Rhabdomyosarcomagenesis—Novel pathway found

Rhabdomyosarcoma (RMS) is the most common soft tissue sarcoma in children. Recent work presented in this issue of *Cancer Cell* by Fleischmann et al. on gene knockout mice revealed a remarkable molecular pathway for rhabdomyosarcomagenesis, in which *Trp53*/*Fos* double mutant mice developed RMS of the facial and orbital regions with high penetrance. This finding may provide novel molecular mechanisms for Rhabdomyosarcomagenesis and therapeutic implications for RMS patients.

Rhabdomyosarcoma (RMS) is a family of soft tissue tumors that generally occur in the pediatric population. The developmental origin of RMS is unclear. However, RMS cells express a number of skeletal muscle markers such as desmin, sarcomeric actin, sarcomeric myosin heavy chain, and muscle-specific transcription factor MyoD. In addition, cytoplasmic striations, a sarcomeric muscle-specific characteristic, can be detected in RMS cells. These observations suggest myogenic cell origin of RMS (Merlino and Helman, 1999).

RMS can be categorized as two different types: embryonal and alveolar RMS. Embryonal RMS is characterized by loss of heterozygosity (LOH) at the 11p15 region where it is maternally imprinted in normal tissue and locates a number of imprinted genes such as H19, insulin-like growth factor 2 (IGF2), and $p57^{KIP2}$. LOH at these loci results in transcriptional activation from both maternal and paternal alleles and increases expressions of the imprinted genes such as IGF2 observed in RMS. Alveolar RMS tends to occur in the older age group. 90% of alveolar RMS carry a characteristic chromosomal translocation of DNA binding domain of the Pax3 or Pax7 gene to the transactivation domain of FKHR gene at t(2;13)(q35;q14) or t(1;13)(p36;q14), respectively (Merlino and Helman, 1999). Pax3 and Pax7, a member of the Paired box transcription family, are involved in skeletal muscle development. Gene mutant mice demonstrate that Pax3 is essential for embryonic skeletal muscle development by regulating gene expression of c-met, a hepatocyte growth factor/scatter factor (HGF/SF) receptor, required for myogenic cell migration (Buckingham, 2001). In contrast, Pax7 is required for specification of muscle satellite cells and myogenic stem cells and essential for postnatal muscle growth and regeneration (Seale et al., 2000). FKHR is a member of the forkhead/HNF-3 transcription factor family. The chimeric protein of Pax3-FKHR is a more potent transcriptional activator than wild-type Pax3. Ectopic expression of the chimeric gene converts fibroblasts to myogenic cells by activation of a number of muscle-specific genes (Khan et al., 1999). These observations indicate that overexpression of growth factors such as IGF2 or activation of Pax genes results in RMS.

RMS is also generated in Li-Fraumeni syndrome associated with *Trp53* germline mutations. In addition, some sporadic cases of RMS involved Trp53 germline mutations (Merlino and Helman, 1999). Trp53, a transcription factor, is a tumor suppressor gene product involved in cell apoptosis, cell cycle withdraw, differentiation, and cellular senescence (Kirkwood, 2002). Trp53 is activated by a number of cellular stresses such as DNA damages. The transcriptional activity of Trp53 was first found in skeletal muscle-specific gene muscle creatine kinase (MCK) enhancer. However, mice carrying null mutations in both Trp53 alleles ($Trp53^{-/-}$) seem to exhibit normal skeletal muscle development (White et al., 2002). $Trp53^{-/-}$ mice increase tumor susceptibility in adulthood. The majority of tumors are lymphomas and RMS is very rare in these mice. However, Trp53 heterozygotes ($Trp53^{+/-}$) develop mostly sarcomas and 19% of sarcomas are RMS. These results suggest implication of Trp53 gene in rhabdomyosarcomagenesis (Merlino and Helman, 1999).

Here, Fleischmann et al. (2003) described remarkable results in which *Trp53*/*Fos* double knockout mice developed highly proliferative and invasive rhabdomyosarcomas of the facial and orbital regions, which is a similar location as seen in human rhabdomyosarcoma patients. Fos is a zinc finger transcription factor consisting of AP-1 and regulates various biological processes by transcriptional activation of a number of target genes downstream of signaling pathways such as Protein Kinase C (PKC) (Acquaviva et al., 2002). $Fos^{-/-}$ mice display osteopetrosis due to lack of osteoclasts. Overexpression of Fos in transgenic mice causes them to develop chondrosarcoma and osteosarcoma. Fos is also known to regulate apoptotic signaling pathways. Fleischmann et al. generated *Trp53*/*Fos* compound mutant mice ($Trp53^{-/-}$;$Fos^{-/-}$), and these mice initially developed osteopetrosis similar to the phenotype seen in $Fos^{-/-}$ mice. However, $Trp53^{-/-}$;$Fos^{-/-}$ mice started to develop tumors of the facial and orbital regions at 10 weeks of age and the tumor penetrance was over 90% at 25 weeks. These tumor cells with polygonal or elongated shape expressed a cell cycle-associated protein and a number of skeletal muscle markers such as desmin and MyoD. These characteristics are very similar to human RMS. Cell lines isolated from the RMS-like tumors expressed desmin and MyoD. Differentiation condition such as low serum induced myotube formation of the $Trp53^{-/-}$;$Fos^{-/-}$ RMS cells, in which cytoplasmic cross-striations were observed. Reexpression of Trp53 or Fos in the $Trp53^{-/-}$;$Fos^{-/-}$ RMS cells induced apoptotic cell death. Interestingly, while reexpression of Fos in the $Trp53^{-/-}$;$Fos^{-/-}$ RMS cells did not affect expression of pro- or antiapoptotic genes such as Bcl-2 family members and TNF-α/Fas pathways, Pax7 gene expression was significantly reduced in these cells. Reexpression of Fos in the $Trp53^{-/-}$;$Fos^{-/-}$ RMS cells also downregulated muscle-specific gene expression and inhibited myogenic differetiation. In addition, overexpression of Fos in the primary myoblasts also downregulated muscle-specific gene expression, and Pax7 gene expression was decreased to below the detection limit. These results suggest the interesting molecular mechanisms in which Fos is the repressor for Pax7 gene transcription and upregulation of Pax7 gene expression may result in increased myoblast proliferation and prevent apoptosis. Finally, Fleischmann et al. concluded that Fos proto-oncogene possesses a novel tumor-suppressive function and provide an important mouse model for human RMS.

The molecular mechanisms by which Fos suppresses Pax7 gene tran-

scription are still unknown. However, upregulation of Pax gene expression is clearly involved in rhabdomyosarcomagenesis. Pax3 is known to regulate c-met gene transcription, overexpression of which is occasionally seen in human RMS. Overexpression of HGF/SF in transgenic mice also caused RMS to develop in 4% of mice (Merlino and Helman, 1999). More recently, RMS is frequently observed in mice carrying overexpression of HGF/SF gene in an *Ink4a/Arf* null background (Sharp et al., 2002). These reports strongly suggest that rhabdomyosaromagenesis implicates Fos, Pax, c-met, and HGF/SF signaling pathways. Although the origin of RMS cells is assumed to be from myogenic cells such as satellite cells, recent work demonstrates existence of novel stem cells in muscle, which give rise to myogenic cells during muscle regeneration (Asakura et al., 2002; Polesskaya et al., 2003). Therefore, the novel stem cells in muscle such as side population (SP) cells may be a developmental origin for RMS cells, in which Fos/Pax/c-met pathway is involved. In the near future, finding the molecular mechanisms of Fos/Pax/c-met pathway involved in rhabdomyosarcomagenesis and the developmental origin of RMS would not only facilitate our understanding of the mechanisms of rhabdomyosarcomagenesis but also develop novel therapeutic applications for human RMS patients.

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Defective autophagy leads to cancer

Cellular proteins are degraded within two distinct compartments: the proteasome and the lysosome. Alterations in proteasomal degradation can contribute to carcinogenesis. In contrast, alterations in autophagic protein degradation through the lysosome have not been linked to cancer. Now two reports demonstrate that the autophagic gene, Beclin 1, is a haploinsufficient tumor suppressor gene. These new data suggest that autophagic degradation provides an important mechanism to prevent cellular transformation.

The accumulation of individual proteins within eukaryotic cells reflects a tightly controlled balance between protein synthesis and degradation. Two major pathways for the degradation of proteins have been described: cytosolic degradation by the proteasome and autophagic degradation of proteins and organelles within the lysosome (Klionsky and Emr, 2000). Considerable evidence suggests that the proteasome is responsible for the regulated degradation of short-lived proteins involved in cell cycle control as well as proteins that participate in stress responses such as DNA damage-induced cell cycle arrest or adaptation to hypoxia. A number of examples linking alterations in proteasomal protein degradation to the pathogenesis of cancer exist (Pagano and Benmaamar, 2003). In contrast, alterations in autophagic degradation of cellular proteins has not been linked to the causation of cancer. Rather, autophagy has been demonstrated to be important in developmental/differentiative remodeling of cells. Autophagy is also required for the cellular adaptation to nutrient deprivation and the elimination of damaged organelles (Figure 1; Klionsky and Emr, 2000).

Regulation of autophagy

During autophagic degradation, cytosolic proteins and/or organelles are first sequestered within double membrane vesicles, which are then fused to the lysosome (Klionsky and Emr, 2000). The vesicular contents are broken down by pH-sensitive lysosomal hydrolases, and the degradation products are recycled for use in macromolecular synthesis and/or bioenergetics. Relatively little is known about how protein complexes and organelles are specifically targeted for degradation through autophagy. However, much of the molecular machinery required for autophagic vacuole formation and fusion with the lysosome has been identified through genetic screens