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AUTHOR'S VIEWS

The perfect PTEN – transcriptional regulation by PTEN dictates sarcoma identity

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ABSTRACT

Fusion-negative rhabdomyosarcoma (FN-RMS) is molecularly heterogeneous with few universal alterations except for *Phosphatase and tensin homolog* (*PTEN*) promoter hypermethylation. We demonstrate that losing *Pten* in FN-RMS engages an aberrant transcriptional program key in tumor maintenance and cell identity. These results highlight the importance between transcriptional state, cell of origin, and genetic perturbation in tumorigenesis.

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Rhabdomyosarcoma (RMS) is the most common pediatric soft tissue sarcoma. Survival has not improved for patients with RMS for nearly four decades, emphasizing the need to understand the underlying RMS biology. Fusion-negative RMS (FN-RMS) is defined by the lack of a PAIRED BOX 3/PAIRED BOX 7-FORKHEAD BOX O1 (PAX3/7-FOXO1) fusion oncoprotein and represents a molecularly diverse cancer with many putative driver mutations. Unifying molecular features are not seen across FN-RMS patient tumors[1] with the exception of *Phosphatase and tensin homolog* (*PTEN*) promoter methylation that is seen in approximately 90% of FN-RMS tumors.[2] This suggests a necessity to downregulate PTEN expression in FN-RMS tumors.

PTEN is a lipid and protein phosphatase that is found both in the cytoplasm and nucleus.[3] PTEN's role as a tumor suppressor is well known and thought to function by negatively regulating the phosphatidylinositol-3,4,5-triphosphate kinase (PI3K) pathway. Increasing evidence suggests a diverse myriad of nuclear functions for PTEN, including regulating DNA damage and repair as well as transcriptional regulation.[3] Therefore, a more comprehensive analysis of the functional consequences of PTEN loss in cancer is necessary to potentially uncover novel mechanistic and therapeutic insights.

Pediatric cancers are highly enriched for genetic dependencies involving oncogenic transcription factors, such as *ISL LIM homeobox 1* (*ISL1*) and *GATA binding protein 3* (*GATA3*) in neuroblastoma and *Myogenic differentiation 1* (*MYOD1*) and *Myogenin* (*MYOG*) in rhabdomyosarcoma cell lines.[4] Understanding how these lineage factors, many of which are associated with core regulatory circuits (CRCs), can impart their oncogenic effects remains elusive. Furthermore, since pediatric cancers are more dependent on these lineage-specific transcription factors than adult cancers, it is possible that the therapeutic regimens needed to treat these tumors will be entirely different from those used in the adult oncology.[4] Now, with the advent of induced proximity (protein degradation) therapies, such as proteolysis-targeting chimeras (PROTACs) or molecular glues, targeting these transcription factors becomes more feasible. Understanding how oncogenic transcriptional networks are regulated will be critical for implementing these therapies or for identifying additional therapeutic targets.

Previously, we characterized a Hedgehog-driven murine model of FN-RMS, *aP2-Cre;Smo*^{M2} (*adipose protein 2-Cre recombinase; Smoothened*^{M2}) originating from non-myogenic cells.[5] Activation of the Hedgehog pathway in an endothelial progenitor cell results in transdifferentiation into a muscle-like cell or FN-RMS.[6] This cell reprogramming event in transformation gives us a unique model to identify tumor cell fate determinants. *Pten* loss in this model (*aP2-Cre;Smo*^{M2}*Pten*$^{flox/flox}$, ASP^{cKO}) produced a tumor with faster onset and higher proliferative index.[7] This was specific to conditional *Pten* deletion as mice with conditional *Cyclin-dependent kinase inhibitor 2A* (*Cdkn2a), Transformation-related protein 53* (*Trp53*, also known as p53), or *RB transcriptional corepressor 1* (*Rb1*, also known as RB) deletion did not phenocopy *Pten* loss. Additionally, the histology of ASP^{cKO} tumors more closely resembled the human disease with less skeletal muscle differentiation than their wild-type counterparts. Interestingly, although AKT phosphorylation was increased in the ASP^{cKO} tumors, there was no change in signaling downstream of mammalian target of rapamycin (mTOR). Furthermore, ASP^{WT} tumors exhibited nuclear staining for PTEN, suggesting possible PI3K pathway-independent nuclear tumor suppressive functions.

To further investigate these PI3K pathway-independent functions of PTEN, we profiled the transcriptomes of ASP^{WT} and ASP^{cKO} tumors and found that our *Pten*-deficient tumors had higher expression of two transcription factors – *Developing brain homeobox 1* (*Dbx1*) and *Pax7*. DBX1 had not been functionally described in cancer. PAX7 is a marker of satellite cells, the resident stem cell within the skeletal muscle niche, and our group has

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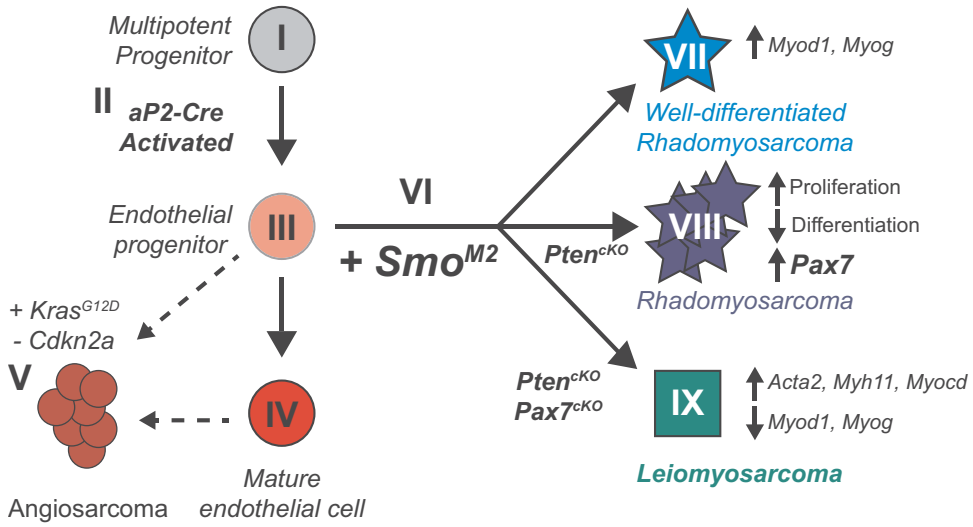

Figure 1. Interplay between the tumor cell of origin, genetic alterations, and transcriptional programs dictates sarcoma identity. A multipotent progenitor (i) in the branchial arches, structures that give rise to head and neck cells, of mice expresses *aP2/Fabp4* (*Adipose protein 2/Fatty acid binding protein 4*) (II) by day E10.5 of embryogenesis. In our model, this endothelial progenitor population (III) will express *aP2-Cre*, and it can then go on to develop into mature endothelial cells (IV) under wild-type conditions. However, following oncogenic insult with concomitant *Kras*^{G12D} (*Kirsten rat sarcoma viral oncogene homolog*) activation and *Cdkn2a* (*Cyclin dependent kinase inhibitor 2a*) loss in these *aP2-Cre*-expressing endothelial progenitors or mature endothelial cells (v), these cells will ultimately initiate angiosarcoma formation, a deadly vascular sarcoma.[6] Activation of the sonic hedgehog pathway by transgenic conditional *Smo*^{M2} (*Smoothened*^{M2}) (VI) expression in the E14.5 endothelial progenitor population in *aP2-Cre;Smo*^{M2} mice results in a well-differentiated rhabdomyosarcoma (RMS) expressing skeletal myogenic transcription factors MYOD1 (MYOGENIC DIFFERENTIATION 1) and MYOG (MYOGENIN) (VII). However, when *Pten (Phosphatase and tensin homolog)* is conditionally deleted (*Pten*^{cKO}) in the *aP2-Cre;Smo*^{M2} tumors, a more malignant RMS develops with increased proliferative index, decreased skeletal myogenic differentiation, and increased expression of the satellite cell marker *Pax7* (*Paired box 7*) (VIII). With concomitant deletion of *Pten* and *Pax7* (*Pten*^{cKO} and *Pax7*^{cKO}) in the *aP2-Cre;Smo*^{M2} tumors, we no longer see RMS but a switch to a smooth muscle differentiated tumor such as leiomyosarcoma expressing smooth muscle-related genes (*Acta2, Actin alpha 2, smooth muscle; Myh11, Myosin heavy chain 11; Myocd, Myocardin*) and not skeletal myogenic genes (*Myod1* and *Myog*) (IX).[7]

shown that PAX7 is important in maintaining the dedifferentiated state of FN-RMS.[8] Both *DBX1* and *PAX7* were necessary for human FN-RMS growth as depleting either *DBX1* or *PAX7* slowed the growth of human FN-RMS cell lines and patient-derived xenografts. Furthermore, in human neural stem cells, *PAX7* expression was increased and caused a more glioblastoma-like state when *PTEN* was deleted possibly indicating this PTEN-PAX7 axis is a more general tumor promoting mechanism across cancers.[9]

To determine PAX7's role in FN-RMS, we concomitantly deleted *Pax7* in our ASP^{cKO} mice (*aP2-Cre;Smo;*^{M2}*Pten*$^{flox/flox}$; *Pax7*$^{flox/flox}$, ASP^{cKO}P7^{cKO}) and found that *Pax7* loss rescued the effects of *Pten* loss in our FN-RMS model. Intriguingly, AKT phosphorylation was elevated in both ASP^{cKO}P7^{cKO} and ASP^{cKO} tumors compared to ASP^{WT} tumors indicating PI3K pathway activity was decoupled from the rescued phenotype. Histological analysis indicated that the ASP^{cKO}P7^{cKO} tumors no longer resembled the immature skeletal muscle indicative of FN-RMS without MYOD1, MYOGENIN, and DESMIN expression. Immunohistochemical, ultrastructural, and transcriptomic analyses revealed that the ASP^{cKO}P7^{cKO} tumors were smooth muscle differentiated tumors including leiomyosarcoma. This indicated that not only did PAX7 regulate tumor maintenance but was a central node in the specification of FN-RMS tumor identity.

Our model highlights a stepwise iteration of determining the factors important in defining the essential components of FN-RMS tumorigenesis and fate determination (Figure 1). Furthermore, this work extends previous work in the lab showing how the context between cell of origin and genetic context is critical in tumor fate determination.[6] The central role of PAX7 in FN-RMS maintenance and tumor fate is buoyed by recent work indicating PAX7 is a key component in the CRC.[10] CRCs are key transcriptional feed-forward loop originally discovered in embryonic stem cells with recent work indicating a major role in tumorigenesis, especially for pediatric tumors.[4,10] This suggests that PAX7 may be a crucial potential therapeutic target for FN-RMS. Partnering PAX7-targeted agents (PROTAC, molecular glues, etc.) with other compounds known to functionally disrupt CRCs, such as histone deacetylase inhibitors, may be a rational combination regimen to begin preclinical validation.[10] Indeed, these therapies may have therapeutic benefit in alveolar RMS patients that harbor PAX7-FOXO1 fusion oncoproteins. Understanding the underlying mechanistic determinants of tumor fate and the genetic alterations that dictate those determinants will be key to deciphering FN-RMS and cancer at-large.

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References

1. Shern JF, et al. Genomic classification and clinical outcome in rhabdomyosarcoma: a report from an international consortium. J Clin Oncol. 2021;Jco2003060. doi:10.1200/jco.20.03060.
2. Seki M, Nishimura R, Yoshida K, Shimamura T, Shiraishi Y, Sato Y, Kato M, Chiba K, Tanaka H, Hoshino N, et al. Integrated genetic and epigenetic analysis defines novel molecular subgroups in rhabdomyosarcoma. Nat Commun. 2015;6:7557. doi:10.1038/ncomms8557.
3. Lee YR, Chen M, Pandolfi PP. The functions and regulation of the PTEN tumour suppressor: new modes and prospects. Nat Rev Mol Cell Biol. 2018;19:547–3. doi:10.1038/s41580-018-0015-0.
4. Dharia NV, Kugener G, Guenther LM, Malone CF, Durbin AD, Hong AL, Howard TP, Bandopadhayay P, Wechsler CS, Fung I, et al. A first-generation pediatric cancer dependency map. Nat Genet. 2021;53:529–538. doi:10.1038/s41588-021-00819-w.
5. Hatley ME, Tang W, Garcia M, Finkelstein D, Millay D, Liu N, Graff J, Galindo R, Olson E. A mouse model of rhabdomyosarcoma originating from the adipocyte lineage. Cancer Cell. 2012;22:536–546. doi:10.1016/j.ccr.2012.09.004.
6. Drummond CJ, Hanna JA, Garcia MR, Devine DJ, Heyrana AJ, Finkelstein D, Rehg JE, Hatley ME. Hedgehog pathway drives fusion-negative rhabdomyosarcoma initiated from non-myogenic endothelial progenitors. Cancer Cell. 2018;33:108–124 e105. doi:10.1016/j.ccell.2017.12.001.
7. Langdon CG, et al. Synthetic essentiality between PTEN and core dependency factor PAX7 dictates rhabdomyosarcoma identity. Nat Commun. 2021;12:5520. doi:10.1038/s41467-021-25829-4.
8. Hanna JA, et al. PAX7 is a required target for microRNA-206-induced differentiation of fusion-negative rhabdomyosarcoma. Cell Death Dis. 2016;7:e2256. doi:10.1038/cddis.2016.159.
9. Duan S, Yuan G, Liu X, Ren R, Li J, Zhang W, Wu J, Xu X, Fu L, Li Y, et al. PTEN deficiency reprogrammes human neural stem cells towards a glioblastoma stem cell-like phenotype. Nat Commun. 2015;6:10068. doi:10.1038/ncomms10068.
10. Gryder BE, Pomella S, Sayers C, Wu XS, Song Y, Chiarella AM, Bagchi S, Chou H-C, Sinniah RS, Walton A, et al. Histone hyperacetylation disrupts core gene regulatory architecture in rhabdomyosarcoma. Nat Genet. 2019;51:1714–1722. doi:10.1038/s41588-019-0534-4.