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Review

Placenta growth factor mediated gene regulation in sickle cell disease

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ABSTRACT

Sickle cell anemia (SCA) is an autosomal recessive disorder caused by mutation in the β-globin gene. Pulmonary hypertension (PH), a complication of SCA, results in severe morbidity and mortality. PH is a multifactorial disease: systemic vasculopathy, pulmonary vasoconstriction, and endothelial dysfunction and remodeling. Placenta growth factor (PlGF), an angiogenic growth factor, elaborated from erythroid cells, has been shown to contribute to inflammation, pulmonary vasoconstriction and airway hyper-responsiveness (AH) in mouse models of sickle cell disease. In this review, we summarize the cell-signaling mechanism(s) by which PlGF regulates the expression of genes involved in inflammation, PH and AH in cell culture and corroborate these findings in mouse models of SCA and in individuals with SCA. The role of microRNAs (miRNAs) in the post-transcriptional regulation of these genes is presented and how these miRNAs located in their host genes are transcriptionally regulated. An understanding of the transcriptional regulation of these miRNAs provides a new therapeutic approach to ameliorate the clinical manifestations of SCA.

1. Sickle cell anemia

Sickle cell anemia (SCA) and its variants are genetic disorders of the hemoglobin molecule. These disorders include sickle cell disease (HbSS), sickle β-thalassemia syndromes (HbS-thal) and hemoglobin S associated with other hemoglobinopathies. The hemoglobin S (Hb S) molecule contains a mutation in the β-globin chain, wherein the glutamic acid residue at the 6th position is replaced by a non-polar valine residue. At low oxygen tensions polymerization of HbS occurs forming long fibers, resulting in the sickled morphology of RBCs and contributing to reduced deformability of sickle RBC (SS RBC). These features of SS RBCs result in shortened red cell survival, culminating in chronic hemolytic anemia and vaso-occlusion [1–3]. Vaso-occlusion of the microvasculature occurs by trapping of the less deformable SS RBCs, adhesion of SS RBCs to the vascular endothelium, activation and adhesion of neutrophils to endothelium, leukocytosis, inflammation and thrombosis [4–9]. The clinical manifestations of SCA include chronic anemia, episodic vaso-occlusive painful events, acute chest syndrome, splenic sequestration, chronic leg ulcers, stroke, pulmonary hypertension and asthma/reactive airway disease [3,6,7,10]. Recurrent vaso-occlusions cause ischemia/reperfusion injury that ultimately results in tissue/organ damage and the chronic morbidity and foreshortened life-span associated with SCA [3]. In fact, cardiopulmonary complications are the most common cause of adult mortality in SCA.

1.1. Pulmonary hypertension in SCA

Pulmonary hypertension (PH), a complication of SCA, results in severe morbidity and mortality [11]. The pathogenesis of PH is likely multi-factorial: including hemolysis-mediated impaired nitric oxide bioavailability, chronic thromboembolic disease from a pro-coagulant state, systemic vasculopathy from hypoxia and inflammation, pulmonary vasoconstriction, and endothelial dysfunction and remodeling [8,10,12,13].

Nitric oxide (NO) and endothelin-1 are opposing vasoactive factors that regulate the pulmonary vascular tone [14,15]. During the last decade a number of studies, both experimental and clinical, have focused on the depletion of nitric oxide by elevated levels of hemoglobin in plasma in the clinical manifestations of PH in SCASCA [10,12,16–19]. However, the role of NO in the pathophysiology of PH in SCA has been challenged [13]. Studies show plasma levels of endothelin-1 are significantly higher in SCA patients [20,21], and elevated levels of ET-1 are associated with endothelial dysfunction [22]. Our studies show high levels of placenta growth factor (PlGF) in plasma of SCA patients is associated with elevated plasma ET-1 levels, and elevated pulmonary artery pressure, reflective of pulmonary hypertension [23]. In this article, we will address how placenta growth factor (PlGF) regulates transcription of ET-1 and the role of microRNAs (miRNAs) in regulating expression of ET-1. Recent comprehensive

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reviews summarize the biosynthesis of endothelin, the structure and function of endothelin receptors, genetic endothelin knockout mouse models, and the role of endothelin in human pharmacology [24].

1.2. Asthma and reactive airway disease in SCA

Asthma is a disease of the airways characterized by airway obstruction, airway hyper-responsiveness/reactivity, and inflammation leading to intermittent respiratory symptoms [25]. Leukotrienes (LT) are lipid mediators that play an important role in the pathophysiology of asthma, allergic inflammation and innate immunity [26]. Since inflammation has an important role in the pathogenesis of vaso-occlusion and asthma augments the pro-inflammatory state, a vicious cycle of inflammation, asthma and vaso-occlusion develops in SCA [27]. Asthma among children and adults with SCA is associated with increased incidence of SCA-related morbidity and mortality [27,28]. Studies show elevated levels of leukotrienes in plasma in SCA individuals, which correlates with increased vaso-occlusion and acute chest syndrome (ACS) [29,30].

The biosynthesis of leukotrienes predominantly occurs in leukocytes, and the first step of the process is activation of membrane phospholipids by phospholipase A2 to yield arachidonic acid (AA; C20-fatty acid). Next, AA is converted to leukotriene A4 (LTA4) by 5-lipoxygenase (5-LO) in concert with 5-lipoxygenase activating protein (FLAP) [31,32]. LTA4 is converted either to leukotriene B4 (LTB4) by LTA4 hydrolase or conjugated with reduced glutathione by leukotriene C4 (LTC4) synthase to yield LTC4 [33]. LTC4 is subsequently converted to LTD4 and then to LTE4; these three LTs are collectively referred to as cysteinyl leukotrienes (cyst LTs). Leukotriene B4 is among the most potent inflammatory and chemotactic molecule for neutrophils, while Cyst LTs promote airway hyper-responsiveness (AHR). We show placenta growth factor induces the synthesis of leukotrienes in vitro and in mouse models of SCA, and correlates with airway hyper-responsiveness in SCA [34,35]. We will address how enzymes involved in LT synthesis are transcriptionally and post-transcriptionally regulated in this article. The biochemical and clinical aspects of leukotrienes are reviewed elsewhere [36–38].

1.3. Placenta growth factor in the pathophysiology of SCA

Placenta growth factor (PlGF), an angiogenic growth factor, belonging to the vascular endothelial growth factor (VEGF) family, was originally discovered in 1991 [39]. The human PlGF gene (PGF) encodes four isoforms PlGF 1–4, which are generated by alternative mRNA splicing [40]. PlGF was originally identified in the placenta, where it regulates growth and differentiation of trophoblasts. It is also expressed in umbilical vein endothelial cells and other non-placental tissues, like the thyroid gland and developing lung tissue [41,42]. Tordjman et al. discovered erythroid cells produce angiogenic growth factors, including PlGF [43]. Our studies show increased plasma levels of PlGF in SCA individuals is a consequence of increased erythropoiesis, resulting from hemolytic anemia seen in these patients [44]. The expression of PlGF is enhanced by erythropoietin (Epo) in $CD34^+$ progenitor cells of bone marrow (Fig. 1) [44]. PlGF binds exclusively to VEGFR-1 receptor, while VEGF binds to both VEGFR-1 and VEGFR-2 receptors. PlGF has pleiotropic effects on different cell types and regulates various biological activities, e.g. cell proliferation and migration [41,45,46]. Studies utilizing PlGF gain of function and loss of function approaches in mouse models, revealed the roles of PlGF in specific tissues and cells. These effects contribute to disease pathophysiology as described in the review by Dewerchin and Carmeliet [41]. For example, PlGF-deficient mice show reduced angiogenesis and inflammation of the infarcted myocardium [47] and delivery of either PlGF gene or protein in infarcted mice led to cardiac improvement [48]. Our studies show overexpression of PlGF in erythroid cells via lentiviral vector delivery in C57 mice (normal mice) to the PlGF levels seen in Berkeley Sickle mouse (BK-SS) resulted in augmented ET-1 plasma levels. This was manifested by increased right ventricular hypertrophy and right ventricular pressures, both features of pulmonary hypertension [23]. Furthermore, overexpression of PlGF in normal mice induces PAI-1 levels and promoted a prothrombic state [49]. Thus unregulated PlGF expression is conducive to several pathophysiologic changes associated with SCA. A brief review summarizes the role of PlGF in the pathobiology of blood diseases, including hemoglobinopathies and hematologic malignancies [50].

2. PlGF-mediated transcription of cytochemokines and inflammation in sickle cell disease

Vascular occlusion leads to recurrent episodes of painful crises and damage to the various end organs and is the major cause of morbidity and mortality in SCA [4,51,52]. The classical studies of Hebbel and his coworkers [53] show that the extent of endothelial cell adherence by SS RBCs to cultured endothelial cells appears to be comparable to the clinical severity of vaso-occlusive events in SCA. However, SCA individuals with an identical alteration in the β-globin genes show distinct variability in the severity and frequency of vaso-occlusive episodes, suggesting that factors other than sickle RBCs may be playing a role in the pathophysiology of vaso-occlusion in SCA.

A salient feature of SCA is leukocytosis, which occurs even in the absence of infection or inflammation. The activation of monocytes, neutrophils, endothelial cells and coagulation cascade observed in SCA at steady state is presumed to be secondary to the sickling phenomena [4,54–56]. Although activated leukocytes can promote vaso-occlusion [57], the mechanistic activation of monocytes and neutrophils, and leukocytosis remained enigmatic. Studies by Kalra and Malik, and their coworkers [44,58] show mononuclear cells (MNCs) from SCA patients at steady state are highly activated as these MNCs exhibit higher mRNA levels of cytochemokines (IL-1β, IL-8, MIP-1β, MCP-1 and VEGF) compared to MNCs isolated from healthy normal individuals. The MNCs of normal individuals show an absence or trace mRNA levels of these same cytochemokines [44].

Since SCA patients have higher circulating levels of PlGF, it has been hypothesized that PlGF may be responsible for activation of MNCs [44]. Studies of MNCs isolated from normal healthy individuals (AA) upon treatment with PlGF show significantly increased mRNA levels of pro-inflammatory cytochemokines, as observed in MNCs of SCA individuals (Fig. 1) [44]. Peripheral blood monocytes isolated from SCA patients are in an activated state for inflammation as exemplified by increased mRNA levels of cytochemokines (TNF-α, IL-1β, MIP-1b,MCP-1, IL-8), compared to monocytes isolated from healthy individuals [58]. The activation of monocytes by PlGF is mediated by VEGFR-1 and occurs via activation of PI-3 kinase/AKT and ERK-1/2 pathways [58]. These studies show mononuclear cells/monocytes from SCA patients are activated for expression of cytochemokines as these cells are in the milieu of circulating PlGF, thus contributing to the inflammatory state observed in SCA patients. These cytochemokines released by monocytes have a potential to augment expression of adhesion molecules (VCAM-1, ICAM-1, P-selectin and E-selectin) in the vascular endothelium. These adhesion molecules are involved in adhesion of sickle RBCs and monocytes via corresponding surface ligands which lead to vaso-occlusion in small capillaries and ischemia in SCA.

2.1. PlGF-mediated transcription of ET-1, and post-transcriptional regulation of ET-1 by miRNAs

Since erythropoiesis is expanded in SCA individuals, resulting in elevated plasma PlGF [44], it has been hypothesized that PlGF may be a key mediator in activation of endothelial cells to augment expression of ET-1, a potent vasoconstrictor. Studies show PlGF increases mRNA and protein expression of ET-1 in cultured human pulmonary microvascular endothelial cells (HPMVEC) via activation of PI-3 Kinase and NADPH-