

Myeloproliferative disorders: let the partner guide!

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Myeloproliferative disorders (MPD) are clonal proliferative diseases of the hematopoietic stem cell with expansion of one or several blood cell lineages. Many of the characterized molecular alterations found in MPD are mutations and translocations of tyrosine kinase genes. Translocations lead to fusion of tyrosine kinase genes to partner genes and to production of oncogenic fusion kinases. The number of fusion kinases involved in MPD increases slowly but regularly as new cases are discovered and characterized. Three new fusions have been reported recently.¹ They involve the PDGFRB kinase receptor and three different new partners. This brings the number of different protein partners fused to tyrosine kinases to 26, with the following distribution: 15 for PDGFRB, seven for FGFR1, four for PDGFRA, three for JAK2, two for ABL1 and one for FLT3 (Figure 1). While ETV6 and BCR may be partners of several tyrosine kinases, all others fuse only to a specific one. This increase in partner number and variety raises questions about their role. So far, the partner moiety has been simply viewed as a vector of oligomerization, necessary to activate the kinase but having no other function. Most partner proteins do indeed have dimerization domains, or at least dimerization motifs. Coiled-coil motifs, in particular, are frequent in partner structure but various other types of protein-protein interaction regions are encountered. Mediating dimerization is certainly a major role for the partner. However, to limit the partner to this neutral activity is perhaps a bit short-sighted.

The partner may not be so passive. We can propose other additional functions for the partner moiety of the fusion protein.

First, the partner drives. The partner gene promoter drives the expression of the fusion protein in the cell. The various partner genes are expressed in the cell-of-origin of the disease, which is thought in the case of MPD to be the hematopoietic stem cell. The partner genes are expressed ubiquitously or in stem cells. However, tyrosine kinases such as ABL1, JAK2 and FLT3 are also expressed in hematopoietic progenitors where they exert their normal functions. Rather than ectopic expression of a kinase, its level of expression and timing throughout cell cycle may be important.

Second, the partner guides. The partner may direct the fusion to a specific subcellular localization. We have shown that FOP addresses the FOP-FGFR1 fusion kinase to the centrosome.² CDK5RAP2, CEP1, NDE1, NIN, PCM1, PDE4DIP, and TRIP11, which are centrosomal proteins, could do the same with their respective fusions. The centrosome is an organelle that regulates

cell division, cell polarity, and cell migration. At this organelle, the constitutively-activated tyrosine kinase may unleash a cascade of unregulated phosphorylation and perturb centrosome function and cell cycle regulation.³ This targeting may be important because concentration of signaling substrates, phosphatidylinositol-3 kinase for example, can be increased in specific subcellular areas such as the centrosome.

In addition, the partner itself may be involved in normal centrosome function and in the regulation of cell division. For example, the partner may be part of protein complexes involved in centrosome duplication and/or G1 checkpoints.⁴ In stem cells, a centrosomal partner may play a role in asymmetric division. The activated kinase may directly phosphorylate the partner and thus modify its normal function. A dominant negative effect of the truncated partner is also possible. Thus, by directly targeting a kinase to a cellular command center and close to its inner circuits the partner is far from being just a passive dimerization trigger.

Not all partners are centrosomal proteins. However, what is true for the centrosome may be true for other cell compartments, which may also play important regulatory roles in cell division. Several partners are involved in intracellular trafficking (GIT2, HIP1, RABEP1, TRIP11), nuclear functions (ETV6, FIP1L1, TRIM24, ZNF198) or other regulatory processes (BCR, PRKG2, TPM3). They may target their respective fusion kinases to specific subcellular control areas.

Third, the partner may modulate. The structure of the partner may influence kinase activity. Inhibition of the juxta-membrane domain function, which has auto-inhibitory activity, is an important requirement for the activation of a tyrosine kinase receptor. Because of the localization of the translocation breakpoint in the *FGFR1* gene, the juxta-membrane region is disrupted in FGFR1 fusions. In leukemias, this mechanism may also be at work for FLT3, for which internal tandem duplications may interfere with correct juxta-membrane function. As emphasized in recent works on PDGFRB,^{1,5} the breakpoint in the *PDGFRB* gene occurs in such a way that the transmembrane and juxta-membrane regions of the receptor are usually preserved in the fusion protein. However, since no signal peptide is preserved in the fusion, the latter cannot be localized at the cell surface and must be trapped within the cell where it may be targeted to specific compartments, as discussed above. In the case of PDGFRB fusions, one role of the partner might be to inhibit the juxta-membrane domain function by folding or steric hindrance. The same could be true for PCM1 with regard to the JAK2 catalytically-

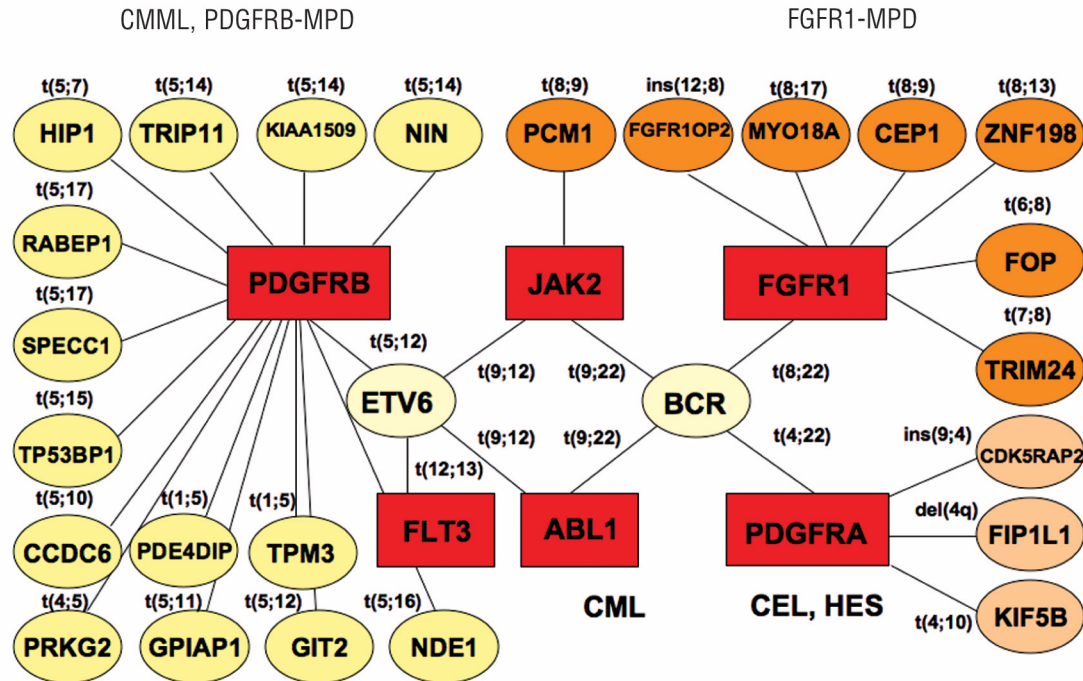


Figure 1. Schematic summary of fusions between genes involved in translocations associated with myeloproliferative disorders (MPD). Tyrosine kinases are represented by red squares, and protein partners by ovals. The *FGFR1* gene is fused to seven partners, including *BCR*. Reciprocally, *BCR* is the partner of four tyrosine kinase genes. Fifteen different rearrangements of PDGF receptor B, and four of PDGF receptor A, have been characterized so far. *FLT3* receptor and non receptor-type tyrosine kinases *ABL1* and *JAK2* are also involved in MPD. CML: chronic myeloid leukemia; CMML: chronic myelomonocytic leukemia; CEL: chronic eosinophilic leukemia; HES: idiopathic hypereosinophilic syndrome.

inactive domain in the PCM1-JAK2 fusion.⁶⁷ To date, this remains speculative but could be tested. In the case of the FIP1L1-PDGFRB fusion, the juxta-membrane domain is disrupted. The role of FIP1L1 has been shown to be dispensable.⁸ In this case, the *FIP1L1* locus seems to have a limited participation in the fusion oncogenicity. It may provide a specific eosinophilic gene promoter and the first amino acid residues. FIP1L1-PDGFRB is not created by a deletion but by an 800-kb deletion at 4q12; only a few genes are contained in this genomic segment. *FIP1L1* could simply be the first gene upstream of *PDGFRB* with both the correct orientation and the appropriate promoter. It is not understood whether the other known PDGFRB partners (*BCR*, *CDK5RAP2* and *KIF5B*) may be similarly dispensable but they all have dimerization potential. The juxta-membrane domain is disrupted in the *CDK5RAP2*-*PDGFRB* fusion while it seems to be, in great part, preserved in the *KIF5B*-*PDGFRB* protein.^{9,10} It is probable that a partner is required when the juxta-membrane domain is present whereas it may be dispensable if the domain is disrupted. However, it is surprising that *CDK5RAP2* is a coiled-coil centrosomal protein strongly related to myomegalin, the latter being found fused to *PDGFRB* with pre-

served transmembrane and juxta-membrane domains. For some unknown reasons, both an active oligomerization function of *CDK5RAP2* and juxta-membrane disruption may be required in this case.

In nature, activation is often achieved by relieving an inhibitory mechanism. Disruption of the juxta-membrane inhibitory domain of tyrosine kinase receptors, mutation of the *JAK2* pseudo-kinase inhibitory domain, and disruption of the microRNA repression of the *HMG2* locus in some MPD with translocations that do not involve tyrosine kinases¹¹ show that this is also frequently the case in pathological processes.

In conclusion, the role of the fusion partner, which was regarded as merely passive, might actually be more diverse and more complex. It might even be considered as a potential therapeutic target. MPD fusion kinases may be efficient missile-like molecules endowed not only with explosive capacity but also optimal guidance and control systems. Several questions remain unanswered. What particular feature do *ETV6* and *BCR* share to be so promiscuous? Reciprocally, what features make the other partners specific to one kinase? What is the impact of different partners and targeting on signaling and on disease features and outcome?

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