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RESEARCH ARTICLE

Protein tyrosine phosphatase PTPN9 regulates erythroid cell development through STAT3 dephosphorylation in zebrafish

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ABSTRACT

Protein tyrosine phosphatases (PTPs) are involved in hematopoiesis, but the function of many PTPs is not well characterized *in vivo*. Here, we have identified Ptpn9a, an ortholog of human PTPN9, as a crucial regulator of erythroid cell development in zebrafish embryos. *ptpn9a*, but not *ptpn9b*, was expressed in the posterior lateral plate mesoderm and intermediate cell mass – two primitive hematopoietic sites during zebrafish embryogenesis. Morpholino-mediated knockdown of *ptpn9a* caused erythrocytes to be depleted by inhibiting erythroid cell maturation without affecting erythroid proliferation and apoptosis. Consistently, both dominant-negative PTPN9 (with mutation C515S) and siRNA against *PTPN9* inhibited erythroid differentiation in human K562 cells. Mechanistically, depletion of *ptpn9* in zebrafish embryos *in vivo* or in K562 cells *in vitro* increased phosphorylated STAT3, and the hyper-phosphorylated STAT3 entrapped and prevented the transcription factors GATA1 and ZBP-89 (also known as ZNF148) from regulating erythroid gene expression. These findings imply that PTPN9 plays an important role in erythropoiesis by disrupting an inhibitory complex of phosphorylated STAT3, GATA1 and ZBP-89, providing new cellular and molecular insights into the role of *ptpn9a* in developmental hematopoiesis.

KEY WORDS: Erythroid cell development, PTPN9, STAT3, K562 cell

INTRODUCTION

Vertebrate hematopoiesis is a sequential process that occurs in primitive and definitive waves in anatomically distinct sites (Orkin and Zon, 2008). In mammals, primitive hematopoiesis occurs in the yolk-sac blood island, and definitive hematopoiesis originates in a distinct region known as the aorta-gonad-mesonephros (Cumano et al., 1996; Müller et al., 1994). In zebrafish, the primitive wave occurs in two intraembryonic regions, known as the anterior lateral plate mesoderm (ALPM) and the posterior lateral plate mesoderm (PLPM) that subsequently form the intermediate cell mass (ICM), whereas definitive hematopoiesis begins in the ventral wall of the dorsal aorta (de Jong and Zon, 2005). The first wave produces primitive erythrocytes and macrophages, and the second wave generates hematopoietic stem cells (HSCs), which are self-renewing and pluripotent. HSCs migrate to the fetal liver and then to the bone marrow in mammals, but migrate to the caudal hematopoietic tissue and finally colonize at the kidney in zebrafish.

Primitive hematopoiesis is regulated by several transcription factors, including the stem cell leukemia gene (*SCL/TAL1*), the lim-only 2 gene (*LMO2*), *PU.1* (also known as *SPI1*), *ZBP-89* (also known as *ZNF148*) and *GATA1*. By contrast, definitive hematopoiesis is initiated by the transcription factors RUNX1 and c-MYB (Paik and Zon, 2010). The importance of these factors has been demonstrated in cell-based *ex vivo* assays, as well as in knockout mouse models. SCL, a transcription factor with a basic helix-loop-helix motif, is a central regulator of hematopoietic differentiation. LMO2, a LIM domain transcription factor, acts in the same manner as SCL also in embryonic hematopoiesis (Gering et al., 2003; Shivdasani et al., 1995; Warren et al., 1994). The zinc finger protein ZBP-89 regulates hematopoietic progenitor cell differentiation (Li et al., 2006; Woo et al., 2008). Another zinc finger protein, GATA1, is specifically required for the maturation of proerythroblasts (Pevny et al., 1991). PU.1, a transcription factor that contains an ETS domain, plays an indispensable role in myeloid cell development (Scott et al., 1994). RUNX1, a runt domain transcription factor, is essential for the generation of HSCs in the ventral wall of the dorsal aorta (Chen et al., 2009; Kalev-Zylinska et al., 2002; Okuda et al., 1996). The myb-family member c-MYB maintains the population of HSCs in definitive hematopoiesis (Mucenski et al., 1991). These transcription factors form a sophisticated network in order to control the processes of primitive and definitive hematopoiesis.

Protein tyrosine kinases and protein tyrosine phosphatases (PTPs) play important roles in cell proliferation, differentiation, and migration through delicate regulation of signaling pathways. Human PTPN9 is a cytoplasmic phosphatase that is hyper-activated in erythroid progenitors in the bone marrow disorder polycythemia vera (Xu et al., 2003). Previous studies have shown that PTPN9 dephosphorylates NSF, FOXO1, ERBB2, EGFR and VEGFR2 for different functions, such as promoting homotypic vesicle fusion (Huynh et al., 2004), mediating insulin signaling in hepatocytes (Cho et al., 2006), suppressing breast cancer cell growth (Yuan et al., 2010) and regulating endothelial cell function (Hao et al., 2012). Recently, we have shown that PTPN9 directly interacts with STAT3 and mediates its dephosphorylation in breast cancer cells (Su et al., 2012). Overexpression of *PTPN9* decreases tyrosine phosphorylation of STAT3, whereas depletion of *PTPN9* increases its phosphorylation. Here, we show that PTPN9 has undergone duplication into *ptpn9a* and *ptpn9b* in

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