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Dock10 regulates CD23 expression and sustains B-cell lymphopoiesis in secondary lymphoid tissue

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ABSTRACT

Dock10, a guanine nucleotide exchange factor for the Rho GTPases Rac1 and Cdc42, affects cell morphology, membrane protrusive activity, and cell movement. Dock10 is prominently expressed in lymphoid tissue and upregulated by IL-4 in B cells. To investigate the physiological role of Dock10, WT mice and Dock10 KO mice were used. KO mice showed decreased numbers of B cells in spleen, both follicular B cells and marginal zone B cells, and in peripheral blood, but not in bone marrow. The antiapoptotic effect of IL-4 in vitro, the migratory response to CXCL13 or CCL21 in vitro, and the whole genome expression profile were intact in spleen B cells from KO mice. CD23, the low-affinity receptor for immunoglobulin E, was overexpressed on follicular B cells from KO mice, suggesting that Dock10 negatively regulates membrane CD23 expression. Negative regulation of CD23 expression by Dock10 could play a role in B cell maturation and function.

1. Introduction

Dedicator of cytokinesis 10 (Dock10, Zizimin3) belongs to the Dock family of guanosine nucleotide exchange factors (GEFs) for small Rho GTPases (Yelo et al., 2008; Gadea and Blangy, 2014). Dock10 targets and activates Rac1 and Cdc42 (Ruiz-Lafuente et al., 2015; Jaudon et al., 2015; Gadea et al., 2008). Rho GTPases play essential roles in actin cytoskeleton dynamics and cell motility (Wennerberg and Der, 2004; Heasman and Ridley, 2008). Rac1 stimulates lamellipodia and membrane ruffles formation, and Cdc42 regulates cell polarity and induces filopodia (Aspenström et al., 2004; Chhabra and Higgs, 2007). Rac1 and Cdc42 regulate the actin cytoskeleton through interactions with Wiskott–Aldrich syndrome proteins, diaphanous-related formins, and p21 protein activated kinases. Dock10 is expressed as two forms with divergent amino termini, named Dock10.1 and Dock10.2, expressed preferentially by T cells and B cells, respectively (Fig. 1A). Dock10 expression, especially that of Dock10.2, is strongly upregulated in B cells by IL-4 (Yelo et al., 2008; Alcaraz-García et al., 2011; Ruiz-Lafuente et al., 2014). IL-4 is a pleiotropic cytokine that induces maturation of B cell precursors into Ig-secreting cells, isotype switching toward IgE, and has a potent antiapoptotic effect in vitro (Okada et al., 2003).

Rho GTPases are required for normal lymphopoiesis (Tybulewicz and Henderson, 2009; Mulloy et al., 2010). B-cell lymphopoiesis begins in the bone marrow (BM), where the immunoglobulin (Ig) genes are rearranged. B cells expressing

Abbreviations: ANOVA, analysis of variance; BCR, B-cell receptor; BM, bone marrow; CM, complete medium; DN, double negative; DP, double positive; FO, follicular; GC, germinal center; GEF, guanine nucleotide exchange factor; GEP, gene expression profile; IB, isolation buffer; LF, lymphoid follicle; MZ, marginal zone; NK, natural killer; PB, peripheral blood; qPCR, quantitative PCR; SP, single positive.

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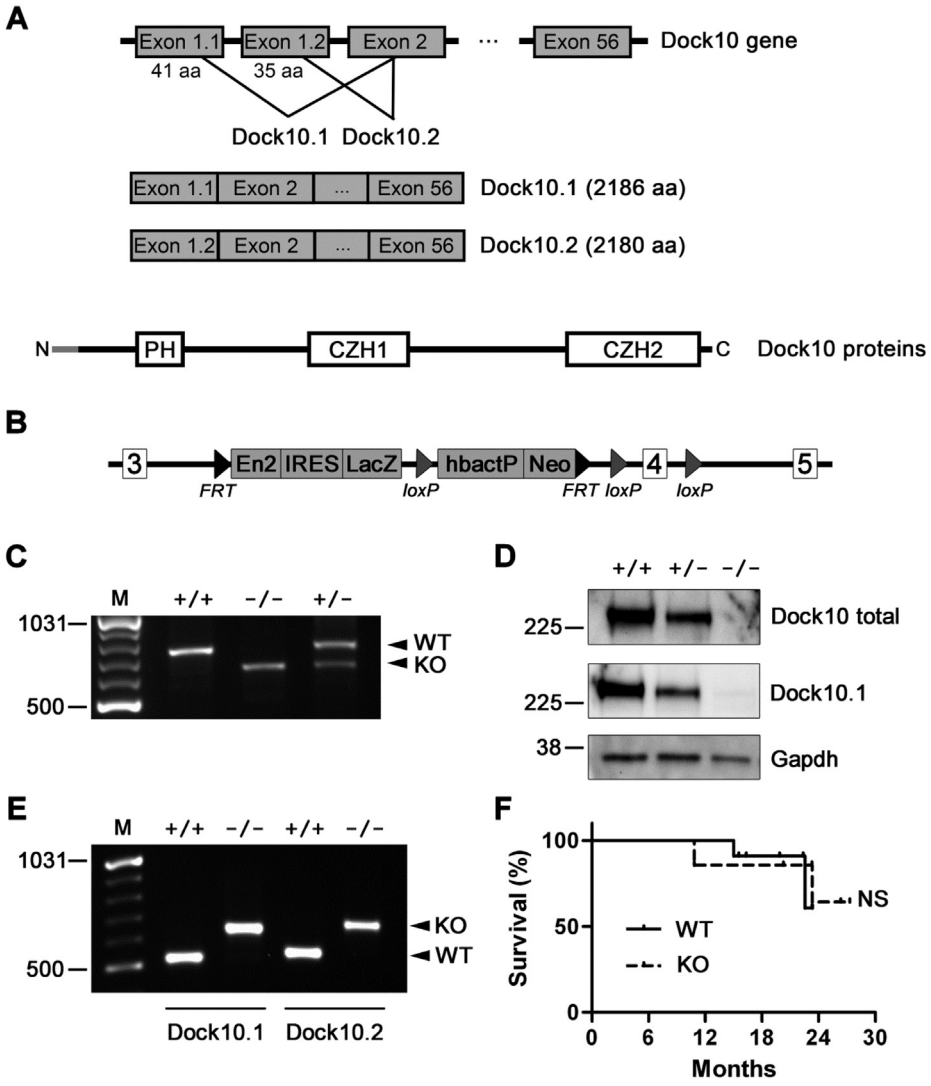

Fig. 1. Panel A, schematic representation of the Dock10 gene, its two alternative first exon isoforms, and the Dock10 proteins. The latter depicts the approximate localization of the pleckstrin homology (PH) domain, the Dock family homology domains 1 and 2 (CZH1 and CZH2), and in grey, the divergent segment in the alternative first exon isoforms. Panel B, schematic representation of the Dock10 KO first allele. The L1L2_Bact_P cassette, flanked by FRT sequences, is inserted into intron 3 next to exon 4, which in turn is flanked by loxP sequences. Panel C, genotype assessment of the mice by duplex PCR. Fragments of different size were generated from the WT allele (740 bp) and the KO allele (631 bp). Panel D, western blot analysis of whole-cell protein extracts from splenocyte suspensions. The positions of the size markers are indicated in kDa to the left. Panel E, expression of mutated Dock10 transcripts in spleen cells by RT-PCR. The bands of a larger size in the KO mice correspond to the insertion of the 115-bp En2 exon between exons 3 and 4 of the Dock10 transcripts. Panel F, survival analysis of two cohorts of WT mice (N = 11) and KO mice (N = 7). +/+, homozygous WT mice; +/−, heterozygous mice; −/−, homozygous KO mice. Aa, amino acids. N, amino terminus. C, carboxy terminus. M, size markers. NS, nonsignificant.

surface IgM migrate to the spleen, where they maturate into two main subpopulations, the follicular (FO) B cells and the marginal zone (MZ) B cells, which make up the two separate areas of the lymphoid follicles (LFs), i.e., the germinal center (GC) and the MZ, respectively. The FO B cells, characterized by membrane CD23 expression, recirculate between secondary lymphoid organs. T-cell lymphopoiesis begins in the thymus, where CD4−CD8− double negative (DN) thymocytes differentiate into CD4+CD8+ double positive (DP) cells. DP thymocytes go through positive selection into either CD4+CD8− or CD4−CD8+ single positive (SP) thymocytes. SP thymocytes recirculate between secondary lymphoid organs. Rac1 and Cdc42 global KO mice are embryonic lethal. B cell-specific double deletion of Rac1 and Rac2 results in reduced numbers of both FO B cells and MZ B cells (Walmsley et al., 2003). T cell-specific double deletion of Rac1 and Rac2 blocks thymic differentiation resulting in decreased numbers of DP and SP thymocytes, and of T cells in peripheral blood (PB) and spleen (Guo et al., 2008; Dumont et al., 2009). B cell-specific deletion of Cdc42 impairs B-cell receptor (BCR) signaling and compromises B-cell development in BM and spleen, resulting in fewer and smaller LFs (Guo et al., 2009; Burbage et al., 2015). Specific deletion of Cdc42 in mature B cells impairs their adhesion capacity, ability to activate T cells, antibody affinity maturation and homing to the B cell follicles of the spleen (Gerasimcik et al., 2015). T cell-specific deletion of Cdc42 blocks thymic differentiation resulting in a reduction in the number of SP thymocytes (Guo et al., 2011).

Other Dock proteins significantly expressed in lymphoid tissue, such as Dock2, Dock8, and Dock11, have diverse effects in lymphocyte development (Gadea and Blangy, 2014). Dock2 is a Rac specific GEF that regulates lymphocyte trafficking in and out of the secondary lymphoid organs. In the Dock2 KO mice, there is an impaired homing of T and B lymphocytes in spleen and lymph nodes, and GCs are poorly structured (Fukui et al., 2001). Dock8 is a Cdc42 specific GEF whose mutations in humans cause a rare immune deficiency