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Epigenetic Changes of Brca1 and S100a4 Genes During Ovarian Cancer Progression

Medical Science

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ABSTRACT

Hypoxia is known to play important roles in the development and progression of tumors. We previously demonstrated that BRCA1 and S100A4, critical molecules for tumor initiation and metastasis, were differential expressed in ovarian cancer cells. Therefore, we examined the mechanisms of the BRCA1 and S100A4 expression in ovarian carcinoma cells, with particular attention paid to the effects of hypoxia. The expression levels of S100A4 and BRCA1 were found to correlate with the invasiveness of ovarian carcinoma cells in vitro and in vivo, and the BRCA1 and S100A4 expression was associated with epigenetic changes in the regulatory regions of BRCA1 or S100A4 cording genes in ovarian carcinoma cell lines and tissues. The expression of S100A4 was increased under hypoxia and was associated with elevated invasiveness. In addition, exposure to hypoxia reduced the methylation of hypoxia-response elements (HRE) of the S100A4 gene in a time-dependent fashion, in association with the increased binding of HIF-1 α to a methylation-free HRE in ovarian carcinoma cells. These findings indicate that hypoxia-induced hypomethylation plays an essential role in S100A4 over expression and the epigenetic transformation of ovarian carcinoma cells into the ''metastatic phenotype''.

Introduction

Breast cancer type 1 susceptibility protein (BRCA1) is expressed in the cells of breast and other tissue, where it helps repair damaged DNA, or destroy cells if DNA cannot be repaired. If BRCA1 itself is damaged, damaged DNA is not repaired properly and this increases risks for cancers. S100A4 (also known as mts1, pEL-98, 18A2, p9Ka, CAPL, calvasculin and FSPl) belongs to the S100 family of calcium binding proteins.1,2,3 The indication that the expression of *BRCA1* and *S100A4* gene in different tumor cells is strongly correlated with tumor progression and aggressive metastatic phenotype.4

Later on, a potent metastasis regulating role of S100A4 was demonstrated convincingly by use of various approaches and animal models in different laboratories and the data have been summarized in several reviews.1,3 More recent data associate the up-regulation of S100A4 both in tumor and stroma cells with poor prognosis and survival of patients with different types of cancer.5-9 Various immune cells, macrophages, neutrophils, certain types of lymphocytes, dendritic and mast cells and human endothelial cells express and release S100A4 into the extracellular space.10 Specifically, we focus on the mammalian methylation and demethylation activities in the ovarian carcinoma progression, and the problem of transmitting epigenetic information across tumor cell divisions and generations.

The epigenetic changes of tumor suppressor gene, BRCA1 in ovarian cancer.

The epigenetic changes regulate gene expression. Although the importance of DNA methylation in the transcriptional silencing of tumor suppressor genes is recognized, genome-wide hypomethylation has also been reported in human malignancy and has been associated with genetic instabilitv.11,12 The epigenetic changes of tumor suppressor gene, BRCA1 and hypomethylation of metastasis associated gene, S100A1 are focused to clarify biological characteristics of ovarian cancer. BRCAl is a tumor suppressor, which plays a crucial role in the repair of DNA damages and its abnormality is responsible for hereditary ovarian cancer syndrome.13,14,15,16 To clarify possible involvement of BRCRl in the development of sporadic ovarian neoplasms, we analyzed the BRCAl expression, loss of heterozygosity (LOH) and it`s promoter methylation in normal ovarian surface epithelium and 119 epithelial ovarian tumors.17 The decreased-expression of BRCA l was observed in carcinoma, the differential expression of BRCA1, which correlated protein along with epigenetic changes, might plays a key role in development of sporadic ovarian carcinomas.17 From prognostic analysis, ovarian carcinoma patients negative for BRCA l expression showed favorable prognosis. To address if BRCAl expression plays a role in the chemotherapeutic response, we analyzed the effect of BRCAl suppression by siRNA on the sensitivity to cisplatin and paclitaxel in ovarian cancer cells. We found the reduced expression of BRCAl enhances the cisplatin sensitivity and apoptosis.17 Accordingly expression of BRCAl might be an important biomarker for cisplatin resistance in ovarian carcinoma.

The epigenetic changes of metastasis associated gene, S100A1 in ovarian cancer.

The S100A4 protein, which belongs to calcium binding S100 protein family, has reportedly been associated with cell motility and invasion.3,4 The hypoxia reportedly attenuates the "metastatic phenotypes" of ovarian carcinoma cells and found the increased expression of S100A4 under hypoxia.18,19,20 We investigated expression of S100A4 and subcellular localization in 113 epithelial ovarian neoplasms and analyzed its prognostic significance in patients with ovarian carcinoma. Pathological analysis showed that both cytoplasmic and nuclear expressions of S100A4 were significantly stronger in carcinomas than those in benign and borderline tumors. Ovarian carcinoma patients with strong nuclear S100A4 expression showed a significantly shorter survival than those without. Moreover, the in vivo analysis showed that the nuclear expression of S100A4 is involved in the aggressive behavior of ovarian cancer cells. Bisulfite sequence experiment demonstrated that hypomethylation of S100A4-encording gene was associated with over expression of S100A4.21,22 Since hypoxia increased ovarian cancer invasiveness with increased S100A4 expression, we examined the change of methylation status of S100A4 under hypoxia. Hypoxia increases hypomethylation of S100A4 1st intron and increased bindings between HIF-1α and hypomethylated HREs in S100A4 1st intron.21,22 From our findings up-regulation of S100A4 expression was associated with hypomethylation, along with increased the malignancy during the ovarian cancer progression.

Discussion

Level of BRCA1 expression is also relevant to ovarian cancer treatment. Patients having sporadic ovarian cancer who were treated with platinum drugs had longer median survival times if their BRCA1 expression was low compared to patients with higher BRCA1 expression. Our previous study showed that the nuclear expression of S100A4 was an independent prognostic factor in patients with ovarian cancer.21,22 In addition, the recent report showed that the nuclear expression of S100A4 in combination with nuclear HIF-1α protein is a marker of poor prognosis. Accordingly, the presence of hypoxic conditions might upregulate S100A4 expression, producing an unfavorable prognosis. Although S100A4 was first identified as a cytoplasmic protein, its translocation between the cytoplasm and the nucleus has been reported in human cells.23,24 The nuclear expression of S100A4 has been reported to be implicated in the

regulation of gene transcription either through direct DNA binding or its interaction with other DNA-binding proteins.23,24

Our findings suggest that the nuclear expression of S100A4 combined with HIF-1α is an important biological marker and could be a molecular target for ovarian cancer treatment. The upregulation of S100A4 expression was associated with hypomethylation, along with increased malignancy during ovarian cancer progression. The hypoxia-induced hypomethylation plays an important role in gene expression during ovarian cancer progression.

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