Aberrant expression of hSef and Sprouty4 in endometrial adenocarcinoma

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Abstract. Fibroblast growth factor (FGF) 2-mediated signaling of the mitogen-activated protein kinase/RAS/extracellular signal-regulated kinase 1/2 pathway is a critical modulator in angiogenesis and is therefore essential for the pathogenesis of endometrial carcinoma. Human similar expression to FGFs (hSef) and Sprouty4 have each been reported to be negative regulators of FGF signaling. The aim of the present study was to investigate the expression of hSef and Sprouty4 in human endometrial adenocarcinoma. Using immunohistochemistry analysis, the expression of hSef and Sprouty4 was detected in human endometrial adenocarcinomas. Increased hSef expression was found to be present in endometrial adenocarcinomas. In addition, decreased hSef expression was identified in the blood vessels of endometrial adenocarcinoma samples. However, the expression of Sprouty4 was downregulated in human endometrial adenocarcinoma. Aberrant expression of hSef and Sprouty4 are involved in the pathogenesis of human endometrial adenocarcinoma.

Introduction

Fibroblast growth factor (FGF) was one of the first confirmed angiogenesis-associated growth factors (1) that play a critical role in the neovascularization of various solid tumors (2-5). By binding to FGF receptors, FGF2 activates the mitogen-activated protein kinase (MAPK) pathway (6,7). As a characteristic MAPK, extracellular signal-regulated kinase 1/2 (ERK1/2) plays a central role in mitogenic signaling, and activated ERK1/2 triggers a series of responses in target cells, including the proliferation and migration of cells (8).

Similar expression to FGF (hSef) is a negative feedback regulator of FGF-mediated MAPK/ERK1/2 signaling (9-11) and exerts an inhibitory function by affecting the FGF signaling cascade at multiple levels (9). Sprouty genes have been reported to act as an additional negative feedback regulator of FGF signaling (12) by interrupting the interaction of the growth factor receptor-bound protein 2/son of sevenless complex with fibroblast growth factor receptor substrate 2 and Src homology 2 domain-containing tyrosine phosphatase (13,14), preventing Raf activation (15,16).

Aberrant FGF signaling has been identified in human endometrial carcinoma, and this aberrant expression facilitates the growth and invasion of cancer cells (17). In a previous study, hSef was identified as a negative feedback regulator of FGF-mediated MAPK/ERK1/2 signaling in endometrial cancer cells (11). Plasmid-driven hSef expression has also been revealed to significantly downregulate the growth of endometrial carcinoma Ishikawa cells (11). However, the expression pattern of these negative regulators in endometrial cancers has not yet been investigated. In the current study, the aberrant expression of hSef and Sprouty4 was explored in endometrial adenocarcinoma.

Materials and methods

Tissue collection and immunohistochemical analysis. In the present study, immunohistochemical analysis was performed on the normal tissue samples obtained from endometrial biopsies performed on 27 women of reproductive age at Shandong Provincial Hospital Affiliated to Shandong University (Jinan, China), with 15 tissues excised in the proliferative phase and 13 in the secretory phase, and cancer tissues obtained from 28 patients with endometrial adenocarcinoma. The diagnosis of endometrial adenocarcinoma was confirmed by histological examination. None of the participants received any hormonal therapy throughout the 3 months prior to the surgical procedure. The present study was approved by the Institutional Review Board of Shandong Provincial Hospital Affiliated to Shandong University and written informed consent was obtained from all participants.

The immunohistochemical analysis was performed as previously described (18). Briefly, the fresh tissues were washed with phosphate-buffered saline (PBS) and then fixed in 4% paraformaldehyde. Subsequent to dehydration and paraffin-embedding, the samples were cut into 5-µm sections and mounted onto glass...
slides. Deparaffinized and rehydrated sections were incubated with 100 µl 3% H$_2$O$_2$ for 10 min at room temperature and antigen retrieval was performed. Subsequent to blocking, the sections were incubated overnight with goat anti-human hSef primary polyclonal antibody diluted in phosphate-buffered saline (PBS; dilution, 1:100; cat no. AF2275; R&D Systems, Inc., Minneapolis, MN, USA) or rabbit anti-human Sprouty4 primary monoclonal antibody diluted in PBS (dilution, 1:100; cat no. ab103114; Abcam, Cambridge, UK) overnight in a wet chamber at 4˚C. Horseradish peroxidase-conjugated rabbit anti-goat or goat anti-rabbit IgG was used as secondary antibody. Tissue sections incubated with non-immune serum instead of primary antibody were used as a negative control. The experiments were repeated in duplicate or triplicate.

The immunohistochemical score was evaluated as previously described (18). Two sections per sample were evaluated for immunohistochemistry in a blind manner, without any knowledge of the clinical or pathological data.

**Statistical analysis.** Statistical analyses were performed using SPSS software, version 11.5 (SPSS, Inc., Chicago, IL, USA). The data were expressed as the mean ± standard deviation. Differences between two and multiple groups were determined by one-way analysis of variance. *P*<0.05 was considered to indicate a statistically significant difference.

**Results**

**Expression of hSef is upregulated in human endometrial adenocarcinoma.** The endothelial expression of hSef in human endometrial adenocarcinoma was determined using immunohistochemical analysis. The immunostaining of hSef in endometrial adenocarcinoma tissues was strong and confined to the cytoplasm of the cancer cells (Fig. 1A-C and F-H). As shown in Fig. 1, in the normal endometrium, the immunostaining of hSef was present or null and mostly confined to the cytoplasm of epithelial cells (Fig. 1D, E, I and J). However, no significant difference was identified between the endometrial tissue samples in the proliferative and secretory phases (Fig. 2B; *P>*0.05). Compared with normal endometrial tissue, endometrial carcinoma demonstrated increased hSef expression (Fig. 2A, C and D; *P*<0.05). These data suggest the possible role of hSef overexpression in the pathogenesis and development of human endometrial adenocarcinoma.

**Loss of Sprouty4 expression occurs in human endometrial adenocarcinoma.** The expression of Sprouty4, another
negative regulator of FGF signaling, was also detected in human endometrial carcinoma tissue samples. To determine the expression of Sprouty4 in human endometrial adenocarcinoma, immunohistochemical analysis was performed. Endometrial cancer tissue samples obtained from 31 patients with endometrial adenocarcinoma were used in the analysis. Normal endometrial tissue samples obtained from 30 women of reproductive age were used as a control. As demonstrated by the immunohistochemical analysis results, the immunostaining for Sprouty4 was strong and constricted to the cytoplasm of glandular cells in normal endometrial tissues (Fig. 4A, B, E and F). In endometrial adenocarcinoma tissues, the staining for Sprouty4 revealed absent or weak expression, either in the cancer tissues or...
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Figure 4. Immunohistochemical analysis of Sprouty4 expression in endometrial cancer tissues. Decreased expression of Sprouty4 was identified in endometrial cancer tissues compared with normal endometrium. The expression of Sprouty4 in (A and B) normal endometrial and (C and D) endometrial adenocarcinoma tissues, observed at a magnification of x200. The expression of Sprouty4 in (E and F) normal endometrial and (G and H) endometrial adenocarcinoma tissues, observed at a magnification of x400.

Figure 5. Decreased Sprouty4 expression was identified in endometrial cancer tissues compared with normal endometrial tissues. (A) Detailed data of the immunoscore for Sprouty4 expression in endometrial adenocarcinoma and (B) normal endometrial tissues. (A) The surgical-pathological stage of endometrial carcinomas is reported. (C) The immunoscore data expressed as the mean ± standard deviation. Each experiment was performed in duplicate.

The detailed immunostaining scores are reported in Fig. 5. As shown in Fig. 5, the Sprouty4 immunostaining was significantly decreased in endometrial adenocarcinoma tissues ($P<0.01$). These data suggest that the loss of Sprouty4 expression is involved in the pathogenesis and development of human endometrial adenocarcinoma.

Discussion

Abnormal angiogenesis has been reported to participate in the pathogenesis of human endometrial carcinoma, which is one of the most common malignancies of the female genital tract (19). FGF signaling is closely associated with neovascularization in numerous human carcinomas (2-5). In the present study, hSef
expression was observed to be upregulated in endometrial carcinoma tissues, while the expression of Sprouty4 was downregulated in these tissues. However, the blood vessels in these tissue specimens exhibited decreased hSef expression.

As a negative feedback regulator of FGF/MAPK signaling, hSef exerts an inhibitory effect by acting on multiple points of the FGF/RAS/MAPK cascade. hSef has been found to inhibit RAS (20) and FGF-induced phosphorylation of FGF receptors (20), and block the nuclear translocation of activated ERK1/2 (21). hSef expression is induced by FGF signaling (11), and therefore, hSef is a negative feedback mechanism and self-restricting element of FGF signaling. Activated MAPK/ERK1/2 signaling is involved in caryomtosis, gene expression, and cell proliferation and survival, which indicates that FGF/MAPK signaling is significant in the genesis and development of tumors.

FGF-mediated signaling is involved in the pathogenesis and development of endometrial carcinoma (17). Elevated FGF expression and secretion have been reported to be present in endometrial cancers (17). Plasmid-driven overexpression of FGF in endometrial adenocarcinoma cells has been revealed to promote the formation and growth of tumors in nude mice (22), while administration of the FGF antibody attenuates this process (23). Thus, as a negative feedback regulator of FGF signaling, hSef plays an important role in the pathogenesis of carcinoma. The loss of hSef expression may lead to over-activated FGF signaling, and therefore, to the development of endometrial carcinoma.

Loss of hSef expression is considered to be a common mechanism in epithelial neoplasia. Decreased hSef expression was identified in prostate cancer cells and advanced prostate cancer, and siRNA-mediated hSef downregulation enhanced FGF-induced cell migration and invasion in prostate cancer cells (24,25). In in vitro studies, hSef overexpression was found to inhibit cell proliferation, migration and invasive potential in prostate cancer cells (26). Loss of hSef expression was also observed in breast, thyroid and ovarian carcinomas. The downregulation of hSef expression was found to be associated with tumor progression (27). In addition, ectopic hSef expression reduced the growth of breast cancer cells, while inhibition of hSef expression accelerated FGF-induced growth in cervical cancer cells (27).

In the current study, an unexpected increase in hSef expression was observed in endometrial adenocarcinomas. As hSef is inducible by FGF-mediated MAPK signaling, the upregulation of hSef expression may be due to the elevated activity of MAPK. In previous studies, plasmid-driven hSef expression has been demonstrated to reduce FGF2-mediated MAPK/ERK signaling and cell growth in Ishikawa cells, a well-differentiated endometrial adenocarcinoma cell line (11). This indicates that hSef may function as a tumor suppressor in endometrial cancer cells. However, considering the current study, it is hypothesized that hSef may be also involved in other mechanisms. These data indicate the potential roles of hSef in the pathogenesis of endometrial carcinomas.

In the present study, the blood vessels in endometrial cancer tissues were also found to demonstrate decreased hSef expression. The human endometrium undergoes cyclic changes of degradation and reestablishment, which results in angiogenesis being of considerable significance in the physiology and carcinogenesis of the endometrium. Considering the critical role of FGF signaling in the neovascularization of various solid tumors (2-5), the loss of hSef expression may facilitate the activity of FGF signaling, subsequently promoting the neovascularization in endometrial cancers. The potential role of hSef in the neovascularization of tumors requires additional investigation.

The loss of Sprouty expression has been identified in a series of human carcinomas, including prostate cancer (28-30), breast carcinoma (31,32), hepatocellular cancer (33,34), lung carcinoma (35), melanoma (36), colon cancer (37) and liver cancer (38). In addition, the downregulation of Sprouty2 has been identified in endometrial carcinoma tissue (39). In the present study, the loss of Sprouty4 expression was also identified in human endometrial adenocarcinoma tissue samples, indicating the possible role of Sprouty4 in endometrial carcinogenesis.

Overall, to the best of our knowledge, the present study demonstrated that increased hSef expression and decreased Sprouty4 are present in endometrial carcinoma tissue, while decreased hSef expression is observed in the blood vessels of endometrial carcinoma. These data indicate the various roles of negative regulators of FGF signaling in the pathology and genesis of human endometrial carcinoma tissues. The present study provides novel insight into the pathogenesis of endometrial cancer, and suggests a potential therapeutic target for gene-target therapy of the disease.

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