Kindlin-3 in hematopoietic integrin activation: Absence in leukocyte adhesion deficiency type III

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CHAPTER 1

General introduction
GENERAL INTRODUCTION

In 1997, our group reported for the first time a syndrome affecting a 5-yr old boy who was hospitalized with a history of nonpussing inflammatory lesions, leukocytosis (i.e. an increased number of circulating white blood cells) and a clinically overt bleeding tendency. Apart from the platelet aggregation defect, similar leukocyte defects are seen in the classical Leukocyte Adhesion Deficiency type 1 (LAD-I) syndrome. We hence designated the novel combination of leukocyte and platelet defects Leukocyte Adhesion Deficiency type-1/variant (LAD-1/v), which was later termed LAD-III. Over the past 15 years, approximately 30 cases of LAD-III have been reported, in which a severe bleeding tendency, delayed wound healing and recurrent infections are often preceded by delayed separation of the umbilical cord. In vitro experiments showed defective adhesion and aggregation of leukocytes and platelets. In LAD-I, the defect lies in the absence of β2 integrins, due to mutations in the ITGB2 gene. Integrins form the major family of adhesion molecules on blood cells. In LAD-III, all integrins are normally present but fail to be activated during leukocyte or platelet activation.

The aim of the current project was to reveal the genetic as well as cell biological defect underlying the LAD-III syndrome. We report mutations in FERMT3, the gene encoding kindlin-3, as the genetic cause for LAD-III. Kindlin-3 is a regulatory protein involved in the activation of integrins, and is thereby a key player in adhesion of hematopoietic cells. We studied patient material and made use of cell lines and molecular approaches to unravel the mechanism by which kindlin-3 acts on a clinical, cellular and molecular level.

ADHESION OF HEMATOPOIETIC CELLS

Adhesion contributes to several functions of the different hematopoietic cell types. Leukocytes are key players in the immune system, as they recognize and destroy pathogens such as bacteria or fungi. Circulating cells are recruited to the sites of inflammation, following a gradient of locally generated chemoattractants. Adhesion is essential for the leukocytes to slow down and migrate through the vessel wall, in a process called extravasation, and subsequently through the tissue. Initial rolling along the vessel wall is mediated by selectins and their fucosylated ligands, whereas firm adhesion occurs through activated integrins.

Phagocytic leukocytes, i.e. neutrophils, eosinophils, monocytes and macrophages, use integrins to actively bind and engulf pathogens in an adhesion-dependent manner, in a process called phagocytosis. The pathogens within the leukocytes are then killed by reactive oxygen species and enzymes stored in intracellular granules, and are subsequently digested. Increased release of chemoattractants at the site of inflammation leads to amplification of the immune response. Lymphocytes contribute to the clearance of pathogens in several ways, including the generation of pathogen-binding antibodies.

Platelets circulate in the blood to maintain hemostasis by the formation of platelet clots in case of vessel injury. Integrins expressed on platelets mediate adhesion to collagen in disrupted vessel walls, and to soluble fibrinogen, which forms bridges between platelets within a platelet clot. Platelets also contribute to vessel wall integrity by filling up weak spots, and lack of platelets leads to more fragile capillaries and increased probability of spontaneous blood loss.
Erythrocytes, which are the most abundant blood cell type, are of major importance as they are responsible for transport of oxygen throughout the body. Erythropoiesis occurs in the bone marrow and is dependent on integrin-mediated adhesion to sustain specific ‘erythroid islands’. Integrin expression, and major adhesive capacity, is lost during erythrocyte maturation. Mature erythrocytes are non-nucleated red cells, which show a characteristic biconcave shape and transport oxygen from the lungs to the body tissues as well as carbon dioxide from the tissues back to the lungs.

**INTEGRINS**

Integrins are ubiquitously expressed transmembrane receptors consisting of an α and a β chain. They represent the major class of adhesion receptors on hematopoietic cells. In mammals, 18 α and 8 β subunits form 24 known combinations, each of which can bind to a specific repertoire of cell-surface, extracellular matrix, or soluble ligands. Different hematopoietic cell types and tissues express different integrins. On leukocytes, α4β1 (VLA-4), α5β1 (VLA-5), αLβ2 (LFA-1) and αMβ2 (Mac-1, CR3) are the most prominent family members, whereas αIIbβ3 and α2β1 are the predominant integrins expressed on platelets.

A hallmark of integrins is their ability to shift between different conformational states (Figure 1). On circulating hematopoietic cells, integrins have an inactive conformation.

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**Figure 1. Leukocyte integrin activation.** Upon cell stimulation via e.g. GPCRs, inside-out signaling results in recruitment of talin-1 and kindlin-3, which act in concert to induce conformational changes in integrins from a low ligand-binding affinity towards a intermediate and subsequent high-affinity state. Talin-1 binds to a membrane-proximal NPxY/F motif whereas kindlin-3 binds to a membrane-distal NxxY/F motif of the β integrin cytoplasmic tails. (Adapted from Van de Vijver E, van den Berg TK, Kuijpers TWK. Leukocyte Adhesion Deficiencies. Hematol Oncol Clin N Am. 2012 Feb; 27(1):101-116; chapter 3 of this thesis.)
Injury and infection induce activating signals in the extracellular environment, such as collagen exposure or cytokine production. Chemattractants signal through G protein-coupled receptors (GPCRs). The resulting intracellular signaling leads to extracellular conformational changes from a bended, inactive integrin towards an extended conformation with low or intermediate ligand affinity, a process known as ‘inside-out’ signaling. Subsequent ligand-mediated ‘outside-in’ signaling leads to further integrin activation towards a high-affinity conformation with a fully unclasped integrin ligand-binding headpiece. High-affinity ligand binding triggers downstream signaling cascades that regulate cell shape, spreading and motility, and alter gene expression, cell proliferation, differentiation and apoptosis. The majority of these events depends on the modulation of the cell cytoskeleton.

These cascades are complex and involve several ways of signal transduction, such as (de)phosphorylation of plasma membrane phosphoinositides, and interactions with many cytoplasmic proteins, among which are the small GTPase Rap1, talin-1 and the kindlin proteins. Talin-1 and kindlins act in concert to support the final step of integrin activation.

**KINDLIN-3**

Both kindlins and talin-1 contain a FERM domain (called after the homologous 4.1 protein, ezrin, radixin, moesin), which is commonly involved in protein-protein and protein-lipid interactions and can regulate subcellular localization and activity, as well as recruitment into larger protein complexes. The FERM domain of kindlins and talin-1 bears a phosphotyrosine binding (PTB) site specific for NxxY/F motifs in the integrin β chains. Talin-1 binds to the NxxY/F motif in the membrane-proximal region of integrin β chains, whereas kindlins bind to a membrane-distal NxxY/F motif (Figure 1). Of many of these FERM domain-containing proteins, the expression and function in hematopoietic cells is unclear, as for instance for talin-2.

The FERM domain of kindlins consists of two parts surrounding a so-called pleckstrin homology (PH) domain. PH domains are known for their ability to interact with phosphoinositol phosphates (PIPs), for instance in the inner leaflet of the plasma membrane. The availability of specific PIPs depends on a dynamic process of phosphorylation and dephosphorylation of phosphoinositides in membrane lipids. A recent study on the PH domain of kindlin-2 suggests that it indeed binds to PIPs directly and that this interaction regulates the activation of integrins.

The kindlin family consists of fibroblast-specific kindlin-1, ubiquitously expressed kindlin-2 and hematopoietic kindlin-3, with high homology between them. Loss of kindlin-1 leads to the Kindler syndrome, a hereditary genodermatosis characterized by skin blistering and cutaneous atrophy, first described by Theresa Kindler in 1954. Absence of kindlin-2 is embryonically lethal in mice, corresponding to its ubiquitous expression. Kindlin-3−/− mice were first described in 2008, and are characterized by a severe bleeding tendency, anemia and defective leukocyte function. The phenocopy of LAD-III symptoms in the kindlin-3−/− mice contributed to the discovery of kindlin-3-deficiency as the cause of LAD-III by us and by others.
SCOPE OF THIS THESIS

The research described in this thesis was designed to unravel the genetic defect in LAD-III, and to reveal the molecular mechanism of hematopoietic integrin activation affected in this syndrome. In Chapter 2, we describe mutations in FERMT3, the gene encoding kindlin-3, as the cause of the disease. The current clinical prospect of LAD-III patients is reviewed in Chapter 3, whereas the genetic context of the deficiency is given in Chapter 4.

Kindlin-3 is a hematopoietic regulator of integrin activation, thereby playing a crucial role in the regulation of blood cell adhesion. We focused on αMβ2 integrin on neutrophils to study the role of kindlin-3 in a more mechanistic approach. Although the relevance of kindlin-3-dependent activation of neutrophil integrins is clearly illustrated in the patients, kindlin-3-independent activation was also observed, as is described in Chapter 5. The contribution of the membrane-binding PH domain and the integrin-binding PTB domain of kindlin-3 to integrin activation are described in Chapter 6.

We describe our studies on erythrocytes of LAD-III patients in Chapter 7 and reveal contribution of kindlin-3 to α4β1 integrin-mediated erythropoiesis in the bone marrow. The importance of kindlin-3 in platelet aggregation is reported in Chapter 8, where we used a new flow cytometry-based assay to analyze the individual contribution of α2β1 and αIIbβ3 integrin to platelet aggregation.

In Chapter 9, we summarize our results and discuss our findings in a broader clinical perspective and in the scope of current knowledge on hematopoietic integrin activation.

REFERENCE LIST

INTRODUCTION


