Roles of RUNX in Solid Tumors

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Abstract

All *RUNX* genes have been implicated in the development of solid tumors, but the role each RUNX gene plays in the different tumor types is complicated by multiple interactions with major signaling pathways and tumor heterogeneity. Moreover, for a given tissue type, the specific role of each RUNX protein is distinct at different stages of differentiation. A regulatory function for RUNX in tissue stem cells points sharply to a causal effect in tumorigenesis. Understanding how RUNX dysregulation in cancer impinges on normal biological processes is important for identifying the molecular mechanisms that lead to malignancy. It will also indicate whether restoration of proper RUNX function to redirect cell fate is a feasible treatment for cancer. With the recent advances in RUNX research, it is time to revisit the many mechanisms/pathways that RUNX engage to regulate cell fate and decide whether cells proliferate, differentiate or die.

Keywords

RUNX • Solid tumors • Tumor suppressor • Oncogene • Wnt • TGFβ • RAS • Senescence • Protein-protein interaction • Stress-inducible gene • Precancerous state • Intestinal metaplasia

19.1 Introduction

The past decade has seen considerable progress in our understanding of the RUNX family of transcription factors in solid tumors. There are three mammalian *RUNX* genes and all have been directly implicated at various stages of tumor development, including initiation, progression and invasion. Animal knockout models of individual *Runx* genes revealed distinct developmental

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defects in hematopoiesis, bone development, the gastronintestinal tract and neurogenesis. While these findings reflect the tissue-specific expression and function of RUNX1 in the hematopoietic system, RUNX2 in bone, and RUNX3 in the gastrointestinal tract, lymphocytes as well as the dorsal root ganglion (DRG) neuron, it is noteworthy that all RUNX proteins are expressed in a broad range of tissues (Ito et al. 2015). Moreover, RUNX expression patterns are extremely dynamic and depend on the stage of differentiation as well as developmental and environmental cues. Because of the conserved Runt and the divergent C-terminal domains, RUNX proteins act redundantly in some cellular contexts and exert unique effects in others. This review summarizes the role of RUNX in solid tumors. This is by no means an exhaustive review for many types of cancer. Because of the focus of our laboratory, we will describe in detail our analyses of *RUNX3* function in tumors of the gastrointestinal tract. Through key examples, we will discuss how RUNX proteins engage different signaling pathways and biological processes to regulate proliferation, determine cell identity and influence tumor progression in solid tumors.

19.2 Ancient Origin of RUNX

RUNX genes have been uncovered in most, if not all, metazoans. Although *RUNX* was previously believed to be metazoan-specific, the discovery of two *Runx* genes in the holozoan *Capsaspora owczarzaki* indicates that *RUNX* originated in a unicellular organism, well before the emergence of multicellular metazoans (Sebe-Pedros et al. 2011). While the primordial role of *RUNX* remains unclear, it is reasonable to hypothesize that *RUNX* controls cell growth by orchestrating transcriptional programs in response to environmental cues. All other attributes of *RUNX* (eg. lineage specification) that were acquired later in evolution would likely reflect this original role. The extent to which the roles of multiple *RUNX* family members overlap, are inter-dependent or antagonistic is however unclear.

19.3 Dysregulation of RUNX in Solid Tumors

Although *RUNX1* aberrations are heavily implicated in leukemogenesis, recent studies have indicated causative roles for *RUNX1* in solid tumors.

RUNX1 is one of the significantly mutated genes in luminal breast cancer (Banerji et al. 2012; Ellis et al. 2012). Missense mutations at the Runt domain of RUNX1 and its binding partner, *CBFB*, are clear indications that the DNA binding ability/transcriptional activity of RUNX1, and perhaps other RUNX proteins, influence breast cancer growth (Banerji et al. 2012; Ellis et al. 2012). Yet, RUNX1 is highly expressed in a broad range of epithelial tumors, such as those of the skin, oesophageal, lung, colon, and interestingly, the breast (Scheitz et al. 2012). It has been proposed that leukemia, breast and skin cancers are stem cell disorders. Tight regulation of *RUNX1* expression appears to be necessary for proper stem cell function and differentiation.

RUNX2 has not been shown to be significantly mutated in cancer. Rather, RUNX2 mutation is identified as the main cause of the heritable dominant skeletal disorder cleidocranial dysplasia (CCD). Overexpression of RUNX2 is frequently observed in bone, breast and prostate cancers, suggesting that enhanced RUNX2 activity contributes to oncogenic growth in such tissues (Pratap et al. 2008; Akech et al. 2010) (Fig. 19.1). For example, human tissue microarray revealed that RUNX2 expression is elevated in triple negative (i.e. oestrogen receptor (ER)/progesterone receptor (PR)/HER2 negative) breast cancers and associated with a poor survival rate (McDonald et al. 2014). The chromosomal region *6p12-p21* where RUNX2 is located has been shown to be amplified in osteosarcoma (Sadikovic et al. 2010).

Unlike *RUNX1* and *RUNX2*, no familial disorder has been linked to *RUNX3* inactivation. *RUNX3* is located at *1p36*, a chromosomal region that is frequently deleted in a diverse range of cancers, including breast, lung, colorectal, neuroblastomas, hepatocellular carcinoma and pancreatic cancer (Nomoto et al. 2000; Mori et al. 2005; Nomoto et al. 2008; Henrich et al.

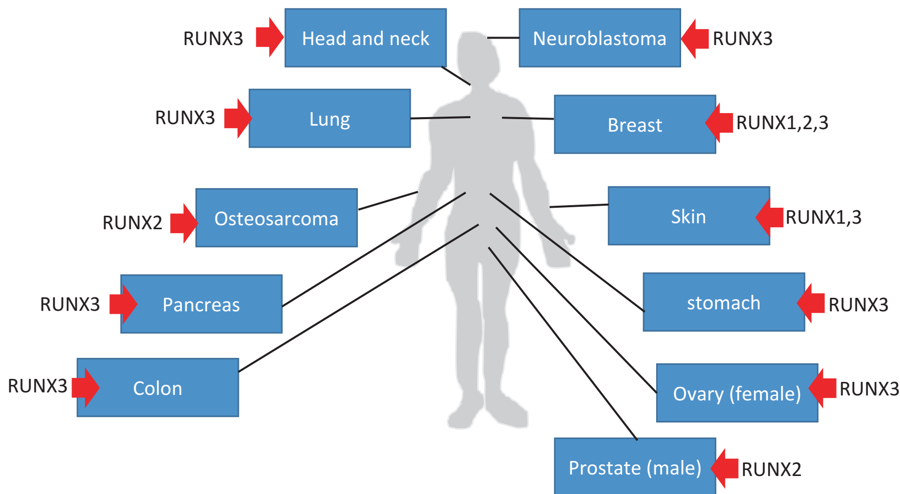


Fig. 19.1 The links of RUNX to solid tumors. Solid tumors with alterations of *RUNX* expression and activity are shown

2012). Moreover, *RUNX3* is silenced by hypermethylation of its promoter in cancers such as colon, lung, bladder, lung and bone (Chuang and Ito 2010) (Fig. 19.1). In fact, aberrant hypermethylation/inactivation of *RUNX3* is a very frequent event in the CpG island methylator phenotype (CIMP) subtype of colon cancer (Weisenberger et al. 2006). Mislocalisation of the RUNX3 protein in the cytoplasm has also been reported in gastric and breast cancer (Ito et al. 2005; Lau et al. 2006). The cytoplasmic localization of RUNX3 has been attributed to Src tyrosine kinase activation in the cancer cell lines (Goh et al. 2010) as well as defective TGFβ signaling (Ito et al. 2005). The crosstalk between epithelial cells and the microenvironment is a strong determinant of epithelial cancer initiation and progression. RUNX3 is one of the genes represented in a stroma-derived prognostic predictor – its expression in breast tumor stroma is associated a good clinical outcome (Finak et al. 2008).

19.4 RUNX in Developmental and Oncogenic Signaling Pathways

A central question is why the dysregulation of different *RUNX* genes led to distinct cancer types. For example, *RUNX1* haploinsufficiency caused predisposition to leukemia but its overexpression is necessary for skin tumors; RUNX2 overexpression is associated with bone cancer, as well as breast and prostate metastasis; RUNX3 inactivation is associated with multiple solid tumors, yet it is overexpressed in ovarian cancer (Nevadunsky et al. 2009; Lee et al. 2011). RUNX dosage therefore plays a critical role in determining proper cell growth. RUNX genes control cell fate through their ability to modulate the signaling outputs of major developmental pathways such as TGFβ, Wnt, Hippo, Hedgehog, Notch and Receptor tyrosine kinases (Ito et al. 2015) (Fig. 19.2).

If we could harness this potential by regulating RUNX expression, it would be a major advancement in cancer prevention or treatment.

19.5 Gastric Cancer: Proliferation, Survival and Invasion

A comprehensive molecular evaluation of gastric adenocarcinoma revealed key dysregulated pathways and putative drivers of various subtypes of gastric cancer (Cancer Genome Atlas Research 2014). The list of significantly mutated genes included those in the KRAS, β-catenin, TGFβ signaling, p53, Fanconi anemia and mitotic pathways (Cancer Genome Atlas Research 2014). As

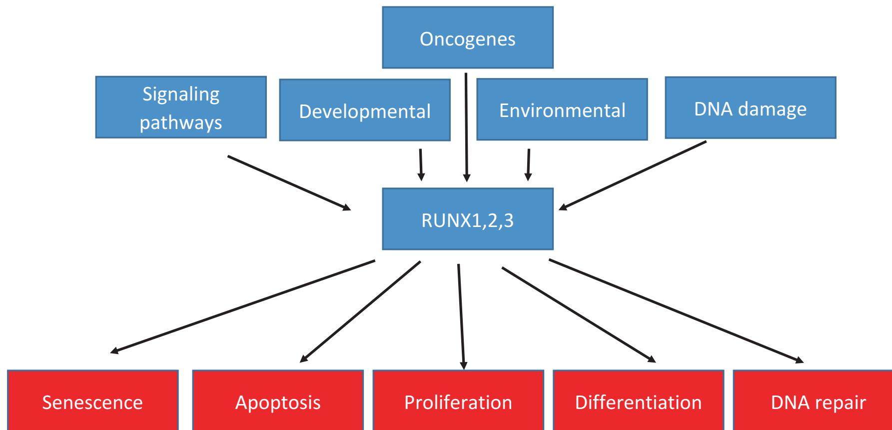


Fig. 19.2 RUNX integrates stimuli from the environment to influence cell fate. The *RUNX* family is regulated by signaling pathways, developmental stimuli, environmental cues, DNA damage, and oncogenic/hyperproliferative signals. In response to the diverse signals, RUNX proteins initiate transcription programs leading to senescence, DNA repair, apoptosis, differentiation or proliferation

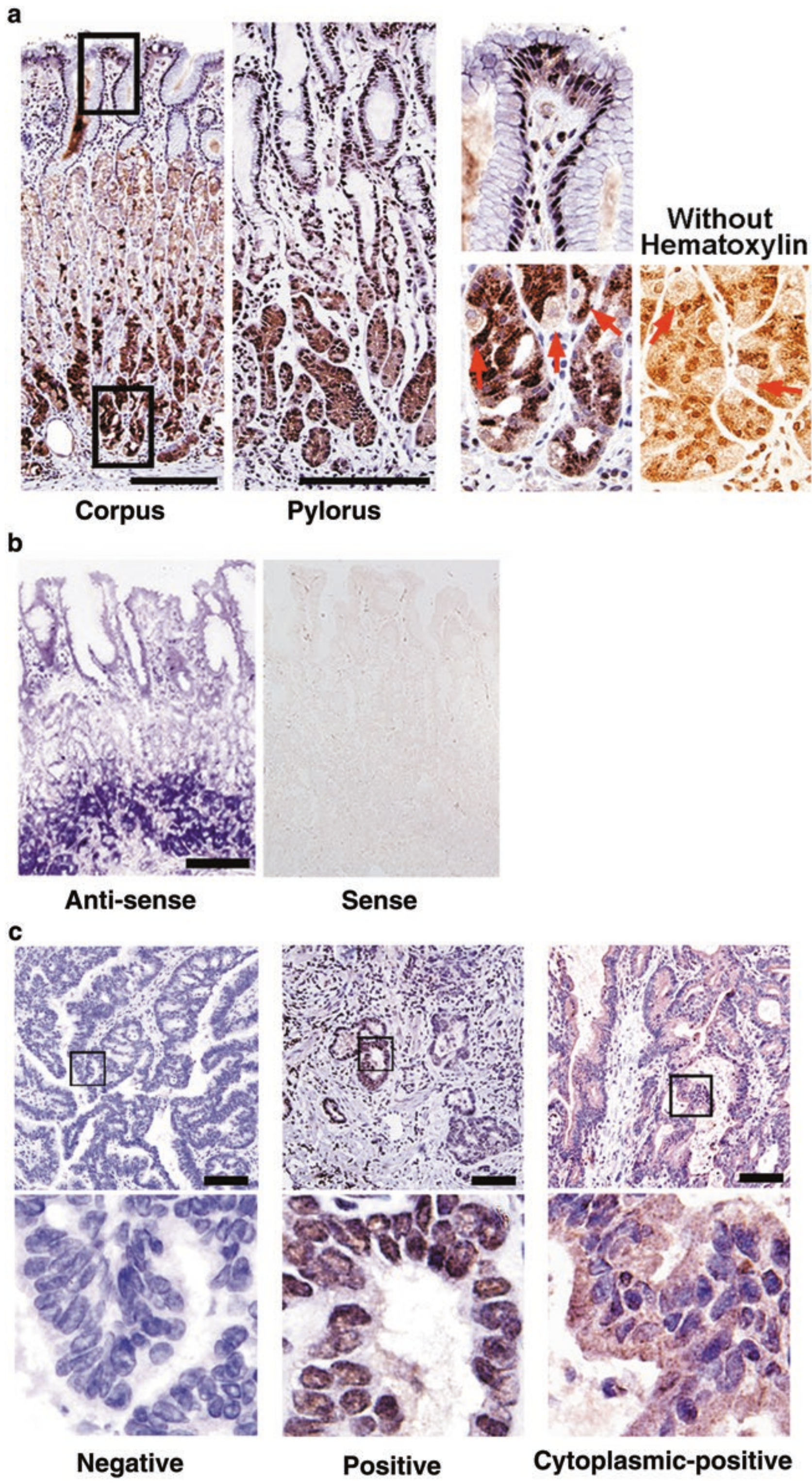
discussed below, RUNX3 is a direct participant in most of these pathways.

In normal mouse and human stomach corpus, RUNX3 is expressed in the lower part of the epithelium, primarily in chief cells but not parietal cells (Ito et al. 2005; Ogasawara et al. 2009) (Figs. 19.3 and 19.4).

It is notable that 45–60% of human gastric cancer do not express RUNX3 – this is mainly due to RUNX3 promoter methylation/epigenetic silencing (Li et al. 2002). In addition, hemizygous deletion of the RUNX3 gene has also been identified. Mislocalisation of the RUNX3 protein in the cytoplasm was also reported to be a major form of RUNX3 inactivation (Fig. 19.3c) (Ito et al. 2005).

Helicobacter pylori infection is considered to be the primary cause of human gastric cancer. Chronic inflammation of stomach caused by *H. pylori* infection is associated with the loss of acid-producing parietal cells and pepsinogen-producing chief cells in the main body of the stomach (as known as corpus). Loss of parietal cells after *H pylori* infection is the main cause of oxyntic atrophy, a precancerous stomach condition (Weis and Goldenring 2009). The host inflammatory response, coupled with *H. pylori* virulence factors,resulted in promoter methylation and silencing of many tumor suppressor genes. *RUNX3* is one of them. The *CpG* island at the 5′ end of the P2 (proximal) promoter of *RUNX3* is frequently methylated in *H. pylori*-infected stomach and gastric cancer tissues (Katayama et al. 2009). The silencing of the *RUNX3* gene is therefore key epigenetic event during the development of gastric cancer.

Fig. 19.3 Comparison of RUNX3 expression in normal human gastric epithelial cells with cancer cells (Figures adapted from Ito et al. 2005, with permission from the American Association for Cancer Research). (**a**) Immunodetection of RUNX3 in the corpus and pyloric antrum of normal gastric epithelial cells. *Upper* and *lower boxed* regions are enlarged on the *right*. Counterstaining was done with hematoxylin. *Far right*, immunostaining without counterstaining in a section similar to the *lower* enlarged region is shown. *Arrows* indicate parietal cells with weaker immunoreactivity than the adjacent chief cells. (**b**) Detection of *RUNX3* mRNA by *in situ* hybridization with a *RUNX3* anti-sense or sense probe in normal gastric epithelial cells. (**c**) Immunodetection of RUNX3 in gastric cancer cells. Sections were prepared from differentiated (intestinal) gastric cancers. Three types of staining patterns for RUNX3 were observed: negative (44%; n = 43/97), positive (18%; n = 17/97), and cytoplasmic-positive (38%; n = 37/97). The *boxed* regions in the *upper panels* are enlarged *below*. Counterstaining was done with hematoxylin. Scale bars, 200 μm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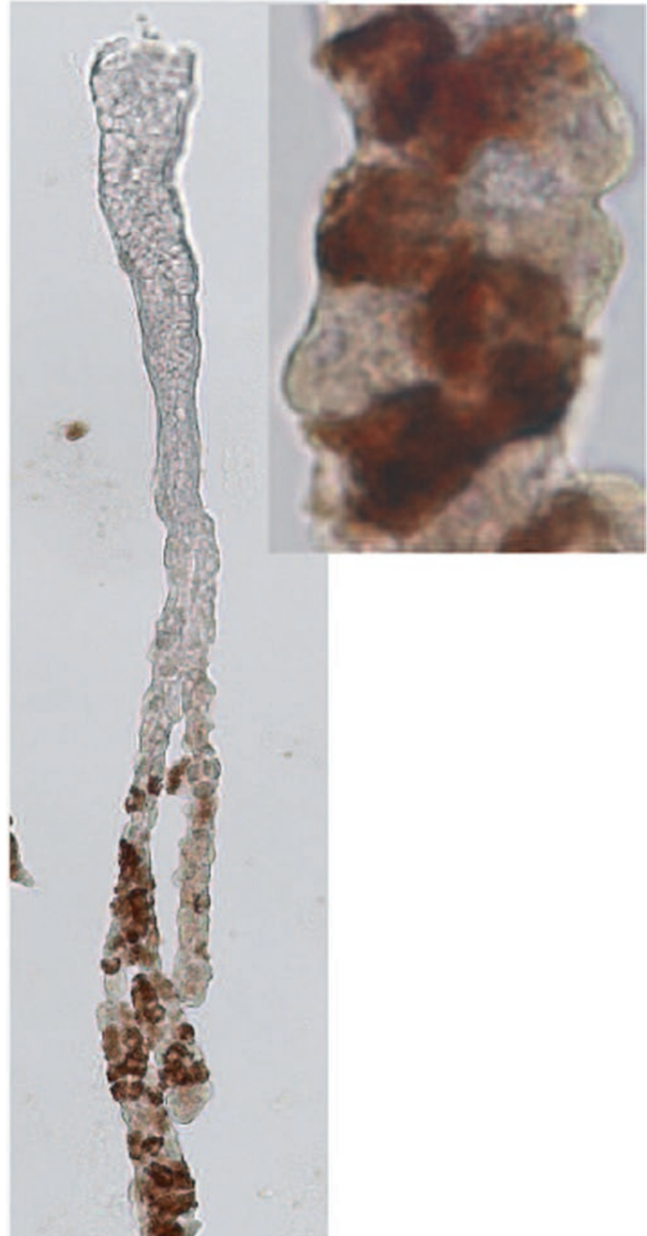

Fig. 19.4 RUNX3 is localized in the basal region of isolated fundic glands. Glands were isolated from the fundic mucosa of normal portions of resected human epithelium and evaluated for RUNX3 expression by immunohistochemistry. Original magnification: 100x; *inset*, 640x. (Figure adapted from Ogasawara et al. 2009, with permission from Histology and Histopathology)

From the gastric cancer-derived tissue, we found a *RUNX3* mutation, R122C, within the highly conserved Runt domain (Li et al. 2002). The oncogenic mutation R122C was also found in head and neck cancer (based upon data generated by the TCGA Research Network: http://cancergenome.nih.gov/) and thus represented a major breakthrough in understanding RUNX3's anti-tumor properties (Li et al. 2002). When we exogenously expressed RUNX3 in cultured cell lines, strong growth inhibition is often observed. Similar phenomenon has been reported for well-known tumor suppressors such as p53. We therefore suspected that RUNX3 may have tumor suppressor activity during the early stages of our studies on RUNX3. Likewise, when wild type RUNX3 is stably expressed in gastric cancer cell line, MKN28, a RUNX3-deficient gastric cancer

cell line, tumorigenicity in immune-compromised mice is strongly inhibited compared with parental cell line. This is a strong indication that RUNX3 functions as a tumor suppressor in the stomach. When similar experiments were performed with the RUNX3^{R122C} mutant, tumor growth was not inhibited. Rather tumorigenic activity was enhanced beyond the level of the parental gastric cancer cell line: apparently, a single amino acid substitution R122C was sufficient to convert a strong tumor suppressor to an oncogene (Li et al. 2002). As we begin to understand the molecular mechanisms underlying growth inhibitory functions of wild-type RUNX3, how R122C provoked oncogenic activities becomes clear (see below).

In addition to clinical samples or molecular characterization, mouse models have also linked RUNX3 inactivation to gastric cancer. As with most animal models, we found interesting phenotypic variations among different mouse strains used to generate *Runx3* knockout. Conventional knockout of *Runx3* in C57BL/6 mice led to multiple developmental defects and early death – mostly within 24 h after birth. This is primarily due to a defective glosso-pharyngeal nervous system which resulted in an inability to control tongue and pharynx action to suck milk (unpublished data). In other strains such as BALB/c and ICR, a significant number of mice survived until adulthood. Groner's group reported that although *Runx3*$^{-/-}$ mice bred on ICR or MF1 background exhibited defects such as congenital sensory ataxia, reduced growth, and high mortality rates during the first 2 weeks after birth, some knockout mice survived and lived till old age (24 months) (Levanon et al. 2002; Brenner et al. 2004).

The strong influence of genetic variation on tumor development in epithelial tissues was also observed for different strains of Runx3 knockout mice. The gastric mucosa of the *Runx3*-null mice exhibited hyperplasia, due to increased proliferation and decreased sensitivity to transforming growth factor 1 (TGF-1) mediated growth suppression and apoptosis (Li et al. 2002). We had earlier reported that direct interaction with the effectors of TGFβ pathway, the SMAD proteins, is a common feature of all mammalian RUNX proteins (Hanai et al. 1999) (Fig. 19.5).

In response to TGFβ, RUNX3 directly activated the transcription of growth inhibitor *CDKN1A* (also known as *p21*^{CIP}) (Chi et al. 2005) and pro-apoptotic *BIM* in the stomach (Yano et al. 2006). Besides *BIM*, re-introduction of RUNX3 in a gastric cancer cell line also induced other genes involved in apoptosis, including those for Fas-associated death domain (FADD) and death-receptor mitochondria mediated apoptosis (Nagahama et al. 2008).

We investigated how *Runx3*-deficiency contributed to the distinct morphological changes in precancerous gastric epithelium. Conventional knockout of *Runx3* was generated with the BALB/c strain (Ito et al. 2011). These mice survived for 10–12 months and could be studied at the adult stage. The gastric epithelia in *Runx3*$^{-/-}$ mice show gradual loss of chief cells that express pepsinogen and the stomachs of 6-month-old *Runx3*$^{-/-}$ mice show nearly complete loss of pepsinogen expression, suggesting the loss of chief cells. Importantly, the population of Muc6- and TFF2-positive mucous neck cells was significantly elevated (Ito et al. 2011). Since chief cells are known to be derived from mucous neck cells, it is likely that chief cells trans-differentiated or retro-differentiated back to the precursor cells. This phenomenon is very similar to Spasmolytic Polypeptide Expressing Metaplasia (SPEM), a precancerous condition associated with 90% of resected gastric cancers (Weis and Goldenring 2009).

The *Runx3*$^{-/-}$ mouse stomach also showed intestinal metaplasia, characterized by the presence of intestinal goblet cells (indicated by the expression of intestine specific mucin Muc2) and Alcian Blue staining, which normally marks intestinal epithelium.

Stem cells in the stomach corpus epithelium are normally located in the isthmus. In the case of *Runx3*$^{-/-}$ stomach epithelium, bromodeoxyuridine (BrdU) incorporation – indicating the presence of rapidly growing cells – is observed throughout the epithelium suggesting that stem cell activity is enhanced. Organization or differentiation of epithelial cells might also be dysregulated

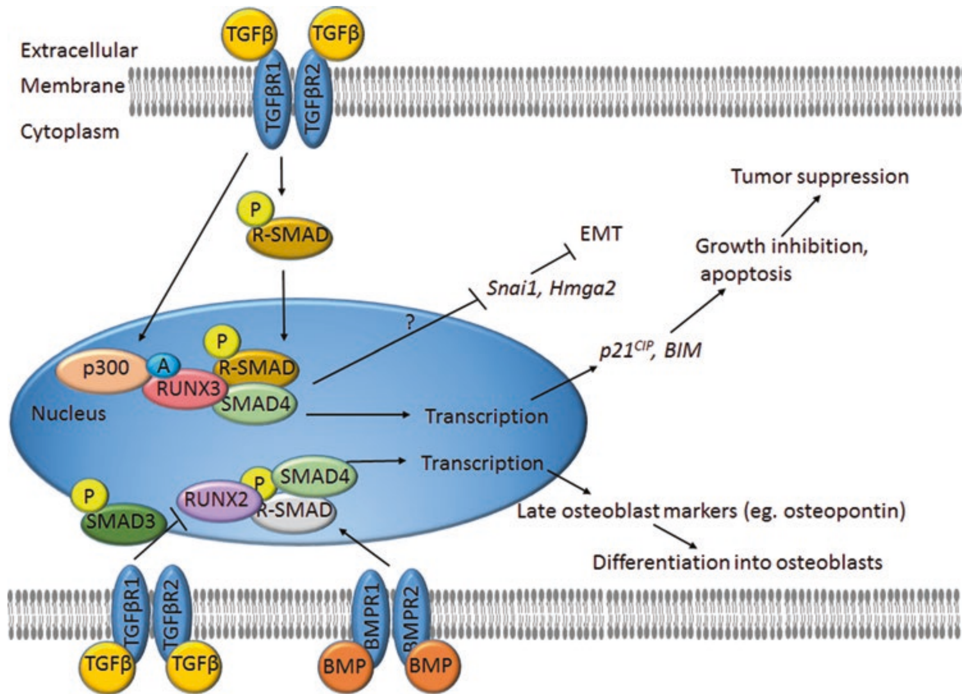

Fig. 19.5 Reciprocal interactions between RUNX and key elements of the TGFβ pathway. Activation of TGFβ receptors 1 and 2 (TGFβR1 and R2) by the TGFβ cytokine lead to phosphorylation of R-SMAD proteins. Phosphorylated R-SMAD translocates into the nucleus and forms a multiprotein complex with SMAD4 and RUNX3. This synergistic cooperation with RUNX3 and the SMAD proteins – key effectors of TGFb pathway – strongly induces transcription of *p21*^{CIP} and *BIM*, which are respectively associated with growth inhibition and apoptosis as well as tumor suppression. RUNX3-SMAD complex also inhibits *Snai1*, *Hmga2* and EMT by unknown mechanisms. Activation of TGFβR1 and R2 also enhance p300-mediated acetylation of RUNX3, resulting in increased stability of RUNX3, thereby promoting complex formation with SMAD effectors. Conversely, activation of TGFβR1 and R2 phosphorylates SMAD3 which inhibits RUNX2. Instead, the BMP pathway promotes interaction of RUNX2 with SMAD proteins, leading to transcription of late osteoblast markers (eg. Osteopontin) and differentiation to osteoblasts

(Ito et al. 2011). This observation unequivocally indicates strong tumor suppressor activity of Runx3 in the stomach epithelium.

Importantly, we detected a marked elevation of Wnt activity in the *Runx3*$^{-/-}$ gastric epithelium, suggesting that oncogenic Wnt drives the development of intestinal metaplasia in *Runx3*$^{-/-}$ stomach. This observation is consistent with the finding that RUNX3 inhibits the DNA binding activity of the TCF4/β-catenin complex (Ito et al. 2008) (see intestinal cancer section). The Runt domain of RUNX3 protein interacted with the DNA binding domain of TCF4, thereby inhibiting the DNA binding of both proteins. Indeed, Wnt activity is up-regulated in *Runx3*$^{-/-}$ mouse stomach (Ito et al. 2011).

Although reported mouse models of gastric cancer rarely develops invasive carcinoma, a low dose of the chemical carcinogen N-methyl-N-nitrosourea (MNU), which did not affect the stomach of wild-type mice, induced invasive gastric cancer in *Runx3*$^{-/-}$ mice at 52 weeks of age (Fig. 19.6c–e) (Ito et al. 2011). This observation suggests that RUNX3 protects against DNA damage-induced stress and tumorigenesis in the stomach. Indeed, a non-transciptional role for RUNX3 in the Fanconi anemia DNA repair pathway has been described (Wang et al. 2014) and is discussed in detail elsewhere in this book.

The susceptibility to carcinogen-induced gastric cancer may also indicate mitotic defects in *Runx3*$^{-/-}$ mice. Key regulators of mitosis Aurora

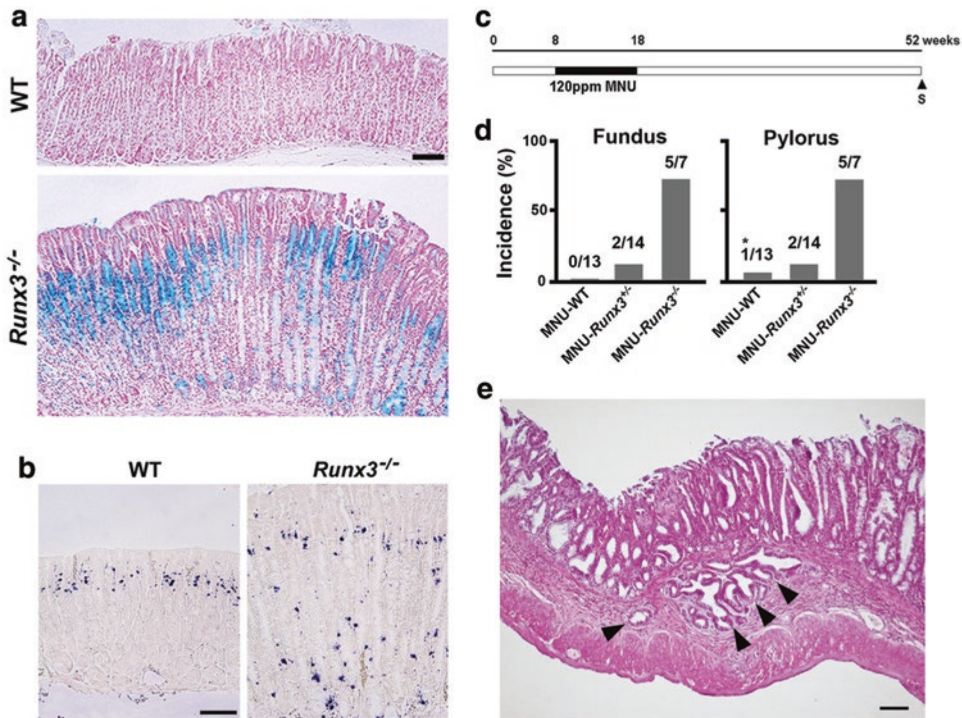

Fig. 19.6 Low dose of MNU induced cancer development in precancerous *Runx3*$^{-/-}$ stomach (Figures adapted from Ito et al. 2011, with permission from the Gastroenterology, Elsevier). (**a**) Alcian blue staining of wild-type (WT) and *Runx3*$^{-/-}$ fundic glands. (**b**) Detection of replicating cells by BrdU incorporation in WT and *Runx3*$^{-/-}$ fundic glands. (**c**) Mice were treated with 120 p.p.m. *N*-methyl-*N*-nitrosourea (MNU) through their drinking water for 10 weeks (from 8 to 18 weeks of age) and were sacrificed at 52 weeks (1 year) of age. (**d**) Frequency of adenocarcinoma development in the fundus and pylorus of MNU-treated WT, *Runx3*$^{+/-}$, and *Runx3*$^{-/-}$ mice at 52 weeks of age. All adenocarcinomas except one in WT (*) migrated into the submucosa. (**e**) Morphology of fundic glands in MNU-treated *Runx3*$^{-/-}$ mice at 52 weeks of age, stained by hematoxylin and eosin. Cancerous glands that invaded the submucosa are indicated by arrow heads. Scale bars, 100 μm

kinases induced phosphorylation of RUNX3 at G2/M transition (Chuang et al. 2016). It is possible that phosphorylation of RUNX triggers mitotic entry. Phosphorylation of threonine 173 in the Runt domain of RUNX3 (and its equivalent in RUNX1 and RUNX2) detaches RUNX from the DNA and promotes its localization at the mitotic apparatus such as the centrosome and midbody (Chuang et al. 2016). Currently, the biological consequence of T173 phosphorylation is unclear but not likely to involve DNA binding or transcription regulation. The identification of T173I mutation in colon cancer suggests the importance of this residue in cancer development (cancer.sanger.ac.uk/cosmic). As indicated above, RUNX protein can exert its tumor suppressor activity through protein-protein interaction without binding to DNA. The involvement of other RUNX proteins in mitosis has been reported. RUNX2 ensures transmission of parental epigenetic memory to daughter cells (Young et al. 2007a, b); RUNX1 contributes to the spindle assembly checkpoint (Ben-Ami et al. 2013). How much functional overlap of RUNX activities in mitosis remains to be seen.

Inflammation is well established as an oncogenic factor in the stomach. RUNX3 cooperates with TNF-α/NF-kB pathway and *Helicobacter pylori* infection to directly upregulate *IL23A* in gastric epithelial cells (Hor et al. 2014), However, *IL23B* is not produced and the Interleukin-23 (IL-23) heterodimeric cytokine cannot be formed. Why IL23A alone is secreted from gastric

epithelial cells is unknown. However, the ability of RUNX3 to induce *IL23A* is strongly suggestive of a RUNX3 role in the innate immunity of gastric epithelial cells, where it enhances pathogen clearance during infection and inflammation. It may also be protecting stomach epithelium from inflammation, hence development of cancer.

Epithelial-mesenchymal transition (EMT) is one of the important factors for solid tumor progression and invasion. We found that the loss of Runx3 in gastric epithelial cells led to the induction of EMT, resulting in a subpopulation of cells which acquired tumorigenic, stem cell-like properties (Voon et al. 2012). RUNX3 therefore protects gastric epithelial cells from aberrant TGFβ signaling and subsequent reprogramming by EMT (Fig. 19.5). Involvement of RUNX in EMT is described in more detail elsewhere in this book.

The nuclear effector of the Hippo pathway, YAP-TEAD4, functions as an oncogene in several cancer types including gastric cancer. Increased TEAD-YAP expression is significantly correlated with poor survival of gastric cancer patients. We found that RUNX3 negatively regulates the oncogenic TEAD–YAP complex in gastric cancer (Qiao et al. 2015). The Runt domain of RUNX3 interacts with the DNA binding domain of TEAD4, resulting in attenuation of TEAD4 DNA binding activity and downregulation of TEAD-YAP mediated transcription. Various YAP-TEAD target genes (eg. *collagen type XII* and *calpain 6*) that were involved in metastasis and apoptosis were suppressed by RUNX3 (Qiao et al. 2015).

As discussed earlier, the cancer-derived mutation R122C has oncogenic effects. The RUNX3^{R122C} mutant showed severe defects in DNA binding and could not induce *p21*^{CIP1} transcription (Chi et al. 2005). RUNX3^{R122C} is also defective in tumor suppressor activity mediated by protein-protein interactions: RUNX3^{R122C} did not interact with Wnt effector TCF4 and failed to suppress oncogenic Wnt (Ito et al. 2011). RUNX3^{R122C} also failed to interact with TEAD4 and, therefore, cannot suppress the oncogenic activity of TEAD4-YAP complex (Qiao et al. 2015).

The tumor suppressor function of RUNX3 in gastrointestinal tumors is much debated for many years, because of the low expression level of Runx3 in normal gastric epithelium. Expression of RUNX3 in normal human stomach epithelium was shown by in situ hybridization and immunohistochemistry (Fig. 19.3a, b) (Ito et al. 2005). Co–expression of RUNX3 and pepsinogen in isolated human gastric unit of corpus was also reported (Ogasawara et al. 2009). At issue is the expression level of Runx3 in the stomach epithelial cells of normal mice kept in specific pathogen free (SPF) facility and this presented challenges when interpreting Runx3's role in epithelial tumors (Levanon et al. 2011). Although *Runx3* expression is easily detected in the stomach epithelium of wild mice (ie. mice caught in the field), *Runx3* expression in mice kept in modern mouse facilities are generally quite low, requiring highly sensitive detection techniques. However, it has become increasingly apparent that Runx3 expression is highly dynamic (Whittle et al. 2015) and changes according to environmental cues. In response to oxidative and osmotic stress, the *Caenorhabditis elegans* RUNX homolog RNT-1 protein is rapidly stabilized in the intestine (Lee et al. 2012). In humans, chemotherapeutic and DNA damaging agent doxorubicin induced RUNX3 expression in different cultured cell lines (Yamada et al. 2010). Oncogenic stress, such as expression of mutant *K-Ras*, induced Runx3 expression in human embryonic kidney HEK293 cells (Lee et al. 2013). This observation reinforced the notion that stress response is a fundamental, as well as evolutionarily conserved, function of RUNX3. In this study, RUNX3 was found to be transcriptionally activated by oncogenic K-Ras to mediate the expression of p53. Conceptually, RUNX3 may serve as a monitor of the level of K-Ras activity and other oncogenic signals (Lee et al. 2013). The fact that RUNX2 was strongly induced in Ha-ras transformed NIH3T3 indicates a close relationship between RUNX induction and proliferation (Ogawa et al. 1993). It might be that all *RUNX* genes are activated by stress or other oncogenic signals to serve a fundamental function – protect normal cells from tumorigenesis.

The molecular identification of tissue stem cells, which can give rise to stomach cancer, will offer mechanistic insights on how gastric tumor is initiated, sustained or metastatic. We have successfully generated mice with *Runx1* or *Runx3* knockout in hematopoietic stem cells (Wang et al. 2014) and will be able to target tissue stem cells in the near future. Stem cell specific knockout of *Runx* genes singly or in combination will reveal more precise roles of individual *Runx* genes in the stomach epithelium.

As discussed above, completely different approaches have converged on the tumor suppressor function of RUNX3 in the stomach. Its abilities to engage multiple signaling pathways to suppress growth, attenuate oncogenic signaling, induce apoptosis and antagonize EMT have important implications in cancer treatment. A cell permeable RUNX protein was developed with promising results – locally administered RUNX3 suppressed the growth of subcutaneous human gastric tumor xenografts with increased $p21^{CIP1}$ and decreased VEGF expression – consistent with the interaction of RUNX3 with TGFβ signaling (Lim et al. 2013). Moreover, *RUNX3* is frequently epigenetically silenced in cancer, its expression, and perhaps anti-tumor activity, can be pharmacologically restored by inhibitors of DNA methyltransferases and histone deacetylases.

Given the prominent role of RUNX3 in the stomach, a pertinent question would be whether RUNX1 and RUNX2 functionally compensate for the anti-tumor activity of RUNX3. However, mutational analysis of RUNX1 in laser-captured gastric cancer cells of 44 patients did not reveal any significant mutation. Moreover, RUNX1 mRNA was detected in many gastric cancer cell lines and cancer tissues, suggesting that RUNX1 might not play a major role in suppression of most gastric cancers (Usui et al. 2006). It is important to note that while all RUNX proteins have the ability to regulate $p21^{CIP1}$ transcription through the multiple RUNX consensus binding sequence in the $p21^{CIP1}$ promoter, the downstream effects are different. RUNX1 regulates the $p21^{CIP1}$ promoter in a cell type dependent manner, transactivating the $p21^{CIP1}$ promoter in myeloid leukemia cells and repressing the $p21^{CIP1}$ promoter in NIH3T3 fibroblasts (Lutterbach et al. 2000). RUNX2 repressed the *CDKN1A* promoter and attenuated TGFβ1-mediated growth inhibition and apoptosis in vascular endothelial cells (Sun et al. 2004).

19.6 Intestinal Cancers: Interaction with Wnt Signaling

Studies on genetic alterations have overwhelmingly implicated the Wnt signaling pathway as the main player in colon cancer pathogenesis. Adult *Runx3*$^{-/-}$ BALB/c mice exhibited increased proliferation and hyperplasia in the epithelia of the jejunum and colon (Ito et al. 2008) (Fig. 19.7a, b).

Furthermore, Wnt target genes such as *CD44*, *cyclin D1*, *c-Myc*, *conductin* and *EphB2* were upregulated in the intestine of *Runx3*$^{-/-}$ mice. The adenomatous polyposis coli (APC) gene is well established as a major regulator of Wnt signaling in colorectal cancers. We found that at 65 weeks of age, *Runx3*$^{+/-}$ mice developed small intestinal adenomas at a frequency similar to that of *Apc*$^{Min/+}$ mice with the same BALB/c background (Fig. 19.7c) (Ito et al. 2008). Strikingly, adenomatous polyps were obtained from *Runx3*$^{+/-}$ *Apc*$^{Min/+}$*mice*, suggesting that the combined effects of defective *Runx3* and *Apc* genes drive progression from adenoma to adenocarcinoma (Fig. 19.7c). Since the analysis of the very small adenomas from *Runx3*$^{+/-}$*Apc*$^{Min/+}$ mice revealed either downregulation *Runx3* expression or nuclear accumulation of β-catenin, but not both phenotypes (Fig. 19.7d), it is possible that the adenomas developed because of biallelic inactivation of either *Apc* or *Runx3*. Interestingly, the large adenomas or adenocarcinomas of the *Runx3*$^{+/-}$*Apc*$^{Min/+}$ mice showed β-catenin accumulation suggesting that defects in both genes contribute to heightened activation of the oncogenic Wnt pathway. Mechanistically, RUNX3 formed a ternary complex with the Wnt effector complex TCF4-β-catenin, which resulted in attenuation of

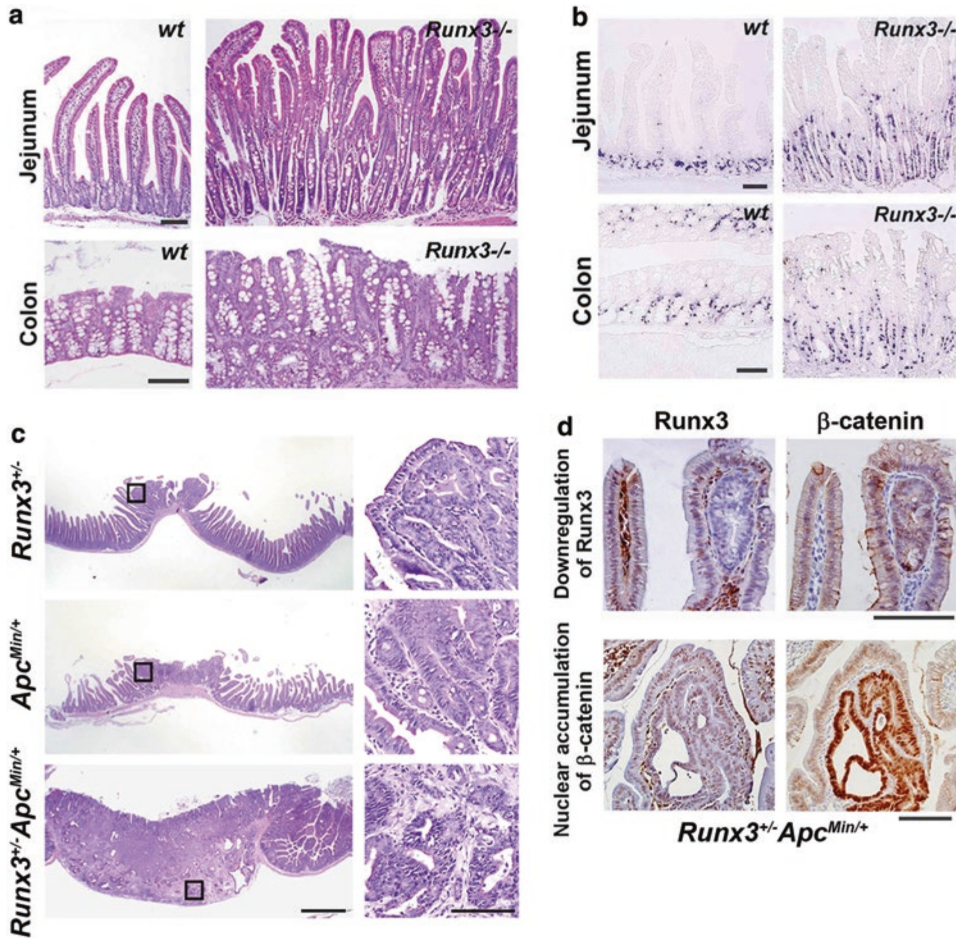

Fig. 19.7 Development of adenomatous polyps in *Runx3*$^{+/-}$ and adenocarcinoma in *Runx3*$^{+/-}$*Apc*$^{Min/+}$ BALB/c mice (Figures adapted from Ito et al. 2008, with permission from the Cell Press, Elsevier). (**a**) Morphology of jejunum and proximal colon of wild-type (WT) and *Runx3*$^{-/-}$ mice at 40 weeks of age. Tissues were stained by hematoxylin and eosin. (**b**) Detection of proliferating cells in WT and *Runx3*$^{-/-}$ jejunum and proximal colon by BrdU incorporation (adult mice at 40 weeks of age). (**c**) *Left*, Hematoxylin and eosin staining of small intestines in *Runx3*$^{+/-}$, *Apc*$^{Min/+}$ and *Runx3*$^{+/-}$*Apc*$^{Min/+}$ mice at 65 weeks of age. Adenomatous polyps in *Runx3*$^{+/-}$ and *Apc*$^{Min/+}$ mice were compared with adenocarcinomas in *Runx3*$^{+/-}$*Apc*$^{Min/+}$. *Right*, Boxed regions in *left* panel are enlarged. (**d**) Analysis of very small adenomas formed in the jejunum of *Runx3*$^{+/-}$*Apc*$^{Min/+}$ mice. Downregulation of Runx3 was not associated with activation of β-catenin (*upper panels*). In contrast, nuclear accumulation and activation of β-catenin were not associated with down-regulation of Runx3 (*lower panels*). Counterstaining was done with hematoxylin. Scale bars correspond to 100 μm (**a**–**d**) and 1 mm (*left* in **b**)

TCF4-β-catenin DNA binding ability and suppression of oncogenic Wnt mediated transcription (Ito et al. 2008) (Fig. 19.8).

Moreover, the adenomatous polyps of *Runx3*$^{+/-}$ mice acquired *CpG* island methylation of the *Runx3* promoter, suggesting that DNA methylation is responsible for the downregulation the remaining *Runx3* gene. This reflects the human scenario and points to DNA methylation as major mechanism of *Runx3* inactivation in cancer. Given that Wnt signaling drives proliferation of intestinal epithelial cells, and that overactive Wnt has a causal role in intestinal tumor formation, it is not surprising that RUNX3, through its modulation of the Wnt pathway, is critical for the homeostatic regulation of growth and differentiation in the intestinal epithelia.

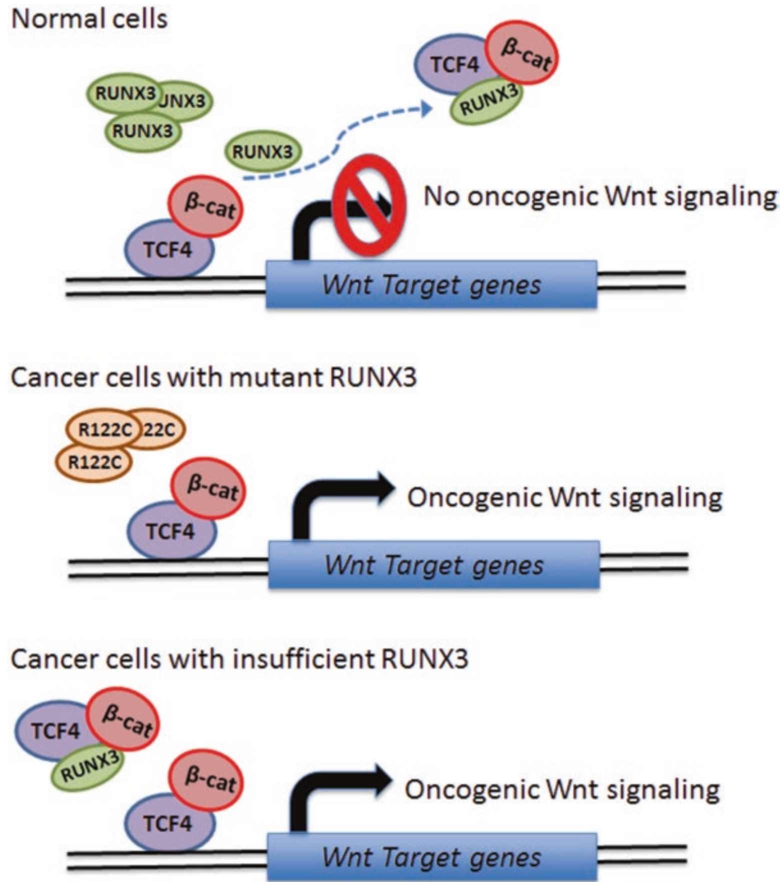

Fig. 19.8 Dysregulation of RUNX3 affects oncogenic Wnt signaling. *Top*, in normal cells with sufficient amounts of RUNX3, RUNX3 interacts with the TCF4-β-catenin complex and prevents the Wnt effector from binding to DNA; transcription of downstream genes of Wnt signaling is attenuated. *Middle*, in cancer cells with *RUNX3*^{R122C} mutation, the mutant RUNX3 fails to bind to TCF4-β-catenin. TCF4-β-catenin induces transcription of oncogenic Wnt downstream genes to promote tumorigenesis. *Bottom*, in cancer cells with insufficient RUNX3 levels (e.g. due to epigenetic silencing of *RUNX3*), excess TCF4-β-catenin proteins induce oncogenic Wnt transcriptional program

Recently, a different aspect of RUNX-TCF4-β-catenin interaction in the *Drosophila* embryonic midgut was reported (Fiedler et al. 2015). Instead of displacing TCF4 from the DNA, Runt functioned as a key component of the multi-protein Wnt enhanceosome that was tethered to TCF enhancers. Simultaneous interactions of RUNX with Groucho/TLE (through its C-terminal WRPY motif), TCF and a protein complex called ChiLS [composed of Chip/LDB ((Lin-11 Isl-1 Mec-3-) LIM-domain-binding protein) and single-stranded DNA binding protein (SSDP)] fine-tune the Wnt output (Fiedler et al. 2015). Human RUNX1/2/3 directly interacted with ChiLS, thereby suggesting the conservation of an ancient RUNX-Wnt link for a pivotal role in gut development. Whether RUNX3 suppress or activate the Wnt signaling pathway is thus context-dependent. Because the ChiLS complex also regulates Notch responsive enhancers, defining the factors that determine the function of the RUNX in this complex might help explain the interplay of RUNX, Notch and Wnt in intestinal cancer.

Bacteria *Citrobacter rodentium* infection is associated with epithelial cell hyperproliferation in mice. Moreover, it induced mucosal hyperplasia and adenoma formation in the colon of *Apc*$^{Min/+}$ mice; it has thus been proposed that infectious colitis is a risk factor for colon cancer (Newman 2001). RUNX3 is involved in the development of innate lymphoid cells (ILC), which reside on the intestinal mucosa to direct immune defenses against pathogenic infection (Ebihara et al. 2015). *Runx3* knockout in ILC cells resulted in impaired immune response against *Citrobacter rodentium* infection – the mice exhibited prolonged epithelial injury, crypt hyperplasia and increased inflammatory cell infiltration (Ebihara et al. 2015). It is possible that RUNX3 deficiency and ensuing

defective immune response are risk factors to tumorigenesis.

RUNX1 also contributes to intestinal homeostasis. RUNX1 expression is highly dynamic in the intestine, with weak expression in a few stem cells at the base of the crypt, and strong expression in the transit amplifying cells at the upper crypt (Scheitz et al. 2012). Conditional knockout of *Runx1* in the mouse colon using the inducible Mx-Cre system revealed that RUNX1 upregulates *Klf4* transcription to induce goblet cell differentiation in the mouse intestine (Buchert et al. 2009). Conditional knockout *Runx1*$^{-/-}$ C57BL/6 J mice (with intestine-specific Villin-Cre) showed increased adenoma formation in the duodenum by 12 months of age. In addition, *Runx1*$^{-/-}$ mice in *Apc*^{min} background led to further increases in tumor size and formation in the small and large intestines (Fijneman et al. 2012). This suggests that RUNX1 is a tumor suppressor in the intestine. Knockdown of *RUNX1* in colon cancer cell lines, however, did not reveal any changes in cell growth (Scheitz et al. 2012). It is possible that RUNX1 tumor suppressor activity in the intestine stems, in part, from its role in promoting terminal differentiation.

In contrast to RUNX1 and RUNX3, RUNX2 promoted oncogenic activity in the human colon carcinoma cells; depletion of RUNX2 resulted in decreased proliferation, migration and invasion abilities of colorectal cell lines SW480 and DLD1 (Sase et al. 2012). Mechanistically, RUNX2 directly upregulated the metastatic gene osteopontin by binding to the RUNX binding sites in its promoter (Wai et al. 2006). In human colon carcinoma patients, elevated RUNX2 expression was significantly correlated with tumor stage and liver metastasis, further indicating its oncogenicity and as well as potential as prognostic factor (Sase et al. 2012).

A role for RUNX in gut development is conserved from *Caenorhabditis elegans* to *Drosophila* to human (Nam et al. 2002). One RUNX gene in the *Caenorhabditis elegans* is sufficient to dictate gut development and acquisition of multiple RUNX genes, while advantageous for complex organisms, might be inherently dangerous. Clearly, the imbalance of the RUNX1, RUNX2 and RUNX3 expression contributes to gastrointestinal cancers in humans – how RUNX2 activity impinges on that of RUNX1 and RUNX3 to activate an oncogenic transcriptional program in the intestine remains unknown.

19.7 Neuroblastoma: RUNX3 Inhibits MYCN

RUNX3 is involved in dorsal root ganglion neurogenesis. 20–40% of neuroblastoma cases exhibit loss of heterozygosity at *1p36*. Chromosomal deletion at *1p36* is reported to be one of the reasons why *RUNX3* expression is reduced in advanced neuroblastoma (Yu et al. 2013). Amplification of *MYCN* was significantly associated with reduced *RUNX3* expression in neuroblastoma patients. High *RUNX3* expression is associated with a more favorable prognosis in neuroblastoma patients. RUNX3 binds directly to MYCN to promote ubiquitin-mediated degradation of MYCN, thereby suppressing the oncogenic effects of MYCN transcriptional activity (Yu et al. 2013).

19.8 Dualistic Roles of RUNX3 in Pancreatic Cancer

The ability of RUNX3 to suppress proliferation and the discovery of a cancer mutation RUNX3^{R122C} that promotes oncogenic growth, would reasonably have led to the view of RUNX3 as a classical tumor suppressor. This is however a simplistic assumption. Increasingly, we note that precise expression levels of RUNX proteins are critical for proper growth. A recent study on pancreatic ductal adenocarcinoma (PDA) revealed that the regulation of RUNX3 expression is highly complex and dependent on multiple inputs from the gene expression landscape of the cancer cells (see relevant chapter in book). Hingorani's group explored RUNX3's activity in the context of key genetic changes such as activating *KRAS* mutation, *p53* point mutation and loss of *SMAD4* expression. They showed that SMAD4 regulates *RUNX3* expression in a biphasic, dose-dependent

manner. Total loss of SMAD4 is correlated with elevated RUNX3 expression, which in turn promotes cell migration and metastasis of PDA cells (Whittle et al. 2015). Mechanistically, overexpression of RUNX3 upregulated osteopontin transcription to facilitate distant colonization. And yet, RUNX3 still possesses the ability to stimulate p21^{CIP1} expression and suppress proliferation in PDA cells, as previously observed in gastric cell lines. The role of RUNX3 in PDA is therefore dualistic. Depending on inputs from TGFβ and possibly p53, RUNX3 can function as both tumor suppressor and metastasis promoter in PDA (Whittle et al. 2015).

19.9 Skin Cancer: Stem Cells and Differentiation

It is not surprising, therefore, that RUNX genes can also function as potent oncogenes. An interesting example is skin cancer. All three *Runx* genes are expressed in the hair follicle (Levanon et al. 2001; Raveh et al. 2006). While some functional compensation of the three genes is likely, mouse knockout studies showed obvious phenotypes for single *Runx* knockout mice. Depletion of *Runx1* affects hair structure. *Runx1* expression is highly dynamic as cells progress through the different stages of hair follicle development, indicating that RUNX1 dosage is necessary for normal differentiation. *Runx2* knockout mice are impaired in follicle maturation. It was suggested that the dynamic expression of *Runx2* in the hair follicle cooperates with the hedgehog pathway to regulate skin thickness and hair follicle development (Glotzer et al. 2008). *Runx3* knockout mice showed changes in hair type composition and intrinsic shape of the hair (Raveh et al. 2005). In the human, immunohistochemistry revealed that RUNX3 is expressed all epidermal layers of normal skin – in particular, the number of RUNX3 expressing cells is prominent in the basal cell layer and hair shaft (Salto-Tellez et al. 2006).

Several lines of study have implicated RUNX proteins in skin tumor. Immunohistochemistry revealed that RUNX3 is overexpressed in basal cell carcinoma (BCC), when compared to normal epidermis. No mutation was found in the *RUNX3* gene, suggesting that it is fully functional and contributes as an oncogene to BCC pathogenesis (Salto-Tellez et al. 2006). Perhaps the most compelling evidence for a RUNX role in skin tumor come from the adult hair follicle stem cells (HFSC) in mice with conditional knockout of *Runx1* (using keratin 14 driven Cre on BL6 and/or CD1 backgrounds). These mice showed defective de novo production of hair shafts and differentiated hair lineages, which was attributed to a prolonged quiescent phase during the first hair cycle (Osorio et al. 2008). RUNX1 promoted proliferation of the HFSC, in part through transcriptional repression of cell cycle inhibitor *CDKN1A* and STAT inhibitors *SOCS3/4* (Hoi et al. 2010; Scheitz et al. 2012). RUNX1-mediated stimulation of Stat3 signaling is likely to be a major factor for cancer growth and survival in the skin. In fact, strong RUNX1 expression was observed in the skin epithelium, papillomas and squamous cell carcinomas derived from chemically induced skin tumors (Hoi et al. 2010). A two-step carcinogenic treatment on the *Runx1* conditional knockout mice revealed that RUNX1 expression was required for tumor initiation (Scheitz et al. 2012). The finding that RUNX1 is frequently overexpressed in human epithelial cancers, and necessary for the growth and survival of skin squamous cell carcinoma (SCC) and oral SCC (Scheitz et al. 2012), suggests that overexpression of Runx1 plays a key role in solid tumor initiation.

19.10 Bone Cancer and Metastasis

The frequent overexpression of RUNX2 in osteosarcoma suggests that it might promote bone cancer (Martin et al. 2011). RUNX2 expression is dynamically regulated during bone development. Its levels are low in mesenchymal progenitor cells, where it suppresses growth. During osteoblast differentiation, RUNX2 levels are upregulated and its synergistic cooperation with Smad proteins promotes bone specific gene transcription. The interaction of RUNX2 with the TGFβ/BMP pathway is critical for bone formation

(Fig. 19.5). Bone formation also requires the crosstalk of TGFβ/BMP with other pathways such as Wnt (Zhou et al. 2008), MAPK, Indian Hedgehog (Yoshida et al. 2004), Notch and Akt/mTOR – these signaling inputs have been linked to RUNX2 (Martin et al. 2011). While it is conceivable that dysregulated RUNX2 levels adversely affect the output of the above signaling pathways, the mechanistic link between RUNX2 and osteosarcoma remains unclear.

RUNX2 is also heavily implicated in metastasis to the bone from breast and prostate cancer. Runx2 is a direct upstream activator of genes involved in angiogenesis, survival, invasion and metastasis (Akech et al. 2010). These genes which include *VEGF*, *survivin* and *osteopontin* are associated with EMT. While normal prostate epithelial cells show negligible RUNX2 expression, advanced prostate tumors and metastatic prostate cancer cell lines are associated with high RUNX2 levels (Akech et al. 2010). The Runx2-Smad complex was shown to promote metastasis to distal sites; Runx2 expressing prostate cancer cells generated mixed osteolytic and osteoblastic lesions, which further metastasized to the lung (Zhang et al. 2015).

19.11 Breast Cancer: An Imbalance of RUNX Dosage

Since the involvement of RUNX in breast cancer is reviewed in detail elsewhere in this book, below is the brief comparison of the phenotypes of mice with altered Runx expression in the breast. The identification of RUNX1 as a significantly mutated gene in human luminal breast cancer suggests that RUNX1 plays a causal role in the pathogenesis of breast cancer (Ellis et al. 2012). In the mammary gland, the two major epithelial cell types are the luminal and the myoepithelial lineages. In adult virgin mice, the luminal and basal cells both show predominant *Runx1* expression, as compared to *Runx2* and *Runx3* (van Bragt et al. 2014). During pregnancy, *Runx1* expression is found mainly in the myoepithelial cells and absent from the alveolar luminal cells. Conditional knockout (KO) of *Runx1* using MMTV-Cre transgenic mice, which mainly targeted luminal epithelial cells, did not exhibit any gross morphological abnormalities. However, lactating Runx1 KO mice were distinguished by milk stasis and reduction in the luminal population. *Runx1* KO luminal cells exhibited a gene expression signature resembling progenitor cells in the luminal lineage (van Bragt et al. 2014). Elf5, a transcription factor which marks luminal progenitor cells and is critical for the alveolar cell lineage, was upregulated in *Runx1* KO luminal cells; conversely, expression of ductal luminal transcription factors, such as Foxa1, ERα and Cited1, were reduced (van Bragt et al. 2014). Importantly, the ER+ mature luminal cell population was decreased in the Runx1 KO mice and this phenotype that could be rescued if either *p53* or *RB1* was mutated. Moreover, *RUNX1* mutations frequently co-occur with *p53* or *RB1* mutations in breast cancer (van Bragt et al. 2014). Therefore, *RUNX1* mutation, in conjunction with the acquisition of oncogenic mutations in *p53* or *RB1*, are likely to play important roles in the pathogenesis of ER+ luminal breast cancer.

RUNX2 was also shown to play an important role in epithelial cancers originating from the breast. Mammary-specific Runx2 transgenic mouse models studies directly implicated RUNX2 in breast differentiation and cancer progression (McDonald et al. 2014; Owens et al. 2014; Ferrari et al. 2015). Runx2 is required for adult mammary stem/progenitor cell function (Owens et al. 2014; Ferrari et al. 2015) and is expressed in the basal as well as luminal cell lineages. Runx2 expression in the breast epithelium of pregnant mice is regulated in a temporal and hormonal manner (Owens et al. 2014). Ectopic expression of Runx2 disrupted lobular alveolar differentiation during pregnancy. Moreover, overexpression of Runx2 led to EMT-like changes, suggesting that Runx2 can promote metastasis (Owens et al. 2014). Conversely, *Runx2* deficiency was associated with reduced proliferation, delayed onset of breast cancer and better survival rates. The fact that RUNX2 expression is dynamically regulated and that its overexpression leads to impaired differentiation and cancer formation indicate that breast

tumorigenesis stems, in part, from defective control of RUNX2 expression and deregulated differentiation in breast progenitor cells.

20% of *Runx3*$^{+/-}$ BALB/c female mice developed mammary ductal carcinoma (Huang et al. 2012). Expression of RUNX3 in ERα-positive MCF-7 cells resulted in inhibition of estrogen-dependent proliferation and transformation potential. This is due to the ability of RUNX3 to induce proteasome-specific degradation of ERα (Huang et al. 2012). Importantly, RUNX3 expression inversely correlates with the levels of ERα in human breast cancer tissues (Huang et al. 2012). RUNX3 is therefore associated with suppression of tumorigenesis of ERα-positive breast cancer cells.

It is clear that all RUNX proteins are involved in normal growth of breast tissues and it is tempting to speculate that RUNX1 and/or RUNX3 suppress the oncogenic tendencies of RUNX2. In other words, an imbalance of RUNX1/2/3 activities contribute to breast cancer progression.

19.12 Lung Cancer: RUNX3-p53-p14ARF Axis

The varied oncogenic outputs of mutated KRAS includes enhanced cell proliferation, suppression of apoptosis and modulation of the tumor microenvironment (eg. promotion of the angiogenesis and alteration of host immune response) (Pylayeva-Gupta et al. 2011). RAS mutations are frequently observed in lung cancer. We next investigated the relationship between oncogenic RAS activation and RUNX in the lung. Loss of *Runx3* resulted in hyperproliferation of bronchiolar epithelial cells and development of lung adenomas (Lee et al. 2013), suggesting that Runx3 deficiency might predispose lung epithelial cells to tumorigenesis. Using an oncogenic *KRAS*^{G12D} mouse cancer model, we demonstrated that targeted inactivation of *Runx3* in the lung resulted in accelerated lung adenocarcinoma formation. Furthermore, RUNX3 protects against oncogenic *KRAS* by collaborating with co-activator BRD2 to activate the p53-p14^{ARF} pathway (Lee et al. 2013). Intriguingly, the interaction of BRD2 with RUNX3 is enhanced by acetylation of the lysine 171 residue, which at the −2 position of the phosphorylation site T173, is part of the Aurora kinase b consensus motif (Chuang et al. 2016). How acetylation affects the phosphorylation of T173 remains unclear. Nevertheless, this finding draws attention to the exciting notion that post-translational modification of ancient, highly conserved motifs in the Runt domain is necessary for modulating the contact of the Runt domain with DNA (Bravo et al. 2001; Tahirov et al. 2001).

In normal cells, the persistent mitogenic stimulation induced by mutant RAS results in an irreversible cell cycle arrest also known as oncogene induced senescence – a crucial fail-safe mechanism that is activated by p53 and retinoblastoma protein. The p53-p14^{ARF} pathway protects against the consequences of replicative stress – namely genomic instability and malignant transformation – induced by oncogenic *RAS* mutations (Pylayeva-Gupta et al. 2011). Similarly, all RUNX family members induced senescence in mouse embryonic fibroblasts (Kilbey et al. 2007; Wolyniec et al. 2009). Ectopic expression of RUNX1 and RUNX1-ETO are associated with increases in reactive oxygen species, suggesting that the induction of senescence might be due in part to oxidative stress. In addition, RUNX proteins possess the capability to act upstream of p53. *P14*^{ARF}, which functions to stabilize the p53 protein, possesses RUNX binding sites in its promoter and is directly induced by RUNX1 as well as RUNX3 (Linggi et al. 2002; Lee et al. 2013).

19.13 Direct Transcription Regulation Versus Protein-Protein Interactions

RUNX genes function as tumor suppressors in some cancer types but act as oncogenes in other cases (Ito et al. 2015). As discussed above, the multiple mechanisms underlying the tumor suppressor activity of RUNX3 involve direct transcriptional regulation of growth inhibitory genes and/or disruption of DNA binding ability of oncogenic effectors through protein-protein interaction. Transcriptional regulation represents the classical RUNX3 tumor suppression scenario:

RUNX3 interacts with SMAD proteins and functions as an integral component of TGFβ tumor suppressor pathway at early stages of carcinogenesis (Ito et al. 2015). The other mechanism – tumor suppression through protein-protein interaction – has not been appreciated until recently. In addition to interacting with the DNA binding domains of TCF4 and TEAD4 and disrupting their activities, the Runt domain also binds the DNA binding domain of STAT5 (Ogawa et al. 2008). Complex formation between STAT5 and RUNX nullifies DNA binding of both proteins. Therefore, depending on the concentration of each protein, the level of oncogenic or tumor suppressive output would be significantly affected. For example, at elevated RUNX3 proteins levels (relative to the other three proteins), DNA binding activities of TCF4, TEAD4 and STAT5 would be significantly reduced. On the other hand, if TCF4 protein level is elevated due to enhanced Wnt activity, the amount of RUNX3 that interacts with DNA would be significantly reduced. This means that RUNX3 may simultaneously inhibit multiple oncogenic pathways and depending on cell context, the inactivation of RUNX3 would be pleiotropic. Another interesting aspect of this mechanism is that the DNA binding domains of RUNX3, TCF4, TEAD4, and STAT5 are highly conserved in the respective family. Therefore, RUNX1, RUNX2 and RUNX3 may interact with most, if not all, members of TCF, TEAD and STAT family. This possibility is extremely interesting and further studies will yield insights on the crosstalk of RUNX proteins with oncogenic effectors in cancer. In this light, the behavior of the oncogenic mutant $RUNX3^{R122C}$ becomes clear. Not only is $RUNX3^{R122C}$ unable to activate the transcription of growth inhibitory genes, it fails to interact with members of TCF and TEAD family and "resists" any existing oncogenic stimuli.

19.14 Conclusion

RUNX proteins are involved in many diverse mechanisms that cells employ to thwart malignant transformation. The interaction between RUNX and the TGFβ superfamily indicates how RUNX interact with a developmental pathway to direct differentiation and adult homeostasis (Fig. 19.5). The varied responses of RUNX to oncogenic signals (eg. Wnt, c-myc and mutant RAS) indicate how RUNX might influence or react to different oncogenic activities. Since many of these pathways crosstalk, a central question is how RUNX coordinate their crosstalk and integrate the signals to reach a cell fate decision. Aside from its classical role as transcription regulator, emerging evidence has indicated that the RUNX proteins directly participate in fundamental biological processes such as mitosis, centrosome function and DNA repair (Ito et al. 2015).

It is likely that the ability of RUNX to respond to diverse stimuli and regulate cell fate led to its evolutionary recruitment as developmental regulator as well as potent cancer gene. This is a double-edged sword. Depending on interacting proteins and post-translational modification, RUNX functions as either a transcriptional activator or repressor; if RUNX can function as a tumor suppressor by limiting proliferation and regulating differentiation, it should also be capable of promoting tumorigenesis in other cellular contexts. Understanding how RUNX dysregulation in cancer impinges on normal biological processes is important for identifying the molecular mechanisms that lead stepwise to malignancy.

Mounting evidence showed that tight regulation of RUNX expression is important for normal differentiation whereas dysregulated RUNX expression can lead to deregulated differentiation, tumor initiation and progression. Expression of RUNX or its downstream targets might therefore serve as biomarkers for early cancer detection and prognosis. Moreover, a pertinent question is whether we can augment RUNX tumor suppressor activity while decreasing its oncogenic potential. This will indicate whether restoration of proper RUNX expression to redirect cell fate or differentiation pathway is a feasible treatment for cancer. For example, the pro-tumorigenic activity of RUNX1 in stem cells might be suppressed by the restoration of RUNX3. In other words, the antidote to RUNX-induced tumors might well be its own family members.

This is an exciting period for RUNX research. The RUNX field is rapidly expanding into previously unanticipated directions – in the coming years we will gain a clearer understanding of the major theme underlying RUNX's pleiotropic properties for implementation in cancer therapy.

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