Journal of Dermatological Science 70 (2013) 182–189

Contents lists available at SciVerse ScienceDirect

Journal of Dermatological Science

journal homepage: www.elsevier.com/jds

CD147 promotes MTX resistance by immune cells through up-regulating ABCG2 expression and function

Shuang Zhao [a], Chen Chen [b], Shuang Liu [a], Weiqi Zeng [a], Juan Su [a], Lisha Wu [a], Zhongling Luo [a], Shuangyuan Zhou [a], Qingling Li [a], Jianglin Zhang [a], Yehong Kuang [a,*], Xiang Chen [a,**]

[a] Department of Dermatology, XiangYa Hospital, Central South University, Changsha, Hunan, People's Republic of China
[b] Department of Nephrology, XiangYa Hospital, Central South University, Changsha, Hunan, People's Republic of China

ARTICLE INFO

Article history:
Received 29 September 2012
Received in revised form 5 February 2013
Accepted 14 February 2013

Keywords:
CD147
ABCG2
MTX
PBMCs
Multidrug resistance
Apoptosis

ABSTRACT

Background: Methotrexate (MTX) is a drug used to treat psoriasis due to inducing immune cell apoptosis. However, certain patients show MTX resistant. CD147, highly expressed by psoriatic PBMCs, is assumed to regulate MTX sensitivity. The underlining mechanism is still relatively understudied.
Objective: To understand the mechanisms of that CD147 promotes MTX resistance in immune cells.
Methods: The expression of CD147 and ABCG2 in PBMCs from psoriatic patients, cellular apoptosis and intracellular MTX amount were measured. We also checked the cellular drug sensitivity of CHO (Chinese Hamster Ovary) cell lines with introduced CD147 and Jurkat T cells depeleted CD147. By immunoprecipitation, we detected the interaction between CD147 and ABCG2.
Results: Both ABCG2 and CD147 are highly expressed in psoriatic PBMCs. Cultured in vitro, the PBMCs from psoriatic patients were more resistant to MTX-induced apoptosis comparing to PBMCs from healthy people. Further studies demonstrated that exogenous overexpression of CD147 in CHO cells increased ABCG2 protein level. After MTX treatment, CD147 overexpressing CHO cells showed lower apoptosis rate and lower intracellular MTX concentration. On the contrary, knockdown of CD147 by shRNA in Jurkat T cells decreased ABCG2 expression, as well as increased MTX-induced apoptosis and decreased MTX efflux. Immunoprecipitation experiment revealed that the trans-membrane domain of CD147 conferred its' interaction with ABCG2.
Conclusion: Our study suggests a role of CD147 in regulating ABCG2 transportation of MTX in immune cells. Strategies involving targeting CD147 could be considered in clinical treatment of psoriatic patients resistant to MTX.
Crown Copyright © 2013 Japanese Society for Investigative Dermatology. Published by Elsevier Ireland Ltd. All rights reserved.

1. Introduction

MTX, a traditional anti-cancer chemotheraputic drug, was first found during leukemia treatment programs in the 1940's. MTX has since been widely used in treatment of a variety of malignant tumors and inflammatory skin diseases [1–3]. In clinical applications, MTX usually has two kinds of dose levels; high doses of MTX inhibits dihydrofolate reductase, interfering with cellular DNA and RNA synthesis and therefore leading to anti-tumor effects, while low-dose MTX interferes with a variety of inflammatory process by inducing apoptosis of inflammatory cells and obstructing the generation of inflammatory factors, and is mainly used for the treatment of psoriasis, rheumatoid arthritis and other inflammatory diseases in clinic [2–4]. MTX is an inexpensive and effective drug. MTX is well accepted by many doctors and patients. However, a number of adverse reactions, such as bone marrow suppression, as well as liver and kidney damage, limits the clinical application of MTX. Therefore, increasing the sensitivity of diseased cells to MTX and reducing the clinical dose thus reducing the side effects, are becoming the main topics of research in MTX for inflammatory disease.

A large number of studies have found that the intracellular MTX was extruded from cells, mainly through the ABC (ATP-binding cassette) transporters pumps (including the ABCC family and ABCG2 family) thus reducing the original pharmacological effects [5,6]. These ABC transporter are widely expressed on the cell membrane of a variety of inflammatory cells, such as in peripheral blood mononuclear cells (PBMCs), bone marrow, liver and kidney [7]. They exert function of inhibition of inflammatory cell

* Corresponding author at: Department of Dermatology, XiangYa Hospital, Central South University, 87 XiangYa Road, Changsha, Hunan 410008, People's Republic of China. Tel.: +86 731 8432 7128; fax: +86 731 8975 3406.
** Corresponding author at: Department of Dermatology, XiangYa Hospital, Central South University, 87 XiangYa Road, Changsha, Hunan 410008, People's Republic of China. Tel.: +86 731 8432 7377; fax: +86 731 8432 8478.
E-mail addresses: yh_927@yahoo.com.cn (Y. Kuang), chenxck@yahoo.com (X. Chen).

0923-1811/$36.00. Crown Copyright © 2013 Japanese Society for Investigative Dermatology. Published by Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.jdermsci.2013.02.005

apoptosis induced by MTX, and increase adverse effects [8–11]. Some studies including that of our group have confirmed that ABCG2 (the second member of G sub-family of the ABC transporter system), a half-transporter, is the main transporter of MTX and certain folates. The activity of ABCG2 must depend on its homodimer formation [12]. Despite several studies reporting ABCG2 expression in normal and malignant tissues, no study has addressed the role of ABCG2 in the anti-inflammatory effects of MTX.

CD147 (basigin, EMMPRIN), a member of the immunoglobulin superfamily, has been initially thought to play an important role in tumor cell invasion and metastasis through the induction of MMPs production [13]. In recent years, more and more evidence has proved that CD147 also plays a pivotal role in tumor cell angiogenesis, energy metabolism, and MDR (multidrug resistance) [14–17]. CD147 is highly expressed on T-lymphoma cells and activated T and B lymphocytes, dendritic cells, monocytes, and macrophages. CD147 regulates the activation and function of these inflammatory cells [18,19]. Yang et al. found in the year 2003 that the expression of CD147 in multidrug resistance (MDR) cells is significant higher than that in its parental drug sensitive cell lines, suggesting that CD147 is involved in drug sensitivity. There were reports that CD147 increased the expression of ABC transporter families, such as MDR1 [20] and CD147 affects drug resistance of MDR tumor cells by regulating their susceptibility to apoptosis in a hyaluronan dependent manner that was mediated *via* the PI3K and Erk1/2 pathways [21–23]. Our findings also demonstrated that inhibition of CD147 and subsequent XIAP depletion might have an anti-tumor effect through enhancement of the susceptibility of cancer cells to apoptosis, which provide new understanding that CD147 plays a crucial role in MDR by regulating tumor cell apoptosis [17]. However, CD147 in the context of the T cells drug sensitivity is still undetermined.

Our study has revealed that CD147 was overexpressed in psoriatic PBMCs and contributed to T cell activation. We determined that CD147 was a gene susceptible to psoriasis [19]. In the present study, we found that the expression of ABCG2 is also increased in psoriatic PBMCs – the same trend as CD147. Therefore, we sought to determine whether CD147 and ABCG2, either alone or through interdependent interactions, regulate inflammatory cells resistant to MTX. We established CD147 overexpressing CHO (Chinese Hamster Ovary) cell lines and CD147 depeletion Jurkat T cells for this study.

2. Materials and methods

2.1. Subjects

Twenty eight psoriasis patients of Chinese Han population (average age: 36 years, range: 11–72 years) were recruited from the Department of Dermatology in XiangYa Hospital, Changsha city, Hunan province. All patients filled out a clinical questionnaire, received a skin examination and were diagnosed independently by two senior dermatologists. The control group consisted of 24 healthy adult donors (average age 26.6 years, range: 24–30 years). Controls were matched with cases by age and ratio of gender. Informed consent was obtained from each participant. The study was approved by the Ethic Committee of XiangYa Hospital.

2.2. Cell cultures and preparations

The CHO cells and Human Embryonic Kidney HEK293 cells were purchased from Shanghai Institute of Cell Biology, Chinese Academy of Sciences (Shanghai, China). The human leukemia Jurkat T cell line, was provided by the Cell Center of Xiangya Medical School of Central South University of China (Changsha, China). The cells were routinely cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS), 100 units/mL penicillin-G, and 100 μg/mL streptomycin at 37 °C in a humidified atmosphere containing 5% CO_2.

2.3. Preparation of PBMCs

PBMCs were isolated from freshly heparinized/EDTA blood by gradient centrifugation using Histopaque®-1077 (Sigma-Aldrich Co, MO, USA) according to the manufacturer's instructions. The heparinized blood samples were centrifuged at 400 g for 30 min and plasma was removed. The remaining blood samples were either washed with phosphate-buffered saline (PBS) (pH 7.4) and extracted with total protein for Western blot analysis, or resuspended with RPMI 1640 supplemented with 10 mM HEPES, 100 units/mL penicillin-G, 100 μg/mL streptomycin, and 1% human serum (Invitrogen, NY) for cell culture [19].

2.4. Western blot analysis

Protein samples (35 μg) were separated by 10% SDS–PAGE and transferred onto PVDF membranes (Pierce Chemical, Rockford, IL). Nonspecific reactivity was blocked by 5% non-fat dry milk in TBST (10 mM Tris–HCl, 150 mM NaCl, 0.05% Tween 20, pH 7.5) for 1 h at room temperature. Membranes were incubated overnight with the primary mouse anti-CD147 antibody diluted to 1:1000 (Abcam Co, Cambridge, UK), the primary rat anti-ABCG2 (1:200,Abcam Co., Cambridge, UK), or the primary rabbit anti-cleaved-PARP antibody (1:10,000,Abcam Co, Cambridge, UK). Membranes were subsequently incubated with a secondary HRP conjugated goat anti-mouse, anti-rat, or anti-rabbit IgG antibody (Santa Cruz Biotech, Santa Cruz, CA) respectively at 1:10,000 dilution. The reactions were visualized with ECL detection system (Pierce Chemical, Rockford, IL). Anti-β-actin antibody at 1:10,000 dilution (Sigma–Aldrich, St. Louis, MO) was used to ensure equal loading of each sample. The Western blot analyses were repeated at least three times. The grayscale ratio of each molecule to β-actin were calculated.

2.5. Cell viability assay

Cell viability was tested by CCK8 method assays. For CCK8 assays, cells were seeded into 96-well plates at a density of 5.0×10^3 per well and cultured for 24 h, then treated with MTX for indicated time. Cells were switched to 10 μL CCK8 solutions (5 mg/mL in PBS) and incubated for 4 h at 37 °C. DMSO (150 μL) was added to each well. The plate was gently rotated on an orbital shaker for 10 min to completely dissolve the precipitation. The absorbance was detected at 490 nm with a Microplate Reader (Beckman Coulter-DTX 800). Each well had three repeats of counting and the experiment was repeated three times.

2.6. Flow cytometric analysis

A total of at least 5.0×10^5 cells were incubated in individual well of 100 mm cell culture dish treated with MTX for 24 h. Then, the cells were washed with PBS and resuspended in binding buffer (Genzime, Cambridge, MA). FITC Annexin V was added at a final concentration of 1 mg/mL. After 30 min of incubation in the dark at room temperature, cells were analyzed by FACS (Becton Dickinson Co., NJ).

3. Construction of CD147 overexpressing CHO cell lines and CD147 depeletion

3.1. Jurkat T cells

The cDNA containing the whole region of human CD147 was prepared by Agel enzyme digestion (Genechenm Co. Ltd., Shanghai,

Download English Version:

https://daneshyari.com/en/article/3212969

Download Persian Version:

https://daneshyari.com/article/3212969

Daneshyari.com