様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９　（共通）

科学研究費助成事業　　研究成果報告書

平成　２９　年　　５　月　２４　日現在

機関番号：　１０１０１
研究種目：　若手研究(B)
研究期間：　2015～2016
課題番号：　１５Ｋ１８４３４

研究課題名（和文）Identification of new therapeutic targets to sensitize chemoresistant tumor microenvironment to chemotherapy

研究課題名（英文）Identification of new therapeutic targets to sensitize chemoresistant tumor microenvironment to chemotherapy

研究代表者

バグダーディ　ムハンマド（Baghdadi, Muhammad）

北海道大学・遺伝子病制御研究所・助教

研究者番号：６０７１１５７０

交付決定額（研究期間全体）：（直接経費）　　3,200,000円

研究成果の概要（和文）：抗がん剤耐性となったヒト肺がん細胞がIL-34を産生することを発見。IL34はがん組織内に免疫抑制型マクロファージを増加させるとともに、がん細胞自身にも働き、がん細胞の抗がん剤耐性を強めていることを発見。IL34を高発現するヒト肺がん患者では低発現である場合に比べ生命予後が不良であることを発見。IL34阻害により抗がん剤耐性となったがんに対しても奏功する治療として期待できる。

研究成果の概要（英文）：The ability of tumor cells to escape immune destruction and their acquired resistance to chemotherapy are major obstacles to effective cancer therapy. Although immune checkpoint therapies such as anti-PD-1 address these issues in part, clinical responses remain limited to a subpopulation of patients. In this report, we identified IL34 produced by cancer cells as a driver of chemoresistance. In particular, we found that IL34 modulated the functions of tumor-associated macrophages to enhance local immunosuppression and to promote the survival of chemoresistant cancer cells by activating AKT signaling. Targeting IL34 in chemoresistant tumors resulted in a remarkable inhibition of tumor growth when accompanied with chemotherapy. Our results define a pathogenic role for IL34 in mediating immunosuppression and chemoresistance and identify it as a tractable target for anticancer therapy.

研究分野：　腫瘍免疫学分野

キーワード：　Tumor microenvironment　Chemoresistance　Macrophages　Immunosuppression　Interleukin 34

１．研究開始当初の背景

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.

２．研究の目的

identification and clarification of the role of IL-34 in tumor microenvironment, which may help to understand mechanisms of chemoresistance, and contribute to the improvement of current chemotherapy regimens.

３．研究の方法

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.

４．研究成果

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.

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 can 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.

５．主な発表論文等
（研究代表者、研究分担者及び連携研究者には下線）

〔雑誌論文〕（計 1 件）

Chemotherapy-induced IL34 enhances immunosuppression by tumor-associated macrophages and mediates survival of chemoresistant lung cancer cells

Muhammad Baghdadi, Haruka Wada, Sayaka Nakanishi, Hirotake Abe, Nanumi Han, Wira Eka Putra, Daisuke Endo, Hidemichi Watari, Noriaki Sakuragi, Yasuhiro Hida, Kichizo Kaga, Yohei Miyagi, Tomoyuki Yokose, Atsushi Takano, Yataro Daigo, Ken-ichiro Seino

Cancer Research 76 (20): 6030-6042 (2016)
PMID: 27550451

Peer Reviewed

〔学会発表〕（計 6 件）

1. Muhammad Baghdadi, Sayaka Nakanishi, Haruka Wada, Ken-ichiro Seino
   Lung cancer cells-derived IL34

contributes to chemoresistance through CSF1R-mediated autocrine and paracrine signaling.
The 20th Korea-Japan Cancer Research Workshop
Tokyo 2015

2. Chemotherapy-induced IL34 accelerates the formation of tumor-associated macrophages (TAMs) in human tumor microenvironment.
International Conference of Cancer Immunotherapy and Macrophages
Tokyo 2015

3. Muhammad Baghdadi, Sayaka Nakanishi, Haruka Wada, Ken-ichiro Seino
IL34 accelerates the formation of immunosuppressive macrophages in chemoresistant tumors.
The 44th Annual meeting of the Japanese Society of Immunology
Sapporo 2015

4. Muhammad Baghdadi
Chemotherapy-induced IL34 enhances immunosuppression by tumor-associated macrophages and mediates survival of chemoresistant lung cancer cells.
平成 27 年度　がん研究分野の特性等を踏まえた支援活動　公開シンポジウム
東京　2016

5. Muhammad Baghdadi, Haruka Wada, Ken-ichiro Seino
A Novel pathogenic role of IL34 in cancer chemoresistance
The 45th annual meeting of the Japanese Society for Immunology
Okinawa 2016

6. Muhammad Baghdadi, Hiraku Endo, Kozo Ishikawa, Haruka Wada, Ken-ichiro Seino
IL34-modified tumor-associated macrophages suppress anti-tumor T cell response at the local tumor microenvironment.
The 7th International Workshop of Kyoto T Cell Conference
Kyoto 2017

〔図書〕（計 0 件）

〔産業財産権〕

○出願状況（計 0 件）

名称：
発明者：
権利者：
種類：
番号：
出願年月日：
国内外の別：

○取得状況（計　　件）

名称：
発明者：
権利者：
種類：
番号：
取得年月日：
国内外の別：

〔その他〕
ホームページ等

６．研究組織

(1)研究代表者
バグダーディー・ムハンマド
（Muhammad Baghdadi）
北海道大学遺伝子病制御研究所
助教
研究者番号：60711570

(2)研究分担者
(　　)

研究者番号：

(3)連携研究者
(　　)

研究者番号：

(4)研究協力者
(　　)