PEDF gene therapy in ovarian cancer

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Abstract
Ovarian cancer-associated ascites is generally considered non-beneficial for disease status. However, our group was able to identify PEDF in the acellular fraction of ascites, which shows antiangiogenic and proapoptotic effects on tumor cells. Thus, we propose inducing PEDF gene expression using the Sleeping Beauty transposon (SBT) system may potentially emerge as a new therapeutic tool in ovarian cancer treatment.

Reference
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Pigment Epithelium Derived Factor (PEDF)

PEDF Gene Therapy in Ovarian Cancer Cells

Ovarian cancer-associated ascites is generally considered non-beneficial for disease status. However, our group was able to identify PEDF in the acellular fraction of ascites, which shows antiangiogenic and proapoptotic effects on tumor cells. Thus, we propose inducing PEDF gene expression using the Sleeping Beauty transposon (SBT) system may potentially emerge as a new therapeutic tool in ovarian cancer treatment.

Ascites is generally considered a contributor to disease progression by facilitating ovarian cancer cell dissemination, and by its components such as growth factors, cytokines, chemokines and extracellular matrix components known to be involved in cell proliferation and invasion. Moreover, in vitro studies demonstrated that the acellular fraction of ascites can stimulate epithelial-mesenchymal transition in ovarian cancer cell lines and may contribute to increased transmesothelial ovarian cancer cell invasion. On the other hand, we demonstrated that the acellular fraction of ascites was able to potentize the inhibitory effects of paclitaxel on cell viability in vitro by increasing cell apoptosis via Fas-FasL(igand) pathway and to decrease tumor growth in vivo by reducing tumor angiogenesis, suggesting that ascites also contains potent therapeutic proteins.

Identification of a therapeutic protein in ascites

Using ascites fractionation by gel filtration, cell viability assay to identify active fractions and mass spectrometry to compare the protein contents in active and inactive fractions, we could identify PEDF as the factor responsible for the antiangiogenic and proapoptotic properties of ascites. Regarding the literature about PEDF, it is well described that this protein regulates angiogenesis by negating vascular endothelial growth factor (VEGF) activity. It was thus first studied as anti-cancer agent able to target tumor vasculature. Nevertheless, it is also able to exert a direct anti-tumor role by inhibiting cancer cell proliferation and invasion whilst promoting cancer cell apoptosis.

PEDF level in ovarian cancer cells and serum

Interestingly, studying allelic deletion in early ovarian cancer, Philipps et al. described the loss of PEDF in early ovarian cancer. We thus examined the PEDF level in ovarian cancer patients. We confirmed that PEDF mRNA level was decreased in high grade serous ovarian cancer cells compared to healthy ovarian cells (0.0001+/−0.0002 and 0.02+/−0.04, respectively; p<0.001). Moreover, the circulating level of PEDF is also diminished in ovarian cancer patients compared to healthy patients. Its level in malignant ascites is higher than in ovarian cancer serum, suggesting that cells in tumoral microenvironment are able to secrete PEDF, probably as a defense mechanism. Considering these roles of PEDF, loss of PEDF in ovarian cancer cells could thus participate in disease progression.

Development of a PEDF gene therapy

Two main strategies have already been considered for testing PEDF as a therapeutic agent: the PEDF gene therapy using viral vectors and systemic administration of PEDF proteins or peptides. All these approaches could demonstrate that PEDF was effective as anti-cancer agent in different types of cancer. However, they have not been tested on ovarian cancer and are not usable for clinical application. We thus decided to develop a cost-effective therapeutic strategy to induce and sustain PEDF expression in tumor environment. As proof of concept, we used the SBT system to stably introduce PEDF in the genome of ovarian cancer cells. The SBT is a non-viral vector which combines the advantages of both viral and naked DNA (table 1). Indeed, as a non-viral vector, it is easy to handle and the cost of production is low.

KEYPOINTS

- Ascites from ovarian cancer patients can enhance the inhibitory effect of paclitaxel on ovarian cancer cell viability and tumor growth.
- PEDF is responsible for the effects of ascites on paclitaxel-induced apoptosis in vitro and tumor growth in ovo.
- PEDF expression is decreased in ovarian cancer cells compared to normal ovarian cells.
- PEDF in ascites may be secreted by cells present in the tumor microenvironment.
- PEDF-SBT allows to sustain PEDF secretion in the tumoral microenvironment at an effective concentration to significantly inhibit tumor growth, highlighting its potential use for ovarian cancer gene therapy.
and as viral vector, it can stably introduce the gene of interest in the genome of target cells. The SBT consists of two plasmids, one SB transposase plasmid and one SB transposon plasmid in which the transposon is inserted between two inverted terminal repeat sequences (IRS) recognized by the transposase (fig. 1). The transposase binds the two inverted IRS and catalyzes a cut-and-paste of the transposon from the SB transposon plasmid into the genome. Using PEDF-SBT to transfect ovarian cancer cells, we were able to demonstrate that this system allows to stably increase PEDF mRNA expression in ovarian cancer cells (by about 3500-fold) and PEDF secretion (by 10-fold) compared to non-transfected cells. It thus permitted to sustain PEDF expression/secretion in the tumoral microenvironment at an effective concentration, and to significantly inhibit tumor growth in ovo (by decreasing the tumor size by 2-fold, compared to control ovarian cancer cell-derived tumor after three days of tumor development).

In conclusion, we found that malignant ascites contains high amount of PEDF that can significantly impact tumor growth. The SBT is a suitable system to induce and sustain PEDF expression in tumors, eventually decreasing tumor growth. Thus, it is suggested that PEDF-SBT is a promising tool for ovarian cancer gene therapy.

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