The Not-so-Skinny on Muscle Cancer

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The childhood cancer embryonal rhabdomyosarcoma can arise in tissue without skeletal muscle elements. In this issue of *Cancer Cell*, Hatley and colleagues report that non-skeletal muscle progenitors can be a cell of origin for Sonic Hedgehog-driven embryonal rhabdomyosarcoma in an adipocyte-restricted conditional mouse model.

One of the most intriguing clinical and scientific questions in pediatric oncology is how rhabdomyosarcoma, a tumor with a myogenic phenotype, can arise in tissue without hypaxial-like skeletal muscle elements. This conundrum is especially evident for the embryonal subtype of rhabdomyosarcoma (eRMS), which can arise from the salivary glands, skull base (parameninges), biliary tree, and genitourinary tract (bladder/prostate) (Gurney et al., 1999; Shapiro and Bhattacharyya, 2006). The work by Hatley et al. (2012) in this issue of *Cancer Cell* begins to address this conundrum by highlighting the plasticity of cells traditionally thought to be in the adipogenic lineage.

In this paper, Hatley et al. (2012) conditionally activate a mutant *Smoothened* (Smo^{M2}) allele to drive Hedgehog signaling in the adipogenic lineage using a 5.4 kb promoter/enhancer fragment from the *adipocyte fatty acid binding protein 4* gene (*Fabp4*, also called *aP2*) to drive Cre. Eighty percent of *aP2-Cre;* $Smo^{M2/+}$ mice developed eRMS in the head and ventral neck by 2 months of age. *aP2-Cre* $Cdkn2a^{Flox/Flox}$ mice did not develop eRMS, whereas deletion of *Cdkn2a* in *aP2-Cre;*$Smo^{M2/+}$ mice decreased latency and increased tumor penetrance, with all mice having tumors by 55 days. This finding implicates *Cdkn2a* locus loss as a secondary factor (disease modifier) of eRMS progression. The authors validated tumors as true eRMS by histology and immunohistochemistry (Desmin, MyoD, and Myogenin). Similarly, an embryonal muscle gene signature (*MyoD1*, *Myogenin*, *Pax7*, *Myf5*, *Myh3*, and *Myh8*) was evident by RT-PCR assay. To further affirm the diagnosis, Hatley et al. (2012) performed gene expression profiling of *aP2-Cre;*$Smo^{M2/+}$ tumors in comparison to human eRMS and tumors of previously reported mouse eRMS models. Overall, 67% of the probe pairs and 58% of the ortholog gene pairs showed agreement in gene expression between their mouse tumors and previously published eRMS models (Rubin et al., 2011) or human tumors, respectively. Hatley et al. (2012) concluded that, despite being of an adipogenic lineage of origin, *aP2-Cre;*$Smo^{M2/+}$ mouse tumors are an accurate preclinical eRMS model based on these histological and transcriptome parameters.

The most interesting aspect of this paper is that these tumors originated from aP2 expressing cells. Previously, activity of the *aP2* promoter in transgenic mice has been documented as specific for the adipocyte lineage and to be distinct from any skeletal muscle lineage (Urs et al., 2006). Moreover, the specific *aP2-Cre* transgenic line used by Hatley et al. (2012) was shown to be active in adipose tissue but not in skeletal muscle, at least as evidenced from recombination in the sternocleidomastoid muscle (SCM) of *aP2-Cre;R26-LacZ* reporter mice and *aP2-Cre;R26-YFP* mice and whole-tissue examination. Interestingly, it was found that eRMS tumors in situ were completely surrounded by non-neoplastic adipose tissue adjacent to and clearly separated from the SCM at P14.

To compare the tumorigenic potential of Smo^{M} in myogenic lineages, Hatley et al. (2012) activated the Smo^{M2} allele in early muscle development, employing *Pax3*-, *Myf5*-, or *MyoD1-Cre*. All of these crosses resulted in embryonic lethality without tumor formation. However, activation of Smo^{M2} with *Myogenin-Cre*, which is specific for myoblast-stage muscle differentiation, resulted in tongue tumors in 100% of the mice. Mice with activation of Smo^{M2} in terminally differentiated skeletal muscle (*MCK-Cre*) were viable with no evidence of tumorigenesis. This result is the best by far in asking whether differentiated versus differentiating myofibers can transform into rhabdomyosarcoma. To address the tumorigenic potential of Smo^{M2} in postnatal muscle stem cells (satellite cells), a *Pax7-*$CreER^{T2}$ was employed, and mice were aged until 150 days—without development of tumors. It would have been intriguing to see if prior reports of non-myogenic sarcomas arising from a satellite cell lineage (Rubin et al., 2011) would have been observed if the mice had been aged longer. Another caveat of these findings is that the promoter of Smo^{M2} in this system was *Rosa26*, not the native *Smo* promoter, and it is therefore difficult to say that this is a molecularly physiological eRMS model. Furthermore, given that most Hedgehog pathway inhibitors currently in clinical trials are Smoothened antogonists, this model may have highest value as a genetic model of eRMS for the time being and as a preclinical therapeutic model only when GLI inhibitors emerge as clinical candidate agents.

Taking a look onto mesenchymal progenitor cell biology, the plasticity of these stem cells may not be so surprising. It is known that Myf5 expressing cell can develop into both muscle and brown fat tissue via PRDM16 and PPARγ signaling (Seale et al., 2008). Furthermore, satellite cells can undergo adipogenesis under certain experimental conditions (Asakura et al., 2001; Shefer et al., 2004) or may apparently undergo transdifferentiation into fibroblasts (Brack et al., 2007). Hedgehog activation can also reduce fat

formation via PPARγ (Suh et al., 2006). Such reports lend one to speculate that the origin of *aP2-Cre; Smo*$^{M2/+}$ mice tumors might be cells that have undergone a transdifferentiation event from the adipocyte to the myogenic lineage (Figure 1).

Hatley et al. (2012) recapitulate another important finding by gene expression profiling that *aP2-Cre;Smo*$^{M2/+}$ tumors show an activated satellite cell phenotype reflective of the eRMS tumor pathology rather than the lineage of origin for the tumor. Certainly, activation of Hedgehog signaling was evident by RT-PCR studies of *aP2-Cre;Smo*$^{M2/+}$ tumors, yet, by contrast, less than 30% of human eRMS exhibit a gene expression signature consistent with a Hedgehog pathway on overdrive state (Rubin et al., 2011), and only rarely is Hedgehog overdrive a solitary signaling abnormality. Instead, p53 loss of function was most frequent (Rubin et al., 2011)—implying that p53 loss precedes Hedgehog overdrive temporally in rhabdomyosarcomagenesis. The p53 status of *aP2-Cre;Smo*$^{M2/+}$ tumors was unexplored, but it would have been of interest to have known the p53 status of these tumors, particularly given the 1969 historical precedent of Li and Fraumeni describing a familial eRMS syndrome now known to be attributable to germline *p53* mutation.

In the Hatley model, eRMS tumors occurred from only the head and neck, yet human eRMS occur also in the urogenital tract, extremities, and retroperitoneum. Nevertheless, this cranial-oriented eRMS model may be of specific interest for the subset of head and neck eRMS patients it models, and closer examination of the preneoplastic and early neoplastic lesions will invariably help us understand the microenvironment in which these tumor initiating cells transform.

Overall, the heterogeneity in human rhabdomyosarcoma phenotypes may result from the balance between genetic factors (mutational profile of the tumor including the initial mutation[s] and modifiers) and epigenetic factors (the cell of origin). In an era of precision medicine, this transgenic model representing the subset of human head and neck ERMS not derived from myogenic precursors may be particularly valuable in defining therapeutic targets for select patients. Applying knowledge from these specialized preclinical models to clinical use will no doubt require novel statistical designs for clinical trials, given that the overall annual incidence of pediatric rhabdomyosarcoma is only in the hundreds, not thousands. Personalized rhabdomyosarcoma science inches ever closer.

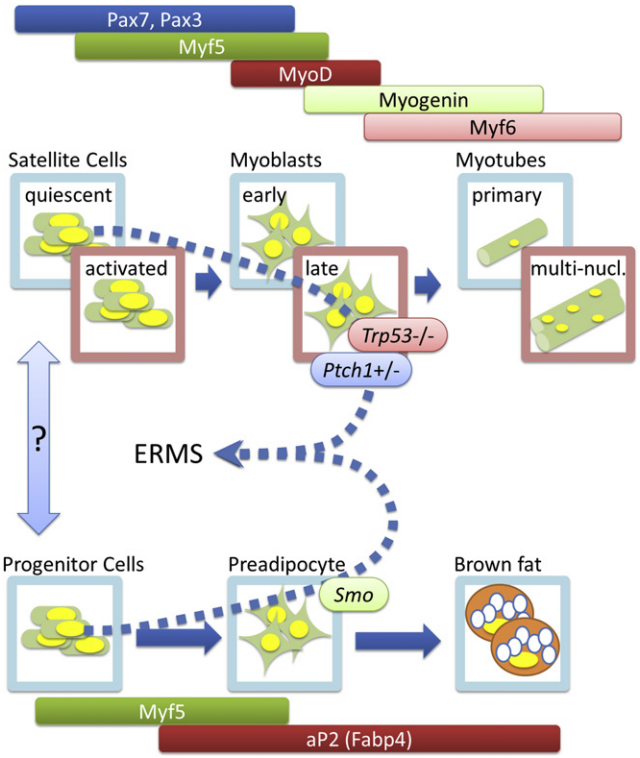

Figure 1. Model of Skeletal Myogenesis and Possible Cellular Origins of eRMS

Postnatal muscle maintenance and regeneration are regulated by Pax3, Pax7, and muscle regulatory factors (MyoD, Myf5, Myogenin, and Myf6). Studies described in the text suggest that the cells of origin for eRMS include differentiating myoblasts and adipocytes.

REFERENCES

Asakura, A., Komaki, M., and Rudnicki, M. (2001). Differentiation *68*, 245–253.

Brack, A.S., Conboy, M.J., Roy, S., Lee, M., Kuo, C.J., Keller, C., and Rando, T.A. (2007). Science *317*, 807–810.

Gurney, J.G., Young, J.L., Jr., Roffers, S.D., Smith, M.A., and Bunin, G.R. (1999). Soft Tissue Sarcomas. In Cancer Incidence and Survival among Children and Adolescents: United States SEER Program 1975–1995, National Cancer Institute, SEER Program. NIH Pub. No. 99–4649. Ries LAG, SM, J.G. Gurney, M. Linet, T. Tamra, J.L. Young, and G.R. Bunin, eds. (Bethesda, MD: National Cancer Institute).

Hatley, M.E., Tang, W., Garcia, M.R., Finkelstein, D., Millay, D.P., Liu, N., Graff, J., Galindo, R.L., and Olson, E.N. (2012). Cancer Cell *22*, this issue, 536–546.

Rubin, B.P., Nishijo, K., Chen, H.I., Yi, X., Schuetze, D.P., Pal, R., Prajapati, S.I., Abraham, J., Arenkiel, B.R., Chen, Q.R., et al. (2011). Cancer Cell *19*, 177–191.

Seale, P., Bjork, B., Yang, W., Kajimura, S., Chin, S., Kuang, S., Scimè, A., Devarakonda, S., Conroe, H.M., Erdjument-Bromage, H., et al. (2008). Nature *454*, 961–967.

Shapiro, N.L., and Bhattacharyya, N. (2006). Otolaryngol. Head Neck Surg. *134*, 631–634.

Shefer, G., Wleklinski-Lee, M., and Yablonka-Reuveni, Z. (2004). J. Cell Sci. *117*, 5393–5404.

Suh, J.M., Gao, X., McKay, J., McKay, R., Salo, Z., and Graff, J.M. (2006). Cell Metab. *3*, 25–34.

Urs, S., Harrington, A., Liaw, L., and Small, D. (2006). Transgenic Res. *15*, 647–653.