Pathology – Research and Practice 213 (2017) 773–777

Contents lists available at ScienceDirect

Pathology – Research and Practice

journal homepage: www.elsevier.com/locate/prp

Effect of plasma-activated medium on the decrease of tumorigenic population in lymphoma☆

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ARTICLE INFO

Article history:
Received 11 February 2017
Received in revised form 8 April 2017
Accepted 9 April 2017

Keywords:
Plasma-activated medium
Lymphoplasmacytic lymphoma
CD138
Differentiation

ABSTRACT

Nonequilibrium atmospheric pressure plasma (NEAPP) is a novel approach for blood coagulation, wound healing, and tumor elimination. NEAPP not only directly but also indirectly affects living cells via the medium exposed to NEAPP-yielding devises, called plasma-activated medium (PAM). The conservable and portable PAM serves as an alternative and advantageous approach over direct NEAPP. Here we examined the effect of PAM on lymphoplasmacytic lymphoma (LPL) cell lines. We found that PAM induced plasma cell differentiation and reduced tumorigenic population. PAM increased the expression level of PRDM1α, which is a transcription factor promoting plasma cell differentiation, suggesting that plasma cell differentiation of LPL might be mediated by PRDM1α. We previously reported that plasma cell component of LPL is vulnerable to apoptosis and less tumorigenic. These findings suggested that PAM treatment might become a novel therapy against LPL by inducing the transition from tumorigenic to non-tumorigenic population.

1. Introduction

Plasma has been used for sterilization of medical equipment, packaging in the food industry, implants and blood coagulation, mainly via the thermal effects sustained for a long period of time [1]. In nonequilibrium atmospheric pressure plasma (NEAPP), also known as low-temperature plasma or non-thermal plasma, the temperature at the point of application was less than 40 °C. Since the development of NEAPP devises, medical applications of plasma for living cells have been addressed, such as blood coagulation, wound healing and tumor elimination without thermogenesis [1–6]. Recent findings revealed that the biological effects of NEAPP is mediated by a large number of reactive species such as reactive oxygen and nitrogen species [6–9]. NEAPP is usually yielded from devices, which are inserted into body cavity to elicit biological effects. Recently, alternative way to apply NEAPP to living cells has been established; the medium exposed to NEAPP-yielding devises, called plasma-activated medium (PAM). PAM contains a large number of reactive species, and can be stored at 4 °C. The conservable and portable PAM serves as an alternative and advantageous approach over NEAPP-yielding devises [10].

Tumors derive from a single clone, but consist of heterogenous subpopulations whose features and functions are diverse. Immature cells with tumorigenic potential are limited to a small subpopulation, called cancer-initiating cells (CICs), in several types of tumors, such as leukemia, lymphoma, brain tumor, breast cancer and colon cancer [11–22]. CICs show anti-apoptotic ability and become one of the causes of tumor recurrence [11–23]. In contrast to CICs, non-CICs are vulnerable to various therapies. Therefore, the transition from CICs to non-CICs, leading to elimination of CICs, would be a novel anti-tumor therapy. However, the efficient way of transition has yet been unknown.

Waldenström macroglobulinemia is defined as lymphoplasmacytic lymphoma (LPL) with bone marrow involvement and an

Abbreviations: NEAPP, nonequilibrium atmospheric pressure plasma; PAM, plasma-activated medium; CICs, cancer-initiating cells; LPL, lymphoplasmacytic lymphoma; FBS, fetal bovine serum; APC, Allophycocyanin; PE, Phycoerythrin; PI, propidium iodide; FSC-A, forward-scatter-area; SSC-A, side-scatter-area; qRT-PCR, quantitative reverse transcription-polymerase chain reaction; SE, standard error; BCR, B cell receptor.

☆ This work was supported by grants from the Ministry of Education, Culture, Sports, Science and Technology, Japan (A15H008940, T15K083630, T16K086490, and T264604700).

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http://dx.doi.org/10.1016/j.prp.2017.04.003
0344-0338/

immunoglobulin M monoclonal gammopathy [24,25]. LPL is an indolent non-Hodgkin's lymphoma and consists of tumor cells with relatively diverse surface markers, which shows a spectrum of small B lymphocytes ($CD20^+$ $CD138^-$), plasma cells ($CD20^-$ $CD138^+$) and lymphoplasmacytoid cells ($CD20^+$ $CD138^+$) [21,25–28]. Because of the diverse phenotypes of lymphoma cells and the presence of cell lines such as MWCL-1 and RPCI-WM1 [29,30], LPL is suitable for the study about heterogeneity of cellular subpopulations in lymphoma cells. We previously demonstrated that a small population without any B and plasma cell markers ($CD20^-$ $CD138^-$) is more tumorigenic and shows phenotypes like CICs [21]. From this CIC-like subpopulation, other subpopulations with B/plasma cell markers ($CD20^+$ $CD138^-$ and $CD20^+$ $CD138^+$) are yielded [21]. In contrast to this CIC-like subpopulation, $CD20^+$ $CD138^+$ subpopulation is vulnerable to apoptosis and shows senescent phenotype [21]. These findings suggest that the transition process from immature CIC-like population ($CD20^-$ $CD138^-$) to mature non-CIC-like population ($CD20^+$ $CD138^+$) is observed in LPL, and that this process might be useful for studying the way of efficient transition from CICs.

Since reactive oxygen and nitrogen species affect signaling pathways regulating differentiation, NEAPP might have the potential to control cellular differentiation. In fact, NEAPP inhibits mouse embryonic stem cell differentiation to mesoderm and endoderm, but promotes ectoderm differentiation [9]. In the present study, we investigated whether PAM, which contained a large number of reactive species, promoted cellular differentiation of LPL, and was useful for the elimination of tumorigenic subpopulation.

2. Materials and methods

2.1. Cell lines and flow cytometric analysis

MWCL-1 was provided from Mayo Foundation for Medical Education and Research, and RPCI-WM1 from Mayo Clinic Cancer Center. They were cultured in IMDM + GlutaMAX™-I (Gibco by Life Technologies, Carlsbad, USA) supplemented with 10% fetal bovine serum (FBS, Central America Origin, Biosera, Kansas City, USA). Cells were stained with anti-CD20-Allophycocyanin (APC) (clone 2H7, BD Biosciences, San Jose, USA) and/or anti-CD138-Phycoerythrin (PE) (clone MI15, BD Biosciences) antibodies, and their immunophenotypes of CD20 and/or CD138 were examined with FACS Canto II (BD Biosciences). We reacted MWCL-1 cells in the presence of human FcR blocking reagent (Miltenyi Biotec, Auburn, USA). The dot blot pattern obtained with FcR blocking was comparable to that without blocking [21]. To remove dead cells, propidium iodide (PI) staining and gating of forward-scatter-area (FSC-A) versus side-scatter-area (SSC-A) were performed. The proportion of dead cells removed by PI staining and gating of FSC-A versus SSC-A was similar to that removed only by gating of FSC-A versus SSC-A [21]. Then, in the following experiments, FcR blocking and PI staining were omitted. Since RPCI-WM1 cells were negative for CD20 unlike MWCL-1 cells [30], only immunophenotype of CD138 was examined. Data were analyzed by Cell Quest software (BD Biosciences).

2.2. Experimental setup of NEAPP and generation of PAM

3 ml IMDM + GlutaMAX™-I without FBS in 6-well plate was treated with NEAPP provided from National Institute of Advanced Industrial Science and Technology as previously described [3,4,31–33]. Briefly, the peak-to-peak voltage, $V_{p\text{-}hp}$, applied to the electrode was within the range of 6–10 kV, the frequency range of the sinusoidal wave was between 10 and 70 kHz, the flow rate of helium gas was set at 2 standard liters/min, and the distance between the plasma source and the medium was fixed at 15 mm. Exposure time of NEAPP treatment was 3 min. Since PAM stored at 4 °C retained the effectiveness for a maximum of 5 days [10], PAM was stored until the experiments under optimal conditions. The resultant PAM was diluted with normal medium, and cells were cultured in medium containing various amounts of PAM (0, 20, 40, 60, and 80%) in the presence of 10% FBS.

2.3. Quantitative reverse transcription-polymerase chain reaction (qRT-PCR)

Total RNA was extracted from cells using the RNeasy RNA extraction kit (Qiagen, Valencia, CA, USA), according to the manufacturer's protocol, and cDNA was synthesized using oligo (dT) primers and SuperScript III reverse transcriptase (Invitrogen). The qRT-PCR was performed with a StepOnePlus™ Real-Time PCR System (Applied Biosystems, Foster City, USA) using Taqman probe/primer sets specific for human PRDM1α and XBP1. GAPDH was used as a reference for gene amplification (Applied Biosystems).

2.4. Immunoblotting

Intracellular proteins were prepared using NE-PER Nuclear and Cytoplasmic Extraction Reagents based on the manufacturer's instruction (Pierce Biotechnology, Rockford, USA). Electrophoresis was performed with 10% sodium dodecyl sulfate–polyacrylamide gels (ATTO, Tokyo, Japan) and proteins were transferred to polyvinylidene fluoride membranes (Merck Millipore, Billerica, USA). Anti- PRDM1 antibody (clone 646702, R&D Systems, Minneapolis, USA, dilution at 1.5:1000) and anti-α-actin antibody (Sigma-Aldrich, St. Louis, USA, dilution at 1:1000) were used as the primary antibodies. HRP-conjugated anti-mouse IgG (H + L chain)

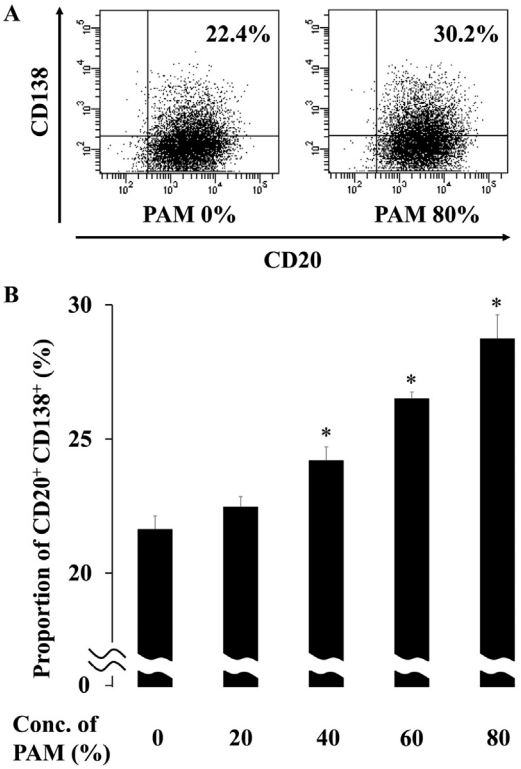

Fig. 1. Immunophenotypes of MWCL-1 cells cultured for 24 h with PAM. (A) Two-dimensional plot displaying flow cytometric data with CD20 and CD138 antibodies. The proportion values of number of $CD20^+$ $CD138^+$ cells were shown. (B) Bar graphs showing the proportion of $CD20^+$ $CD138^+$ in MWCL-1 cells cultured for 24 h with 0%, 20%, 40%, 60% and 80% PAM. * $p < 0.05$ when compared to 0% PAM.