Blood Spotlight

Platelets in tissue repair - control of apoptosis and interactions with regenerative cells

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Abstract

Besides mediating primary hemostasis and thrombosis, platelets play a critical role in tissue repair and regeneration. They regulate fundamental mechanisms involved in the healing process including cellular migration, proliferation, and angiogenesis. Control of apoptosis/cell survival and interaction with progenitor cells, which are clinically relevant but poorly understood aspects of platelets in tissue repair, will be highlighted in this review. Gaining deeper insight into the less well characterized molecular mechanisms is necessary to develop new therapeutic platelet-based options.

Platelet - the healer of damaged tissue

For a long time platelets have been used to treat patients with thrombocytopenia or bleeding events to restore hemostasis. However, platelets also function as circulating cellular sensors that provide a unique link to immune responses and tissue repair\(^1\). Wound repair indeed is inseparably associated with inflammation and requires a finely tuned interplay of mechanisms regulating cellular migration, extracellular matrix organization/remodeling, cell proliferation, differentiation, and angiogenesis/neovascularization\(^2\). Platelets have been recognized to be majorly involved in all these cellular events which is reviewed elsewhere\(^3\)\(^-\)\(^6\). This review gives an update on the intensively investigated role of platelets in tissue regeneration and highlights clinically relevant but still poorly characterized mechanisms, namely interactions with progenitors and control of apoptosis/cell survival.

Besides the fact that platelet-rich plasma (PRP) has increasingly gained attention to seal wounds and enhance wound healing\(^7\), experimental and clinical data clearly indicate that platelets are fundamentally involved in repair and regeneration of damaged tissues and preservation of organ function. During tissue injury, for example caused by trauma or local ischemia as seen with myocardial infarction or stroke, the coagulation system as well as immune responses become activated very early, initiating the process of wound healing. Platelets are the first cells that accumulate at sites of the lesion and, on activation, release a multitude of biologically active mediators into their microenvironment\(^5\). Various cytokines, chemokines and growth
factors including CXCL12 (stromal-derived growth factor 1, SDF-1)\(^8\-\text{9}\) and hepatocyte growth factor (HGF)\(^\text{10}\-\text{11}\) have been identified to be secreted from platelets. Platelet-derived mediators induce and modulate activation of fibroblasts and recruitment of leukocytes, first neutrophils, followed by macrophages, resulting in elimination of dead cells and cellular debris\(^2\). Moreover, platelet-released factors induce and control proliferation and migration of other cell types that are critically involved in tissue repair such as smooth muscle cells (SMC)\(^\text{12}\) and mesenchymal stem cells (MSC)\(^\text{13}\). Angiogenesis in damaged tissue, another pivotal mechanism for recovery of tissue function, is also substantially regulated by platelets due to release of a multitude of pro- and anti-angiogenic mediators upon platelet activation\(^5\) (Figure 1).

Nowadays, platelets and their secretory products may successfully be utilized as feasible therapeutic tools, facilitating repair of injured tissues and organs. For instance, autologous platelet releasate\(^\text{14}\) as well as recombinant platelet-derived growth factors\(^\text{15}\) may enhance healing of chronic lower extremity diabetic ulcers. Moreover, regeneration of cutaneous wounds\(^\text{16}\), retina\(^\text{17}\) and peri-implant bone\(^\text{18}\) by platelets has been reported. However, treatment of surgical lesions with PRP has also generated controversial results in clinical trials\(^\text{19}\). In 10 patients with chronic liver disease, platelet transfusion improved distinct parameters of liver function, although adverse events related to platelet transfusion could be seen as well\(^\text{20}\).

One major achievement in the understanding of platelets and their defects in terms of tissue repair has been made in the field of liver pathophysiology. In a mouse model, Lesurtel et al. identified platelet-derived serotonin as key player for hepatic regeneration\(^\text{21}\). Interestingly, thrombocytopenia as well as impaired platelet activity in mice abrogated cellular proliferation in the liver substantially. Conversely, thrombopoietin-induced thrombocytosis resulted in strong accumulation of platelets in the sinusoids of liver and induction of hepatocyte proliferation shortly after hepatectomy in mice\(^\text{22}\). Moreover, platelets have been shown to be involved in postnatal occlusion of the ductus arteriosus and vessel remodeling\(^\text{23}\). Malfunctioning platelet adhesion/aggregation and defective platelet biogenesis was associated with impaired postnatal occlusion of the ductus in
neonatal mice. Moreover, preterm human newborns with thrombocytopenia showed increased risk of persistent open ductus. The impact of abnormal platelet function on tissue repair has also been investigated in atypical hemolytic uremic syndrome (HUS) with dysfunctional platelet-derived complement factor H. Besides its function as a complement regulatory protein, factor H exerts anti-inflammatory activity, and its mutations contribute substantially to HUS and glomerular membrane damage resulting in membranoproliferative glomerulonephritis type II.

Thus, growing evidence indicates that platelets or platelet-derived factors play a pivotal role in determining the balance between tissue repair and tissue damage and may therefore successfully be utilized for regenerative care. However, the underlying molecular mechanisms involved in platelet-mediated tissue repair are less well characterized and further experimental and clinical studies are needed to define specific targets for future therapeutic interventions.

**Platelet/progenitor cell interaction and regeneration**

Activated platelets release a whole range of chemokines and promote recruitment, adhesion and proliferation of adult stem cells including CD34-positive progenitor cells, MSC, SMC progenitors and endothelial progenitors (Figure 1). The multipotency of these stem cell types and their ability to augment vascular and tissue repair due to paracrine mechanisms make them promising therapeutic vehicles in regenerative medicine. Moreover, tissue damage itself generates strong chemoattractive signals for stem cells, providing the basis for their regenerative activity. Platelet-regulated recruitment of adult stem cells towards injured cells may therefore be a substantial mechanism in exerting regenerative cellular responses.

After myocardial infarction and intramyocardial, intracoronary or intravenous transplantation of MSC, the cells have been shown to migrate towards the injured heart, prevent ventricular remodeling and significantly restore cardiac function. Various clinical studies could confirm beneficial effects of MSC after myocardial damage even though the clinical benefit occasionally turned out to be moderate. Recently, we could demonstrate
that only apoptotic, but not necrotic or vital cardiomyocytes induced recruitment of MSC via HGF/MET-receptor interaction, providing a link between apoptotic cell death and the recruitment of cells with regenerative potential\textsuperscript{36}. HGF is a growth factor known to be produced after myocardial ischemia, as investigated in animals\textsuperscript{37} as well as humans\textsuperscript{38}. With its anti-apoptotic\textsuperscript{39}, pro-angiogenetic\textsuperscript{40}, and immunosuppressive\textsuperscript{41} activity it exerts cardioprotection\textsuperscript{42}. Platelets are also known to release, upon activation, HGF\textsuperscript{11} and have been described to promote recruitment of MSC to human arterial endothelial cells\textsuperscript{13}. Modulation of HGF-mediated migration of MSC to apoptotic tissue cells by platelets is therefore likely and may become a potent therapeutic tool to improve cardiac function after myocardial infarction.

As like HGF, SDF-1, another important mediator involved in stem cell trafficking, is also upregulated after myocardial ischemia. SDF-1/CXCR4 has been shown to induce recruitment of bone marrow-derived progenitors to the left ventricle after intravenous administration of the cells in a mouse model\textsuperscript{43}. In clinical trials, CD34-positive progenitor cells have been reported to be critically involved in myocardial repair and regeneration, contributing to preserved cardiac function\textsuperscript{44}. Moreover, injection of recombinant SDF-1 into the left ventricular cavity of mice before coronary occlusion significantly decreased infarct size compared with control groups\textsuperscript{45}. To improve efficacy of SDF-1-mediated cardioprotection, we have established a bifunctional protein consisting of an SDF-1 domain and a glycoprotein (GP)VI domain with high binding affinity to CXCR4 as well as to extracellular matrix proteins that become exposed after tissue injury\textsuperscript{46}. After experimental myocardial infarction administration of SDF1-GPVI had significant cardioprotective effects, promoting migration of CXCR4-positive bone marrow-derived progenitors, enhancing endothelial differentiation of the latter, preserving cell survival, and revealing proangiogenic effects. Platelets have an influence on these SDF-1-mediated progenitor cell activities. SDF-1 secreted by activated platelets supported CD34-positive progenitor cell recruitment to arterial thrombi and differentiation of the cells to endothelial progenitor cells \textit{in vivo}\textsuperscript{8-9}. In patients with myocardial infarction, platelet-derived SDF-1 correlated with the number of circulating progenitor cells and was associated with restoration of left ventricular function and improved prognosis\textsuperscript{47-48}. Moreover, formation of
circulating platelet/CD34-positive progenitor cell coaggregates has been described in patients with acute coronary syndromes, which was associated with a significantly decreased myocardial infarct size and better left ventricular function as seen with cardiac MRI at a 3-month follow-up. However, platelet-induced differentiation of CD34-positive progenitors into mature foam cells and endothelial cells has been described in an in vitro co-culture system, which may be of particular relevance for development of atherosclerotic vascular lesions.

**Platelets regulate apoptosis and survival of cells - mechanisms for regeneration**

Apoptosis is a precisely executed mode of cell death that sets off processes to limit further tissue damage and is generally associated with immunological tolerance. Increasing evidence indicates that regulation of the balance between apoptosis and cell survival, which determines fate of the injured tissues, is a process that is controlled by platelets (Figure 1). Induction of apoptosis is regulated by a diverse range of cell signals, which may originate either extracellularly (extrinsic) or intracellularly (intrinsic). One prominent extrinsic apoptotic pathway involves death receptors that are members of the tumor necrosis factor (TNF) receptor gene superfamily. TNF-alpha is a major cytokine regulating apoptosis. Although the presence of TNF-alpha in platelets is debatable, they store and secrete a variety of TNF-alpha-related ligands such as CD95 (Fas-L), CD154 (CD40L), Apo2-L (TRAIL), Apo3-L (TWEAK), and LIGHT, which have the potential of regulating apoptosis through paracrine signalling.

In the field of sepsis pathophysiology, pivotal insights could be gained about the significance of platelet-induced apoptosis. Incubation of endothelial cells and SMC with platelet-derived microparticles from septic patients resulted in strong induction of apoptosis in the cells due to production of reactive oxygen species, suggesting a central mechanism in the pathogenesis of septic vascular dysfunction. However, platelet microparticles have also been shown to phosphorylate and activate Akt, a serine-threonine kinase that inactivates the pro-apoptotic BCL-2 family member BAD, and exert anti-apoptotic activity in THP-1 cells, a human...
monocytic leukemia cell line, in a P-selectin-dependent manner. Interestingly, distinct microparticle types induced differential monocyte responses in terms of intracellular calcium fluxes and release of complement factor C5a as well as TNF-alpha. Another group demonstrated that platelets from septic mice induced apoptosis in mouse CD4-positive splenocytes via microparticle-independent mechanism. In this study, apoptosis was mediated by the serine protease granzyme B which was upregulated in megakaryocytes from the septic mice. Later, the same group demonstrated that platelet granzyme B-mediated apoptosis occurs in spleen and lung depending on direct cell-cell contacts and proper GPIIb/IIIa-function (Figure 1).

On the other hand, platelets are capable of executing anti-apoptotic mechanisms, shifting the balance toward cell survival and tissue repair (Figure 1). In neural stem cells, platelet-derived microparticles induced phosphorylation of Akt which was associated with neuronal cell proliferation, survival, and differentiation. Platelet microparticle-mediated phosphorylation of Akt has also been observed in endothelial cells, and improved endothelial regeneration took place after injection of microparticle-treated angiogenic early outgrowth cells in a mouse carotid artery wire denudation injury model. Moreover, platelets secret, upon activation, mediators with anti-apoptotic activity, such as HGF, SDF-1, serotonin, adenosine diphosphate, and sphingosine-1-phosphate, promoting survival signals for vascular endothelial cells and SMC at sites of vascular injury. High mobility group box 1 (HMGB1), a nuclear protein passively released by necrotic cells during tissue injury or actively secreted by innate immune cells, has been identified as a danger-signal that activates immune responses and regulates cell death and survival, as it has been shown for tumor cells, depending on HMGB1-redox status or formation of complexes with p53-protein. Platelets contain endogenous HMGB1, which is exported to the cell surface upon activation, making it another candidate for platelet-mediated regulation of cell death and survival.

The target cell type as well as regional distribution and intensity of surface expression of the respective death/survival receptors may define the ultimate outcome of pro- and anti-apoptotic function of platelets. Further
experimental and clinical studies have to be carried out to offer a better understanding of the crosstalk between platelets and mechanisms that control tissue repair, including less well characterized processes such as recruitment of cells with regenerative potential and regulation of apoptosis/cell survival. Such new insights will help us find better therapeutic platelet-based options to facilitate repair and regeneration of injured tissues and organs.
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Author Contribution Statement

Both authors M.G. and S.V. made significant contributions to the manuscript. M.G. and S.V. analyzed and interpreted the literature, designed the focus of the article, and wrote the manuscript.

Disclosure of Conflicts of Interest

The authors declare no competing financial interests.
References


Figure Legends

Figure 1. Mechanisms governing platelet-mediated tissue repair. Platelets are cellular mediators which orchestrate clinically relevant but still poorly understood mechanisms of tissue repair. They release cytokines, chemokines and growth factors such as SDF-1 and HGF that control recruitment, proliferation and activation of fibroblasts, neutrophils, monocytes, SMC, MSC and other cell types critically involved in wound healing. Platelets also regulate angiogenesis in damaged tissue which is another important mechanism for recovery of tissue function. Recruitment of progenitor cells including MSC, SMC, endothelial progenitors, and CD34-positive progenitors is influenced by platelets, as well, promoting wound repair at least partially due to paracrine mechanisms. Moreover, platelets are capable of modulating the balance between apoptosis and cell survival, which determines the pathophysiology of damaged tissues. They can release pro-apoptotic (Fas-L, CD40L, TRAIL, TWEAK, LIGHT) as well as anti-apoptotic (HGF, SDF-1, serotonin, adenosine diphosphate, sphingosine-1-phosphate) mediators. Moreover, microparticles derived from platelets can regulate apoptosis in endothelial cells and SMC as well as provide survival signals to monocytic cells, endothelial cells and neural stem cells. Granzyme B is a mediator of platelet-induced apoptosis in spleen and lung. HMGB1, a danger signal that is exported to the cell surface by platelets upon activation, regulates apoptosis as well as autophagy in tumor cells depending on its redox status. Therefore, platelets control complex mechanisms of tissue repair. Abbreviations: ADP - adenosine diphosphate; CD62P - P-selectin; CM - cardiomyocytes; EC - endothelial cells; MØ - macrophages; MP - microparticles; MSC - mesenchymal stem cells; NSC - neural stem cells; pAkt - phosphorylated Akt; PC - progenitor cells; ROS - reactive oxygen species; Ser - serotonin; SMC - smooth muscle cells; SP-1 - sphingosine-1-phosphate; TC - tumor cells.
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