

Epigenetic Changes Of Oncogenic And Anti-Oncogenic Factors Encoding Gene During Gynaecological Tumour Progression

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Abstract: Hypoxia is known to play key roles in the development and progression of tumours. We previously demonstrated that breast cancer type 1 susceptibility protein (BRCA1) and S100 calcium-binding protein A4 (S100A4), critical molecules for tumour initiation and metastasis, were differentially expressed in ovarian cancer. 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 BRCA1 and S100A4 were found to correlate with the invasiveness of ovarian carcinoma cells *in vitro* and *in vivo*, and the expression of BRCA1 and S100A4 gene was associated with epigenetic changes in the regulatory regions of BRCA1 and S100A4 coding genes in ovarian carcinoma cell and tissues. The expression of S100A4 was increased under hypoxia, and then 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 enhancing binding activity of hypoxia inducible factor-1 (HIF-1) 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'.

Keywords: S100A4, BRCA1, Hypoxia, hypomethylation, Ovarian carcinoma

I. Introduction

Breast cancer type 1 susceptibility protein (BRCA1) and BRCA2 are normally expressed in the cells of breast and other tissue, where they help repair damaged DNA, or destroy cells if DNA cannot be repaired. If BRCA1 or BRCA2 itself is damaged by a BRCA mutation, damaged DNA is not repaired properly, and this increases the risk for breast cancer. S100 calcium-binding protein A4 (S100A4) belongs to the S100 family of calcium binding proteins¹⁻³. S100A4 may function in motility, invasion, and tubulin polymerization. The indication that the expression of BRCA1 and S100A4 gene in different malignant tumour cells is strongly correlated with tumour progression and aggressive metastatic phenotypes⁴.

Later on, a potent metastasis regulating role of S100A4 was demonstrated convincingly by use of various approaches and animal models in different research facilities^{1,3}. More recent data associates the up-regulation of S100A4 both in ovarian tumour, and stroma cells with poor prognosis and survival of patients with different types of cancer⁵⁻⁹. Various immune cells, macrophages, neutrophils, certain types of lymphocytes, dendritic and mast cells and human endothelial cells express and release S100A4 into the extracellular space¹⁰. Specifically, we focus on the mammalian methylation and demethylation activities in the ovarian carcinoma progression, and the problem of transmitting epigenetic information across tumour cell divisions and generations. In this report, we will discuss the emerging importance of the epigenetic alterations of BRCA1 and S100A4 genes in the management of gynaecological tumours, ovarian cancer.

II. The Epigenetic Changes Of Anti-Oncogenic Factor BRCA1 Gene In Gynaecological Tumours

The epigenetic changes regulate gene expression. Although the physiological significance of DNA methylation in the transcriptional silencing of anti-oncogenic factor coding gene is recognized, 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 is anti-oncogenic factor, which plays a crucial role in the repair of DNA damages and its abnormality is responsible for hereditary ovarian cancer syndrome ¹³⁻¹⁶. To clarify possible involvement of BRCA1 in the development of sporadic ovarian neoplasms, we analyzed the BRCA1 expression, loss of heterozygosity (LOH) and its promoter methylation in normal ovarian surface epithelium and 119 epithelial ovarian tumours ¹⁷. The lowered expression of BRCA1 was normally observed in carcinoma, the differential expression of BRCA1, which correlated protein along with epigenetic changes, might play a key role in development of sporadic ovarian carcinomas ¹⁷. From prognostic analysis, patients with ovarian carcinoma negative for BRCA1 expression showed favorable prognosis. If anything, the cisplatin sensitivity and apoptosis would be observed among patients with ovarian carcinoma negative for BRCA1 expression ¹⁷. According expression of BRCA1 might be an important biomarker for cisplatin resistance in ovarian carcinoma.

III. The Epigenetic Changes Of Metastasis Associated Factor S100A4 Gene In Gynaecological Tumours

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 enhancing 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 showed that both cytoplasmic and nuclear expressions of S100A4 were significantly stronger in carcinomas than those in benign and borderline tumours. Patients with ovarian carcinoma, which markedly expressed nuclear S100A4, showed a significantly shorter survival than lowered expression of nuclear S100A4. Moreover, the *in vivo* analysis demonstrated that the nuclear expression of S100A4 is involved in the aggressive behavior of ovarian cancer cells. Bisulfite sequence experiment demonstrated that hypomethylation of *S100A4* encoding gene was associated with over expression of S100A4 ^{21,22}. Since hypoxia increased ovarian cancer invasiveness with enhance S100A4 expression, we examined the change of methylation status of S100A4 under hypoxia. Hypoxia increases hypomethylation of 1st intron of *S100A4* gene and increased bindings between hypoxia inducible factor-1 (HIF-1) and hypomethylated hypoxia response elements (HREs) in 1st intron of *S100A4* gene ^{21,22}. On the other hand, in case of myometrium, certain mesenchymal tumour cells that express caveolins, which are a family of integral membrane proteins, have been shown to be more aggressive and metastatic, because of a potential for anchorage-independent growth. From our findings up-regulation of S100A4 and/or integral membrane proteins expression might be associated with hypomethylation, along with increased the malignancy during the gynaecological tumour progression.

IV. Discussion

Expression level of BRCA1 is also relevant to clinical treatment against ovarian cancer. Patients with lowered BRCA1 expressing sporadic ovarian cancer, had longer median survival times under the treatment with platinum drugs in comparison 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}. Further report showed that the expression of nuclear S100A4 in combination with nuclear HIF-1 α is a potential biomarker of poor prognosis. Accordingly, the presence of hypoxic conditions might upregulate S100A4 expression, producing an unfavorable prognosis. The translocation of S100A4 between the cytoplasm and the nucleus has been reported in human normal, mesenchymal tumour and malignant tumour cells ^{23,24}. The expression of nuclear 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 ^{23,24,25}. The upregulation of S100A4 expression was associated with hypomethylation, along with increased malignancy during gynaecological tumours; ovarian cancer and mesenchymal tumour progression ²⁶. Research findings suggest that the nuclear expression of S100A4 combined with HIF-1 α may be a significant biomarker and could be a molecular target for clinical treatment against ovarian cancer.

The significant activity of poly (ADP-ribose) polymerase (PARP) inhibitors in the treatment of germline *BRCA* epigenetic character-associated ovarian carcinoma, which represents ~15% of high-grade serous cases, has recently led to European Medicines Agency and food and drug administration approval of olaparib. Further research addressing the optimal material for *BRCA* and S100A4 epigenetic analyses in gynaecological tumours; ovarian cancer, uterine mesenchymal tumours is needed.

V. Conclusion

Recent research report showed that the upregulation of S100A4 expression was associated with hypomethylation, along with increased malignancy during gynaecological tumours; especially ovarian cancer

progression. We also found that exposure to hypoxia increased the hypomethylation of the first intron of S100A4 and increased the binding of HIF-1 α in gynaecological tumours. These results indicate that hypoxia-induced hypomethylation plays an important role in gene overexpression during gynaecological tumour progression.

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