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Doxorubicin effect is enhanced by sphingosine-1-phosphate signaling antagonist in breast cancer

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ABSTRACT

Background: Doxorubicin is one of the most commonly used chemotherapeutic drugs for breast cancer; however, its use is limited by drug resistance and side effects. We hypothesized that adding FTY720, a sphingosine-1-phosphate (S1P) receptor functional antagonist, to doxorubicin would potentiate its effects by suppression of drug-induced inflammation.

Materials and Methods: The Cancer Genome Atlas, Gene Expression Omnibus data sets, and National Cancer Institute-60 panel were used for gene expressions and gene set enrichment analysis. E0771 syngeneic mammary tumor cells were used. OB/OB mice fed with western high-fat diet were used as an obesity model.

Results: STAT3 expression was significantly increased after doxorubicin treatment in human breast cancer that implicates that doxorubicin evokes inflammation. Expression of sphingosine kinase 1, the enzyme that produces S1P and links inflammation and cancer,

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Sphingosine-1-phosphate
Mouse model

tended to be higher in doxorubicin-resistant human cancer and cell lines. In a murine breast cancer model, sphingosine kinase 1, S1P receptor 1, interleukin 6, and STAT3 were overexpressed in the doxorubicin-treated group, whereas all of them were significantly suppressed with addition of FTY720. Combination therapy synergistically suppressed cancer growth both *in vitro* and *in vivo*. Furthermore, combination therapy showed higher efficacy in an obesity breast cancer model, where high body mass index demonstrated trends toward worse disease-free and overall survival, and high-serum S1P levels in human patients and volunteers.

Conclusions: We found that FTY720 enhanced the efficacy of doxorubicin by suppression of drug-induced inflammation, and combination therapy showed stronger effect in obesity-related breast cancer.

Introduction

Doxorubicin, an anthracycline antibiotic, is one of the most commonly used chemotherapeutic agents for breast cancer. It is regarded as one of the most potent chemotherapeutic drugs[1,2] and its response rate for metastatic lesions is approximately 25%-40%.[3] Despite its therapeutic effects, there are limitations to its use; one of them is drug resistance, and another is cardiotoxicity, which is a crucial anthracycline specific side effect.[4] Cumulative anthracycline dose results in doxorubicin-induced cardiotoxicity.[5] Therefore, doxorubicin dose reduction in combination with another compound is expected to be an important strategy to overcome these limitations.

Sphingosine-1-phosphate (S1P) is a bioactive lipid mediator generated by sphingosine kinases, SphK1, and SphK2.[6] Activated SphK1 increases intracellular S1P, which is secreted out of the cell[7] and acts extracellularly by binding to and signaling through S1P receptors (S1PRs) in autocrine and/or paracrine manners as "inside-out" signaling.[8] This regulates cell proliferation, invasion, and angiogenesis in cancer cells[6,8,9] as well as in patients.[10] We have established methods to measure the levels of S1P in tumor interstitial fluid[11] and lymphatic fluid[12] and demonstrated that S1P is associated with lymphangiogenesis and lymph node metastasis both in an animal model[13,14] and in patients,[15] which suggests that S1P in the tumor microenvironment worsens cancer progression.[9,13,16-18] Furthermore, we recently found that S1P signaling plays an even more important role in metastatic triple-negative breast cancers.[19] Given that S1P signaling is related to cancer malignant potential and progression, we hypothesized that S1P may contribute to doxorubicin resistance. FTY720, a functional antagonist of S1PRs, is clinically used for the treatment of multiple sclerosis; thus, its phase 1 trials are completed.[8] FTY720 also demonstrates proven efficacy in multiple *in vitro* and *in vivo* cancer models, suggesting a potential therapeutic role in cancer patients.[20]

A feature of obesity-related cancer is low-grade inflammation which increases cancer malignant potential.[21,22] S1P is known to play significant roles in inflammation,[23-26] and we recently reported that S1P links inflammation and cancer progression, stimulates tumor-associated inflammation, and increases cytokine levels.[20,27] We hypothesized that the addition of FTY720, which blocks S1P signaling and thus suppresses the effect of obesity-mediated inflammation, should enhance the anticancer effects of doxorubicin in this setting. Therefore, we investigated the efficacy of this combination therapy in obesity-related breast cancer.

Materials and Methods

Gene expression before and after doxorubicin treatment

DNA microarray gene expression data of humans before and after doxorubicin treatment were obtained through the Gene Expression Omnibus (GEO) database (GSE28844).[28] Of 33 participants, 17 cases that were not treated with doxorubicin were excluded from our analysis. Of the remaining 16 cases, 12 cases had both pretreatment and posttreatment gene expression data. Two cases were classified as good response, five cases were midresponse, and five cases were bad response using Miller and Payne grades.[28]

Human samples

Eighty-six cases of The Cancer Genome Atlas (TCGA) contributor from the Roswell Park Cancer Institute had body mass index (BMI) data that allowed survival analysis.

For serum S1P level analysis, blood was taken from 12 healthy volunteers at the Virginia Commonwealth University Medical Center under the approval from its Institutional Review Board. The power analysis was based on our previous observation that obese patients have a higher serum S1P, with an estimated effect size of about 1.5. Based on this assumption, a total of 12 patients (6 in each of high- and low-BMI groups) will provide a power of 78% at 0.05 significant levels using one side test. One, seven, and four individuals were found to be in the BMI <20, 20-24.9, and 25-29.9 groups, respectively. Serum was separated by centrifugation and preserved at $-80^{\circ}C$. Lipids were extracted from blood and sphingolipids quantified by liquid chromatography, electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS, 4000 QTRAP, AB Sciex) as previously described.[13]

Gene set enrichment analysis (GSEA) of TCGA database

GSEA was performed on TCGA database using software provided by the Broad Institute (http://software.broadinstitute.org/gsea/index.jsp). We classified the patients into two groups according to BMI; high (BMI $>$ 27) and low (BMI $<$ 27).