

Tryptophan-induced pathogenesis of breast cancer

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Abstract

Background: The pathogenesis of breast cancer remains unclear.

Aims: To investigate the pathogenesis of breast cancer through targeted metabolomics of amino acids components in serum of patients with breast cancer.

Methods: Patients with breast cancers were enrolled in our hospital between year January 1st, 2013 and December 31st, 2014. Targeted analysis of amino acids was performed using ESI-QTOF-MS instrument. In vitro experiment was performed to determine the influence of tryptophan towards interleukin-10 (IL-10) secretion by CD4+ T cell.

Results: Targeted metabolomics of amino acids showed that the level of tryptophan significantly ($p < 0.05$) increased in patients with breast cancer. Furthermore, the biological function of tryptophan was determined through determining the influence of tryptophan towards IL-10 secretion using in vitro method. The addition of tryptophan (100 μ M) in the cell medium can significantly inhibited the secretion of IL-10 by CD4+ T cells, as indicated by the mRNA level and protein concentration.

Conclusion: The inhibition of IL-10 secretion by CD4+ T cells is a potential pathogenesis of breast cancer.

Keywords: breast cancer, interleukin-10 (IL-10), pathogenesis, T cell

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Introduction

Breast cancer, developing from breast tissue, remains the top reason for death of women. Unclear target information leads to the limited therapeutic drugs. Therefore, more and more studies are carried out to find new therapeutic targets. The long noncoding RNA SPRY4-IT1 has been demonstrated to be a novel prognostic biomarker and a potential therapeutic candidate for breast cancer¹. The experiment performed by Finlay et al. showed that RNA-based TWIST1 inhibition can reduce breast cancer metastasis through dendrimer complex². However, all these reasons can not completely ex-

plain the pathogenesis of breast cancer. The elucidation of a new mechanism for breast cancer pathogenesis is important and necessary.

Metabolomics analysis is an art-of-work technology for profiling all the small molecules in the biosamples (e.g., urine, serum, etc.). Metabolomics has been successfully applied to explain the pathogenesis of diseases, and the efficiency, pharmacokinetic behavior, and toxicity of xenobiotics³. Metabolomics studies indicated the importance of intestinal farnesoid X receptor signaling in the pathogenesis of nonalcoholic fatty liver disease⁴. The experiment performed by Zhang et al. showed that persistent organic pollutants can modify gut microbiota-host metabolic homeostasis in mice through aryl hydrocarbon receptor activation⁵.

The present study aims to compare the metabolomics profile of serum between health volunteers and patients with breast cancer to find the key inducing factor for breast cancer.

Materials and methods

Patients and biosamples preparation

Healthy volunteers (n=50, age, 18-70 years; body weight,

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45-90kg) and patients with breast cancers (n=20, age, 18-68 years; body weight, 40-85kg) were enrolled in our hospital between year January 1st, 2013 and December 31st, 2014. The patients were confirmed using histological and cytological examination methods. The patients with a second primary malignancy, distant metastasis, or any serious concomitant systemic disorders were excluded from this study. The blood samples were taken 3 months from the beginning, and stored at -80°C.

Targeted analysis of amino acids components

10 uL of serum was added in the 190 uL 95% acetonitrile to deproteinization. After 20-min centrifugation at 14,000*g, the aliquots (10 uL) were dropped on the filter paper. The filter paper was put into a 96-well polypropylene micro titer plates. 100 µL of methanol solution containing isotope labeled amino acid and acylcarnitine internal standards (NSK-A, NSK-B, Cambridge Isotope Laboratories, Andover, USA). 30 min gentle shaking at room temperature was performed. 60 µL of 3n butanolic-HCL was added for derivatisation at 65°C after evaporation at 70°C for 50 min. After a second evaporation step the samples were reconstituted with 150 µL of the mobile phase (1/1 v/v isopropanol/water) and analyzed by flow injection analysis (FIA)-MS/MS using a API 4000 triple quadrupole mass spectrometer (AB SCIEX, Darmstadt, Germany). The mobile phase is 80/20 (v/v) acetonitrile/water, and changed flow rate was used as followed: 0.01-0.09 min, 0.26 mL/min; 0.11-0.45 min, 0.03 mL/min; 1-1.13 min, 0.1-0.6 mL/min; 1.13-1.4 min, 0.6 mL/min; 1.41-2 min, 0.26 mL/min. The following mass conditions were used: Curtain gas, 20 psi; Collision gas, 8 psi; Ionspray voltage, 4500

V; Temperature, 350°C; Ion source gas (GS) 1 and 2, 35 psi. Neutral loss (NL) of 102.1 Da and precursor ion monitoring of 85.1 Da were used to monitoring the amino acids and their corresponding internal standard.

Determination of tryptophan-induced IL-10 secretion

CD4+CD25- T cells for cell culture were separated by EasySep™ Mouse CD4+CD25+ Regulatory T Cell Isolation Kit were from Stemcell Technologies, and were cultured with plated bound anti-CD3 (5 µg/ml) and soluble anti-CD28 (2 µg/ml) antibodies in the presence or absence of tryptophan for 24 hours. After that, cells were collected for IL-10 mRNA expression. IL-10 protein in culture supernatants was determined by ELISA analysis.

Quantitative polymerase chain reaction (qPCR) analysis

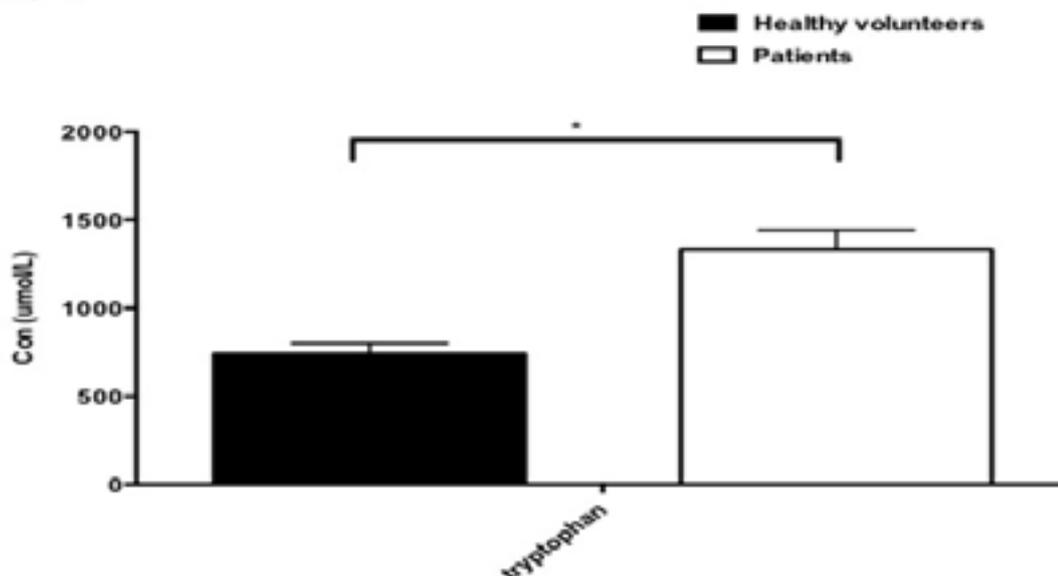
A total of liver RNA was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA), and cDNA was generated from 1 µg RNA with a SuperScript IITM Reverse Transcriptase kit and random oligonucleotides (Invitrogen, Grand Island, NY). The primer pairs were designed using qPrimerDepot, and messenger RNA quantitation was performed using the comparative cycle threshold (CT) method. The expression of mRNA was normalized to mouse 18S rRNA.

Results

Targeted metabolomics of amino acids showed that the level of tryptophan significantly (p<0.05) increased in patients with breast cancer (Figure 1).

Figure 1. Comparison of concentration of tryptophan in patients with breast cancer. *, p<0.05.

Figure 1

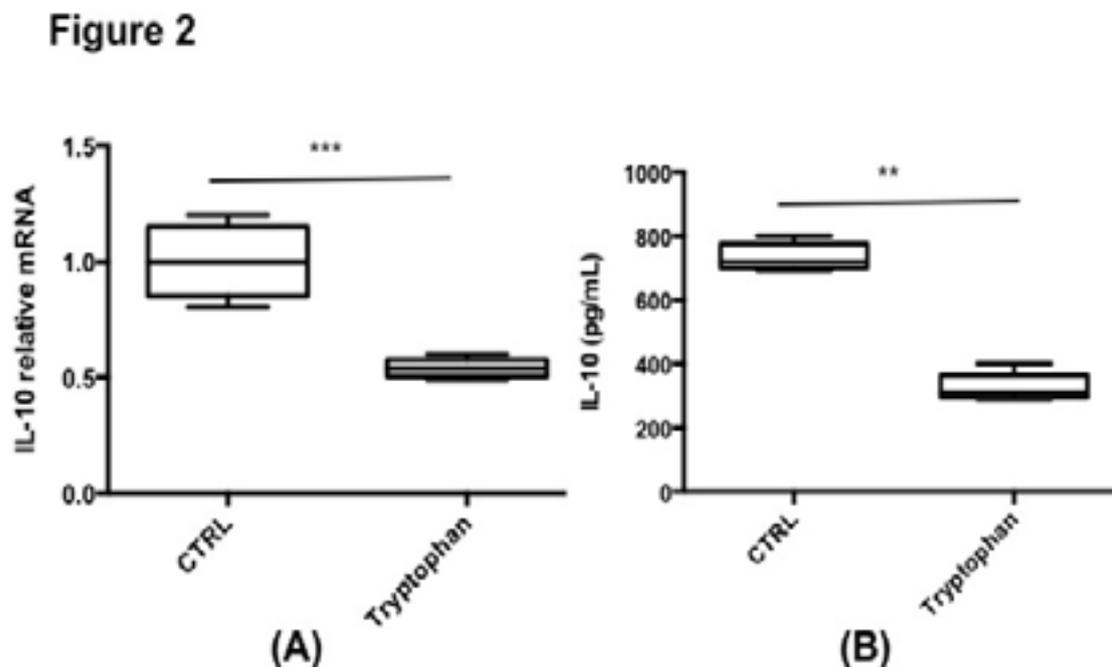


The blood sample was taken 3 months since the beginning of the experiment.

Furthermore, the biological function of tryptophan was determined through determining the influence of tryptophan towards IL-10 secretion using in vitro method.

The addition of tryptophan (100 μ M) in the cell medium can significantly inhibited the secretion of IL-10 by CD4+ T cells, as indicated by the mRNA level (Figure 2A) and protein concentration (Figure 2B).

Figure 2. The inhibition of tryptophan towards IL-10 secretion by CD4+ T cells.



CD4+ Naive T cells were isolated from mice splenocytes and cultured with anti-CD3 plus anti-CD28 for 48hrs. (A) Bar graph shows IL-10 mRNA expression in T cells cultured with or without tryptophan. (B) Bar graph shows IL-10 production in the culture media. **, $p < 0.01$.

Discussion

Interleukin-10 (IL-10) is a multifunctional cytokine which exerts the ability to inhibit the activation and effector function of T cells. Generally speaking, the function of IL-10 is to limit and even terminate the immune reaction⁶. The relationship between IL-10 and pathogenesis of cancers has been reported in previous literature. For example, the experiment performed by Chard et al. has shown that a vaccinia virus armed with IL-10 is a promising therapeutic agent for pancreatic cancer⁷. IL-10 promoter genotypes are reported to be associated with lung cancer⁸.

The present study used metabolomics method to find the significant elevation of tryptophan in serum, and

no change for other amino acids. In vitro experiment showed the inhibition of tryptophan towards the IL-10 by T cells, indicating that inhibition of IL-10 secretion by elevated tryptophan was a potential pathogenesis of breast cancer. This phenomenon has been previously reported in previous literature⁹.

Conclusion

The present study gives a short communication on the pathogenesis of breast cancer, which will be beneficial for the understanding of mechanism of breast cancer and the discovery of new drugs for breast cancer.

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