ETV6 and *PDGFRB*: a license to fuse

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In 1994 the *ETV6-PDGFRB* (*TEL-PDGFRB*) fusion gene was identified by Todd Golub and Gary Gilliland in patients with chronic myelomonocytic leukemia (CMML) and t(5;12)(q33;p13).[1] The implications of the molecular characterization of this translocation go far beyond the identification of the *ETV6-PDGFRB* oncogene. The identification of the *ETV6* gene on chromosome 12p13 has led to the discovery of a large number of important fusion genes such as the *ETV6-RUNX1* fusion that is present in up to 25% of childhood B-cell acute lymphocytic leukemia (B-ALL).[2] In addition, a wide variety of other fusion partners of *PDGFRB* have also been identified. In this issue of the journal Walz and colleagues report on the identification of yet three more novel *PDGFRB* fusion genes in chronic myeloproliferative disorders.[3] The identification of a *PDGFRB* fusion gene in the leukemic cells of a patient is of major importance since these patients can be treated with the small molecule kinase inhibitor imatinib (STI-571, Gleevec®, Glivec®).[4] All patients with myeloproliferative disorders characterized by expression of a *PDGFRB* fusion gene respond well to imatinib therapy, with rapid and complete hematologic and molecular remissions observed in these patients. Thus, the identification of a translocation involving the *PDGFRB* gene in patients with myeloproliferative disorders is a clear marker that predicts response to imatinib therapy.

Translocations involving the ETV6 gene

Since the initial identification of *ETV6* as a gene rearranged by the t(5;12)(q33;p13), a large number of variant translocations that also involve *ETV6* have been reported (Table 1). Some of these translocations result in fusions of *ETV6* to other tyrosine kinase genes such as *JAK2*,[5] *ABL1*,[6] *NTRK3*,[7] and *FLT3*.[8] The common theme here is the fusion of the homodimerization domain of ETV6 to a tyrosine kinase domain, resulting in the generation of a constitutively active kinase. These activated kinases phosphorylate themselves as well as a variety of downstream signaling proteins, which leads to the stimulation of proliferation and survival pathways. Most of these translocations are rare, but remain interesting to detect, as these patients are likely to respond to treatment with small molecule inhibitors interfering with the activity of these oncogenic tyrosine kinase genes. The fusion of *ETV6* to tyrosine kinase genes is, however, not the only mechanism by which ETV6 contributes to leukemogenesis. Different parts of ETV6 can also be fused to transcription factors, such as RUNX1 (AML1),[9] EVI1,[10] and MN1.[11] The most important translocation in this subgroup is the cryptic t(12;21)(p13;q22) that is found in 25% of childhood B-ALL cases, and results in the fusion of ETV6 to RUNX1.[2] The t(12;21) is frequently associated with deletion or inactivation of the other *ETV6* allele, and is believed to confer a favorable prognosis. ETV6-RUNX1 (TEL-AML1) acts as an aberrant transcription factor that is believed to be involved in the expansion of hematopoietic progenitors,[12] but the exact way *ETV6-RUNX1* alters the differentiation and self-renewal pathways remains unclear.

Most of the chromosomal translocations involving *ETV6* result in the generation of some kind of fusion gene, but it has not always been clear whether these fusions are the relevant oncogenic events or not.[13,14] In fact, other consequences of the chromosomal translocations may also be important. In this context, it was proposed that translocations involving *ETV6* could also result in a deregulated expression of nearby oncogenes, as in the case of the translocations t(4;12) and t(5;12), resulting in expression of *GSH2* and *IL3* respectively.[15] Finally, *ETV6* was also found to be mutated in acute myeloid leukemia,[16] revealing yet another mechanism by which *ETV6* can be implicated in the pathogenesis of hematologic malignancies. For a complete description of all aberrations involving *ETV6* we refer the reader to a detailed review written by Stefan Bohlander.[17]

Translocations involving PDGFRB

When the *ETV6-PDGFRB* fusion gene was identified in 1994, it was not immediately clear that this would have tremendous therapeutic implications. However, during the development of imatinib, it was observed that this small molecule inhibitor of *BCR-ABL* also inhibited the activity of PDGFRα and PDGFRβ. Perhaps the most important consequence of this observation was the clinical application of imatinib for the treatment of chronic eosinophilic leukemia with the *FIP1L1-PDGFRA* fusion, and CMML/myeloproliferative diseases with *ETV6-PDGFRB* or variant *PDGFRB* fusions.[4,18]

To date, 11 additional fusion partners of *PDGFRB* have been identified (Table 2), and most patients with *ETV6-PDGFRB* or variant *PDGFRB* fusion genes were reported to respond extremely well to imatinib therapy. In this issue of the journal, Walz and co-workers report the identification of three more novel *PDGFRB* fusion genes.[3] Two of the three patients in their study had marked eosinophilia, as is frequently observed in patients with myeloproliferative diseases and PDGFRα or PDGFRβ fusions. All three patients also showed good

Table 1. Translocations involving the *ETV6* gene.

Translocation	*Molecular consequence*	*Type of oncogene*	*Disease*
t(5;12)(q35;p13)	*ETV6-PDGFRB* fusion	Tyrosine kinase	CMML
t(9;12)(p24;p13)	*ETV6-JAK2* fusion	Tyrosine kinase	ALL, MPD
t(12;15)(p13;q26)	*ETV6-NTRK3* fusion	Tyrosine kinase	AML
T(12;13)(p13;q12)	*ETV6-FLT3* fusion	Tyrosine kinase	MPD
t(12;21)(p13;q22)	*ETV6-RUNX1* fusion	Transcription factor	B-ALL
t(12;22)(p13;q11)	*MN1-ETV6* fusion	Transcription factor	AML, MDS
t(3;12)(q26;p13)	*ETV6-MDS1/EVI1* fusion	Transcription factor	MPD
t(4;12)(q12;p13)	*CHIC2-ETV6* fusion, deregulated *GSH2* expression	Transcription factor (*GSH2*)	AML
t(5;12)(q31;p13)	*ETV6-ACS2* fusion, deregulated *IL3* expression	Growth factor (*IL3*)	AML, MPD
t(12;13)(p13;q12)	*ETV6-CDX2* fusion, deregulated *CDX2* expression	Transcription factor (*CDX2*)	AML
t(10;12)(q24;p13)	*ETV6-GOT1* fusion	Unknown	MDS
t(1;12)(p36;p13)	*ETV6-MDS2* fusion	Unknown	MDS

Note: not all translocations involving ETV6 are shown in this table. For a complete overview we refer the reader to reference 17.

Table 2. Translocations involving the *PDGFRB* gene in myeloproliferative disorders.

Fusion partner of PDGFRB	*Chromosomal location*
TPM3	1q21
PDE4DIP	1q12
*PRKG2**	4q21
HIP1	7q11
CCDC6 (H4)	10q21
*GPIAP1**	11p13
ETV6 (TEL)	12p13
*GIT2**	12q24
NIN	14q24
TRIP11 (CEV14)	14q32
KIAA1509	14q32
TP53BP1	15q15
NDE1	16p13
SPECC1 (HCMOGT-1)	17p11
RABEP1 (RABAPTIN-5)	17p13

Note: The fusion partners identified in the study by Walz et al. in this issue of the journal are indicated with an asterisk.

responses to imatinib therapy, and achieved complete hematologic and molecular remissions.[3]

In most of the chromosomal aberrations affecting *PDGFRB*, the breakpoint in the *PDGFRB* gene is located in the intron upstream of the exon encoding the transmembrane region of PDGFRβ. In these fusions, the fusion partner of *PDGFRB* is believed to provide a homodimerization domain, which is required to dimerize the kinase domain of PDGFRβ, subsequently leading to its catalytic activation. Although this has not always been experimentally verified, most of the fusion partners of PDGFRβ do indeed contain domains that are known to possess potential to form homodimers. Interestingly, the breakpoint in *PDGFRB* in the *PRKG2-PDGFRB* fusion reported in this issue of Haematologica/The Hematology Journal has an unusual breakpoint that falls within the exon of *PDGFRB* that encodes the juxtamembrane region (WW-motif).[3] This breakpoint resembles the breakpoints in *PDGFRA* in chronic eosinophilic leukemia cases with the *FIP1L1-PDGFRA* fusion.[18] In the case of the *FIP1L1-PDGFRA* fusion, the breakpoints in *PDGFRA* disrupt the exon encoding the juxtamembrane region, and as a consequence, the juxtamembrane region of *PDGFRα* is only partially present in the *FIP1L1-PDGFRα* fusion protein. We have recently shown that the interruption of the juxtamembrane region is strictly required to activate the kinase activity of FIP1L1-PDGFRα, whereas the presence of the full juxtamembrane region inhibits kinase activity and transforming potential of FIP1L1-PDGFRα. The predicted PRKG2-PDGFRβ fusion protein described here by Walz *et al.*[3] also lacks part of the juxtamembrane region of PDGFRβ, which could indicate that in this particular fusion, the interruption of the juxtamembrane region is required for kinase activity of PRKG2-PDGFRβ.

Future perspectives

Whatever the mechanism of activation, the type of fusion, or the fusion partner, the most important message from the work by Walz and co-workers is that patients with a myeloproliferative disease and a chromosomal translocation involving the *PDGFRB* gene respond well to imatinib therapy.[3] As it is clear that there are a large number of translocations involving *PDGFRB*, with a different partner gene being involved each time, it may not be strictly required for diagnostic purpose to identify the fusion partner. It may be sufficient in these patients to prove by fluorescence *in situ* hybridization that *PDGFRB* is rearranged. It should be noted, however, that the identification of the exact fusion gene

remains extremely valid for both research purposes and to enable molecular follow-up of the response of a patient to therapy. *PDGFRB* may have a license to fuse, imatinib still has its license to kill the leukemic cells with *PDGFRB* fusions. We will need to follow-up patients to determine whether imatinib as single agent therapy is sufficient to achieve long-term responses in patients with myeloproliferative diseases and translocations involving *PDGFRB*. If not, a number of other small molecule inhibitors that inhibit ETV6-PDGFRβ kinase activity at low nanomolar concentrations have already been identified.[20,21]

References

1. Golub TR, Barker GF, Lovett M, Gilliland DG. Fusion of PDGF receptor β to a novel ets-like gene, tel, in chronic myelomonocytic leukemia with t(5;12) chromosomal translocation. Cell 1994;77:307-16.
2. Romana SP, Poirel H, Leconiat M, Flexor MA, Mauchauffe M, Jonveaux P, et al. High frequency of t(12;21) in childhood B-lineage acute lymphoblastic leukemia. Blood 1995;86:4263-9.
3. Walz C, Metzegeroth G, Haferlach C, Schmitt-Graeff A, Fabarius A, Hagen V, et al. Characterization of three new imatinib-responsive fusion genes in chronic myeloproliferative disorders generated by disruption of the platelet-derived growth factor receptor β gene. Haematologica 2007;92:163-9.
4. Apperley JF, Gardembas M, Melo JV, Russell-Jones R, Bain BJ, Baxter EJ, et al. Response to imatinib mesylate in patients with chronic myeloproliferative diseases with rearrangements of the platelet-derived growth factor receptor β. N Engl J Med 2002;347:481-7.
5. Peeters P, Raynaud SD, Cools J, Wlodarska I, Grosgeorge J, Philip P, et al. Fusion of TEL, the ETS-variant gene 6 (ETV6), to the receptor-associated kinase JAK2 as a result of t(9;12) in a lymphoid and t(9;15;12) in a myeloid leukemia. Blood 1997;90:2535-40.
6. Papadopoulos P, Ridge SA, Boucher CA, Stocking C, Wiedemann LM. The novel activation of ABL by fusion to an ets-related gene, TEL. Cancer Res 1995;55:34-8.
7. Eguchi M, Eguchi-Ishimae M, Tojo A, Morishita K, Suzuki K, Sato Y, et al. Fusion of ETV6 to neurotrophin-3 receptor TRKC in acute myeloid leukemia with t(12;15)(p13;q25). Blood 1999;93:1355-63.
8. Vu HA, Xinh PT, Masuda M, Motoji T, Toyoda A, Sakaki Y, et al. FLT3 is fused to ETV6 in a myeloproliferative disorder with hypereosinophilia and a t(12;13)(p13;q12) translocation. Leukemia 2006;20:1414-21.
9. Golub TR, Barker GF, Bohlander SK, Hiebert SW, Ward DC, Bray-Ward P, et al. Fusion of the TEL gene on 12p13 to the AML1 gene on 21q22 in acute lymphoblastic leukemia. Proc Natl Acad Sci USA 1995; 92:4917-21.
10. Peeters P, Wlodarska I, Baens M, Criel A, Selleslag D, Hagemeijer A, Van den Berghe H, et al. Fusion of ETV6 to MDS1/EVI1 as a result of t(3;12)(q26;p13) in myeloproliferative disorders. Cancer Res 1997;57:564-9.
11. Buijs A, Van Rompaey L, Molijn AC, Davis JN, Vertegaal AC, Potter MD, et al. The MN1-TEL fusion protein, encoded by the translocation (12;22)(p13;q11) in myeloid leukemia, is a transcription factor with transforming activity. Mol Cell Biol 2000;20:9281-93.
12. Sabaawy HE, Azuma M, Embree LJ, Tsai HJ, Starost MF, Hickstein DD. TEL-AML1 transgenic zebrafish model of precursor B cell acute lymphoblastic leukemia. Proc Natl Acad Sci USA 2006;103:15166-71.
13. Janssen H, Wlodarska I, Mecucci C, Hagemeijer A, Vandenberghe P, Marynen P, et al. Fusion of ETV6 to GOT1 in a case with myelodysplastic syndrome and t(10;12)(q24;p13). Haematologica 2006;91:949-51.
14. Odero MD, Vizmanos JL, Roman JP, Lahortiga I, Panizo C, Calasanz MJ,et al. A novel gene, MDS2, is fused to ETV6/TEL in a t(1;12)(p36.1;p13) in a patient with myelodysplastic syndrome. Genes Chromosomes Cancer 2002; 35:11-9.
15. Cools J, Mentens N, Odero MD, Peeters P, Wlodarska I, Delforge M, et al. Evidence for position effects as a variant ETV6-mediated leukemogenic mechanism in myeloid leukemias with a t(4;12)(q11-q12;p13) or t(5;12)(q31;p13). Blood 2002;99:1776-84.
16. Barjesteh van Waalwijk van Doorn-Khosrovani S, Spensberger D, de Knegt Y, de Knegt Y, Tang M, Lowenberg B, et al. Somatic heterozygous mutations in ETV6 (TEL) and frequent absence of ETV6 protein in acute myeloid leukemia. Oncogene 2005;24:4129-37.
17. Bohlander SK. ETV6: a versatile player in leukemogenesis. Semin Cancer Biol 2005;15:162-74.
18. Cools J. The hypereosinophilic syndrome: idiopathic or not, that is the question. Haematologica 2005;90:582-4.
19. Stover EH, Chen J, Folens C, Lee BH, Mentens N, Marynen P, et al. Activation of FIP1L1-PDGFRα requires disruption of the juxtamembrane domain of PDGFRα and is FIP1L1-independent. Proc Natl Acad Sci USA 2006;103:8078-83.
20. Stover EH, Chen J, Lee BH, Cools J, McDowell E, Adelsperger J, et al. The small molecule tyrosine kinase inhibitor AMN107 inhibits TEL-PDGFRβ and FIP1L1-PDGFRα in vitro and in vivo. Blood 2005; 106:3206-13.
21. Lierman E, Lahortiga I, Van Miegroet E, Mentens N, Marynen P, Cools J. The ability of sorafenib to inhibit oncogenic PDGFRβ and FLT3 mutants and overcome resistance to other small molecule inhibitors. Haematologica 2007;92: 27-34.