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The Expression and Clinical Significance of Sema4A in Triple Negative Breast Cancer

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Abstract: ***Objective:*** To explore the expression and clinical significance of Sema4A in triple-negative breast cancer. ***Methods:*** Eighty patients with invasive ductal carcinoma of the breast and 40 normal tissues adjacent to cancer were selected. Immunohistochemical methods were used to detect the expression of Sema4A in breast cancer and normal tissues adjacent to cancer, and its relationship with breast cancer clinicopathological features and prognosis. ***Results:*** The expression of Sema4a in the serum of BCA patients was significantly higher than that of healthy controls. In addition, the expression of sema4a in BCA cells and in the cell supernatants was also up-regulated under hypoxia. ***Conclusion:*** Exogenous Sema4A can protect BCA cells from hypoxia-induced cytotoxicity, inhibit cell apoptosis and promote cell proliferation.

Keywords: Sema4A; Triple Negative Breast Cancer; Hypoxia-induced cytotoxicity

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1 Introduction

BCA is one of the most common malignant tumors in women, accounting for 25% of cancer cases and 15% of cancer-related mortality. BCA is a complex heterogeneous cell disease, and its diagnosis and treatment face huge challenges.

Breast cancer is a multifactorial disease, and many risk factors cannot be changed. For example, pre-menopausal and postmenopausal reproductive factors will expose breast tissue due to increased estrogen and progesterone, increasing the risk of cancer. As a member of the signaling family, Sema4a can be expressed as an extracellular ligand. Sema4a is regulated by breast cancer cell hypoxia. The regulation of BCA cell activity indicates that Sema4a is a potential target for BCA therapy, which can help in the diagnosis and treatment of breast cancer[1].

Sema4A is a member of the signal transduction family and exists in a transmembrane form. The extracellular form can be cleaved and expressed as a soluble ligand. Sema4A is involved in the development of tissues and organs and is expressed in the brain, lungs, kidneys, testes and spleen of adults. Studies have shown that Sema4A can participate in the pathological process of various diseases by affecting the activity of T-cells and macrophages (viral bronchitis, asthma and retinal degenerative diseases)[2].

In this paper, by clarifying the regulating effect of Sema4A on BCA cell activites, the relationship between Sema4A and hypoxia and whether Sema4A is involved in the regulation of BCA cell activities were explored, hereby reported as follow.

2 Materials and Methods

2.1 Study Subjects

From September 2018 to October 2019, BCA and surrounding tissues (5 cases) were collected from the hospital, and serum samples were collected in the hospital from September 2018 to October 2019. All patients signed the informed consent form for MDA-MB231 and MCF-7 cells. Mcc-7 cells were purchased from ATCC, Manassas, Virginia, USA,

and cultured in dmemrpmi1640 medium and 10% inactivated serum.

2.2 Methods

(1) RT-qPCR and Western-blot were used to detect the expression of Sema4A in BCA tissues. RT-qPCR and Western-blot were used to detect the expression of Sema4A in normal cells and BCA tissues.

(2) Enzyme-linked immunosorbent assay (ELSA) is used to detect the expression of 4a in the serum of BCA patients and normal controls.

(3) RT-qPCR and Western blot were used to detect the expression of Sema4A in BCA cells. Then ELISA was used to detect the expression of Sema4A in the supernatant of BCA cells.

(4) HIF-1a regulates the expression of Sema4A in BCA cells. Under hypoxic conditions, sRNA silences the expression of HIF-1a and hf-2a. RT-qPCR detects the expression of Sema4A in BCA cells.

(5) Chromatin immunoprecipitation method was used to detect the binding of HIF-1a and Sema4A.

2.3 Results Assessment

The experiment was repeated more than 3 times, and the two groups of independent samples were compared and tested. The test is used to compare two independent samples. $P<0.05$ was considered statistically significant.

2.4 Statistical Processing

SPSS 16.0 was used to perform statistical analysis.

3 Results

3.1 Increased Expression of Sema4A in Breast Cancer

In order to clarify the role of Sema4A in BCA, the expression of Sema4A in BCA and non-cancerous tissues were first analyzed. The results showed that the expression of Sema4A at the mRNA level (Figure 1A) and protein level (Figure 1B) in breast cancer tissues was higher than that of the corresponding normal tissues. According to reports, Sema4A is degraded and released into cells, where it can act through receptors. In this study, the ELSA method was used to detect the expression of Sema4A in the serum of BCA patients. The results showed that the expression of Sema4A in the serum of BCA patients was significantly higher than that of healthy controls.

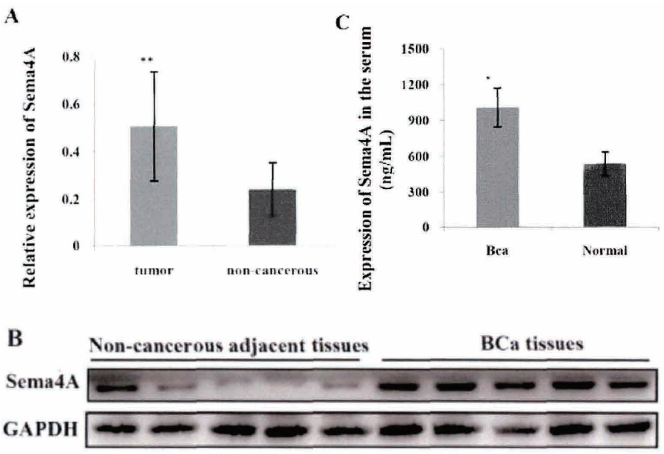

Figure 1. Increased expression of Sema4A in breast cancer.

In Figure 1, the expression of Sema4A in breast cancer was detected by qPCR and Western blot. ELISA method was used to determine the serum 4a level of BCA patients (Figure. 1C). (*) $P<0.05$, *$P<0.05$).

3.2 Hypoxia(1% and 0.2%02) can Induce the Up-regulation of Expression of Sema4A in B0a Cells

Taking into account the high expression of Sema4A in BCA cells and the important role of hypoxia in BCA, MDA-MB-231 and MCF-7 cells were used to clarify the relationship between hypoxia and Sema4A. A model was used to study the relationship between hypoxia and BCA. The results showed that hypoxia induced the up-regulation of Sema4A expression in BCA cells (Figure 2A, B), and the level of Sema4A in the cell supernatant was also up-regulated (Figure 2C, D).

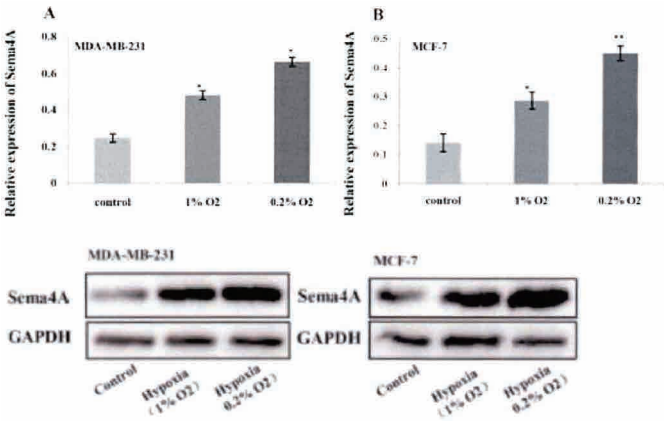

Figure 2. Expression of Sema4A is regulated by HIF-1a

4 Discussion

BCA is the most common malignant tumor in women. Cell testing is complicated by high morbidity and mortality. As a member of the signal transduction family, Sema4A exists in a transmembrane form. Sema4A contains 761 amino acids and has a

molecular weight of 150 kda. It is composed of NH2 terminal signal peptide, SEMA domain, C2 Ig type region, hydrophobic transmembrane region and cytoplasmic tail. Currently, there is no accurate information about the possible phosphorylation sites of the short cytoplasmic tail of Sema4A, and the crystal structure of Sema4A has not been clearly defined. It was found that human and mouse Sema4A homologues have 82% amino acid homology[3].

Studies have shown that this molecule can affect the activity of T-cells and macrophages, and is involved in the pathogenesis of viral bronchitis, asthma and retinal degeneration diseases. The expression of Sema4A in synovial tissue and synovial fluid is significantly increased, and its expression is up-regulated by lipopolysaccharide; exogenous Sema4A stimulates synovial cells and macrophages to significantly up-regulate the expression and secretion of inflammatory factors in cells, Sema4A acts on plexin1 receptor[4].

This paper tested the expression level of Sema4A in peripheral normal tissues and BCA tissues. The results showed that the expression of Sema4A in BCA tissues was higher than that in normal tissues. The results showed that the expression of Sema4A in the serum of BCA patients was significantly higher than that of healthy controls. It suggests that Sema4A may be involved in the progression of BCA.

Hypoxic conditions can regulate the expression of Sema4A in BCA cells, and can be used as a direct downstream regulation target gene of hf-1a transcription factor to regulate the activity of BCA cells. Hypoxia (1% and 0.2% O_2) can induce BCA cells to express Sema4A, and the level of Sema4A in the cell supernatant will also increase after hypoxia. The effect of HIF-1a and HF2a knockout on the expression of Sema4A in BCa cells was analyzed.

Hif-1a instead of hif-2a can inhibit the basic expression of Sema4A in MDA-MB-231 and MCF-7 cells. Hif-1a may reduce the production of Sema4A due to hypoxia in BCA cells, but hif-2a cannot.

The expression of Sema4A in the supernatant of BCA cells was analyzed. Consistent with the changes in the expression of endogenous Sema4A in BCA cells, soluble Sema4A increased significantly after hypoxia (1% O_2) and the up-regulation of HIF-1a silent antagonist. By studying the effect of Sema4A on the hypoxic activity of BCA cells, it was found that the phosphorylation levels of Akt and STAT3 in MDA-MB-231 and MCF-7 cells were significantly increased. The results showed that hypoxia treatment can significantly increase the production of VDA and the phosphorylation of Akt and STAT3 in MDA-MB-231 and MCF-7 cells. However, the silent Sema4A can alleviate the above-mentioned increase caused by hypoxia. The effect of recombinant human Sema4A (rhsema4a) on the expression of v-egf under hypoxic conditions has been studied, and it has been found that recombinant human sema 4a can increase the production of endothelial growth factor and promote the phosphorylation of frk, Akt and Stat3. In other words, the silent Sema4A can inhibit the proliferation of BCA cells and induce tumor cell apoptosis under hypoxic conditions. In other words, the expression of silent Sema4A can significantly inhibit the proliferation of BCA cells, and the inhibitory effect is more obvious under hypoxic conditions.

The effects of Sema4A on cell apoptosis under hypoxia (0.2% O_2). Annexin V data show that under normal circumstances, silma4a silencing does not significantly affect cell apoptosis. However, Annexin V data show that under normal circumstances, silencing Sema4A does not significantly affect cell apoptosis. However, hypoxia can also induce apoptosis, but when Sema4A gene knockout increases significantly, rhsema4a can protect BCA cells from hypoxia-induced apoptosis. To explore the role of soluble Sema4A in BCA cells, RH- Sema4A can be used to detect the effects on the biological activity of BCA cells and promote the proliferation of BCA cells.

In conclusion, Sema4A is closely related to tumors. In BCA, Sema4A regulates the movement of BCA cells by interacting with the receptors. However, the exact role and pathological mechanism of Sema4A in BCA are still unclear. Hypoxia plays a very important role in the pathogenesis of BCA.

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