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The tyrosine phosphatase SHP2 is required for cell transformation by the receptor tyrosine kinase mutants FIP1L1-PDGFRα and PDGFRα D842V

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ABSTRACT

Activated forms of the platelet derived growth factor receptor alpha (PDGFRα) have been described in various tumors, including FIP1L1-PDGFRα in patients with myeloproliferative diseases associated with hypereosinophilia and the PDGFRα^{D842V} mutant in gastrointestinal stromal tumors and inflammatory fibroid polyps.

To gain a better insight into the signal transduction mechanisms of PDGFRα oncogenes, we mutated twelve potentially phosphorylated tyrosine residues of FIP1L1-PDGFRα and identified three mutations that affected cell proliferation. In particular, mutation of tyrosine 720 in FIP1L1-PDGFRα or PDGFRα^{D842V} inhibited cell growth and blocked ERK signaling in Ba/F3 cells. This mutation also decreased myeloproliferation in transplanted mice and the proliferation of human CD34^{+} hematopoietic progenitors transduced with FIP1L1-PDGFRα. We showed that the non-receptor protein tyrosine phosphatase SHP2 bound directly to tyrosine 720 of FIP1L1-PDGFRα. SHP2 knock-down decreased proliferation of Ba/F3 cells transformed with FIP1L1-PDGFRα and PDGFRα^{D842V} and affected ERK signaling, but not STAT5 phosphorylation. Remarkably, SHP2 was not essential for cell proliferation and ERK phosphorylation induced by the wild-type PDGF receptor in response to ligand stimulation, suggesting a shift in the function of SHP2 downstream of oncogenic receptors. In conclusion, our results indicate that SHP2 is required for cell transformation and ERK activation by mutant PDGF receptors.

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Abbreviations: ERK, extracellular signal-regulated kinase; FBS, fetal bovine serum; FLT3, Fms-like tyrosine kinase 3 receptor; FPα, FIP1L1-PDGFRα; GIST, gastrointestinal stromal tumors; MAPK, mitogen-activated protein kinases; PDGF, platelet-derived growth factor; PI3K, phosphatidylinositol-3-kinase; PLCγ, phospholipase C gamma; PKB, protein kinase B; PTPN11, protein tyrosine phosphatase, non-receptor type, 11; SCF, stem cell factor; ShRNA, short hairpin RNA; SH2, SRC homology 2; SHP2, SH2 domain-containing phosphatase 2; SRE, serum response element; STAT, signal transducer and activator of transcription.

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1. Introduction

The fusion of the *FIP1L1* gene with *PDGFRA* is generated by a cryptic deletion on chromosome 4q12 and is responsible for the development of myeloid neoplasms associated with hypereosinophilia, a disease that is also referred to as chronic eosinophilic leukemia (Vardiman et al., 2009).

FIP1L1 (Factor interacting with Pap1-like 1) is the homologue of a yeast gene *FIP* required for mRNA polyadenylation (Ezeokonkwo et al.). *PDGFRA* encodes the platelet-derived growth factor receptor α chain (PDGFRα), which belongs to the receptor-tyrosine kinase family (Andrae et al., 2008; Toffalini and Demoulin, 2010a,b). All breakpoints identified to date in *PDGFRA* are located within exon 12, which encodes the juxtamembrane domain, an inhibitory sequence located between the transmembrane and the kinase domains (Cools et al., 2003a). A partial deletion of this domain is sufficient to constitutively activate the tyrosine kinase activity of PDGFRα (Stover et al., 2006). Most patients respond well to the tyrosine kinase inhibitor imatinib mesylate (Glivec), which blocks PDGF receptors as well as ABL and c-KIT (Gleich et al., 2002; Metzgeroth et al., 2008). Nevertheless, some patients acquire imatinib-resistant mutations, such as T674I or D842V (Lierman et al., 2009).

Expression of FIP1L1-PDGFRα (FPα) in the Ba/F3 hematopoietic cell line and in $CD34^+$ human hematopoietic progenitors promotes cytokine-independent cell growth (Buitenhuis et al., 2007; Cools et al., 2003a; Montano-Almendras et al., 2012). In Ba/F3 cells, the FIP1L1 part can be replaced by a simple tag, suggesting that it is dispensable for FPα activation (Stover et al., 2006). By contrast, deletion of the FIP1L1 part decreased the impact of the oncoprotein in human hematopoietic progenitors (Buitenhuis et al., 2007). We observed that FPα escapes the normal degradation of activated receptors, leading to the accumulation of the oncoprotein and an enhanced transformation potential (Toffalini et al., 2009).

In addition to fusion genes, point mutations in *PDGFRA* were identified in various cancers, including gastrointestinal stromal tumor (GIST), glioma, FPα-negative hypereosinophilic syndrome and inflammatory fibroid polyps (Elling et al., 2011; Heinrich et al., 2003; Huss et al., 2012; Velghe et al., 2013). The most common activating mutation is D842V, which is located in the activation loop of PDGFRα (Dewaele et al., 2008). It is present in 8% of all patients with GIST and is resistant to imatinib (Corless et al., 2005; Dewaele et al., 2008; Elling et al., 2011). Recently, this mutation was reported in a few patients diagnosed with multiple myeloma (Mulligan et al., 2013).

Signal transduction by wild-type PDGFRα has been extensively studied (Heldin et al., 1998). The activated kinase domain phosphorylates at least ten tyrosine residues within the cytosolic part of the receptor. These phosphorylated tyrosines act as docking sites for the Src homology 2 (SH2) domains of multiple signaling mediators, including SRC kinases, the SHP2 phosphatase, the signal transducers and activators of transcription (STAT), phospholipase Cγ, phosphatidylinositol-3 kinase (PI3K) and adaptor proteins such as GRB2, SHC and NCK (Heldin et al., 1998). Much redundancy has been found among phosphorylated tyrosines and signaling molecules as these pathways regulate broadly overlapping sets of genes, which promote cell survival and proliferation (Fambrough et al., 1999).

SHP2, encoded by the *PTPN11* gene, is a ubiquitously expressed non-receptor protein tyrosine phosphatase, which contains two N-terminal SH2 domains and a C-terminal protein tyrosine phosphatase domain. Germline *PTPN11* mutations were reported in Noonan and LEOPARD syndromes, whereas somatic mutations occur in several neoplasms, such as juvenile myelomonocytic leukemia (Chan et al., 2008). The full activation of SHP2 requires the binding of the two SH2 domains to a doubly phosphorylated peptide (Heldin et al., 1998; Pluskey et al., 1995). In this respect, tyrosine residues 720 and 754 in PDGFRα have been described to bind SHP2 and could have a role in SHP2 activation (Bazenet et al., 1996; Rupp et al., 1994). A second possible activation mechanism implicates the association between the SH2 domains and one or two phosphorylated tyrosines located in the C-terminal tail of SHP2 (Lu et al., 2001; Neel et al., 2003). SHP2 regulates many signaling pathways such as JAK/STAT, PI3K/PKB and RAS/mitogen-activated protein kinases (MAPK). Besides its catalytic role, SHP2 also plays an adaptor role by recruiting signaling molecules such as STAT, GAB1/2 and GRB2, which is an essential component of the MAPK pathway (Kallin et al., 2004; Liu and Qu, 2011; Neel et al., 2003). SHP2 controls the activation of the RAS/MAPK pathway by PDGF at least in some cell types (Araki et al., 2003; Bennett et al., 1994; Ronnstrand et al., 1999; Zhang et al., 2004). Two reports also suggested that SHP2 is required for chemotaxis but not for proliferation induced by PDGF (Bazenet et al., 1996; Ronnstrand et al., 1999).

While signaling by wild-type PDGF receptors has been intensively studied for almost three decades, surprisingly little is known about the pathways required for cell transformation by oncogenic PDGF receptor mutants. In the present study, we identified tyrosine 720 as a critical site for SHP2 recruitment by FPα, activation of ERK and transformation of hematopoietic cells. SHP2 was similarly important for the D842V mutant but not for the wild-type receptor.

2. Material and methods

2.1. Antibodies, inhibitors and constructs

Anti-PDGFRα (951), anti-phosphotyrosine (PY99) and anti-STAT5 (C-17) antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz). Anti-phospho-PLCγ1 (Tyr783), anti-phospho-ERK1/2 (Thr202/Tyr204), anti-phospho-SHP2 (Tyr542), anti-SHP2 and anti-PLCγ antibodies were purchased from Cell Signaling. The anti-ERK2 (EET) rabbit polyclonal antiserum was previously described (Leevers and Marshall, 1992). Anti-phospho-STAT5 (Tyr694) antibodies were purchased from Cell Signaling and Signalway Antibody (SAB). A mouse monoclonal antibody against β-actin (clone AC-15) was purchased from Sigma. Imatinib was purchased from LC laboratories (Woburn, MA, USA). PDGF-BB, stem cell factor (SCF) and FLT3 ligand (FLT3L) were obtained from PeproTech.

The RNAi Consortium lentiviral mouse PTPN11/SHP2 shRNA was obtained from Thermo scientific. Three constructs (TRCN0000029875, TRCN0000029877 and TRCN0000029878) were used for this study. The negative pLKO.1-puro shScramble

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