PROSPECTS
Journal of Cellular Biochemistry 112:387–393 (2011)

Journal of Cellular Biochemistry

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-β (TGF-β)-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. J. Cell. Biochem. 112: 387–393, 2011.

KEY WORDS: RUNX3; ONCOGENE; HEAD AND NECK CANCER

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/Pebpb2*, 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 observed in various cancers including lung cancer [Araki et al., 2005; Yanada et al., 2005], esophageal cancer [Hiramatsu et al., 2005; Long et al., 2007; Sakakura et al., 2007; Tonomoto et al., 2007; Sugiura et al., 2008], 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 [Li et al., 2004a; 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, 2008a; Wolff 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], york 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

The authors have declared that no competing interests exist.
Grant sponsor: Ministry of Education, Science and Culture of Japan.
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Received 18 November 2010; Accepted 18 November 2010 • DOI 10.1002/jcb.22967 • © 2010 Wiley-Liss, Inc.
Published online 6 December 2010 in Wiley Online Library (wileyonlinelibrary.com).

than that in normal tissues by a previously published microarray dataset of 41 HNSCC patients and 13 normal controls [Tsunematsu et 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. [2007] 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. In salivary adenoid cystic carcinoma and nasopharyngeal carcinoma, which arise in head and neck region, RUNX3 acts as a tumor suppressor gene [Tan et al., 2006; He et al., 2008]. 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 [Salto-Tellez et al., 2006; Tsunematsu et al., 2009] (Fig. 1). On the other hand, the upregulated RUNX3 in ovarian 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; Long et al., 2007; Sakakura et al., 2007; Tonomoto 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*

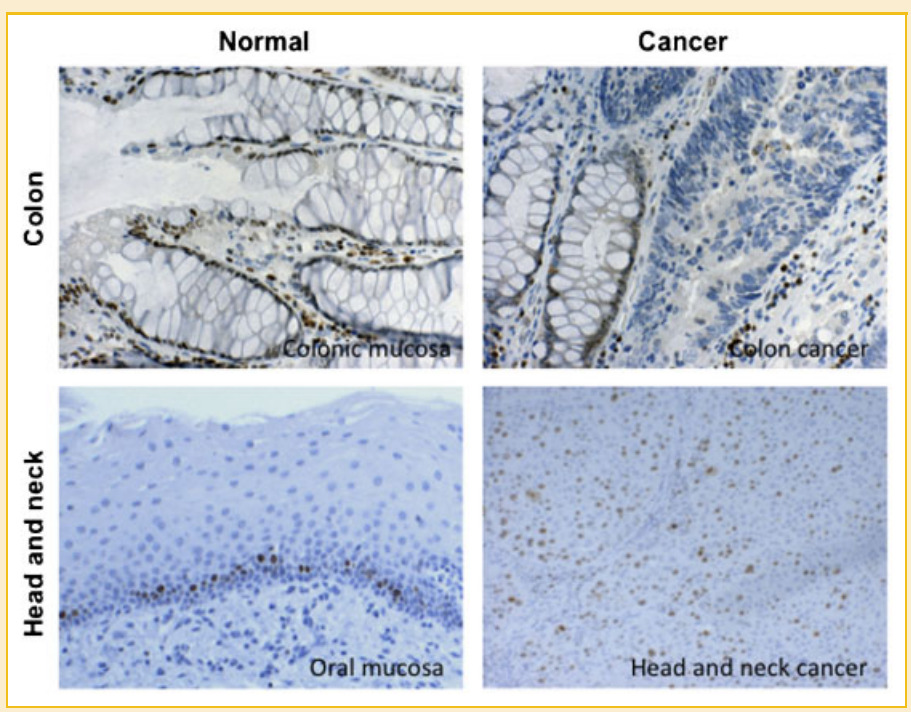

Fig. 1. Immunohistochemical expression of RUNX3 in colon cancer and HNSCC. Representative cases of RUNX3 expression in normal colon mucosa, colon cancer, normal oral mucosa and HNSCC are shown.

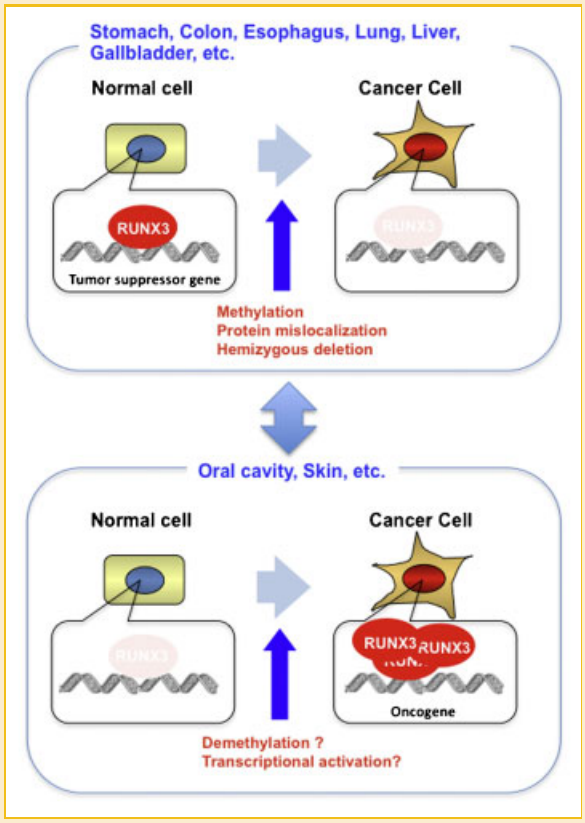

Fig. 2. RUNX3 acts as tumor suppressor gene or oncogene. In stomach, colon, esophagus, lung, liver, gallbladder, etc., epithelial cells express RUNX3 and that acts as a tumor suppressor gene. RUNX3 expression is reduced in cancer cells. On the other hand, RUNX3 expression is low in normal oral epithelial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

gene, hypermethylation, and protein mislocalization. The location of *RUNX3* gene at chromosome 1p36, a deletion hotspot in various cancers [Bagchi and Mills, 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., 2004b; 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, 2008a; Wolff et al., 2008], ovarian cancer [Zhang et al., 2009], prostate cancer [Richiardi et al., 2009], brain cancer [Mueller et al., 2007], and york 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 et al., 2008b, 2009]. Thus, RUNX3 is inactivated in various types of cancer.

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. [2006] 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. 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 [Miething et al., 2007]. Oncogenic role of RUNX3 may be associated with the promotion 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 upregulated and down-regulated [Tsunematsu et al., 2009]. CXCL14, IGFBP2, and EphA4 receptor were upregulated 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 transforming growth factor-β (TGF-β) 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, 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 effecter of the TGF-β/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-β, 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-β1, 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., 1983; Kim et al., 1994], and (ii) demethylation of individual CpG

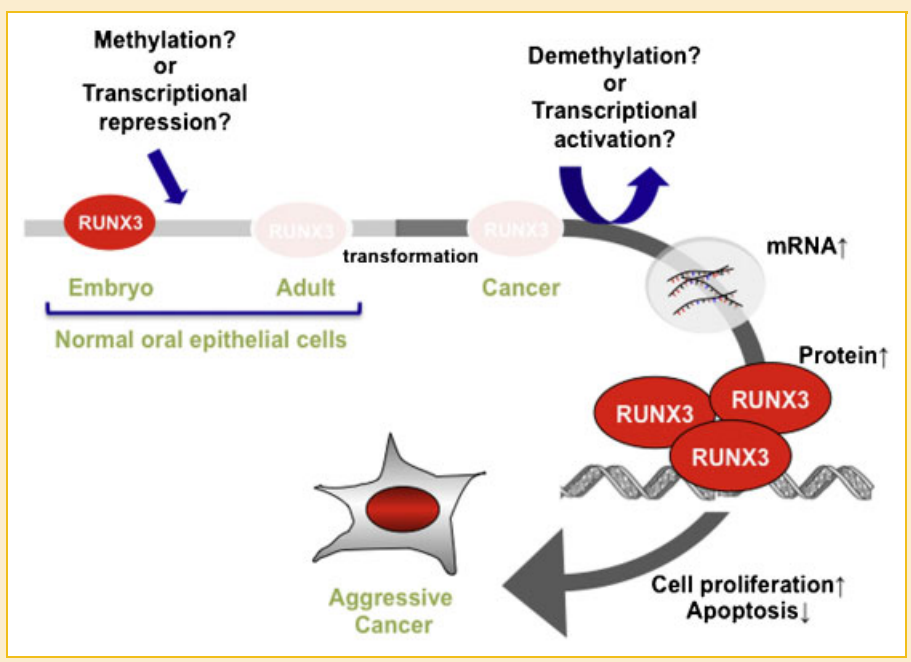

Fig. 3. A schematic model of RUNX3 overexpression in HNSCC. In oral epithelium of embryo, RUNX3 is expressed and may be induced by some signaling pathways such as BMP/TGF-β, Shh, and FGF. After birth, RUNX3 may be methylated of its promoter and/or transcriptional repression in oral epithelial cells.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

dinucleotides located in C-MYC, Ha RAS, and ERB-A1 proto-oncogene was revealed in human tumors [Fienberg and Vogelstein, 1983; Cheah et al., 1984; Lipsanen et al., 1988; Borrello et al., 1992]. Recent report shows that EZH2 binds to the RUNX3 promoter, resulting in upregulation of H3K27 methylation and concomitant down-regulation 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 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.

REFERENCES

Ahlquist T, Lind GE, Costa VL, Meling GI, Vatn M, Hoff GS, Rognum TO, Skotheim RI, Thiis-Evensen E, Lothe RA. 2008. Gene methylation profiles of normal mucosa, and benign and malignant colorectal tumors identify early onset markers. Mol Cancer 7:94.

Araki K, Osaki M, Nagahama Y, Hiramatsu T, Nakamura H, Ohgi S, Ito H. 2005. Expression of RUNX3 protein in human lung adenocarcinoma: Implications for tumor progression and prognosis. Cancer Sci 96:227–231.

Bae SC, Takahashi E, Zhang YW, Ogawa E, Shigesada K, Namba Y, Satake M, Ito Y. 1995. Cloning, mapping and expression of PEBP2aC, a third gene encoding the mammalian runt domain. Gene 159:245–248.

Bagchi A, Mills AA. 2008. The quest for the 1p36 tumor suppressor. Cancer Res 68:2551–2556.

Bangsow C, Rubins N, Glusman G, Bernstein Y, Negreanu V, Goldenberg D, Lotem J, Ben Asher E, Lancet D, Levanon D, Groner Y. 2001. The RUNX3 gene-sequence, structure and regulated expression. Gene 279:221–232.

Borrello MG, Pierotti MA, Tamborini E, Biassoni D, Rizzetti MG, Pilotti S, Della Porta G. 1992. DNA methylation of coding and non-coding regions of the human H-RAS gene in normal and tumor tissues. Oncogene 7:269–275.

Cameron ER, Neil JC. 2004. The Runx genes: Lineage-specific oncogenes and tumor suppressors. Oncogene 23:4308–4314.

Caro I, Low JA. 2010. The role of the hedgehog signaling pathway in the development of basal cell carcinoma and opportunities for treatment. Clin Cancer Res 16:3335–3339.

Cheah MS, Wallace CD, Hoffman RM. 1984. Hypomethylation of DNA in human cancer cells: A site-specific change in the c-myc oncogene. J Natl Cancer Inst 73:1057–1065.

Chi XZ, Kim J, Lee YH, Lee JW, Lee KS, Wee H, Kim WJ, Park WY, Oh BC, Stein GS, Ito Y, van Wijnen AJ, Bae SC. 2009. Runtrelated transcription factor RUNX3 is a target of MDM2-mediated ubiquitination. Cancer Res 69:8111–8119.

Fidler IJ. 1990. Critical factors in the biology of human cancer metastasis: Twenty-eighth GHA Clowes Memorial Award Lecture. Cancer Res 50:6130–6138.

Fienberg AP, Vogelstein B. 1983. Hypomethylation of ras oncogenes in primary human cancers. Biochem Biophys Res Commun 111:47–54.

Francis-West P, Ladher R, Barlow A, Graveson A. 1998. Signaling interactions during facial development. Mech Dev 75:3–28.

Friedrich MJ, Rad R, Langer R, Voland P, Hoefler H, Schmid RM, Prinz C, Gerhard M. 2006. Lack of RUNX3 regulation in human gastric cancer. J Pathol 210:141–146.

Fujii S, Ito K, Ito Y, Ochiai A. 2008. Enhancer of zeste homologue 2 (EZH2) down-regulates RUNX3 by increasing histone H3 methylation. J Biol Chem 283:17324–17332.

Gama-Sosa MA, Slagel VA, Trewyn RW, Oxenhandler R, Kuo KC, Gehrke CW, Ehrlich M. 1983. The 5-methylcytosine content of DNA from human tumors. Nucleic Acids Res 11:6883–6894.

Gao F, Huang C, Lin M, Wang Z, Shen J, Zhang H, Jiang L, Chen Q. 2009. Frequent inactivation of RUNX3 by promoter hypermethylation and protein mislocalization in oral squamous cell carcinomas. J Cancer Res Clin Oncol 135:739–747.

Goel A, Arnold CN, Tassone P, Chang DK, Niedzwiecki D, Dowell JM, Wasserman L, Compton C, Mayer RJ, Bertagnolli MM, Boland CR. 2004. Epigenetic inactivation of RUNX3 in microsatellite unstable sporadic colon cancers. Int J Cancer 112:754–759.

Goh YM, Cinghu S, Hwee Hong ET, Lee YS, Kim JH, Zhang JW, Li YH, Chi XZ, Lee KS, Wee H, Ito Y, Oh BC, Bae SC. 2010. Src kinase phosphorylates RUNX3 at tyrosine residues and localizes the protein in the cytoplasm. J Biol Chem 25:10122–10129.

He JF, Ge MH, Zhu X, Chen C, Tan Z, Li YN, Gu ZY. 2008. Expression of RUNX3 in salivary adenoid cystic carcinoma: Implications for tumor progression and prognosis. Cancer Sci 99:1334–1340.

Hiramatsu T, Osaki M, Ito Y, Tanji Y, Tokuyasu N, Ito H. 2005. Expression of RUNX3 protein in human esophageal mucosa and squamous cell carcinoma. Pathobiology 72:316–324.

Hoeflich A, Reisinger R, Lahm H, Kiess W, Blum WF, Kolb HJ, Weber MM, Wolf E. 2001. Insulin-like growth factor-binding protein 2 in tumorigenesis: Protector or promoter? Cancer Res 61:8601–8610.

Homma N, Tamura G, Honda T, Matsumoto Y, Nishizuka S, Kawata S, Motoyama T. 2006. Spreading of methylation within RUNX3 CpG island in gastric cancer. Cancer Sci 97:51–56.

Hsu PI, Hsieh HL, Lee J, Lin LF, Chen HC, Lu PJ, Hsiao M. 2009. Loss of RUNX3 expression correlates with differentiation, nodal metastasis, and poor prognosis of gastric cancer. Ann Surg Oncol 16:1686–1694.

Iizumi M, Hosokawa M, Takehara A, Chung S, Nakamura T, Katagiri T, Eguchi H, Ohigashi H, Ishikawa O, Nakamura Y, Nakagawa H. 2006. EphA4

receptor, overexpressed in pancreatic ductal adenocarcinoma, promotes cancer cell growth. Cancer Sci 97:1211–1216.

Imamura Y, Hibi K, Koike M, Fujiwara M, Kodera Y, Ito K, Nakao A. 2005. RUNX3 promoter region is specifically methylated in poorly-differentiated colorectal cancer. Anticancer Res 25:2627–2630.

Ito Y. 2004. Oncogenic potential of the RUNX gene family: 'Overview'. Oncogene 23:4198–4208.

Ito K, Liu Q, Salto-Tellez M, Yano T, Tada K, Ida H, Huang C, Shah N, Inoue M, Rajnakova A, Hiong KC, Peh BK, Han HC, Ito T, Teh M, Yeoh KG, Ito Y. 2005. RUNX3, a novel tumor suppressor, is frequently inactivated in gastric cancer by protein mislocalization. Cancer Res 65:7743–7750.

Ito K, Lim AC, Salto-Tellez M, Motoda L, Osato M, Chuang LS, Lee CW, Voon DC, Koo JK, Wang H, Fukamachi H, Ito Y. 2008. RUNX3 attenuates beta-catenin/T cell factors in intestinal tumorigenesis. Cancer Cell 14:226–237.

Jiang Y, Tong D, Lou G, Zhang Y, Geng J. 2008. Expression of RUNX3 gene, methylation status and clinicopathological significance in breast cancer and breast cancer cell lines. Pathobiology 75:244–251.

Kato N, Tamura G, Fukase M, Shibuya H, Motoyama T. 2003. Hypermethylation of the RUNX3 gene promoter in testicular yolk sac tumor of infants. Am J Pathol 163:387–391.

Kim YI, Giuliano A, Hatch KD, Schneider A, Nour MA, Dallal GE, Selhub J, Mason JB. 1994. Global DNA hypomethylation increases progressively in cervical dysplasia and carcinoma. Cancer 74:893–899.

Kim TY, Lee HJ, Hwang KS, Lee M, Kim JW, Bang YJ, Kang GH. 2004. Methylation of RUNX3 in various types of human cancers and premalignant stages of gastric carcinoma. Lab Invest 84:479–484.

Kim WJ, Kim EJ, Jeong P, Quan C, Kim J, Li QL, Yang JO, Ito Y, Bae SC. 2005. RUNX3 inactivation by point mutations and aberrant DNA methylation in bladder tumors. Cancer Res 65:9347–9354.

Kim EJ, Kim YJ, Jeong P, Ha YS, Bae SC, Kim WJ. 2008a. Methylation of the RUNX3 promoter as a potential prognostic marker for bladder tumor. J Urol 180:1141–1145.

Kim HR, Oh BC, Choi JK, Bae SC. 2008b. Pim-1 kinase phosphorylates and stabilizes RUNX3 and alters its subcellular localization. J Cell Biochem 105:1048–1058.

Kim JH, Choi JK, Cinghu S, Jang JW, Lee YS, Li YH, Goh YM, Chi XZ, Lee KS, Wee H, Bae SC. 2009. Jab1/CSN5 induces the cytoplasmic localization and degradation of RUNX3. J Cell Biochem 107:557–565.

Kitago M, Martinez SR, Nakamura T, Sim MS, Hoon DS. 2009. Regulation of RUNX3 tumor suppressor gene expression in cutaneous melanoma. Clin Cancer Res 15:2988–2994.

Ku JL, Kang SB, Shin YK, Kang HC, Hong SH, Kim IJ, Shin JH, Han IO, Park JG. 2004. Promoter hypermethylation downregulates RUNX3 gene expression in colorectal cancer cell lines. Oncogene 23:6736–6742.

Lau QC, Raja E, Salto-Tellez M, Liu Q, Ito K, Inoue M, Putti TC, Loh M, Ko TK, Huang C, Bhalla KN, Zhu T, Ito Y, Sukumar S. 2006. RUNX3 is frequently inactivated by dual mechanisms of protein mislocalization and promoter hypermethylation in breast cancer. Cancer Res 66:6512–6520.

Levanon D, Negreanu V, Bernstein Y, Bar-Am I, Avivi L, Groner Y. 1995. AML1, AML2, and AML3, the human members of the runt domain gene-family: cDNA structure, expression, and chromosomal localization. Genomics 23:425–432.

Li QL, Ito K, Sakakura C, Fukamachi H, Inoue K, Chi XZ, Lee KY, Nomura S, Lee CW, Han SB, Kim HM, Kim WJ, Yamamoto H, Yamashita N, Yano T, Ikeda T, Itohara S, Inazawa J, Abe T, Hagiwara A, Yamagishi H, Ooe A, Kaneda A, Sugimura T, Ushijima T, Bae SC, Ito Y. 2002. Causal relationship between the loss of RUNX3 expression and gastric cancer. Cell 109:113–124.

Li J, Kleeff J, Guweidhi A, Esposito I, Berberat PO, Giese T, Büchler MW, Friess H. 2004a. RUNX3 expression in primary and metastatic pancreatic cancer. J Clin Pathol 57:294–299.

Li QL, Kim HR, Kim WJ, Choi JK, Lee YH, Kim HM, Li LS, Kim H, Chang J, Ito Y, Youl Lee K, Bae SC. 2004b. Transcriptional silencing of the RUNX3 gene by CpG hypermethylation is associated with lung cancer. Biochem Biophys Res Commun 314:223–228.

Lipsanen V, Leinonen P, Alhonen L, Janne J. 1988. Hypomethylation of ornithine decarboxylase gene and erb-A1 oncogene in human chronic lymphatic leukemia. Blood 72:2042–2044.

Long C, Yin B, Lu Q, Zhou X, Hu J, Yang Y, Yu F, Yuan Y. 2007. Promoter hypermethylation of the RUNX3 gene in esophageal squamous cell carcinoma. Cancer Invest 25:685–690.

Mao L, Hong WK, Papadimitrakopoulou VA. 2004. Focus on head and neck cancer. Cancer Cell 5:311–316.

Miething C, Grundler R, Mugler C, Brero S, Hoepfl J, Geigl J, Speicher MR, Ottmann O, Peschel C, Duyster J. 2007. Retroviral insertional mutagenesis identifies RUNX genes involved in chronic myeloid leukemia disease persistence under imatinib treatment. Proc Natl Acad Sci U S A 104:4594–4599.

Michimukai E, Kitamura N, Zhang Y, Wang H, Hiraishi Y, Sumi K, Hayashido Y, Toratani S, Okamoto T. 2001. Mutations in the human homologue of the Drosophila segment polarity gene patched in oral squamous cell carcinoma cell lines. *In Vitro* Cell Dev Biol Anim 37:459–464.

Miyagawa K, Sakakura C, Nakashima S, Yoshikawa T, Kin S, Nakase Y, Ito K, Yamagishi H, Ida H, Yazumi S, Chiba T, Ito Y, Hagiwara A. 2006. Down-regulation of RUNX1, RUNX3 and CBFbeta in hepatocellular carcinomas in an early stage of hepatocarcinogenesis. Anticancer Res 26:3633–3643.

Mori T, Nomoto S, Koshikawa K, Fujii T, Sakai M, Nishikawa Y, Inoue S, Takeda S, Kaneko T, Nakao A. 2005. Decreased expression and frequent allelic inactivation of the RUNX3 gene at 1p36 in human hepatocellular carcinoma. Liver Int 25:380–388.

Mueller W, Nutt CL, Ehrich M, Riemenschneider MJ, von Deimling A, van den Boom D, Louis DN. 2007. Downregulation of RUNX3 and TES by hypermethylation in glioblastoma. Oncogene 26:583–593.

Nevadunsky NS, Barbieri JS, Kwong J, Merritt MA, Welch WR, Berkowitz RS, Mok SC. 2009. RUNX3 protein is overexpressed in human epithelial ovarian cancer. Gynecol Oncol 112:325–330.

Nishimaki H, Kasai K, Kozaki K, Takeo T, Ikeda H, Saga S, Nitta M, Itoh G. 2004. A role of activated Sonic hedgehog signaling for the cellular profileration of oral squamous cell carcinoma cell line. Biochem Biophys Res Commun 314:313–320.

Nomoto S, Kinoshita T, Mori T, Kato K, Sugimoto H, Kanazumi N, Takeda S, Nakao A. 2008. Adverse prognosis of epigenetic inactivation in RUNX3 gene at 1p36 in human pancreatic cancer. Br J Cancer 98:1690–1695.

Ogasawara N, Tsukamoto T, Mizoshita T, Inada KI, Ban H, Kondo S, Takasu S, Ushijima T, Ito K, Ito Y, Ichinose M, Ogawa T, Joh T, Tatematsu M. 2009. RUNX3 expression correlates with chief cell differentiation in human gastric cancers. Histol Histopathol 24:31–40.

Osaki M, Moriyama M, Adachi K, Nakada C, Takeda A, Inoue Y, Adachi H, Sato K, Oshimura M, Ito H. 2004. Expression of RUNX3 protein in human gastric mucosa, intestinal metaplasia and carcinoma. Eur J Clin Invest 34: 605–612.

Oshimo Y, Oue N, Mitani Y, Nakayama H, Kitadai Y, Yoshida K, Ito Y, Chayama K, Yasui W. 2004. Frequent loss of RUNX3 expression by promoter hypermethylation in gastric carcinoma. Pathobiology 71:137–143.

Park WS, Cho YG, Kim CJ, Song JH, Lee YS, Kim SY, Nam SW, Lee SH, Yoo NJ, Lee JY. 2005. Hypermethylation of the RUNX3 gene in hepatocellular carcinoma. Exp Mol Med 37:276–281.

Razin A, Riggs AD. 1984. DNA methylation and gene function. Science 210:604–610.

Razin A, Shemer R. 1995. DNA methylation in early development. Hum Mol Genet 4:1751–1755.

Richiardi L, Fiano V, Vizzini L, De Marco L, Delsedime L, Akre O, Tos AG, Merletti F. 2009. Promoter methylation in APC, RUNX3, and GSTP1 and mortality in prostate cancer patients. J Clin Oncol 27:3161–3168.

Sakakura C, Miyagawa K, Fukuda KI, Nakashima S, Yoshikawa T, Kin S, Nakase Y, Ida H, Yazumi S, Yamagishi H, Okanoue T, Chiba T, Ito K, Hagiwara A, Ito Y. 2007. Frequent silencing of RUNX3 in esophageal squamous cell carcinomas is associated with radioresistance and poor prognosis. Oncogene 26:5927–5938.

Salto-Tellez M, Peh BK, Ito K, Tan SH, Chong PY, Han HC, Tada K, Ong WY, Soong R, Voon DCC, Ito Y. 2006. RUNX3 protein is overexpressed in human basal cell carcinomas. Oncogene 25:7646–7649.

Sato K, Tomizawa Y, Iijima H, Saito R, Ishizuka T, Nakajima T, Mori M. 2006. Epigenetic inactivation of the RUNX3 gene in lung cancer. Oncol Rep 15:129–135.

Soong R, Shah N, Peh BK, Chong PY, Ng SS, Zeps N, Joseph D, Salto-Tellez M, Iacopetta B, Ito Y. 2009. The expression of RUNX3 in colorectal cancer is associated with disease stage and patient outcome. Br J Cancer 100:676–679.

Stewart M, Mackay N, Cameron ER, Neil JC. 2002. The common retroviral insertion locus Dsi1 maps 30 kilobases upstream of the P1 promoter of the murine Runx3/Cbfa3/Aml2 gene. J Virol 76:4364–4369.

Subramaniam MM, Chan JY, Soong R, Ito K, Yeoh KG, Wong R, Guenther T, Will O, Chen CL, Kumarasinghe MP, Ito Y, Salto-Tellez M. 2009. RUNX3 inactivation in colorectal polyps arising through different pathways of colonic carcinogenesis. Am J Gastroenterol 104:426–436.

Sugiura H, Ishiguro H, Kuwabara Y, Kimura M, Mitsui A, Mori Y, Ogawa R, Katada T, Harata K, Fujii Y. 2008. Decreased expression of RUNX3 is correlated with tumor progression and poor prognosis in patients with esophageal squamous cell carcinoma. Oncol Rep 19:713–719.

Tan SH, Ida H, Goh BC, Hsieh W, Loh M, Ito Y. 2006. Analyses of promoter hypermethylation for RUNX3 and other tumor suppressor genes in nasopharyngeal carcinoma. Anticancer Res 26:4287–4292.

Tanji Y, Osaki M, Nagahama Y, Kodani I, Ryoke K, Ito H. 2007. Runt-related transcription factor 3 expression in human oral squamous cell carcinomas; implication for tumor progression and prognosis. Oral Oncol 43:88–94.

Tonomoto Y, Tachibana M, Dhar DK, Onoda T, Hata K, Ohnuma H, Tanaka T, Nagasue N. 2007. Differential expression of RUNX genes in human esophageal squamous cell carcinoma: Downregulation of RUNX3 worsens patient prognosis. Oncology 73:346–356.

Torquati A, O'rear L, Longobardi L, Spagnoli A, Richards WO, Daniel Beauchamp R. 2004. RUNX3 inhibits cell proliferation and induces apoptosis by reinstating transforming growth factor beta responsiveness in esophageal adenocarcinoma cells. Surgery 136:310–316.

Tsunematsu T, Kudo Y, Iizuka S, Ogawa I, Fujita T, Kurihara H, Abiko Y, Takata T. 2009. RUNX3 has an oncogenic role in head and neck cancer. PLoS ONE 4e:5892.

Varambally S, Cao Q, Mani RS, Shankar S, Wang X, Ateeq B, Laxman B, Cao X, Jing X, Ramnarayanan K, Brenner JC, Yu J, Kim JH, Han B, Tan P, Kumar-Sinha C, Lonigro RJ, Palanisamy N, Maher CA, Chinnaiyan AM. 2008. Genomic loss of microRNA-101 leads to overexpression of histone methyltransferase EZH2 in cancer. Science 322:1695–1699.

Wada M, Yazumi S, Takaishi S, Hasegawa K, Sawada M, Tanaka H, Ida H, Sakakura C, Ito K, Ito Y, Chiba T. 2004. Frequent loss of RUNX3 gene expression in human bile duct and pancreatic cancer cell lines. Oncogene 23:2401–2407.

Wei D, Gong W, Oh SC, Li Q, Kim WD, Wang L, Le X, Yao J, Wu TT, Huang S, Xie K. 2005. Loss of RUNX3 expression significantly affects the clinical outcome of gastric cancer patients and its restoration causes drastic suppression of tumor growth and metastasis. Cancer Res 65:4809–4816.

Wente MN, Mayer C, Gaida MM, Michalski CW, Giese T, Bergmann F, Giese NA, Büchler MW, Friess H. 2008. CXCL14 expression and potential function in pancreatic cancer. Cancer Lett 259:209–217.

Wolff EM, Liang G, Cortez CC, Tsai YC, Castelao JE, Cortessis VK, Tsao-Wei DD, Groshen S, Jones PA. 2008. RUNX3 methylation reveals that bladder tumors are older in patients with a history of smoking. Cancer Res 68:6208–6214.

Xiao WH, Liu WW. 2004. Hemizygous deletion and hypermethylation of RUNX3 gene in hepatocellular carcinoma. World J Gastroenterol 10:376–380.

Yamamoto H, Ito K, Kawai M, Murakami Y, Bessho K, Ito Y. 2006. Runx3 expression during mouse tongue and palate development. Anat Rec A Discov Mol Cell Evol Biol 288:695–699.

Yamamura Y, Lee WL, Inoue K, Ida H, Ito Y. 2006. RUNX3 cooperates with FoxO3a to induce apoptosis in gastric cancer cells. J Biol Chem 281:5267–5276.

Yanada M, Yaoi T, Shimada J, Sakakura C, Nishimura M, Ito K, Terauchi K, Nishiyama K, Itoh K, Fushiki S. 2005. Frequent hemizygous deletion at 1p36 and hypermethylation downregulate RUNX3 expression in human lung cancer cell lines. Oncol Rep 14:817–822.

Yanagawa N, Tamura G, Oizumi H, Kanauchi N, Endoh M, Sadahiro M, Motoyama T. 2007. Promoter hypermethylation of RASSF1A and RUNX3 genes as an independent prognostic prediction marker in surgically resected non-small cell lung cancers. Lung Cancer 58:131–138.

Yoshizaki T, Enomoto T, Fujita M, Ueda Y, Miyatake T, Fujiwara K, Miyake T, Kimura T, Yoshino K, Kimura T. 2008. Frequent inactivation of RUNX3 in endometrial carcinoma. Gynecol Oncol 110:439–444.

Zhang S, Wei L, Zhang A, Zhang L, Yu H. 2009. RUNX3 gene methylation in epithelial ovarian cancer tissues and ovarian cancer cell lines. OMICS 13: 307–311.