A Molecular Take on Malignant Rhabdoid Tumors

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The molecular basis for the clinical heterogeneity observed in patients with malignant rhabdoid tumors is unknown. Recently, two reports revealed molecular intertumor 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.

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 functions [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, miRNA, 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, and revealed distinct molecular subgroups and potential therapeutic targets.

Johann *et al.* identified three distinct molecular subtypes of ATRTs based on the 450 k DNA methylation profile of a cohort of 192 tumors: (i) ATRT-TYR, (ii) ATRT-SHH, and (iii) 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, seven soft tissue, and two liver) together with tumor and normal samples from TCGA on the basis of their miRNA expression profiles. ecMRTs were 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 that were not fully concordant with miRNA subgroups. mRNA subgroup 1 consisted of ecMRTs from liver, soft tissues, and kidney, and carried an expression signature more similar to ATRT, while MRT subgroup

2 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 whole-genome 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 with the other. Both subgroups showed CpG island hypermethylation compared with neural progenitor cells and embryonic stem cells. 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 ecMRT-specific super-enhancers targeted the same *HOX* gene cluster. 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
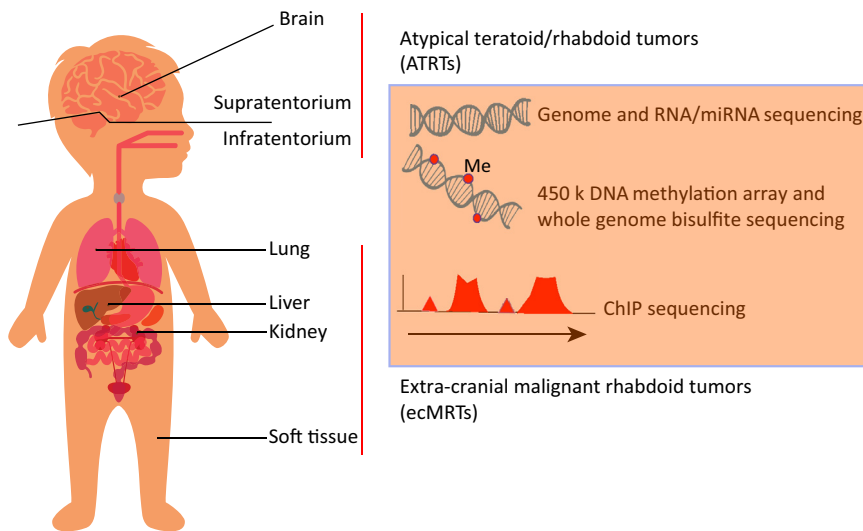
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Figure 1. Schematic Representation of Different Types of Malignant Rhabdoid Tumors (MRTs) and Molecular Assays Used to Profile Them.

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 SPECC1L and KCNJ3 genes that affect the expression levels of these genes. Integration of noncoding mutations and transcriptomic profiles from both datasets may provide additional evidence for the role of SPECC1L 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 the role of SMARCB1 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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