How a rare pediatric neoplasia can give important insights into biological concepts: a perspective on juvenile myelomonocytic leukemia

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Representing as little as 2% of hematopoietic malignancies in childhood, juvenile myelomonocytic leukemia (JMML) has nevertheless formed the basis of a fascinating chapter of biomedical research on hematopoietic neoplasia over the past two decades. It not only furthered our understanding of one of the most important oncogenic processes in human cells, the hyperactivation of the RAS signal transduction machinery, it also yielded surprising insight into the mutual relationship between inherited predisposition syndromes and the development of myeloid leukemia. JMML is a stem cell disorder characterized by clonal hyperproliferation of monocytes and granulocytes without differentiation arrest.[1] The affected children are diagnosed at a median age of 2 years and typically present with hepatosplenomegaly and lymphadenopathy accompanied by infiltration of other organs, especially lung and skin.[2] If splenomegaly is not apparent at diagnosis, it invariably develops rapidly in the course of the disease, creating abdominal distension and often considerable discomfort (Figure 1A). The lung infiltrates generally cause a dry cough and tachypnea and account for part of the mortality due to respiratory insufficiency (Figure 1B). The skin lesions are pleomorphic and occasionally pose significant diagnostic problems, especially as aleukemic presentations have been reported (Figure 1C). However, a skin biopsy usually reveals the myelomonocytic nature of the infiltrates. Involvement of the central nervous system is rare in JMML. The white blood cell count is elevated in the majority of cases (median, 33000/μL) but, in contrast to in chronic myelogenous leukemia (CML) rarely exceeds 100000/μL. The bone marrow findings fit with the diagnosis but are rather non-specific. By contrast, the morphologic evaluation of peripheral blood smears is an important diagnostic step: most cases exhibit notable monocytosis with immature and dysplastic forms (Figure 1D); an absolute monocyte count ≥1000/μL is a prerequisite for the diagnosis.[3] In addition, there are circulating immature granulocytes and nucleated red cells. Blasts may be present in the peripheral blood but their percentage seldom exceeds 10–15%.[2] Generally there is some degree of thrombocytopenia and anemia. An interesting feature of two-thirds of cases (specifically, those without chromosome 7 abnormality; *see below)* is the reversal to fetal red cell characteristics, including increased levels of hemoglobin F, expression of the i antigen and low carbonic anhydrase levels.[4] Therefore, hemoglobin electrophoresis may help establish the diagnosis. JMML responds poorly to chemotherapy regardless of its intensity,[5] and presently allogeneic hematopoietic stem cell transplantation (HSCT) is the only curative therapeutic modality.[6] This perspective article will briefly review the classification of JMML, its molecular pathogenesis and current therapeutic concepts.

Diagnosis and delineation from other myeloproliferative disorders in childhood

In the past, the classification and differential diagnosis of JMML have sparked a fair amount of debate. Early descriptions referred to the condition as *subacute/chronic myelomonocytic leukemia of childhood* or *juvenile chronic myeloid leukemia*.[7,8] However, receptor tyrosine kinase gene fusions characteristic of adult-type chronic myelomonocytic leukemia (e.g., *TEL-PDGFRB*) are not known in JMML, and in contrast to chronic myeloid leukemia, JMML lacks the translocation t(9;22) and the *BCR-ABL1* fusion. In 1996 an international consensus was reached to name this unique disorder JMML. To aid with the differential diagnosis of JMML, which involves other myeloproliferative disorders (MPD) or myelodysplastic syndromes (MDS) as well as non-malignant conditions such as hematotropic virus infections, guidelines were drawn up by an international working group.[3] In brief, absence of *BCR-ABL1*, absolute monocyte count ≥1000/μL in peripheral blood and bone marrow blasts <20% are obligatory criteria for the diagnosis; in addition, at least two of the following are required: elevated hemoglobin F (adjusted for age), presence of myeloid precursor cells in blood, white blood cell count >10000/μL, evidence of a clonal genetic abnormality, or granulocyte-macrophage colony-stimulating factor (GM-CSF) hypersensitivity (*see below*). The current WHO classification of hematopoietic malignancies lists JMML in the group of mixed myelodysplastic/myeloproliferative disorders to account for the impracticality of clearly categorizing JMML as the former or the latter.[9] There is still some puzzlement about the significance of clonal monosomy 7 or deletion 7q in childhood myeloid disorders including JMML. The frequent occurrence of this cytogenetic lesion in MPD and MDS prompted some investigators in the 1980s to propose a separate entity termed infantile monosomy 7 syndrome.[10] However, it is now felt that there is little justification to consider monosomy 7 as a specific subgroup within JMML because the presence or absence of the lesion does not change the clinical presentation or alter the prognosis.[2,11] It seems at present that a conclusive appraisal of monosomy 7 must be deferred until the molecular basis of this chromosomal aberration is clarified.

Molecular pathogenesis: is it all about the RAS pathway?

Two important features characterize the disordered hematopoiesis in JMML. First, myeloid progenitor cells

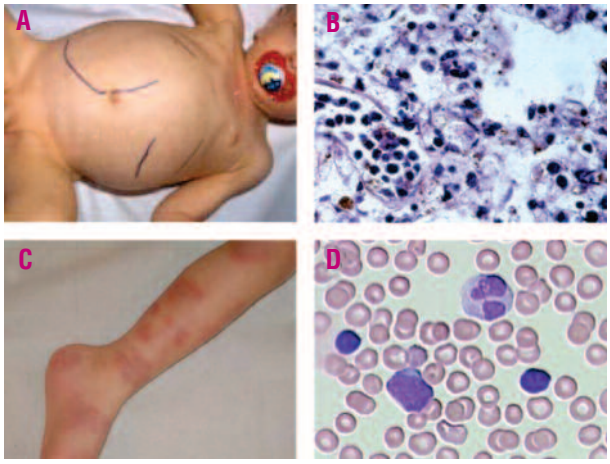

Figure 1. Characteristic findings in children with JMML. (A) Hepatosplenomegaly in a 2-year old boy at the time of diagnosis. (B) Pulmonary interstitial infiltration by leukemic cells. (C) Skin infiltration by leukemic cells. (D) Peripheral blood smear with a dysplastic monocyte with nuclear bridging and a blast cell.

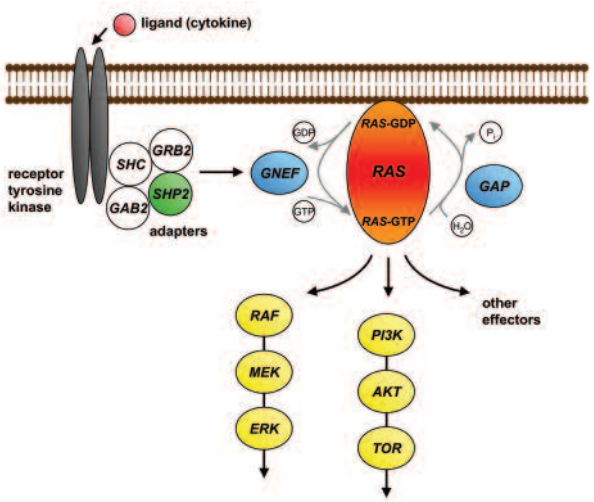

Figure 2. Schematic diagram of the RAS signal transduction pathway. Upon binding of cytokines to receptor tyrosine kinases, several adapter molecules (such as Src homology 2 domain-containing proteins [SHC], Src homology 2 domain-containing protein tyrosine phosphatase 2 [SHP2], growth factor receptor-bound protein 2 [GRB2] and GRB2-associated binding protein 2 [GAB2]) are activated and stimulate guanosine nucleotide exchange factors (GNEF) such as son-of-sevenless homolog 1 (SOS1). GNEF transform RAS into its active GTP-bound state. RAS signaling is terminated by intrinsic RAS-GTP hydrolysis (accelerated by GTPase activating proteins [GAP] such as neurofibromin). Active RAS interacts with several effector pathways. v-raf murine sarcoma viral oncogene homolog (RAF), mitogen-activated protein kinase kinase (MEK) and extracellular signal-regulated kinase (ERK) are serially activated by phosphorylation reactions; active ERK is transferred to the nucleus and regulates cell cycle progression. PI3K is a lipid kinase catalyzing the formation of phosphatidylinositol-triphosphate, a second messenger molecule with activating effect on v-akt murine thymoma viral oncogene homolog (AKT). This protein kinase interacts with target of rapamycin (TOR), a regulator of apoptosis and cell cycle. Other abbreviations: GTP, guanosine triphosphate; GDP, guanosine diphosphate.

derived from peripheral blood or bone marrow of JMML patients form excessive numbers of granulocyte-macrophage colonies *in vitro* even when cultured without exogenous cytokines.[12] This phenomenon is known as spontaneous proliferation and depends on the presence of leukemic monocytes.[13] Second, dose-response experiments demonstrated that JMML myeloid progenitor cells are hypersensitive to GM-CSF.[14] It is likely that constitutive low-level secretion of cytokines, primarily GM-CSF, by leukemic monocytes, coupled with the hypersensitivity of granulocyte-macrophage colony-forming units to these stimuli, is sufficient to drive the excessive myeloproliferation. Although not absolutely specific for JMML, GM-CSF hypersensitivity is a key feature of the disease and has become a valuable diagnostic tool. In addition, it pointed the way to major advances in our understanding of the molecular pathogenesis of JMML. Today, gene mutations interfering with downstream components of the GM-CSF signal transduction pathway can be defined in approximately 70% of children with this disorder.

The transmission of GM-CSF signals from its receptor to the nucleus occurs mainly through the sequential phosphorylation of a series of intracellular proteins centered around the RAS signal transduction pathway,[15] as reviewed in more detail by Downward.[16] Functioning as cellular master switches, RAS proteins bind guanosine triphosphate (RAS-GTP) in their active configuration and guanosine diphosphate (RAS-GDP) when inactive.[17] The cellular levels of active RAS-GTP are balanced within tight boundaries by the concurrent action of guanine nucleotide exchange factors (GNEF) and GTPase-activating proteins (GAP).[18] Extracellular stimuli, such as GM-CSF binding to its receptor, activate adaptor molecules (e.g., GRB2, SHC, GAB2) which recruit GNEF (e.g., SOS1) to turn on RAS by displacing GDP and allowing GTP to bind (Figure 2). Active RAS forwards the signal to RAF1, PI3K, RALGDS and other effector molecules.[19] RAS itself possesses intrinsic GTPase activity which hydrolyses RAS-GTP to RAS-GDP and thus terminates the signal.[20] GAP, most notably the *NF1* gene product, neurofibromin, dramatically accelerate this process, serving as molecular brakes on the RAS pathway.[19,20] The following paragraphs will briefly describe how the various genetic lesions observed in JMML result in deregulation of RAS signaling. There are three prominent *RAS* proto-oncogenes in the human genome, termed *NRAS, KRAS* and *HRAS*, which were originally identified as orthologs of rat sarcoma virus oncogenes. Point mutations at codons 12, 13 or 61 of *NRAS, KRAS* or *HRAS* are commonly encountered in a broad spectrum of human cancers and leukemias.[21] The mutations affecting these residues lead to RAS proteins with reduced capacity for GTP hydrolysis and resistance to GAP, resulting in overstimulation of RAS-dependent target molecules in the nucleus. In JMML, somatic mutations of the *NRAS* or *KRAS* (but not *HRAS*) genes are found in leukemic cells at diagnosis in 20–25% of cases.[22,23] A number of recent exciting findings augment the understanding of how *RAS* genes contribute to disordered myelopoiesis. First was the discovery of a germline $KRAS^{T58I}$ mutation in a girl with Noonan syndrome who developed an MPD which resembled JMML but was less aggressive.[24] The $KRAS^{T58I}$ allele was found to have inter-

mediate biochemical (e.g. ability to hydrolyse Ras-GTP) and biological (e.g. colony growth in response to cytokines after retroviral transduction) properties when compared with wildtype *KRAS* or the classic oncogenic mutant *KRAS*^{G12D}.[24] In an attempt to take the idea of genotype-phenotype correlation further, Matsuda *et al*. recently compared the clinical presentation and disease progression between JMML patients with different *RAS* mutations.[25] They reported long-term survival despite no therapy in three patients in whom glycine at position 12 of KRAS or NRAS was replaced specifically with serine. However, those cases also had other favorable prognostic features and it remains to be determined more conclusively whether or not the consensus genotype was merely coincidental. In a paper that appears in this issue of the journal, the same group describes the complete loss of wildtype *NRAS* from leukemic cells of a child with JMML at the time of transition into blast crisis.[26] As in other types of neoplasia, oncogenic *RAS* mutations in JMML are usually heterozygous, indicating that the expression of the protein from one altered allele is sufficient for the deregulation of the RAS pathway even in the presence of a second wildtype allele. The case description by Matsuda *et al*.[26] raises the provocative possibility that the mutant RAS protein is capable not only of driving excessive proliferation but also, in the absence of wildtype RAS, of arresting differentiation. It cannot be ruled out, however, that an unknown neighboring gene was the actual target of the partial 1p isodisomy observed by the authors.

Elevent percent of children with JMML have constitutional neurofibromatosis type 1 (NF-1). These patients carry one intact and one deficient allele of the NF1 tumor suppressor gene in the germline. The deficient allele is inherited or results from *de novo* mutation. Neurofibromin, the protein encoded by NF1, functions as a GAP and negatively regulates RAS.[18,27] In genetically engineered mice, loss of Nf1 gives rise to an aberrant pattern of hematopoietic progenitor colony growth with selective GM-CSF hypersensitivity.[28] Homozygous inactivation of NF1 in the leukemic clone, resulting from somatic inactivation of the normal NF1 allele, was demonstrated in some children with NF1 and JMML.[29] However, those early studies were hampered by technical limitations in the detection of small intragenic NF1 lesions. Two recent studies have confirmed the somatic loss of heterozygosity (LOH) at the *NF1* locus in eight out of nine children with NF-1 and JMML.[30,31] Surprisingly, in seven of these eight patients with LOH at *NF1*, the lesion was not restricted to a small segment around the *NF1* locus but rather involved almost the entire long arm of chromosome 17. In all cases, the chromosomal arm carrying the wildtype *NF1* allele was not only deleted, but also replaced by a second copy of the 17q arm bearing the *NF1* mutation. These findings unexpectedly identified mitotic recombination, an otherwise rare genetic event, as a recurrent underlying mechanism. Interestingly, functional neurofibromin was also eliminated in one case without LOH at NF1. Here, each NF1 allele carried a distinct truncating mutation, inactivating the gene in a compound-heterozygous fashion.[31] Together, these data confirm the concept that a somatic *second hit* to the remaining wildtype *NF1* allele in an early hematopoietic progenitor cell is the basis for the emergence of JMML in children with NF-1.

Somatic mutations in *PTPN11*, the gene encoding the protein tyrosine phosphatase SHP2, represent the most frequent molecular lesion in JMML, accounting for approximately 35% of cases of JMML.[32] The key to this discovery was the observation that a JMML-like myeloproliferative disorder occurred in some children with Noonan syndrome,[33] a developmental disorder characterized by cardiac defects, short stature, dysmorphic facial features, and skeletal abnormalities.[34] About half of children with Noonan syndrome are known to carry specific germline mutations in *PTPN11*.[35] The mutations disturb the inhibitory interaction between the enzyme's src homology 2 and tyrosine phosphatase domains, causing a gain of phosphatase activity.[36] SHP2 is recruited to the intracellular portion of various cytokine receptors and regulates several downstream responses including proliferation.[37] In contrast to other cytosolic tyrosine phosphatases, SHP2 functions as a positive stimulus on downstream signal transduction molecules. Although a direct interaction of SHP2 with the RAS pathway has yet to be demonstrated, the assumption that SHP2 signaling is relayed at least in part through RAS is backed by strong evidence. First, when *PTPN11* mutations typical of JMML were expressed in experimental cell systems, the growth of granulocyte-macrophage colonies became hypersensitive to GM-CSF and the proliferation of immature progenitors with high replating potential was enhanced.[38] Second, with the exception of rare case, the mutational analysis of leukemic cells typically identifies clonal alterations at *NF1*, *PTPN11*, or *KRAS/NRAS*, but not at more than one of these loci in a given patient with JMML,[32] consistent with the idea that these molecules form parts of the same regulatory pathway. Third, some cases of Noonan syndrome are caused by germline *KRAS* mutations, again indicating that the functional consequences of alterations in the *PTPN11* or *RAS* genes overlap.[24]

Taken together, 70% of children with JMML carry somatic clonal gene lesions that interact with the RAS signaling pathway, cause a gain of activity and thus explain the proliferative phenotype. It is obvious to speculate that the patients in whom no *RAS, PTPN11* or *NF1* abnormality is present might harbor defects in other components of the pathway. This hypothesis recently gained impetus when it was reported that the *SOS1* gene, encoding a GNEF for RAS, was affected by missense mutations in approximately 20% of cases of Noonan syndrome.[39] However, a mutational analysis of *SOS1* in 49 children with JMML who lacked alterations in the *RAS* or *PTPN11* genes and did not have NF-1 or Noonan syndrome revealed no *SOS1* abnormality.[40] The study also excluded *SHC1, GRB2, GAB1, MAP2K1 (MEK1)* and *MAP2K2*

(*MEK2*) as candidate JMML genes, with the caveat that the latter five genes were examined in smaller numbers of patients (between 9 and 17) and only mutational hotspots were screened.[40] The RAF family of kinases, encoded by the *ARAF, BRAF* and *CRAF (RAF1)* genes, are direct effector molecules for RAS, serving as connectors to the mitogen-activated protein kinase cascade by phosphorylating MEK1 and MEK2 (Figure 2). *BRAF* is a well-known proto-oncogene affected by somatic mutations in 5–8% of human malignancies, with amino-acid residue 600 being the target in 90% of cases. However, a letter in this issue of Haematologica contributes another piece to the mosaic by reporting that *BRAF* mutations do not occur in JMML.[41] *CRAF* was recently described as another Noonan syndrome-causing gene.[42] To our knowledge, data on *CRAF* alterations in JMML have not yet been published.

Clonality studies have shown that malignant transformation in JMML takes place at the level of stem cells or early myeloid progenitor cells.[23,43] Even though the genetic lesions perturbing the functional state of the RAS pathway can be traced back to clonal cells at the earliest stages of differentiation,[43] it is still an unanswered question whether the RAS pathway alteration is actually the primary event initiating transformation. The alternative hypothesis supposes the RAS-deregulating mutation to be a secondary lesion that co-operates with an undefined primary alteration by conferring a proliferative advantage on the malignant clone. The question is not merely academic: if hyperactive RAS is by itself responsible for the initiation and maintenance of JMML, then targeted therapies aimed at the inhibition of RAS signaling would not just restrain marrow hyperproliferation but would also have the potential to cure the disease by eradicating the transformed clone. Data from genetically engineered mouse models indicate that experimental deregulation of Nf1, Kras or Shp2 is sufficient to induce a myeloproliferative disorder.[44-46] On the other hand, an extrapolation from acute myeloid leukemia and/or myelodysplastic syndrome would suggest that *RAS* mutations are co-operating secondary events which may be functionally equivalent to the deregulation of other cytokine receptor signaling pathways (e.g., KIT or FLT3).[47] It is interesting in this regard that a study by de Vries *et al.*, published in this issue of Haematologica, found no evidence of FLT3 activation in JMML, whether by genetic mutation, aberrant expression or autocrine stimulation.[48] One might interpret these results as an indication that the contribution of hyperactive RAS to the pathogenesis of JMML cannot be substituted by just another signaling pathway. As a concluding remark, when debating the role of RAS pathway perturbation in JMML, it should not be overlooked that cytogenetic studies of leukemic cells reveal chromosomal abnormalities, in particular monosomy 7 or large 7q deletions, in 35% of cases of JMML .[2] These aberrations occur independently of the mutational status of *PTPN11, RAS* or *NF1*. Although the nature of their contribution to the pathogenesis of JMML is largely unclear, the frequency of their occurrence makes it difficult to believe that they arise without purpose.

Current treatment

The natural course of JMML is rapidly fatal with 80% of patients surviving less than 3 years. Progression to blast crisis is infrequent; most children die from progressive respiratory and organ failure. Thrombocytopenia, older age and elevated fetal hemoglobin levels were identified as major prognostic factors for short survival.[2] It is important to rule out or confirm the possibility of Noonan syndrome in newly-diagnosed children with JMML under the age of 1 year by careful clinical examination and, if a *PTPN11* mutation is present, by determining whether the mutation is somatic or in the germline. The JMML-like disorder may spontaneously disappear in patients with Noonan syndrome, so close observation without therapy is reasonable for these children. Systematic evaluation of chemotherapeutic approaches in JMML is difficult due to the lack of standardized response criteria and the heterogeneity of response (i.e., clearance of leukemic cells from bone marrow but not solid organs, or vice versa). It appears that mercaptopurine is an agent that consistently produces clinical and/or hematologic improvement, either alone or combined with cytarabine or etoposide,[49] but it should be stressed that the induction of a durable remission with this type of treatment is rare and that it is also doubtful whether low-dose chemotherapy has any effect on the overall duration of survival.[5] Published data do not support the use of more intensive AML-type regimens because remission is attained only in a fraction of cases,[50] is mostly temporary and comes at the cost of a high rate of complications, most notably the induction of long-term, and sometimes fatal, marrow aplasia. In a cohort of 121 JMML patients without HSCT evaluated in 2003, the survival rate at 10 years was only 5%, irrespective of intensive chemotherapy.[1] In summary, there is no rationale for a universal recommendation of chemotherapy in JMML, although it may have a role in selected cases with life-threatening organ infiltration prior to allogeneic HSCT. Allogeneic HSCT is the only curative treatment modality for JMML, with current regimens achieving long-term event-free survival in about half of the children. A large prospective HSCT trial which incorporated 100 children and used unmanipulated grafts and a standardized preparative regimen consisting of busulfan, cyclophosphamide and melphalan was conducted by the European Working Group on MDS in Childhood (EWOG-MDS).[6] The authors reported no significant difference in event-free survival or transplantation-related mortality between grafts from matched family donors versus matched unrelated donors, and no divergence in the two parameters between grafts from bone marrow, mobilized peripheral blood or cord blood. Although the removal of an enlarged spleen before HSCT has the possible benefits of lowering transfusion requirements, accelerating hematologic recovery and reducing the risk of hemorrhage, the question

whether or not splenectomy had been performed prior to HSCT had no influence on outcome.[6] These observations, the rapid lethality in the absence of treatment and the lack of other efficient modalities translate into the general recommendation to initiate a timely HSCT procedure. HSCT in JMML is, however, still haunted by relapse rates of 30–40%. Remarkably, though, a sustained remission can be induced in about half of relapsed children by means of a second transplant, suggesting that the reduced immunosuppressive therapy applied during the second procedure leads to a stronger graft-versus-leukemia effect.[51]

A number of approaches other than chemotherapy have been investigated in JMML. 13-*cis* retinoic acid (isotretinoin) inhibits spontaneous colony growth of JMML progenitor cells *in vitro* and was, therefore, put to the clinical test in a pilot study with ten children and subsequently in a phase II study with 22 children.[52] Although some complete or partial responses were observed, the delineation of a true drug effect from other clinical factors was difficult.[53] The utility of isotretinoin in JMML remains to be determined. With so many details about the mode of RAS operation now unraveled, it is not surprising that newer therapy concepts aim at bringing leverage to specific components of the RAS pathway. Farnesyl transferase inhibitors (FTI), which block a post-translational prenylation reaction critical for the membrane anchorage of RAS, have attracted much attention because of their promising *in vitro* activity against JMML colony formation.[54] However, several issues with FTI, concerning resistance (i.e., the ability of cells to use alternative prenylation reactions) and unspecificity (i.e., interference with farnesylation of proteins other than RAS), remain unsolved. A recent trial of the Children's Oncology Group in the United States evaluated FTI in JMML as an up-front therapeutic window before HSCT.[55] Other experimental strategies include antagonizing GM-CSF,[56] suppressing an endoprotease reaction that directs RAS to membranes,[57] or harnessing the activity of certain bisphosphonates against RAS prenylation.[58] Synthetic inhibitors targeting downstream RAS effectors such as RAF1, MEK or TOR have become available although none of these substances has yet reached the stage of clinical testing in JMML. Interested readers may wish to refer to a recent review covering the subject of targeted therapies for JMML in more detail.[59] A general obstacle when evaluating any type of innovative therapy in JMML is the lack of accepted clinical response parameters, a problem created by the variability of individual response with respect to leukocyte count, platelet count or organomegaly, and by the difficulty in defining complete hematologic remission in a setting with excessive myeloproliferation but low blast percentages. Promising research is under way to develop methods to measure minimal residual disease by quantitatively assaying clone-specific mutations of the *RAS* or *PTPN11* genes.[60]

In summary, recent advances in researching the genetic basis of a common inherited disorder, Noonan syndrome, greatly improved our understanding of the pathogenesis of JMML. *PTPN11*, the major disease gene in Noonan syndrome, is not only mutated in the rare pediatric neoplasia JMML, but also in several cases of acute myeloid and lymphoblastic leukemia, leading to the understanding that the RAS pathway is a common backbone of hyperactive cytokine signaling in hematopoietic cells. It is reasonable to speculate that future discoveries of yet unknown genetic lesions in inherited predisposition disorders will also provide novel insights into leukemogenesis.

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References

1. Niemeyer CM, Locatelli F. Chronic myeloproliferative disorders. In: Pui CH, editor. Childhood Leukemia. New York: Cambridge University Press; 2006.571-98.
2. Niemeyer CM, Aricò M, Basso G, Biondi A, Cantù-Rajnoldi A, Creutzig U, et al. Chronic myelomonocytic leukemia in childhood: a retrospective analysis of 110 cases. Blood 1997;89:3534-43.
3. Niemeyer CM, Fenu S, Hasle H, Mann G, Stary J, van Wering E. Differentiating juvenile myelomonocytic leukemia from infectious disease. Blood 1998;91:365-7.
4. Weatherall DJ, Edwards JA, Donohoe WT. Haemoglobin and red cell enzyme changes in juvenile myeloid leukaemia. Br Med J 1968;1:679-81.
5. Bergsträßer E, Hasle H, Rogge T, Fischer A, Zimmermann M, Nöllke P, et al. Non-hematopoietic stem cell transplantation treatment of juvenile myelomonocytic leukemia: A retrospective analysis and definition of response criteria. Pediatr Blood Cancer 2007;49:629-33.
6. Locatelli F, Nöllke P, Zecca M, Korthof E, Lanino E, Peters C, et al. Hematopoietic stem cell transplantation (HSCT) in children with juvenile myelomonocytic leukemia (JMML): results of the EWOG-MDS/EBMT trial. Blood 2005; 105:410-9.
7. Bernard J, Seligmann M, Acar J. La leucémie myeloide chronique de l'enfant. Étude de vingt observations. Arch Fr Pediatr 1962;19:881-4.
8. Hardisty RM, Speed DE, Morwenna T. Granulocytic leukemia in childhood. Br J Haematol 1964;10:551-66.
9. Vardiman JW. Myelodysplastic / myeloproliferative diseases. In: Jaffe ES, Harris NL, Stein H, Vardiman JW, eds. World Health Organization Classification of Tumours of Haematopoietic and Lymphoid Tissues. Lyon: IARC Press; 2001. 47-59.
10. Sieff CA, Chessells JM, Harvey BAM, Pickthall VJ, Lawler SD. Monosmy 7 in childhood: a myeloproliferative disorder. Br J Haematol 1981;49:235-49.
11. Hasle H, Arico M, Basso G, Biondi A, Cantù-Rajnoldi A, Creutzig U, et al. Myelodysplastic syndrome and acute myeloid leukemia associated with complete or partial monosomy 7. Leukemia 1999;13:376-85.
12. Altman AJ, Palmer CG, Baehner RL. Juvenile "chronic granulocytic" leukemia: a panmyelopathy with prominent monocytic involvement and circulating monocyte colony-forming cells. Blood 1974;43:341-50.
13. Emanuel PD, Bates LJ, Zhu SW, Castleberry RP, Gualtieri RJ, Zuckerman KS. The role of monocyte-derived hemopoietic growth factors in the regulation of myeloproliferation in juvenile chronic myelogenous leukemia. Exp Hematol 1991;19:1017-24.
14. Emanuel PD, Bates LJ, Castleberry RP, Gualtieri RJ, Zuckerman KS. Selective hypersensitivity to granulocyte-macrophage colony-stimulating factor by juvenile chronic myeloid leukemia hematopoietic progenitors. Blood 1991;77:925-9.
15. Lanfrancone L, Pelicci G, Brizzi MF, Arouica MG, Casciari C, Giuli S, et al. Overexpression of Shc proteins potentiates the proliferative response to the granulocyte-macrophage colony-stimulating factor and recruitment of Grb2/SoS and Grb2/p140 complexes to the β receptor subunit. Oncogene 1995;10:907-17.

16. Downward J. Targeting RAS signalling pathways in cancer therapy. Nat Rev Cancer 2003;3:11-22.
17. Bourne HR, Sanders DA, McCormick F. The GTPase superfamily: a conserved switch for diverse cell functions. Nature 1990;348:125-32.
18. Boguski MS, McCormick F. Proteins regulating Ras and its relatives. Nature 1993;366:643-54.
19. Repasky GA, Chenette EJ, Der CJ. Renewing the conspiracy theory debate: does Raf function alone to mediate Ras oncogenesis? Trends Cell Biol 2004;14:639-47.
20. Donovan S, Shannon KM, Bollag G. GTPase activating proteins: critical regulators of intracellular signaling. Biochim Biophys Acta 2002;1602:23-45.
21. Bos JL. Ras oncogenes in human cancer: a review. Cancer Res 1989;49:4682-9.
22. Kalra R, Paderanga DC, Olson K, Shannon KM. Genetic analysis is consistent with the hypothesis that NF1 limits myeloid cell growth through p21ras. Blood 1994;84:3435-9.
23. Flotho C, Valcamonica S, Mach-Pascual S, Schmahl G, Corral L, Ritterbach J, et al. Ras mutations and clonality analysis in children with juvenile myelomonocytic leukemia (JMML). Leukemia 1999;13:32-7.
24. Schubbert S, Zenker M, Rowe SL, Böll S, Klein C, Bollag G, et al. Germline KRAS mutations cause Noonan syndrome. Nat Genet 2006;38:331-6.
25. Matsuda K, Shimada A, Yoshida N, Ogawa A, Watanabe A, Yajima S, et al. Spontaneous improvement of hematologic abnormalities in patients having juvenile myelomonocytic leukemia with specific RAS mutations. Blood 2007;109:5477-80.
26. Matsuda K, Nakazawa Y, Sakashita K, Shiohara M, Yamauchi K, Koike K. Acquirement of loss of the wild-type NRAS locus with aggressive disease progression in a patient with juvenile myelomonocytic leukemia and a heterozygous NRAS mutation. Haematologica 2007;1576-8.
27. Xu GF, O'Connell P, Viskochil D, Cawthon R, Robertson M, Culver M, et al. The neurofibromatosis type 1 gene encodes a protein related to GAP. Cell 1990;62:599-608.
28. Bollag G, Clapp DW, Shih S, Adler F, Zhang YY, Thompson P, et al. Loss of NF1 results in activation of the Ras signaling pathway and leads to aberrant growth in haematopoietic cells. Nat Genet 1996;12:144-8.
29. Side L, Taylor B, Cayouette M, Conner E, Thompson P, Luce M, et al. Homozygous inactivation of the NF1 gene in bone marrow cells from children with neurofibromatosis type 1 and malignant myeloid disorders. N Engl J Med 1997;336:1713-20.
30. Stephens K, Weaver M, Leppig KA, Maruyama K, Emanuel PD, Le Beau MM, et al. Interstitial uniparental isodisomy at clustered breakpoint intervals is a frequent mechanism of NF1 inactivation in myeloid malignancies. Blood 2006; 108:1684-9.
31. Flotho C, Steinemann D, Mullighan CG, Neale G, Mayer K, Kratz CP, et al. Genome-wide single nucleotide polymorphism analysis in juvenile myelomonocytic leukemia identifies uniparental disomy surrounding the NF1 locus in cases associated with neurofibromatosis but not in cases with mutant RAS or PTPN11. Oncogene 2007;26:5816-21.
32. Tartaglia M, Niemeyer CM, Song X, Buechner J, Hählen K, Hasle H, et al. Somatic PTPN11 mutations in juvenile myelomonocytic leukemia, myelodysplastic syndromes and acute myeloid leukemia. Nat Genet 2003;34:148-50.
33. Bader-Meunier B, Tchernia G, Mielot F, Fontaine JL, Thomas C, Lyonnet S, et al. Occurrence of myeloproliferative disorder in patients with Noonan syndrome. J Pediatr 1997;130:885-9.
34. Noonan JA. Hypertelorism with Turner phenotype. A new syndrome with associated congenital heart disease. Am J Dis Child 1968;116:373-80.
35. Tartaglia M, Mehler EL, Goldberg R, Zampino G, Brunner HG, Kremer H, et al. Mutations in PTPN11, encoding the protein tyrosine phosphatase SHP-2, cause Noonan syndrome. Nat Genet 2001;29:465-8.
36. Niihori T, Aoki Y, Ohashi H, Kurosawa K, Kondoh T, Ishikiriyama S, et al. Functional analysis of PTPN11/SHP-2 mutants identified in Noonan syndrome and childhood leukemia. J Hum Genet 2005;50:192-202.
37. Neel BG, Gu H, Pao L. The 'Shp'ing news: SH2 domain-containing tyrosine phosphatases in cell signaling. Trends Biochem Sci 2003;28:284-93.
38. Schubbert S, Lieuw K, Rowe SL, Lee CM, Li X, Loh ML, et al. Functional analysis of leukemia-associated PTPN11 mutations in primary hematopoietic cells. Blood 2005;106:311-7.
39. Roberts AE, Araki T, Swanson KD, Montgomery KT, Schiripo TA, Joshi VA, et al. Germline gain-of-function mutations in SOS1 cause Noonan syndrome. Nat Genet 2007;39:70-4.
40. Kratz CP, Niemeyer CM, Thomas C, Bauhuber S, Matejas V, Bergsträßer E, et al. Mutation analysis of Son of Sevenless in juvenile myelomonocytic leukemia. Leukemia 2007;21:1108-9.
41. de Vries ACH, Stam RW, Kratz CP, Zenker M, Niemeyer CM, van den Heuvel-Eibrink MM. Mutation analysis of the BRAF oncogene in juvenile myelomonocytic leukemia. Haematologica 2007;92:1574-5.
42. Pandit B, Sarkozy A, Pennacchio LA, Carta C, Oishi K, Martinelli S, et al. Gain-of-function RAF1 mutations cause Noonan and LEOPARD syndromes with hypertrophic cardiomyopathy. Nat Genet 2007;1007-12.
43. Busque L, Gilliland DG, Prchal JT, Sieff CA, Weinstein HJ, Sokol JM, et al. Clonality in juvenile chronic myelogenous leukemia. Blood 1995;85:21-30.
44. Jacks T, Shih TS, Schmitt EM, Bronson RT, Bernards A, Weinberg RA. Tumor predisposition in mice heterozygous for a targeted mutation in NF1. Nat Genet 1994;7:353-61.
45. Braun BS, Tuveson DA, Kong N, Le DT, Kogan SC, Rozmus J, et al. Somatic activation of oncogenic Kras in hematopoietic cells initiates a rapidly fatal myeloproliferative disorder. Proc Natl Acad Sci USA 2004;101:597-602.
46. Araki T, Mohi MG, Ismat FA, Bronson RT, Williams IR, Kutok JL, et al. Mouse model of Noonan syndrome reveals cell type- and gene dosage-dependent effects of Ptpn11 mutation. Nat Med 2004;10:849-57.
47. Downing JR, Shannon KM. Acute leukemia: a pediatric perspective. Cancer Cell 2002;2:437-45.
48. de Vries ACH, Stam RW, Schneider P, Niemeyer CM, van Wering E, Haas OA, et al. Role of mutation independent constitutive activation of FLT3 in juvenile myelomonocytic leukemia. Haematologica 2007;1557-60.
49. Lutz P, Zix-Kieffer I, Souillet G, Bertrand Y, Dhooge Y, Rubie H, et al. Juvenile myelomonocytic leukemia: analyses of treatment results in the EORTC childrens leukemia cooperative group (CLCG). Bone Marrow Transplant 1996;18:1111-6.
50. Woods WG, Barnard DR, Alonzo TA, Buckley JD, Kobrinsky N, Arthur DC, et al. Prospective study of 90 children requiring treatment for juvenile myelomonocytic leukemia or myelodysplastic syndrome: a report from the Children's Cancer Group. J Clin Oncol 2002;20:434-40.
51. Yoshimi A, Mohamed M, Bierings M, Urban C, Korthof E, Zecca M, et al. Second allogeneic hematopoietic stem cell transplantation (HSCT) results in outcome similar to that of first HSCT for patients with juvenile myelomonocytic leukemia. Leukemia 2007;21:556-60.
52. Castleberry RP, Emanuel PD, Zuckerman KS, Cohn S, Strauss L, Byrd RL, et al. A pilot study of isotretinoin in the treatment of juvenile chronic myelogenous leukemia. N Engl J Med 1994; 331:1680-4.
53. Pui CH, Aricò M. Isotretinoin for juvenile chronic myelogenous leukemia. N Engl J Med 1995;332:1520-1.
54. Emanuel PD, Snyder RC, Wiley T, Gopurala B, Castleberry RP. Inhibition of juvenile myelomonocytic leukemia cell growth in vitro by farnesyltransferase inhibitors. Blood 2000;95:639-45.
55. Castleberry RP, Loh ML, Jayaprakash N, Peterson A, Casey V, Chang M, et al. Phase II window study of the farnesyltransferase inhibitor R115777 (Zarnestra®) in untreated juvenile myelomonocytic leukemia (JMML): a Children's Oncology Group study. Blood 2005;106:727a[abstract].
56. Bernard F, Thomas C, Emile JF, Hercus T, Cassinat B, Chomienne C, et al. Transient hematologic and clinical effect of E21R in a child with end-stage juvenile myelomonocytic leukemia. Blood 2002;99:2615-6.
57. Aiyagari AL, Taylor BR, Aurora V, Young SG, Shannon KM. Hematologic effects of inactivating the Ras processing enzyme Rce1. Blood 2003;101:2250-2.
58. Shimada H, Shima H, Shimasaki N, Yoshihara H, Mori T, Takahashi T. Little response to zoledronic acid in a child of juvenile myelomonocytic leukemia (JMML) harboring the PTPN11 mutation. Ann Oncol 2005;16:1400.
59. Flotho C, Kratz CP, Niemeyer CM. Targeting RAS signaling pathways in juvenile myelomonocytic leukemia. Curr Drug Targets 2007;8:715-25.
60. Archambeault S, Yoshimi A, Kratz C, Reising M, Fischer A, Noellke P, et al. Development of an allele-specific minimal residual disease assay for patients with juvenile myelomonocytic leukemia-moving beyond clinical assessment. Blood 2006;108:758a[abstract].