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RUNX: A trilogy of cancer genes

The RUNX family of transcription factors plays pivotal roles during normal development and in neoplasias. Recent data involve RUNX3 as an important tumor suppressor in gastric cancers and pose interesting questions about how perturbed levels and interspecific competition among RUNX family members may contribute to tumorigenesis.

On a worldwide basis, gastric cancer is a major cancer-related killer. In Japan and certain other Asian countries gastric cancer tops the list of cancer-induced deaths. While clear links have been discovered between environmental factors, such as *Helicobacter pylori* infection, dietary components, and gastric cancer frequencies, the genetic basis for gastric cancer development is still largely unclear. In the April 5 issue of *Cell*, Li and coworkers (2002) describe the uncovering of RUNX3/AML2/CBFA3/PEBP2αC as a candidate tumor suppressor in gastric cancer development. RUNX3 belongs to the Runt domain family of transcription factors, which consists of 3 DNA binding α subunits, RUNX1, RUNX2, and RUNX3 (see Table 1 for alternative names), each of which is capable of forming heterodimers with the common β subunit CBFβ. RUNX heterodimers are relatively weakly acting transcriptional regulators, the potency of which can be induced by associations with transcriptional (co)activators, such as MYB, ETS, and p300/CBP, or corepressors such as TLE1 and mSin3A (Perry et al., 2002). The main family feature, the 128 amino acid runt domain named for its high homology to the *Drosophila* pair-rule protein runt, facilitates dimerization and DNA binding. Like their counterparts in *D. melanogaster* and *C. elegans,* mammalian RUNX family transcription factors play important roles in cell fate determination during development.

The work of Li and coworkers (2002) completes the mouse knockout analyses of the *Runx* family and underscores the role of Runt family members as cancer-related genes. As previously found for *Runx1* (Okuda et al., 1996), *Runx2* (Otto et al., 1997), and *Cfb*β (Wang et al., 1996), genetic ablation of *Runx3* has profound effects. While *Runx3* knockout mice are born in Mendelian ratios, they die soon after birth probably due to starvation. *Runx3* is strongly expressed in gastrointestinal organs in the developing embryo and throughout adult life of the mouse and the gastric epithelium in *Runx3* knockouts displays hyperplasia and a reduced apoptotic rate. Interestingly, when analyzed in primary cultures *Runx3*$^{-/-}$ gastric epithelial cells are less sensitive to TGF-β-mediated growth inhibition, based on a marked failure to enter apoptosis when treated with TGF-β. To investigate the potential connection between RUNX3 and gastric cancers in humans, Li et al. (2002) analyze a series of gastric cancer cell lines and primary human gastric tumors. Out of 46 primary human tumors, 30% displayed hemizygosity of *RUNX3* with a significant correlation between *RUNX3* loss and gastric cancer progression stage. Furthermore, RUNX3 expression analysis revealed that on average 60% of the analyzed primary human gastric tumors exhibited reduced RUNX3 levels rising to nearly 90% among the late stage, representing highly metastatic tumors. Upon examination of 119 tumor samples, Li et al. were able to identify only a single nucleotide transition causing an arginine-to-cytosine conversion within the conserved Runt domain, which did not strengthen the tumor suppressor argument much. However, the high CpG nucleotide content triggered analysis of DNA methylation status, and interestingly *RUNX3* hypermethylation in a large number of primary tumor samples was found to correlate with gene silencing, which indicates an unusual strong prevalence for epigenetic gene silencing. To further establish a causal link between RUNX3 expression and oncogenesis, Li et al. injected gastric cancer cells engineered to overexpress either wild-type or Runt domain mutated RUNX3 into nude mice. While RUNX3 mediated a significant reduction in tumorigenicity, the Runt domain mutant aggravated tumor formation. Moreover, gastric epithelial cells immortalized by loss of p53 only were able to form tumors in nude mice when Runx3 was also deleted. Together, these results underline the role of RUNX3 as a bona fide tumor suppressor.

It is now clear that all three Runx family members play important roles in normal developmental processes as well as in cancers (Table 1). RUNX1, perhaps better known as AML1, plays a critical role in hematopoietic development, and genetic ablation of either *Runx1* or *Cbf*β results in embryonic

Table 1. Functions and oncogenesis of Runt-related transcription factors[a]

Gene	Alternative gene names	Proposed essential function	Mouse (–/–) phenotype	Tumorigenesis
RUNX1	*AML1/CBFA2/ PEBP2αB*	Definitive hematopoiesis	Embryonic lethal. Absence of fetal liver hematopoiesis.	Hemizygocity in humans predisposes to acute myeloid leukemia. Frequent translocation breakpoint. Common insertion site in retrovirus-induced mouse leukemia.
RUNX2	*AML3/CBFA1/ PEBP2αA*	Bone ossification	Dies at birth from respiratory failure.	Transgenic Runx2 overexpression predisposes to T-cell lymphomas. Common insertion site in retrovirus-induced mouse leukemia.
RUNX3	*AML2/CBFA3/ PEBP2αC*	Development of the gastrointestinal tract	Dies soon after birth. Hyperplastic gastric epithelium.	Frequently inactivated in human gastric cancers. Common insertion site in retrovirus-induced mouse leukemia.

[a]See text for references

lethality and a complete lack of fetal liver hematopoiesis (Okuda et al., 1996; Wang et al., 1996). *RUNX1* has long been recognized as an important translocation breakpoint in human leukemias, with the TEL-AML1 t(12;21) fusion accounting for 20% of acute lymphoblastic leukemia (ALL) cases and the AML1-ETO t(8;21) fusion accounting for 12% of acute myeloid leukemias (AML) (Look, 1997). Importantly, a number of the translocation breakpoint products involving RUNX1 have been shown to have *trans*-dominant effects and block the normal functions of the RUNX1/CBFB heterodimer (reviewed in Perry et al., 2002). The hypothetical function of RUNX1 as a tumor suppressor for AML was further strengthened by the finding that familial mutations with RUNX1 cause thrombocytopenia and predispose to acute myeloid leukemia (Song et al., 1999). The *Runx2* gene product is pivotal for osteoblast differentiation and bone ossification (Otto et al., 1997). Furthermore, RUNX2 is haploinsufficient, and heterozygous RUNX2 loss of function gives rise to cleidocranial dysplasia in both humans and mice, a disorder characterized by multiple skeletal abnormalities (Otto et al., 1997; Mundlos et al., 1997). The oncogenic properties of Runx2 were demonstrated in transgenic mice where Runx2 overexpression pertubates T cell development and synergizes strongly with c-*myc* in lymphomagenesis (Vaillant et al., 1999). Interestingly, all three *Runx* family members have been identified as putative proto-oncogenes in mouse insertional mutagenesis leukemia models (Li et al., 1999; Stewart et al., 1997; A.H.L. and M.v.L., submitted), hence further indicating that absolute levels of the *Runx* heterodimers are important and need to be tightly controlled.

How can we seek to understand the apparently opposing effects of RUNX family members as, on the one hand dominant-acting oncogenes, while on the other hand being important tumor suppressors? One possible explanation could be that since all three RUNX heterodimers are known to recognize the same consensus DNA core motif (TGt/cGGT), erroneous expression of RUNX members outside of their normal tissues might exert oncogenic effects through competitive interference with the RUNX heterodimer species normally present within that particular tissue. Also, RUNX members differ in their associations with coacting factors; RUNX3 for instance entirely lacks a domain present in RUNX1 and RUNX2 responsible for interaction with Ets family members (Bae et al., 1995). Along the same lines, the "wiring" of RUNX members, in terms of activating and repressing signaling pathways, may differ in different cells types. It will be of obvious importance to use expression profiling and other techniques to find the relevant downstream targets for each of the *Runx* heterodimeric transcription factors.

The recognition of *Helicobacter pylori* as a major causative agent in gastric cancer development has led to the classification of *H. pylori* as a Class I carcinogen by the World Health Organization. *H. pylori* infection causes release of reactive oxygen species, mitogenic stimulation, and constitutive inflammatory response in gastric epithelium (Peek and Blaser, 2002; Karin et al., 2002). Early steps in gastric neoplasias involve transition of normal epithelial mucosa to gastritis, a condition promoted by *H. pylori*, implicating *H. pylori* in tumor initiation and promotion (Peek and Blaser, 2002). Later stages involve many of the "common themes" in tumorigenesis: p53 loss of function, K-*ras* activation, and E-cadherin loss or mutation (Kuniyasu et al., 2000). So where does RUNX3 fit into the picture? On the one hand, *Runx3* loss may be an early event, as is suggested by the hyperplasia in the *Runx* knockout mice; on the other hand, the finding of Li and coworkers (2002) that RUNX3 loss increases with cancer progression suggests a role in tumor progression. This dual action of *Runx* loss may be explained by its effects on inducing both cell proliferation (inducing hyperplasia) and on preventing apoptosis (which could be selected for in tumor progression). Indeed, a role for RUNX3 in proapoptotic pathways is sustained by the fact that *Runx3*$^{-/-}$ gastric epithelial cells display apoptotic defects in response to TGF-β. In support of a role for RUNX family members in TGF-β and bone morphogenic protein signaling pathways are the findings that all three RUNX members have been found to bind R-Smads (Hanai et al., 1999) and that thymocytes from *Runx2* transgenic mice are hypersensitive to TGF-β (Vaillant et al., 1999).

Interestingly, *Runx3* loss appears specifically to predispose to gastric cancer but not to colon cancer, despite equally high expression of *Runx3* in both gastric and colonic epithelial cells. Among several other speculative explanations, one is that this could relate to the unique milieu and cofactors in the stomach, such as low pH, dietary factors and *H. pylori*. Given the capability of both *H. pylori* and loss of *Runx3* to induce initial lesions and hyperplasia, an intriguing possibility could be that *Runx3* is one of the downstream "targets" of *H. pylori* action. Having the *Runx3* knockout mice in hand, this and several other important questions can now be investigated directly. While the early death of *Runx3*$^{-/-}$ mice precludes tumorigenesis experiments, careful monitoring of *Runx3*$^{+/-}$ mice, perhaps in combination with other

genetic lesions such as p53 ablation, is expected to reveal gastric cancer predisposition and could provide more detailed insight into the roles of Runx3 in tumor initiation and progression and its possible interactions with cofactors. Alternatively, a conditional *Runx3* knockout mouse may serve this purpose. In addition, such mouse models will allow investigation of the extent to which loss of RUNX3 complements or substitutes for other lesions known from gastric cancers, such as E-cadherin loss, RAS mutations, and loss of DCC. It can be expected that this mouse model for gastric cancer will greatly assist unraveling of the underlying molecular causes for this important widespread disease in the coming years.

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The secrets of selective estrogen receptor modulation: Cell-specific coregulation

A specific increase in the level of a single coactivator appears to enhance estrogen action with tamoxifen at some gene targets in uterine cells but not breast cells.

The discovery and development of antiestrogens as treatments for estrogen receptor (ER) positive breast cancer (Lerner and Jordan, 1990) introduced a new approach for targeted therapy with few side effects compared to traditional cytotoxic chemotherapy. The novel so-called nonsteroidal antiestrogens, initially investigated during the 1960s, were all classified as partial estrogen agonists in the rat uterus but with a predominantly antiestrogen action. This pharmacologic activity in the laboratory extrapolated to antitumor action by blocking estrogen-stimulated breast tumor growth at the ER. Tamoxifen was introduced clinically during the 1970s, and the drug has had a profound effect on patient survival. It is estimated that 400,000 women are alive today because of the success of tamoxifen treatment. However, tamoxifen is a pioneering medicine over and above its ability to save lives.

The recognition of selective estrogen receptor modulation in the laboratory during the 1980s (Jordan, 2001) has had important implications not only for the evaluation of the side effects associated with tamoxifen, but also has established the rationale for a new class of drugs, the selective estrogen receptor modulators (SERMs). It is now clear that SERMs have potential as multifunctional medicines. The SERMs express estrogen-like actions in bone, lower circulating cholesterol, but produce an antiestrogenic action in the breast. The actions of tamoxifen in the endometrium are important because they illustrate the concept of selective ER modulation. When both breast and endometrial tumors are implanted into immune deficient mice (Gottardis et al., 1988), tamoxifen enhances the growth of endometrial cancer but prevents the growth of breast cancer. The tamoxifen ER complex is perceived as an estrogen in endometrial cancer cells but as an antiestrogen in breast cancer. This concept translated to the clinic with a predicted modest rise in the incidence of endometrial cancer in postmenopausal women during tamoxifen therapy. The question is, how is the pharmacology of tamoxifen reversed in