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Expression of small glutamine-rich TPR-containing protein A (SGTA) in Non-Hodgkin's Lymphomas promotes tumor proliferation and reverses cell adhesion-mediated drug resistance (CAM-DR)

Yuchan Wang[a,1], Yuejiao Huang[b,1], Xiaohong Xu[a], Jie Tang[b], Xianting Huang[b], Junya Zhu[b], Jing Liu[b], Xiaobing Miao[a], Yaxun Wu[a], Fan Yang[c], Lili Ji[b,*], Song He[a,**]

[a] Department of Pathology, Affiliated Cancer Hospital of Nantong University, Nantong 226361, Jiangsu, China
[b] Department of Pathology, Medical College, Nantong University, Nantong 226001, Jiangsu, China
[c] Department of Pediatrics, Medical College, Nantong University, Nantong 226001, Jiangsu, China

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ABSTRACT

The expression and biologic function of SGTA in Non-Hodgkin's Lymphomas (NHL) was investigated in this study. Clinically, by immunohistochemistry analysis we detected SGTA expression in both reactive lymphoid tissues and NHL tissues. In addition, we also correlated high expression of SGTA with poor prognosis. Functionally, SGTA expression was positively related with cell proliferation and negative related with cell adhesion. Finally, SGTA knockdown induced adhesion-mediated drug resistance. Our finding supports a role of SGTA in NHL cell proliferation, adhesion and drug resistance, and it may pave the way for a novel therapeutic approach for CAM-DR in NHL.

1. Introduction

Non-Hodgkin's Lymphomas (NHL) is a heterogeneous group of solid tumors that derives from lymphocytes and involves many kinds of lymphoma except Hodgkin's lymphoma [1]. The classification of NHL is diverse, including diffuse large B-cell lymphoma (DLBCL), follicular lymphoma (FL), extranodal lymphoma of mucosa-associated lymphoid tissue (MALT), and Nasal natural killer (NK)/T-cell lymphoma (NK/T), etc. [2–4]. Significant improvements have been made in the treatment of NHL where event-free survival rate has increased from 30% in the 1970s to 60–95% nowadays. The outcome for people with relapsed or refractory disease, however, remains poor [5,6]. About an additional 10–20% of NHL patients can be rescued with high-dose chemotherapy following stem cell transplantation, implicating drug resistance as a significant reason of treatment failure in lymphoma [7].

Stromal cells are essential for bone marrow microenvironment that regulate tumor cell survival. Bone marrow stroma has been regarded as a 'sanctuary site' for lymphoma cells during traditional chemotherapy [8,9]. Mounting evidence suggests that interaction between the bone marrow microenvironment and lymphoma cells may play an essential role in tumor development [10]. Previous studies have indicated that adhesion to bone marrow stromal cells or fibronectin (FN)-coated surface can protect malignant lymphoma cells from apoptosis induced by chemotherapy drugs [cell adhesion-mediated drug resistance (CAM-DR)] [8,11–13]. However, how bone marrow stroma regulates CAM-DR in lymphoma, and the underlying molecular mechanisms involved, are unclear to date. In this article, we used NHL as a disease model to characterize the mechanism by which bone marrow stroma regulates chemotherapeutic drug resistance.

Small glutamine-rich tetratricopeptide repeat (TPR)-containing protein A (SGTA, also known as SGT, hSGT or Vpu-binding protein), was originally discovered as a binding partner of the non-structural protein of autonomous parvovirus H-1[14–16]. The TPR motifs are involved in a variety of processes, such as cell cycle, protein folding, transcription, protein transport, hormone receptor signaling

* Corresponding author at: Department of Pathology, Medical College, Nantong University, 19 Qixiu Road, Nantong 226001, Jiangsu, China.
** Corresponding author at: Department of Pathology, Affiliated Cancer Hospital of Nantong University, 30 Tongyang Road, Nantong 226361, Jiangsu, China.
Tel.: +86 513 85051999.
E-mail addresses: liliji79@ntu.edu.cn (L. Ji), hesong2009@126.com (S. He).
[1] These authors contributed equally to this work.

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and several other pathways [17–19]. It has been reported that knockdown of SGTA results in the suppression of androgen and phosphatidylinositol 3 kinase (PI3K)/Akt signaling and inhibition of prostate cancer cell proliferation [20]. Also, previous studies have reported that SGTA down-regulate receptor tyrosine kinases (RTK) signaling and might participate in a regulatory loop acting to enhance sensitivity of cancer cells to chaperone inhibitors [21]. Deficiency of SGTA might lead to partial cell cycle arrest in G2/M and inability to complete cell division due to mitotic arrest [22]. In NHL, it has been reported that cell adhesion to bone marrow stroma induces a reversible cell-cycle arrest. Therefore, it is of interest to investigate whether SGTA can also affect cell proliferation and cell cycle arrest of NHL.

In our previous study, it has been showed that SGTA is highly expressed in several solid neoplasms such as pulmonary carcinoma, esophageal squamous carcinoma and human hepatocellular carcinoma [14,15,18]. In this study, we aimed to investigate SGTA expression in NHL and to explore the relationship between SGTA expression and cell proliferation or CAM-DR. We also investigated its association with clinical and pathologic factors, as well as prognosis after chemotherapy. Our study first reported that SGTA expression promoted tumor proliferation and reversed CAM-DR in NHL, and it may provide a novel perspective for a better understanding of the mechanism of drug resistance in NHL.

2. Materials and methods

2.1. Patients and tissue samples

This study was carried out on a total of 96 B-cell lymphomas and 19 reactive lymphadenopathy samples, which were histopathologically and clinically diagnosed at Affiliated Cancer Hospital of Nantong University, during the period of January 1, 1993, to April 1, 2 005. All of these tissue samples were new diagnosed and classified according to the WHO criteria, which included: 37 diffuse large B-cell lymphomas (DLBCL), 16 follicular lymphomas (FL), 28 mucosa-associated lymphoid tissue B cell lymphomas (MALT), 15 Nasal natural killer (NK)/T-cell lymphoma (NK/T), and 19 reactive lymphoid tissues (RL). A written consent form was obtained from all patients.

2.2. Antibodies

Western Blot was performed according to methods described previously [23,24]. The antibodies used for immunohistochemistry and Western blotting were obtained from Santa Cruz Biotechnology, USA. The primary antibodies in this study included: SGTA (1:1000), cyclin A (1:500), $P2^{Kip1}$ (1:1000), PCNA (1:1000), and GAPDH (1:1000).

2.3. Immunohistochemical staining and evaluation

The procedures were carried out similarly to previously described methods [25,26]. Three independent observers (YJH, YCW and LLJ) evaluated the immunostaining results. Cells with brown-colored staining were considered as positive. Staining intensity was graded according to the following criteria: 0 (no); 1 (weak); 2 (moderate) and 3 (strong).

The percentage of staining tumor cell was scored as follows: 0 (no positive tumor cells); 1 (1%-25% positive tumor cells); 2 (25–49% positive tumor cells) and 3 (50–100% positive tumor cells). The staining index (SI) was used to calculate the staining intensity score and the percentage of positive tumor cells score. Using this method, we assessed SGTA expression by the SI with scores of 0, 1, 2, 3, 4, 6 or 9. For statistical analysis: an SI score of $\geq$4 was used to define tumors with high SGTA expression, and an SI score of $\leq$3 was used to indicate low SGTA expression.

2.4. Cell cultures and transient transfection

The human NHL cell line Daudi and the human bone marrow stromal cells (BMSC) line HS-5, were both obtained from Jiangsu Institute of Hematology, China. The NHL cells were cultured in suspension in RPMI 1640 (Sigma–Aldrich, Rehovot) while HS-5 were cultured in F12 (Sigma–Aldrich, Rehovot) with 10% fetal bovine serum at 37 °C and 5%CO_2.

The SGTA-siRNA and control-siRNA were obstained from Biomices Biotechnologies Co. Ltd. The siRNA targeting SGTA sequences were 1#: 5′-CGUGCAUUUCUACGGAAAA-3′; 2#: 5′-AAGCACGUGGAGGCCGUGG-3′, and 3#: 5′-CUUCGAACCUAAUGAACAA-3′. Cells were seeded in susupension in 1640 medium with 10% FBS without antibiotics before transfection. The siRNA transfection was performed using lipofectamine 2000 in accordance with the manufacturer's protocol. Cells were cultured at 37 °C in 1640 with no serum or antibiotics for 6 h at 10^5/ml. Transfected cells were harvested 48 h after transfection.

2.5. Cell cycle analysis and viability assay

After cells were harvested, they were fixed in 70% ethanol at −20 °C and then incubated with 1 mg/ml RNase A for 30 min. Afterwards, cells were collected by centrifugation at 2000 rpm for 5 min and stained with propidium iodide (50 μg/ml PI; Becton–Dickinson, San Jose, CA, USA) in phosphate-buffered saline (PBS), 0.5% Tween-20. After that, cells were analyzed using a Becton–Dickinson BD fluorescence activating cell sorter (FACScan) flow cytometer.

Cell viability was measured using the Cell Counting Kit-8 (CCK-8) assay (Dojindo Molecular Technologies, Gaithersburg, MD, USA) according to the manufacturer's instructions. Frist, all the cells were inoculated into a 96 well plate at the density of 10^5 cells/well. Then, cells were treated with Doxorubicin or Mitoxantrone for 48 or 72 h. CCK-8 reagents were added to each well at due time, incubated for an additional 1 h at 37 °C, and the absorbance at 450 nm was read in an automated plate reader. Each experiment was repeated three times at least.

2.6. Cell co-culture and adhesion assay

Firstly, the dishes were mantled overnight at 37 °C with HS-5 cells or 40 μg/mL human FN (Sigma–Aldrich, Rehovot) in a final volume of 1 mL PBS. Secondly, Daudi cells (10^5 cells/mL) were adhered to pre-established monolayers of HS-5 or FN for 2–4 h. Lastly, adherent cells were carefully removed for next experiments, with the HS-5 monolayer kept intact.

The ability of cell adhesion was assessed by staining lymphocytes with calcein (Santa Cruz Biotechnology) for 30 min according to the manufacturer's protocol and then incubating them in 96-well plates with a FN-coated surface or pre-established monolayers of HS-5 in RPMI 1640 medium. After 2 h of co-culture, the non-adherent cells were washed off twice with 1 ml of PBS and the number of adherent cells was measured with an automated plate reader.

2.7. Statistical analysis

The calculations were analyzed using the Statistical Package for the Social Sciences SPSS 13.0 software. The association between SGTA expression and clinicopathological features was analyzed using the χ^2 test. As the data were not normally distributed, SGTA and Ki-67 expressions were studied using the Spearman rank correlation test. Multivariate analysis was performed using Cox's proportional hazards model. Statistical significance was determined using the Student's *t*-test. All data shown represent the results of three independent experiments at least and *P*–values <0.05 were considered significant.

3. Results

3.1. SGTA was expressed in reactive lymphoid tissues and human B-cell Non-Hodgkin's Lymphoma

In our previous study, SGTA was found to be highly expressed in several solid neoplasms [14,15,18]. But whether this is the case for hematologic malignancies remains still unclear. Hence, immunohistochemical assay was performed to investigate the expression of SGTA in vivo in clinical NHL specimens including FL, DLBCL, MALT and NK/T except for RL tissues. In non-tumor RL tissues, SGTA was found to be expressed predominantly in proliferating germinal centers (Fig. 1a and b). Moreover, in lymphoma issues other than MALT, overexpression of SGTA was observed. SGTA immunoreactivity was seen primarily localized in the follicular mantle zones of FL (Fig. 1c and d), while in DLBCL and NK/T tissues SGTA was diffusely strong expressed (Fig. 1e–h). However, compared with FL and DLBCL tissues, the expression of SGTA in MALT tissues was much weaker (Fig. 1i and j). Finally, we used PBS-based control as an alternative approach to evaluate the specificity of IHC signaling (Fig. 1k and l).

3.2. SGTA expression was associated with high-risk clinical parameters in NHL

The level of SGTA and Ki-67 expression was divided into high group and low group according to the cuf-off value stated in aforementioned methods. Pearson χ^2 test was performed to analyze the association of SGTA expression with clinicopathologic variables including Ki-67 expression (Table 1). The expression of SGTA was