New Insights into the Origin and the Genetic Basis of Rhabdomyosarcomas

Vahab D. Soleimani[1,2] and Michael A. Rudnicki[1,2,*]
[1]Sprott Center for Stem Cell Research, Ottawa Hospital Research Institute, Ottawa, ON, Canada
[2]Department of Medicine, University of Ottawa, Ottawa, ON, Canada
*Correspondence: mrudnicki@ohri.ca
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In this issue of *Cancer Cell*, Rubin et al. (2011) describe using various conditional mouse models to trace the developmental origin and genetic basis of rhabdomyosarcomas. Their work provides a genetic dissection underlying rhabdomyosarcomas development and unveils unexpected relationship between various soft-tissue tumor types.

Rhabdomyosarcomas (RMSs) are the predominant soft-tissue tumors of children and adolescents (Arndt and Crist, 1999). These tumors are generally divided into alveolar rhabdomyosarcoma (aRMS) and embryonal rhabdomyosarcoma (eRMS) subtypes. Most aRMSs are the result of chromosomal translocations between *PAX3* or *PAX7* and *FOXO1A* genes, resulting in Pax3-FKHR and Pax7-FKHR fusion proteins (Tiffin et al., 2003). These fusion proteins have potent transcriptional activity leading to cellular transformation and oncogenesis into aRMS. On the other hand, a wide range of causative mutations have been implicated in eRMS, such as the loss of heterozygosity at 11p15.5 locus (Anderson et al., 1999), mutations in tumor suppressor p53 (Felix et al., 1992), retinoblastoma (Rb1) (Kohashi et al., 2008), N- and K-ras genes (Stratton et al., 1989), and *PTCH1* haploinsuficiency (Hahn et al., 1998).

It is thought that eRMSs develop from cells residing within the muscle tissue, partly because eRMSs express markers of muscle cells such as MyoD, Myogenin, and Desmin. Furthermore, these tumors can also occur where muscle tissue resides. However, muscle tissue contains a heterogeneous population of muscle stem cells and downstream myogenic progenitors as well as nonmyogenic cells (Kuang et al., 2007). To study the potentials of individual subpopulations of muscle cells in eRMS development, Rubin et al. (2011) deleted *p53* either with or without *Ptch1* haploinsuficiency. They then used various Cre drivers to inactivate these genes in muscle stem cells and in proliferating and maturing myoblasts. In a 600 day follow-up period, they observed that all mouse Cre lines developed tumors at the penetrance rate of 13%–56%. Upon histological examination of these tumors, they found a spectrum of malignancies ranging from alveolar and embryonal RMS to undifferentiated pleomorphic sarcomas (UPSs). They observed that rhabdomyosarcomas developed from all subpopulations of muscle cells, including muscle satellite cells and differentiating myoblasts (Figure 1). More importantly, they found that the cell of origin and the mutational profile of the tumors were important in determining the proportion of rhabdomyosarcomas versus undifferentiated spindle cell sarcomas (i.e., UPSs). Loss of p53 in maturing myoblasts (Myf6+ cells) gave rise to the highest percentage of eRMSs. These tumors showed the highest degree of myogenic differentiation potential, while those derived from satellite cells (Pax7+ cells) had the lowest rate of myogenic differentiation in in vitro differentiation assays.

To study the effect of retinoblastoma (*Rb1*) mutation on eRMS development in combination with the loss of p53, Rubin et al. (2011) inactivated both *p53* and *Rb1* with or without *Ptch1* haploinsuficiancy. Loss of *Rb1* alone did not lead to tumor initiation. However, unlike *Rb1* loss, *Ptch1* haploinsuficiency contributed to tumor initiation at every level of cellular differentiation. To further explore the role of *Rb1* in rhabdomyosarcomas development, they inactivated both *Rb1* and *p53* in various subpopulations of muscle progenitor cells using different Cre drivers. Surprisingly, they observed that combination of *Rb1* and *p53* loss was generally associated with an undifferentiated phenotype in the resulting tumors. *Rb1* deletion reduced the myodifferentiation potentials of *p53* null tumor cells. Based on these observations, Rb1 seems to act as a modifier of tumor phenotype, in part by regulating the proliferation rate of sarcoma cells. Interestingly, analysis of the global gene expression profiling showed a marked difference between tumors with intact versus mutant Rb1. These findings further support the conclusion that Rb1 may act as modifier in sarcoma development, potentially by regulating a broad range of genetic and transcriptional networks.

Perhaps one of the most intriguing aspects of this study by Rubin et al. (2011) is that at least a subset of eRMS and UPS tumors seem to share a common cell of origin. This is interesting, as UPS tumors, a broad range of heterogeneous neoplasms including malignant fibrous histiocytomas or undifferentiated spindle cell sarcomas have a poorly defined etiology. These tumors also show no obvious signs of differentiation by immunohistochemical and molecular criteria. On the other hand, eRMSs express a broad range of muscle cell markers and possess myodifferentiation potentials. Their data further show that while maturing myoblasts are more prone to giving rise to eRMS tumors, UPS tumors are more likely to develop from Pax7 expressing muscle satellite cells. Furthermore, irrespective of the cell of origin, Rb1 modifies tumor phenotype to mimic UPS (Figure 1). Therefore, UPSs and eRMSs may constitute a continuum of the same disease.

By comparative analysis and global gene expression profiling, Rubin et al. (2011) delineate gene expression signature for UPS and eRMS and show that

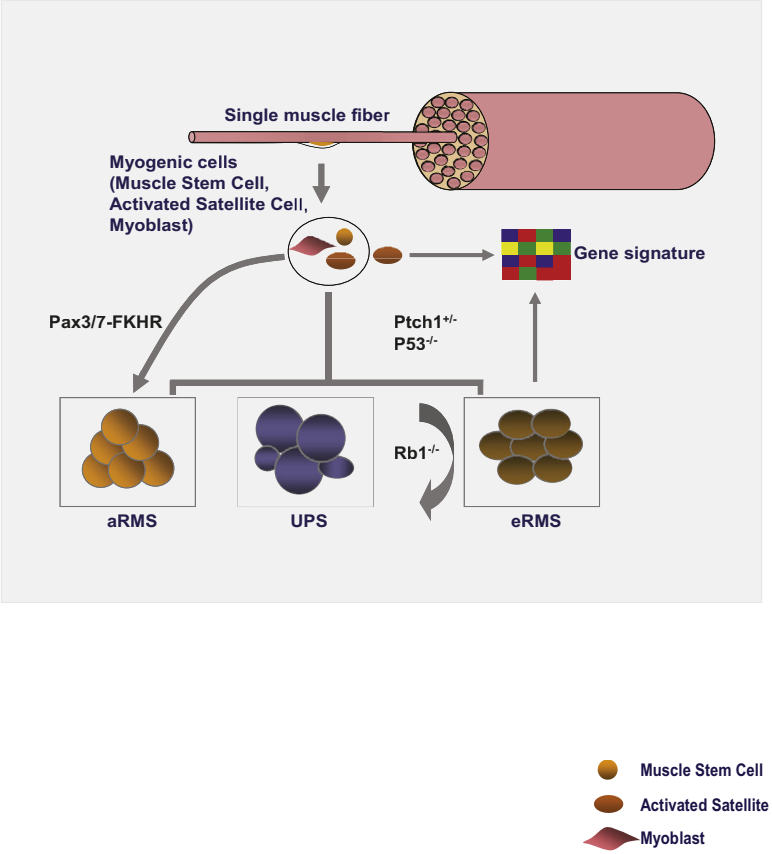

Figure 1. The Developmental Origin and Mutational Profile of the Tumor Determine the Proportions of RMS

Different subpopulations of myogenic precursor cells give rise to RMS. While *p53* deletion and *Ptch1* haploinsufficiency are important players in cellular transformation and myodifferentiation potential of the resulting tumor, *Rb1* deletion acts as a modifier of tumor phenotype in that context. Tumors (i.e., eRMSs) arising from different cells of origin exhibit the same gene expression profile as that of the activated muscle satellite cells.

eRMSs have a similar gene signature with that of the activated muscle satellite cell (Figure 1). This finding is interesting for two reasons: first, it shows that gene expression pattern is not a predictor of the cell of origin, as eRMSs develop from a range of muscle cells, including muscle satellite cells and downstream myogenic progenitors such as maturing myoblasts, as shown in this study. Second, it implies that the cohort of mutations giving rise to eRMS likely results in a broad reprogramming of the transcriptional network of the transformed cells, making it to resemble gene signature of the activated satellite cells.

The study by Rubin et al. (2011) provides important new insight into the genetic basis of rhabdomyosarcomas in the context of *p53*, *Rb1*, and *Ptch1* mutational pathways, and shows the potential of various subpopulations of muscle stem cells and downstream myogenic precursors in rhabdomyosarcomas development. Importantly, this study opens a forum for addressing other fundamental questions in the future. For example, to assess the relevance of their mouse sarcoma models to the human disease, Rubin et al. (2011) studied the gene expression profile of 111 primary human fusion-negative rhabdomyosarcomas from public databases and found that in at least 29% of cases they were unable to identify a gene signature in line with their mouse sarcoma models. The authors rightly argue that there might be additional mutations involving other tumor suppressors that may be involved in rhabdomyosarcomas development. Indeed, there is recent evidence indicating that other tumor suppressor genes such as PTEN may also play a role in sarcoma development (Gibault et al., 2011). In addition, given the observation that muscle stem cells and the downstream myogenic precursors can give rise to eRMS as demonstrated in this study does not preclude the possibility of other nonmuscle cells to contribute to the disease, as also emphasized by the authors. The conclusions from this study and previous work suggest that the genetic basis of rhabdomyosarcomas, especially that of the eRMS, is complex and is likely defined by a wide range of genetic and epigenetic factors. The heterogeneity in rhabdomyosarcomas phenotype may therefore be the result of the balance between the mutational profile of the tumor and the cell of origin. The involvement of tumor modifiers such as Rb1 in changing sarcoma phenotype as shown in this study raises many interesting questions about the possibility of yet other unknown modifiers and the genetic context in which these modifiers exert their effect on shaping the tumor phenotype. Future studies involving comparative genetic and epigenetic analysis of these tumors may provide a more concrete understanding of a cohort of potential players in rhabdomyosarcomas development. The feasibility and relative affordability of large scale genomic sequencing platforms provide opportunities to perform comparative genome-wide analysis in large sets of tumor samples in search of these genetics or epigenetic factors. In addition, a role for posttranscriptional gene regulation by microRNAs in rhabdomyosarcomas development has also been demonstrated (Wang et al., 2008). Further studies on the role of microRNAs in sarcomas development are another avenue that is important to pursue.

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One NOTCH Further: Jagged 1 in Bone Metastasis

Jianning Tao,[1] Ayelet Erez,[1] and Brendan Lee[1,2,*]
[1]Department of Molecular and Human Genetics
[2]Howard Hughes Medical Institute
Baylor College of Medicine, Houston, Texas 77030, USA
*Correspondence: blee@bcm.edu
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The outgrowth of metastatic cells to bone depends on the interaction between multiple intrinsic and host factors. In this issue of *Cancer Cell*, Sethi and colleagues report Notch signaling in bone cells as responsible for promoting this outgrowth and provide evidence for a beneficial treatment effect of NOTCH inhibitors.

Metastasis, the last and most devastating stage of tumor progression, remains the cause of 90% death in cancer patients. The development of metastases requires a series of sequential rate-limiting steps through which malignant tumor cells from the primary site invade into blood and lymphatic vasculature, survive in the circulation, lodge at distant organs, and outgrow. The revised "seed and soil" theory, originally proposed by Steven Paget a century ago, hypothesizes that the outcome of metastasis depends on crosstalk between predetermined cancer cells (the "seeds") and specific organ microenvironments (the "soil"), which release homeostatic factors (Fidler and Poste, 2008). In the soil organ, the seed cancer cells can enter either latent phase or outgrowth phase.

The skeletal system is recognized as the habitat of the hematopoietic stem cell as well as the most common metastatic site for breast cancer. Recently, a positive feedback loop causing a "vicious cycle" has been identified, in which the outgrowth phase of the bone metastases is determined by a bidirectional interaction between the cancer cells and the bone microenvironment. This crosstalk involves growth factors and cytokines derived from both host and cancer cells (Figure 1) (Kang et al., 2003; Zhang et al., 2009).

Seventy percent of breast cancer patients are affected by bone metastasis, manifested by skeletal-related events such as severe bone pain and pathological fractures (Mundy, 2002). There is much evidence that metastatic cancer cells usurp the normal process of bone remodeling through stimulation of both osteoclasts that resorb bone and osteoblasts that deposit bone, and the net outcome of lesions depends on the relative contribution of each cell type. In the outgrowth (or osteolytic) phase, multiple growth factors, including transforming growth factor β (TGFβ) and insulin-like growth factor 1 (IGF1), are released from the degraded bone matrix. TGFβ and IGF1 both enhance the growth of cancer cells and stimulate them to produce several cytokines, including parathyroid hormone-related protein (PTHrP), connective tissue growth factor (CTGF), and interleukin 11 (IL11). PTHrP and IL11 regulate the expression of osteoclastogenic factors receptor activator of nuclear factor-κB ligand (RANKL) and osteoprotegerin (OPG) in osteoblasts, whereas CTGF mediates both angiogenesis and invasion (Massague, 2008). Additional cells, such as bone borrow-derived stromal, endothelial, and hematopoietic cells, have all been shown to contribute to the development of the macrometastases and the production of prometastatic factors (Joyce and Pollard, 2009). On the other hand, the processes by which metastatic cancer cells directly communicate with various types of cells in the bone and bone marrow remains an enigma in the field of bone metastasis. Undoubtedly, fully answering such questions will provide the insight necessary for the development of effective therapies against bone metastasis.

Sethi et al. (2011) now provide both experimental and preclinical evidence that the Notch ligand Jagged1 plays a critical role in the promotion of bone metastatic outgrowth of breast cancer. Using a bioinformatic approach that correlates the gene expression pattern of Notch signaling pathway components (ligands, receptors, and downstream targets) to bone metastasis, the authors indentified a unique upregulation of Jagged1, which was highly correlated with human breast cancer metastases to bone. To investigate the functional role of Jagged1 in the development of bone metastasis, the authors applied two different types of Jagged1-expressing human breast cancer cell lines in a xenograft mouse model. In the strongly bone tropic cell lines with high levels of Jagged1 expression, stable knockdown of Jagged1 resulted in a reduction of