FGFR1OP2-FGFR1 induced myeloid leukemia and T-cell lymphoma in a mouse model

Myeloproliferative neoplasms (MPN) associated with chromosome translocations involving the fibroblast growth factor receptor 1 (FGFR1) gene is a distinct disease entity in the current WHO classification[1,2] (hereafter MPN-tFGFR1). These patients have a poor outcome with a 5-year survival rate of < 20%. MPN-tFGFR1 patients typically present with bilineage disease (myeloid and lymphoid) and frequently (~80%) progress to AML.[3] There have been at least 15 different chromosome rearrangements described, which have been shown to or are predicted to lead to ligand-independent, constitutive activation of the FGFR1 kinase.[3] Even though activation of FGFR1 is seen in all cases, there is variability in the clinical presentation of the disease, depending on the specific chromosome translocations that are present.[3] Because this is a rare disease, developing better therapies requires models that show the same phenotypic and genetic characteristics. One aggressive variant of this disease shows a t(8;12)(p12;p11) chromosome translocation that generates the chimeric FGFR1OP2-FGFR1 protein.[4] We have used a mouse bone marrow transduction and transplantation approach to develop a model for FGFR1OP2-FGFR1 disease where, consistent with the human disease, the mice concurrently developed rapid onset CD4$^+$ T-cell lymphoblastic lymphoma and acute myeloid leukemia (cKit$^+$Gr1$^+$Mac1$^+$CD19$^+$). Molecular analysis demonstrates that the T-cell lymphomas are associated with activating mutations of Notch1, and the progression of AML is associated with dysregulation of genes involved in multiple signaling pathways related to myeloid cell development and differentiation, including KIT, CSF1R, SPI1, IRF8 and KLF4. This highly representative mouse model will allow evaluation of potential therapies against this almost invariably lethal disease.

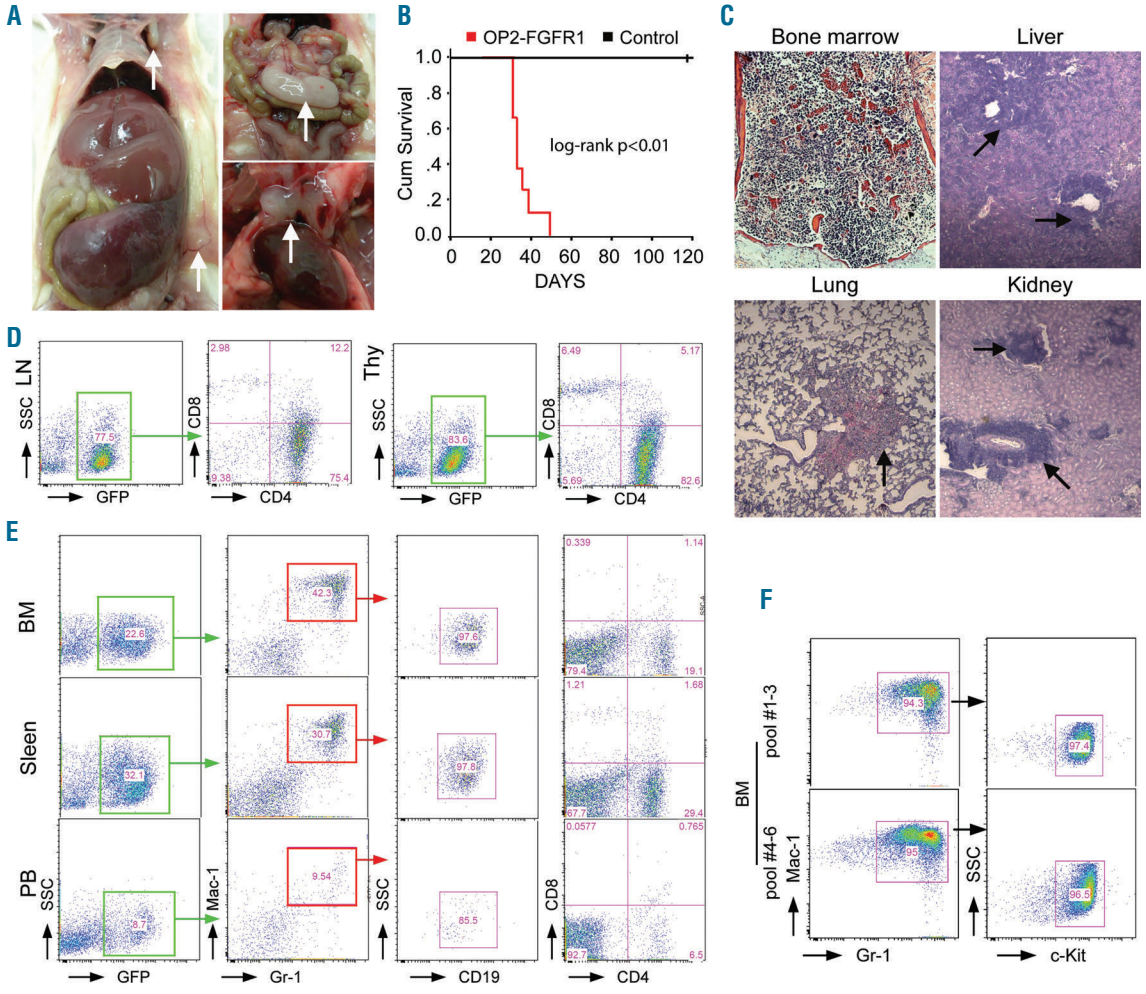

Figure 1. FGFR1OP2-FGFR1 induces myeloid leukemia and T-cell lymphomas in a mouse model. (A) FGFR1OP2-FGFR1 induced myeloid leukemia and T-cell lymphomas, as evidenced by hepatosplenomegaly (left) and enlarged lymph nodes (arrows). (B) Kaplan-Meier survival analysis of mice with FGFR1OP2-FGFR1 T-lymphomas. The difference in survival between primary (n=8) recipients and control MIEG3 recipients (n=5) is significantly different (P<0.01, log-rank test). (C) Histologic analysis shows a hypercellular bone marrow and frequent infiltration of lymphocytes into various organs of FGFR1OP2-FGFR1 recipients. (D) Flow cytometric analysis of GFP$^+$ cells from thymus (Thy) and lymph nodes (LN) show a predominantly CD4$^+$ phenotype. (E) Myeloid (Mac1$^+$Gr1$^+$) cells from the bone marrow (BM), spleen and peripheral blood (PB) from a representative mouse show a CD19$^+$ phenotype and a subset shows a CD4$^+$ phenotype. F) Analyses of pooled myeloid leukemia cells (GR1$^+$Mac1$^+$) show high expression levels of c-Kit.

To investigate the transforming potential of FGFR1OP2-FGFR1, we isolated the fusion gene from the KG1 cell line and, as described previously,[5] cloned it into the murine stem cell virus vector (MIEG3) which co-expresses enhanced green fluorescent protein (EGFP). Expression of this chimeric gene in IL3 dependent BaF3 pro-B cells transformed then to IL3 independence (*data not shown*), demonstrating the oncogenic potential of the FGFR1OP2-FGFR1 gene. To determine whether FGFR1OP2-FGFR1 induces leukemia/lymphoma *in vivo*, BM cells from 10 normal male BALB/c donors were transduced independently with MIEG3-FGFR1OP2-FGFR1 and cultured in vitro with stem cell growth factors.[6] Five days post-infection, each of the transduced BM samples was transplanted into lethally irradiated (850 cGy) female recipients. Although two mice died from the irradiation after 6 days, the remaining mice (n=8) showed > 5% GFP$^+$ cells in the peripheral blood (PB) after two weeks, indicating successful engraftment (*data not shown*). Four to five weeks post-transplantation, the primary recipients developed overt leukemia and lymphoma, as evidenced by enlarged lymph nodes and/or thymuses and hepatosplenomagaly (Figure 1A) with >20% GFP$^+$ cells in the spleen and liver (Figure 1). The median survival time in this cohort of mice was only one month post-transplantation (Figure 1B), where all mice died from multiple organ failure due to the infiltration of leukemic cells into the lung, liver and kidney (Figure 1C). Flow cytometric analysis was performed on cells from PB, BM, spleen and lymph nodes (LN) which identified both myeloid leukemia and T-lymphomas in all animals. Eosinophilia, sometimes associated with this syndrome was not noted.

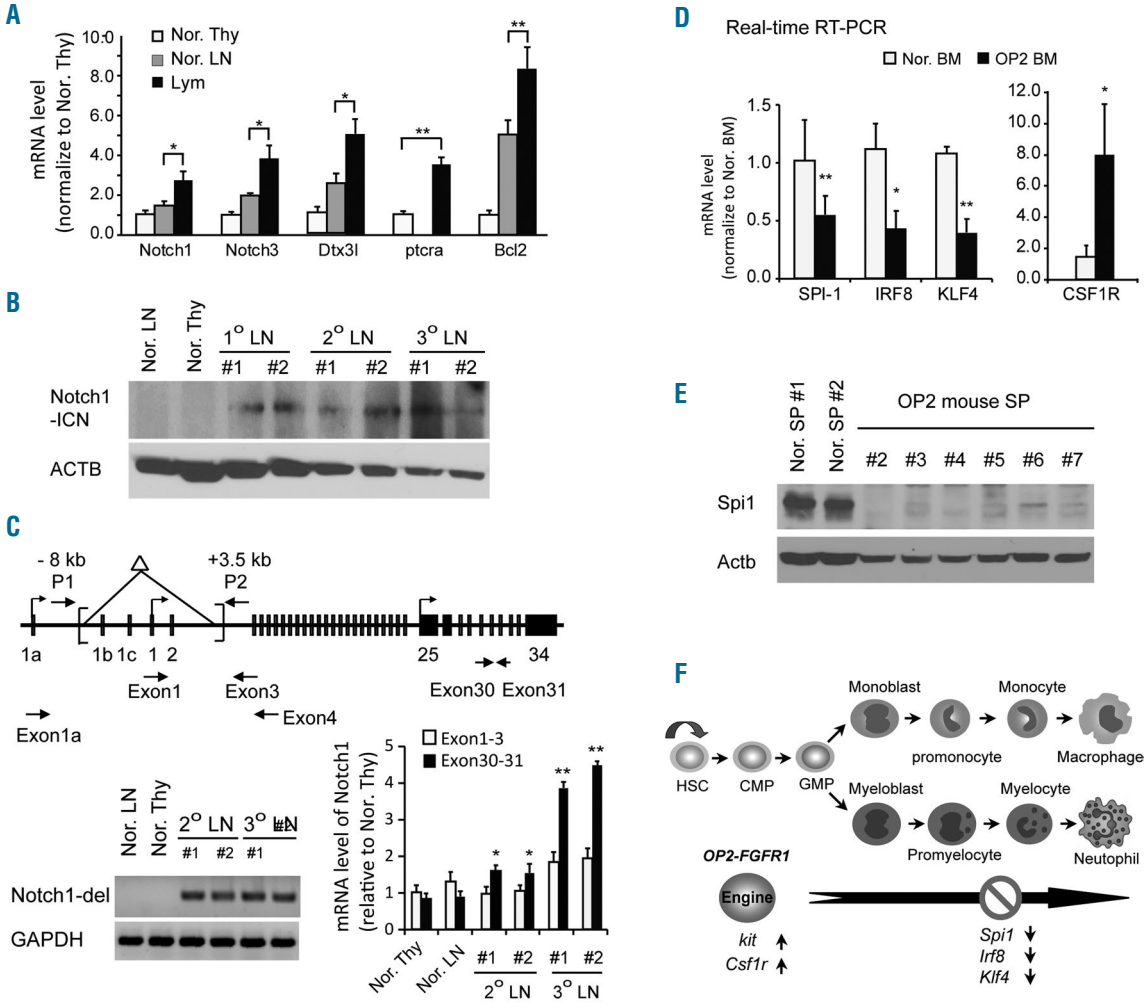

Figure 2. Dysregulation of multiple signaling pathways in myeloid leukemia and T-lymphoma induced by FGFR1OP2-FGFR1. (A) RT-PCR shows that Notch1 and several direct target genes are up-regulated in T-lymphomas from FGFR1OP2-FGFR1 mice. (B) Western blot analysis, using activated Notch1 antibodies (Val 1774), shows that activated Notch1 is present in lymph nodes (LN) isolated from diseased mice. (C) Schematic of the Notch1 gene showing the relative locations of the primers used to analyze the deletion mutants (above). A 5 Kb deletion (brackets) creates a 500-base pair fragment using the P1/P2 primers, as shown in LN from diseased mice (below left). In normal LN and thymus (Thy) cells, the wild-type 11.5-kb fragment cannot be amplified. RT-PCR shows that the alternative Notch1 transcript (exon 1a-exon 4) was only found in tumor samples (above), whereas real-time RT-PCR showed that Notch1 transcription levels for exons 30-31 was higher than that for exons 1-3 (below) in the majority of lymphomas, compared with normal cells (below right). (D) Quantitative RT-PCR analysis shows the comparison of gene expression levels in bone marrow cells (BM) from FGFR1OP2-FGFR1 mice (OP2, n=3) compared with normal BALB/c mice (Nor, n=3). (E) Western blot analysis shows Spi1 protein levels in leukemic mouse spleens compared with normal BALB/c mouse spleens. (F) Schematic summary of differentially expressed genes related to stages of myeloid development. * =P<0.05; ** =P<0.01.

The immunophenotype of the LN cells was predominantly CD4+, which was also seen in the thymus (Figure 1D). Although the majority (>70%) of T-lymphoma cells were CD4+, some cells were CD4+CD8+. All T-lymphoma cells displayed persistent expression of CD25 (*data not shown*). Unlike previous models of FGFR1-driven T-lymphoma,[6] lymphomas involving the Peyer's patches were not observed. The immunophenotype of the myeloid leukemia was Gr1+Mac1+CD19+, indicating immature myeloid cells (Figure 1E). These cells also expressed high levels of cKit (Figure 1F), suggesting that these myeloid cells were blocked at an early stage of myeloid development and resulted in AML. In this respect, the myeloid disease in the FGFR1OP2-FGFR1 mouse model is similar to the CNTRL-FGFR1 mouse model.[5] Due to the early onset of AML in these mice a defined chronic myeloproliferative disorder could not be demonstrated. Serial transplantation of the bone marrow cells from the primary recipients resulted in the full spectrum of MPD, AML and T-lymphoma in successive transplants.

Notch1 is a critical regulator of normal T-cell development, and promotes the transition through early developmental stages of multipotential precursor cells.[7] Sustained activation of Notch1 has been consistently shown in human T-ALL patients,[8] suggesting it is an important event in maintaining an immature phenotype that promotes lymphomagenesis. We, therefore, investigated Notch signaling in these T-lymphomas. Quantitative RT-PCR demonstrated that the Notch1 mRNA levels were upregulated in the T-lymphoma cells. Direct target genes of Notch1 that are specifically related to T-lineage commitment, such as *Ptera, Dtx3l* (deltex 3-like), *Bcl2* and *Notch3*, were also highly up-regulated in the T-lymphomas compared with normal LN and thymus (Figure 2A). We further identified activated Notch1 (containing the intracellular domain) using the cleaved Notch1 (Val1744) antibody (Cell Signaling) in the T-lymphomas from serially transplanted mice (Figure 2B), indicating progressive accumulation of immature T-lymphoma cells during disease development.

We, and others, previously demonstrated that Notch1 activation results from the deletion of the primary promoter in murine T-ALL.[9,10] To determine whether this is also the case for FGFR1OP2-FGFR1 lymphomas, we analyzed T-cell lymphoma samples from two serially transplanted mice and showed that tumors from the secondary transplanted mice harbored exon 1b/exon 2 Notch1 deletions (Figure 2C). These deletions were identical to those seen in T-lymphomas that developed in a ZMYM2-FGFR1 mouse model.[9] Exon 1 deletions prevent wild-type Notch1 transcription and promote alternative promoter activation (transcripts from exon 1a) from the Notch1 locus in tumor samples (Figure 2C).[9] RT-PCR showed that the alternative Notch1 transcript (exon 1a-exon 4) was found in tumor samples, but not in normal LN and thymus. Furthermore, we used 2 different sets of primers to amplify within the C- and N- terminal regions of Notch1, respectively. Quantitative RT-PCR demonstrated that carboxyl-terminal transcript levels (exons 30-31) were higher than N-terminal (exon 1-3) levels (Figure 2C, lower panel) in the lymphomas, whereas this difference was not observed in normal lymph nodes and thymus. These results indicate that constitutive activation of Notch1 in T-lymphoma from FGFR1OP2-FGFR1 mice is due mainly to deletion mutations, whereas the aberrant increase in transcription may be through the use of an alternative Notch1 promoter. Furthermore, analysis of *Tcrb* rearrangements in the T-cell lymphomas, demonstrated multiple subclones in the primary, secondary and tertiary recipients (*data not shown*), suggesting that expression of the FGFR1OP2-FGFR1 fusion kinase alone cannot induce T-cell lymphoma development. It is not clear, however, whether Notch1 mutations also occur in primary human FGFR1OP2-FGFR1 associated disease, since there are still too few clinical reports for this rare subtype. In our previous study of the human KG1, AML cell line, which carries the FGFR1OP2-FGFR1 rearrangement[11] however, we also demonstrated mutational activation of Notch1.[9] Interestingly, a derivative subline, KG1a, which had lost myeloid features seen in the parental cells, had acquired new immunophenotypic characteristics associated with immature T-cells.[12] This observation suggests that expression of FGFR1OP2-FGFR1, in combination with Notch1 mutations, can drive the stem cells to a committed T-cell lineage, leading to lymphoma.

Previously, we showed that several signaling pathways related to myeloid cell development were aberrantly dysregulated in FGFR1-induced AML.[5] To determine whether this is also the case for FGFR1OP2-FGFR1 mice, we used quantitative RT-PCR to compare gene expression levels between spleen cells from FGFR1OP2-FGFR1 and normal BALB/c mice. This analysis showed an increased expression of Csf1r, which is related to early myeloid lineage commitment, whereas the expression of genes related to myeloid differentiation, *Irf8, Klf4,* and *Spi1*, were decreased compared with normal BM myeloid cells (Figures 2D-F).

Since all the fusion genes in MPN-tFGFR1 have the identical truncated FGFR1 protein, it appears that the FGFR1 partners have an important role in the development of the diseases with different lineages. The N-terminal BCR component of the BCR-FGFR1 fusion protein, for example, has a serine/threonine protein kinase function as well as guanine nucleotide exchange factor (GEF) and GTPase-activating domains.[13] These motifs may account for the more aggressive disease associated with this particular chimeric kinase. Inactivation of the BCR kinase function, however, leads to delayed disease development in a mouse model,[14] and the phenotype changes from a CML-like disease to MPD, coincident with T-cell leukemia/lymphoma rather than B-lymphoma. Of particular note, unlike other FGFR1-driven AML, which have a long latency period,[5] AML induced by FGFR1OP2-FGFR1 arise within only 5-6 weeks, possibly suggesting that this particular chimeric protein partner can also promote the aggressive development of AML. The function of the FGFR1OP2 gene, however, is still unclear, but in fibroblasts it appears to associate with the cytoskeleton and is implicated in cell movement.[15] Apart from this suggestion, beyond the coiled-coil motif that is retained in the chimeric protein and presumably facilitates dimerization,[4] exactly how FGFR1OP2 influences FGFR1 kinase function in MPN-tFGFR1 is not clear, since little else is known about this protein.

In summary, we have developed a syngeneic mouse model of MPN-tFGFR1 following the expression of the FGFR1OP2-FGFR1 chimeric kinase in hematopoietic stem cells. This disease shows identical features of the primary human disease and therefore provides the opportunity not only to contribute further to our understanding of the underlying molecular etiology of this syndrome, but also provides a model to evaluate the impact of FGFR1 and Notch inhibitors in suppressing the different manifestations of the disease.

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