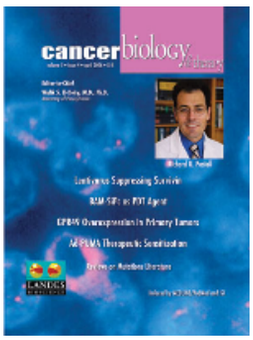

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Journal Club

How Does the Human RUNX3 Gene Induce Apoptosis in Gastric Cancer?

Latest Data, Reflections and Reactions

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KEY WORDS

Runt-related (RUNX), RUNX3, FoxO3a/FKHRL1, tumor suppressor, gastric cancer

ABSTRACT

RUNX3 is the oldest known gene in the RUNX family. Data have demonstrated its function to be thoroughly involved the neurogenesis of the dorsal root ganglia, T-cell differentiation and tumorigenesis of gastric epithelium. As a TGF-β target, RUNX3 protein is believed to be involved in TGF-β-mediated tumor suppressor pathway; however, little is known about its role in apoptosis. According to recent data reported by Yamamura et al., (*J Biol Chem* 2006; 281:5267-76), RUNX3 interacts with FoxO3a/FKHRL1 expressed in gastric cancer cells to activate *Bim* and induce apoptosis. The cooperation between RUNX3 and the PI3K/Akt signaling pathway component FoxO3a/FKHRL1 suggests the putative role of *RUNX3* in the homoeostasis of gastric cells and in stomach cancer control. Here we discuss recent breakthroughs in our understanding of the mechanisms of *RUNX3* in gastric malignancy and comment on possible future trends and perspectives.

INTRODUCTION

Gastric cancer is a leading cause of death from cancer worldwide.[1] Although many scientific advances have been made in this research arena, major questions are still unresolved.

It is a particularly exciting time for research on the *RUNX3* gene, with as many as 153 papers published to date, of which 42 focus on its relationship with gastric tumor. The novel finding of cytoplasmic retention of RUNX3 needs to be emphasized. While previous reports focused on RUNX3 genetic and epigenetic alterations, Ito et al.[2] posed further interesting questions about "how" and "where" altered *RUNX3* gene or protein levels may contribute to gastric tumorigenesis. Also, Chi et al.[3] suggested that at least part of the tumor suppressor activity of *RUNX3* is due to its ability to induce *CDKN1A* (*$p21^{WAF1/Cip1}$*) expression. Finally, Yamamura et al.[4] showed that RUNX3 works together with FoxO3a/FKHRL1 in the induction of apoptosis by activating *Bim* and may play an important role in tumor suppression of gastric cancer (Fig. 1).

RUNX3 encodes the DNA binding subunit of the heterodimeric transcription factor PEBP2/CBF, and is regulated by the TGF-β/Smad pathway. RUNX3 has frequently been reported to be epigenetically silenced in many types of cancers,[5-16] including stomach cancer. Ito et al.[2] discovered that RUNX3 is inactivated in more than 80% of gastric cancers not only by gene silencing but also by protein mislocalization.

Notably, in 1994, Levanon et al.[17] shed light on the human runt-related transcription factor 3 (RUNX3) gene, and one year later Bae et al.[18] better defined its chromosomal locus at 1p36.13-p36.11, a region that has been suggested to be a tumor suppressor locus in various cancers. The same year, Avraham et al.[19] mapped the homologous gene to mouse chromosome 4. In 2002, it was reported that *RUNX3* may harbor dominant-oncogene properties in T-cell lymphoma,[20] while it seems to exert tumor suppressor properties in gastric cancer.[5,21,22]

RUNX3 belongs to a small family of transcription factors (RUNX), whose name originates from the "runt domain" (RD), found in the *runt* gene of *Drosophila*.[23] The RUNX mammalian family includes three genes, *RUNX1*, *RUNX2* and *RUNX3*; by sequence analysis *RUNX3* was demonstrated to be the oldest of the three genes, and is found in Cnidarians, the most primitive animals, where it regulates the growth of their primitive gastrointestinal system. *RUNX3* is the smallest in size and simplest gene in the RUNX family, and maintains extensive structural similarities with the other family members. All RUNX genes are regulated at the transcriptional level by two promoters, P1 at 3', and P2 at 5', are TATA box-less, harbor multiple TSSs and share the runt domain. The P2 promoter of *RUNX3* is almost ubiquitously expressed, including in stomach cancer cells, whereas P1 is only known to be expressed in a few tissues, such as the thymus and ovary. This may generate confusion with the widely used Rel homology domain and, in fact, it

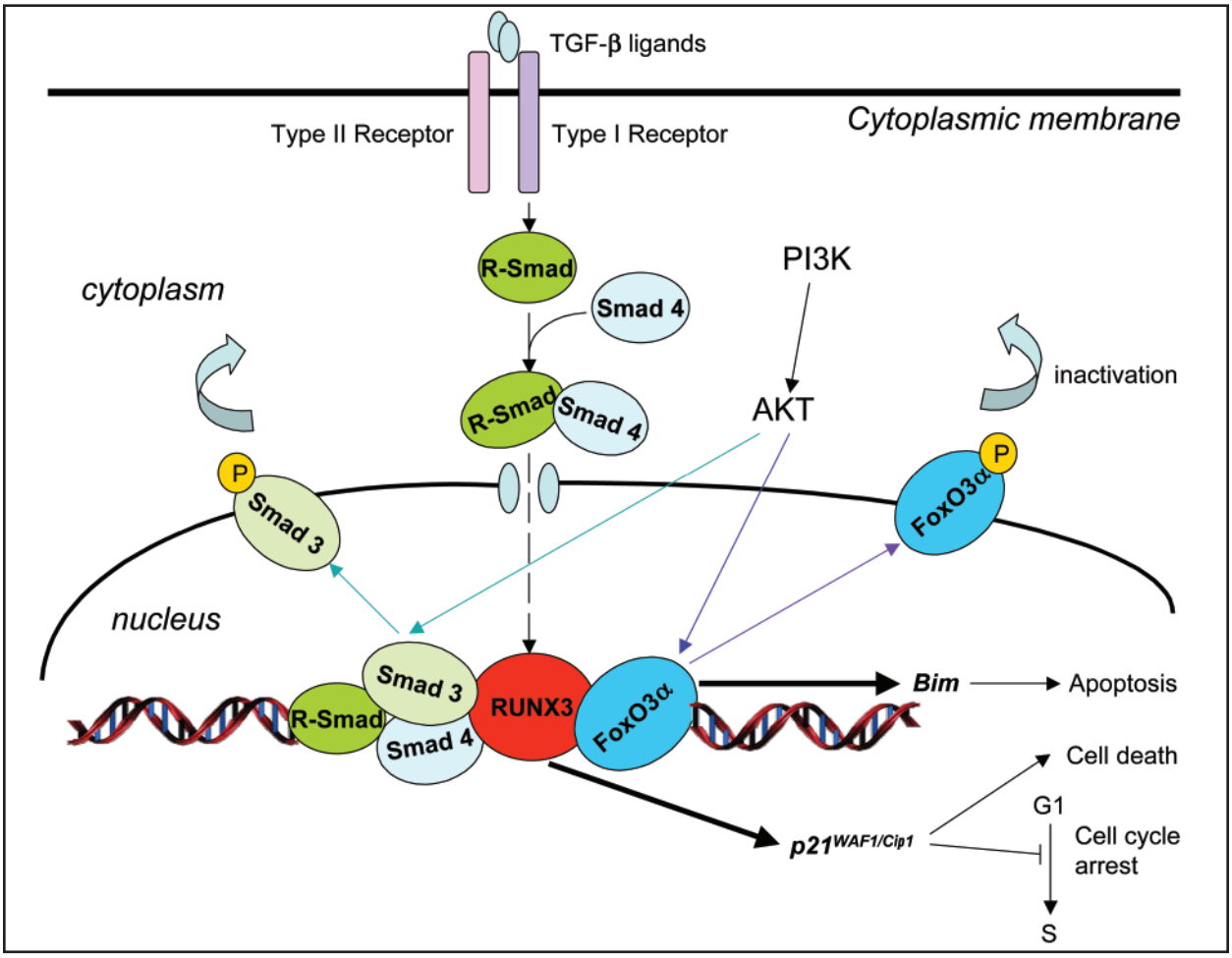

Figure 1. Epithelial growth suppression induced by TGF-β signaling pathway in gastric cancer. The receptor complex consists of two types of transmembrane serine/threonine kinases (type I and type II). R-Smad complexes (receptor-regulators Smad 2/3 with common-partner Smad or Smad 4) are released by the receptors of type I, and translocate into the nucleus, where they cooperate with sequence-specific transcription factors to regulate gene expression. Here emphasis is placed on RUNX3, which cooperates with the transcriptional factor FoxO3α to induce apoptosis in gastric cancer cells. Akt phosphorylates Fox03α and exports it from the nucleus to the cytoplasm. On the other hand, Akt phosphorylates also Smad3, sequestering it in the cytoplasm. It is also indicated here that RUNX3 overexpression may stimulate TGF-β-dependent endogenous p21 induction, inhibiting cell growth and causing arrest of the cell cycle in early G1 phase.

must be noted that some researchers use the term RHD erroneously in reference to the Runt domain (RD). The RUNX proteins, with their runt domain, bind to the RUNX DNA-motif of the promoter of target genes and allow protein-protein interactions with core-binding factor-β (CBF-β). In this way, RUNX proteins either activate or repress transcription through interactions with other transcription factors and co-activators or co-repressors.

RUNX1 is involved in the process of hematopoiesis and individuals who inherit heterozygous mutations in this gene develop acute myeloid leukemia. *RUNX2* is required for osteogenesis. *RUNX3* instead appears to be expressed in many cell types including mesenchymal cells, blood cells, and dorsal root ganglion neurons; it seems to be especially prominent in epithelial cells of the adult gastrointestinal tract and in hematopoietic cells.

Intestinalization of gastric epithelium is closely associated with gastric carcinogenesis. In this respect, it is worth noting that *RUNX3* is downregulated in intestinal metaplasia.[5] A critical question is whether *RUNX3* inactivation induces intestinal metaplasia.

The *RUNX3* gene is located on 1p36, where loss of heterozygosity (LOH) is often reported in many types of cancers including stomach, colon and pancreatic cancer. Therefore, a major tumor suppressor has been predicted to reside on 1p36. On the other hand, the TGF-β-mediated signal transduction pathway is regarded as a tumor suppressor pathway, since receptors and Smad proteins are often altered in many different types of cancers (reviewed by Akhurst, Derynck).[24] Therefore, *RUNX3* may be the long sought-after tumor suppressor on 1p36 and the RUNX3 protein may be a target of the TGF-β-mediated tumor suppressor pathway (reviewed by Ito and Miyazono).[25] If this model is correct, *RUNX3* could be involved in many cancers in addition to gastric cancer. Studies to further examine this possibility are under way in various laboratories.

Many findings support the role of *RUNX3* as a tumor suppressor gene in gastric tumors even though it does not correspond to the traditional view of tumor suppressor genes such as Rb1 and p53, which follow the "two hit hypothesis" of Knudson.[26] Knudson's model supposes that a gene's mutation of both alleles is required to cause a tumor. Recent reports describe haplo-insufficient genes requiring inactivation of only one allele, and genes inactivated not by mutation but rather epigenetic hypermethylation. Also, cytosolic accumulation can be an alternative way of inactivation, and this may

be the case of *RUNX3*.[2] Better understanding nuclear-cytoplasmic shuttling could offer useful molecular markers and potential therapeutic drugs, and may open new horizons in cancer therapy.

These findings are extremely intensive; nevertheless, results from another group exclude *RUNX3* as a tumor suppressor gene in early-onset gastric carcinomas, which may display molecular characteristics distinct from gastric carcinomas occurring at a later age.[27]

RUNX3 is expressed by normal gastric epithelial cells, but expression is undetectable in all gastric cancer cell lines tested, and it is reduced in primary gastric cancer samples. *RUNX3* expression is reduced in 40% of early-stage carcinomas, and in nearly 90% of advanced cases by hemizygous deletion and hypermethylation of its promoter.[5]

IS *RUNX3* A TUMOR SUPPRESSOR?

When subcutaneously injected into nude mice, $RUNX3^{-/-}$ $p53^{-/-}$ gastric epithelial cells developed tumors, while $RUNX3^{+/+}$ $p53^{-/-}$ cells did not. This is in agreement with the hypothesis that *RUNX3* is a tumor suppressor gene whose loss of function may cause tumorigenesis (reviewed by Fukamachi).[28] However, one of the pitfalls of this experiment is the lack of mice injected with $RUNX3^{-/-}$ $p53^{+/+}$ cells since the authors reported that $p53^{+/+}$ cells exhibited senescence in early passages. More robust data could have been achieved by inclusion in the experiment of a $RUNX3^{-/-}$ cell line with an inducible p53.

This "new generation" of tumor suppressor genes, when characterized could be possible drug targets of small molecules or demethylating agents to guide protein reexpression.[21] A variety of detection methods of DNA methylation (e.g., original MSP, quantitative MSP, COBRA, bisulfite sequencing, and so on) may allow investigation of a larger number of CpG islands. But, to prevent confusion, a certain degree of uniformity is necessary for the evaluation of the experimental results, as is the use of gene-specific, not global demethylating agents with gene-specific control of DNA methylation.

Additionally, new question marks in this field of research originate from the observation that a retrovirus activates RUNX3 in T-cell lymphomas in mice.[29]

There are many uncertainties when inferring gene function through the interpretation of knockout phenotypes. For example, the association of *RUNX3* deficiency with defects in cytotoxic T-cell development[30] may also cause secondary phenotypes that are not linked to RUNX3 activity (reviewed by Levanon et al.).[31]

However, a widespread involvement of *RUNX3* in different cancers is certain. Therapeutic approaches focused on the cross-talk between RUNX3 and PI3K/Akt and between RUNX3 and TGF-β might contribute to improve gastric cancer control. Future studies aimed to focus more closely on RUNX3 in the TGF-β pathway, and to investigate the gene in "Smad-dependent" and "Smad-independent" signaling pathways should be encouraged.

Future perspectives and challenges will be provided by the investigation of the interaction between *RUNX3*, the *Drosophila caudal*-related homeobox transcription factor, *Cdx2* and Wnt signaling.[28]

Another recent study in Drosophila shows the interaction between the GATA factor (Serpent) and RUNX factor (Lozenge).[32] This finding defines a new perspective for research using human homologues.

Other unresolved question is how RUNX3 regulates gastric epithelial differentiation through other genes such as *hedgehog*, *neurogenin 3* and *intestinal trefoil factor* which, when deleted, result in intestinal metaplasia of gastric epithelial cells in mice (reviewed by Yuasa).[33]

From the first gastric biopsy by Billroth to the recent molecular findings on gastric malignancy, a great deal has been learned; however, the mechanisms characterizing the relationship between gene expression and gastric cancer still need to be deciphered. In the plethora of interacting signaling factors in stomach cancer, recent technical advances such as microarray technology, proteomics, and gene therapy could offer real hope to patients and families for a personalized diagnosis and therapy.

The mechanisms by which *RUNX3* exhibits its functional properties are slowly on their way to being clarified. Gene therapy is a new therapeutic tool but for the moment with relatively poor therapeutic outcome, and it is not restricted to a specific tumor. Anti-sense RNA for *RUNX3* may be of potential clinical value in the gene therapy of gastric cancer.

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