Activating Mutations of the Noonan Syndrome-Associated SHP2/PTPN11 Gene in Human Solid Tumors and Adult Acute Myelogenous Leukemia

Mohamed Bentires-Alj,1,2 J. Guillermo Paez,3 Frank S. David,1,5 Heike Keilhack,1 Balazs Halmos,1 Katsuhiko Naoki,3 John M. Maris,4 Andrea Richardson,5 Alberto Bardelli,6 David J. Sugarbaker,7 William G. Richards,8 Jinyan Du,9 Luc Girard,10 John D. Minna,10 Mignon L. Loh,11 David E. Fisher,9 Victor E. Velculescu,12 Bert Vogelstein,12 Matthew Meyerson,3 William R. Sellers,3,13 and Benjamin G. Neel1

1Cancer Biology Program, Department of Medicine, Harvard Medical School, Beth Israel Deaconess Medical Center, Boston, Massachusetts; 2Laboratory of Medical Chemistry and Human Genetics, Center for Cellular and Molecular Therapy, University of Liège, Liège, Belgium; 3Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, Massachusetts; 4Division of Oncology, Children’s Hospital of Philadelphia, Philadelphia, Pennsylvania; 5Department of Pathology, Brigham and Women’s Hospital, Boston, Massachusetts; 6The Oncogenomics Center, Institute for Cancer Research and Treatment, University of Torino Medical School, Turin, Italy; 7Department of Surgical Services, Dana-Farber Cancer Institute, Boston, Massachusetts; 8Department of Surgery, Brigham and Women’s Hospital, Boston, Massachusetts; 9Department of Pediatric Hematology/Oncology, Dana-Farber Cancer Institute and Children’s Hospital, Boston, Massachusetts; 10Human Center for Therapeutic Oncology Research and Departments of Internal Medicine and Pharmacology, University of Texas Southwestern Medical Center, Dallas, Texas; 11Department of Pediatrics and Comprehensive Cancer Center, University of California, San Francisco, San Francisco, California; 12The Howard Hughes Medical Institute and the Sidney Kimmel Comprehensive Cancer Center, John Hopkins University Medical Institutions, Baltimore, Maryland; and 13Department of Medicine, Harvard Medical School, Broad Institute of Harvard and Massachusetts Institute of Technology, Boston, Massachusetts

Abstract

The SH2 domain-containing protein-tyrosine phosphatase PTPN11 (Shp2) is required for normal development and is an essential component of signaling pathways initiated by growth factors, cytokines, and extra-cellular matrix. In many of these pathways, Shp2 acts upstream of Ras. About 50% of patients with Noonan syndrome have germ-line PTPN11 gain of function mutations. Associations between Noonan syndrome and an increased risk of some malignancies, notably leukemia and neuroblastoma, have been reported, and recent data indicate that somatic PTPN11 mutations occur in children with sporadic juvenile myelomonocytic leukemia, myelodysplastic syndrome, B-cell acute lymphoblastic leukemia, and acute myelogenous leukemia (AML). Juvenile myelomonocytic leukemia patients without PTPN11 mutations have either homozygotic NF-1 deletion or activating RAS mutations. Given the role of Shp2 in Ras activation and the frequent mutation of RAS in human tumors, these data raise the possibility that PTPN11 mutations play a broader role in cancer. We asked whether PTPN11 mutations occur in other malignancies in which activating RAS mutations occur at low but significant frequency. Sequencing of PTPN11 from 13 different human neoplasms including breast, lung, gastric, and neuroblastoma tumors and adult AML and acute lymphoblastic leukemia revealed 11 missense mutations. Five are known mutations predicted to result in an activated form of Shp2, whereas six are new mutations. Biochemical analysis confirmed that several of the new mutations result in increased Shp2 activity. Our data demonstrate that mutations in PTPN11 occur at low frequency in several human cancers, especially neuroblastoma and AML, and suggest that Shp2 may be a novel target for antineoplastic therapy.

Introduction

Protein-tyrosine phosphatases (PTPs) have key positive (signal-enhancing) or negative (signal-attenuating) roles in a variety of normal signal transduction pathways. Mutations in PTPs and/or altered expression of PTPs can contribute to disease, including cancer, autoimmune disorders, inflammation, and/or developmental defects (1). The nonreceptor PTP Shp2, encoded by the gene PTPN11, is a positive (signal-enhancing) signaling component downstream of growth factor, cytokine, and extracellular matrix receptors and plays an important role in regulating cell growth, transformation, differentiation, and migration. Genetic and biochemical analysis have established that Shp2 is required for normal Ras activation in many of these pathways (1).

Dominant mutations in PTPN11 cause ~50% of cases of the developmental disorder Noonan syndrome (NS). Furthermore, associations between NS and increased risk of malignancy, notably leukemia (2) and possibly neuroblastoma (3), were reported in early studies. Subsequently, somatic PTPN11 mutations were found in ~35% of juvenile myelomonocytic leukemias (JMMs), 10% of childhood myelodysplastic syndromes, and at a lower incidence in other childhood hematopoietic disorders, including B-cell precursor acute lymphoblastic leukemia (~7%) and acute myelogenous leukemia (AML) (~4%) (4–6).

Shp2 has two Src homology 2 domains at its NH2 terminus (N-SH2 and C-SH2, respectively), a catalytic (PTP) domain, and a COOH terminus containing tyrosyl phosphorylation sites. In the basal state, the PTP domain is inhibited by intramolecular interaction with NSH2. Phosphotyrosyl peptide binding to the N-SH2 domain induces a conformational change that reverses this inhibition and activates Shp2 (1, 7). Most PTPN11 mutations in NS and leukemia affect N-SH2 or PTP domain residues involved in basal inhibition of Shp2 (4). The location of these mutations, the crystal structure of Shp2, our work on activated mutants of Shp2 (1), molecular dynamic simulations (8), and functional and biochemical analysis (4) suggest that NS/leukemia mutations are “activated mutants.”

Nearly all JMML cases without PTPN11 mutations have either an activating RAS mutation or homozygotic inactivation of the neurofibromatosis type-1 (NF1) gene, whose protein product, neurofibromin, is a Ras-GTPase activating protein (RasGap). Given the role of Shp2 in Ras/extracellular signal-regulated kinase (ERK) activation, these findings raise the possibility that Shp2 alterations play a role in other human malignancies that have a low frequency of RAS mutations but

Received 6/1/04; revised 9/29/04; accepted 10/22/04.

Grant support: Supported by National Institutes of Health grants R01 CA9152 (B. Neel) and CA34660 (B. Vogelstein) and grants from the International Agency for Research on Cancer and European Molecular Biology Organization (M. Bentires-Alj) and partially supported by the Dana-Farber/Harvard Specialized Programs of Research Excellence in breast cancer.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Note: M. Bentires-Alj is a Research Assistant at the National Fund for Scientific Research.

Requests for reprints: Mohamed Bentires-Alj, Cancer Biology Program, New Research Building 1038, 77 Louis Pasteur Avenue, Boston, MA 02215. Phone: 617-667-6889; Fax: 617-667-6810; E-mail: mbentire@bidmc.harvard.edu.

©2004 American Association for Cancer Research.
demonstrate an activated Ras/ERK pathway (9). Here, we screened such tumors for PTPN11 mutations.

Materials and Methods

Complementary RNA and Genomic DNA. Generation of primary lung adenocarcinoma cRNAs was described previously (10). Genomic DNA was extracted from previously characterized human lung cancer cell lines using standard techniques. Genomic DNA from human breast cancer specimens was obtained from the Dana-Farber/Harvard Cancer Center Breast Program Tissue Resource. Paraffin-embedded samples of human gastric cancer were obtained from the Department of Pathology at the Brigham and Women’s Hospital (Boston, MA), and DNA was purified using the QIAamp DNA extraction kit (Qiagen, Valencia, CA). Neuroblastoma DNAs were obtained from the Children’s Oncology Group Neuroblastoma Nucleic Acids Bank (Children’s Hospital of Philadelphia). DNA samples from human colon tumors were described previously (11). DNA samples from prostate cancers were obtained from the Department of Medical Oncology at the Dana-Farber Cancer Institute (Boston, MA); AML, acute lymphoblastic leukemia (ALL), and polycythemia vera (PV) DNAs were provided by the Department of Medicine at the Brigham and Women’s Hospital, and melanoma DNAs were from the Department of Pediatric Hematology/Oncology at the Dana-Farber Cancer Institute and Children’s Hospital (Boston, MA). DNA from astrocytomas and medulloblastomas was from the Department of Cancer Biology at the Dana-Farber Cancer Institute, and glioblastoma cell lines were from the American Type Culture Collection.

All studies were approved by the institutional review boards of the Beth Israel-Deaconess Medical Center and other participating institutions.

Reverse Transcription-Polymerase Chain Reaction, Genomic DNA Polymerase Chain Reaction, and DNA Sequencing. For sequencing cRNAs, reverse transcription-polymerase chain reaction (RT-PCR) was performed using Superscript One-Step RT-PCR and the Platinum Taq kit (Life Technologies). PCR was performed using M13-flanked primers (Table 1). Reactions were performed in a 25-μL volume containing 5 ng of genomic DNA, 0.1 μL (0.5 unit) of Platinum Taq DNA polymerase (Life Technologies, Inc.), 1 μL of 10 μmol/L stock solution of each primer, 1 μL of 50 mmol/L MgCl2, 0.5 μL of 10 mmol/L deoxynucleotide triphosphate mix, and 2.5 μL of 10× PCR buffer. Cycling parameters were as follows: 8 minutes at 94°C, 34 cycles of amplification consisting of 45 seconds at 94°C, 30 seconds at 60°C (exons 2, 3, 5, 10, 11, 13, 14, and 15) or 30 seconds at 57°C (exons 4, 6, 7, 8, 9, and 12) and 45 seconds at 72°C, followed by a final extension step of 72°C for 10 minutes. Amplified DNAs were sequenced by Agencourt Bioscience Corp. (Beverly, MA).

Protein-Tyrosine Phosphatase Assays. PTP activity assays were performed using the artificial substrate RCM-lysozyme, essentially as described previously (13).

Results and Discussion

Genomic DNA was obtained from 65 lung cancer cell lines, 9 prostate cancer cell lines, 15 prostate tumors, 100 breast tumors, 40 gastric tumors, 189 colon tumors, 65 AMLs, 11 ALLs, 5 PVs, 10 melanomas, 9 astrocytomas, 9 glioblastomas, 9 medulloblastomas, and 89 neuroblastomas. We also analyzed cRNAs from 118 well-characterized lung tumors (10). The whole PTPN11 coding region of 118 lung, 24 colon, and 40 gastric carcinomas was sequenced. For the remaining samples, we sequenced either exon 3 alone (165 colon cancers) or exons 2, 3, 4, 5, 7, 8, and 13, which comprise the SH2 and PTP domains (all other neoplasms). These regions were chosen for more intensive sequencing because nearly all reported associated PTPN11 mutations lie within them (2, 4–6).

Eleven somatic missense mutations of PTPN11 were found in these samples (Table 2). Five of these are mutations known or predicted to result in an activated form of Shp2. Six of the mutations have not been described previously. We found an additional 87 silent nucleotide changes (Table 3), all of which have been reported previously as single nucleotide polymorphisms (SNPs) in control individuals (2). These changes include 81 intronic SNPs and 6 synonymous changes in exon 3.

Table 1 Exon-specific and M13 flanked nested PCR primers used in amplification of PTPN11 genomic DNA

<table>
<thead>
<tr>
<th>Exon</th>
<th>Primer Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>CTGATCAGATCCGAGACTGCA</td>
</tr>
<tr>
<td>3</td>
<td>TGTGTCGGCTTCTTTCAGTAC</td>
</tr>
<tr>
<td>4</td>
<td>GATCACTGTCAGGTTGAGGAG</td>
</tr>
<tr>
<td>5</td>
<td>AGTCTGGTTCATTTTTCAGT</td>
</tr>
<tr>
<td>6</td>
<td>TCGATTACACCTTTTCAACT</td>
</tr>
<tr>
<td>7</td>
<td>AGTCTGGTTCATTTTTCAGT</td>
</tr>
<tr>
<td>8</td>
<td>GCATTGCCAGATCGCACCG</td>
</tr>
<tr>
<td>9</td>
<td>ACATCACCCGACTCATCAGCT</td>
</tr>
<tr>
<td>10</td>
<td>TTCCTTCTGCTTCTATCTTCT</td>
</tr>
<tr>
<td>11</td>
<td>CTGATCAGATCCGAGACTGCA</td>
</tr>
<tr>
<td>12</td>
<td>CTGATCAGATCCGAGACTGCA</td>
</tr>
<tr>
<td>13</td>
<td>CTGATCAGATCCGAGACTGCA</td>
</tr>
<tr>
<td>14</td>
<td>CTGATCAGATCCGAGACTGCA</td>
</tr>
<tr>
<td>15</td>
<td>CTGATCAGATCCGAGACTGCA</td>
</tr>
</tbody>
</table>

D61Y, a known mutation in childhood leukemia, and three new missense mutations in the N- or K-RAS genes occur in 15% to 30% of AML patients. In 65 adult AML samples, we found four PTPN11 mutations: D61Y, a known mutation in childhood leukemia, and three new
mutations, E69V, R289G, and G503V. Although E69V has not been reported previously, E69Q and E69K have been found in NS and JMML, respectively (4, 14). Asp61 and Glu69 are located within the reported previously, E69Q and E69K have been found in NS and mutations, E69V, R289G, and G503V. Although E69V has not been in parentheses.

whether these lines have unclear. Two lie within the N-SH2 domain: Y62C, a new mutation in the wild-type (WT) PTPN11 mutations described herein. PTP assays carried out with the artificial substrate RCM-lysozyme revealed that the N-SH2 mutations and instead showed decreased catalytic activity in this assay. Additionally, the C-SH2 does not make significant contact with the N-SH2/PTP domain interface. Asn58 is a key residue in the N-SH2/PTP interface hydrogen bonding network (1, 7), pocket rather than the N-SH2/PTP domain interface. Asn58 is a key residue in the N-SH2/PTP interface hydrogen bonding network (1, 7), so N58S is probably an activating mutant. Consistent with our findings in neuroblastomas, the H661 cell line has neither BRAF nor RAS mutations (18). HCC1171 has a G12C RAS mutation, however, suggesting that in some cases, PTPN11 mutations may collaborate with other activating mutations in the Ras/ERK pathway. Although Shp2 is clearly required for Ras/ERK activation, several studies indicate actions downstream or parallel to Ras as well (1).

We also found one known PTPN11 mutation in a colon tumor: E76G. This residue, which maps to the NS-H2/PTP interface, is a hot spot for JMML mutations. The corresponding normal DNA of this sample lacked this mutation, demonstrating its somatic nature. This particular tumor exhibits “nucleotide instability” with an increased frequency of somatic alterations, but without microsatellite instability. It has WT RAS, but a mutated B-RAF (R461I).

In melanoma, wherein B-RAF, N-RAS, or K-RAS mutations occur in >60% of cases (19), we found one new mutation (R138Q), located in phosphotyrosyl peptide binding pocket of the C-SH2 domain. This motif is critical for the binding of SH2 domains to tyrosine-phosphorylated residues. The corresponding normal DNA of this sample lacks this mutation, demonstrating that it is not a polymorphism. Because the C-SH2 does not make significant contact with the N-SH2/PTP domain interface, and its role in activation remains controversial, additional experiments are required to address the mechanistic significance of this mutation.

No mutations were found in astrocytoma, glioblastoma, medulloblastoma, ALL, PV, and breast, prostate, and gastric cancers. This could be explained by the low number of samples tested (glioma, PV, and ALL) and/or by the possibility that other oncogenic changes, such as ERBB2 amplification (breast cancer), can activate the Ras/ERK pathway in these tumors.

Finally, we tested the biochemical effects of several of the new PTPN11 mutations described herein. PTP assays carried out with the artificial substrate RCM-lysozyme revealed that the N-SH2 mutations V45L (3.5 X), Y62C (2.5 X), and E69K (15.5 X) all were basally activated compared with WT Shp2 (Fig. 2C). In contrast, the PTP domain mutation R289G found in an AML patient was not activated and instead showed decreased catalytic activity in this assay. Additional studies will be required to determine whether this mutant is less stable under these conditions, whether it is activated only against

<table>
<thead>
<tr>
<th>Tumor type</th>
<th>No. of cases</th>
<th>Exon</th>
<th>Mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung adenocarcinoma (1)</td>
<td>118</td>
<td>3</td>
<td>E76V</td>
</tr>
<tr>
<td>Lung cancer cell lines (2)</td>
<td>65</td>
<td>2</td>
<td>V45L</td>
</tr>
<tr>
<td>Colon cancer (1)</td>
<td>196</td>
<td>3</td>
<td>E76G</td>
</tr>
<tr>
<td>AML (4)</td>
<td>65</td>
<td>3</td>
<td>D61Y</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>E69V</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8</td>
<td>R289G</td>
</tr>
<tr>
<td></td>
<td></td>
<td>13</td>
<td>G503V</td>
</tr>
<tr>
<td>Neuroblastoma (3)</td>
<td>89</td>
<td>3</td>
<td>Y62C</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>E69K</td>
</tr>
<tr>
<td></td>
<td></td>
<td>13</td>
<td>T507K</td>
</tr>
<tr>
<td>Melanoma (1)</td>
<td>10</td>
<td>4</td>
<td>R138Q</td>
</tr>
</tbody>
</table>

NOTE. The total number of nucleotide changes is indicated in parentheses.
some substrates (and not the artificial substrate tested here), and/or whether PTPN11 mutations can contribute to oncogenesis by mechanisms other than increased basal PTP activity. Furthermore, because we do not have DNA from the normal tissue of this patient, we cannot exclude that R289G is a rare, not previously reported SNP. Notably, V45L, which does appear to be a functionally significant PTPN11 mutation, is encoded by exon 2, which is often excluded from screens for disease-associated PTPN11 mutations. This finding, together with data indicating that T42A, a NS-associated mutant, also is enzymatically activated, argues for caution in interpreting negative findings from sequencing only the more commonly affected exons 3 and 13 of PTPN11.

RAS mutations are found in many human malignancies (9). Other tumors exhibit ERK activation but have normal RAS. Such tumors can have mutations in other Ras/ERK pathway components. For example, >60% of melanomas have B-RAF mutations (19), and >60% of colorectal cancers have either RAS or B-RAF mutations that occur in a mutually exclusive fashion (20). Taken together, the previously described studies of childhood leukemias, the known role of Shp2 as a regulator of the Ras/ERK pathway, and the present findings provide...
evidence that sporadic PTPN11 mutations contribute to the pathogenesis of other human tumors. Indeed, in preliminary studies, we have found that small interfering RNA-mediated knockdown of Shp2 (WT and N58S mutant) impairs basal and EGF-induced ERK activation in H661 cells (data not shown). Further work is needed to determine the effects of selective elimination of the mutant Shp2 protein in these cells.

Although PTPN11 mutations are rare, alterations in other signaling molecules have recently been shown to have dramatic pathophysiological significance. For example, activating EGFR mutations are also infrequent but predict clinical response of NSCLC to the EGFR inhibitor gefitinib (Iressa) (16, 17). Thus, Shp2 may be a novel target for antineoplastic therapy, particularly in AML and neuroblastoma.

Acknowledgments

We thank Dr. Gordon Chan for help with the figures and the Children’s Oncology Group for providing the neuroblastoma DNAs.

References

Activating Mutations of the Noonan Syndrome-Associated SHP2/PTPN11 Gene in Human Solid Tumors and Adult Acute Myelogenous Leukemia

Mohamed Bentires-Alj, J. Guillermo Paez, Frank S. David, et al.

Cancer Res 2004;64:8816-8820.

Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/64/24/8816

Cited articles
This article cites 20 articles, 8 of which you can access for free at:
http://cancerres.aacrjournals.org/content/64/24/8816.full#ref-list-1

Citing articles
This article has been cited by 59 HighWire-hosted articles. Access the articles at:
http://cancerres.aacrjournals.org/content/64/24/8816.full#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.