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# RAS and ROS in Rhabdomyosarcoma

**Minsi Zhang**[1,2], **Corinne M. Linardic**[1,3], and **David G. Kirsch**[1,4,*]

[1]Department of Pharmacology and Cancer Biology, Duke University Medical Center, Durham, NC 27710, USA

[2]Program in Molecular Cancer Biology, Duke University, Durham, NC 27710, USA

[3]Department of Pediatrics, Division of Hematology-Oncology, Duke University Medical Center, Durham, NC 27710, USA

[4]Department of Radiation Oncology, Duke University Medical Center, Durham, NC 27710, USA

## Summary

The 5-year survival for localized rhabdomyosarcoma is over 70%, but only 30% for patients presenting with metastatic disease. In this issue of *Cancer Cell*, Dyer and colleagues performed whole-genome and RNA sequencing on human rhabdomyosarcoma and identified RAS mutations and oxidative stress as potential therapeutic targets for high-risk embryonal rhabdomyosarcoma.

Rhabdomyosarcoma (RMS) is the most commonly diagnosed soft tissue sarcoma in children. The two major subtypes, alveolar (ARMS) and embryonal (ERMS), differ in their histological features, genetic mutations, age of onset, and prognosis (Parham and Ellison, 2006). ERMS, more commonly diagnosed in children less than 10 years of age, is characterized by the presence of rhabdomyoblasts, cells with eccentric nuclei and eosinophilic cytoplasm, embedded in a myxoid stroma. ARMS, more commonly diagnosed in children over 10, is distinguished histologically by clusters of small, round, blue cells in a highly cellular background. Most tumors with ARMS histology have chromosomal translocations t(2;13)(q35;q14) or t(1;13)(p36;q14), resulting in the expression of either PAX3-FOXO1 or PAX7-FOXO1 fusion proteins, with few other chromosomal alterations. In contrast, ERMS frequently possesses multiple chromosomal alterations. Although most cases of RMS occur sporadically, inherited syndromes with germline mutations associated with developing RMS include Neurofibromatosis Type I (*NF1*) (Ferrari et al., 2007), Li-Fraumeni (*TP53*) (Diller et al., 1995), Costello (*HRAS*) (Kratz et al., 2011), Noonan (*PTPN11*, *SOS1*, *RAF1*, *KRAS*, *NRAS*, *BRAF*) (Kratz et al., 2011), and Gorlin syndromes (*PTCH1*) (Gorlin, 1987).



*Correspondence: david.kirsch@duke.edu.

The current clinical criteria for classification of RMS into low-, intermediate-, or high-risk groups depends on primary tumor site, size, surgical resectability, and metastasis to regional lymph nodes or distant sites (Malempati and Hawkins, 2012). While the high-risk group encompasses metastatic RMS of both alveolar and embryonal subtypes regardless of primary tumor size or site, ARMS patients generally have worse clinical outcome than ERMS patients. Importantly, ARMS that is diagnosed at the histological level and lacking PAX3-FOXO1 or PAX7-FOXO1 translocation is more similar to ERMS in both gene expression and clinical outcome (Williamson et al., 2010). Treatment for RMS is multi-modal and includes chemotherapy, radiation therapy, and surgery. Although patients with localized RMS who receive combination therapy have a 5-year survival greater than 70%, relapse and poor outcome are common for patients with metastasis at the time of diagnosis. Thus, it is important to gain a better understanding of these aggressive tumors at the molecular level and insight into how they are biologically different from those with better clinical outcomes. This information may inform risk stratification for current therapies and the development of novel treatments. In this issue of *Cancer Cell*, Chen et al. (2013) performed genomic and RNA sequencing of human ERMS and ARMS to identify novel differentiating characteristics for high-risk ERMS.

Using data from 29 ERMS and 17 ARMS specimens, Dyer and colleagues identified recurrent genetic lesions, including single nucleotide variations, indels, and structural variants, in 8 consensus cancer genes for ERMS (*NRAS*, *KRAS*, *TP53*, *NF1*, *RARA*, *CTNNB2*, *CARD11*, *PIK3CA*) and 2 translocations for ARMS (*PAX3-FOXO1*, *PAX7-FOXO1*). They identified no mutations in the SHH pathway. *TP53* gene mutations were associated with concurrent *FGFR4* mutations, and mutations in the p53 pathway were overall more frequent in ERMS than in ARMS. The RAS pathway - specifically RAS family members *NRAS*, *KRAS*, and *HRAS*, and the RAS-GAP *NF1* - were identified as the most commonly mutated genes in ERMS. Importantly, RAS pathway mutation status was significantly associated with ERMS risk group classification, with mutations found in 75% of high-risk ERMS, 45% of intermediate-risk ERMS, and none of low-risk ERMS. Moreover, none of the ARMS samples had RAS pathway mutations. These findings are consistent with prior studies that identified RAS pathway mutations in ERMS (Paulson et al., 2011). If this correlation with ERMS risk groups is validated in a larger dataset, RAS pathway mutations genotyping could potentially be used to improve ERMS risk stratification.

In this study, all current RAS pathway inhibitors tested - including 13 MEK/ERK inhibitors and 17 PI3K inhibitors - failed to show significant activity on patient-derived NRAS mutant xenografts. The only exception was BGT-226. Treatment with this dual PI3K/mTOR inhibitor led to tumor responses in the 100–1000 nM range. The efficacy of this agent may be due in part to inhibition of other PI3K-related kinases, such as ATM and/or DNA-PKcs. Therefore, novel small molecules that directly inhibit mutant RAS itself may be needed to treat patients with aggressive ERMS with a confirmed RAS mutation. The authors failed to find an association of RAS pathway mutations with mutations in the p53, RB, or SHH pathways as previously suggested (Rubin et al., 2011), but epigenetic modifications may be contributing to these previously reported associations at the transcriptome level.

Chen et al. (2013) also detected more G→T transversions in RMS than in both T-ALL and medulloblastoma combined. Thus, although RMS is a childhood cancer, it is subjected to more oxidative damage-induced mutations than other childhood tumor types. Interestingly, the rate of G→T transversions was more common in ERMS than ARMS, but not as common as in lung cancer. The increase in G→T transversions in ERMS correlated with increased p38 MAPK activity as measured by up-regulation of *MAPK12* and *MAP2K6*. Prior studies reported that RAS pathway mutations, which are frequently observed in adult cancers of the lung, colon, and pancreas, increase oxidative stress in tumors to fuel RAS-driven oncogenesis (Weinberg et al., 2010). Together these data suggest that ERMS, especially those classified as higher-risk ERMS, may behave more biologically like adult cancers, which are more commonly associated with oxidative damage. These findings should prompt biochemical analyses to quantify the extent of oxidative stress in ERMS in different risk-groups as compared to ARMS and other cancers. Interestingly, patient-derived ERMS xenografts responded to drugs that modify oxidative stress. If validated, regulators of oxidative stress and ROS production may provide new therapeutic options for higher-risk ERMS.

To gain insight into the temporal dynamics of gene mutation in RMS, the authors obtained 3 recurrent RMS from 2 separate ERMS patients. The sequencing analyses comparing the diagnostic sample with the recurrent tumor(s) led to a number of clinically relevant findings. Gene clusters showed that all tumors contained more than one clone, and in each of the 2 diagnostic tumors, the major clone was eliminated after combination therapy. The minor clone in each tumor accumulated further mutations generating 2 subclones within the recurrent tumor of one patient and 6 subclones between the two recurrent tumors of the other patient. Therefore, repeated biopsy at each instance of recurrent or metastatic disease may identify new molecular targets to guide therapy. Moreover, these results may explain why patients with multiple metastatic ERMS lesions show a mixed response to systemic therapy.

In summary, Chen et al. (2013) performed whole-genome and RNA sequencing on human ARMS and ERMS samples and identified frequent p53 and RAS pathway mutations in ERMS. These mutations may inform ERMS risk stratification and provide important, but potentially challenging, targets for future drug development. The authors also found the oxidative stress pathway was upregulated in ERMS, and correspondingly, drugs that modify oxidative stress showed activity in patient-derived ERMS xenograft models suggesting that further investigation of this drug class is warranted. Finally, as the authors noted dramatic differences in the genetic landscape between primary diagnostic samples and recurrent tumors, clinical trials testing molecularly targeted agents in patients with progressive disease should be based on biopsies of metastatic lesions rather than original diagnostic biopsies. Combined, these findings provide a better understanding of the genetic landscape of RMS and suggest that future advances in the treatment of ERMS will be through RAS and ROS.

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