YAPping About Differentiation Therapy in Muscle Cancer

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http://dx.doi.org/10.1016/j.ccr.2014.07.011

Overcoming a presumed differentiation block in the childhood muscle cancer embryonal rhabdomyosarcoma is often thought to hold promise as an approach to replace cytotoxic chemotherapy with molecularly-targeted differentiation therapies. In this issue of *Cancer Cell*, Tremblay and colleagues implicate YAP1 and the Hippo signaling pathway in the maintenance of differentiation-arrested and proliferative phenotypes for embryonal rhabdomyosarcoma.

Differentiation therapy for the muscle cancer rhabdomyosarcoma (RMS) has been thought to hold promise for replacing cytotoxic chemotherapy with molecularly targeted therapies. Such a targeted therapy might restore the terminal myogenic differentiation program to the rhabdomyosarcoma cells and (potentially) reduce life-long chemotherapy related sequelae for the patient. Indeed, differentiation therapy has been used successfully in the treatment of acute promyelocytic leukemia and neuroblastoma (Reynolds and Lemons, 2001). Embryonal rhabdomyosarcoma (ERMS), an RMS subtype thought to have an activated satellite cell phenotype and an arrested myogenic differentiation program, displays the greatest tendency toward myodifferentiation and may be amenable to differentiation therapy. However, no successful differentiation therapies for RMS have entered the clinic. Recently, there has been renewed interest in differentiation therapy for solid tumors, the development of which will depend on understanding the molecular mechanisms involved in suppressing differentiation and identifying targets for therapeutics. In the work presented in this issue of *Cancer Cell*, Tremblay et al. (2014) implicate YAP1 and the Hippo signaling pathway in the differentiation-arrested and proliferative phenotypes of ERMS.

Tremblay at al. (2014) first explored the expression and cellular compartment localization of YAP1 in human RMS samples and found that YAP1 was overexpressed in ERMS tumors and was predominately nuclear localized. YAP1 immunostaining correlated with Ki-67 positivity. These results are in accord with a recent report in which the YAP1 oncoprotein was found to be overexpressed in both the cytoplasmic and nuclear compartments in alveolar RMS and ERMS tumor samples (Crose et al., 2014). Furthermore, a number of patient-derived ERMS samples also exhibited a recurrent *YAP1* locus copy number gain.

To examine the functional relevance of these findings, Tremblay et al. (2014) conditionally activated a doxycycline (DOX)-inducible *hYAP1 S127A* transgene to drive YAP1 overexpression in specific lineages: Pax7-creERT2 (activated and quiescent satellite cells), Myf5-Cre (prenatal and postnatal lineages of very early myogenic progenitors/activated satellite cells and early myoblasts), and Myod1-iCre (early myogenic progenitors/activated satellite cells and early and late myoblasts). Myf5-Cre also marks an adipose lineage. Myf5-Cre and Myod1-iCre mice developed ERMS-like tumors in the interstitial compartment of all muscles. These tumors demonstrated positive desmin and myogenin immunostaining, although no tumors developed in the brown fat pads of Myf5-Cre mice. Pax7 mice whose limbs were cardiotoxin-injured developed tumors arising from the Pax7-creERT2 lineage; no tumors developed in the contralateral uninjured limbs of these mice, suggesting that activated satellite cells and their progeny, not the quiescent population, may be the cell-of-origin in this YAP1-driven model of ERMS.

In this genetic system, the tumors were transplantable—and yet this tumorigenicity was reversible. Primary cell cultures established from explant secondary tumors were able to proliferate in the presence of DOX, but spontaneously differentiated when withdrawn from DOX and subjected to low-serum culture conditions. In vivo, mice bearing secondary tumors experienced spontaneous regression and differentiation of their tumors when withdrawn from DOX, demonstrating that YAP1 overexpression drives proliferation and may have a role in arresting the terminal differentiation program. It is perhaps not surprising then that the genes preferentially downregulated following YAP1 normalization included the early myogenic lineage markers Pax7 and Myf5 with concomitant upregulation of the differentiation markers Myod1 and Myh4. Tremblay et al. (2014) also found that YAP1 globally regulates gene expression, maintaining the pro-proliferation phenotype through direct transcriptional repression of myogenic regulatory factors and gene expression upregulation of known inhibitors of Myod1 and Mef2 (i.e., Id2, Twist1, and Snai1/2). Correlatively, *YAP1* expression declines in differentiating mouse and human fetal myoblasts.

It should be noted that murine primary tumors in this model have only one genetic lesion, and YAP1 overexpression is linked to not only the *Rosa26* promoter, but also a tetracycline-responsive element, resulting in a perhaps nonphysiological level of (over)expression. While Tremblay et al. (2014)

demonstrate that activated YAP1 expression can be a sufficient transformational event in the murine myoblast cell line C2C12, human ERMS is more heterogeneous with a mutational landscape known to be considerably more complex with multiple copy number variants, a nonmodest background mutation rate, and recurrent activating *RAS* mutations (Shern et al., 2014). The exact role of YAP1 in the context of oncogenic RAS signaling for ERMS is, as of now, unexplored. However, recent reports suggest that YAP1 and KRAS converge in other forms of cancer. The same may be true in ERMS, for which Tremblay et al. (2014) provide evidence that a YAP1 overexpression signature is associated with higher stage tumors and worsened prognosis.

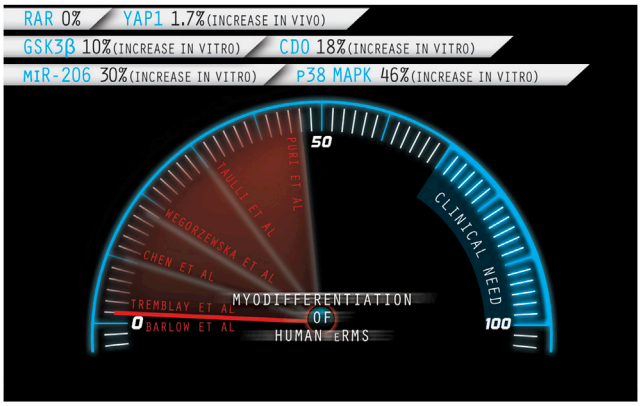

Figure 1. Benchmarking Myogenic Differentiation in Human ERMS
Representative interventions reported as percentage increase of MHC positive cells in vitro or in vivo (Barlow et al., 2006; Chen et al., 2014; Puri et al., 2000; Taulli et al., 2009; Wegorzewska et al., 2003). (In the case of RAR, MHC was not done but the authors reported no differentiation by morphology or by Troponin-T immunocytochemistry in response to retinoic acid.) Corresponding targets are noted. For consistency, only studies of the prototypic RD cell line (generated in 1968) are included. Some of these pathways may be interlinked (e.g., GSK3β and YAP1 have been reported to be coassociated on the Axin scaffold, regulating β-catenin and YAP1 signaling in parallel). Illustration by Nick Escobar.

The most poignant result of these studies was the attempt to translate from a murine genetic proof-of-concept system to a human tumor system, as measured by the differentiation effect on the human ERMS cell line RD in a xenograft system. Knockdown of YAP1 in overexpressing RD cells resulted in a reduced tumorigenicity, but only a 1.7% increase in differentiation ability (and overall, no more than a 3% differentiation of tumor cells was seen). Thus, the reversibility of YAP1 driven tumors was less impressive in human RD tumor cells. Unfortunately, too, only one human ERMS cell culture was tested. The results presented by Tremblay et al. (2014), while novel and exciting, raise an important question about the feasibility of differentiation therapy: is complete differentiation of nearly all ERMS cells within a tumor really possible (Figure 1), if not only in the setting of microscopic residual disease? The authors suggest in their Highlight that "YAP1 inhibition is a promising strategy for differentiation therapy of ERMS." We ask for caution on this point. In the context of the mouse model studies, their approach is *interesting*; however, their experimental evidence is insufficient and inadequate in the context of a therapeutic strategy for human patients. The same concern raised in recent commentaries on the rigorousness of preclinical studies (Macleod, 2014) should be embraced here, so that unjustified clinical trials are not initiated—and so that families of children affected by ERMS are not given false hope. Nonetheless, one might say this approach is worthy of deeper study—potentially by means of targeting several pathways simultaneously. We have known since the earliest chemotherapy clinical trials that combination therapies are more effective than single agents. In RMS, differentiation therapy may be no different.

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