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Visions & Reflections (Minireview)

How do Shp2 mutations that oppositely influence its biochemical activity result in syndromes with overlapping symptoms?

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Abstract. Activating and inactivating mutations of SHP-2 are responsible, respectively, for the Noonan (NS) and the LEOPARD (LS) syndromes. Clinically, these developmental disorders overlap greatly, resulting in the apparent paradox of similar diseases caused by mutations that oppositely influence SHP-2 phosphatase activity. While the mechanisms remain unclear, recent functional analysis of SHP-2, along with the identification of other genes involved in NS and in other related syndromes (neurofibromatosis-1, Costello and cardio-facio-cutaneous syndromes), strongly suggest that Ras/MAPK represents the major signaling pathway deregulated by SHP-2 mutants. We discuss the idea that, with the exception of LS mutations that have been shown to exert a dominant negative effect, all disease-causing mutations involved in Ras/MAPK-mediated signaling, including SHP-2, might lead to enhanced MAPK activation. This suggests that a narrow range of MAPK signaling is required for appropriate development. We also discuss the possibility that LS mutations may not simply exhibit dominant negative activity.

Keywords. SHP-2, *PTPN11,* Noonan syndrome, LEOPARD syndrome, Gab1, Ras.

Visions

SHP-2 is a ubiquitous protein tyrosine phosphatase (PTP) that plays a major role in the biological activities of numerous growth factors, cytokines, and hormones acting through receptors that mobilize protein tyrosine kinases. Most notably, it is one of the few PTPs that seems to favor promotion rather than downregulation of tyrosine kinase-dependent signaling, in contrast to its close relative SHP-1, an important component of immunomodulation. Supporting this view, disruption of *PTPN11*, the gene encoding SHP-2, results in early embryonic lethality in mice and generates profound developmental defects in lower organisms. This mimics the loss of essential receptor tyrosine kinases (RTKs) [see refs. 1, 2 for reviews]. More recently, experiments using tissue-specific *PTPN11* knockout mice have demonstrated critical functions for SHP-2 during liver regeneration, mammary gland development and central control of metabolism [3–5]. It is now well established that an essential component of the biological function of SHP-2 is mediated by its capacity to enhance the activation of the so-called Ras/mitogen-activated protein kinase (MAPK) signaling cascade. This pathway controls cell proliferation, differentiation and survival in response to various growth factors, hormones and cytokines [1, 2].

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Recently, germline missense mutations in the PTPN11 gene have been found in ~50% of patients affected by the Noonan syndrome (NS; MIM#163950) and also in >80% of cases with the LEOPARD (multiple Lentigines, Electrocardiographic conduction abnormalities, Ocular hypertelorism, Pulmonary stenosis, Abnormal genitalia, Retardation of growth, sensorineural Deafness) syndrome (LS; MIM#151100). These mutations are regarded as the causative agents responsible for these diseases [see ref. 6 for latest review]. NS is a relatively frequent (estimated ≈1/2000 births) autosomal dominant disorder that is primarily characterized by facial dysmorphia, heart defects, short stature and increased risk of leukemia. LS is a more rare autosomal dominant syndrome associating the same symptoms with more specific clinical manifestations: multiple lentigines (scattered lentil-shaped pigmentation) are typically present and 'café au lait' spot can be observed along with sensorineural deafness, which is specifically to LS. When considering congenital heart defects among patients with PTPN11 mutations, pulmonary stenosis is most common in NS, while hypertrophic cardiomyopathy prevails in LS patients [7]. In LS as in NS, there is substantial heterogeneity of the occurrence of the various symptoms between patients and a distinction between NS and LS may be difficult in certain cases, especially in childhood [8]. In addition to germline mutations, somatic missense PTPN11 mutations are also responsible for juvenile myelomonocytic leukemia (JMML) and for other forms of leukemia, albeit to a lesser extent, as well as for particular solid tumors [2, 9, 10].

To understand how *PTPN11* mutations instigate these diseases, it is necessary to investigate the function of SHP-2 at the molecular level. In brief, the structure of SHP-2 consists of two Src-homology 2 (SH2) domains and a single PTP domain (Fig. 1). SHP-2 displays a low basal catalytic activity due to close interactions between the N-terminal SH2 (N-SH2) and the PTP domains. These interactions function to keep the phosphatase in an autoinhibited, closed conformation. Its catalytic activation requires 'opening' the molecule which involves suspension of these interactions. This occurs when the SH2 domains of the molecule bind to specific phosphotyrosine motifs found on SHP-2 'activators,' components that represent upstream signaling partners. One of these activators is the multiadapter protein Gab1, which is rapidly tyrosine phosphorylated in response to stimulation of epidermal growth factor (EGF) or cytokine receptors. Once activated, SHP-2 plays a crucial role in enhancing the activation of Ras/MAPK, this latter pathway being initiated following recruitment of the Ras activation complex Grb2/Sos to activated receptors. This occurs simultaneously with the Gab1/SHP-2 pathway. Several mechanisms have been proposed to illustrate how SHP-2 facilitates Ras/MAPK activation, including dephosphorylation of binding sites on receptors for RasGAP (a Ras-inactivating protein), dephosphorylation and inactivation of the Grb2/Sos inhibitor Sprouty, and activation of Src family kinases resulting in sustained Ras activation on endomembranes [1, 11, 12]. However, as these mechanisms await further validation, this question remains a matter of debate. In our laboratory, we described a novel mechanism whereby SHP-2 regulates the docking of the Ras inhibitor RasGAP on Gab1 [13]. Recently, a study investigating the hepatic function of Gab1 and SHP-2 using liver-specific knockout mice offered, among other possibilities, significant support in favor of this model [4].

In NS, >40 mutations of SHP-2 have been reported to date, affecting ≈30 different residues located in or close to the N-SH2 and PTP interaction domains. These mutations are thought to disrupt the autoinhibitory closed SHP-2 conformation, resulting in an increased PTP activity of most NS-associated SHP-2 mutants observed *in vitro* [14, 15]. Thus, NS pathogenesis is due without doubt to activating, gain-of-function, mutations of SHP-2, which explains why NS is a dominant disorder. Similarly, *PTPN11* mutations involved in JMML are the most stimulatory for SHP-2 catalytic activity among all disease-causing *PTPN11* mutations [2, 14]. Since wild-type (WT) SHP-2 promotes MAPK activation, it is not surprising to observe that NS- or leukemia-associated SHP-2 mutations have a general tendency to sustain MAPK activation in transfected cells or in animal models [15–22]. However, additional studies are still necessary to explore the deregulation of cell signaling induced by these mutations, since the mechanisms involved remain obscure. While it seems plausible that the mutations involved in 'opening' the molecule, as described above, facilitate SHP-2 docking with its upstream scaffolding partners such as Gab1 or Gab2, further clarification is required to explain exactly how Ras/MAPK activation is increased [15, 16, 19].

Nevertheless, the notion that Ras/MAPK most likely represents the major signaling target deregulated by SHP-2 mutants has been reinforced by recent investigations that demonstrate that NS is also caused by activating mutations of *KRAS*, a gene encoding a protein of the Ras family, and of *SOS1,* whose protein product is an important activator of Ras [23–26]. Moreover, other syndromes displaying similar symptoms to NS, i.e. Costello and cardio-facio-cutaneous (CFC) syndromes, are due to mutations of H- or K-Ras, B-Raf and MEK1 and MEK2, four critical components of the Ras/MAPK pathway [27–31].

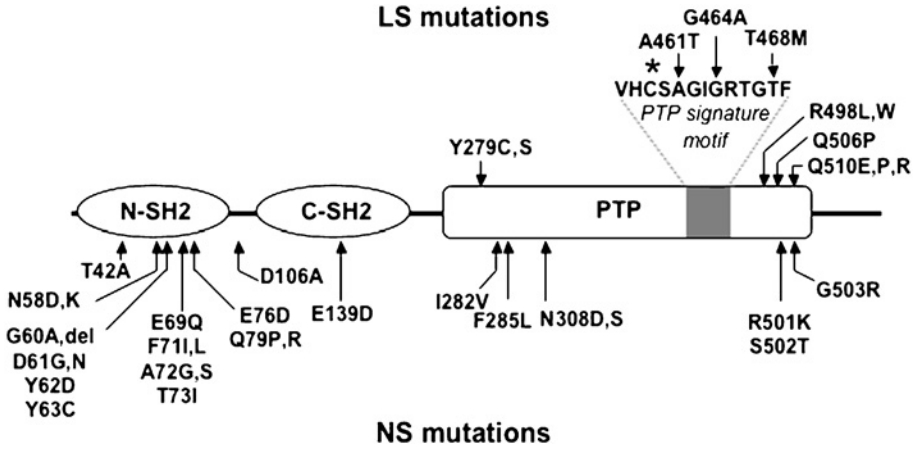

Figure 1. Schematic representation of SHP-2 showing the distribution of mutations identified in NS (below) and LS (above). The asterisk indicates Cys459 [6].

Similarly, neurofibromatosis type I, which also clinically overlaps with NS, results from an inactivating mutation of a RasGAP protein called neurofibromin [32–34]. However, this does not exclude the possibility that SHP-2 mutations could disrupt alternative signaling pathways. Indeed, the PI3K/Akt or STAT pathways have both been found to be overactivated by leukemia-associated mutations, whereas an NS-causing mutation has been reported to disrupt the Ca^{2+} oscillatory control of NFAT in cardiomyocytes. In both cases, the mechanism underpinning these molecular events is at best poorly understood, thus meriting further investigation [18, 19, 35].

In LS, the mutations described so far affect seven different amino acids that are all located in the PTP domain (Fig. 1). In-depth structural analysis revealed that these residues are concentrated within the catalytic core of the enzyme, three residing within the 'signature motif' that defines the PTP family. This is never the case for NS-causing mutations [36–38]. This motif [(I/V)HCxAGxGR(S/T)GT] includes critical residues involved in the catalytic activity, notably Cys459 that carries out the nucleophilic attack on the phosphotyrosine substrate. This suggested that, unlike NS-associated mutations that act to modify SHP-2's activation state, LS-associated mutations might alter SHP-2's catalytic properties. Biochemical evidence pertaining to this idea, recently performed by independent research groups, confirmed the hypothesis by showing that LS-causing mutations induce a dramatic decrease in SHP-2 catalytic activity *in vitro* [36–38]. At first, it seemed possible that LS pathogenesis could be due to a loss of function of the product of one *PTPN11* allele. However, genetic data argue against this view since it was shown in two independent 'knockout' mice models that loss of one allele is well tolerated and fails to produce any developmental defect reminiscent of LS symptoms [1]. In addition, none of the LS-causing mutations identified thus far consist of either nonsense, frameshift or splice mutations which would suppress SHP-2 expression from the mutated allele. This therefore does not favor the possibility that LS pathogenesis is caused by haploinsufficiency. Consequently, the suggestion that LS could result from dominant negative SHP-2 mutations emerged. In view of this concept, the group of B. Neel offered strong support by demonstrating that LS mutants exert a dominant negative effect on MAPK activation in transfected cells stimulated with various growth factors. This effect is in accordance with similar findings observed for the well-known 'phosphatase-dead' SHP-2 construct which is experimentally mutated on Cys459 [36].

Reflections

These findings have raised an apparent paradox where either activating or dominant negative mutations of the same signaling molecule result in very similar disorders. Classically, impaired or excessive activation of a component of a signaling pathway results in distinct diseases. For example, in the case of RTK, Kit or Ret gain-of-function mutations favor cell proliferation and thereby predispose or indeed cause cancer, whereas inhibitory mutations induce defects due to underdevelopment of tissues or organs [39]. Nevertheless, the situation where higher-than-normal activation or inhibition of a particular signaling pathway result in the same pathophysiological outcome is not unique. For example, it has been recently reported that both overexpression or suppression of the adapter protein insulin receptor substrate-1 can promote mammary tumor metastasis [40, 41].

In NS and related syndromes, the biochemical or structural data available to date suggest that all the disease-causing mutations of SHP-2, neurofibromin, Ras, B-Raf, MEK1/2 and SOS1 lead to enhanced

MAPK activation [6, 23–31, 34]. Some CFC-related mutations of B-Raf attenuate its catalytic activity, but one could suggest that these mutants most likely promote MAPK activation through increased heterodimerization-mediated allosteric activation of C-Raf. Although such a mechanism remains to be demonstrated in the case of CFC mutations, it has already been reported for other catalytically-impaired B-Raf mutants involved in tumor development [28, 42]. Thus, the case of LS-associated SHP-2 mutants repressing MAPK activation could represent an interesting exception to this rule. However, considering that the Ras/MAPK pathway can control antagonistic processes essential for tissue development, i.e. cell proliferation and differentiation, the idea that developmental diseases could be caused by distinct mutations resulting in opposite effects on the activation of this pathway is not altogether surprising. Indeed, studies have suggested that the appropriate level or duration of MAPK activity is required for eliciting a specific proliferative or differentiating response [43]. Therefore, altering positively or negatively the fine-tuned regulation of the MAPK pathway might indeed result in similar developmental defects. This hypothetical concept is even easier to imagine in the case of SHP-2 which controls the recruitment of various positive or negative regulators of Ras activation (e.g. PI3K, RasGAP, Src), thereby playing the role of a 'pacemaker' rather than a direct activator of this pathway [11, 13, 44].

To address the concept that MAPK signaling is required 'at the right place and at the right time', Kontaridis et al. brilliantly suggested a hypothetical explanation for the generation of cardiac valve stenosis frequently associated with LS and NS [36]. In summary, valve development requires first proliferation of specialized endothelial cells, followed by a morphogenesis of these cells that necessitates an arrest in proliferation. The activation of the Ras/MAPK pathway seems to promote these two stages. Consequently, one could envisage a scenario where NS mutations responsible for excessive MAPK activation during the first period, or LS mutations that block during the second phase, could result in excessive cell proliferation and therefore in stenosis. While attractive, this hypothesis has yet to be demonstrated.

It may also be worthwhile considering the possibility that LS-associated mutations might not merely exhibit a simple dominant negative effect. Kontaridis et al. [36] suggested that, in addition to suppressing the catalytic activity, LS mutants are particularly active as dominant negatives since some LS mutations also alter the interaction between the N-SH2 and PTP domain. Indeed, this would produce an inactive protein in an 'open' conformation, more prone for binding to upstream activators, and thereby a more efficient competitor for WT SHP-2 than a mutant Cys459 counterpart that resides in a 'closed' conformation unable to interact with the N-SH2 domain [36]. Nevertheless, one cannot exclude the possibility that LS mutations might result in differential dephosphorylation capabilities of SHP-2 depending on its substrate rather than simply suppressing its catalytic activity altogether. It is interesting to note that no mutation was reported to affect residue Cys459, a mutation that would completely suppress SHP-2 catalytic activity, whereas several LS mutations (A461T, G464A and T468M) have been identified within the vicinity of this residue, which represents the core of the catalytic site (Fig. 1). This allows one to speculate that, *in vivo*, a germline mutation of this residue is probably lethal, because it would generate a dominant negative mutant blocking all SHP-2 functions (except hypothetical docking functions [45]), probably constituting an intolerable environment for a developing organism. Consequently, LS mutants, despite having no activity on substrates regulating the MAPK pathway, might retain a significant catalytic activity oriented toward SHP-2 substrates that are not directly involved in MAPK activation. Such mutants would exert differential dominant negative effects on the distinct SHP-2-dependent signaling pathways, which could explain the substantial heterogeneity of symptoms associated with LS. Consistent with this idea, when assayed using a *bona fide* SHP-2 substrate, i.e. a binding site of Gab1 for PI3K, we revealed a significant activity of the T468M mutant [38]. This suggests that, although T468M cannot promote MAPK activation, as shown by Kontaridis et al. [36], it can still, in contrast with a mutant of Cys459, control PI3K activation in a similar fashion to WT SHP-2. In addition, other mechanisms can be proposed to explain the functional consequences of SHP-2 mutations, although these hypotheses are highly speculative due to the still very limited number of studies addressing this question. Nevertheless, based on the observation that LS mutations seem to display enhanced association with Gab1 [36], one may propose that mutations might have a gain-of-function effect on the docking function of Shp2, which may participate in Grb2 recruitment through direct binding [45]. Obviously, additional studies are necessary to test these various hypotheses.

In conclusion, the understanding of NS and LS pathogenesis will require solving the paradox that mutations oppositely influencing the biochemical activity of SHP-2 result in similar syndromes. To this aim, detailed examination of the molecular functions of SHP-2 mutants and of their consequences for

cellular responses is required. Reciprocally, the development of animal and cellular disease models is pertinent and will help provide further insights into molecular mechanisms underlying the role of SHP-2 in cell signaling.

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