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A Molecular Take on Malignant Rhabdoid Tumors

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Abstract

The molecular basis for the clinical heterogeneity observed in patients with malignant rhabdoid tumors is unknown. Recently, two reports revealed molecular inter-tumor heterogeneity in teratoid/rhabdoid tumors (ATRTs) and extra-cranial MRTs (ecMRTs) using genomic, transcriptomic and epigenomic profiling. Distinct molecular subgroups were identified and new therapeutic targets were revealed.

Keywords

malignant rhabdoid tumors; sequencing; molecular analysis; pediatric cancer

Malignant rhabdoid tumors (MRTs) are a rare but aggressive form of pediatric solid tumor with a 5-year overall survival rate of 23%[1]. MRTs can be broadly classified into atypical teratoid/rhabdoid tumors (ATRTs), which are found in the brain, and extra-cranial MRTs (ecMRTs), which can be found in a variety of tissues including rhabdoid tumors of the kidney, liver, lung and soft tissues (Figure 1). All types of MRTs are driven by *SMARCB1* loss [2]. This gene is a core member of the SWI/SNF chromatin remodeling complexes whose components are frequently mutated or silenced in cancer and have tumor suppressing function [3]. Previous studies showed that MRTs harbor very few mutations and have relatively homogeneous genomes [4]. However, variability in clinical outcomes including long-term survival in a subset of patients has been reported suggesting that additional but not yet understood factors impact disease progression. Using whole-genome DNA, RNA, microRNA, ChIP sequencing and genome-wide DNA methylation assays (Figure 1), Johann et al. [5] and Chun et al. [6] provided new insights into the molecular heterogeneity of ATRTs and ecMRTs, respectively, revealed distinct molecular subgroups and potential therapeutic targets.

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Johann et al. identified three distinct molecular subtypes of ATRTs based on the 450k DNA methylation profile of a cohort of 192 tumors: 1. ATRT-TYR, 2. ATRT-SHH and 3. ATRT-MYC, which were further confirmed through clustering of 67 mRNA expression profiles. Subgroup names were coined by dominant proteins/signaling pathways within those clustered tumors, such as the expression of melanosomal markers (*MITF*, *TYR* and *DCT*) in ATRT-TYR; SHH and NOTCH signaling pathway genes in ATRT-SHH; and *MYC* in ATRT-MYC. Genetic aberrations in *SMARCB1* differed within ATRT subtypes. Broad *SMARCB1* deletions were often observed in ATRT-TYR (77%); focal *SMARCB1* deletions were more prevalent in the ATRT-MYC subgroup (79%), while focal gains were noted in a minority of ATRT-SHH tumors (23%). Chun et al. similarly set out to define molecular subgroups of ecMRTs by unsupervised clustering of 66 primary tumors (57 kidney, 7 soft tissue and 2 liver) together with tumor and normal samples from TCGA on the basis of their miRNA expression profiles. ecMRTs segregated into two groups: one large subgroup (n=57) that clustered closely with normal cerebellum and with paragangliomas/pheochromocytomas, cancers of neural crest origin, and a smaller group (n=9) that clustered closely with synovial sarcomas, tumors that are thought to be of neural origin. This grouping provides intriguing suggestions on differences between the cell of origin of ecMRTs. In addition to miRNA profile based grouping, clustering of mRNA profiles in ecMRTs revealed the existence of two subgroups which were not fully concordant with miRNA subgroups. mRNA subgroup1 consisted of ecMRTs from liver, soft tissues and kidney, and carried an expression signature more similar to ATRT, while MRT subgroup2 was dominated by kidney rhabdoid tumors and resulting gene expression patterns.

ATRT whole-genome sequencing did not reveal disease-relevant mutations other than *SMARCB1*, and Johann et al. extended their analysis by performing wholegenome bisulfite sequencing (WGBS) of 17 ATRTs. WGBS showed genome wide DNA hypermethylation in ATRT-TYR and ATRT-SHH but not in ATRT-MYC tumors, as compared with other brain tumors and normal cerebellum. The global level of DNA methylation of ATRT-TYR and ATRT-SHH resembled that of IDH-mutated glioblastoma, a type of adult glioblastoma for which hypermethylation is a defining feature [7]. Similarly, Chun et al. analyzed WGBS data from 40 ecMRTs and uncovered two DNA methylation subgroups with one subgroup displaying global higher methylation levels compared to the other. Both subgroups showed CpG island hypermethylation compared to NPCs and ESCs. Whether ecMRT DNA methylation subgroups overlap with those identified through miRNA and mRNA profiling is a topic for future investigation.

Using H3K27ac ChIP-seq data, enhancers and super-enhancers were identified in both studies. Both ATRT-MYC specific super-enhancers and ecMRTs specific super-enhancers targeted the same *HOX* gene locus. Epigenetic activation of homeobox genes, which are anatomical development morphogenesis regulators, may contribute to MRT progression by deregulating developmental processes. By constructing subgroup-specific regulatory networks, Johann et al. identified *MITF* as a master regulator of the ATRT-TYR subgroup. *MITF* is a transcription factor (TF) significantly amplified in malignant melanoma [8]. While not amplified in ATRT, inhibiting *MITF* in BT12 ATRT cells using a small molecule led to apoptosis and reduced cell viability, nominating *MITF* as an actionable target in ATRT-TYR tumors. Inhibiting *MITF*-related genes, such as *PAX3*, was found to sensitize

melanoma cells to MEK inhibitors [9] and such alternative strategies could be explored for ATRT-TYR tumors as well.

Additional insights into the molecular mechanisms of MRTs can be obtained through meta-analysis of these datasets and additional molecular MRT studies. For example, ecMRTs from liver, soft tissues and a subset of kidney showed gene expression patterns resembling those of ATRTs, whereas ATRTs themselves can be further classified into molecular subgroups potentially related to cell of origin. The similarities of transcriptomic and epigenetic MRT classifications observed in the ATRT analysis from Johann *et al.* and the ecMRT analysis by Chun *et al.* may reflect cell of origin footprints that supersede site of tumor origin and an integrated analysis may be able to provide substantial new insights. Future work is also needed to demonstrate the clinical relevance of the subtypes identified, including a review of survival time and treatment response.

Chun *et al.* identified intronic mutations in *SPECCIL* and *KCNJ3* genes that affect the expression levels of these genes. Integration of non-coding mutations and transcriptomic profiles from both datasets may provide additional evidence for the role of *SPECCIL* and *KCNJ3* mutations or may help identify additional recurrent abnormalities.

Reanalyzing the data generated in these two studies from a different perspective could play a major role in deciphering the mechanisms by which *SMARCB1* mutations initiate MRTs. Given *SMARCB1*'s role in nucleosome remodeling [10], we postulate that nucleosome positioning in the MRT genome is disrupted. Computational methods that use ChIP-seq data to infer nucleosome positioning have been proposed and these methods can be used to test this hypothesis using the ChIP-seq data generated in both studies. Such analysis and comparisons to data from normal tissues as well as tumors lacking SWI/SNF alterations may help to clarify whether aberrant *SMARCB1* activation results in epigenetic dysregulation.

The publications by Chun *et al.* and Johann *et al.* provide a substantial advance to improve our understanding of this pediatric carcinoma where few therapeutic modalities have been able to make an impact. While the analysis described in both papers is sophisticated and had led to new insights, the immense data resources that are provided invite a plethora of additional and follow-up studies. As exemplified by Chun *et al.*, which is part of the Therapeutically Applicable Research To Generate Effective Treatments (TARGET) effort to find molecular drivers of pediatric cancers by comprehensive genomic profiling, advances of this magnitude can be made only through extensive collaborations. The surface of the molecular landscape of MRT has been scratched. It is now time to capitalize on this seminal work and translate these and follow-up findings towards new clinical approaches.

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Figure 1. Schematic representation of different types of Malignant Rhabdoid Tumors (MRTs) and molecular assays used to profile them.