Juvenile myelomonocytic leukemia (JMML) is a relentless myeloproliferative disorder (MPD) of young children characterized by over-production of myeloid lineage cells that infiltrate hematopoietic and non-hematopoietic tissues including skin, lung, and spleen. The median survival is < 1 year without hematopoietic stem cell transplantation (HSCT). A cellular hallmark of JMML is that primary blood or bone marrow cells form abnormal numbers of colony forming unit granulocyte-macrophage (CFU-GM) colonies in methylcellulose cultures containing low concentrations of the growth factor granulocyte-macrophage colony-stimulating factor (GM-CSF). Children with neurofibromatosis type 1 (NF1) and Noonan syndrome (NS) are at increased risk of developing JMML and studies of these inherited cancer predispositions implicate hyperactive Ras signaling as an initiating event in leukemogenesis.

Ras proteins regulate cell fates by cycling between an active guanosine triphosphate (GTP)-bound and an inactive guanosine diphosphate (GDP)-bound conformations (Ras-GTP and Ras-GDP). Ras-GTP interacts productively with downstream effectors including phosphoinositide-3-OH (PI3) kinase, Raf1, and Ral-GDS to regulate multiple cellular processes including proliferation, survival, and differentiation. The intrinsic Ras GTPase terminates signaling by hydrolyzing Ras-GTP to Ras-GDP. This slow “off” reaction is greatly augmented by GTPase activating proteins (GAPs), which bind to the effector domain of Ras-GTP and accelerate its conversion to Ras-GDP by stabilizing a transition state between Ras-GTP and Ras-GDP. Somatic point mutations in the NRAS and KRAS2 genes occur in myeloid malignancies and many other human cancers, including about 25% of JMML cases. These mutations introduce amino acid substitutions at codons 12, 13, and 61 and encode proteins that accumulate in the GTP-bound conformation due to defective intrinsic GTPase activity and resistance to GAPs. Neurofibromin, the NF1 gene product, is a GAP for Ras.

Children (but not adults) with NF1 are strongly predisposed to JMML and other myeloid malignancies. The dominantly-inherited cancer predisposition in persons with NF1, the frequent occurrence of NRAS and KRAS2 in human malignancies, and the identification of neurofibromin as a GAP for Ras suggested that NF1 might function as a tumor suppressor gene (TSG) in JMML. Consistent with the model, JMML bone marrows from children with NF1 frequently show loss of constitutional heterozygosity (LOH) at the NF1 locus, which invariably involved deletion of the normal parental allele in familial cases. A study that demonstrated homozygous inactivation of NF1 in JMML bone marrows provided formal genetic proof that the NF1 gene functions as a TSG. In addition, JMML samples from children with NF1 showed a reduction of neurofibromin GAP activity, elevated levels of Ras-GTP, and activation of the Raf1 effector ERK. Together these data identified NF1 as a myeloid TSG that functions by negatively regulating Ras signaling.

NS is a dominant developmental disorder characterized by cardiac defects, facial dysmorphism, and skeletal malformations. Clinical reports described a spectrum of hematologic abnormalities including isolated monocytosis, a, CMML-like disorder that remits spontaneously, and JMML. As in NF1, the discovery of germline missense mutations in the PTPN11 gene as the underlying cause of ~50% of NS cases by Marco Tartaglia, Bruce Gelb and their colleagues proved crucial for unraveling the mechanism of aberrant myeloid proliferation in NS patients and unexpectedly identified PTPN11...
as the most common target of somatic mutations in JMML. *PTPN11* encodes SHP-2, a cytosolic non-receptor tyrosine phosphatase that includes two Src homology 2 (SH2) domains (termed N-SH2 and C-SH2) and a catalytic protein tyrosine phosphatase (PTP) domain. The SHP-2 N-SH2 domain inhibits phosphatase activity by blocking the active site of the PTP domain. The SHP-2 PTPase becomes activated when its SH-2 domains bind an appropriate tyrosine phosphorylated ligand, which induces a conformational shift that "opens" the active site. In contrast to many other PTPases, SHP-2 generally plays a positive role in transducing signals from these receptors, which is mediated, at least in part, through Ras-GTP. SHP-2 is expressed at high levels in hematopoietic cells and undergoes rapid tyrosine phosphorylation upon activation of the c-kit, interleukin 3 (IL-3), GM-CSF, and erythropoietin receptors.

Most of the germline *PTPN11* mutations found in NS constitutively activate SHP-2 phosphatase activity by destabilizing auto-inhibitory interactions between the N-SH2 and PTP domains of the protein. The association between NS and JMML and the key roles of SHP-2 in Ras signaling and hematopoiesis suggested that *PTPN11* might be mutated in patients with JMML. Indeed, this hypothesis was confirmed by studies that identified novel heterozygous germline *PTPN11* mutations in patients with NS and JMML. Importantly, somatic mutations in *PTPN11* occur in approximately 35% of all patients with JMML. *PTPN11*, *KRAS*, *NRAS*, and *NF1* mutations are rarely detected in the same patient, which is consistent with the fact that these genes encode components of the same growth control pathway. Although all of the somatic *PTPN11* mutations identified in JMML are missense changes, both the spectrum and distribution of these mutations differs from NS. These leukemia-associated mutations encode stronger gain-of-function mutations than those found in NS and it is therefore likely that they would not be tolerated in the germline. The less severe biochemical phenotype of the mutations found in children with NS likely explains clinical observation that many of the hematological abnormalities detected in infants with NS resolve without specific treatment. Most recently, a child with NS and JMML was identified with a novel germline *KRAS* mutation that deregulates the growth of primary hematopoietic progenitors and induces elevated levels of phosphorylated MEK and Akt (p MEK and pAkt) in cultured macrophages.

Genetically engineered strains of mutant mice have been extremely valuable for analyzing the functional consequences of genes that are mutated in JMML. Although homozygous inactivation of *NF1* is lethal in embryonic life, fetal hematopoietic cells demonstrate a similar pattern of hypersensitive CFU-GM colony growth in response to GM-CSF as human JMMLs and adoptive transfer into irradiated recipients induces a JMML-like MPD with hyperactive Ras. Studies in this mouse model also showed that *NF1* inactivation leads to deregulated growth in multiple hematopoietic compartments and confers a durable proliferative advantage in competitive repopulation assays. A cross between *NF1* and *Gmcsf* mutant mice also showed that aberrant GM-CSF signaling plays a central role in initiating and maintaining the JMML-like MPD. The JMML model that was developed by injecting *Nf1*-deficient fetal liver cells into irradiated recipient mice was also used to perform a preclinical trial that evaluated the efficacy of an inhibitor of the Ras processing enzyme farnesyltransferase, which included pharmacodynamic monitoring in primary hematopoietic cells. This study showed no inhibition of K-Ras and N-Ras processing at the maximal tolerated dose, and no improvement in the MPD. A conditional mutant allele of *NF1* was generated to overcome the embryonic lethality that results from homozygous *NF1* inactivation. Somatic inactivation of this *Nf1*flox allele in hematopoietic cells and found that that this consistently results in a JMML-like MPD that is associated with leukocytosis, splenomegaly, hyperproliferation, impaired apoptosis, and in vitro hypersensitivity to GM-CSF. Using the same strategy to activate a conditional "knock-in" allele to induce expression of an oncogenic K-RasG12D protein from the endogenous locus by excising a loxP-stop-loxP (LSL) cassette. Compound *Mx1-Cre LSL-Kras* mutant mice also develop an aggressive MPD with leukocytosis and death at a median 105 days of age. Bone marrow cells and splenocytes demonstrate profound hypersensitivity in response to GM-CSF and form
many large CFU-GM in methylcellulose cultures containing low concentrations of GM-CSF and other myeloid growth factors. Finally, a mouse model of NS colleagues was generated by constitutively expressing an amino acid substitution (D61G) identified in human patients from the endogenous murine Ptpn11 locus. Heterozygous Ptpn11 D61G mutant mice showed cardiac and skeletal defects and develop a subacute MPD that models some aspects of JMML. Interestingly, D61G is the only PTPN11 mutation that is detected in NS patients with and without leukemia as well as in sporadic JMML.

Together, the germline and somatic mutations found in children with JMML and evidence from strains of Kras, Nf1, and Ptpn11 mutant mice strongly support the hypothesis that genetic lesions that result in hyperactive Ras play a central role in the pathogenesis of JMML. Understanding the association of JMML with NF1 and NS has helped to uncover genes and proteins that are critical for normal growth control and, when deregulated, contribute to leukemia. The studies performed to raise a number of biologic and clinical questions. An intriguing question is why the risk of leukemia in persons with NF1 and NS is confined to a narrow developmental window. Children with these diseases who do not develop a myeloid malignancy during the first few years of life do not appear to be at increased risk of developing hematologic malignancies later in life despite the fact that the bone marrow remains highly proliferative. This clinical observation, in turn, suggests something unique about fetal hematopoietic stem cells and/or the fetal microenvironment that increases the probability that a clone that has sustained a mutation that deregulates Ras signaling will persist and ultimately cause JMML. Finally, genetic and biochemical data have identified hyperactive Ras as a rational biochemical target for treating children with JMML. Unfortunately, the inhibiting aberrant signaling at the level of Ras itself requires restoring enzymatic activity (i.e. accelerating the intrinsic GTPase), which is an exceedingly difficult pharmacologic problem. For this reason, efforts have focused on discovering small molecules that inhibit components of effector kinase cascades that are activated downstream of Ras-GTP. Kras and Nf1 mutant mice and cells from these animals provide robust reagents for testing novel therapeutic strategies that may ultimately improve the treatment of children with JMML.

References

