149. 化学療法耐性に対する腫瘍関連免疫細胞の役割の解明

Baghdadi　Muhammad

北海道大学 遺伝子病制御研究所

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緒　言

Cancer therapy is generally associated with limited clinical benefits without combined strategies to overcome the immunosuppressive microenvironment induced by tumors. Myeloid cells, in particular tumor-associated macrophages (TAMs) are considered as critical cellular drivers of immune escape in the tumor microenvironment. TAMS play important roles in the suppression of antitumor immunity, and contribute to tumor metastasis and resistance against cytotoxic therapies. Recent progress has unveiled the importance of colony-stimulating factor 1 receptor (CSF1R) in the differentiation and function of TAMs at tumor microenvironment. M-CSF is a ligand of CSF1R that has been found to be secreted by various tumors and correlated to TAMs differentiation and function. Importantly, we have found that under chemotherapeutic conditions, tumor cells also secrete another ligand of CSF1R, known as IL-34. IL-34 has been recently identified as a second ligand of CSF1R, which is involved in the survival and differentiation of human monocytes into immunosuppressive macrophages. This suggests that IL-34 produced by tumor cells under chemotherapeutic conditions may be involved in the generation of immunosuppressive macrophages that contribute to chemoresistance. Taken together, the identification and clarification of the role of IL-34 in tumor microenvironment can help to understand mechanisms of chemoresistance, and contribute to the improvement of current chemotherapy regimens.

方　法

In this research project, we hypothesize that IL-34 has a great impact on the differentiation status of myeloid cells in chemotherapy-treated tumor microenvironments, and thus affects the therapeutic effects mediated by anticancer cytotoxic agents. To evaluate this hypothesis, we established various tumor cell lines that have acquired resistance against anticancer cytotoxic agents. The supernatants of chemo-resistant cell lines were utilized to examine its effects on myeloid cells differentiation in comparison with chemo-sensitive cell lines. Furthermore, we utilized CRISPR-CAS9 system to generate IL-34 knock out cell lines. Additionally, These cell lines were transplanted into immunodeficient nude mice, and utilized to evaluate the infiltration and differentiation of myeloid cells, tumor growth and response to chemotherapy. We also examined signal pathways that contribute to IL-34 expression in tumor cells. Finally, we examined the expression of IL-34 in samples obtained from cancer patients, and evaluated the correlation between IL-34 expression and phenotype of tumor-associated macrophages, and its consequences on tumor progression and prognosis.

結　果

Regarding the role of IL-34 in chemotherapy-treated tumor microenvironment, we first identified a paracrine effect of IL-34 represented by recruiting high frequencies of M2-polarized TAMs and enhancing its immunosuppressive phenotype. Chemoresistance is significantly enhanced when chemotherapy increase – directly or indirectly – the proportion of M2-like TAMs, which in turn limit the efficacy of chemotherapy. Indeed, IL-34

secreted by chemoresistant lung cancer cells enhanced monocytes differentiation into M2-polarized macrophages *in vitro*.

Additionally, in a humanized mouse model, IL-34-producing chemoresistant tumors were infiltrated with increased frequencies of M2-like TAMs compared to IL-34-deficient chemoresistant tumors, which was negatively correlated with the frequencies of tumor-infiltrating cytotoxic $CD8^+$ T cells, indicating the importance of IL-34 in recruiting M2-like TAMs with potent abilities to suppress antitumor immune response under chemotherapeutic condition. Furthermore, TAMs showed enhanced expression levels of immunosuppressive and other chemo-protective factors in IL-34-producing chemoresistant tumors. The second role of IL-34 in chemoresistance is the autocrine effect on activating Akt signal pathway downstream of CSF1R expressed in chemoresistant cancer cells. Akt pathway is a signal transduction pathway that promotes survival and growth in response to external signal. In our experiments we found that the expression of CSF1R is upregulated and can be detected at protein levels in chemoresistant lung cancer cells. Upon binding to IL-34, CSF1R increases the phosphorylation of Akt, providing a critical signal that help cancer cells to survive under chemotherapeutic conditions. In addition to IL-34 induction by chemotherapy treatment, IL-34 can also be detected in lung cancer tissues of cancer patients with some variations, and correlated with poor prognosis when highly expressed (Figure.1).

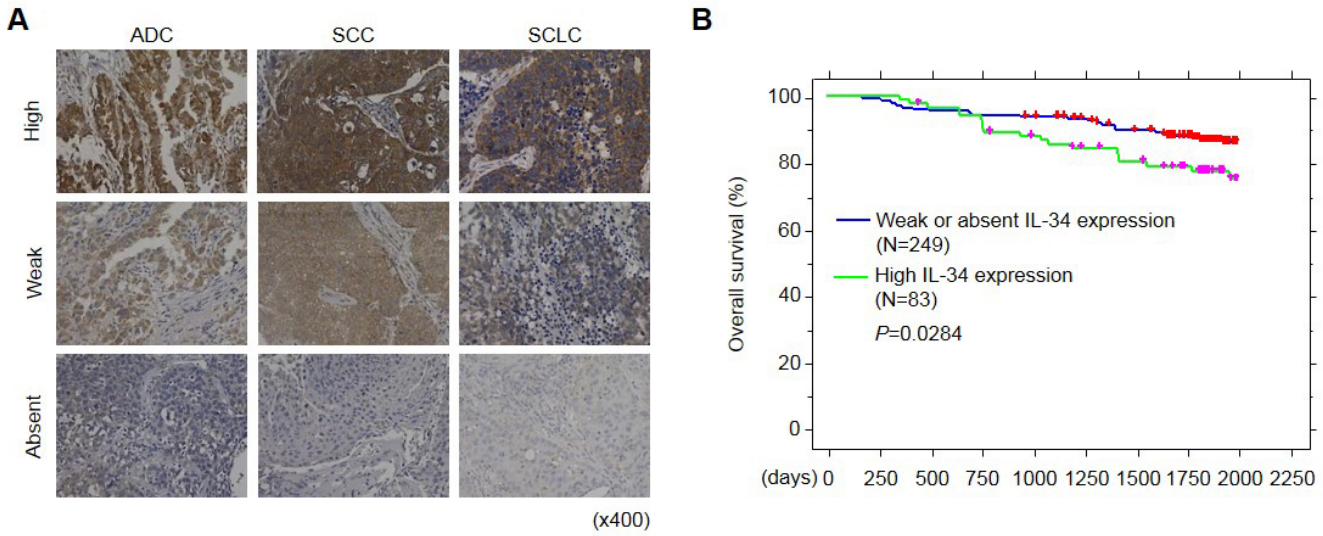

Fig. 1. IL-34 expression in lung cancers

IL34 expression in primary human lung cancers correlates with poor prognosis. (A) Immunohistochemistry staining of IL-34 in cancer tissues from lung cancer patients diagnosed with adenocarcinoma (ADC), squamous cell carcinoma (SCC) or small cell lung carcinoma (SCLC). (B) Kaplan-Meier analysis of overall survival in 332 lung cancer patients stratified as high (green line, n=83) or weak/absent (blue line, n=249).

考　察

Recent progress in understanding the relation between macrophage function and therapeutic resistance has helped to improve new therapeutic strategies based on the characteristics of tumor microenvironment. In this report, we provide evidence that chemotherapy-treated tumor microenvironments adopt a novel strategy to develop chemoresistance and suppress antitumor immunosurveillance by triggering IL-34 production in tumor cells.

IL-34 is a newly discovered cytokine, which share a common receptor (CSF1R) with M-CSF. Both cytokines mediate monocytes/macrophages survival and proliferation but also have distinct features. IL-34 plays important

roles in the pathogenicity of diseases associated with chronic inflammation such as viral infections and inflammatory bowel disease. In cancer, IL-34 was found to promote tumor progression and metastatic process of osteosarcoma via promotion of angiogenesis and macrophage recruitment.

Regarding the role of IL-34 in chemotherapy-treated tumor microenvironment, we first identified a paracrine effect of IL-34 represented by recruiting high frequencies of M2-polarized TAMs. IL-34 has the ability to induce monocytes differentiation into macrophages that exhibit M2 phenotype characterized by IL-10^{high} IL-12^{low} expression, low levels of the costimulatory molecules CD80 and CD86, and potent properties to suppress T cell response. Chemoresistance is significantly enhanced when chemotherapy increase – directly or indirectly – the proportion of M2-like TAMs, which in turn limit the efficacy of chemotherapy. Indeed, IL-34 secreted by chemoresistant lung cancer cells enhanced monocytes differentiation into M2-polarized macrophages in vitro. Additionally, in a humanized mouse model, IL-34-producing chemoresistant tumors were infiltrated with increased frequencies of M2-like TAMs compared to IL-34-deficient chemoresistant tumors, which was negatively correlated with the frequencies of tumor-infiltrating cytotoxic $CD8^+$ T cells, indicating the importance of IL-34 in recruiting M2-like TAMs with potent abilities to suppress antitumor immune response under chemotherapeutic condition. Furthermore, TAMs showed enhanced expression levels of immunosuppressive and chemo-protective factors in IL-34-producing chemoresistant tumors, suggesting that IL-34 also modulates TAMs functions in chemoresistant tumors in a way that benefit tumor survival under chemotherapeutic conditions.

The second role of IL-34 in chemoresistance is the unexpected autocrine effect on activating Akt signal pathway downstream of CSF1R expressed in chemoresistant cancer cells. Akt pathway is a signal transduction pathway that promotes survival and growth in response to external signal. Akt signaling was found to play critical roles in chemoresistance to cytotoxic agents such as paclitaxel and cisplatin in human cancers. Akt acts downstream of CSF1R to transduce signals from M-CSF and IL-34. In addition to myeloid cells, recent reports have suggested that CSF1R mRNA can be detected in some cancers like lung and breast cancer cells. In our experiments we found that the expression of CSF1R is upregulated and can be detected at protein levels in chemoresistant lung cancer cells. Upon binding to IL-34, CSF1R increases the phosphorylation of Akt, providing a critical signal that help cancer cells to survive under chemotherapeutic conditions.

In summary, we here identify a novel role for IL-34 produced by cancer cells following chemotherapy treatment in the formation of chemo-protective niche in a paracrine manner through the recruitment of pro-tumor M2-polarized TAMs, and autocrine effect via the enhancement and elongation of Akt-mediated survival signal downstream of CSF1R, and thus help to maintain chemoresistance in cancer cells, suggesting IL-34 as promising target in future therapeutic strategies.

文　献

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