The sum is greater than the *FGFR1* partner

Cancer-associated chromosomal translocations create chimeric oncoproteins that contribute to aberrant growth by dominant or dominant negative mechanisms. Interestingly, genes such as *MLL*, *RARA*, and *EWS* are fused to multiple partners. This molecular promiscuity can provide important functional information, as specific translocations may be associated with discrete clinical and molecular features. In this issue of *Cancer Cell*, Roumiantsev et al. (2004) use a murine retroviral transduction/transplantation system to analyze two *FGFR1* fusions found in hematologic malignancies. Their results show that these chromosomal rearrangements play a central role in pathogenesis, underscore the role of partner genes in modulating disease phenotypes, and uncover potential therapeutic targets.

As the final episode of *Sex and the City* drew ever closer, the legion of viewers who had followed the tempestuous relationships of Carrie Bradshaw focused on a single question. Would she end up with Mr. Big, Petrovsky, a dark horse, or none of the clever and handsome men who populated her universe? Carrie and the fans knew that her choice of partner was what ultimately mattered. In this issue of *Cancer Cell*, Roumiantsev and coworkers find that this principle also explains major biologic and phenotype features of leukemia-associated *FGFR1* fusions. The *FGFR1* gene, which encodes the fibroblast growth receptor-1 tyrosine kinase, is located on chromosome band 8p11. Translocations that join *FGFR1* with a number of binding partners generate fusion proteins with aberrant tyrosine kinase activity in patients with "8p11 myeloproliferative syndrome" (EMS). Affected individuals develop a myeloproliferative disorder (MPD), and are strongly predisposed to lymphoblastic T cell lymphoma bearing the same 8p11 translocation, implying a common stem cell origin. In addition to this association with EMS, a few patients have been described in whom *FGFR1* is fused to the *BCR* gene. *BCR* is famous for another role in leukemia biology—it causes chronic myeloid leukemia (CML) with another partner gene, the *ABL* tyrosine kinase. Interestingly, patients with *BCR-FGFR1* fusions develop a CML-like MPD, but lymphomas have not been reported.

This new work provides a satisfying molecular explanation for these clinical differences, and identifies key amino acids within BCR-FGFR1 and the EMS-associated ZNF198-FGFR1 protein that contribute to specific disease phenotypes. Roumiantsev et al. utilized retroviral gene transfer into mouse bone marrow followed by adoptive transfer into irradiated recipients to investigate how the *ZNF198-FGFR1* and *BCR-FGFR1* fusion genes perturb hematopoietic growth control. Mice transplanted with cells engineered to express a chimeric ZNF198-FGFR1 protein consistently developed both overproliferation of myeloid cells and T cell lymphomas of the gastrointestinal tract that closely model human EMS disease. These data and a recent report in which a FOP-FGFR1 protein was expressed in murine bone marrow (Guasch et al., 2004) firmly establish a central pathogenic role of EMS-associated fusions.

Importantly, Southern blot analysis revealed that *ZNF198-FGFR1*-infected marrow generated clonal or oligoclonal tumors. This indicates intense in vivo selection, as the fatal malignancies that arise in recipient animals are derived from one (or a few) of many thousands of infected cells. The most likely explanation is that *ZNF198-FGFR1* requires one or more additional mutations to induce leukemia, which is also a feature of transgenic and "knockin" strains of mice that express many other leukemia-associated fusions such as PML-RARA and AML1-ETO (Grisolano et al., 1997; Higuchi et al., 2002). An intriguing possibility is that retroviral insertions contribute directly to leukemogenesis. This idea has been used with great success to perform forward genetic screens in mice to uncover genes that are mutated in human hematologic malignancies (Li et al., 1999). A recent analysis of two children with X-linked severe combined immunodeficiency who developed T cell leukemia after receiving autologous hematopoieitc cells that had been transduced ex vivo with a retrovirus containing the *IL2RG* gene found LMO1 insertions in both cases (Hacein-Bey-Abina et al., 2003). Based on an analysis of retrovirally induced murine T cell leukemias, Dave and associates (Dave et al., 2004) proposed that high level *IL2RG* expression from the retroviral long terminal repeat rendered the vector oncogenic, and that insertion into *Lmo1* provided a cooperating mutation. By analogy, cloning *ZNF198-FGFR1* insertions from murine leukemias and lymphomas may identify genes and pathways that contribute to EMS.

Compared with transgenic or gene-targeting technologies, both of which require extensive screening and breeding of mice, retroviral vector strategies sacrifice precise oncogene regulation for experimental efficiency. The tractable nature of marrow transduction/transplantation systems permits investigators to generate allele series in order to analyze functional domains that are required for transformation in vivo. Roumiantsev et al. employed this strategy to show that FGFR1 kinase activity is essential for leukemogenesis, and that a phospholipase C-γ1 (PLCγ1) binding site located at Tyr-766 contributes to the EMS phenotype. Interestingly, mice transplanted with bone marrow cells engineered to express the latter mutation show enhanced survival and less myeloproliferation, and succumb from lymphomas with a more mature immunophenotype. Whereas primary lymphoma cells from mice transplanted with *ZNF198-FGFR1*-expresing cells showed elevated levels of activated (phosphorylated) PLCγ1, this was not true of lymphomas from recipients that expressed the Tyr-766 mutation. Together, these studies identify FGFR1 and PLCγ1 as rational biochemical targets for small molecule therapeutics in EMS.

The authors next found that mice transplanted with bone marrow engineered to express the *BCR-FGFR1* fusion, which is associated with a CML-like disease in patients, succumbed from an aggressive polyclonal MPD that closely mimics the disease induced by *BCR-ABL*. Thus, the *FGFR1* partner gene has a major effect on disease phenotype. Over a decade ago, Tyr-177 of BCR was identified as important for BCR-ABL-induced transformation of cultured cells by regulating phosphorylation of Grb-2 and Ras activation (Pendergast et al., 1993). Recent work

in hematopoietic cells showed that the Gab-2 adaptor is recruited to Tyr-177, and is essential for efficiently phosphorylating Grb-2, for activating signaling cascades downstream of Ras-GTP, and for induction of MPD by BCR-ABL (Sattler et al., 2002). Similarly, mutating Tyr-177 in the *BCR-FGFR1* fusion markedly attenuated the MPD, and transplanted mice died from a clonal EMS-like disease with modest leukocytosis and T cell lymphoma. Interestingly, somatic activation of a latent mutant *Kras* allele also induces a fulminant MPD (Braun et al., 2004). The aggressive MPDs that result from expressing *BCR-ABL*, *BCR-FGFR1*, or *Kras* in primary mouse bone marrow cells argue that high level, constitutive hyperactivation of Ras signaling is critical for this phenotype. This idea does not exclude a role for hyperactive Ras in the EMS-like disease; indeed, Guasch et al. (2004) have reported that the FOP-FGFR1 fusion activates the ERK and phosphatidylinositol-3-kinase cascade in cultured cells. Instead, the specific disease phenotype may be dictated, in part, by the degree to which Ras and its effectors are deregulated.

Beyond providing mechanistic insights regarding the role of *FGFR1* fusions in hematologic malignancies, Roumiantsev et al. have generated reagents that might be harnessed to develop treatments for EMS. The Ba/F3 system has been used to test inhibitors of BCR-ABL, FLT3, and FIP1L1-PDGFRA and to characterize mutations that confer resistance to targeted therapeutics (Cools et al., 2003; Levis et al., 2002; Shah et al., 2002). Similarly, Ba/F3 cells engineered to express various EMS-associated FGFR1 chimeric proteins will be useful for screen for inhibitors of FGFR1 or PLCγ1. Promising compounds could be further tested in mice transplanted with ZNF198-FGFR1-expressing bone marrow cells to assess therapeutic index in vivo.

How might candidate small molecule inhibitors be identified for testing? Because EMS is an uncommon cancer, no commercial efforts will be launched to discover FGFR1 or PLCγ1 inhibitors for this indication. However, pharmaceutical companies are performing large-scale screens to identify kinase inhibitors. Small molecules that block the FGFR1 kinase might emerge from these efforts, which could be developed further in partnership with academic investigators and/or the National Cancer Institute. Moreover, a molecule that is marketed for another indication might also inhibit FGFR1 due to the extensive structural similarity between the active sites of many tyrosine kinases. For example, imatinib mesylate was developed as a BCR-ABL inhibitor but also blocks pathogenic kinases in gastrointestinal stromal tumors and idiopathic hypereosinophilic syndrome (Cools et al., 2003; Joensuu et al., 2001). Indeed, imatinib mesylate is now a first line treatment for these rare malignancies. The tractable experimental systems developed by Roumiantsev et al. now set the stage for translating mechanistic insights into improved therapies for individuals with EMS.

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Selected reading

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