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Review Article

Platelet functions and clinical effects in acute myelogenous leukemia

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Summary

Platelets interact with normal peripheral blood cells via adhesion as well as soluble mediators, and platelet released mediators can affect hematopoietic stem and progenitor cells. Interactions may also be involved between platelets and circulating malignant cells, which is suggested by the effects platelets seem to have on metastasis and the various platelet abnormalities observed in various malignant disorders, including acute myelogenous leukemia (AML) and other leukemias. It is only recently that the interactions between platelets and AML cells have been characterized in detail, and studies show that; i) platelets and AML blasts can affect functional characteristic of each other, ii) chemotherapeutic drugs frequently used in AML therapy can alter several platelet functions, iii) the systemic levels of various cytokines are enhanced during AML chemotherapy, including cytokines known to affect both leukemic blasts and platelet activation, and iv) platelet secretion of growth factors are clearly detected in peripheral blood stem cells autografts. In this review we describe platelet interactions with normal leukocytes, normal hematopoietic and leukemic cells and the possible clinical relevance of these interactions in AML.

Keywords

Platelets, hematopoiesis, AML, chemotherapy, PBSCT

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Introduction

Platelets normally play a role in hemostasis (1) and are involved in the activation of immunocompetent cells (2). Thus, platelets interact with normal blood cells during physiological processes. Similarly, platelets can also interact with malignant cells including leukemic blasts (3, 4) and have been indicated to play a significant role in tumour metastasis (5). However, it has not yet been characterized in detail whether platelet interactions are important for carcinogenesis or treatment responsiveness of malignancies. Previous studies suggest that platelets interact with acute myelogenous leukemia (AML) blasts both *in vitro* and *in vivo* (3, 4, 6–8), and may be important during conventional chemotherapy as well as peripheral blood stem cells transplantation (PBSCT) (9–11). Here we describe platelet interactions with normal leukocytes, normal hematopoietic and leukemic cells and discuss the possible clinical relevance of these interactions in AML.

Platelets

Platelets store several biologically active molecules, including platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), transforming growth factor-β (TGF-β) platelet factor-4 (PF-4) (Table 1) and Angiopoietin-1 (Ang-1) (12). During in-vivo vessel wall rupture and bleeding platelets become adherent and activated. Platelet activation is among other things characterized by secretion of the biologically active molecules from α-granula and dense bodies (Table 1) (1, 2). Activated platelets also release smaller membrane vesicles, the platelet-derived microparticles (PDMP) (2), which at least express tissue factor (TF) and seem to be involved in the regulation of thrombus formation (13). The activated platelets increased stickiness is due to expression of activation dependent adhesion molecules including GPIIb/IIIa (CD41/CD61, fibrinogen receptor) and CD62P (1, 2). These molecules induce platelet aggregation and adhesion with subendothelium, endothelial cells and leukocytes (1, 2) and thereby participate in the mechanism of bleeding arrest.

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Table 1: Platelet granula contents.

Granula	Substance group	Molecule
α-granula	Growth factors	Platelet-derived growth factor (PDGF)
		Transforming growth factor-β (TGF-β)
		Epidermal growth factor (EGF)
		Vascular endothelial growth factor (VEGF)
		Thrombopoietin (TPO)
	Platelet specific proteins	β-thromboglobulin (β-TG)
		Platelet factor-4 (PF-4)
	Coagulation factors	Factor V, VII, XI, XIII
		Fibrinogen
	Glycoprotein	Thrombospondin (TSP)
		Fibronectin
		vWF
Dense bodies		Serotonin
		ATP, ADP
		Ca^{2+}

vWF, von Willebrandt factor; ATP, adenosine triphosphate; ADP, adenosine diphosphate. Table is based on (1, 2, 164).

Table 2: Adhesion molecules involved in platelet adhesion to normal leukocytes and bone marrow cells.

Platelet adhesion molecule	Ligand-expressing cells	Ref
CD62P	Monocytes	23, 25
	Neutrophils	25
	Eosinophils	25
	Basophils	25
	Polymorphonuclear leukocytes (PMN)	23
	T-cell subpopulations	25, 26
CD41a / fibrinogen	Polymorphonuclear leukocytes	27
Thrombospondin (TSP)	Bone marrow cells	28
Fibronectin	Bone marrow cells	29

Thrombin, collagen, adenosine diphosphate (ADP), thromboxane A_2 (TXA_2) and von Willebrand factor are all physiological platelet activators (1, 2). In addition to physiological mediators, various forms of stress, including centrifugation and exposure to artificial surfaces, can also induce platelet activation. This is observed during clinical procedures that involve extracorporeal circulation including collection of peripheral blood hematopoietic stem cells (10, 11, 14, 15).

The role of platelets in specific variants of AML

Later on in this review we will go on to discuss the possible importance of platelets in AML in general. However, two specific variants of AML clearly differ from other subsets, namely the acute promyelocytic variant and the acute megakaryoblastic leukemia, and the role of platelets may thus be different for these two subsets.

Acute promyelocytic leukemia (APL)

The acute promyelocytic variant of AML is characterized by specific genetic abnormalities, predominant signs of promyelocytic differentiation in the leukemic cells and a dramatic clinical presentation different from other AML patients (16). A high proportion of patients with APL has coagulation problems. As described in detail in a recent review (16), the pattern of coagulation abnormalities in most patients at presentation is consistent with intravascular coagulation activation, and thrombocytopenia is a part of this. The molecular mechanisms behind the activation are not known in detail, but TF is expressed by the promyeloblastic cells, and the cancer procoagulant cystein protease is also expressed at higher levels in APL cells than by other AML cell subsets (16, 17). All these observations suggest that the role of platelets in APL differ from the other subtypes, and the guidelines for platelet transfusions also differ. The British Socitey for Haematology Guidelines state that platelet counts should be kept above 20 x 10^9/l also in the absence of fever or bleedings, and other authors have suggested that maintaining platelet counts above 50 x 10^9/l during the first 10 days of treatment, especially in those who are actively bleeding, will reduce the risk of early haemorrhagic death (16).

Acute megakaryoblastic leukemia (AML-M7)

AML-M7 occurs in two age groups, young children and adults. AML-M7 seems to be a heterogeneous group; Down syndrome associated, t(1;22) associated and those with other abnormalities (18). The two first are childhood disorders. The disease is often characterized by organomegaly, thrombocytosis may be seen, the bone marrow often shows fibrosis and associated myelodysplastic changes, and as explained above for many patients leukemogenesis depends on specific genetic abnormalities (i.e. trisomy8, t(1;21), mutations of the GATA1 transcription factor) (18, 19). As would then be expected, gene expression profiles are also different for AML-M7 compared with other subsets, class-discriminating genes have been identified but in addition there seems to be a heterogeneity with different subsets within the M7 group (20, 21). Taken together, these observations suggest that AML-M7 is a specific entity, functional characteristics of these leukemic cells probably differ from other AML subtypes and the role of platelets in this variant is possibly also different.

Platelet interactions with normal and malignant cells

Platelet interactions with normal leukocytes

Several studies have shown that normal platelets and leukocytes adhere well to each other and interfere functionally (2) (Table 2). Here we review some of these interactions. Thrombin activated

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platelets adhere well to monocytes and polymorphonuclear leukocytes (PMN) (22), and platelet adhesion to monocytes seems to be favoured over neutrophils (23) and is mediated via the CD62P membrane molecule (24). Adhesion is also observed for eosinophils, basophils and subpopulations of T-lymphocytes (25, 26). However, other membrane molecules may also be involved, including CD41a and fibrinogen (27). In addition, both thrombospondin-1 (TSP) and fibronectin bind bone marrow cells (28, 29) and may therefore be involved in normal platelet-leukocyte adhesion.

Leukocytes can affect platelet aggregation, induce intraplatelet Ca^{2+} fluctuations and increase platelet thromboxane B_2 production by adhesion or via released mediators (30). In addition, leukocyte released interleukin (IL)-1 and interferon-γ induce platelet expression of CD62P that enhance platelet-leukocyte adhesion (31). Platelets, on the other hand, can release NAP-2 that indirectly increases the intracellular calcium concentration in PMN's (32), and platelets together with fibrinogen can activate neutrophil cells (33). Furthermore, CD62P can prime monocytes to increase the production of platelet activating factor (PAF), which indicates that CD62P on platelets serves to localize monocytes at inflammatory sites and prime these cells for inflammatory responses (34). CD62P dependent adhesion between platelets and neutrophils and monocytes induces both cells to produce extracellular superoxide anion (O_2^-) (31), which is further potentiated by IL-8 (CXCL8) and granulocyte-colony stimulating factor (G-CSF). Activated platelets also induce the expression of monocyte chemotactic protein-1 (CCL2) and IL-8 by monocytes that require the CD62P antigen on platelets (35). Soluble mediators released by activated platelets can also modulate leukocyte functions, e.g. PDGF and PF-4-induced activation of human peripheral blood neutrophils (36, 37). Thus, normal functional interactions between platelets and normal leukocytes are complex and involve both adhesion and soluble mediators.

Platelet effects on normal hematopoietic cells

Platelets can also bind (38), and via released mediators, affect hematopoietic stem cells (Table 3). PDGF can alter proliferation of hematopoietic progenitors (39), including enhance expansion of $CD34^+$ cells (40). However, PDGF may not have direct effects on $CD34^+$ cells because this progenitor subset seems to lack its receptor (41) or develops it later in culture (40). TGF-β (Transform growth factor β) divergently affects hematopoietic stem cells (42), but is stored in platelets as a poorly active complex (43). TSP and Fibronectin adhere to bone marrow cells (28, 29) and can alter megakaryocytopoiesis and hematopoietic stem cells, respectively (44, 45). Fibrinogen can also affect the growth of bone marrow cells (46). VEGF possibly regulates hematopoietic stem cell survival (47) and can affect hematopoietic cells by suppressing apoptosis (48) and divergently alter colony growth (49). PF-4, β-thromboglobulin (β-TG) and Serotonin can all affect megakaryocyte cell growth (50–52), and PF-4 in addition supports the survival of myeloid progenitors (53) and promotes the adhesion of hematopoietic stem cells (54). The fact that $CD34^+$ cells themselves produce and respond to VEGF and PF-4, amongst several other growth factors, indicates that these factors do play a physiological role in normal hematopoiesis (55). Furthermore, PDMP's seem to alter several functions of hematopoietic cells, including survival, proliferation and adhesion (56).

To conclude so far, platelets can interact both with mature myeloid cells and immature hematopoietic stem or progenitor cells through several molecular mechanisms. The clinical importance of these interactions in AML is not yet known. Patients with AML may have increased levels of circulating activated platelets and possible effects of local platelet activation are: i) altered function of immunocompetent cells in these severely immunocompromised patients, or ii) modulation of remaining normal hematopoiesis in the bone marrow compartment that is slowed by the dominating AML cell population and a minor cell subset of normal hematopoietic cells (for further discussion, continue).

Table 3: Effects of different platelet-released mediators on hematopoietic cells.

Platelet released mediator	Hematopoietic cell type	Effect	Ref.
Platelet-derived growth factor (PDGF)	Progenitors	Cell growth	39
	$CD34^+$ cells	Expansion	40
Transforming growth factor-β (TGF-β)	Stem cells	Cell growth (divergently effects)	42
Thrombospondin (TSP)	Megakaryocytic cells	Inhibiting colony formation	44
Fibronectin	Stem cells	Increase CFU-GEMM	45
Fibrinogen	Bone marrow $CD34^+$ cells	Increase BFU-E	46
Vascular endothelial growth factor (VEGF)	Stem cells	Cell survival	47
		Apoptosis	48
		Colony growth	49
Platelet factor-4 (PF-4)	Megakaryocytic cells	Cell growth	50, 51
	Myeloid progenitors	Survival	53
β-thromboglobulin (β-TG)	Megakaryocytic cells	Cell growth	51
Serotonin	Megakaryocytic cells	Cell growth	52

CFU-GEMM, colony forming unit-granulocyte-erythroid-macrophage/monocyte-megakaryocyte; BFU-E, burst-forming unit-erythroid.

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Platelet interactions with neoplastic hematopoietic cells

There is clear evidence that platelets are altered in several malignancies and interfere with malignant cells. At least three types of platelet-malignancy interactions occur: i) altered platelet activation (57–59); ii) platelet effects on malignant cell growth (60), and iii) platelet enhancement of metastasis (5). Here we briefly describe some of these interactions.

Abnormally high levels of the platelet specific proteins β-TG and PF-4 are detected in patients with active or progressive malignant disease (61), and elevated in-vivo levels of PDMP are described for gastric cancer (57) and myeloproliferative syndromes (58). Platelet activation is further observed during tumour development (59). This indicates that there might be an ongoing platelet activation in patients with malignant diseases. In addition, malignant cells can induce platelet aggregation and activate platelets by ADP secretion (62). On the contrary, decreased and abnormal platelet aggregation is described for chronic myeloproliferative disorders and acute lymphoblastic leukemia (63, 64). However, it is uncertain whether this is directly caused by the malignant cells.

Platelets can affect tumour cells via their release of soluble mediators (65). VEGF is stored in and released by activated platelets (2) and may affect tumour growth via direct effects on malignant cells, or indirectly via its effects on angiogenesis (66–68). PDGF, also released by platelets (1), can affect malignant cells. PDGF receptors are expressed by several tumours and are related to both tumour growth directly and indirectly via angiogenesis (60). Such receptors are among others described on chronic lymphocytic leukemia cells (69). The widespread expression of PDGF receptors in tumours makes it a possible target for cancer drugs (70) which suggests that PDGF plays an important role in malignancies and that platelets might affect these functions. Other platelet-released mediators that can affect malignant cells are: i) TGF-β that can affect leukemic HL-60 myeloid cells (71); ii) PF-4 that may affect tumour invasion (72), and interestingly iii) TSP and fibronectin that are suggested to be involved in cancer metastasis (73, 74).

The aforementioned observations clearly illustrate the wide range of molecular mechanisms that can be involved in the interactions between circulating platelets and malignant cells.

Clinical importance of platelets in AML

AML is characterised by malignant myeloid cells that show neoplastic proliferation with a differentiation block. This results in the accumulation of immature blasts in the bone marrow that frequently leads to hematopoietic insufficiency with peripheral blood cytopenia as well as functional alterations of the remaining cells.

Platelet abnormalities in AML

It has been known since the early 70's that platelets may show a wide range of defects in acute leukemias, including abnormal metabolism, lifespan and aggregation (see [75, 76] and references therein). Several groups have verified these results and characterized additional platelets dysfunctions in AML. These include abnormal platelet aggregation (77–81), abnormal platelet factor-3 (PF-3) activity (78), dysfunction in the release reaction and thromboxane B_2 production (79), abnormal plasma PF-4 and serotonin levels (80, 82), abnormal platelet volumes (83), dense granula abnormalities (83, 84), abnormal clot retraction and prolonged bleeding time (77). Hemorrhage and thrombosis are well known in acute promyelocytic leukemia (85, 86). In addition, platelet abnormalities/activation have also been detected for chronic myelogenous and lymphocytic leukemia (58, 87) suggesting that platelet abnormalities might be a more general phenomenon in leukemias. However, these abnormalities may be of particular importance in AML where they usually occur in combination with a rapidly progressive malignancy with a severe quantitative platelet defect.

Platelet – AML blast interactions *in vitro*

Native myeloid leukemic cells and cells from the AML cell line HL-60 can alter platelet function by inhibiting aggregation (88). In addition, native AML blasts seem to increase the platelet PDGF and soluble CD62P secretion *in vitro* (4). Thus, leukemic blasts are suggested to alter platelet activation *in vitro*. On the other hand, normal platelets can alter AML blast proliferation and constitutive cytokine secretion *in vitro* (3). This is caused both via direct adhesion (3) and via platelet release of soluble mediators, including PDGF, PF-4 and VEGF (3, 6, 7). These observations are further supported by studies showing that these mediators affect the function of other malignant and normal hematopoietic cells: PDGF that is released by platelets in co-culture with AML blast (4) is a growth factor for normal hematopoietic and chronic myelogenous leukemia cells (39, 89). PF-4 regulates apoptosis and supports the survival of normal myeloid cells (53), including megakaryocytopoiesis (50, 51). It is also elevated in peripheral blood of AML patients (80, 82). AML cells can express VEGF receptors (90) and VEGF can affect both normal and leukemic hematopoiesis (47–49, 91). The clinical importance of VEGF is also shown by its prognostic impact in AML patients receiving intensive chemotherapy (92–94).

Possible effects of complex cytokine networks *in vivo*

The clinical importance of platelets and platelet released mediators in AML is not well characterized. Previous observations have shown that both acute and chronic lymphoid and myeloid leukemia cells can release PAF in the active phase of the disease (95), thus possibly affecting platelet activation *in vivo*. Other in-vitro studies suggest that platelet interactions with AML blasts may be important in clinical settings. Via adhesion and/or activation platelets may create or modulate local as well as systemic cytokine networks that affect AML blast functions. We previously showed that the cytokines thrombopoietin (TPO), G-CSF and Flt3-L are elevated in AML patients with chemotherapy induced cytopenia (96). The increased levels of TPO and the high levels of SCF in AML (96) may affect platelet activation in AML patients (97–99) and thereby the secretion of various growth factors capable of altering AML blasts. In addition, TPO and IL-6 can increase the number of released PDMP (100), which can alter normal hematopoietic cell functions (56) and possibly also leukemic cells characteristics. Furthermore, various studies show increased systemic levels of cytokines in AML, including intercellular adhesion molecule-1 (ICAM-1) (101,

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102), IL-6 (101–104), IL-2, TNF-α (105), EPO (106), IL-8 (103), G-CSF (103, 104) and IL-10 (104). Thus, growth factors released by activated platelets together with disease- and chemotherapy-induced modulation of local and systemic cytokine levels create a unique cytokine network in AML patients. This network may then affect disease development as well as chemosensitivity in human AML.

Effects of chemotherapeutic drugs on platelet functions

Patients with malignant diseases can develop quantitative platelet defects due to chemotherapy-induced bone marrow toxicity. Cytotoxic drugs may also induce qualitative platelet defects with abnormalities both in platelet secretion and aggregation (107) (Table 4). Studies performed in the beginning of the 70's showed that various cytotoxic drugs, including cytarabine and doxorubicin, could alter platelet functions (108, 109). The antracycline doxorubicin can also inhibit platelet aggregation (110), platelet release reaction and affect protein phosphorylation (111). Platelet aggregation is also inhibited by vincristin and epirubicin (110), whereas daunorubicin can induce platelet and mitochondrial swelling, vacuole formation, decreased aggregation, decreased serotonin release, inhibition of platelet prostaglandin pathway and decreased availability of PF-3, and for high concentrations even platelet lysis has been observed (111–113).

We previously studied the effects of cytarabine and various antracyclines on normal whole blood platelet activation using flow cytometry. Both daunorubicin and higher doses of idarubicin enhanced platelet expression of GPIIb/IIIa, CD62P and CD63, indicating that platelets were highly activated (8). Both these antracyclins were also absorbed by the platelets. Although the liposomal form of daunorubicin was absorbed by platelets as well, it did not enhance platelet activation in our study. Thus, several cytotoxic agents commonly used in AML therapy seem to affect various platelet functions.

Table 4: Effects of chemotherapeutic drugs on platelet functions.

Drug	Effect	Ref.
Doxorubicin	Inhibit platelet aggregation	110
	Inhibit platelets release reaction	111
	Affect protein phosphorylation	111
Vincristine	Inhibit platelet aggregation	110
Epirubicin	Inhibit platelet aggregation	110
Daunorubicin	Platelet swelling	113
	Vacuole formation	113
	Decreased aggregation	112, 113
	Decreased serotonin release	111
	Platelet lysis (high concentrations)	113
	Increased expression of CD62P *	8
	Increased expression of CD63 *	8
	Increased expression of GPIIb/IIIa *	8
Idarubicin (high doses)	Increased expression of CD62P *	8
	Increased expression of CD63 *	8
	Increased expression of GPIIb/IIIa *	8

* = in-vivo studies.

Platelet activation and adhesion in AML patients *in vivo*

Although high systemic levels of the platelet-activating cytokine TPO is detected (96) and chemotherapeutic drugs relevant for AML can affect platelet functions, a general activation pattern of circulating platelets derived from AML patients during chemotherapy was not detected in our previous study (8). These findings are later confirmed where the expression of platelet activation markers actually was decreased compared to healthy volunteers (81). However, elevated CD63 expression, which is usually expressed on highly activated platelets (114, 115), was detected for a subset of our AML patients prior to chemotherapy, an expression heterogeneity of platelet activation markers that is later confirmed (81). The absence or low levels of fibrinogen receptor and CD62P expression suggest that this CD63 expression should be regarded as a single platelet dysfunction in AML and possibly not as a sign of true platelet activation, just like the low expression of fibrinogen receptor and CD62P (81). But these results may also be explained by reversal of fibrinogen receptor expression (116) and CD62P release (117) after activation (8). It is worth noting that our observations seem to be in contrast to previous reports describing drug-induced modulation of platelet activation in AML (107–113, 118). However, these studies and the previous studies that describe platelet dysfunctions in AML (77–80) are mainly in-vitro investigations, whereas our study address the in-vivo status of the platelets. Another possible explanation for the conflicting observations could be that activated platelets adhere to AML blasts in our patients with high levels of circulating blasts (8). Thus, platelet abnormalities can be detected in AML, but these abnormalities do not represent a conventional activation pattern with development of the normal activated platelet phenotype.

Clinical importance of platelets in stem cell transplantation

Another important setting where platelets may play a significant role is during stem cell transplantation. In a previous study, both PDMP and platelets released mediators, including TPO, increased after auto- and allo-transplantation (119) possibly indicating platelet activation *in vivo*. Furthermore, both platelets and leukocytes become activated during the collection of mobilized stem cells (14, 120, 121) partly detected by elevated levels of P-selectin, TPO and PDMP (11). In addition, platelets and leukocytes are increased in PBSCT autografts (9, 10, 15, 122, 123). Malignant cells may also contaminate autografts, and AML blast contamination may occur in PBSC grafts even when it cannot be detected in the bone marrow collection (124–127). Importantly, graft contaminating malignant cells may be responsible for post-transplant relapse (128–130). We have previously described that the platelet released mediators PF-4, β-TG and PDGF-AB are increased in the autografts compared with peripheral blood, which demonstrates that platelets are activated and make a detectable contribution to a unique cytokine network in autografts (9, 10). In addition, high levels of the hematopoietic growth factors TPO,

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GM-CSF and Flt3-L were also detected in autografts (10, 131), whereas GM-CSF and Flt3-L levels were increased in peripheral blood (131). Thus, both the peripheral blood and the autografts of these patients seem to contain unique cytokine networks, and platelet released mediators contribute to the cytokine network in the autografts. These observations were made in patients with different malignancies, but similar results were detected for all patients independent of their primary disease (10). Thus, platelet activation is most likely dependent on the apheresis procedure (14, 132, 133), and the results are therefore also relevant for patients with other malignant disorders. It is important to note that different apheresis procedures might yield different amounts of platelets and possibly different levels of platelet released mediators (133). Still, taken together with the in-vitro results (3, 6, 7) our and others observations suggest that normal hematopoietic stem cells and contaminating AML blasts in PBSCT autografts are exposed to a local cytokine network, including platelet released growth factors that may alter their functional characteristics. It is not yet known whether these effects are clinically relevant, but as discussed by Bruserud et al. (9) this may be a mechanism for graft platelets to affect the risk of post-transplant relapse.

Platelet transfusions and bleeding complications in AML

AML patients are at increased risk of bleeding due to the disease-related lack of platelets, platelets dysfunctions and systemic coagulopathy. Platelet transfusion may therefore be life saving therapy to achieve hemostasis in the bleeding patients. The transfusions are traditionally given at a platelet threshold of 20,000/µl, however, lower thresholds have since been established for stable patients who do not have a fever or active hemorrhage (134). Interestingly, due to different methods used in the production of platelet concentrates (135), refractoriness to platelet transfusion due to alloimmunisation (135) and altered functions by stored platelets (136), it may be difficult to predict the effect of platelet transfusions on AML blast functions. To the best of our knowledge, no studies have examined the possible effects of transfused platelets on leukemic cells, and the possibility can not be excluded that transfused platelets will affect AML cell functions, including chemosensitivity.

Thrombosis is not negligible in hematologic malignancies (137, 138), and several thrombogenic factors are present in AML patients. Firstly, platelet activation can be present (see before). Secondly, TF seem to be involved in venous thromboembolism (138), whereas TF-positive PDMP seem to be involved in hemostasis activation in cancer patients (139). Thirdly, circulating CD62P (P-selctin) may also be a risk factor for venous thromboembolism (140). This molecule is released by platelets co-cultured with AML blasts *in vitro* (4) and may also be released *in vivo* by activated platelets (8, 117). Finally, an in-vitro study showed that TF induced procoagulant activity can be affected by cytotoxic drugs used in AML therapy (141). Thus, several mechanisms may contribute to procoagulant activity in AML and TF, which also seems to be involved in the mechanisms of disseminated intravascular coagulation in promyelocytic leukemia (16), might be one of the most important mechanisms (16). Platelets may then contribute via their PDMP (139).

Despite increased procoagulant activity, the major problem in AML patients is severe haemorrhages and not thromboembolic complications (16), even though the AML cells express TF as well as other factors that can activate coagulation (16, 17). The most likely explanation for this discrepancy is that the severe disease- and therapy-induced thrombocytopenia in these patients counteract the procoagulant activity. The most common thrombotic manifestation seems to be central-line associated thrombosis that has been reported to occur in up to 40–50% of patients when using sensitive Doppler-ultrasound of the catheterized vein for visualization, but usually the thromboses are asymptomatic and may even resolve spontaneously (142).

Circulating reticulated platelets as a marker of hematopoietic reconstitution after intensive chemotherapy

Reticulated platelets can be detected in the circulation at low levels during the period of severe treatment-induced thrombocytopenia following intensive chemotherapy, and this is also true in AML (143, 144). However, prior to hematopoietic reconstitution with increasing peripheral blood platelet counts an increase in the absolute count and percentage of reticulated platelets can be detected (143, 144), and results from initial clinical studies suggest that the requirement for prophylactic platelet transfusion can be reduced according to the patients' reticulated platelet increase. The study by Chaoui et al. (144) included patients with various hematologic malignancies treated with autologous stem cell transplantation, and it is unknown whether these observations are relevant for AML patients receiving repeated cycles with conventional intensive chemotherapy.

The role of platelets in AML-M7 and myelofibrosis

Due to the fact that platelets are produced from the megakaryocytes in the bone marrow and that megakaryocytes store biological active mediators as platelets, it is possible that platelets and/or platelet stored mediators may be involved in the AML-M7 and other AML-M7 associated diseases. Whereas AML-M7 is not very common, myelofibrosis, which is associated to most kinds of AML but mostly to de novo AML-M7, is relatively more frequent. Unfortunately, interactions of platelets and cells involved in AML-M7 and myelofibrosis are not well characterized. However, we will present here some possible effects that platelet released mediators may be involved in.

In AML-M7 and other subtypes of AML with the cytogenetic abnormality involving 3q21, increased platelet number and presence of micromegakaryocytes are common (145, 146). Marked polymorphism of blast cells and platelets in peripheral blood and bone marrow with altered platelet aggregation has also been detected in AML-M7 (147). However, whether platelets or platelet stored mediators are directly involved in the evolution of AML-M7 has not previously been described. On the other hand, platelet stored mediators, which is also released by megakaryocytes, might be involved in myelofibrosis via PDGF and TGF-β (148) and in the transformation of AML-M7 into AML-M0 by PF-4 and β-TG (149). As previously described, these mediators are released into autografts during PBSCT (10) and might thereby be involved in the development of myelofibrosis after autotransplantation. As a result of observations that mediators which are stored and released by platelets seem to alter cell func-

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tions in the bone marrow, we are lead to speculate that platelets can be involved in cellular alterations in bone marrows, including myelofibrosis and AML transformations.

Platelet effects on apoptosis

In addition to the effects of platelet released mediators on AML blasts as previously mentioned (3, 6, 7) it is worth noting that several studies hypothesize that platelets can have an affect on apoptosis of normal and malignant myelogenous cells as well (reviewed in [9]). ATP and Serotonin that are stored in platelet dense bodies, can affect apoptosis of the human HL-60 and K562 AML cell lines via receptor ligation (150–154). Furthermore, VEGF, PF-4 and PDGF interfere with the regulation of apoptosis in several cell types, including normal myeloid cells (48, 53, 155, 156), and may also interfere with apoptosis regulation in AML blasts that at least express receptors for PDGF and VEGF (6, 90).

The effects of platelets on cancer-associated angiogenesis

Platelets can release several proangiogenic mediators, including VEGF, PDGF, TGF-β and Ang-1 (see references in [157]). It is also known that TF can contribute to angiogenesis both through coagulation-dependent and independent mechanisms (17). Angiogenesis is also important for both leukemogenesis and chemosensitivity in AML, but at present it is not known whether or how platelets contribute to local angioregulation in the AML bone marrow.

The possible role of platelets during leukemogenesis

The studies reviewed above suggest that platelets can interact with primary AML cells and may contribute to AML cell proliferation, regulation of AML cell apoptosis and susceptibility to intensive chemotherapy. Thus, platelets may have a role in AML after the development of the disease and during treatment. However, the fundamental events in leukemogenesis are induction of genetic abnormalities that causes malignant transformation of these hematopoietic cells. Very few studies have addressed this question, but the current knowledge suggests that platelets may even contribute to the process of leukemogenesis.

Patients who have been recently diagnosed with AML can possibly be cured using conventional intensive chemotherapy, but more that half of those receiving this treatment will later on suffer a leukemia relapse. Relapsed AML shows chemoresistance and the only curative treatment is allogeneic stem cell transplantation. The development from newly diagnosed disease to chemoresistant relapse should be regarded as an additional step in leukemogenesis, and according to this new genetic abnormalities can be detected in relapse cells compared with the original cells. Many of the AML associated genetic abnormalities affect intracellular signalling pathways, including mutations in tyrosine kinase-associated receptor molecules (e.g. Flt3, c-kit) leading to constitutive tyrosine kinase activation (158). Many of these signalling pathways will converge on intracellular STAT molecules known to be important for regulation of proliferation, apoptosis and differentiation. Furthermore, the STAT molecules are also important mediators downstream to a wide range of hematopoietic growth factor receptors, and these growth factors are released in the AML cell microenvironment and affect both proliferation, survival and differentiation of the AML cells.

The next question is whether platelet-derived soluble mediators can interfere with growth-factor initiated, receptor-mediated intracellular signalling through the STAT molecules. We know that activated platelets can release several mediators (e.g. VEGF, PDGF, Ang-1) that can bind to receptors expressed on primary human AML cells and thereby alter the status of the STAT network through activation of receptor-associated tyrosine kinases (159, 160, 161). Thus, platelet-derived mediators can contribute to the overall tyrosine-associated signalling in AML cells, and thereby modulate the effects on the STAT system of exogenous hematopoietic growth factors and constitutively activated/mutated kinases receptors.

The final question remains whether platelet-induced modulation of the intracellular signalling from receptor tyrosine kinases (i.e. the STAT network) can lead to genetic instability and thereby contribute to the risk of inducing genetic abnormalities associated with leukemogenesis. This question has not been resolved, but a recent review described that constitutively activated/mutated tyrosine kinases can fascilitate DNA repair through activation of cell cycle checkpoints and elevate the expression of intracellular antiapoptotic proteins (162). Despite these protective effects that fascilitate malignant cell survival, the constitutive activity stimulates the generation of reactive oxygen species and enhance spontaneous DNA damage that will fascilitate further leukemogenesis. At present it is only known that i) platelets release relevant soluble mediators; ii) AML cells express the corresponding receptors, and iii) the possibility exists that platelets can affect the intracellular signalling network and thereby modulate genetic instability and contribute to leukemogenesis and the development of chemoresistant AML relapse.

Another aspect of leukemogenesis is the development of AML from low-grade malignant diseases like chronic myeloid leukemia and myelodysplastic syndromes. The development of AML from these disorders is also associated with the detection of additional genetic abnormalities (162, 163), and platelets may then interfere with these steps in leukemogenesis through the same molecular mechanisms as previously described.

Conclusion

Platelets are capable of interacting with normal peripheral blood cells both via adhesion and soluble mediators. Similarly, platelet released mediators can affect hematopoietic stem and progenitor cells. These interactions may also be involved between platelets and circulating malignant cells, which is suggested by the effects platelets seem to have on metastasis and the various platelet defects that is detected in AML and other leukemias. However, the interactions between platelets and AML cells have only recently been characterized in detail. In-vitro studies clearly show that platelets and AML blasts can affect functional characteristics of each other. In addition, they also indicate that various chemotherapeutic drugs frequently used in AML therapy can alter platelet activation and other platelet functions. In-vivo studies reveal that the systemic levels of various cytokines are enhanced during AML chemotherapy, including cytokines that are known to affect both leukemic blasts and platelet activation. However, systemic platelet activation was not detectable during AML

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chemotherapy, but another platelet dysfunction was observed prior to chemotherapy for a subset of AML patients. These observations together with the other platelet dysfunctions detected in AML and the possible effects of chemotherapeutic drugs on platelet function cannot exclude the possibility of platelet secretion in AML and thereby the effect of platelets on circulating AML blasts. Platelet secretion of growth factors are clearly detected in PBSCT autografts together with various hematopoietic growth factors. These observations may be of clinical importance because platelet released mediators can affect AML blast functions and might thereby influence the relapse risk after autotransplantation. Thus, platelets are most likely active participants during AML chemotherapy.

The big question remains whether the molecular mechanisms for interactions between platelets and AML cells are also relevant for other malignancies. Although many of the molecular substrates for interactions will also be present in other malignancies, this does not mean that they are operative and mediate clinically relevant effects. For several reasons one should be very careful to generalize to cancer in general from observations in AML:

There is heterogeneity even within the group of AML, and the biological differences will be even larger between AML and other malignancies.

AML cells show molecular and morphological signs of differentiation in various myeloid directions and would therefore be expected to behave differently from malignancies developing from other tissues. Both the cancer cells and the stromal elements will differ.

AML is a more aggressive disorder than may other malignancies, and the genetic abnormalities differ.

The leukemias show diffuse bone marrow infiltration, whereas most other malignancies show localized tumor growth and eventually distant metastasis to organs not usually involved in AML. The cancer cell microenvironment would therefore be expected to be very different.

Severe disease-induced thrombocytopenia is usually not found in solid tumors.

Thus, taken together, the overall data reviewed in this article suggest that platelets can directly or indirectly affect human AML cells and thereby influence disease development and response to therapy in these patients.

References

1. Chaer RA, Graham JA, Mureebe L. Platelet Function and Pharmacologic Inhibition. Vasc Endovasc Surg 2006; 40: 261–267.

2. Jurk K, Kehrel B. Platelets: physiology and biochemistry. Semin Thromb Hemost 2005; 31: 381–392.

3. Bruserud Ø, Foss B, Hervig T. Effects of normal platelets on proliferation and constitutive cytokine secretion by human acute myelogenous leukaemia blasts. Platelets 1997; 8: 397–404.

4. Bruserud Ø, Foss B, Ulvestad E, et al. Effects of acute myelogenous leukemia blasts on platelet release of soluble P-selectin and platelet-derived growth factor. Platelets 1998; 9: 352–358.

5. Gupta GP, Massague J. Platelets and metastasis revisited: a novel fatty link. J Clin Invest 2004; 114: 1691–1693.

6. Foss B, Ulvestad E, Bruserud Ø. Platelet-derived growth factor (PDGF) in human acute myelogenous leukemia (AML): PDGF receptor expression, endogenous PDGF release and responsiveness to exogenous PDGF isoforms by in vitro cultured AML blasts. Eur J Haematology 2001; 67: 267–278.

7. Foss B, Mentzoni L, Bruserud Ø. Effects of vascular endothelial growth factor on acute myelogenous leukemia blasts. J Hematother Stem Cell Res 2001; 10: 81–94.

8. Foss B, Ulvestad E, Hervig T, et al. Effects of cytarabine and various anthracyclins on platelet activation: characterization of in vitro effects and their possible clinical relevance in acute myelogenous leukemia. Int J Cancer 2002; 97: 106–114.

9. Bruserud O, Foss B, Abrahamsen JF, et al. Autologous stem cell transplantation as post-remission therapy in adult acute myelogenous leukemia: does platelet contamination of peripheral blood mobilized stem cell grafts influence the risk of leukemia relapse? J Hematother Stem Cell Res 2000; 9: 433–443.

10. Foss B, Abrahamsen JF, Bruserud Ø. Peripheral blood stem cell grafts contain high levels of platelet-secreted mediators. Transfusion 2001; 41: 1431–1437.

11. Nomura S, Inami N, Kanazawa S, et al. Elevation of Platelet Activation Markers and Chemokines during Peripheral Blood Stem Cell Harvest with G-CSF. Stem Cells 2004; 22: 696–703.

12. Li JJ, Huang YQ, Basch R, et al. Thrombin induces the release of angiopoietin-1 from platelets. Thromb Haemost 2001; 85: 204–206.

13. Biró E, Sturk-Maquelin KN, Vogel GM, et al. Human cell-derived microparticles promote thrombus formation in vivo in a tissue factor-dependent manner. J Thromb Haemost 2003;1: 2561–2568.

14. Gutensohn K, Maerz M, Kuehnl P. Alteration of platelet-associated membrane glycoproteins during extracorporeal apheresis of peripheral blood progenitor cells. J Hematother 1997; 6: 315–321.

15. Saigo K, Kumagai S, Sugimoto T, et al. RANTES and p-Selectin in peripheral blood stem cell harvest. Ther Apher 2001; 5: 517–518.

16. Arbuthnot C, Wilde JT. Haemostatic problems in acute promyelocytic leukaemia. Blood Reviews 2006; 20: 289–297.

17. Lopez-Pedrera C, Barbarroja N, Dorado G, et al. Tissue factor as an effector of angiogenesis and tumor progression in hematological malignancies. Leukemia 2006; 20: 1331–1340.

18. Arber DA. Realistic pathologic classification of acute myeloid leukemias. Am J Clin Pathol 2001; 115: 552–560.

19. Vyas P, Crispino JD. Molecular insights into Down syndrome-associated leukemia. Curr Opin Pediatr 2007; 19: 9–14.

20. Ross ME, Mahfouz R, Onciu M, et al. Gene expression profiling of pediatric acute myelogenous leukaemia. Blood 2004; 104: 3679–3687.

21. Bourquin J-P, Subramanian A, Langebrake C, et al. Identification of distinct molecular phenotypes in acute megakaryoblastic leukemia by gene expression profiling. Proc Natl Acad Sci USA 2006; 103: 3339–3344.

22. Jungi TW, Spycher MO, Nydegger UE, et al. Platelet-leukocyte interaction: selective binding of thrombin- stimulated platelets to human monocytes, polymorphonuclear leukocytes, and related cell lines. Blood 1986; 67: 629–636.

23. Rinder HM, Bonan JL, Rinder CS, et al. Activated and unactivated platelet adhesion to monocytes and neutrophils. Blood 1991; 78: 1760–1769.

24. Moore KL, Stults NL, Diaz S, et al. Identification of a specific glycoprotein ligand for P-selectin (CD62) on myeloid cells. J Cell Biol 1992; 118: 445–456.

25. de Bruijne-Admiraal LG, Modderman PW, Von dem Borne AE, et al. P-selectin mediates Ca(2+)-dependent adhesion of activated platelets to many different types of leukocytes: detection by flow cytometry. Blood 1992; 80: 134–142.

26. Moore KL, Thompson LF. P-selectin (CD62) binds to subpopulations of human memory T lymphocytes and natural killer cells. Biochem Biophys Res Commun 1992; 186: 173–181.

27. Spangenberg P, Redlich H, Bergmann I, et al. The platelet glycoprotein IIb/IIIa complex is involved in the adhesion of activated platelets to leukocytes. Thromb Haemost 1993; 70: 514–521.

28. Long MW, Briddell R, Walter AW, et al. Human hematopoietic stem cell adherence to cytokines and matrix molecules. J Clin Invest 1992; 90: 251–255.

29. Tsai S, Patel V, Beaumont E, et al. Differential binding of erythroid and myeloid progenitors to fibroblasts and fibronectin. Blood 1987; 69: 1587–1594.

30. Del Maschio A, Evangelista V, Rajtar G, et al. Platelet activation by polymorphonuclear leukocytes exposed to chemotactic agents. Am J Physiol 1990; 258(3 Pt 2): H870–879.

31. Tsuji T, Nagata K, Koike J, et al. Induction of superoxide anion production from monocytes an neutrophils by activated platelets through the P-selectin-sialyl Lewis X interaction. J Leukoc Biol 1994; 56: 583–587.

32. Piccardoni P, Evangelista V, Piccoli A, et al. Thrombin-activated human platelets release two NAP-2 variants that stimulate polymorphonuclear leukocytes. Thromb Haemost 1996; 76: 780–785.

33. Ruf A, Patscheke H. Platelet-induced neutrophil activation: platelet-expressed fibrinogen induces the oxidative burst in neutrophils by an interaction with CD11C/CD18. Br J Haematol 1995; 90: 791–796.

This document was downloaded for personal use only. Unauthorized distribution is strictly prohibited.

34. Elstad MR, La Pine TR, Cowley FS, et al. P-selectin regulates platelet-activating factor synthesis and phagocytosis by monocytes. J Immunol 1995; 155: 2109–2122.
35. Weyrich AS, Elstad MR, McEver RP, et al. Activated platelets signal chemokine synthesis by human monocytes. J Clin Invest 1996; 97: 1525–1534.
36. Tzeng DY, Deuel TF, Huang JS, et al. Platelet-derived growth factor promotes polymorphonuclear leukocyte activation. Blood 1984; 64: 1123–1128.
37. Aziz KA, Cawley JC, Zuzel M. Platelets prime PMN via released PF4: mechanism of priming and synergy with GM-CSF. Br J Haematol 1995; 91: 846–853.
38. Dercksen MW, Weimar IS, Richel DJ, et al. The value of flow cytometric analysis of platelet glycoprotein expression of CD34+ cells measured under conditions that prevent P- selectin-mediated binding of platelets. Blood 1995; 86: 3771–3782.
39. Michalevicz R, Katz F, Stroobant P, et al. Platelet-derived growth factor stimulates growth of highly enriched multipotent haemopoietic progenitors. Br J Haematol 1986; 63: 591–598.
40. Su RJ, Zhang XB, Li K, et al. Platelet-derived growth factor promotes ex vivo expansion of CD34+ cells from human cord blood and enhances long-term culture-initiating cells, non-obese diabetic/severe combined immunodeficient repopulating cells and formation of adherent cells. Br J Haematol 2002; 117: 735–746.
41. Trink B, Wang G, Shahar M, et al. Functional platelet-derived growth factor-beta (PDGF-beta) receptor expressed on early B-lineage precursor cells. Clin Exp Immunol 1995; 102: 417–424.
42. Ruscetti FW, Akel S, Bartelmez SH. Autocrine transforming growth factor-beta regulation of hematopoiesis: many outcomes that depend on the context. Oncogene 2005; 24: 5751–5763.
43. Pircher R, Jullien P, Lawrence DA. Beta-transforming growth factor is stored in human blood platelets as a latent high molecular weight complex. Biochem Biophys Res Commun 1986; 136: 30–37.
44. Yang M, Li K, Ng MH, et al. Thrombospondin-1 inhibits in vitro megakaryocytopoiesis via CD36. Thromb Res 2003; 109: 47–54.
45. Sagar BM, Rentala S, Gopal PN, et al. Fibronectin and laminin enhance engraftibility of cultured hematopoietic stem cells. Biochem Biophys Res Commun 2006; 350: 1000–1005.
46. Zhou YQ, Levesque JP, Hatzfeld A, et al. Fibrinogen potentiates the effect of interleukin-3 on early human hematopoietic progenitors. Blood 1993; 82: 800–806.
47. Gerber HP, Malik AK, Solar GP, et al. VEGF regulates haematopoietic stem cell survival by an internal autocrine loop mechanism. Nature 2002; 417: 954–958.
48. Katoh O, Tauchi H, Kawaishi K, et al. Expression of the vascular endothelial growth factor (VEGF) receptor gene, KDR, in hematopoietic cells and inhibitory effect of VEGF on apoptotic cell death caused by ionizing radiation. Cancer Res 1995; 55: 5687–5692.
49. Broxmeyer HE, Cooper S, Li ZH, et al. Myeloid progenitor cell regulatory effects of vascular endothelial cell growth factor. Int J Hematol 1995; 62: 203–215.
50. Gewirtz AM, Calabretta B, Rucinski B, et al. Inhibition of human megakaryocytopoiesis in vitro by platelet factor 4 (PF4) and a synthetic COOH-terminal PF4 peptide. J Clin Invest 1989; 83: 1477–1486.
51. Han ZC, Bellucci S, Tenza D, et al. Negative regulation of human megakaryocytopoiesis by human platelet factor 4 and beta thromboglobulin: comparative analysis in bone marrow cultures from normal individuals and patients with essential thrombocythaemia and immune thrombocytopenic purpura. Br J Haematol 1990; 74: 395–401.
52. Yang M, Srikiatkhachorn A, Anthony M, et al. Serotonin stimulates megakaryocytopoiesis via the 5-HT2 receptor. Blood Coagul Fibrinolysis 1996; 7: 127–133.
53. Han ZC, Lu M, Li J, et al. Platelet factor 4 and other CXC chemokines support the survival of normal hematopoietic cells and reduce the chemosensitivity of cells to cytotoxic agents. Blood 1997; 89: 2328–2335.
54. Zhang J, Lu SH, Liu YJ, et al. Platelet factor 4 enhances the adhesion of normal and leukemic hematopoietic stem/progenitor cells to endothelial cells. Leuk Res 2004; 28: 631–638.
55. Majka M, Janowska-Wieczorek A, Ratajczak J, et al. Numerous growth factors, cytokines, and chemokines are secreted by human CD34(+) cells, myeloblasts, erythroblasts, and megakaryoblasts and regulate normal hematopoiesis in an autocrine/paracrine manner. Blood 2001; 97: 3075–3085.
56. Baj-Krzyworzeka M, Majka M, Pratico D, et al. Platelet-derived microparticles stimulate proliferation, survival, adhesion, and chemotaxis of hematopoietic cells. Exp Hematol 2002; 30: 450–459.
57. Kim HK, Song KS, Park YS, et al. Elevated levels of circulating platelet microparticles, VEGF, IL-6 and RANTES in patients with gastric cancer: possible role of a metastasis predictor. Eur J Cancer 2003; 39: 184–191.
58. Villmow T, Kemkes-Matthes B, Matzdorff AC. Markers of platelet activation and platelet-leukocyte interaction in patients with myeloproliferative syndromes. Thromb Res 2002; 108: 139–145.
59. Yang ZF, Ho DW, Lau CK, et al. Platelet activation during tumor development, the potential role of BDNF-TrkB autocrine loop. Biochem Biophys Res Commun 2006; 346: 981–985.
60. Ostman A. PDGF receptors-mediators of autocrine tumor growth and regulators of tumor vasculature and stroma. Cytokine Growth Factor Rev 2004; 15: 275–286.
61. Al-Mondhiry H. beta-Thromboglobulin and platelet-factor 4 in patients with cancer: correlation with the stage of disease and the effect of chemotherapy. Am J Hematol 1983; 14: 105–111.
62. Grignani G, Pacchiarini L, Ricetti MM, et al. Mechanisms of platelet activation by cultured human cancer cells and cells freshly isolated from tumor tissues. Invasion Metastasis 1989; 9: 298–309.
63. Avram S, Lupu A, Angelescu S, et al. Abnormalities of platelet aggregation in chronic myeloproliferative disorders. J Cell Mol Med 2001; 5: 79–87.
64. Jaime-Perez JC, Cantu-Rodriguez OG, Herrera-Garza JL, et al. Platelet aggregation in children with acute lymphoblastic leukemia during induction of remission therapy. Arch Med Res 2004; 35: 141–144.
65. Ibele GM, Kay NE, Johnson GJ, et al. Human platelets exert cytotoxic effects on tumor cells. Blood 1985; 65: 1252–1255.
66. Verheul HM, Pinedo HM. Tumor growth: A putative role for platelets? Oncologist 1998; 3: ii.
67. Veikkola T, Alitalo K. VEGFs, receptors and angiogenesis. Semin Cancer Biol 1999; 9:211–220.
68. Brown LF, Berse B, Jackman RW, et al. Expression of vascular permeability factor (vascular endothelial growth factor) and its receptors in adenocarcinomas of the gastrointestinal tract. Cancer Res 1993; 53: 4727–4735.
69. Ho C-L, Hsu L-F, Phyliky RL, et al. Autocrine Expression of Platelet-Derived Growth Factor B in B Cell Chronic Lymphocytic Leukemia. Acta Haematol 2005; 114: 133–140.
70. Pietras K, Sjoblom T, Rubin K, et al. PDGF receptors as cancer drug targets. Cancer Cell 2003 5; 3: 439–443.
71. Steinhilber D, Radmark O, Samuelsson B. Transforming growth factor beta upregulates 5-lipoxygenase activity during myeloid cell maturation. Proc Natl Acad Sci USA 1993; 90: 5984–5988.
72. Bikfalvi A, Gimenez-Gallego G. The Control of Angiogenesis and Tumor Invasion by Platelet Factor-4 and Platelet Factor-4-Derived Molecules. Semin Thromb Hemost 2004; 30: 137–144.
73. Silverstein RL, Asch AS, Nachman RL. Glycoprotein IV mediates thrombospondin-dependent platelet-monocyte and platelet-U937 cell adhesion. J Clin Invest 1989; 84: 546–552.
74. Tuszynski GP, Wang TN, Berger D. Adhesive proteins and the hematogenous spread of cancer. Acta Haematol 1997; 97: 29–39.
75. Cowan DH, Haut MJ. Platelet function in acute leukemia. J Lab Clin Med 1972; 79: 893–905.
76. Cowan DH. Platelet metabolism in acute leukemia. J Lab Clin Med 1973; 82: 54–66.
77. Ramos OF, Moron EC, De Castro Arenas R. Platelet function abnormalities in acute leukaemia. Haematologia 1981; 14: 383–391.
78. Tiwari NN, Singh VP, Dube B, et al. Platelet function in leukaemias. J Assoc Physicians India 1984; 32: 805–807.
79. Woodcock BE, Cooper PC, Brown PR, et al. The platelet defect in acute myeloid leukaemia. J Clin Pathol 1984; 37: 1339–1342.
80. Pogliani EM, Colombi M, Cofrancesco E, et al. Platelet dysfunction in acute megakaryoblastic leukemia. Acta Haematol 1989; 81: 1–4.
81. Leinoe EB, Hoffmann MH, Kjaersgaard E, et al. Multiple platelet defects identified by flow cytometry at diagnosis in acute myeloid leukaemia. Br J Haematol 2004; 127: 76–84.
82. Gerrard JM, Israels ED, Bishop AJ, et al. Inherited platelet-storage pool deficiency associated with a high incidence of acute myeloid leukaemia. Br J Haematol 1991; 79: 246–255.
83. Nouvel C, Caranobe C, Sie P, et al. Platelet volume, density and 5 HT organelles (mepacrine test) in acute leukaemia. Scand J Haematol 1978; 21: 421–426.
84. Gerrard JM, McNicol A. Platelet storage pool deficiency, leukemia, and myelodysplastic syndromes. Leuk Lymphoma 1992; 8: 277–281.
85. Tallman MS, Hakimian D, Kwaan HC, et al. New insights into the pathogenesis of coagulation dysfunction in acute promyelocytic leukemia. Leuk Lymphoma 1993; 11: 27–36.
86. Higuchi T, Shimizu T, Mori H, et al. Coagulation patterns of disseminated intravascular coagulation in acute promyelocytic leukemia. Hematol Oncol 1997; 15: 209–217.
87. Naresh KN, Sivasankaran P, Veliath AJ. Platelet function in chronic leukemias. Indian J Cancer 1992; 29: 49–55.
88. Faldt R, Ankerst J, Zoucas E. Inhibition of platelet aggregation by myeloid leukaemic cells demonstrated in vitro. Br J Haematol 1987; 66: 529–534.
89. Katz FE, Michalevicz R, Lam G, et al. Effect of platelet-derived growth factor on enriched populations of haemopoietic progenitors from patients with chronic myeloid leukaemia. Leuk Res 1987; 11: 339–344.
90. Fiedler W, Graeven U, Ergun S, et al. Vascular endothelial growth factor, a possible paracrine growth factor in human acute myeloid leukemia. Blood 1997; 89: 1870–1875.
91. Gabrilovich D, Ishida T, Oyama T, et al. Vascular endothelial growth factor inhibits the development of dendritic cells and dramatically affects the differenti-

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ation of multiple hematopoietic lineages in vivo. Blood 1998; 92: 4150–4166.
92. Hussong JW, Rodgers GM, Shami PJ. Evidece of increased angiogenesis in patients with acute myeloid leukemia. Blood 2000; 95: 309–313.
93. Aguayo A, Estey E, Kantarjian H, et al. Cellular vascular endothelial growth factor is a predictor of outcome in patients with acute myeloid leukemia. Blood 1999; 94: 3717–3721.
94. Aguayo A, Kantarjian H, Manshouri T, et al. Angiogenesis in acute and chronic leukemias and myelodysplastic syndromes. Blood 2000; 96: 2240–2245.
95. Foa R, Bussolino F, Ferrando ML, et al. Release of platelet-activating factor in human leukemia. Cancer Res 1985; 45: 4483–4485.
96. Foss B, Nesthus I, Bergheim J, et al. Serum levels of thrombopoietin and stem cell factor in acute leukemia patients with chemotherapy-induced cytopenia and complicating infections. Platelets 1999; 10: 17–23.
97. Oda A, Miyakawa Y, Druker BJ, et al. Thrombopoietin primes human platelet aggregation induced by shear stress and by multiple agonists. Blood 1996; 87: 4664–4670.
98. Fontenay-Roupie M, Huret G, Loza JP, et al. Thrombopoietin activates human platelets and induces tyrosine phosphorylation of p80/85 cortactin. Thromb Haemost 1998; 79: 195–201.
99. Grabarek J, Groopman JE, Lyles YR, et al. Human kit ligand (stem cell factor) modulates platelet activation in vitro. J Biol Chem 1994; 269: 21718–21724.
100. Nomura S, Nakamura T, Cone J, et al. Cytometric analysis of high shear-induced platelet microparticles and effect of cytokines on microparticle generation. Cytometry 2000; 40: 173–181.
101. Bruserud Ø, Akselen PE, Bergheim J, et al. Serum concentrations of E-selectin, P-selectin, ICAM-1 and interleukin 6 in acute leukaemia patients with chemotherapy-induced leucopenia and bacterial infections. Br J Haematol 1995; 91: 394–402.
102. Bruserud Ø, Halstensen A, Peen E, et al. Serum levels of adhesion molecules and cytokines in patients with acute leukaemia. Leuk Lymphoma 1996; 23 : 423–430.
103. Schonbohn H, Schuler M, Kolbe K, et al. Plasma levels of IL-1, TNF alpha, IL-6, IL-8, G-CSF, and IL1-RA during febrile neutropenia: results of a prospective study in patients undergoing chemotherapy for acute myelogenous leukemia. Ann Hematol 1995; 71: 161–168.
104. Reisbach G, Kamp T, Welzl G, et al. Regulated plasma levels of colony-stimulating factors, interleukin-6 and interleukin-10 in patients with acute leukaemia and non-hodgkin's lymphoma undergoing cytoreductive chemotherapy. Br J Haematol 1996; 92: 907–912.
105. Cimino G, Amadori S, Cava MC, et al. Serum interleukin-2 (IL-2), soluble IL-2 receptors and tumor necrosis factor-alfa levels are significantly increased in acute myeloid leukemia patients. Leukemia 1991; 5: 32–35.
106. Aydogdu I, Ilhan O, Beksac M, et al. Serum erythropoietin levels in patients with leukemia on cytostatic treatment. Haematologica 1998; 29: 133–137.
107. Panella TJ, Peters W, White JG, et al. Platelets acquire a secretion defect after high-dose chemotherapy. Cancer 1990; 65: 1711–1716.
108. Hicsonmez G. The effect of cancer chemotherapy drugs on platelet aggregation. Turk J Pediatr 1974; 16: 1–7.
109. Kubisz P, Suranova J. Influence of cytostatics on some platelet functions in vitro II. Cytosine-arabinoside. Neoplasma 1974; 21: 711–716.
110. Matera C, Falzarano C, Vacca C, et al. Effects of some antineoplastic drugs (vincristine, doxorubicin and epirubicin) on human platelet aggregation. J Med 1994; 25: 2–16.
111. Lanzi C, Banfi P, Ravagnani F, et al. Diversity of effects of two antitumor anthracycline analogs on the pathway of activation of PKC in intact human platelets. Biochem Pharmacol 1988; 37: 3497–3504.
112. Pogliani EM, Fantasia R, Lambertenghi-Deliliers G, et al. Daunorubicin and platelet function. Thromb Haemost 1981; 45: 38–42.
113. Whaun JM, Clarke HD. The effect of daunomycin on platelets in vitro. Arch Int Pharmacodyn Ther 1989; 300: 292–304.
114. Shattil SJ, Hoxie JA, Cunningham M, et al. Changes in the platelet membrane glycoprotein IIb.IIIa complex during platelet activation. J Biol Chem 1985; 260: 11107–11114.
115. Janes SL, Wilson DJ, Chronos N, et al. Evaluation of whole blood flow cytometric detection of platelet bound fibrinogen on normal subjects and patients with activated platelets. Thromb Haemost 1993; 70: 659–666.
116. Ruf A, Patscheke H. Flow cytometric detection of activated platelets: comparison of determining shape change, fibrinogen binding, and P-selectin expression. Semin Thromb Hemost 1995; 21: 146–151.
117. Michelson AD, Barnard MR, Hechtman HB, et al. In vivo tracking of platelets: circulating degranulated platelets rapidly lose surface P-selectin but continue to circulate and function. Proc Natl Acad Sci U S A 1996; 93: 11877–11882.
118. Kumar A, Kumar R, Sandilium A, et al. 5-Fluorouracil induces defects in platelet function. Platelets 1999; 10: 137–140.
119. Nomura S, Ishii K, Kanazawa S, et al. Role of platelet-derived chemokines (RANTES and ENA-78) after stem cell transplantation. Transplant Immunology 2006; 15: 247–253.
120. Singh RK, Ino K, Varney ML, et al. Immunoregulatory cytokines in bone marrow and peripheral blood stem cell products. Bone Marrow Transplant 1999; 23: 53–62.
121. Ageitos AG, Varney ML, Bierman PJ, et al. Comparison of monocyte-dependent T cell inhibitory activity in GM-CSF vs G-CSF mobilized PSC products. Bone Marrow Transplant 1999; 23: 63–69.
122. Stroncek DF, Clay ME, Smith J, et al. Composition of peripheral blood progenitor cell components collected from healthy donors. Transfusion 1997; 37: 411–417.
123. Stroncek DF, Clay ME, Jaszcz W, et al. Collection of two peripheral blood stem cell concentrates from healthy donors. Transfus Med 1999; 9: 37–50.
124. Lemoli RM, Curti A, Tura S. Negative selection of autologous peripheral blood stem cells. Baillieres Best Pract Res Clin Haematol 1999; 12: 57–69.
125. Nagafuji K, Harada M, Takamatsu Y, et al. Evaluation of leukaemic contamination in peripheral blood stem cell harvests by reverse transcriptase polymerase chain reaction. Br J Haematol 1993; 85: 578–583.
126. Miyamoto T, Nagafuji K, Harada M, et al. Significance of quantitative analysis of AML1/ETO transcripts in peripheral blood stem cells from t(8;21) acute myelogenous leukemia. Leuk Lymphoma 1997; 25: 69–75.
127. Testoni N, Lemoli RM, Martinelli G, et al. Autologous peripheral blood stem cell transplantation in acute myeloblastic leukaemia and myelodysplastic syndrome patients: evaluation of tumour cell contamination of leukaphereses by cytogenetic and molecular methods. Bone Marrow Transplant 1998; 22: 1065–1070.
128. Lie AK, To LB. Peripheral Blood Stem Cells: Transplantation and Beyond. Oncologist 1997; 2: 40–49.
129. Deisseroth AB, Zu Z, Claxton D, et al. Genetic marking shows that Ph+ cells present in autologous transplants of chronic myelogenous leukemia (CML) contribute to relapse after autologous bone marrow in CML. Blood 1994; 83: 3068–3076.
130. Rill DR, Santana VM, Roberts WM, et al. Direct demonstration that autologous bone marrow transplantation for solid tumors can return a multiplicity of tumorigenic cells. Blood 1994; 84: 380–383.
131. Bruserud Ø, Foss B, Petersen H. Hematopoietic growth factors in patients receiving intensive chemotherapy for malignant disorders: studies of granulocyte-colony stimulating factor (G-CSF), granulocyte-macrophage colony stimulating factor (GM-CSF), interleukin-3 (IL-3) and Flt-3 ligand (Flt3L). Eur Cytokine Netw 2001; 12: 231–238.
132. Voss R, Scarlat T, Matzdorff A, et al. Flow cytometric detection of platelet activation in patients undergoing diagnostic and interventional coronary angiography. Platelets 1996; 7: 237–241.
133. Saigo K, Hashimmoto M, Kumagai S, et al. Platelet and RANTES contamination in peripheral blood stem cell products: comparison of three different instruments for PBSC harvesting. Vox Sanguinis 2003; 84: 241–242.
134. Wandt H, Ehninger G, Gallmeier WM. New Strategies for Prophylactic Platelet Transfusion in Patients with Hematologic Diseases. Oncologist 2001; 6: 446–450.
135. Stroncek DF, Rebulla P. Platelet transfusions. Lancet 2007; 370: 427–438.
136. Perez-Pujol S, Aras O, Lozano M, et al. Stored platelets contain residual amounts of tissue factor: evidence from studies on platelet concentrates stored for prolonged periods. Transfusion 2005; 45: 572–579.
137. De Stefano V, Sora F, Rossi E, et al. The risk of thrombosis in patients with acute leukemia: occurrence of thrombosis at diagnosis and during treatment. J Thromb Hemost 2005; 3: 1985–1992.
138. Kwaan HC, Vicuna B. Incidence and pathogenesis of thrombosis in hematologic malignancies. Semin Thromb Hemost 2007; 33: 303–312.
139. Hron G, Kollars M, Weber H, et al. Tissue factor-positive microparticles: cellular origin and association with coagulation activation in patients with colorectal cancer. Thromb Haemost 2007; 97: 119–123.
140. Kyrle PA, Hron G, Eichinger S, et al. Circulating P-selectin and the risk of recurrent venous thromboembolism. Thromb Haemost 2007; 97: 880–883.
141. Langer F, Amirkhosravi A, Loges S, et al. An in vitro study on the mechanisms of coagulation activation in acute myelogenous leukemia (AML): role of tissue factor regulation by cytotoxic drugs and GM-CSF. Thromb Haemost 2004; 92: 1136–1146.
142. Ruud E, Holmstrøm H, Natvig S, et al. Prevalence of thrombophilia and central venous catheter-associated neck vein thrombosis in 41 children with cancer – a prospective study. Med Pediatr Oncol 2002; 38: 405–410.
143. Ryningen A, Apelseth T, Hausken T, et al. Reticulated platelets are increased in chronic myeloproliferative disorders, pure erythrocytosis, reactive thrombocytosis and prior to hematopoietic reconstitution after intensive chemotherapy. Platelets 2006; 17: 296–302.
144. Chaoui D, Chakroun T, Robert F, et al. Reticulated platelets: a reliable measure to reduce prophylactic platelet transfusions after intensive chemotherapy. Transfusion 2005; 45: 766–772.
145. Cripe LD, Hromas R. Malignant disorders of megakaryocytes. Semin Hematol 1998; 35: 200–209.
146. Testoni N, Borsaru G, Martinelli G, et al. 3q21 and 3q26 cytogenetic abnormalities in acute myeloblastic

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leukemia: biological and clinical features. Haematologica 1999; 84: 690–694.
147. Shukla J, Rai S, Singh VP. Acute megakaryoblastic leukaemia: a clinico-haematological profile of five cases. Indian J Pathol Microbiol 2004; 47: 266–268.
148. Reilly JT. Idiopathic myelofibrosis: pathogenesis to treatment. Hematol Oncol 2006; 24: 56–63.
149. Shibata K, Nakano S, Watanabe M, et al. Acute megakaryocytic leukaemia (AML-M7) with myelofibrosis terminating in AML-MO with concurrent liver fibrosis. Eur J Haematol 1998; 60: 310–312.
150. Zheng LM, Zychlinsky A, Liu CC, et al. Extracellular ATP as a trigger for apoptosis or programmed cell death. J Cell Biol 1991; 112: 279–288.
151. Murgia M, Pizzo P, Steinberg TH, et al. Characterization of the cytotoxic effect of extracellular ATP in J774 mouse macrophages. Biochem J 1992; 288(Pt3): 897–901.
152. Khan NA, Ferriere F, Deschaux P. Serotonin-induced calcium signaling via 5-HT1A receptors in human leukemia (K 562) cells. Cell Immunol 1995; 165: 148–152.
153. Clifford EE, Parker K, Humphreys BD, et al. The P2X1 receptor, an adenosine triphosphate-gated cation channel, is expressed in human platelets but not in human blood leukocytes. Blood 1998; 91: 3172–3181.
154. Suchanek B, Struppeck H, Fahrig T. The 5-HT1A receptor agonist BAY x 3702 prevents staurosporine-induced apoptosis. Eur J Pharmacol 1998; 355: 95–101.
155. Meeson AP, Argilla M, Ko K, et al. VEGF deprivation-induced apoptosis is a component of programmed capillary regression. Development 1999; 126: 1407–1415.
156. Kim HR, Upadhyay S, Li G, et al. Platelet-derived growth factor induces apoptosis in growth-arrested murine fibroblasts. Proc Natl Acad Sci USA 1995; 92: 9500–9504.
157. Kisucka J, Butterfield CE, Duda DG, et al. Platelets and platelet adhesion support angiogenesis while preventing excessive hemorrhage. Proc Natl Acad Sci USA 2006; 103: 855–860.
158. Kottaridis PD, Gale RE, Langabeer SE, et al. Studies of FLT3 mutations in paired presentation and relapse samples from patients with acute myeloid leukemia: implications for the role of FLT3 mutations in leukemogenesis, minimal residual disease detection, and possible therapy with FLT3 inhibitors Blood. 2002; 100: 2393–2398.
159. Coffer PJ, Koenderman L, de Groot RP. The role of STATs in myeloid differentiation and leukemia Oncogene 2000; 19: 2511–25122.
160. Hirai T, Masaki T, Kuratsune M, et al. PDGF receptor tyrosine kinase inhibitor suppresses mesangial cell proliferation involving STAT3 activation. Clin Exp Immunol 2006; 144: 353–361.
161. Hatfield KJ, Hovland R, Øyan AM, et al. Release of angiopoietin-1 by primary human acute myelogenous leukemia cells is associated with mutations of nucleo-phosmin, increased by bone marrow stromal cells and possibly antagonized by high system angiopoietin-2 levels. Leukemia 2007; October 18 [Epub ahead of print].
162. Penserga ETP, Skorski T. Fusion tyrosine kinases: a result and cause of genomic instability. Oncogene 2007; 26: 11–20.
163. Shih LY, Huang CF, Wang PN, et al. Acquisition of FLT3 or N-ras mutations is frequently associated with progression of myelodysplastic syndrome to acute myeloid leukemia. Leukemia 2004; 18: 466–475.
164. Levi M. Platelets. Crit Care Med 2005; 33: S523–525.

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