Oncogene Mutation Profiling of Pediatric Solid Tumors Reveals Significant Subsets of Embryonal Rhabdomyosarcoma and Neuroblastoma with Mutated Genes in Growth Signaling Pathways

Neerav Shukla1, Nabahet Ameur2, Ismail Yilmaz2, Khedoudja Nafa2, Chyau-Yueh Lau2, Angela Marchetti2, Laetitia Borsu2, Frederic G. Barr4, and Marc Ladanyi2,3

Abstract

Purpose: In contrast to the numerous broad screens for oncogene mutations in adult cancers, few such screens have been conducted in pediatric solid tumors. To identify novel mutations and potential therapeutic targets in pediatric cancers, we conducted a high-throughput Sequenom-based analysis in large sets of several major pediatric solid cancers, including neuroblastoma, Ewing sarcoma, rhabdomyosarcoma (RMS), and desmoplastic small round cell tumor (DSRCT).

Experimental Design: We designed a highly multiplexed Sequenom-based assay to interrogate 275 recurrent mutations across 29 genes. Genomic DNA was extracted from 192 neuroblastoma, 75 Ewing sarcoma, 89 RMS, and 24 DSRCT samples. All mutations were verified by Sanger sequencing.

Results: Mutations were identified in 13% of neuroblastoma samples, 4% of Ewing sarcoma samples, 21.1% of RMS samples, and no DSRCT samples. ALK mutations were present in 10.4% of neuroblastoma samples. The remainder of neuroblastoma mutations involved the BRAF, RAS, and MAP2K1 genes and were absent in samples harboring ALK mutations. Mutations were more common in embryonal RMS (ERMS) samples (28.3%) than alveolar RMS (3.5%). In addition to previously identified RAS and FGFR4 mutations, we report for the first time PIK3CA and CTNNB1 (β-catenin) mutations in 5% and 3.3% of ERMS, respectively.

Conclusions: In ERMS, Ewing sarcoma, and neuroblastoma, we identified novel occurrences of several oncogene mutations recognized as drivers in other cancers. Overall, neuroblastoma and ERMS contain significant subsets of cases with nonoverlapping mutated genes in growth signaling pathways. Tumor profiling can identify a subset of pediatric solid tumor patients as candidates for kinase inhibitors or RAS-targeted therapies.

Clin Cancer Res; 18(3); 748–57. ©2011 AACR.

Introduction

Many different tumor types harbor somatic gene mutations which contribute to tumor development and can also serve as promising therapeutic targets. Examples of therapeutically relevant mutations include KIT and PDGFRA mutations in gastrointestinal stromal tumors, EGFR mutations in lung adenocarcinomas, and BRAF mutations in melanoma (1–4). More recently, activating ALK mutations have been identified in a subset of neuroblastomas, providing a rationale to search for therapeutically susceptible mutations in pediatric tumors (5, 6).

Numerous large-scale gene profiling studies have been conducted to identify novel mutations in various tumor types (7–9). However, broad mutational profiling studies have not been conducted on a large cohort of neuroblastoma and pediatric sarcoma samples. Furthermore, although ALK harbors recurrent mutations in neuroblastoma, ALK-mutated tumors account for less than 15% of neuroblastoma cases (5), raising the possibility of alternative signaling gene mutations in additional subsets of this cancer.

The Sequenom MassARRAY technology uses a mass spectrometry–based genotyping approach which allows for...
more sensitive mutational analysis than traditional Sanger sequencing (8). This platform also does well using DNA extracted from archived paraffin-embedded material. Furthermore, highly multiplexed PCR assays allow for efficient high-throughput screening of large tumor sample sets (9).

To identify novel mutations in pediatric tumors, we used the Sequenom MassARRAY platform to conduct a high-throughput sequencing analysis interrogating 275 point mutations across 29 oncogenes in large sample sets of 4 tumor types: neuroblastoma, Ewing sarcoma, rhabdomyosarcoma (RMS), and desmoplastic small round cell tumors (DSRCT). In addition to known recurrent somatically mutated genes, we included an extended panel of 

**PIK3CA** and **CTNNB1** (β-catenin) mutations in ERMS. Overall, neuroblastoma and ERMS contain significant subsets of cases with nonoverlapping mutations in growth signaling pathways. In particular, a substantial minority of ERMS show mutations in the RAS pathway. As more agents inhibiting mutated oncoproteins and their associated downstream signaling pathways become available, mutational genotyping of pediatric solid tumors such as RMS will serve as an important tool for identifying targeted therapy options for individual patients.

**Materials and Methods**

**Tumor samples**

All frozen tumor samples and formalin-fixed paraffin-embedded (FFPE) samples were procured at Memorial Sloan-Kettering Cancer Center (MSKCC) under Institutional Review Board–approved protocols. Genomic DNA was extracted using the Qiagen DNeasy Kit according to the manufacturer’s directions. DNA from 192 neuroblastoma samples, 75 Ewing sarcoma, 89 RMS, and 24 DSRCT samples and cell lines was collected. Cell lines used in the study are listed in Supplementary Table S1. Testing for EWSR1 and PAX/FKHR rearrangements was conducted by reverse transcriptase PCR or FISH, as previously described (14).

**Curation of oncogene point mutations**

We queried the Sanger Institute COSMIC online database for recurrent nonsynonymous point mutations known to occur in different human cancer types. Mutations were selected on the basis of their susceptibility as a therapeutic target and mutation frequency. Furthermore, an extended list of **BRAF** and **PTPN11** mutations was generated on the basis of known germ line mutations in RAS/MAPK syndromes (10, 11). Insertion and deletion type mutations were not included. A total of 275 point mutations (Supplementary Table S2) across 29 genes were selected for interrogation (Table 1). Input sequence files for assay design were generated by the MSKCC Bioinformatics Core Facility.

**Sequenom-based DNA genotyping**

For these assays, we used the MassARRAY system (Sequenom), which is based on matrix-assisted laser desorption/ ionization—time-of-flight/mass spectrometry (MALDI-TOF/MS). Genotyping by this method relies on the principle that mutant and wild-type alleles for a given point mutation produce single-allele base extension reaction products of a mass that is specific to the sequence of the product. Mutation calls are based on the mass differences between the wild-type product and the mutant products as resolved by MALDI-TOF/MS. Amplification and extension primers were designed using Sequenom Assay Designer v3.1 software to target the curated list of mutations. Amplification primers were designed with a 10-mer tag sequence to increase their mass so that they fall outside the range of detection of the MALDI-TOF/MS. The sequencing of primers are shown in Supplementary Table S3.

The initial PCR amplification was conducted in a 5-μL reaction mixture containing 10 to 20 ng of DNA/1.25× buffer; 1.625 mmol/L MgCl2; 500 μmol/L deoxynucleotide triphosphate (dNTP); 100 nmol/L from each primer; and 0.5 units of HotStarTaq DNA polymerase (Qiagen) under the following conditions: 95°C (15 minutes); 95°C (20 seconds); 56°C (30 seconds); and 72°C (60 seconds) for 45 cycles, and a final extension phase at 72°C (3 minutes). Remaining unincorporated dNTPs were dephosphorylated by adding 2 μL of a shrimp alkaline phosphatase cocktail containing 1.53 μL of water, 0.17 μL of reaction buffer (Sequenom), and 0.3 μL of shrimp alkaline phosphatase (Sequenom). The unincorporated dNTPs were then placed in a thermal cycler under the following conditions: 37°C for 40 minutes, 85°C for 5 minutes, and then held at 4°C indefinitely. A single-base extension reaction was then carried out in a 2-μL TypePLEX reaction mix (Sequenom) consisting of 0.72 μL water, 0.20 μL TypePLEX 10× buffer (Sequenom); 0.10 μL TypePLEX terminator mix
Whole genome amplification

Whole genome amplification (WGA) was conducted on 62 RMS samples and 74 EWS samples for sequencing of FGFR4 and CTNNB1. For frozen tissue, the Repli-G Midi (Qiagen) was used to amplify 100 ng of genomic DNA. WGA DNA was assessed by Picogreen quantification followed by PCR amplification with two control amplicons. For FFPE samples, the Sigma GenomePlex (Sigma-Aldrich) was used to amplify 100 ng of genomic DNA. WGA DNA was assessed by Picogreen quantification followed by PCR amplification with two control amplicons.

PCR amplification and DNA sequencing of WGA samples

All exons of FGFR4 and exon 3 of CTNNB1 were amplified as amplicons of 500 bp or less, covering the exonic regions plus at least 50 bp of intronic sequences (Supplementary Table S4). M13 tails were added to the primers to facilitate Sanger sequencing. RMS samples were evaluated for FGFR4 and CTNNB1 mutations. Ewing sarcoma samples were evaluated for CTNNB1 mutations. PCR reactions were carried out in 384-well plates, in a Duncan DT-24 water bath thermal cycler, with 10 ng of whole genome amplified DNA as template, using a touchdown PCR protocol with PCR reactions carried out in 384-well plates, in a Duncan DT-24 water bath thermal cycler, with 10 ng of whole genome amplified DNA (Repli-G Midi, Qiagen) as template, using a touchdown PCR protocol with HotStart Kapa Fast Taq (Kapa Biosystems). The touchdown PCR method consisted of 1 cycle of 95°C for 5 minutes; 3 cycles of 95°C for 30 seconds, 64°C for 15 seconds, 72°C for 30 seconds; 3 cycles of 95°C for 30 seconds, 62°C for 15 seconds, 72°C for 30 seconds; 3 cycles of 95°C for 30 seconds, 60°C for 15 seconds, 72°C for 30 seconds; 3 cycles of 95°C for 30 seconds, 58°C for 15 seconds, 72°C for 30 seconds; 3 cycles of 95°C for 30 seconds, 45°C for 15 seconds, 72°C for 30 seconds; and 1 cycle of 70°C for 5 minutes. Templates were purified using AMPure (Agencourt Biosciences). The purified PCR reactions were split into two and sequenced bidirectionally with M13 forward and reverse primer and Big Dye Terminator Kit v.3.1 (Applied Biosystems), at Agencourt Biosciences. Dye terminators were removed using the CleanSEQ Kit (Agencourt Biosciences), and sequence reactions were run on ABI PRISM 3730xl sequencing apparatus (Applied Biosystems). Mutations were detected using an automated detection pipeline at the MSKCC Bioinformatics Core. Bidirectional reads and mapping tables (to link read names to sample identifiers, gene names, read direction, and amplicon) were subjected to a QC filter which excludes reads that have an average phred score of <10 for bases 100 to 200. Passing reads were assembled against the reference sequences for each gene, containing all coding and untranslated region exons including 5 Kb upstream and downstream of the gene, using command line Consed 16.0 (PMID: 9521923). All traces for mutation calls were manually reviewed and validated with original non-WGA DNA.

PCR amplification and DNA sequencing for mutation validation

All identified mutations were verified by standard Sanger sequencing. Forward and reverse primers are listed in Supplementary Table S3. Each PCR reaction was carried out in a 50-μL volume mixture containing 100 ng of genomic DNA; forward and reverse primers (20 pmol each); 200 μmol/L of each dNTP; 1.5 mmol/L MgCl₂; 1× Qiagen PCR buffer containing 1.5 mmol/L MgCl₂ and 2.5 units of HotStarTaq DNA polymerase (Qiagen). The PCR amplification was carried out under the following conditions: 1 cycle at 95°C (15 minutes); 40 cycles at 94°C (30 seconds), at 60°C (30 seconds), and at 72°C (60 seconds); and a final extension step at 72°C (10 minutes). Amplified products were purified using Spin Columns (Qiagen) and sequenced in both directions using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems), according to the manufacturer’s protocol, on

---

Table 1. Oncogenes included in the Sequenom panel

<table>
<thead>
<tr>
<th>Gene</th>
<th>Gene</th>
<th>Gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>AKT1</td>
<td>FGFR3</td>
<td>MET</td>
</tr>
<tr>
<td>AKT2</td>
<td>FLT3</td>
<td>NOTCH1</td>
</tr>
<tr>
<td>AKT3</td>
<td>GNAQ</td>
<td>NRAS</td>
</tr>
<tr>
<td>ALK</td>
<td>HRAS</td>
<td>PDGFRA</td>
</tr>
<tr>
<td>BRAF</td>
<td>IDH1</td>
<td>PIK3CA</td>
</tr>
<tr>
<td>CDK4</td>
<td>IDH2</td>
<td>PIK3R1</td>
</tr>
<tr>
<td>CTNNB1</td>
<td>JAK2</td>
<td>PTENN1</td>
</tr>
<tr>
<td>EGFR</td>
<td>KIT</td>
<td>RET</td>
</tr>
<tr>
<td>ERBB2</td>
<td>KRAS</td>
<td>SMO</td>
</tr>
<tr>
<td>FGFR2</td>
<td>MAP2K1</td>
<td></td>
</tr>
</tbody>
</table>

NOTE: The panel included 29 genes with known recurrent oncogenic mutations and 275 nucleotide changes were interrogated.
an ABI3730xl running ABI Prism DNA Sequence Analysis Software (Applied Biosystems).

Results

Mutations were identified by Sequenom analysis in 13% (25 of 192) of neuroblastoma samples, 4% (3 of 75) of Ewing sarcoma samples, 20.2% of RMS samples (18 of 89), and 0% of DSRCT samples (Tables 2–4). Clinical information on the patients with mutated samples is provided in Supplementary Table S6. Mutations were found in 28.3% (17 of 60) of embryonal RMS (ERMS) tumors, with 13 of 60 (21.7%) identified by Sequenom analysis and the remainder identified by direct sequencing of \(FGFR4\) (described below). Mutations were observed in 3.5% (1 of 29) of alveolar RMS (ARMS) samples. The majority of neuroblastoma mutations were activating \(ALK\) mutations (Table 5), accounting for 10.4% of the tumor samples, a proportion consistent with previous reports (15). The remainder of neuroblastoma mutations involved \(BRAF\), \(RAS\), and \(MAP2K1\) genes and were not seen in samples harboring \(ALK\) mutations.

\(RAS\) family mutations were found in a subset of neuroblastoma, RMS, and Ewing sarcoma samples. About 11.7% (7 of 60) of ERMS samples, 3.5% (1 of 29) of ARMS samples, 1% (2 of 192) of neuroblastoma samples, and 1.3% (1 of 75) of Ewing sarcoma samples harbored \(RAS\) mutations on our panel. \(BRAF\) mutations were identified in 1% (2 of 192) of neuroblastoma samples, 1.7% (1 of 60) of ERMS samples, and 1.3% (1 of 75) of Ewing sarcoma samples. The ERMS and Ewing sarcoma samples harbored the activating \(BRAF^{V600E}\) mutation. One neuroblastoma sample carried the \(BRAF^{V559V}\) mutation, whereas the other carried the less common \(BRAF^{D594V}\) mutation. A \(MAP2K1^{K57N}\) mutation was identified in one neuroblastoma sample. \(PIK3CA\) mutations were identified in 5% (3 of 60) of ERMS cases but were not seen in any other tumor type in our

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>MYCN amplification</th>
<th>ALK mutation</th>
<th>\ Gene mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>NB32</td>
<td>No</td>
<td>No</td>
<td>(BRAF^{1783T&gt;C}) (F595L)</td>
</tr>
<tr>
<td>NB158</td>
<td>No</td>
<td>No</td>
<td>(BRAF^{1799T&gt;A}) (V600E)</td>
</tr>
<tr>
<td>NB23</td>
<td>Yes</td>
<td>No</td>
<td>(KRAS^{35G&gt;T}) (G12V)</td>
</tr>
<tr>
<td>NB139</td>
<td>No</td>
<td>No</td>
<td>(MAP2K1^{171G&gt;T}) (K57N)</td>
</tr>
<tr>
<td>NB151</td>
<td>No</td>
<td>No</td>
<td>(NRAS^{181C&gt;A}) (Q61K)</td>
</tr>
</tbody>
</table>

**Table 3.** Summary of identified mutations in neuroblastoma (\(n = 192\); \(ALK\) mutations are not included)
screen (Fig. 1). Two of the 3 mutations were in the helical domain of the gene (E542K and E545K). The other mutation was located in the kinase domain (H1047R).

CTNNB1 mutations were identified in 1.3% (1 of 75) of Ewing sarcoma samples and 3.3% (2 of 60) of ERMS samples (Fig. 2). One of the ERMS samples contained both an NRAS and a CTNNB1 gene mutation.

A PTPN11 mutation was identified in 1.7% (1 of 60) ERMS samples. The tumor was from a patient diagnosed with neurofibromatosis type 1. Sequencing of normal tissue from this patient confirmed the PTPN11 mutation to be somatic.

Full sequencing of the coding exons of FGFR4 and β-catenin was conducted in 43 ERMS and 19 ARMS samples. Similarly, full sequencing of β-catenin was conducted on 76 Ewing sarcoma tumor samples. The V550L FGFR4 mutation was identified in 9.3% (4 of 43) ERMS tumors and none of the ARMS tumors. Sequencing of matched normal tissue, available for 2 of the 4 samples, confirmed the FGFR4 mutations to be somatic in both cases. Of the remaining 2 FGFR4-mutated samples, one lacked corresponding normal tissue and the other was a cell line (RMS 559). Additional CTNNB1 mutations, aside from those detected by the Sequenom assay, were not found in any of the RMS or Ewing sarcoma samples.

Discussion

Multiple large-scale cancer genomics efforts are underway to better characterize tumors and to help identify new therapeutic targets but few of these include pediatric solid tumors. Several genes have been examined for mutations in various pediatric tumor types (5, 16–27), but the present study represents the most expansive "hotspot" mutation profiling for these tumor types that has been reported to date. Here, we used a mass spectrometry–based platform to survey recurrent point mutations in 29 cancer genes in 380 cases of 4 major pediatric solid cancers.

The frequency of mutations found in our study was lower than in most adult onset carcinomas. Given the embryonal origin of these pediatric cancers, it is also possible that different genes are recurrently mutated in these tumor types. More comprehensive approaches such as whole exome sequencing would be needed to identify such mutations. However, a significant proportion of ERMS tumor samples were found to harbor various targetable mutations from our focused screen (Fig. 3).

RAS mutations are known to occur in a minority of patients with ERMS (17, 28). More recently, Paulson and colleagues identified RAS mutations in 11 of 26 (42%) of ERMS samples. Furthermore, they identified NF1 gene deletions in 15% of ERMS samples, representing an alternative mechanism of RAS pathway activation (29).

In our study, 7 cases of ERMS harbored a RAS mutation. A codon 61 mutation of NRAS was found in 4 samples, including the RD cell line, as previously reported (30). Two cases had KRAS codon 12 mutations. The ERMS cell line SMS-CTR was found to have an HRAS Q61K mutation. One of the 29 ARMS samples was found to have a RAS mutation. It was the sole mutation found in our cohort of ARMS samples. Our mutation data suggest that overall, RAS mutations are more typical of translocation-negative RMS but this difference may require a larger sample set to confirm statistically.

To our knowledge, RAS mutations have not been described in neuroblastoma or Ewing sarcoma. Whereas

### Table 4. Summary of identified mutations in Ewing Sarcoma (n = 75)

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Translocation</th>
<th>Gene mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>ES2 (A673 cell line)</td>
<td>EWS-FLI1</td>
<td>BRAF_1799T&gt;A (V600E)</td>
</tr>
<tr>
<td>ES9</td>
<td>EWS-FLI1</td>
<td>CTNNB1_133_135delTCT (S45del)</td>
</tr>
<tr>
<td>ES17</td>
<td>EWS-ERG</td>
<td>NRAS_181C&gt;A (Q61K)</td>
</tr>
</tbody>
</table>

### Table 5. ALK mutations detected by the Sequenom panel

<table>
<thead>
<tr>
<th>Neuroblastoma sample</th>
<th>ALK mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>NB58</td>
<td>F1174C</td>
</tr>
<tr>
<td>NB10</td>
<td>F1174L</td>
</tr>
<tr>
<td>NB174</td>
<td>F1174L</td>
</tr>
<tr>
<td>NB191</td>
<td>F1174L</td>
</tr>
<tr>
<td>NB3</td>
<td>F1174L</td>
</tr>
<tr>
<td>NB30</td>
<td>F1174L</td>
</tr>
<tr>
<td>NB39</td>
<td>F1174L</td>
</tr>
<tr>
<td>NB4</td>
<td>F1174L</td>
</tr>
<tr>
<td>NB8</td>
<td>F1174L</td>
</tr>
<tr>
<td>NB83</td>
<td>F1174L</td>
</tr>
<tr>
<td>NB188</td>
<td>F1174L</td>
</tr>
<tr>
<td>NB62</td>
<td>F1245C</td>
</tr>
<tr>
<td>NB12</td>
<td>F1245V</td>
</tr>
<tr>
<td>NB109</td>
<td>R1275Q</td>
</tr>
<tr>
<td>NB129</td>
<td>R1275Q</td>
</tr>
<tr>
<td>NB134</td>
<td>R1275Q</td>
</tr>
<tr>
<td>NB135</td>
<td>R1275Q</td>
</tr>
<tr>
<td>NB171</td>
<td>R1275Q</td>
</tr>
<tr>
<td>NB2</td>
<td>R1275Q</td>
</tr>
<tr>
<td>NB44</td>
<td>R1275Q</td>
</tr>
</tbody>
</table>

**NOTE:** ALK mutations were identified in 20 of 192 (10.4%) neuroblastoma samples. No other mutations were identified in these 20 tumors. ALK mutations were not identified in RMS, Ewing sarcoma, or DSRCT samples.
two studies have reported completely negative results (16, 19), another study described a rare codon 59 NRAS mutation in a neuroblastoma cell line, which was not seen when the original tumor DNA was tested (18). Our study shows that RAS mutations may play a role in rare cases of Ewing sarcoma and neuroblastoma.

**BRAF** point mutations have been studied in various pediatric solid tumors. For instance, mutations have been described in a significant proportion of low-grade pediatric astrocytomas (31). A **BRAF** mutation was previously identified in the Ewing sarcoma cell line A673 as well as in 2 RMS cell lines not used in our study (1). However, **BRAF** mutations have not been reported in neuroblastoma cases (21). Our study findings indicate that **BRAF** mutations are rare in these tumor types but may play an oncogenic role in a small subset of sarcoma and patients with neuroblastoma. The **BRAF** mutation was identified in a single ERMS tumor sample. Furthermore, 2 of 190 neuroblastoma tumor samples harbored **BRAF** mutations and another neuroblastoma was found to have an activating mutation of **MAP2K1 (MEK1)**, indicating a role of MAPK/ERK pathway activation in a subset of patients with neuroblastoma.

Unlike adult onset tumors, mutations in the **PIK3CA** gene have not been commonly reported in pediatric tumor studies. One report of **PIK3CA** mutations in neuroblastoma samples found 2 point mutations in 69 samples. However, neither of these substitutions were the frequent gain-of-function mutations (E542K, E545K, H1047R) often seen in other solid tumor types. Therefore, the effect on kinase activity of these mutations is unknown (24). We did not identify any **PIK3CA** mutations in our larger set of neuroblastoma cases.

Recently, 18% of myxoid/round cell liposarcomas were found to have **PIK3CA** mutations localized to the helical and kinase domains, and these conferred a worse prognosis than wild-type **PIK3CA** within the same patient population (32). **PIK3CA** mutations have not been previously reported in pediatric sarcomas. We identified **PIK3CA** mutations in 3 of 60 (5%) of ERMS samples, including two mutations in the helical domain and one in the kinase domain. Both mutations are known to be oncogenic (33). Activation of **AKT** through the insulin-like growth factor pathway has been well established in RMS (34–36). Our data suggest the possibility of an additional role of activating **PIK3CA** mutations in activating **AKT** in some patients with ERMS. Furthermore, all 3 patients with **PIK3CA**-mutant ERMS died of relapsed disease suggesting a more aggressive phenotype than is typical for ERMS. An expanded RMS sample set will be necessary to rigorously assess the impact of **PIK3CA** mutations on outcomes. **In vitro** evaluation of novel **PIK3CA** inhibitors in RMS cell lines along with efforts...
to establish RMS cell lines harboring PIK3CA mutations are currently in progress. 

CTNNB1 (β-catenin) mutations have been identified in several embryonal pediatric tumor types. Activating CTNNB1 mutations occur in 10% to 15% of medulloblastoma cases and seem to define a distinct subgroup with improved overall outcomes (27). CTNNB1 mutations have also been well characterized in hepatoblastomas and pediatric Wilms tumors (20, 37). In a recent report, an evaluation of the CTNNB1 gene in 14 RMS samples did not identify any gene mutations (38). In our screen, we identified a single mutated Ewing sarcoma sample and 2 RMS cases with mutations. Because of the heterogeneity of mutations in exon 3 of this gene, many of which were not included in our Sequenom panel, Sanger sequencing of exon 3 was conducted in Ewing sarcoma and RMS samples but this did not identify any additional mutations. However, these findings identify a small subset of patients with RMS in which activation of the CTNNB1 pathway may play a role in tumor development. Intriguingly, both patients with RMS with CTNNB1 mutations are alive and disease free for at least 6 years since diagnosis, including one patient diagnosed with stage IV disease. This raises the possibility of improved outcomes in patients with RMS with CTNNB1 mutations, similar to the patients with CTNNB1-mutated medulloblastoma.

There is an increased incidence of RMS and other childhood cancers in genetic syndromes with germ line RAS/MAPK pathway mutations, including Cardio-facio-cutaneous syndrome, Costello syndrome, and Noonan syndrome.
Along with RAS genes, PTPN11 and BRAF are two of the most frequently mutated genes in this group of syndromes, with the germ line mutations overlapping only partly with recurrent somatic mutations in these genes (10, 11, 13). Therefore, we interrogated an extensive range of possible mutations in these genes to assess the possibility that mutations previously reported as germ line may occasionally be acquired in sporadic RMS cases. However, we identified only one PTPN11 and one BRAF mutation in our survey of RMS samples.

FGFR4 mutations were recently identified in 7.5% of primary RMS tumors with mutations involving amino acids 535 and 550 leading to increased receptor phosphorylation and tumor progression. Mutations involving these amino acids were also most susceptible to FGFR4 inhibition (39). We identified FGFR4 mutations in 4 of 62 tumors tested (6.5%). All 4 samples were ERMS, including the cell line RMS559. Interestingly, all 4 mutations led to a V550L amino acid change.

Our panel included several recently described oncogenes which have not been extensively evaluated in pediatric solid tumors, including the IDH and GNAQ genes. IDH1 and IDH2 mutations were initially identified as frequently mutated in adult onset gliomas (40) and soon after were identified in a significant percentage of cytogenetically normal acute myelogenous leukemia samples (41). Frequent mutations in the GNAQ gene have been recently identified in melanocytic tumors leading to activation of the MAPK pathway (42). A large analysis of glial, epithelial, and stromal type adult onset tumors did not identify any mutations in non-melanocytic tumors (43). Our assay conducted a mutational survey of the primary hotspots of these genes; namely, R132 and R172 of IDH1 and IDH2 and Q209 of GNAQ. We did not identify any mutations in either gene in our samples. Interestingly, IDH mutation studies of pediatric gliomas reveal that they occur in children 14 years of age or older and not in younger children (44). Similarly, IDH mutations are not characteristic of pediatric acute myelogenous leukemia (45), indicating that these mutations may be much more likely to exist in adult onset tumor types. Recently, a large mutational survey of the IDH gene in chondrosarcomas and chondromas revealed mutations in more than 50% of cases, with the most frequent mutation involving the R132 codon (46). Further mutational studies in other sarcoma types are warranted.

This analysis identified several novel occurrences of known oncogenic mutations in pediatric tumors including PIK3CA and CTNNB1 mutations in RMS, CTNNB1 mutations in Ewing sarcoma, and RAS, BRAF, and MAP2K1 mutations in neuroblastoma. No mutations were identified in a relatively large sample size of DSRCT. The paucity of mutations identified in Ewing sarcoma samples and the absence of mutations identified in DSRCT samples may be a function of the characteristic aberrant transcription factors generated by the specific translocations found in the two tumor types. The broad transcriptional dysregulation caused by these chimeric proteins may affect multiple oncogenic pathways, reducing the selective pressure for additional oncogenic mutations for tumor formation and progression. However, whole genome sequencing studies on Ewing sarcoma and DSRCT need to be conducted to confirm this hypothesis. A significant proportion of ERMS harbor oncogenic mutations. Including FGFR4 mutations, 28.3% (17 of 60) of ERMS samples had an identifiable oncogenic mutation (Fig. 3).

Several mutations identified are of present interest in terms of targeted agents in clinical development. MEK inhibition has been recognized as an effective modality against melanomas harboring BRAF mutations (47). Various MEK inhibitors are currently in phase III trials for patients with BRAF-mutant melanoma. Similarly, many different PIK3CA inhibitors are under development with numerous early phase studies open for adult onset tumor types with corresponding mutations (48). ALK inhibition has been recognized as effective across various tumor cell lines with ALK mutations or rearrangements (49). The ALK inhibitor crizotinib is currently in phase I/II studies for refractory pediatric solid tumor patients and phase III studies for patients with lung cancer harboring ALK rearrangements. Recent studies have also shown the activity of MEK inhibitors against a subset of RAS-mutant tumors (50). As more agents inhibiting mutated oncoproteins and their associated downstream signaling pathways become available, mutational genotyping of pediatric solid tumors such as RMS will serve as an important tool for identifying targeted therapy options for individual patients.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

The authors thank Dr. Adriana Heguy and the personnel of the Beene Translational Oncology Core Facility (MSKCC); Alex Lash in the MSKCC Bioinformatics Core Facility, Drs. Leonard Wexler and Christine Pratilas from the MSKCC Department of Pediatrics for long time support and helpful discussions, respectively, and Dr. Jonathan Fletcher from the Dana-Farber Cancer Institute, Boston, MA. Dr. Jun Nishio from the Fukuoka University, Fukuoka, Japan, Dr. Hajime Hossui from the Kyoto Prefectural University of Medicine, Kyoto, Japan, and Dr. Michio Kaneko from the University of Tsukuba, Ibaraki, Japan, for providing cell lines.

Grant Support

The study was supported, in part, by NIH P01 grant CA106450 (M. Ladanyi), the Ewing Sarcoma Research Fund (M. Ladanyi), The Joanna Mcafee Childhood Cancer Foundation (F. G. Barr), The Alveolar Rhabdomyosarcoma Research Fund (F. G. Barr), and by a generous donation from M. B. Zuckerman (M. Ladanyi). The MSKCC Sequenom facility was supported by the Anbinder Fund. 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.

Received August 10, 2011; revised October 14, 2011; accepted November 16, 2011; published OnlineFirst December 5, 2011.
References

Oncogene Mutation Profiling of Pediatric Solid Tumors Reveals Significant Subsets of Embryonal Rhabdomyosarcoma and Neuroblastoma with Mutated Genes in Growth Signaling Pathways

Neerav Shukla, Nabahet Ameur, Ismail Yilmaz, et al.


Updated version
Access the most recent version of this article at:
doi:10.1158/1078-0432.CCR-11-2056

Supplementary Material
Access the most recent supplemental material at:
http://clincancerres.aacrjournals.org/content/suppl/2011/12/08/1078-0432.CCR-11-2056.DC1

Cited articles
This article cites 50 articles, 14 of which you can access for free at:
http://clincancerres.aacrjournals.org/content/18/3/748.full#ref-list-1

Citing articles
This article has been cited by 13 HighWire-hosted articles. Access the articles at:
http://clincancerres.aacrjournals.org/content/18/3/748.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.