Epigenetic changes of pro-oncogenic genes during ovarian cancer progression

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Abstract

Hypoxia is known to play important roles in the development and progression of tumors. We previously demonstrated that breast cancer susceptibility gene 1 (BRCA1) and S100 calcium-binding protein A4 (S100A4), critical molecules for tumor initiation and metastasis, were differential expressed in human ovarian cancer. Therefore, we examined the molecular mechanisms of the BRCA1 and S100A4 expression in ovarian carcinoma, with particular attention paid to the effects of hypoxia. The expression of S100A4 and BRCA1 was found to be involved in the migration and invasiveness of ovarian cancer 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 tissues. Expression of S100A4 is induced in hypoxic environment and is involved in the invasive capacity of ovarian cancer cells. 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 hypoxia inducible factor-1 alpha (HIF-1α) 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 cells of mammary gland and other gynecological tissues, where it helps repair damaged DNA, or if DNA is not repaired, the cell will either die or be transformed. BRCA1 itself is therefore damaged, damaged DNA is not repaired properly and this increases risks for cancers. S100 calcium-binding protein A4 (S100A4) (also known as mts1, pEL-98, 18A2, p9ka, CAPL, calvsulin and FSPl) belongs to the S100 family of calcium binding proteins [1-3]. The indication that the expression of BRCA1 and S100A4 gene in different malignant 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 gene modified animal models in individual 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 malignant tumor [5-9]. Various immune cells, macrophages, neutrophils, certain types of lymphocytes, dendritic and mast cells and human endothelial cells express and release S100A4 molecule into the extracellular space [10]. Specifically, we focus on the mammalian methylation and demethylation activities in human 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 significantly regulate gene expression. Although recent findings demonstrated the importance of DNA methylation in the transcriptional silencing of tumor suppressor genes, genome-wide hypomethylation has also been reported in human malignancy and has been associated with genetic instability [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. BRCA1 reportedly plays as tumor suppressor, which regulates a crucial role in the repair of DNA damages and its abnormality is responsible for hereditary ovarian cancer syndrome [13-16]. To clarify possible physiological involvement of BRCAI in the development of sporadic ovarian neoplasms, we analyzed the BRCAI expression, loss of heterozygozity (LOH) and its promoter methylation in normal human ovarian surface epithelium and 119 human epithelial ovarian tumors [17]. The decreased expression of BRCA1 was observed in malignant tumors, the differential expression of BRCA1, which is depended on epigenetic changes, might plays a key role in development of sporadic ovarian carcinomas [17]. From prognostic analysis, patients with ovarian carcinoma negative for BRCA1 expression showed favorable prognosis. To address if BRCA1 expression plays a role in the chemotherapeutic response, we analyzed the effect of BRCA1 suppression by siRNA on the sensitivity to cisplatin and paclitaxel in ovarian cancer. We found the reduced expression of BRCAI results in enhance of the cisplatin sensitivity.

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and apoptosis [17]. Accordingly expression of BRCA1 might be an important biomarker for cisplatin resistance ovarian carcinoma.

The epigenetic changes of metastasis associated gene, S100A4 in ovarian cancer

The S100A4 protein, which belongs to calcium binding S100 protein family, has reportedly 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 [18-20]. 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 demonstrated that both cytoplasmic and nuclear expressions of S100A4 were significantly stronger in carcinomas than those in benign and borderline tumors. Patients with ovarian cancer that are positive for nuclear S100A4 have a poorer prognosis than patients with negative nuclear S100A4. Moreover, the in vivo experiments showed that the nuclear expression of S100A4 is involved in the aggressive behavior of ovarian cancer cells. Bisulfite sequence experiments demonstrated that hypomethylation of S100A4-encoding gene resulted in 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 hypoxic environment. Hypoxia increases hypomethylation of S100A4 1st intron and enhanced the binding activity between hypoxia inducible factor-1 alpha (HIF-1α) and hypomethylated hypoxia-response elements (HREs) in S100A4 1st intron [21,22]. From our findings, the enhanced 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 clinical treatment for ovarian cancer. Patients with 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 clinical studies 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 may be a marker of poor prognosis. Accordingly, the presence of hypoxic conditions might upregulate expression of S100A4, 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 expression of nuclear S100A4 has been reported to be implicated in the regulation of gene transcription through direct association with DNA by its interaction with other DNA-binding proteins [23-25].

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 enhance of S100A4 expression was associated with hypomethylation in S100A4 cording region of DNA, 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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References


