

## Prospects

# Oncogenic Role of RUNX3 in Head and Neck Cancer

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## **ABSTRACT**

Cumulative evidences show that Runt-related transcription factor 3 (RUNX3) has a tumor suppressive role in various cancers. In particular, RUNX3 appears to be an important component of the transforming growth factor-beta (TGF- $\beta$ )-induced tumor suppression pathway. Contrary to reports on this tumor suppressive role of RUNX3, RUNX3 can also function as an oncogene when overexpressed. Recently, we found that RUNX3 overexpression was frequently observed and was well correlated with malignant behaviors in head and neck cancer, which is one of the most common types of human cancer. Moreover, it has been revealed that RUNX3 overexpression promoted cell growth and inhibited apoptosis in head and neck cancer cells. This review introduces the oncogenic role of RUNX3 in certain types of cancer including head and neck cancer.

**KEY WORDS:** RUNX3; oncogene; head and neck cancer

## INTRODUCTION

RUNX3 (Runt-related transcription factor 3)/AML2/PEBP2C/CBFA3 is a transcription factor and one of the Runt-related (RUNX) family [Levanon et al., 1995]. Three members of the RUNX family genes, *RUNX1*, *RUNX2* and *RUNX3*, and related gene, *CBFB/Pebp2*, are all known as the developmental regulators and have been shown to be important in human cancers [Ito, 2004]. These *RUNX* genes have distinct roles in normal development and tissue-specific patterns of expression. The *RUNX* genes bind DNA through a highly conserved N-terminal Runt domain and share a common heterodimeric binding cofactor, CBF. Cumulative evidences show that RUNX proteins are major players in cancer pathogenesis. RUNX3 was originally cloned as AML2 and is localized on chromosome 1p36.1 [Bae et al., 1995]. Multiple transcript variants encoding different isoforms have been found for *RUNX3* gene [Cameron and Neil, 2004]. Among three members of the RUNX family genes, *RUNX3* is the simplest and smallest of the three genes, with the shortest distances between exons and promoters [Bangsow et al., 2001]. Runx3-null mice exhibit hyperplasia of gastric mucosa as a result of stimulated proliferation and suppressed apoptosis of epithelial cells [Li et al., 2002]. Indeed, RUNX3 is inactivated in more than 80% of human gastric cancer by gene silencing and protein mislocalization [Ito et al., 2005]. In gastric cancer, many reports show that RUNX3 down-regulation is frequently observed and is well correlated with malignant behaviors [Osaki et al., 2004; Oshimo et al., 2004; Wei et al., 2005; Friedrich et al., 2006; Homma et al., 2006; Hsu et al., 2009; Ogasawara et al., 2009]. Besides gastric cancer, it has been reported that reduced expression of RUNX3 was

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observed in various cancers including lung cancer [Yanada et al., 2005; Araki et al., 2005], esophageal cancer [Hiramatsu et al., 2005; Sakakura et al., 2007; Tonomoto et al., 2007; Long et al., 2007; Sugiura et al., 2008], breast cancer [Lau et al., 2006; Jiang et al., 2008], colorectal cancer [Ku et al., 2004; Goel et al., 2004; Ku et al., 2004; Imamura et al., 2005; Ahlquist et al., 2008; Soong et al., 2009; Subramaniam et al., 2009], pancreatic cancer [Li et al., 2004; Wada et al., 2004; Nomoto et al., 2008], hepatocellular carcinoma [Xiao and Liu, 2004; Mori et al., 2005; Miyagawa et al., 2006], bile duct carcinoma [Wada et al., 2004], bladder cancer [Kim et al., 2005; Wolff et al., 2008; Kim EJ et al., 2008], prostate cancer [Richiardi et al., 2009], ovarian cancer [Zhang et al., 2009], endometrial cancer [Yoshizaki et al., 2008], brain cancer [Mueller et al., 2007], yolk sac tumor [Kato et al., 2003] and melanoma [Kitago et al., 2009]. In these tumors, RUNX3 acts as a tumor suppressor. On the other hand, it recently has been shown that RUNX3 has an oncogenic role in certain types of cancer including head and neck squamous cell carcinoma (HNSCC). This review highlights our current understanding of the oncogenic function of RUNX3 in the context of HNSCC development and progression.

## **RUNX3 EXPRESSION IN HEAD AND NECK CANCER**

RUNX3 overexpression is observed in certain types of cancer. We found that RUNX3 expression level in HNSCC tissues was higher than that in normal tissues by a previously published microarray dataset of 41 HNSCC patients and 13 normal controls [Tsunematsu et

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al., 2009]. HNSCC is one of the most common types of human cancer, with an annual incidence of more than 500,000 cases worldwide [Mao et al., 2004]. Like most epithelial cancers, HNSCC develops through the accumulation of multiple genetic and epigenetic alterations in a multi-step process [Fidler, 1990]. Indeed, immunohistochemical study has revealed that overexpression of RUNX3 protein is observed and is well correlated with poorly differentiation, invasion and metastasis in HNSCC tissues [Tsunematsu et al., 2009]. To know if RUNX3 protein in HNSCC cells is functional or not, we performed the sequence analysis in HNSCC cells with RUNX3 expression. In HNSCC cell lines, RUNX3 protein was found to be full-length, intact and no point mutation, indicating that full-length RUNX3 has oncogenic function in HNSCC cells. It is well known that RUNX3 functions as a tumor suppressor in various cancers as mentioned above. By immunohistochemical analysis, HNSCC cells express RUNX3 at higher level in comparison with normal oral epithelium, while colon cancer cells do not express RUNX3 (Fig. 1). Tanji et al. also have reported that the labeling indexes of RUNX3 are highest in the dysplasia, followed by the oral squamous cell carcinomas, and the normal epithelia [Tanji et al., 2007]. In salivary adenoid cystic carcinoma and nasopharyngeal carcinoma, which arise in head and neck region, RUNX3 acts as a tumor suppressor gene [He et al., 2008; Tan et al., 2006]. Like HNSCC, RUNX3 overexpression is observed in basal cell carcinoma of skin [Salto-Tellez et al., 2006] and epithelial ovarian cancer [Nevadunsky et al., 2009]. In HNSCC and basal cell carcinoma of skin, RUNX3 expression is observed in the nuclei of cancer cells [Tsunematsu et al., 2009; Salto-Tellez et al., 2006] (Fig. 1). On the other hand, the upregulated RUNX3 in ovarian

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cancer is cytoplasmic [Nevadunsky et al., 2009]. Despite of distinct localization of RUNX3, RUNX3 overexpression acts as an oncogene in these tumors. Histologically, HNSCC and basal cell carcinoma of skin arise from squamous cell epithelium, but the origin of epithelial ovarian cancer is different. In addition, RUNX3 down-regulation is correlated with malignant behaviors in esophageal cancer, which arise from squamous cell epithelium [Hiramatsu et al., 2005; Sakakura et al., 2007; Tonomoto et al., 2007; Long et al., 2007; Sugiura et al., 2008]. Therefore, RUNX3 function is not dependent on histological type of cancer. Why does RUNX3 function as an oncogene in HNSCC, basal cell carcinoma of skin, and ovarian cancer? In normal oral mucosa, a few epithelial cells in basal cell layer express RUNX3, while most of epithelial cells in colon mucosa expressed RUNX3 (Fig. 1). In skin, the frequency of RUNX3 positive cells in normal mucosa is lower than that in basal cell carcinoma [Salto-Tellez et al., 2006]. Thus, expression level of RUNX3 in normal tissue is organ specific (Fig. 2). Therefore, we suggest that the distinct function as an oncogene or a tumor suppressor gene in cancer cells may be accounted for by the origin of cancer.

## **FUNCTION OF RUNX3 AS AN ONCOGENE IN HNSCC**

RUNX3 acts as a tumor suppressor gene in various cancers. How is RUNX3 inactivated in these cancers? It has been reported that RUNX3 inactivation is caused by hemizygous deletion of *RUNX3* gene, hypermethylation, and protein mislocalization. The location of *RUNX3* gene at chromosome 1p36, a deletion hotspot in various cancers [Bagchi and Mills,

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2008]. Reduced expression of RUNX3 due to hemizygous deletion is observed in tissues and cell lines including gastric cancer [Li et al., 2002], lung cancer [Yanada et al., 2005], hepatocellular carcinoma [Xiao and Liu, 2004; Mori et al., 2005] and bile duct carcinoma [Wada et al., 2004]. Hypermethylation of the CpG island at the RUNX3 promoter is frequently found in various cancer tissues and cell lines including gastric cancer [Li et al., 2002; Oshimo et al., 2004; Homma et al., 2006], esophageal cancer [Long et al., 2007; Sakakura et al., 2007], lung cancer [Li et al., 2004; Sato et al., 2006; Yanagawa et al., 2007], breast cancer [Lau et al., 2006; Jiang et al., 2008], colorectal cancer [Goel et al., 2004; Ku et al., 2004; Imamura et al., 2005; Ahlquist et al., 2008; Soong et al., 2009; Subramaniam et al., 2009], pancreatic cancer [Wada et al., 2004; Nomoto et al., 2008], hepatocellular carcinoma [Kim et al., 2004; Park et al., 2005], bladder cancer [Kim et al., 2005; Wolff et al., 2008; Kim EJ et al., 2008], ovarian cancer [Zhang et al., 2009], prostate cancer [Richiardi et al., 2009], brain cancer [Mueller et al., 2007] and yolk sac tumor [Kato et al., 2003]. RUNX3 protein mislocalization in cytoplasm was reported in gastric cancer [Ito et al., 2005], oral cancer [Gao et al., 2009], breast cancer [Lau et al., 2006] and colorectal cancer [Ito et al., 2008]. It recently has been reported that RUNX3 protein mislocalization is caused by MDM2-mediated ubiquitination of RUNX3 [Chi et al., 2009] and overexpression of activated Src [Goh et al., 2010]. In addition, Jab1/CSN5 and Pim1 induces the cytoplasmic localization of RUNX3 [Kim HR et al., 2008; Kim et al., 2009]. Thus, RUNX3 is inactivated in various types of cancer.

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Paradoxically, RUNX3 is overexpressed and act as an oncogene in certain types of cancer including HNSCC. Oncogenic role of RUNX3 is supported by the evidence from murine retroviral insertional work that Runx3 can act as a Myc-collaborating gene in thymic lymphoma [Stewart et al., 2002]. It is well known that the aberrant activation caused by activating mutations of the hedgehog signaling pathway plays an important role in basal cell carcinoma of skin [Caro and Low, 2010]. Salto-Tellez et al. suggest that high frequency of RUNX3 overexpression in basal cell carcinoma of skin implicate a role for RUNX3 as an oncogene downstream of the Shh pathway [Salto-Tellez et al., 2006]. In HNSCC cell lines, overexpression of Shh is found [Nishimaki et al., 2004] and Shh-patched signaling is involved in the cell growth of oral epithelial cells and in the tumorigenesis of HNSCCs [Michimukai et al., 2001]. Therefore, we examined the effect of Shh on RUNX3 expression, but Shh did not affect to RUNX3 expression in HNSCC cells (unpublished data).

In our previous study, ectopic overexpression of RUNX3 promoted cell proliferation [Tsunematsu et al., 2009]. Similar to our finding, forced RUNX3 expression by lentiviral gene delivery in ovarian cancer cells results in increased cell proliferation [Nevadunsky et al., 2009]. Moreover, ectopic overexpression of RUNX3 inhibited serum starvation induced apoptosis and chemotherapeutic drug induced apoptosis in HNSCC cells. This finding is supported by previous report that Bcr-Abl-positive cell lines with stable or inducible expression of RUNX3 were protected from imatinib-induced apoptosis in chronic myeloid leukemia. Oncogenic role of RUNX3 may be associated with the promotion



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of cell growth and the inhibition of apoptosis. However, the detailed mechanism of the oncogenic function of RUNX3 is still unclear.

Comparing the gene expression profile between control and RUNX3 overexpressing HNSCC cells by microarray analysis have revealed that several genes were selectively up-regulated and down-regulated [Tsunematsu et al., 2009]. CXCL14, IGFBP2 and EphA4 receptor were up-regulated in RUNX3 overexpressing HNSCC cells. Although CXCL14 suppresses tumor growth in some type of cancer, CXCL14 is involved in invasion of pancreatic cancer [Wente et al., 2008]. IGFBP-2 is a highly sensitive marker of malignant progression in different tumors and potentially involved in anti-apoptosis, angiogenesis, and metastasis during cancer progression [Hoeflich et al., 2001]. EphA4 receptor also promotes cancer cell growth [Iizumi et al., 2006]. It is interesting to examine the involvement of these molecules in the promotion of cell growth and the inhibition of apoptosis mediated by RUNX3 overexpression. On the other hand, RUNX3 cooperates with FoxO3a/FKHRL1 to participate in the induction of apoptosis by activating Bim in gastric cancer cells [Yamamura et al., 2006]. In esophageal cancer cells, RUNX3 inhibits cell proliferation and induces apoptosis by reinstating TGF- $\beta$  responsiveness [Torquati et al., 2004]. In each types of cancer, function of RUNX3 may be different. Interestingly, RUNX3 overexpression promoted tumorigenesis, demonstrated by tumorsphere formation assay in HNSCC [Tsunematsu et al., 2009].

In view of the distribution of RUNX3 positive cells in normal oral epithelium (Fig. 1), we thought that RUNX3 might be associated with the regulation of stem cell. In fact,

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RUNX3 overexpressing HNSCC cells evaded from serum starvation induced and chemotherapeutic drug induced apoptosis. To verify this hypothesis, we should examine the expression of RUNX3 in oral keratinocyte progenitor/stem cells and cancer stem cells of HNSCC.

### **MECHANISM OF RUNX3 OVEREXPRESSION IN HNSCC**

RUNX3 is known as a nuclear effector of the TGF- $\beta$ /BMP pathways, and a key tumor suppressor gene in the gastric epithelium [Bae et al., 1995; Li et al., 2002]. In vertebrate facial development, BMPs, TGF- $\beta$ , Shh and FGFs are known to be involved [Francis-West et al., 1998]. In the tongue and palate epithelium of mouse embryos, Runx3 expression is observed [Yamamoto et al., 2006]. These findings make us hypothesis that RUNX3 might be involved in the development of oral mucosa through growth factor signaling pathways. In our previous study, we examined the alteration of RUNX3 expression in HNSCC cells after the treatment with growth factors including TGF- $\beta$ 1, IGF, EGF, bFGF and PDGF-AA. Among these growth factors, EGF significantly enhanced RUNX3 expression in HNSCC cells, and EGF expression was well correlated with RUNX3 expression in HNSCC cell lines [Tsunematsu et al., 2009]. RUNX3 overexpression may be caused by activation of EGF-signaling pathway in HNSCC. However, further experiments are required to demonstrate this observation.

As shown in Fig. 1, a few epithelial cells in basal cell layer express RUNX3 in normal oral mucosa, while HNSCC cells express RUNX3 at higher level. In various types of cancer, reduced expression of RUNX3 is frequently caused by CpG island hypermethylation [Kim et al., 2004]. In our previous study, methylation status of the *RUNX3* promoter region was well correlated with RUNX3 mRNA expression in HNSCC cell lines. A recent report shows that Runx3 is expressed in the tongue and palate epithelium of mouse embryos from embryonic day 12.5 to 16.5, and that Runx3 expression decreases after embryonic day 16.5 and disappears in newborn mice [Yamamoto et al., 2006]. Interestingly, partially or fully methylation at promoter region of *RUNX3* is observed in primary cultured keratinocytes obtained from normal oral mucosa. DNA methylation plays an important role in the establishment and maintenance of the program of gene expression. The pattern of 5-methylcytosine distribution in the genome is unique for each cell type and is established in embryogenesis as a result of balance between DNA methylation and demethylation [Razin and Riggs, 1984; Razin and Shemer, 1995]. We suggest that *RUNX3* might be silenced by methylation in adult oral epithelial cells, and that RUNX3 expression in HNSCC may be caused by demethylation during cancer development (Fig. 3). This hypothesis is supported by the previous findings that i) the level of general demethylation and the frequency of demethylation increase with tumor progression in cancer [Gama-Sosa et al., 1993; Kim et al., 1994], and ii) demethylation of individual CpG dinucleotides located in C-MYC, Ha RAS, and ERB-A1 proto-oncogene was revealed in human tumors [Borrello et al., 1992; Cheah et al., 1984; Fienberg and Vogelstein, 1983; Lipsanen et al., 1988]. Recent

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report shows that EZH2 binds to the RUNX3 promoter, resulting in upregulation of H3K27 methylation and concomitant downregulation of RUNX3 expression [Fujii et al., 2008]. Frequent overexpression of EZH2 in aggressive solid tumors is largely due to the loss of its regulator microRNA-101 [Varambally et al., 2008]. In adult oral epithelial cells, the involvement of EZH2 and microRNA-101 in RUNX3 methylation should be examined. To examine the correlation between RUNX3 expression and methylation status in embryonic and adult cells obtained from different organs may lead to clarify the distinct function of RUNX3 in various cancers. Detailed mechanism of RUNX3 regulation by methylation requires further experiments.

## **CONCLUSIONS**

In HNSCC, RUNX3 overexpression is observed and is well correlated with malignant behaviors through promotion of cell proliferation and inhibition of apoptosis. Moreover, RUNX3 expression observed in HNSCC may be caused in part by demethylation during cancer development. These findings suggest that RUNX3 expression can be a useful marker for predicting malignant behavior and the effect of chemotherapeutic drugs in HNSCC. Contrary to our findings, Gao et al. has reported that RUNX3 is down-regulated in oral cancers due to promoter hypermethylation. However, they did not examine the detailed mechanism of RUNX3 inactivation in oral cancers by *in vitro* study. We should examine the immunohistochemical expression of RUNX3 in a large number of HNSCC cases. As mentioned in this review, there remains much to be investigated regarding the mechanism

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of RUNX3 overexpression and the detailed function of RUNX3 as an oncogene in HNSCC. To solve the oncogenic role in HNSCC, it may be important to generate RUNX3 transgenic mice driven by keratin 14-promoter for transgene expression in oral epithelial cells. In addition, it is still unclear why RUNX3 has opposite activities in different cell type of cancer. Identifying these mechanisms may explore the great clinical relevance of RUNX3.

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## FIGURE LEGENDS

**Fig. 1.** Immunohistochemical expression of RUNX3 in colon cancer and HNSCC.

Representative cases of RUNX3 expression in normal colon mucosa, colon cancer, normal oral mucosa and HNSCC are shown.

**Fig. 2.** RUNX3 acts as tumor suppressor gene or oncogene. In stomach, colon, esophagus, lung, liver, gallbladder, and etc., epithelial cells express RUNX3 and that acts as a tumor suppressor gene. RUNX3 expression is reduced in cancer cells. On the other hand, RUNX3 expression is low in normal oral epithelial and skin epithelial cells. In HNSCC and basal cell carcinoma of skin, RUNX3 overexpression is observed and acts as an oncogene.

**Fig. 3.** A schematic model of RUNX3 overexpression in HNSCC. In oral epithelium of embryo, RUNX3 is expressed and may be induced by some signaling pathways such as BMP/TGF- $\beta$ , Shh and FGF. After birth, RUNX3 may be methylated of its promoter and/or transcriptional repression in oral epithelial cells. RUNX3 may be caused by demethylation or transcriptional modulation during cancer development. In aggressive HNSCC, unknown stimulation may enhance RUNX3 expression.

Figure 1

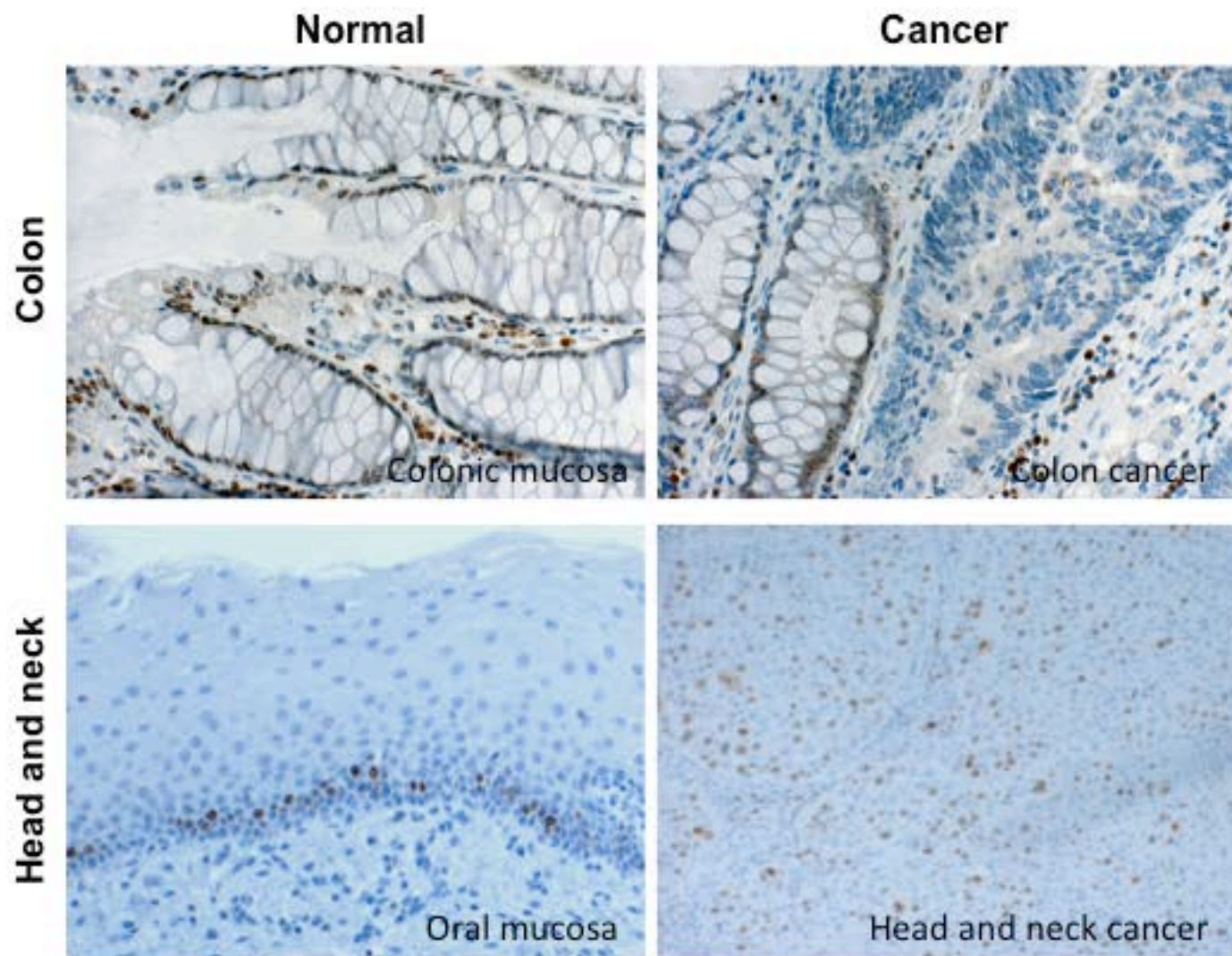


Figure 2

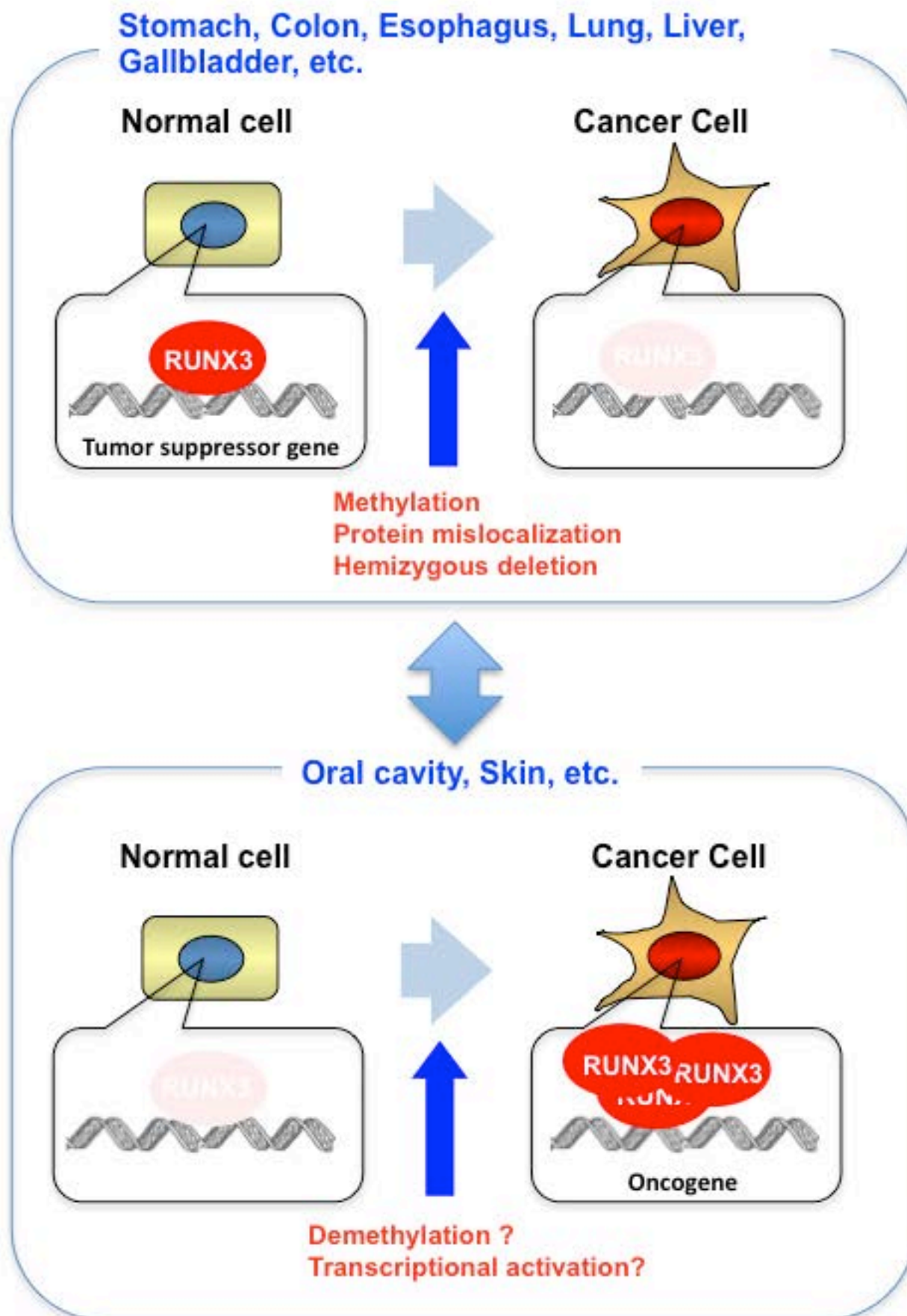


Figure 3

