HOXB9 as a potential target gene for overcoming platinum resistance in mucinous ovarian cancer

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Abstract

We performed a series of in vitro and in vivo studies to find out a tailored strategy of inhibiting HOXB9 expression for overcoming platinum resistance in mucinous epithelial ovarian cancer (EOC).

Introduction

In EOC, there are different types of cancers. For example, in serous cancer, peritoneal carcinomatosis is quite common in advanced stages, however, in mucinous cancer, it is so rare even in advanced stage. And, they are so different in terms of not only histopathologic but also moleculogenetic characteristics. Mucinous cancer was shown to have poorer survival outcome than serous cancer, especially when the operation was suboptimal. Maybe it is because mucinous cancer had poorer response to conventional chemotherapy than serous cancer. However, actually we have used the same primary systemic chemotherapy regimen both for mucinous and serous cancers.

HOX genes are family of transcription factors that play key roles in embryonic development. HOX overexpression was reported in several cancers, such as lung, prostate, breast, and ovarian cancers. Normally, various HOX genes are expressed in mullerian duct axis to preserve a high level of developmental plasticity. HOX9 in fallopian tubes, HOX 10 in uterus, HOX 11 in lower segment and cervix and HOX13 in upper vagina. Notably, there is no HOX expression in normal ovarian surface epithelium. But, it is expressed in EOC tissue. There are many studies that showed the specific HOX expression patterns according to histologic types. For example, HOXA11 is expressed specifically in mucinous cancer, but not in serous or endometrioid cancers. Among the HOX gene functions reported in EOC so far are alteration of phenotype, tumor growth, and cell mobilization and spreading.

We performed this experiment to explore tailored strategy for overcoming the platinum resistance using the histology-specific expression pattern of *HOX* genes.

Methods and Materials

Real time RT-PCR, Western blot, MTT assay, and TUNEL assay were used for in vitro experiment. Immunohistochemical staining was performed to evaluate the expression of HOX genes in human and mouse cancer tissue. siRNA and mammalian expression vector with the CMV Promoter (figure 1) were used in order to suppress and overexpress target genes, respectively. Xenograft mouse model was established by inject manipulated cancer cells into Subcutaneous space and intraperitoneal space.

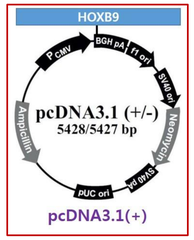


Figure 1. mammalian expression vector with the CMV promoter, **pcDNA™3.1(+)**

Results

HOXA10 and HOXB9 showed exclusively high expression in SKOV-3 and RMUG-S, respectively. Of those 11 HOX genes, we could find out two histology-specific overexpressed HOX genes, HOXA10 in SKOV3 but not in RMUGS. In contrast, HOXB9 in RMUGS but not in SKOV3. When we treated the two cell lines with siRNAs for the overexpressed HOX genes, significant decrease of cell viability was observed only when the corresponding HOX gene was knocked down (Figure 2). When the endogenous HOX genes were knocked down, cell growth markers and apoptotic markers were all changed in the direction of favoring decrease of cell growth and increase of apoptosis. In addition, we conducted migration assay and invasion assay together with western blot for relevant EMT markers. We found that decrease of migration and invasion after the treatment of corresponding siRNA in each cell lines. Here, you can also find out decrease of EMT features as well as the decrease of cancer stem cell markers after the treatment of siRNA. RMUGS shows more resistance to cisplatin compared with SKOV3. SKOV3 showed significant decrease of cell viability after treatment of cisplatin, but no more decrease after additional treatment of siRNA HOXA10. On the contrary, RMUGS initially did not show significant response to cisplatin alone treatment, but there was a significant decrease of cell viability after additional siRNA HOXB9 treatment or siRNA HOXB9 treatment alone (Figure 3). For HOXB9 expression in human cancer patient tissue, patients with platinum resistance had significant higher rate of B9 positivity than those with platinum sensitive tumors.

In vivo mouse xenograft model with SQ injection of tumor cells, we found that tumors from overexpressed HOXB9 cells were significantly heavier and bigger than those from control cells (Figure 4). With intraperitoneal injection of tumor cells, we also found that mice of overexpressed HOXB9 cells had significantly more tumor spreading and ascites production than controls.

Figure 2. Knockdown of cell line specific overexpressed HOX using siRNA

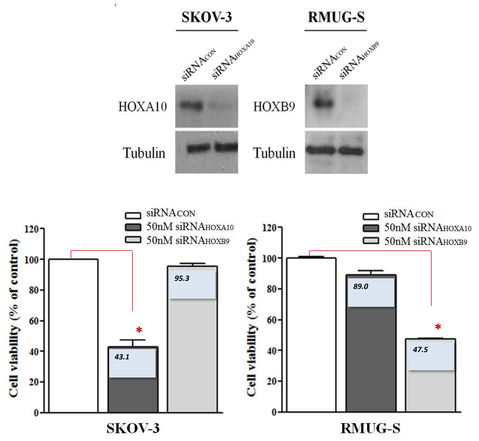
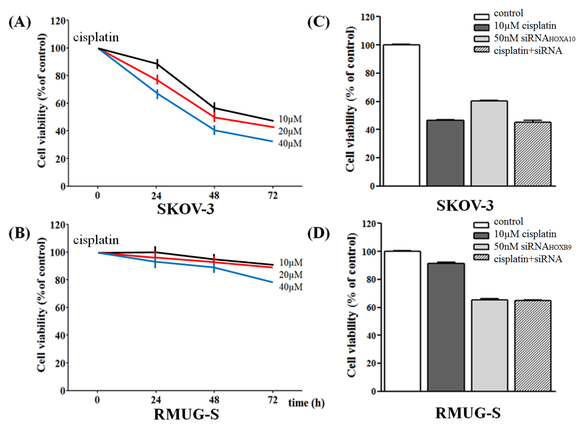


Figure 3. Knockdown of HOXB9 significantly reduces cell viability of RMUGS



Discussion

Regarding cisplatin resistance, we found that SKOV3 cells with overexpressed HOXB9 showed significantly decreased response to cisplatin compared with control cells. PCR results of in vivo tumors harvested from mouse models. From this result, we could suggest that the mechanisms of HOXB9 induced cisplatin resistance includes weakening the cell-to-cell adhesions, enhancing DNA damage repair , and inhibiting apoptosis.

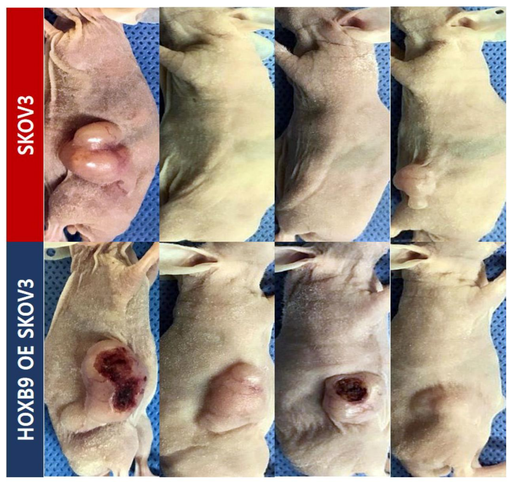
Conclusions

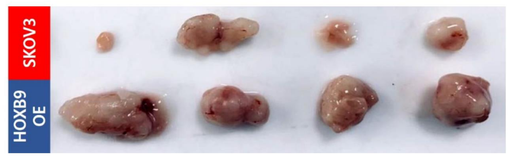
Our findings suggest that platinum-resistance of mucinous ovarian cancer might be defeated by inhibiting HOXB9, which could be a target of tailored strategy for overcoming the resistance to platinum in mucinous EOC.

Future Directions

Association of HOXB9 expression and cancer stem cell could be a next project to find more interesting core mechanisms of HOX gene in chemoresistance in EOC.

Figure 4. Overexpression of HOXB9 promotes tumor growth in vivo





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