Advanced Drug Delivery Reviews 62 (2010) 1196–1203

Contents lists available at ScienceDirect

Advanced Drug Delivery Reviews

journal homepage: www.elsevier.com/locate/addr

Platelets as delivery systems for disease treatments ☆

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ARTICLE INFO

Article history:
Received 30 April 2010
Accepted 29 June 2010
Available online 7 July 2010

Keywords:
Gene therapy
Platelet-specific promoter
Platelet
Hemostasis

ABSTRACT

Platelets are small, anucleate, discoid shaped blood cells that play a fundamental role in hemostasis. Platelets contain a large number of biologically active molecules within cytoplasmic granules that are critical to normal platelet function. Because platelets circulate in blood through out the body, release biological molecules and mediators on demand and participate in hemostasis as well as many other pathophysiologic processes, targeting expression of proteins of interest to platelets and utilizing platelets as delivery systems for disease treatment would be a logical approach. This paper reviews the genetic therapy for inherited bleeding disorders utilizing platelets as delivery system, with a particular focus on platelet-derived FVIII for hemophilia A treatment.

Contents

1. Introduction

Genetic manipulation of cells to produce proteins of interest for disease treatment is an attractive research area. Platelets, which are anucleate blood cells produced by megakaryocytes, are replete with secretory granules. These granules accumulate their stored contents from both megakaryocyte synthesis and endocytosis of plasma proteins. When platelets are activated, a large number of bioactive proteins are released from their granules by exocytosis participating in myriad physiologic and pathologic processes including hemostasis, thrombosis, inflammation, atherosclerosis, wound healing, antimicrobial defense, and malignancy [1]. Given platelets innate storage, trafficking, and release capacities, taking advantage of them as delivery systems for proteins of interest would be a logical and reasonable approach for disease treatment. The feasibility and efficacy of this novel approach, previously carried out in experimental animals, will be reviewed in this article.

2. Overview of platelet production and function

In hematopoietic stem cell linage commitment, two types of blood cell lines are derived: the lymphoid linage, which includes

☆ This review is part of the *Advanced Drug Delivery Reviews* theme issue on "Stem Cell Gene Manipulation and Delivery as Systemic Therapeutics".

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doi:10.1016/j.addr.2010.06.007

lymphocytes, and the myeloid linage, which includes myelocytes, red cells, and megakaryocytes. Pluripotent hematopoietic stem cells produce a progenitor committed to megakaryocyte differentiation that is still capable of mitotic division. Eventually proliferating diploid megakaryocyte progenitors lose their capacity to divide and enter endomitosis. During the endomitotic phase, cells still maintain their ability to replicate DNA and cytoplasmic maturation continues, but neither cytoplasm nor nucleus divides, resulting in cells with ploidy ranges from 8 N to 128 N in a single, highly lobated nucleus and a cytoplasmic mass containing a complex of internal membrane systems, granules, and organelles [2–6]. When the megakaryocyte matures, the polyploid nucleus becomes horseshoe shaped and the cytoplasm expands, resulting in a low nuclear to cytoplasmic ratio, and platelet organelles and the demarcation membrane system are robustly amplified [7–9]. The cytoplasmic mass from differentiated megakaryocytes forms proplatelets and eventually gives rise to circulating platelets [10–12].

Platelets are involved in the cellular mechanisms of primary hemostasis leading to the formation of blood clots, as well as participating in many physiologic and pathologic processes including inflammation, wound healing, atherosclerosis, antimicrobial host defense, angiogenesis, and malignancy [1]. Platelets are produced by megakaryocytes in a process that consumes nearly the entire cytoplasmic complement of membranes, organelles, granules, and soluble macromolecules. Currently, there are two proposed mechanisms for platelet production. In one scenario, platelets are produced by cytoplasmic fragmentation from megakaryocytes [13–18]. The other scenario of platelet biogenesis is that platelets are budded off the tips of proplatelets, which operate like assembly lines, resulting in platelet production at the end of each proplatelet [10–12,19,20]. In either scenario, each megakaryocyte is estimated to give rise to 2000 to 5000 nascent platelets [21–23]. It is estimated that megakaryocytes regenerate at a rate of 10^8 cells per day [24–26]. Thus, each day the adult human produces approximately 2×10^{11} to 5×10^{11} platelets and this number can increase tenfold with demand [24,27]. Production of such a large number of cells circulating in the blood, each with a lifespan of 9 to 10 days, could potentially offer an excellent delivery system for disease treatment.

Platelets contain a large number of biologically active proteins within cytoplasmic granules including α-granules, dense granules, primary lysomes, and peroxisomes [28]. The α-granules are the most abundant granules in platelets. There are about 50 to 80 α-granules per platelet, which is 10-fold more than dense granules. They contain a wide variety of coagulation/adhesive proteins, growth factors and protease inhibitors involved in both primary and secondary hemostatic mechanisms [29,30]. Proteins present in α-granules either arise from megakaryocyte synthesis (e.g. glycoproteins (GP)Ib/V/IX, GPIV, integrin αIIbβ3 (GPIIb/IIIa), von Willebrand factor (VWF), P-selectin, thromboglobulin, platelet-derived factor) or endocytosis through cell surface membrane receptor-mediated uptake from the plasma environment (e.g. fibrinogen, fibronectin, factor V). Dense granules contain ADP, ATP, calcium ions, and serotonin [31]. Besides a vast number of molecules are stored in platelet granules, platelets have several surface receptors [32]. There are two important surface receptors that can bind adhesive glycoproteins (GP) including the GPIb/V/IX complex, which supports platelet adhesive by binding VWF, and the αIIbβ3 receptor, which mediates platelet aggregation by binding fibrinogen, collagen, and VWF. Other receptors include the serpentine receptors for ADP, thrombin, epinephrine, and thromboxane A2 (TXA2) and the Fc receptor FcγRIIA.

Platelets are the principle cells responsible for primary hemostasis. Upon vessel injury, subendothelium portions of the blood vessel wall that normally are concealed from circulating platelets by an intact endothelial lining are exposed, providing the initiation signal for platelet deposition [33]. At sites of injury, platelets adhere to the vessel wall by interactions with VWF and collagen and become activated and undergo degranulation, releasing a variety of potent functional molecules and several mediators that affect platelet function, inflammation, vascular tone, fibrinolysis, and wound healing [34–39]. Thus, platelets are packed with bioactive proteins and circulate in blood, serving as both storage "depot" and trafficking "vehicle" in the circulation. Due to these special characteristics, platelets can be a unique target for gene therapy of diseases that result from defects of proteins that are normally synthesized by megakaryocytes, such as Glanzmann thrombasthenia, which results from deficiency of GPIIb/IIIa, and Bernard–Soulier syndrome, which is caused by defects of GPIb/V/IX. This strategy can also be used for other diseases in which the protein of interest normally circulates in blood and is needed at the sites of vascular injury but is not synthesized by megakaryocytes, e.g. factor VIII (FVIII) for hemophilia A and factor IX (FIX) for hemophilia B. In the later two cases, the protein of interest could be ectopically expressed in platelets where transgene protein could be stored in releasable granules, circulate through the body, and ultimately released locally at sites of platelet activation, such as at sites of vascular injury.

3. Platelet manipulation for disease treatment

Since platelets are anucleate cells with a limited life span, direct molecular manipulation cannot serve as a reliable means for intervention. Although megakaryocytes are amenable to molecular manipulation [40], they also have a finite life span and cannot serve as a means for long-term expression of the target protein. In contrast, hematopoietic stem cells, which give rise to all blood lineages including the megakaryocyte/platelet lineage, are preferable targets for genetic transfer to establish *in vivo* long-term expression of the target protein in platelets. Hematopoietic stem cells are an attractive target for gene therapy because they have a high capacity for clonal expansion including both self-renewal and differentiation into all blood lineages, thus supporting hematopoiesis throughout the lifetime, and because these cells are easily accessible and can be expanded and genetically modified ex *vivo*, and then reimplanted. Since the transgene is introduced by *ex vivo* transduction of hematopoietic stem cells, followed by transplantation, anti-vector immune response should be minimized in contrast to *in vivo* systemic transduction. Gene transfer into hematopoietic stem cells can potentially provide a cure for inherited and acquired diseases of hematopoietic [41,42].

To restrict transcription of the transgene to the platelet/megakaryocyte lineage rather than in all cells, the transgene has to be driven by a platelet lineage-specific promoter. Besides offering tissue-specific gene expression, there are other advantages to use tissue-specific promoters to direct transgene expression. One is that using the promoters of normal cellular genes to direct expression of the therapeutic gene under physiologic regulation, rather than strong viral promoters, may reduce the risks of activating adjacent cellular genes and thereby reduce the potential of insertional onco-mutagenesis, particularly since cell-specific promoters will remain inactivate in the vast majority of transduced cells. The other is that they are less likely to activate the host-cell defense machinery than constitutive viral promoters and therefore, the development of immune response to transgene product may be reduced or circumvented [43]. The promoters that have been employed to direct megakaryocyte/platelet lineage-specific transgene expression include those that control the expression of genes encoding GPIIb (αIIb) [44–54], GPIbα [55–57], GPVI [57], platelet factor 4 (PF4) [58–60], and c-mpl [61,62]. All of these promoters are activate in both megakaryocytes and platelets. Most reported studies have used either the GPIIb promoter or the GPIbα promoter for megakaryocyte/platelet-specific gene expression. The αIIb promoter directs high-level expression of GPIIb, which forms a complex with GPIIIa with a surface density of 80,000 copies per platelets [63]. This promoter is active throughout megakaryocytopoiesis, including very early stage of differentiation [64,65]. Recent studies done by Frampton and co-workers have showed that GPIIb is also expressed on bone marrow mast cells [66]. In contrast, the GPIbα promoter drives expression of GPIbα, which forms a complex with GPIX and V with approximately 25,000 copies per platelet, and