The Role of Platelets in Tumour Growth

Úloha krevních destiček v rozvoji nádoru

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Summary
Platelets, as initial responders to vascular injury, play a very important role in the initial stages of the haemostatic process. While the role of platelets in coagulation has been well studied and documented, their role in other physiological and pathological processes is just emerging. Platelets contain many biologically active molecules and, as they adhere to sites of tumour activated or injured endothelium, many of these molecules are released into the local microenvironment leading to platelet-mediated effects on vascular tone, repair and neo-angiogenesis. Platelets are likely play important roles in the tumour microenvironment that may be thought of as "a wound that never heals".

Key words
blood platelets – angiogenesis – wound healing – tumour growth – neoplasm metastasis

Souhrn
Krevní destičky jako elementy odpovídající v první vlně na poškození cév hrají velmi významnou úlohu v počátečních fázích procesu hemostázy. Zatímco zapojení trombocytů v procesu koagulace je podrobně studováno a popsáno, jejich role v dalších fyziologických a patologických procesech teprve začíná být předmětem zájmu. Krevní destičky obsahují řadu biologicky aktivních molekul a s tím, jak trombocyty adherují na nádorem aktivovaný nebo poškozený endotel, je řada těchto molekul uvolňována do nádorového mikroprostředí, což vede k ovlivnění cévního tonu, reparaci cévy a neoangiogenezi. Destičky pravděpodobně hrají důležitou úlohu v mikroprostředí nádoru, který můžeme považovat za ránu, která se nehojí.

Klíčová slova
trombocyt – angiogeneze – hojení rány – růst nádoru – metastazování nádoru

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The Hypercoagulable State Associated with Malignancy

Numerous clinical and basic science studies corroborate the importance of thrombosis in cancer development [1–7], cancer progression [8–12], and cancer metastasis [8,13–18]. The association is so well known that a deep vein thrombosis (DVT) in a patient without obvious risk factors triggers a search for an occult cancer. Despite this appreciation of a link between DVT and malignancy [19–21], the underlying biology has not been well characterised. The propensity to develop thromboembolic disease varies with the type of cancer [22], suggesting tumour cell-specific or tumour microenvironment-specific pathways to platelet and fibrin aggregation in tumours. Furthermore in some tumours, such as neuroblastoma, high platelet counts are associated with good prognosis [23], whereas in others (lung, colon, cervical, and breast cancers), the finding of high platelet counts implies poor prognosis [24–26].

Even though the association of hypercoagulability in cancer was first documented by Trousseau in 1865 [27], much work remains before we can use this finding therapeutically. There are some encouraging clinical observations. For example, the use of anticoagulants provides cancer patients with a survival advantage over and above that which would be conferred by the treatment of the DVT alone [28–35]. Unfortunately, large studies of the use of anticoagulants in the cancer population have not led to any significant change in the present management of cancer patients_ENREF_194 [36]. Yet both clinicians and basic scientists appreciate that even in patients not presenting with a cancer-associated thrombosis, the coagulation system is activated and platelet turnover increased. The interplay between platelets, coagulation and cancer is yet to be fully explored.

The Role of Platelet in Angiogenesis

The first scientific evidence suggesting that platelets were necessary for vascular integrity was reported in the late 1960's [37]. Organs perfused with platelet poor plasma led to loss of integrity of the endothelial cell layer and haemorrhages, and this effect could be reversed by addition of platelets. Similarly, thrombocytopenia was associated with increase in vascular permeability due to large endothelial wall fenestrations (EC) [38,39]. Based on these and other studies platelets were thought to promote endothelial cell growth [40], even though the mechanism of this trophic effect was unclear.

Platelets contain three types of granules: α-granules, dense granules and lysosomes, but most angiogenesis related proteins are contained in α-granules [41,42]. Tab. 1 lists angiogenesis regulators found in platelets. The presence of proteins with opposing angiogenic functions in platelets suggests that platelets are mediators and their presence can result in different actions depending on the situation. The formation of a clot not only provides a matrix facilitating cell migration, but also leads to a very judicial release of either stimulators or inhibitors of growth. As platelets adhere to activated endothelia or to exposed vascular sub-endothelia, the reciprocal interactions between the cells lead to sequential release of angiogenesis regulators. Platelets in this way serve as potent activators as well as inhibitors of important tissue repair processes such as inflammation [43] and angiogenesis [44].

Platelets in Tumour Angiogenesis

A tumour is a community of cells. There are resident cells (fibroblasts, histiocytes, epithelial and mesenchymal cells) that form the tissues, and cells that are recruited to the site in time of injury or malignant growth (mesenchymal progenitors and inflammatory cells). Platelets are mediators of this community.

Primary tumour growth is facilitated by inflammation and angiogenesis not unlike physiological wound healing [86–88]. However, in cancer, the normal physiological processes of dialing-down angiogenesis as scar tissue develops is prevented by the continuous, oncogene-mediated induction of tumour angiogenesis [89–92]. It has been well described that tumour vasculature is immature, unstable and morphologically different from the normal systemic vasculature. While tumour vasculature is often thought of as abnormal, it is better conceptualised as an unpruned, underdeveloped precursor of mature vessels – a continuously expanding, but not maturing, vascular bed.

Platelets play an important role in modulating tumour dynamics. A large body of evidence spanning at least four decades supports the involvement of platelets in cancer [1,2,13]. The process of sequestration of angiogenesis regulators in platelets is an active and highly selective process [41]. An open-ended proteomic comparison of platelets from mice bearing dormant or fast-growing liposarcoma xenografts revealed significant differences in protein profiles between each of these tumour subtypes [41,93,94], as well as differences when platelets of mice bearing either of the tumour types were compared to platelets of non-tumour-bearing sham-operated controls. Despite the open-ended analysis of all proteins present in platelets, the majority of proteins differentially expressed in platelets of tumour-bearing animals and cancer patients were found to be angiogenesis regulators such as VEGF, bFGF, PDGF, PF4, TSP1, MMP9, endostatin, angiopoietin-1 and -2, etc. While the membership in this "platelet angiogenesis proteome", as well as the concentrations of individual protein members, is fairly stable under physiological conditions [45], it is altered very early in tumour growth [41,93]. The sequestration of angiogenesis regulators in platelets is: i) *active* because it occurs against a concentration gradient in plasma and ii) *highly selective* for angiogenesis regulators, as other very abundant proteins, e.g. albumin or fibrinogen, are not taken up by platelets against a concentration gradient. Interestingly, the sequestration of angiogenesis regulators in platelets occurs very early in primary tumour growth [93]. At a time when tumours are not detectable by conventional methods, and long before the tumour burden results in changes in the levels of angiogenesis regulators in plasma or serum, there are

Tab. 1. Summary of pro- and antiangiogenic actions of platelet cytokines and factors.

ANGIOGENESIS STIMULATORS			
Factor	**Platelet concentration**	**Mechanism of action**	**Ref.**
VEGF (all isoforms, mainly VEGF-A, -B)	0.74 ± 0.37 pg/10^6 PLT	Promotes permeability of the vessel wall and serves as a chemoattractant for EC sprouting in the initial stage of the angiogenic response.	[41,45,46]
PDGF	23 ± 6 pg/10^6 PLT	Stimulates proliferation, differentiation and migration of fibroblasts or smooth muscle cells, providing support for the newly formed angiogenic sprout in the form of the pericyte (advanced stages of the angiogenic process).	[45,47,48]
FGFs (aFGF, bFGF/FGF-2)	bFGF: 0.44 ± 0.15 pg/10^6 PLT	Serve as a chemoattractant for EC and stimulate proliferation of EC.	[45,49]
EGF	1.05 pg/10^6 PLT	EGF binding to specific receptor EGFR induces an EC response leading to increased tubule formation, cell division and movement. EGF can augment the proangiogenic effect of other factors.	[50–52]
HGF	–	HGF is mitogen for different cell types including EC. Mechanisms of its effects include stimulating of secretion of MMP-1, VEGF, HGF itself and its receptor, c-met, in EC. Alternative processing of the HGF α-chain mRNA produces anti-angiogenic fragments.	[53–55]
IGF	–	Stimulates VEGF mRNA synthesis in EC. Facilitates EC motility and tubule formation.	[56–59]
angiopoetins	–	Ang-1 stimulates EC migration, tube formation, sprouting, and survival.	[60–61]
SDF-1/CXCL12	–	SDF-1α expression on activated platelet surface enhances endothelial progenitor cell recruitment to sites of arterial injury.	[62]
CD40L/CD154	–	CD40L binding to CD40 on EC promotes EC proliferation, migration and vessel-like structure formation through activation of the PI3K/Akt signalling pathway.	[63]
MMPs	–	Cleave different components of extracellular matrix (ECM) and basement membrane, which support new vessel development by assisting EC to migrate through the surrounding tissues.	[64]
S1P	–	Stimulates EC proliferation, migration and survival. Stimulates connective tissue growth factor (CTGF) production in ECs.	[65,66]
CTGF	–	Promotes EC growth, migration, adhesion and survival *in vitro*.	[67,68]
heparanase	–	Cleaves heparan sulfate, angiogenesis regulators binding molecule, which increases their bioavailability and facilitating their participation in blood vessel development during wound healing, tumour growth and metastasis.	[6]
ANGIOGENESIS INHIBITORS			
Factor	**Platelet concentration**	**Mechanism of action**	**Ref.**
angiostatin	–	Inhibits proliferation of EC *in vitro*, formation of capillary structures *in vitro* and angiogenesis *in vivo*.	[69–71]
TSP-1	31 ± 12 ng/10^6 PLT	Inhibits EC proliferation and capillary tube formation. It binds CD36 on the endothelial surface and activates a signalling cascade leading to stimulation of caspase-3 and increased EC apoptosis.	[45,72–77]
PF4/CXCL4 and CXCL4L1/PF4var	12 ± 5 ng/10^6 PLT	Inhibits binding of angiogenesis stimulators (e.g. VEGF, FGF) to cells.	[45,78–83]
endostatin	5.6 ± 3.0 pg/10^6 PLT	Inhibits tumour growth and VEGF-induced angiogenesis, but the mechanism of its action remains unclear.	[45,84]
TIMPs (TIMP-4, TIMP-1)	TIMP-4: 120–160 pg/10^6 PLT TIMP-1: < 10 pg/10^6 PLT	Hinder the angiogenic process via neutralization of the activity of different MMPs.	[85]

EC – Endothelial Cell, VEGF – Vascular Endothelial Growth Factor, PDGF – Platelet-Derived Growth Factor, FGFs – Fibroblast Growth Factors, EGF – Epidermal Growth Factor, HGF – Hepatocyte Growth Factor, IGF – Insulin–Like Growth Factor, SDF-1 – Stromal Cell-derived Factor-1, MMPs – Matrix Metalloproteinases, S1P – Sphingosine-1-phosphate, CTGF – Connective Tissue Growth Factor, TSP1 – Thrombospondin-1, PF4 – Platelet Factor 4, TIMPs – Tissue Inhibitors of Metalloproteinases

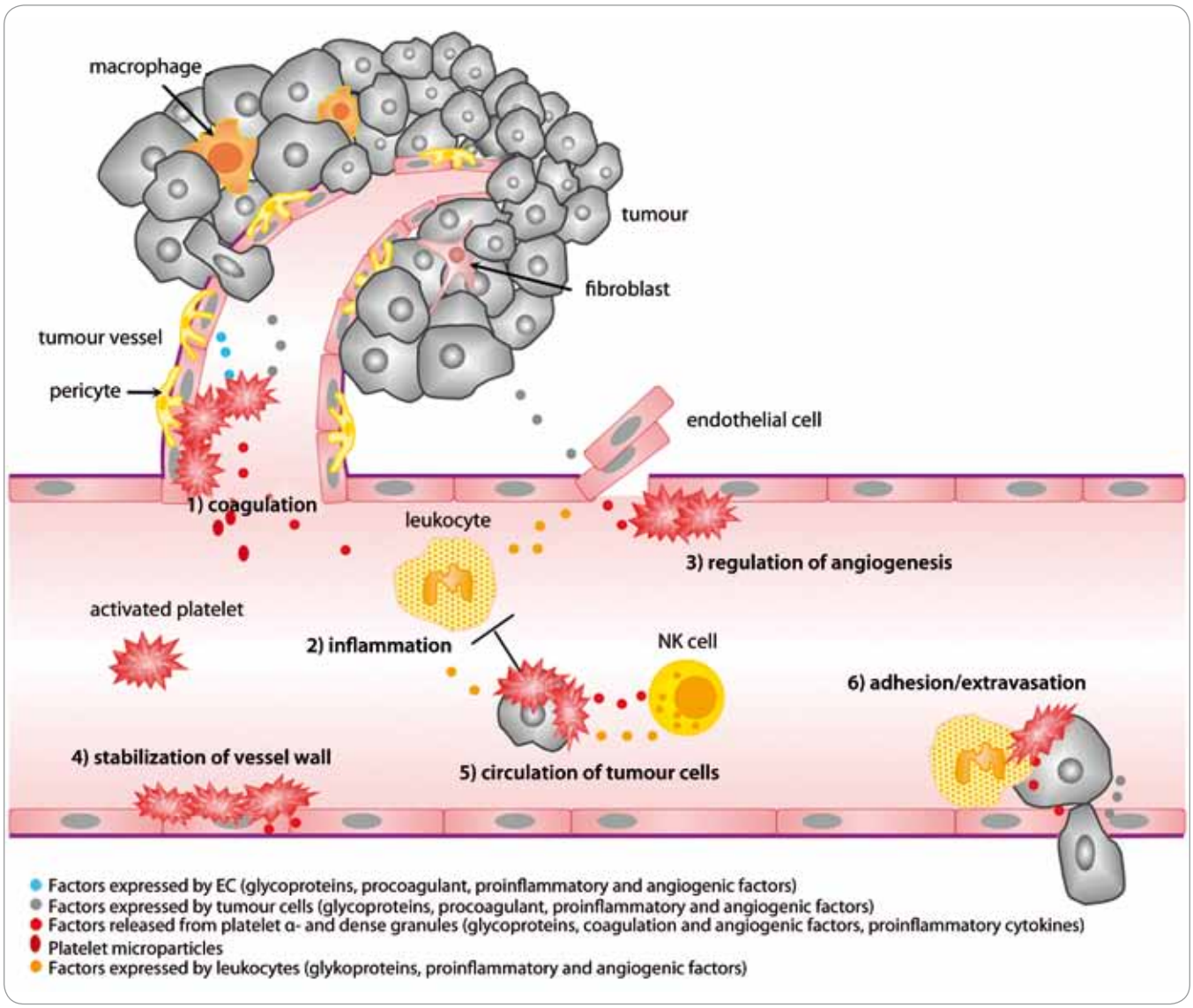

Fig. 1. Platelets contribution to the regulation of tumour angiogenesis and tumour progression. 1. Coagulation: Stimuli for platelet activation come from endothelial cells, as well as tumour stroma itself (expression of tissue factor, thrombin, ADP etc.). After activation, platelets change their shape, release PMP, α- and dense granule content and trigger coagulation cascade [8,11]. 2. Inflammation: Chemokines (IL-8, histamine etc.) released by platelets are chemotactic for leukocytes and precursor cells from bone marrow. These cells also regulate the tumour environment by release of growth and angiogenic factors [12]. 3. Angiogenesis: Platelets participate also in regulation of angiogenesis by releasing pro- and anti-angiogenic factors (VEGF, bFGF, PF-4 etc.), as well as by active sequestering of factors from the circulation [9]. 4. Stabilisation of vessel wall: Platelets stabilise the vessel wall and maintain intercellular connections by releasing factors, such as EGF, S1P, ang-1 etc., to prevent haemorrhage at the site of angiogenesis and inflammation [17]. 5. Circulation of tumour cells: Platelets adhered to tumour cells protect them from immune recognition and the cytotoxic effects of NK cell cytokines, which enables survival in the circulation and migration to distant tissue sites [7,17,18]. 6. Adhesion/extravasation: Aggregates of platelets, leukocytes and tumour cells facilitate adhesion of tumour cells to endothelium and subsequent extravasation into distant tissues. Platelets also release factors promoting cell proliferation and increasing permeability of the vessel wall (e.g. VEGF) [6,17].

detectable changes in platelet levels of angiogenesis regulators [41,93].

Are Platelets Stimulatory or Inhibitory to Tumour Growth?

While postulated many decades ago, the consequences of platelet adhesion to activated endothelium, and their role in early tumour growth and tumour angiogenesis, has been difficult to establish. The main source of the difficulties, similar to the difficulties in establishing their role in wound healing, is the variable method of platelet concentrate preparation. An additional limitation is the animal models, which do not necessarily reciprocate the complexity of platelet receptors and tissue integrins. However, through the use of genetically altered animals, *in vivo* tracking dyes, and three dimensional *in vitro* models, some of the

interactions between platelets, tumour cells, and other inflammatory cells within the tumour microenvironment are beginning to emerge (Fig. 1). The early literature can be very confusing. For example, there is convincing evidence that platelets enhance the development of metastasis [2,13–15,94–97] and primary tumour growth [2,13,15,98], but some studies advocate that the effect of platelets on primary tumour growth is inhibitory [99–101] and that the inhibition of platelet adhesion leads to promotion of metastasis [102]. Similarly, the most abundant proteins in platelets, e.g. PF4 (Tab. 1) are very potent inhibitors of tumour growth [103–109] and other very abundant platelet-associated proteins such as thrombospondin (TSP1) (Tab. 1), previously thought to be inhibitory to angiogenesis [110], may be augmenting the metastatic process under specific conditions [111–112].

One possible explanation for these very contradictory findings may be that platelets are neither inhibitors nor stimulators of tumour growth. Similar to their function in wound healing, they modulate rather than stimulate the malignant process, and the overall result of the platelet effect may depend on the balance of stimulatory and inhibitory signals within the tumour microenvironment. Depending on the reciprocal interaction between the existing host stromal cells, the oncogene-transformed tumour cells, and the recruited progenitors and inflammatory cells, the sum of these communications determines whether the outcome is growth, dormancy, or regression [94]. The final response may be less dependent on platelet numbers than on the specific content of stimulators and inhibitors of angiogenesis in the α-granules of platelets. This content of growth stimulators and inhibitors is continuously modified, a process aided by the short half-life of platelets in circulation (4–7 days in mice and 7–10 days in humans). This theory is informed by the recent finding that there is a higher organization of the opposing angiogenesis-related activities in platelets, enabling a differential release of either stimulators or inhibitors of angiogenesis [113,119]. The stimulators of angiogenesis (e.g., VEGF and bFGF) do not reside in the same granules as the inhibitors (e.g., endostatin) [113].

A widely-held assumption is that platelets degranulate en masse upon activation, and that serum is a good reflection of their content [114–117]. This assumption, which may have hindered the understanding of the reciprocal interaction of platelets and tissues, may not be entirely correct. Angiogenesis regulators associated with α-granules of platelets, unlike the proteins of dense granules, are not indiscriminately released in response to ADP, thrombin or epinephrine [41]. Activation of human platelets with adenosine diphosphate (ADP) stimulates the release of VEGF, but not endostatin whereas, thromboxane A(2) (TXA(2)) releases endostatin but not VEGF [118]. As has been well documented in the setting of gastric ulcers, platelet responses to thrombin are also graded [119–122]. Activation of high-affinity thrombin receptor PAR1 releases stimulators such as VEGF, whereas the low affinity thrombin receptor PAR4 mainly releases inhibitors such as endostatin [121]. Similarly, the increases in acidity and temperature, which are typical in the setting of infection, inflammation, or cancer also change the sequence of release of angiogenesis regulators from platelets [123,124]. This concept may be quite intuitive: if platelets contain both stimulators and inhibitors of angiogenesis, a massive degranulation would be unlikely to provide the sustained and carefully orchestrated signals required for modulation of normal angiogenesis. It is much more advantageous if the majority of angiogenesis regulators sequestered in platelets during early cancer development remain associated with the platelet clot upon coagulation [41]. Some may even be taken up by platelets during activation [125]. This finding may provide some early insights into the mechanisms of tissue/platelet communication. Because the majority of proteins relevant to angiogenesis are retained in the α-granules of platelets, and because the organization of proteins within the α-granules is based on function [113]; the release of angiogenesis regulators, unlike the release of ADP and serotonin from the dense granules, is selective [113,120,121], but also amenable to influences beyond the proteolytic activity of thrombin or environmental influences such as temperature or acidity. In the setting of this new knowledge, a clot, which has been thought of as a simple "plug" to prevent bleeding, now appears to be a sophisticated matrix that is rich in proteins and can regulate angiogenesis and inflammation in a locally-defined, reciprocal fashion.

Platelet-Derived Microparticles in Tumour Progression

Elevation of platelet-derived microparticles (PMP) levels accompanies a number of disorders including cancer, atherosclerosis, sepsis and diabetes [126]. The role of PMP in disease development is unknown but the composition of PMP in the plasma of patients varies considerably depending on the severity of the pathology [127]. The method of cell-cell communication may be dependent on the shedding of PMP upon platelet activation. PMP host a variety of cytokines and growth factors modulating angiogenesis and tissue regeneration. PMP have been shown to promote proliferation of endothelial cells and tubule formation [128] but also survival and proliferation of other cell types [129,130]. Recent evidence suggests that PMP, much like platelets, significantly affect tumour metastasis including modification of angiogenic responses. In gastric cancer, Kim et al showed that PMP levels are better predictors of metastasis than VEGF, IL-6, and RANTES [131]. It has been reported that PMP may serve as chemoattractants to several lung cancer cell lines, activating phosphorylation of ERK and expression of membrane type 1-matrix metalloproteinase [132]. PMP were also shown to stimulate proliferation and adhesion of cancer cells to fibrinogen and EC and enhance the adhesion and chemoinvasion of breast cancer cell lines [130]. PMP can induce secretion of MMP-2 by prostate cancer cells *in vitro*, facilitating their passage through the collagen that is a major component of extracellular matrix [133] contributing to cancer cell spread.

In summary, the systems biology of cancer is not dissimilar from that of a wound. In general, platelets have a pro-angiogenic effect in the setting of early injury, progressive tumour growth, atherogenesis or chronic inflammation, and an anti-angiogenic effect in the setting of a healing wound, dormant tumours, or receding inflammation [94]. Cancer may be thought of as "a wound that never heals" [86,87].

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