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Absence of Oncogenic Canonical Pathway Mutations in Aggressive Pediatric Rhabdoid Tumors

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Abstract

Background—Rhabdoid tumors (also called atypical teratoid/rhabdoid tumor (AT/RT) in the brain), are highly malignant, poor prognosis lesions arising in the kidneys, soft tissues and central nervous system. Targeted therapy in this disease would benefit from advanced technologies detecting relevant actionable mutations.

Procedure—Here we report on the evaluation of twenty-five tumors, all with known SMARCB1/INI1 alterations, for the presence of 983 different mutations in 115 oncogenes and tumor-suppressor genes using OncoMap, a mass spectrometric method of allele detection.

Results—Other than mutations in *SMARCB1*, our results identified a single activating mutation in *NRAS* and complete absence of oncogenic mutations in all other genes tested.

Conclusion—The absence of mutations in canonical pathways critical for development and progression of adult cancers suggests that distinct mechanisms drive these highly malignant pediatric tumors. This may limit the therapeutic utility of available targeted therapies and require a refocusing toward developmental and epigenetic pathways.

Keywords

Rhabdoid; AT/RT; targeted therapy; OncoMap

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INTRODUCTION

Rhabdoid and AT/RT tumors are highly aggressive neoplasms of early childhood that occur in the kidneys or soft tissues and brain, respectively. Nearly all cases share a biallelic mutation or copy number alteration in the *SMARCB1* tumor suppressor gene (*INI1; hSNF5; BAF47*), resulting in the loss of this core subunit of the SWI/SNF chromatin remodeling complex [1]. Diagnosis is based on histopathologic criteria of a poorly differentiated tumor with the variable presence of rhabdoid-appearing cells in conjunction with the loss of SMARCB1 expression in the tumor cells but not in adjacent normal tissues [2]. Treatment is dependent on the location of the tumor, initial staging and age of the patient. While a multimodal approach combining maximal surgery, radiation and chemotherapy is considered optimal for long term cure, the young age of many patients and involvement of the central nervous system limits the utilization of this approach. In spite of this, advances in treatment for this disease have been realized, albeit with significant morbidity [3, 4]. Nonetheless, mortality remains high and new therapeutic approaches are urgently needed.

Most, although not all, rhabdoid and AT/RT tumors [5] demonstrate inactivation of SMARCB1 suggesting a common biology that might provide for therapeutic intervention [6, 7]. Chromosomal imbalances are rare and focused on the *SMARCB1* region of chromosome 22 [8]. Expression profiling has identified distinct gene signatures within AT/RTs and the activation of certain pathways, such as bone morphogenic protein (BMP), has been associated with a poorer prognosis [9]. For rhabdoid tumors, however, most of the molecular information has been derived from a limited number of cases and has focused on the familial association of this mutation [10], gene expression [11] or a limited number of pathways using immunohistochemistry [12, 13]. To advance therapeutic options for patients with rhabdoid and AT/RT tumors, we undertook a comprehensive mutational analysis and target identification in 25 cases in the hopes of identifying targeted therapies that could be rapidly added to the current treatment regimens for this disease.

Advances in molecular biology have resulted in adapted high-throughput genotyping for known oncogene mutations [14]. This method was optimized for both fresh frozen and formalin-fixed paraffin embedded material in an approach called OncoMap [15]. It has been applied to a number of adult and pediatric tumors and resulted in the discovery of the activating BRAF V600E mutation in pediatric gangliogliomas [16], pleomorphic xanthroastrocytomas [17], histiocytosis [18] and diffuse intrinsic pontine gliomas [19]. Ideally suited for oncogenes where a limited number of mutations at specific residues can result in constitutively activated genes products, common mutations in tumor suppressor genes can also be identified, although certain inactivating mutations can be missed.

METHODS

DNA was extracted from FFPE samples obtained at diagnosis from 25 patients with rhabdoid and AT/RT tumors; all samples were obtained on an IRB-approved protocol from patients with documented *SMARCB1* deletions or mutations and histologically consistent rhabdoid or AT/RT lesions. Patient ages ranged from 4 months to 10 years of age (unknown in 7 cases) and had equal sex distribution (12 males, 12 females, one not reported). The majority of the samples were AT/RT from the brain (n=17) and spine (n=2) while rhabdoid tumor of the kidney (n=3), abdomen (n=1) and unknown primary site (n=2) made up the remaining samples. We used approximately 200 nanograms of DNA from each sample in an optimized profiling platform called “OncoMap 3” to interrogate 983 unique mutations in 115 known oncogenes and tumor suppressor genes [15] several of which are targets of existing small molecule inhibitors. DNA was quantified using picogreen analysis, then subjected to whole genome amplification. A subset of samples was evaluated by DNA

fingerprinting to confirm non-biased amplification. Whole genome amplified DNA was used as input for multiplex PCR using primers from OncoMap 3 and OncoMap 3 Extended which together comprise 1047 independent assays interrogating 983 unique mutations across 115 genes (Supplemental Table 1). Single base primer extension was performed using iPlex Gold single base extension enzyme (Sequenom, San Diego, CA) and products were transferred to SpectroCHIPs for analysis by MALDI-TOF mass spectrometry. Allele peaks were flagged using a modified Sequenom algorithm followed by manual review by two independent reviewers of candidate calls which were classified as “aggressive” or “conservative” depending on their apparent robustness. Sample quality was considered adequate for analysis if more than 80% of the attempted genotypes resulted in identifiable products. Candidate mutations were validated using multi-base hME extension chemistry and a bidirectional assay design interrogating both DNA strands independently from unamplified genomic DNA. The proportion of mutant alleles in each sample was determined by dividing the area of the mutant allele peak identified in hME by the sum of the areas of the mutant and wild type allele peaks.

RESULTS

Other than in the *SMARCB1* gene, out of the 115 different genes assayed (Table 1 and Supplemental Table 1) only a single mutation in *NRAS* at the Q61R position [20] was detected in 1/25 patients (a 4 year old male patient with a brain AT/RT). In spite of the highly malignant nature of rhabdoid and AT/RT tumors, none of the remaining samples had cancer-driving mutations detected in pathways that are often implicated in the oncogenic process. In particular, we did not find mutations previously reported in adult or pediatric glial tumors, including TP53, PTEN, RB1, CDKN2A, NOTCH1, MET, BRAF, CTNNB1, H- or K-RAS, MLH1, EPHA genes, or the tyrosine-kinase domain of *KIT, EGFR, PDGFRA/B or PI3KCA*. Similarly, mutations frequently observed in subsets of neural tumors including medulloblastoma, such as *SMO, PTCH1 and SUFU*, were not detected in these samples. Of note, two patients carried the EGFR S703F allele, which, while on OncoMap, was subsequently found to encode a low frequency polymorphism for EGFR, denoted in RefSeq as tx ID NM_201284. This allele is not listed in the catalog of somatic mutations in cancer (COSMIC) database and is likely a germline event.

DISCUSSION

Complex chromosomal alterations combined with point mutations that activate critical oncogenes or inactivate tumor suppressor genes is a common and well documented process in most cancers [21]. The dramatic responses and improved outcomes for patients with defined oncogenic mutations including BCR-ABL [22], *c-kit* dependent gastrointestinal stromal tumor (GIST) [23], hedgehog driven basal cell carcinoma [24] and BRAF V600E mutant malignant melanoma [25] have demonstrated the utility of identifying actionable mutations in specific tumor populations. The diploid and genomically stable nature of aggressive rhabdoid and AT/RT tumors makes them an interesting tumor type in which to search for point mutations, which could potentially be rapidly targeted with available small molecule inhibitors. Surprisingly, other than the *SMARCB1* mutations (see Supplemental Table 1 for those tested), only a single mutation (*NRAS*) was identified. This result strongly suggests that the oncogenic potential of rhabdoid tumors in the context of the mutation of this SWI/SNF chromatin remodeling subunit does not require a succession of classical oncogene or tumor suppressor mutations to propel the highly malignant phenotype. This suggests a novel mechanism of cancer initiation and progression. It will therefore be of critical importance to focus efforts on evaluating mechanisms that can regulate cellular functions in the absence of these abnormalities, such as developmental programs controlled through epigenetic regulation. Since *SMARCB1* was identified as a tumor suppressor for

pediatric RT fourteen years ago, mutations of at least six other genes that encode other subunits of the SWI/SNF chromatin remodeling complex occur at high frequency in a variety of adult cancers. These results support the paradigm first established by characterization of retinoblastoma that studies of pediatric cancers can reveal key pathways relevant to cancer in general. Our findings raise the possibility that mutation of SWI/SNF genes may drive cancer via novel mechanisms that will necessitate development of alternate therapeutic approaches.

The absence of *TP53* abnormalities was unexpected as this has been reported in some rhabdoid and AT/RT tumors samples [12]. While OncoMap assesses for mutational hotspots in a number of tumor suppressor genes including *TP53*, there are a large many nonsense or truncation mutations that could be missed by this technology. Future studies using whole exome sequencing (WES), whole genome sequencing (WGS), and epigenetic profiling will be important in overcoming the limitations of OncoMap in identifying abnormalities in tumor suppressor genes.

The absence of mutations in the common oncogenes and tumor suppressor genes in rhabdoid and AT/RT samples suggests that additional novel mechanisms are driving their oncogenesis; these will need to be evaluated using additional molecular methodologies including next-generation sequencing or epigenetic profiling, both of which are underway. Gene expression profiling may also be used to describe molecular pathways participating in their oncogenesis. The absence of mutations identified in these highly malignant and rapidly fatal tumors was unexpected and suggests that this type of approach can impact our understanding of rare diseases while redirecting efforts to particular pathways and novel tumor cellular mechanisms.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table I

The 115 genes assayed by OncoMap3

ABL1	CUBN	FYN	NF1	ROBO2
ABL2	DBN1	GATA1	NF2	ROS1
ADAMTSL3	DDR1	GNAS	NOTCH1	RUNX1
AKT1	DDR2	GUCY1A2	NPM1	SIX4
AKT2	EGFR	HNF1A	NRAS	SMAD2
ALK	EPHA1	HRAS	NTRK1	SMAD4
APC	EPHA3	IGF1R	NTRK2	SMARCB1
ATM	EPHA4	JAK2	NTRK3	SMO
AURKA	EPHA5	JAK3	PAK7	SPTAN1
AURKB	EPHA8	KIT	PALB2	SRC
AURKC	EPHB1	KRAS	PDGFRA	STK11
AXL	EPHB6	LRP1B	PDGFRB	SUFU
BRAF	ERBB2	LYN	PDPK1	TBX22
BMX	FBXW7	MAP2K4	PIK3CA	TEC
BRCA1	FES	MEN1	PIK3R1	TFDP1
BRCA2	FGFR1	MET	PKHD1	TIAM1
CDH1	FGFR2	MLH1	PTCH1	TP53
CDK4	FGFR3	MLL3	PTEN	TRIM24
CDKN2A	FGFR4	MPL	PTPN11	TRIM33
CEBPA	FLNB	MSH2	RAF1	TSC1
CREBBP	FLT3	MSH6	RB1	TSHR
CSF1R	FLT4	MYC	RET	VHL
CTNNB1	FSCB	MYH1	ROBO1	WT1