Smad4 mediates malignant behaviors of human ovarian carcinoma cell through the effect on expressions of E-cadherin, plasminogen activator inhibitor-1 and VEGF

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INTRODUCTION

Ovarian cancer is the 4th leading cause of cancer death in women accounting for 6% of all cancers, and ranks 2nd among gynecologic cancers and increases dramatically (American Cancer Society, 2008 & 2009, http://www.cancer.org). It is an aggressive and fatal disease with a mortality rate of ~50%. Ovarian carcinoma is expected to develop in 1 out of 70 women in their life times, over one woman out of 100 will ultimately succumb to complications associated with this disease (1-3). It is scarcely detected until its recurrence in the advanced stages. The pathogenesis and malignance of ovarian cancer at molecular level are still poorly understood (1, 2, 4, 5).

Smad4, initially identified as DPC4 (deleted in pancreatic carcinoma, locus 4), belongs to Smad gene family encoding intracellular signaling mediators of the TGF-β superfamily of cytokines (6). It is a central molecule for TGF-β signaling pathway. The interaction of Smad4 with phosphorylated Smad2/Smad3 makes it possible for TGF-β executing its biological activity (7, 8). TGF-β/Smad4 signaling pathway is a potent regulator of cellular adhesion, motility and extracellular matrix. Reduced or lost expression of Smad4 is frequently observed during cancer progression. Smad4 over-expression in Smad4-deficient SW480 colon carcinoma cells suppressed their tumorigenicity in nude mice (9). Smad4 expression negatively correlated with liver metastasis in colorectal cancer patients (10). Loss of Smad4 contributes to the switch of TGF-β from a tumor-suppressive to a tumor-promoting pathway in pancreatic cancer through its interaction with vimentin, beta-catenin and E-cadherin (11). For non-small-cell lung carcinoma cells, Smad4 could prevent their metastasis by inhibiting tumor angiogenesis by decreasing VEGF and increasing TSP1 expressions at both protein and mRNA levels (12). Down-regulated Smad4 was observed in microdissected specimens from patients suffering with advanced-stage ovarian cancers, which suggested that Smad4 might enhance TGF-β signaling (13). However, is Smad4 really associated with the tumor invasion and metastasis of ovarian cancer? Is Smad4 related with other genes, VEGF, E-cadherin and PAI-I, already known relevant to other cancer progressions?

In current study, to gain insights into the potential Smad4-regulated molecules that contribute to ovarian tumor malignancy, two ovarian cancer cell lines originated from the ascitic fluid of a patient with poorly differentiated ovarian papillary serous cyst-adenocarcinoma, HO-8910 with a low metastasis capacity and HO-8910PM with a high metastasis capacity (1, 2, 11, 12, 14-17), were utilized as our initial experimental subjects. Those two cell lines sharing same genetic back-
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RESULTS

Different expressions of Smad4 in HO-8910 and HO-8910PM cells, restoration of Smad4 in HO-8910PM cell

Smad4 showed higher expression in HO-8910 cell relative to HO-8910PM (Fig. 1a, up plot). Smad4 is distributed in the nuclear and cytoplasm of HO-8910 cell. Western blot analysis was consistent with IHC result. The protein level of Smad4 for HO-8910 cell was about 4-fold of that for HO-8910PM (Fig. 1A, middle and bottom plots).

Translent transfections of Smad4 were performed for HO-8910PM cells with the cell density of $4 \times 10^5$/ml in a 24-well plate inoculated with 5 μg pCMV5-Smad4 plasmid plus 5 μl lipofectamine 2000 in Opti-MEM. pCMV5-Smad4 plasmid contains the coding sequence of human Smad4. In 6 h, the transient HO-8910PM/Smad4 cells were continuously cultured in RPMI1640 media for another 24 h and 48 h. Western results indicated that the protein level of Smad4 was increased significantly in the transfected cell (Fig. 1b). The protein levels of Smad4 in HO-8910PM/Smad4 (pCMV5-Smad4-transfected HO-8910PM) were 8.8-fold and 2.6-fold of that in the HO-8910PM control cell at the time points of 24 h and 48 h, respectively. Finally, 24 h culture was performed for further experiments for HO-8910PM/Smad4 cell. The HO-8910PM/Smad4 cells were obtained by pulling of several transfectants.

Loss of Smad4 increases migration and invasion abilities for HO-8910 cell

Cell scratch-wound assay indicated that more HO-8910PM cells passed through the membrane. The relative cell migration capacity of HO-8910PM cell was ~5-fold of that of HO-8910 (Fig. 2a). A modified Boyden chamber assay was performed to examine the effect of Smad4 on the invasion capacities of HO-8910 and HO-8910PM cells. The HO-8910PM cell number in low chamber was 1.8-fold of that of HO-8910 cell (Fig. 2b). The invasive and migratory capacities of HO-8910PM cell were much higher than HO-8910 cell.

Smad4 restoration reduces migration and invasion capacities for HO-8910PM

HO-8910PM cell motility was reduced following the Smad4 restoration. The relative cell migration capacity of HO-8910PM/Smad4 was comparable with that of HO-8910 (Fig. 2a). The invasion ability of HO-8910PM/Smad4 cell was ~45% of that of HO-8910 and ~26% of that of HO-8910PM (Fig. 2b), respectively.