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研究課題名（和文）Effects of sSLAMF7 on NK cells in multiple myeloma patients

研究課題名（英文）Effects of sSLAMF7 on NK cells in multiple myeloma patients

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研究成果の概要（和文）：ヒトNK細胞は、多発性骨髄腫患者と健康なボランティアの末梢血から調製されました。コホートは、20人の多発性骨髄腫患者と10人の健康なドナーで構成されていました。多発性骨髄腫患者は2つのグループに分けられました：sSLAMF7血清レベルが高い10人（新鮮/再発症例）と低レベル（寛解または安定した症例）の10人。 CAGEを使用して、3つのグループ間で遺伝子発現を比較しました。多発性骨髄腫患者のNK細胞の転写プログラムで観察された変化の推定調節因子を同定するために、差次的に発現する遺伝子が同定され、MARAにかけられました。

研究成果の学術的意義や社会的意義
Poor outcome in Multiple Myeloma (MM) patients with high sSLAMF7 serum levels may be linked to changes in NK cell gene expression programs. We aimed to reconstruct the NK cell transcriptional regulatory network that governs MM and is modulated by sSLAMF7, paving the way for novel therapeutic targets

研究成果の概要（英文）：Human primary natural killer cells (NK cells) were prepared from peripheral blood of Multiple Myeloma patients and healthy volunteers. The cohort consisted of 20 Multiple Myeloma patients and 10 healthy donors. The Multiple Myeloma patients were divided into two groups: 10 with high sSLAMF7 serum levels (fresh/relapsed cases) and 10 with low levels (remission or stable cases). RNA was extracted and gene expression was compared between the three groups using Cap Analysis of Gene Expression (CAGE) technology, which allows for base pair resolution detection and expression quantification of promoter regions. Differentially expressed genes were identified and subjected to Motif Activity Response Analysis (MARA) to identify putative regulators of observed changes in the transcriptional program of NK cells of Multiple Myeloma patients.

研究分野：transcriptional regulation

キーワード：multiple myeloma

１．研究開始当初の背景

Multiple Myeloma and immune system

Multiple myeloma is one of the refractory hematological malignancies, derived from plasma cells. Recently, immunomodulating agents are being developed to overcome their resistance to conventional therapies and produced better consequences. Notwithstanding, myeloma is still intractable and one reason is that the precise mechanisms through which these drugs act on myeloma cells are unknown. Furthermore, the impact of myeloma cells on the host immune system still remains to be clarified.

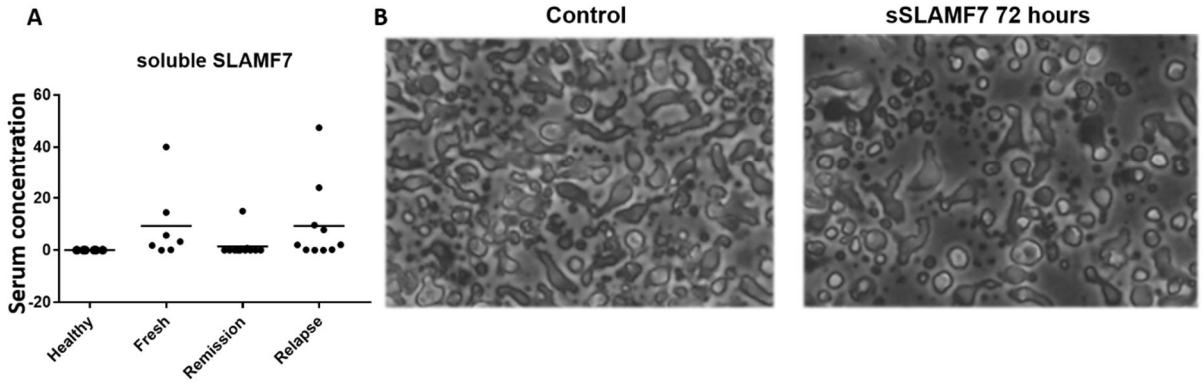
NK cells in Multiple Myeloma

NK cells are thought to play a significant role in the immune defense against myeloma. In a previous study we showed that the number of NK cells in patients was significantly low compared to patients in remission and relapse (Hagiwara S, Sorimachi N. International Myeloma Workshop 2017.). Also the expression of FcγRIII was significantly declined in myeloma patients. Those findings suggest that the function of NK cells was suppressed in patients with multiple myeloma.

SLAMF7 and soluble SLAMF7

SLAMF7 is one of the SLAMF receptors (SLAMF1~9) which belongs to the CD2 subset of the immunoglobulin superfamily. SLAMF7 is widely expressed in myeloma cells and it is common knowledge that targeting SLAMF7 is effective in myeloma treatment. However, the precise role of SLAMF7 in the maintenance of myeloma cells is not known. In addition, it has been reported that the soluble form of SLAMF7 (sSLAMF7) was detected in the serum of multiple myeloma patients (Tai YT, et al.Blood. 2008;112:1329-1337). Also we have observed that sSLAMF7 levels were elevated in the serum of the newly diagnosed and relapsed myeloma patients, but not in the healthy donors (Hagiwara S, Sorimachi N. Unpublished data) (**Fig 1A**).

Figure 1



Toxic effect of sSLAMF7 on human NK cells

Importantly, in our preliminary experiments, the addition of sSLAMF7 to primary human NK cell culture (with sSLAMF7 concentration equivalent to that detected in relapse MM patient sera), triggered apoptosis-like cell death as shown in **Figure 1B**. This observation strongly suggests that increased sSLAMF7 in MM patients exerts an adverse effect on cancer immunosurveillance by NK cells. Since NK cells are expected to be one of the important effector lymphocytes against cancer cells, thanks to their efficient ADCC ability particularly in antibody-based immunotherapy, the understanding of sSLAMF7's significance in the context of NK cell biology is extremely important and urgent issue.

Effect of sSLAMF7 on transcriptome of NK cells

In another (unpublished) pilot study, we hypothesized that sSLAMF7 may affect the NK cells through activation of specific transcriptional programs. To this end we measured changes in the transcriptome of an NK cell line after treatment with sSLAMF7 and could verify that a distinct transcriptional response could be detected. Deeper analysis suggested that the most strongly activated pathways included immune cell related ones such as “immune response”, “immune system process”, and “immune system development”. Following up the results by analyzing primary human NK cells we observed that primary NK cells also change their gene expression patterns in the presence of sSLAMF7, although the genes affected and the magnitude of alterations of gene expression varied between individuals. Therefore, it is of great importance to perform larger scale experiments to pick up a functionally significant target molecules of sSLAMF7 in the primary NK cells of MM patients.

２．研究の目的

AIMEDIn this project, we aimed to obtain insights into the mechanism of sSLAMF7-mediated functional modulation of NK cells. The strategy was to examine, using primary human NK cells, which signaling pathways are activated by sSLAMF7 and which transcriptional programs are affected by the presence of sSLAMF7, and try to understand the mechanism of how sSLAMF7 regulate NK cell effector function in order to identify possible drug targets to recover NK cell activity in MM patient through gene expression analyses. To this end, we set out to analyze the relationship between sSLAMF7 serum levels and NK cell gene expression using NK cells from a cohort of 20 multiple myeloma patients and 10 healthy donors. The MM patients were divided into two groups: 10 with high sSLAMF7 serum levels (fresh/relapsed cases) and 10 with low levels (remission or stable cases). **The central hypothesis** was: *poor outcome in MM patients with high sSLAMF7 serum levels is linked to changes in NK cell gene expression programs*. **Key scientific questions** were: *what are the major differences in the transcriptional program between MM patients with high and low sSLAM7 levels, as well as healthy control subjects? Do sSLAMF7-mediated changes of NK cell gene expression programs affect the anti-MM activity of NK cells?*

３．研究の方法

Human primary NK cells were prepared from peripheral blood of myeloma patients and healthy volunteers by using AutoMACS according to the manufacture’s instruction. A subset of collected cells from each sample was lysed with SDS-sample buffer and western blot analyses were carried out to detect protein expression and to analyze phosphorylation-dependent signaling events. An additional subset was profiled for gene expression using the Cap Analysis of Gene Expression (CAGE) method. In CAGE, the 5’ end of mRNA is sequenced which allows for base pair resolution detection and expression quantification of promoter regions. CAGE captures expression at protein coding as well as non-coding expression, and is a well-established technology that has been used in hundreds of publications including several studies of promoter usage in normal and cancerous blood cells. CAGE libraries were created in multiplex, where each multiplex library contained 8 samples and was sequenced on one lane with illumina HiSeq 2500. Thus, 3 multiplex libraries was sequenced on 3 HiSeq lanes. Sequenced CAGE tags were mapped to the human genome followed by quality control and mapping to genomic regions for promoter and enhancer expression quantification using standard sequence and expression analysis software. Data quality was assessed based on comparisons with in-house as well as publicly available data.

４．研究成果

Using the CAGE data, we subsequently attempted to reconstruct the transcriptional regulatory network (TRN) that governs MM and is modulated by sSLAMF7, using methods previously developed by us that have been successfully used in clinical as well as in vitro settings. For identifying the TRN, promoters of differentially expressed genes were examined for the occurrence of transcription factor binding sites (TFBSs), which makes it possible to infer regulatory edges in the regulatory network. The initial results are currently being evaluated and subjected to validation assays.

５．主な発表論文等

〔雑誌論文〕　計0件

〔学会発表〕　計0件

〔図書〕　計0件

〔産業財産権〕

〔その他〕

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６．研究組織

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７．科研費を使用して開催した国際研究集会

〔国際研究集会〕　計0件

８．本研究に関連して実施した国際共同研究の実施状況

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