Review

Role of Hepatocyte Growth Factor and c-Met Receptor in Neoplastic Conditions of Salivary Glands

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Signaling via hepatocyte growth factor (HGF) and the HGF receptor, c-Met, plays important roles in human salivary glands and salivary gland tumors. Various effects of HGF, including acceleration or inhibition of cell growth, enhancement of cell motility, increase of angiogenesis, inhibition of apoptosis and induction of morphogenesis, have been demonstrated in various epithelial cells. Our recent analysis of HGF regarding saliva, salivary gland and salivary gland tumors demonstrated that: 1) HGF was basically localized in luminal cells of fetal, normal salivary glands and salivary gland

tumors; 2) saliva contained HGF protein on ELISA; 3) HGF tended to be more intensely expressed in benign salivary gland tumors than in malignant salivary gland tumors; 4) HGF/c-Met signaling was associated with cell scattering and invasion into matrix in the ACC3 adenoid cystic carcinoma cell line; 5) prognosis was poor and correlated to metastasis in high-grade salivary gland carcinomas with c-Met overexpression. These profiles suggest that HGF/c-Met systems may play significant roles in differentiation or tumor progression for salivary gland tumors.

Key words: HGF, c-Met, saliva, salivary gland, salivary gland tumor

I. Introduction

The role of the salivary glands is to secrete saliva, which is involved in food digestion, promotion of mastication and antimicrobial activity [40]. The salivary glands may play other important roles, as a variety of substances including cell growth factors are produced by them. In particular, cell growth factors such as epidermal growth factor (EGF) and nerve growth factor (NGF) have been found in the mice salivary glands [8, 9]. Close correlations exist between salivary glands and cell growth factors, and saliva containing

cell growth factors can play important roles in human health. However, little research has been done on the biological roles of salivary cell growth factors with respect to the salivary glands and other organs. Research into the biological phenomena of salivary gland neoplasm is also lacking [45]. Our group has investigated the hypothesis that cell growth factors play important roles in the biological behavior of salivary gland tumors. Hepatocyte growth factor (HGF) is one cell growth factor that is attracting a great deal of attention as a multifunctional cytokine, that not only induces hepatocyte growth, but also displays a wide variety of functions in various epithelial and endothelial cells [25, 37, 40]. In particular, the deregulation of expression and altered function of this factor and its receptor are characteristic of malignant transformation in various mammalian cells [25, 37, 40]. The present review discusses the significance of HGF and its

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receptor, c-Met in salivary glands and salivary gland tumors.

II. Hepatocyte Growth Factor

HGF was originally identified as a protein released by rat platelets after partial hepatectomy, that acted to accelerate liver regeneration rate [23]. This secretory humoral factor synthesized as its single-strand pro-HGF is converted to its double-strand active form comprising α (69 kDa) and β (34 kDa) chains by HGF activator [7, 46]. Structure-function studies have demonstrated that α chains composed of the hairpin loop and kringle domains are important in binding HGF to receptor. In tissues such as the placenta, NK1 and NK2 isoforms of HGF are produced by alternative mRNA splicing [7, 10]. These isoforms demonstrate partial agonist activity without protease activation. Competitive binding of mature-HGF or HGF variants such as NK1 and NK2 for c-Met may regulate HGF functions in tissue [7, 10].

HGF has become known as a multipotential protein that can induce numerous functions, including mitogenesis, cellular motility, angiogenesis and anti-apoptotic activity in normal epithelial or neoplastic cells [15]. HGF is also needed for morphogenesis and homeostasis in various normal developmental stages of the body, and during fetal development [47]. In addition, HGF is typically represented in the induction of tubular forms in neoplasms such as adenocarcinomas [13]. Knockout mice for HGF display placental defects, reduced liver volume and abnormalities of skeletal muscle including the tongue, and invariably die before birth [32, 43]. HGF is predominantly produced and secreted by cells of the mesenchymal/stromal compartments, although recent evidence has shown epithelial expression of HGF in certain instances [42]. Conversely, HGF is reportedly expressed in benign or malignant tumors arising from various organs, including bone marrow, breast, prostate, colon, lung, liver, kidney, gallbladder, uterus, cervix and endometrium [37]. Intra-tissue concentrations of HGF are known to be related to prognosis in breast cancer [38] and malignant glioma [4]. Measurement of HGF may thus contribute to definitions of biological behavior in malignant tumors. In recent years, the HGF variant NK4 has been developed. Since this molecule is an HGF antagonist, inhibition of tumor growth and invasion has been demonstrated in human breast cancers [20]. In future, the clinical applications of NK4 against malignant tumor are sure to spark a great deal of interest.

III. HGF Receptor, c-Met

The HGF receptor was found as a c-Met proto-oncogene from a human osteosarcoma cell line [11, 26]. Certain biological activities of HGF occur only through c-Met. Met knockout mice show the same abnormalities as HGF knockout mice, indicating that HGF acts only through c-Met [29]. The c-Met protein comprises a 50-kDa β chain and a 145-kDa α chain with extracellular, transmembrane and tyrosine kinase domains [29]. c-Met receptor molecule induced the

formation of receptor dimerization and then autophosphorylation [5]. In particular, the autophosphorylation of Y1349 and Y1356 is indispensable to signal transduction. Since Y1349 and Y1356 represent the binding sites of numerous signal transduction molecules, together they are called the multifunctional docking site [28]. Various functions of HGF are known to depend on differences in signal transduction at the intracellular domain of c-Met, but how these differences in signal transduction are regulated is not well understood. Moreover, the physiological significance *in vivo* of c-Met variants is unclear, although some c-Met variants have been found [30]. The c-Met protein is widely present in the epithelial cells, particularly internal organs such as the liver, kidney, thyroid gland and placenta [37]. Moreover, overexpression and amplification of Met genes are identified in many malignant tumors [18]. Point mutations inducing c-Met activation were initially reported in hereditary and sporadic cases of papillary renal cell carcinoma [33], but later also in hepatocellular carcinoma [27] and squamous cell carcinomas of head and neck [1], where the point mutation of met Y1253D inducing persistent receptor activation without ligand was first identified. As for overexpression of c-Met, numerous reports have described correlations with metastasis and malignant tumor size [37]. The molecular mechanisms of metastases displaying c-Met may be expected to attract substantial attention in future.

IV. The Role of HGF in Fetal and Adult Salivary Gland

Human salivary glands basically comprise a secretory portion and a ductal system [6]. Serous and mucous cells are found in the secretory portion, and depending on the composition of these cells, the glands can be classified as serous, mucous or mixed. The ductal system comprises ductal epithelia and can be classified as intercalated, striated or interlobular, based on morphological differences. Intercalated ducts have previously been considered as the site of origin of various salivary gland tumors [34]. Furthermore, myoepithelial cells are stacked in the peripheries of acinar cells and intercalated ducts [6].

In human fetal salivary gland during the early stage, HGF expression is observed in epithelial strands with or without luminal structures [39]. Compared to strands without luminal structure, luminal strands are strongly positive for HGF. HGF expression is considered to be closely associated with the development of ductal structures during maturation in the fetal stage. In adults, HGF-positive reactions for both protein (Fig. 1A) and mRNA are limited to ductal cells of the inner layer [39]. These expression profiles basically agree with previous reports from rodent [2] and monkey [22] salivary glands and adult normal mammary tissues [44]. In collagen gel cultures, HGF is tubulogenic, displays branching morphogenic activities, and induces gland-like structures in cells derived from renal epithelium, hepatic ducts, mammary glands and gastric glands [19]. Furue *et al.* [14] reported that RSMG-1 cells derived from rat submandibular

Fig. 1. Expression profile of HGF in the normal and neoplastic conditions of salivary gland. **(A)** Normal salivary gland. Luminal cells are strongly positive for the HGF, but with little expression in acinar and myoepithelial cells. **(B)** Warthin's tumor. Expression of HGF is observed in the tall columnar cells. **(C, D)** Myoepithelioma. **(C)** Microphotograph showing plasmacytoid type myoepithelioma. **(D)** No apparent HGF expression is identified in neoplastic myoepithelium. **(E)** Adenoid cystic carcinoma, solid type. HGF is faintly expressed in luminal cells.

gland form duct-like structures in collagen gels on the addition of recombinant HGF. HGF plays a role in luminal cell differentiation associated with preserving the ductal structures in human salivary gland. To date, there is no detailed report on the discovery of c-Met in rodent or human salivary gland.

V. Expression of HGF and c-Met in Salivary Gland Tumors

HGF is expressed more strongly in the luminal cells of benign tumors than in malignant tumors (Fig. 1), and may play an important role in ductal differentiation of salivary gland tumors [39]. We reported that ratios of HGF-positive cases were 90.2% for benign tumors and 35.0% for malignant tumors [39]. The histological types of non-ductal benign (Fig. 1C, D) or malignant myoepitheliomas and keratinizing squamous cell carcinomas were completely negative for HGF [39]. Since HGF expression tendencies

are generally characterized by localization in luminal cells indicating highly differentiated nature, high-grade malignant tumors such as solid type adenoid cystic carcinoma (ACC) (Fig. 1E), salivary duct carcinoma have consistently shown less frequent expression of HGF [39]. In other words, the extent of HGF expression seems to reflect the level of tumor differentiation. Immunohistochemical study of HGF expression may offer an ancillary procedure for evaluating grade of malignancy, and numerous immunohistochemical studies have reported that HGF expression is excessive in malignant tumors compared to benign tumors except for breast tumor. Interestingly, this does not seem to hold true for salivary gland tumors. In salivary glands tumor, differentiation induction therapy which uses HGF gene as a target may be effective.

By contrast, Karakida [17] reported the highest expression levels of c-Met and bcl-2 in neoplastic myoepithelium of pleomorphic adenoma, in comparison with basal cell adenoma and myoepithelioma. HGF expression might also par-

Fig. 2. Representative section showing c-Met expression in a high grade mucoepidermoid carcinoma with evidence of lung metastasis.

participate in regulation of apoptosis of salivary gland tumors, since there was a correlation between expressions of c-Met and anti-apoptotic factor bcl-2. Moreover, cyclooxygenase (COX)-2 inhibits apoptosis which is mediated by bcl-2 expression in pleomorphic adenoma [3], and HGF is increased on the addition of rCox-2 in the HSG salivary gland carcinoma cell line (unpublished). These findings suggest that HGF may be the key molecule regulating progression in benign salivary gland tumors.

In ACC, Suzuki *et al.* [35] have reported increased cell scattering and invasive ability on the addition of rHGF to the ACC3 cell line. In a clinicopathological study of high-grade salivary gland carcinoma, c-Met expression of cancer cells

Fig. 3. Kaplan-Meier plots of overall survival of patients of high grade salivary gland carcinomas with/without c-Met expression.

and expression of HGF in fibroblasts adjacent to tumor nests (stromal HGF) were significantly correlated with frequency of regional lymph nodes and distant metastases, but not with HGF expression in cancer cells [41]. Stromal HGF was also correlated with tumor size [41]. Overall survival was significantly worse in patients with c-Met and stromal HGF immunoreactivity than in patients without c-Met and stromal HGF immunoreactivity (Fig. 3) [41]. HGF binds to c-Met in a paracrine fashion, and may induce metastasis of high-grade salivary gland carcinoma. Regulation of c-Met will

A Saliva HGF

B Serum HGF

Fig. 4. HGF concentration in saliva and serum before and after operation. Saliva and serum were obtained from 18 patients with salivary gland tumors (benign: 12 cases; malignant: 6 cases). The Wilcoxon signed-ranks test was employed to determine the differences in saliva or serum between before and after operation. (A) Salivary HGF level is 0.06 ± 0.05 ng/mL (range 0–0.19 ng/mL) before operation and 0.11 ± 0.08 ng/mL (range 0.012–0.276 ng/mL) after operation. (B) Serum level is 0.22 ± 0.08 ng/mL (range 0–0.42 ng/mL) before operation and 0.33 ± 0.14 ng/mL (range 0.174–0.78 ng/mL) after operation. Significant differences exist between pre- and postoperatively in HGF concentrations for both saliva and serum ($p < 0.0005$).

provide a key molecule for the analysis of metastases in salivary gland carcinoma.

VI. HGF in Saliva

Human saliva contains cell growth factors such as insulin-like growth factors I and II [12], transforming growth factor- α [21], vascular endothelial growth factor [36], EGF [24] and fibroblast growth factor-2 [16]. These growth factors have been assumed to play roles in wound healing of the upper digestive tract. We measured concentrations of HGF in saliva and blood before and after an operation for salivary gland tumor using ELISA, and found a significant increase in HGF levels after surgery (Fig. 4) [39]. For patients with salivary gland tumors, no significant relationship has been observed between salivary or serum HGF concentrations and clinicopathological factors such as tumor size, gender, tissue classification or tumor site [39]. Clinically, measurement of salivary and serum HGF cannot be used as a tumor marker for salivary gland tumors. On the other hand, the concentrations of saliva and serum HGF tended to invariably increase for all patients examined after operation. Schmassmann *et al.* [31] reported that in gastric ulcer healing, production and secretion of HGF was induced from various mesenchymal cells such as fibroblasts, endothelial cells and inflammatory cells associated with the regenerative process. Based on this finding, surgical treatment of salivary gland tumor induces HGF production in the damaged tissues. Since the increase of saliva HGF after operation might contribute to wound healing of the oral mucosa [39].

VII. Conclusions

The HGF-Met system performs important roles for constant maintenance and organic formation in the living body, and also participates in the generation and progression of various tumors. We introduced in this review that: 1) HGF was basically localized in luminal cells of fetal, normal salivary glands and salivary gland tumors; 2) saliva contained HGF protein on ELISA; 3) HGF tended to be more intensely expressed in benign salivary gland tumors than in malignant salivary gland tumors; 4) HGF/c-Met signaling was associated with cell scattering and invasion into matrix in the ACC3 adenoid cystic carcinoma cell line; 5) prognosis was poor and correlated to metastasis in high-grade salivary gland carcinomas with c-Met overexpression. These profiles suggest that HGF/c-Met systems may play significant roles in differentiation or tumor progression for salivary gland tumors.

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