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Loss of Runt-Related Transcription Factor 3 Causes Development and Progression of Hepatocellular Carcinoma

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ABSTRACT

Runt-related transcription factor 3 (RUNX3) is reported as a tumor suppressor gene for gastric cancer, and may be important in the development of hepatocellular carcinoma (HCC). RUNX3 expression is frequently lost or decreased by hemizygous deletion or hypermethylation of its promoter lesion in HCC. The significance of decreased expression of RUNX3 in HCC has not been fully elucidated, but is likely related to dysfunction of cell cycle regulation, decrement of apoptosis, enhancement of angiogenesis, and development of epithelial–mesenchymal transition. RUNX3 is a promising candidate as a tumor suppressor gene for HCC. J. Cell. Biochem. 112: 745–749, 2011.

KEY WORDS: APOPTOSIS; ANGIOGENESIS; EPITHELIAL–MESENCHYMAL TRANSITION; TUMOR SUPPRESSOR GENE

HEPATOCELLULAR CARCINOMA

GENERAL ASPECTS OF HEPATOCELLULAR CARCINOMA

Hepatocellular carcinoma (HCC) is the fifth most common cancer worldwide and the third most common cause of cancer mortality [El-Serag and Rudolph, 2007]. HCC has received considerable attention in recent years because of its rapidly increasing incidence. Patients diagnosed with HCC have a poor prognosis because of the aggressive features of the disease [El-Serag and Mason, 1999; El-Serag and Rudolph, 2007]. Surgical resection, ablation therapy, and liver transplantation are effective, but only at an early stage of HCC development. Approximately 70% of HCC patients develop recurrent tumors within 5 years [Nakakura and Choti, 2000]. Systemic chemotherapy has demonstrated a very limited effect on advanced HCC progression [Thomas, 2009].

Molecular target-based drugs are a promising approach to the treatment of HCC. Several such drugs are clinically available [Llovet et al., 2008; Thomas, 2009]; however, their effects are relatively limited. Elucidation of the mechanisms of hepatocarcinogenesis should contribute to the development of molecular target therapies.

GENE ALTERATIONS IN HCC

Molecular mechanisms of hepatocarcinogenesis are not completely understood; however, the current understanding of carcinogenesis mechanisms suggests that development and progression of HCC are caused by accumulated genetic changes. Important genetic mechanisms in HCC involve tumor suppressor genes, activation of growth factors and their receptors, re-activation of developmental pathways, and activation of oncogenes (Fig. 1). Tumor suppressor genes potentially involved in HCC include p53, Rb, and phosphatase and tensin homolog (PTEN). The p53 gene is a regulator of transcriptional activation that controls the cell cycle and cell division. Although p53 mutation is frequently observed in aflatoxin-induced HCC, it is less frequent in Hepatitis B- and Hepatitis C-related HCC (45% and 13%, respectively) [Teramoto et al., 1994]. The role of the Rb gene in carcinogenesis has been extensively investigated; however, Rb gene mutations have been observed in only 15% of HCC cases [Ozturk, 1999]. PTEN is another tumor suppressor gene that negatively regulates the phosphoinositide 3-kinase/Akt signaling pathway. PTEN has been observed in ~40% of HCC cases [Hu et al., 2003]. Although the role of tumor suppressor genes seems to be more important than that of oncogenes in HCC, alterations in p53, Rb, and PTEN genes are observed in less than one half of HCC cases [Tada et al., 1990; Davies et al., 2002; Wang et al., 2002].

Other tumor-related changes in HCC include over-expression of growth factors and their receptors. Transforming growth factor (TGF)-α and insulin growth factor (IGF)-2 expressions are strongly associated with HCC. Over-expression of TGF-α is frequently observed in HCC tissues [Schaff et al., 1994], as is the expression of IGF-2 receptor [De Souza et al., 1995; Yamada et al., 1997]. Aberrant re-activation of the developmental pathway is another

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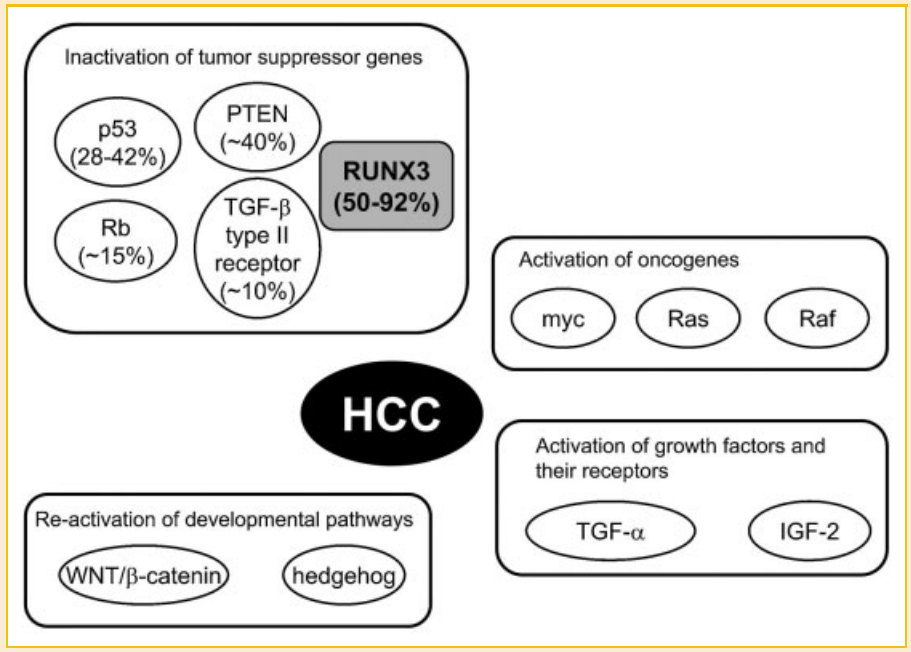

Fig. 1. Gene alteration in HCC. Gene alterations in HCC are inactivation of tumor suppressor genes with percentages showing approximate frequency, re-activation of developmental pathways, activation of oncogenes, and activation of growth factors and their receptors. The inactivation of tumor suppressor genes is relatively infrequent in HCC. RUNX3 can be a major tumor suppressor gene in HCC.

tumor-related change reported for HCC, including re-activation of the hedgehog signaling pathway [Huang et al., 2006] and the WNT/β-catenin pathway [de La Coste et al., 1998].

RUNT-RELATED TRANSCRIPTION FACTOR 3 (RUNX3)

FUNCTIONS OF RUNX3

RUNX3 was identified as core binding factor alpha subunit 3 by Levanon et al. [1994]. RUNX3 was first reported as a tumor suppressor gene for gastric cancer [Li et al., 2002]. RUNX3 expression is decreased by about 45–60% in human gastric cancer [Li et al., 2002; Wei et al., 2005], and its reduced expression has been significantly associated with decreased survival [Wei et al., 2005]. Decreased expression of RUNX3 has also been detected in other human cancers, such as those of the lung, pancreas, colon, and bile duct [Ku et al., 2004; Li et al., 2004; Wada et al., 2004; Araki et al., 2005].

The function of RUNX3 in solid tumors has been intensively investigated in gastric cancers. In gastric cancer cell lines, studies have demonstrated lost or decreased expression of RUNX3, restoration of RUNX3-induced cell cycle arrest, apoptosis, and down-regulation of cyclin D1 expression [Li et al., 2002; Chi et al., 2005; Wei et al., 2005].

RUNX3 IN HCC

RUNX3 is located in chromosome 1p36, which frequently loses heterozygosity in HCC [Buendia, 2000]. Loss of RUNX3 expression by hemizygous deletion was found in 35–40% of HCC cases [Xiao and Liu, 2004; Mori et al., 2005]. It has been suggested that RUNX3 might function as a tumor suppressor in HCC. Decreased mRNA expression of RUNX3 has been observed in 50–92% of HCC cases [Mori et al., 2005; Miyagawa et al., 2006]. The studies involving RUNX3 and HCC were mainly performed in methylation analyses of the RUNX3 promoter lesion. Hypermethylation of the RUNX3 promoter lesion was generally in the range of 41–76% [Kim et al., 2004; Xiao and Liu, 2004; Mori et al., 2005; Park et al., 2005; Nomoto et al., 2007; Moribe et al., 2009]. Hypermethylation of the RUNX3 promoter lesion in hepatitis C virus-related HCC might be more frequent (81.8%) [Nishida et al., 2008].

The significance of decreased RUNX3 expression in HCC has not been fully elucidated. Li et al. [2008] reported that restoration of RUNX3 in a HCC cell line with decreased expression of RUNX3 suppressed cell proliferation and cell cycle progression. They also reported that RUNX3 expression induced the cytotoxic effect of adriamycin [Li et al., 2008]. Gao et al. [2010] reported that exogenous RUNX3 expression decreased Notch signaling in a HCC cell line.

Li et al. [2002] generated RUNX3-knockout mice; however, the effect of RUNX3 knockout in the liver was not elucidated because the knockout mice died soon after birth [Fukamachi and Ito, 2004].

PROSPECTIVE CONCEPT OF FUTURE STUDIES WITH RUNX3 IN HCC

The significance of the decreased expression of RUNX3 in HCC remains unclear. The ideas discussed in this review can be summarized in a set of propositions. First, RUNX3 plays a role in the regulation of cell proliferation, cell survival, and apoptosis. Second, angiogenesis is induced by the loss of RUNX3 expression. Third, the

loss of RUNX3 causes epithelial–mesenchymal transition (EMT). Through these mechanisms, the loss of RUNX3 expression could contribute to the development and progression of HCC (see Fig. 2).

DYSFUNCTION OF CELL CYCLE REGULATION AND APOPTOSIS

Abrogation of cell cycle checkpoints is one of the features of hepatocarcinogenesis. Four major checkpoints are affected in HCC, including processes involving the p53, Rb, and p27 genes [Armengol et al., 2003] and the TGF-β/IGF-2 receptor [El-Serag and Rudolph, 2007]. As discussed earlier, gene alterations are relatively infrequent in HCC. In studies of gastric epithelial cells, the interaction of RUNX3 with cell cycle processes involved the p21, p27, and cyclin D1 molecules. RUNX3 requires TGF-β induced p21 expression that inhibits cell cycle progression via inhibition of cyclin-dependent kinases [Chi et al., 2005]. Exogenous RUNX3 expression suppressed cell growth by inducing p27 [Chen et al., 2010]. As discussed earlier, Wei et al. [2005] also reported that cyclin D1 expression is down-regulated by exogenous RUNX3 expression. RUNX3 may be a major cell cycle regulator in hepatocarcinogenesis.

Insufficient apoptosis is associated with the development of cancers including HCC [Thorgeirsson et al., 1998; Guicciardi and Gores, 2005]. In studies of RUNX3-knockout mice, hyperplasia due to insufficient apoptosis was observed in gastric mucosa epithelial cells. The gastric epithelial cells in RUNX3 null mice did not demonstrate TGF-β induced apoptosis [Li et al., 2002]. As dysregulation of TGF-β induced apoptosis is frequently observed in HCC [Thorgeirsson et al., 1998], RUNX3 could be a major apoptosis HCC regulator. Li et al. [2008] already reported that exogenous expression of RUNX3 enhanced adriamycin induced apoptosis in one HCC cell line. Further study is necessary to elucidate the involvement of RUNX3 in apoptosis and drug resistance in HCC.

ANGIOGENESIS

Angiogenesis is essential for the growth of solid tumors, including HCC. Angiogenesis facilitates progressive tumor growth by providing adequate oxygen and nutrition to the tumor cells. It is also associated with the progression of the metastatic phenotype. RUNX3 might be involved in the regulation of angiogenesis in HCC. Since Notch signaling is closely related with angiogenesis [Noguera-Troise et al., 2006; Ridgway et al., 2006], decreased expression of RUNX3 might be one of the angiogenic mechanisms in HCC. Another possible RUNX3-related angiogenic factor is vascular endothelial growth factor (VEGF). In one study of gastric cancer, exogenous RUNX3 expression suppressed VEGF expression [Peng et al., 2006]. VEGF stimulates proliferation and motility of vascular endothelial cells and capillary differentiation. Although the source of VEGF in HCC is still controversial [Mathonnet et al., 2006], VEGF plays an important role in the angiogenesis of HCC [Park et al., 2000]. VEGF is also believed to enhance the tumorigenicity and metastasis of HCC [Yamaguchi et al., 1998].

EMT

EMT is involved in the progression of non-invasive tumor cells into malignant metastatic carcinomas. Loss of E-cadherin expression is one of the most common indicators of EMT onset [Thiery, 2003]. In studies of breast cancer, Xue et al. [2003] observed E-cadherin-negative cells at the sites of tumor invasive fronts, and considered

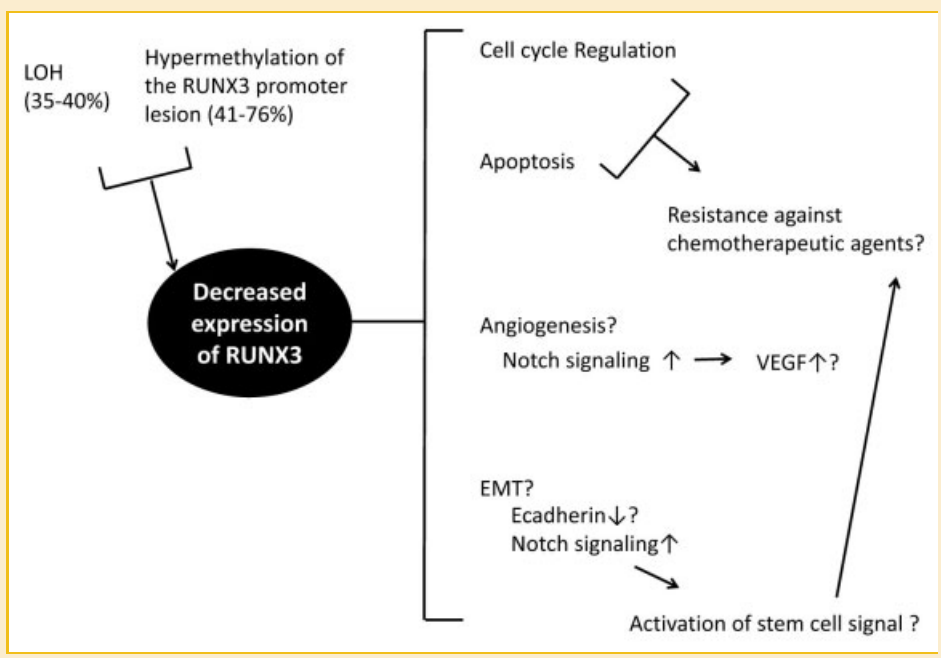

Fig. 2. Overview of the decreased expression of RUNX3 in HCC. Loss or decreased expression of RUNX3 was caused by hemizygous deletion and hypermethylation of its promoter lesion (percentages refer to the frequencies in HCC). The loss of RUNX3 expression can induce dysfunction of cell cycle regulation and apoptosis, enhancement of angiogenesis, and development of EMT (arrows refer to the causal relationships).

these cancer cells to progressively become more metastatic. A number of studies have focused on transcriptional regulation of E-cadherin in the processes of malignant transformation in carcinomas. TGF-β, closely interacting with RUNX3, was reported to regulate E-cadherin expression and distribution. To our knowledge, there is no direct evidence that RUNX3 regulates E-cadherin. Nevertheless, in a study of gastric epithelial cells, Chang et al. [2010] reported that RUNX3 inhibits the expression of claudin, another cell–cell junction protein involved in the development of EMT. As both E-cadherin and claudin are negatively regulated in EMT, RUNX3 could be an EMT regulator.

Interestingly, in a study of breast cancer, Mani et al. [2008] found that cancer cells acquire a stem cell profile by over-expression of either twist or snail, both of which are strong EMT inducers; in other words, the EMT regulator determined the stem cell signal. Gao et al. [2010] reported that RUNX3 controlled Notch signaling, which is closely linked to cancer stem cells (CSCs). Treatment with conventional chemotherapeutic agents can induce tumor re-growth or resistance to the chemotherapeutic agents, and accumulating evidence suggests that these processes are mediated by CSCs. The CSC model suggests that the elimination of CSCs is mandatory for inhibiting tumor re-growth. Consequently, RUNX3 and its related signaling molecules could be the therapeutic target for cancer treatment by CSC elimination.

CONCLUSION

RUNX3 is a promising tumor suppressor gene candidate for HCC, as RUNX3 possibly regulates cell growth and differentiation through multiple mechanisms. RUNX3 and its related signaling molecules could be a therapeutic target for HCC treatment. Further studies are required to elucidate the precise function of RUNX3 in the development and progression of HCC.

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