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Hepatocyte growth factor secreted by ovarian cancer cells stimulates peritoneal implantation via the mesothelial–mesenchymal transition of the peritoneum

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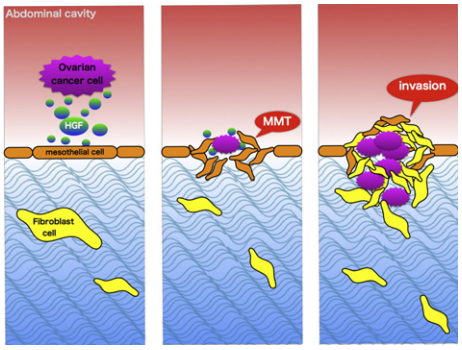
HIGHLIGHTS

- HGF expression status of ovarian cancer significantly correlated with histological subtypes and cancer stages.
- HGF secreted by ovarian cancer cells transforms the mesothelial cells to more suitable state for cancer cell invasion.
- Manipulating the HGF activity affected the degree of dissemination and ascite formation in in vivo ovarian cancer model.

GRAPHICAL ABSTRACT



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ABSTRACT

Objective. A current working model for the metastatic process of ovarian carcinoma suggests that cancer cells are shed from the ovarian tumor into the peritoneal cavity and attach to the layer of mesothelial cells that line the inner surface of the peritoneum, and several studies suggest that hepatocyte growth factor (HGF) plays an important role in the dissemination of ovarian cancer. Our objectives were to evaluate the HGF expression of ovarian cancer using clinical data and assess the effect of HGF secreted from human ovarian cancer cells to human mesothelial cells.

Methods. HGF expression was immunohistochemically evaluated in 165 epithelial ovarian cancer patients arranged as tissue microarrays. HGF expression in four ovarian cancer cell lines was evaluated by using semi-quantitative polymerase chain reaction, Western blotting and enzyme-linked immunosorbent assay. The effect of ovarian cancer cell derived HGF to the human mesothelial cells was assessed by using morphologic analysis, Western blotting and cell invasion assay. The effect of HGF on ovarian cancer metastasis was assessed by using in vivo experimental model.

Results. The clinical data showed a significantly high correlation between the HGF expression and the cancer stage. The in vivo and in vitro experimental models revealed that HGF secreted by ovarian cancer cells induces the mesothelial-to-mesenchymal transition and stimulates the invasion of mesothelial cells. Furthermore, manipulating the HGF activity affected the degree of dissemination and ascite formation.

Conclusions. We demonstrated that HGF secreted by ovarian cancer cells plays an important role in cancer peritoneal implantation.

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1. Introduction

Epithelial ovarian cancer has the highest mortality rate of all gynecologic malignancies diagnosed in women in the United States [1]. The 5-year survival rate of patients with advanced ovarian cancer is under 25% [2]. The lethality of ovarian cancer is mainly attributed to the advanced stage of the disease at the time of initial diagnosis. It typically presents with symptoms such as abdominal bloating, distension, weight loss, and fatigue [3] and approximately 70% of patients present with disease that has spread beyond the ovaries [1], resulting in the majority of patients being diagnosed with advanced disease. A current working model for the metastatic process of ovarian carcinoma suggests that cancer cells are shed from the ovarian tumor into the peritoneal cavity and attach to the layer of mesothelial cells that line the inner surface of the peritoneum. The cancer cells invade the superficial layers of abdominal organs and may then spread to retroperitoneal lymph nodes and the pleural cavity [4,5]. This seeding of the peritoneal cavity is frequently associated with ascite formation and is the most widely recognized characteristic of ovarian carcinomas, particularly serous carcinoma.

Generally, it is believed that the activation of a wide variety of ligands, including fibroblast growth factor (FGF), transforming growth factor (TGF)-beta, epidermal growth factor (EGF), vascular endothelial growth factor (VEGF) and hepatocyte growth factor (HGF), plays an important role in tumorigenesis. The overexpression of c-MET, the receptor for HGF, has been reported in many cancers of epithelial cell origin including ovarian cancer [6,7]. By binding with HGF, c-MET undergoes dimerization and auto-phosphorylation at specific tyrosine residues within the cytoplasmic domain, creating docking sites for intracellular signal transducers that activate the Ras/mitogen-activated protein kinase (MAPK), phosphatidylinositol 3-kinase (PI3K), and the signal transducers and activators of transcription (STAT) signaling pathways [8]. In several epithelial and mesenchymal cancers, high expression of c-Met protein is reported to be an independent prognostic factor associated with an adverse outcome [9–13]. Several studies suggest that HGF plays an important role in the dissemination of ovarian cancer. For instance, HGF is highly expressed in ovarian cancer ascites, inducing the proliferation of cultured ovarian cancer cells [14], or the expression of c-Met is maintained in cultured ovarian surface epithelial cells and its expression level is increased in ovarian cancer [15]. Therefore, c-MET/HGF signaling has been an attractive therapeutic target in ovarian cancer. Few c-MET inhibitors (PF-2341066 and foretinib) have been reported to effectively inhibit ovarian cancer development and metastases in animal models implicating that the c-MET/HGF signaling pathway is a promising target in human ovarian cancer [16]. Sawada et al. reported an 85% inhibition in the number of tumor nodules, tumor weight, and ascites in mice inoculated with ovarian cancer cells which were injected with c-Met siRNA [13]. However, it is still unclear whether or how c-MET/HGF signaling in ovarian cancer cells affects the peritoneal wall.

The peritoneal cavity is lined by a continuous single layer of mesothelial cells, a unique cell type that covers only peritoneal, pleural, and pericardial serosal surfaces [17]. It has been reported that inflammatory mediators and low pH can induce peritoneal mesothelial cells to lose certain epithelial characteristics, and they progressively acquire a fibroblast-like phenotype. This so-called mesothelial-to-mesenchymal transition (MMT) serves as a trigger for peritoneal fibrosis and angiogenesis and is considered to be an important potential therapeutic target in sclerotic peritonitis syndromes [18]. We recently reported that the MMT of peritoneal mesothelial cells was induced by HGF secreted from estradiol-treated endometriosis cells and it plays a crucial role in the peritoneal implantation of human endometriotic cells [19].

In the present study, we evaluated the HGF expression of ovarian cancer using clinical data. In addition, we assessed the effect of HGF secreted from human ovarian cancer cells to human mesothelial cells in order to understand the pathogenesis of peritoneal implantation by ovarian cancer cells using in vivo and in vitro experimental models.

2. Materials and methods

2.1. Tissue samples

Tissue samples and relevant clinical data were obtained from 165 Japanese patients who underwent surgical resection for primary epithelial ovarian cancer at Osaka Medical College. The Institutional Review Board of Osaka Medical College approved this study, and informed consent was obtained from all patients. The specimens were fixed in 10% formalin and embedded in paraffin. Serial sections cut from the paraffin-embedded blocks were used for routine histopathology. Four-μm-thick sections were cut from the tissue microarray block and immunohistochemically analyzed for the expression of HGF.

2.2. Reagents and antibodies

Rabbit polyclonal anti-human Met, rabbit polyclonal anti-human phospho-Met, rabbit polyclonal anti-human E-cadherin, rabbit polyclonal anti-human vimentin and rabbit monoclonal anti-human β-actin antibodies used for immunoblotting and immunohistochemistry were all purchased from Cell Signaling Technology, Inc. (Danvers, MA, USA). Rabbit polyclonal anti-human HGF (ab24865) and rabbit polyclonal anti-human N-cadherin antibodies (ab18203) used for immunoblotting and immunohistochemistry and a goat polyclonal anti-human HGF antibody (ab10679) used as HGF neutralizing antibodies were purchased from Abcam (Cambridge, MA, USA). A rabbit polyclonal anti-human pan-cytokeratin antibody used for immunoblotting and immunohistochemistry was purchased from Thermo Fisher Scientific, Inc. (Waltham, MA, USA). Recombinant mouse HGF was purchased from R&D systems (Minneapolis, MN, USA).

2.3. Cell lines and conditioned media

Human ovarian cancer cell lines A2780, CaOV3, SKOV3 and RMG-1, which were purchased from the American Type Culture Collection (Rockville, MD) in 2013, were utilized in this study. ATCC routinely authenticate their cell lines by Short Tandem Repeat (STR) polymorphism profiling analysis. All experiments were performed at passages <5. Human adult mesothelial cells, MES-F, were purchased from Zen-Bio, Inc. (Research Triangle Park, NC, USA). Ovarian cancer cells were grown in DMEM supplemented with 10% fetal bovine serum (FBS) (Equitech-Bio, Kerrville, TX), and MES-F cells were grown in mesothelial cell growth medium (Zen-Bio) in an atmosphere of 5% CO_2 at 37 °C.

In order to obtain CaOV3 and A2780 cell conditioned medium (CaOV3-CM and A2780-CM), the cells were seeded and cultivated until 60% confluency. The medium was then replaced with DMEM serum-free medium and the supernatants were collected after 48 h of further incubation.

2.4. RNA extraction, reverse transcription and semi-quantitative polymerase chain reaction (RT-PCR)

Total RNA was obtained from the cultured cells using the RNeasy Mini kit (Qiagen, Germantown, MD, USA), and 2 μg of RNA was subsequently reverse transcribed with Superscript II reverse transcriptase (Invitrogen, Carlsbad, CA, USA) using random primers according to the manufacturer's instructions. cDNA was then amplified using Taq DNA polymerase (Roche Diagnostics, Mannheim, Germany). The primers were as follows: *HGF*, forward: 5′-GCTTGCTCCTCCCTTCCTTAC-3′, and reverse: 5′-AGTTTGGTCACCCACATGGT-3′, *GAPDH*, forward: 5′-AGCC ACATCGCTCAGACA-3′ and reverse: 5′-GCCCAATACGACCAAATCC-3′.

2.5. Western blot analysis

A Western blot analysis was performed as previously described [20]. Briefly, total proteins were prepared using Pierce RIPA Buffer (Thermo