression of CCL5 receptors which further allows for the recruitment of leukocytes to the site [120]. Leukocytes are found to be involved in causing metastasis as well enhanced survival of the tumor in case of lung cancer [65]. This was further proved as inhibition of CCL5 caused inhibition of metastasis [120]. Furthermore, PGE2, MMP, LPA and other factors derived from both the platelets as well as leukocytes, which as described earlier were found to aid in inducing EMT and tumor intravasation, also assist in weakening the endothelium for tumor extravasation. Myeloid cells also activate the endothelium via TNF-α, IL1α, IL-1β [121].

TCIPA causes the release of α-granules by platelet. These granules contain TGF-β and PDGF in a higher concentration as compared to other cells [31]. TGF- β1 with other contributing factors released by platelets plays a vital role in extravasation by helping in binding of tumor to the surface of endothelial cells and initiates EMT required for metastasis [33]. The physical changes through which an endothelial cell passes during EMT are reversible and for short period of time. EMT is basically epithelial to mesenchymal transition in which the phenotype of tumor is altered in order to promote its dissemination and motility away from tumor micro environment [122]. TGF-β, when released, enhances the differentiation of CTC into more mesenchymal like phenotype by activating Smad pathway [33]. This transition induced by TGF-β1, released from the platelets, also include activation of NFκB signaling pathways of tumor [33]. In an experiment carried out on TGF-β1 deficient mice indicated diminished metastasis and extravasation mechanism; hence, indicating an obvious role of TGF-β1 in promoting invasiveness as well as metastatic activity of tumor cell [33]. Thus, we can conclude that an interaction between NFκB and TGF-β/Smad is an absolute necessity for efficient metastasis.

PDGF also helps in invasion of cancer and promote angiogenesis. In Prostate cancer increased amount of released PDGF-D helps in promoting EMT via activating downstream targets of rapamycin i.e. S6K and 4E-BP1. Cross talk between EMT and PDGF via NF-Kb and CXCR4 further proves the role of PDGF in EMT [123,124]. When HCC was specifically observed, PDGF was found to be involved in metastasizing the tumor via TGF-β-induced EMT [125]. TGF-β activates β-catenin and STAT3 which ultimately increased the PDGFR and PDGF expression [126]. We can conclude that TGF-β and PDGF from platelets were involved in induction of EMT in CTC; hence, allowing it to escape apoptosis and metastasize. Its role in metastasis was further validated as inhibition of TGF-β decreased metastasis [33].

The sequence of events that follow this interaction include attachment of tumor cell to the endothelial cells of vascular wall, EMT of tumor cell, passing both the endothelium and basement membrane and dissemination to distant sites. Recent data reported suggests that ADP production might be more essential for process of extravasation rather than TCIPA [127] as platelets also assist in extravasation by producing ADP. ADP increases the permeability of the barrier of endothelial cells; hence, permitting the trans endothelial migration [127]. Lack of P2Y2 receptors in mice or diminished ability to secrete ATP by platelets caused a reduction in metastatic potential of tumor cells; hence,

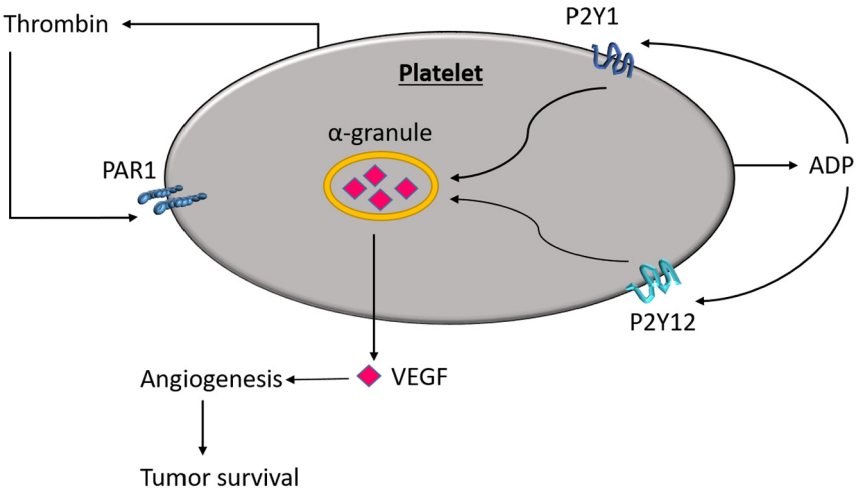

Fig. 4. Role of platelets in tumor proliferation: Platelets release Thrombin and ADP that causes the release of VEGF and induces angiogenesis.

indicating their potent role in metastasis and trans endothelial cell migration via receptors P2Y2 [127].

Moreover, proteases and heparinases from platelets help in retraction of endothelium and dissemination of tumor to the underlying tissues [113]. During the dissemination, the interaction between the tumor and endothelial cells is maintained by GPIIb/IIIa and P-selectins [99,83]. Platelets utilize p selectin to adhere to tumor cell via mucins. This interaction promotes not only attachment of tumor with endothelium but also EMT for establishing a pre-metastatic niche [83,99]. This extravasation and metastatic cascade also involves granulocytes. Chemokines released by platelets help in recruitment of leukocytes during aggregate formation, seeding, trans endothelial migration and metastasis.

8. Platelets role in creation of favorable microenvironment at secondary site of tumor

In addition to the enhanced expression of several genes that are pro metastatic in tumor cells, the pro angiogenic factors present in platelets help in tumor survival and dissemination. VEGF in particular is critical for angiogenesis [128,129].

According to a study platelets residing on the surface of endothelial cells [130], when get activated, release VEGF directly into the malignant tissue; hence, promoting angiogenesis [131,132]. In contrast to VEGF platelets also contain antigenic proteins i.e. thrombospondin-1, Platelet factor-4 and endostatin. These antigenic proteins are known to inhibit vascularization and proliferation of endothelial cells [7,133]. The decision of whether anti-angiogenic or pro-angiogenic factors are to be released depend upon the stimuli received by platelets which contain these factors in separate compartmentalized α-granules [7]. It has been found that thrombin receptors selectively stimulate the release of α-granules to release antigenic or proangiogenic factors on demand [7]. PAR-1 was seen involved in selective release of VEGF and not endostatin, whereas PAR-4 was involved in release of endostatin, yet it does not stimulates the release of VEGF [7,133]. However, platelets release both the factors, yet the net result favors angiogenesis [134,135,136].

Apart from VEGF itself, thrombin released by platelets also assist in angiogenesis by promoting the release of VEGF and also initiates the growth of endothelial cells. Thrombin has high affinity for PAR-1 rather than PAR-4 as a result platelets release pro-angiogenic factor proteins. P2Y1 and P2Y12 receptors help in the release of proangiogenic factors but it was observed that the VEGF released from this pathway produces lesser amount of VEGF as compared to activation mediated by thrombin [137,138]. Activation of platelets by ADP causes an increase in the amount of VEGF released but had zero effect on the release of endostatin, whereas inhibition of release of VEGF was also observed through the use P2Y12 inhibitors. This proves the participation of ADP in neoangiogenesis around the tumor [137].

Platelet microparticles on the other hand are also observed to assist in angiogenesis [139] by forming a network of capillaries [140,141] and stimulate expression of pro angiogenic factors on tumor cells [142]. In addition, microparticles also express tissue factors in cancer patients [143]; hence, further promoting angiogenesis and metastatic cascade.

These all above mentioned observations indicate that platelets do contribute in neovascularization so that the tumor can metastasize as described by the Fig. 4.

9. Use of platelets in liquid biopsy

Although the amount of mRNA in platelets is low when compared to the rest of cells in blood but independent studies i.e. Next Generation Sequencing have suggested that platelets have a rich mRNA repertoire. It is estimated that there are approximately 3000 to 6000 mRNA in platelets [144,145]. This mRNA repertoire can be exploited for their use in liquid biopsy.

It has been established through different studies that cell types other than platelets have the ability to exchange their genetic material via exosome mediated transfer [146]. Platelets are also known to have the ability to internalize proteins [147] and recent studies have shown the capability of platelets to transfer their mRNA to monocytes which brightens the possibility that they may also be active recipients of mRNA by endocytosis [148]. Platelets may also undergo splicing in response to the signals released by tumor. Interaction of platelets with certain growth factors released by tumors also changes the expression of mRNA present in platelets i.e. VEGF, PGDF and PF4 helping in tumor proliferation [149]. Therefore, a potentially differentiated mRNA repertoire exists between healthy and cancer patients [149].This differential expression of mRNA within platelets due to interaction of platelets with tumors can be exploited to be used as a biomarker for early cancer detection or to indicate cancer metastasis. These platelets are known to be educated by tumor, and, hence, are called as Tumor educated platelets and could be potentially used as biomarkers [150]. In addition to change in mRNA several other characteristics of platelets i.e. protein content, platelet count and volume, also differ in patients affected by cancer. Therefore, all of these features either alone or in combination could be used for early detection of cancer, which can improve prognosis of cancer as well as effectiveness of available treatments [149].

10. Conclusion

Platelets poses dynamic biological functions from the array of integral blood component to a prognostic marker for cancer development. Platelets facilitate proliferation of tumors by detaching tumor from its primary site, establishing TCIPA and masking the tumor cells from the immune surveillance. Furthermore, Platelets help in metastasis; thereby, promoting tumor development to distant body parts in addition, it is also suggested that differentiated mRNA profile for the platelets can be effectively targeted as a tool for the early cancer detection.

Taken together, platelets are considered to be enriched in biological information that can be hunted for the prognostic measures' thereby, making it much translational.

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