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Mast Cells, Hypoxia and Structure of the Vascular Bed

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Abstract: Mast cells represent a heterogeneous and multifunctional cells population distributed throughout tissues. Their participation in the response to chronic hypoxia is discussed in consideration to their role in the angiogenesis and remodeling of pulmonary vasculature, including relevance of proangiogenic factors, mediators and proteolytic enzymes released by activated mast cells. Possible mechanism of mast cells activation by hypoxia is considered.

Introduction

The supply of oxygen and nutrients to every cell in the organism is essential for the normal function of tissues. Decreased oxygenation of arterial blood triggers a complex response of the organism aimed at the preservation of sufficient O_2 delivery to the cells. While an acute phase of this reaction typically involves changes in the function of the ventilatory and circulatory systems and metabolic adjustment, in chronic hypoxia morphological changes are also taking place. Our review is concerned with the role of mast cells in two processes affecting the structure of blood vessels – angiogenesis and remodeling of pulmonary vasculature in adults. These processes affect two values in the Krogh's idealized model of O_2 supply to tissue – intercapillary distance and capillary PO_2 (partial pressure O_2) [1].

Intercapillary distance is derived from capillary density and, therefore, from the intensity of angiogenesis. Capillary PO_2 is markedly affected by oxygen transport in the lungs and therefore by the structure of pulmonary vessels.

Angiogenesis is a limiting factor for pre– as well as postnatal growth, but in adult tissues it is normally restricted to reactions such as wound healing and inflammation. It could be, however, initiated when oxygen (and nutrients) supply fails to meet the demands of the cells (i.e. during cancerogenesis).

Similarly, a fully developed vessel wall structure is stable, but in specific situations it may undergo remodeling.

Characterization of mast cells

Mast cells (MCs), first described by Paul Ehrlich [2], were extensively studied mainly for their role in the immune response, particularly in the case of an allergic reaction. They are distributed in almost all organs except compact bone, cartilage and cornea. The highest concentrations are in the tissues that are in contact with the external environment – the skin and mucosa. MCs are derived from pluripotent stem cells and carried in the blood stream in an immature form to the tissues, where they differentiate and mature under the control of numerous growth factors and cytokines [3]. MCs in different tissues may therefore differ in their morphology as well as in function.

Human mast cells are classified according to their production of serine proteases chymase and tryptase into group named MC_T (expressing mostly tryptase) and MC_{TC} (expressing both tryptase and chymase). On the other hand, rodent MCs are classified according to tissue distribution into mucosal MC (MMC) and connective tissue MC (CTMC). MCs usually look like bags of granules containing mediators. The mediators are typically classified into two main groups: 1. Preformed (secretory granules associated), such as histamine, serotonin proteoglycans, proteases, chemotactic factors, and 2. Newly generated – prostaglandins, tromboxane, leukotriens, interleukins (IL), tumor necrosis factor-alpha (TNF_a) and growth factors. Some mediators of both groups are released readily others slowly. More detailed description of MCs biology can be found in recent reviews by Galli and Costa [4], and Metcalfe et al. [5].

Mast cells and angiogenesis

Angiogenesis, resulting in increased density of the capillary network and in the shorter distance for O_2 diffusion from the blood to the cell, can substantially affect the availability of oxygen for cell metabolism. Angiogenesis therefore represents a beneficial reaction to hypoxemia. The involvement of MCs in angiogenesis has been suspected for more than 20 years [6] and was clearly demonstrated in elegant experiments of Norby and coworkers who developed a specific technique for angiogenetic studies (mesenteric window angiogenesis assay) [7]. Using this method they found that angiogenesis could be induced in the mesentery of adult rats and mice (that lack physiologic angiogenesis) by application of a specific activator of MCs – compound 48/80. Compound 48/80 (a micture of polymers derived from N-methyl-p-methoxy-phenylethylamine) is a mast cell secretagogue. It acts at the cell surface and activates G proteins. In guinea pigs, whose MCs do not respond to 48/80, this compound failed to induce such response [8].

Angiogenesis involves series of complex and sequential events: the activation of endothelial cells, the enzymatic degradation of their basal membrane, the degradation of other components of extracellular matrix (ECM), the migration and proliferation of endothelia toward the angiogenic stimulus, the lumen formation, the branching of new microvessel and the formation of vessel network [9]. Each step in this process is triggered and controlled by mediators from which many are produced by MCs. Some of the mediators formed and released by MCs are the direct angiogenic factors like growth factors: vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), transforming growth factor-beta (TGF_β) or tumor necrosis factor-alpha (TNF_α) and interleukin-8 (IL-8) [9]. Others (histamine, prostaglandin D_2, leukotriens B_4 and C_4) increase vascular permeability, which then has a pro-angiogenic effect [10]. Tryptase seems to

stimulate directly the endothelial cells to form vascular tubes [11] and activates the metalloproteinases and plasminogen activator, which degrade the extracellular matrix. Chymase locally generates angiotensin II and stimulates bFGF-induced angiogenesis [12]. bFGF-induced angiogenesis is activated also by heparanase, which clears the growth factor bound to heparan sulfate.
On the other hand, activation of MCs can have also an inhibitory effect on angiogenesis, because stimulation of tromboxane A2 receptors has an antiangiogenic effect [13].

The release of MCs mediators also stimulates other cells important in the process of angiogenesis. These cells (macrophages, leukocytes, endothelial cells, fibroblasts) then multiply the release of proangiogenic factors by their own production or by the production of enzymes that mobilize endogenous factors bound to the extracellular matrix or cell surface (VEGF, bFGF, TGF_{β}). These factors then cause migration of MCs into affected region. Proteolytic enzymes may, however, mobilize also anti-angiogenic factors such as thrombospondins [14], angiostatin [15] or endostatin [14]. In summary, adequate evidence exists that MCs are able to trigger and promote angiogenesis. However, neither their precise role in this process, nor the ability of hypoxia to stimulate MCs are yet clear.

Mast cells and remodeling of pulmonary vessels

In response to acute hypoxia pulmonary vessels contract in the so-called hypoxic pulmonary vasoconstriction. This reaction restricts the blood flow to the hypoxic regions in the lungs and in this sense is beneficial. When hypoxia is sustained for weeks, it initiates thickening of the walls of pulmonary vessels, resulting in hypoxic pulmonary hypertension (HPH) and increasing the right heart afterload. The thickening of pulmonary vessel walls is reversible, over days to weeks, after return to normoxic conditions [16].

The thickening, as well as restoration of normal vessel wall thickness, involves important changes in the extracellular matrix. The ECM serves as a supporting network keeping tissues together and, concurrently, it is an important environment for intercellular signalization. The ECM is ubiquitous and a dynamic structure made from collagens, elastin, proteoglycans and glycoproteins. An imbalance between the formation and breaking of the ECM is inevitable in any process of tissue remodeling. The balance between the two processes is controlled among others by proteolytic enzymes – tissue metalloproteinases (MMPs) and their specific tissue inhibitors of metalloproteinases (TIMPs). MCs are an important source of proteases and, therefore, they are able to enhance the breakdown of ECM.

In rats the remodeling of pulmonary vessels in hypoxia starts with degradation of ECM, followed by proliferation of fibroblasts and smooth

muscle cells. The crucial role in the degradation of the extracellular matrix is played by the enzyme metalloproteinase MMP-13 (rodent-type interstitial collagenase), which starts the cleavage of native collagen [17]. This enzyme is produced in an inactive form (pro-MMP-13) by MCs and is activated by serine proteases (α-chymase and tryptase), which are also a product of MCs [5]. It has been shown that hypoxia increases the number of MMP-13 containing MCs and that these cells surround the peripheral lung vessels [18]. Moreover, the exposure of MCs, isolated from lung tissue, to hypoxia *in vitro* increased the MMPs activity in them [19].

MCs apparently also play an important role during restoration of the lung vascular architecture during recovery from chronic hypoxia. The re-establishment of normoxia also induces an increase in collagenolytic activity in MCs [20]. Tozzi et al. [21] showed significantly higher number of MCs persisting in the main trunk pulmonary arteries after one week of recovery.

Both phases of pulmonary vessels remodeling are characterized by high collagenolytic activity. Surprisingly, interstitial collagenase (MMP-13) cleaves the collagen I in a different manner during hypoxic conditions than during the recovery from hypoxia. During chronic hypoxia MMP-13 cleaves collagen I by triple helicase activity at the 3:1 site. In the recovery phase it is the peptidolytic activity dominates cleaving collagen at the telopeptidase site [22]. This difference is probably related to the alteration of the C-terminal domain of MMP-13, which is a major determinant for substrate specificity [17].

Mast cells activation

The text has shown that the activated MCs are able to trigger angiogenesis as well as a remodeling of pulmonary vessels. The fact that hypoxia affects MCs has been shown by Haas and Bergovsky [23] and Kay et al. [24] in the seventies. More recently Dix et al. [25] found that systemic hypoxia activates MCs in the rat cremaster and we found that hypoxia increases the synthesis of metalloproteinases in isolated rat lung MCs [19]. However, the mechanism of MCs activation by hypoxia is far from being clear.

MCs activation is usually classified as immunologic or non-immunologic. While the immunologic activation mediated by $Fc_{\varepsilon}RI$ receptor and IgE antibody has been extensively studied and is understood, the non-immunologic, relevant in the case of hypoxia, is still unclear. Most of the classical, non-immunologic, stimuli basic compounds (48/80, mastoparan, polymyxin B), peptides (mellitin, somatostatin, vasoactive intestinal polypeptide), IL-8, platelet-activating factor (PAF), complement fragments or dextran [5] – are not released by hypoxia. The rapid and sustained MCs degranulation in rat cremaster muscle [25] resulted from systemic but not local hypoxia and was seen also in normoxic animals after the application of

plasma from conscious hypoxic rats [26]. Thus the results suggest that hypoxia releases a mediator that activates MCs. The nature of this mediator was not established. Problems in the search for such a mediator can be illustrated on one of many candidates – substance P. It is released during hypoxia in some tissues [27] and it is one of the basic compounds that stimulate MCs [5], but the MCs response in different organs are inconsistent. The response of lung MCs is less pronounced than that of skin MCs [28]. Another candidate for the same role – VEGF- is up regulated in hypoxia by hypoxia-inducible factor 1 (HIF-1) [29], but its exact role in the activation of MCs has to be established.

As mentioned above, hypoxia increased the synthesis of metalloproteinases in isolated rat lung mast cells [19]. Direct activation of the formation of these enzymes is therefore likely. Interestingly enough such activation is suppressed by an antioxidant N-acetylcysteine [30] suggesting a role as a reactive oxygen species (ROS). On the other hand we showed that hydrogen peroxide alone didn't affect MMPs production in MCs [31]. ROS are involved in the immunologic activation of MCs (see instructive review by Suzuki et al. [32]), but their role in non-immunologic stimulation is not clear. Swindle et al. [33] reported that macrophage derived hydrogen peroxide inhibited antigen induced MCs degranulation. This finding suggests that extracellular ROS do not activate MCs, but hypoxia increases ROS production intracellularly and such production may have different effects. ROS can act also indirectly by the activation or inactivation of other mediators. For example, NO was shown to inhibit MCs degranulation [33]. However, an increase in the production of ROS that readily react with NO (forming peroxynitrite) could decrease NO concentration, which could relieve the inhibitory effect of NO.

Conclusion

Hypoxia stimulates mast cells and activated mast cells play an important role in the anatomical reconstructions of blood vessels. The elucidation of mechanisms involved in these processes might open new possibilities for the therapy of serious disorders caused by hypoxia in chronic lung diseases or during a stay at high altitude.

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