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

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研究課題名（和文）Functional study of a novel homodimeric IL23A produced by epithelial cells

研究課題名（英文）Functional study of a novel homodimeric IL23A produced by epithelial cells

研究代表者
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研究成果の概要（和文）：IL23Aは炎症応答を制御するIL-23の構成分子であり、主に白血球が産生する。最近、申請者は、上皮細胞もIL23Aを産生・分泌することを見出したが、この上皮細胞に由来するIL23A（eIL23A）の機能は不明である。
マウス由来白血球を用いた実験から、炎症誘導型サイトカインを刺激する古典的IL‐23の活性は、eIL23Aの投与により増強することが分かった。このeIL23Aの機能は、腫瘍免疫応答の増強に付与していることを動物レベルで明らかにした。これらの結果より、eIL23Aは将来的に免疫治療法の改善に貢献する重要な分子であることが強く期待される。

研究成果の学術的意義や社会的意義
慢性炎症はクローン病、乾癬、炎症性腸疾患、リウマチ疾患などの自己免疫疾患の原因である。また、慢性炎症は胃がんなどの難治性疾患の原因であることが知られている。免疫応答で中心的な役割を果たすIL-23の活性制御に関わるeIL23Aの機能を解明により、免疫応答異常に起因する様々な疾患の治療戦略の開発に貢献する。

研究成果の概要（英文）：The cytokine IL23A is usually produced by leukocytes to promote inflammation. However, we discovered that IL23A is also produced by epithelial cells. Moreover, the epithelial form of IL23A (eIL23A) is different from conventional IL-23. We have purified eIL23A and here we proposed to study its immune functions.
Using mouse leukocytes, we found that eIL23A strongly enhances the effects of canonical IL-23 to cause peritoneal exudate cells (PEC) to produce more IL17A/F, a powerful inducer of inflammation. We observed that when human colorectal cancer cells are activated or carry certain mutations, they will produce eIL23A. This is a definitive evidence for a new form of IL23A. Moreover, when we forced expression of IL-23 in cancer cells, tumor immunity is activated. This is further enhanced when eIL23A is also provided. These data means eIL23A can be used to increase tumor immunity. We believe the findings of this project provide a strong basis to improve immunotherapy in the future.

研究分野：Inflammation and cancer

キーワード：Inflammatory cytokine　tumorigenesis　Tumor immunity

様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９（共通）

１．研究開始当初の背景

The proinflammatory cytokine IL23A is a central mediator of tissue inflammation. Its causal importance to human autoimmunity and autoinflammatory conditions, such as psoriasis, Crohn's Disease, IBD, rheumatoid arthritis and celiac disease is well supported by extensive mouse data; as well as the strong genetic association between IL23R polymorphism and human patients. A major caveat in existing mouse genetics experimental data is that they were performed using conventional (whole body) *Il23a* knockout mice with the assumption that IL23A is produced exclusively by macrophages and dendritic cells. However, this assumption is inaccurate as mounting evidence from our group and others that IL23A is also produced by epithelial cells during inflammation, infection and tissue injury. Additionally, our group showed previously that IL23A is secreted in a novel form distinct from canonical IL-23 (i.e. IL23A/IL12B) heterodimer. Anti-IL23A antibodies are current the frontline treatment for psoriasis in the clinics and in clinical trials for the treatment of other conditions. In view of the pathological and therapeutic importance of IL-23/IL23A pathway, there is an urgent need to better understand the function of epithelial-derived IL23A (eIL23A).

２．研究の目的

We aimed to clarify the immunological function of eIL23A through a combination of in vitro and ex vivo approaches. We are especially curious as to whether eIL23A would augment or antagonize canonical IL-23. We are also interested in identifying the target immune cells of eIL23A. Lastly, we posit that eIL23A produced by tumor cells bearing an activating mutation would alter tumor immunity in the tumor microenvironment, and wish to test this.

３．研究の方法

In the first part, we aim to generate and purify recombinant eIL23A. Next, we seek to establish suitable cell models, using cultured human IL23R-positive cells or primary mouse leukocytes, for the testing of eIL23A function. In the case of the latter, we plan to identify the target cells of eIL23A. Lastly, we planned to investigate the effects of eIL23A on tumor immunity by transplanting syngeneic mouse intestinal cancer cells or cancer organoids in wild type immune-competent mice to address the question of whether eIL23A affects tumor immunity.

４．研究成果

1) We confirm the secretion of endogenous eIL23A by activated human colorectal cancer (CRC) cell lines in a novel form that is independent of its canonical partner protein, IL12B. We identified two important upstream signals necessary to induce its secretion, namely the proinflammatory TNF/NF-κB and mitogenic MAPK pathways. As these two pathways are frequently activated by gene mutations in human CRC, we explored and demonstrated the notion that CRC cells with activating BRAF mutations have higher eIL23A production that can be attenuated by small chemical inhibitors against either pathway. Lastly, we elucidated the molecular mechanisms for their strong cooperation, namely that they synergistically facilitate the formation of a transcriptional enhancer complex at the proximal *IL23A* gene promoter. This work was published in the *Journal of Biological Chemistry* [1];

2) Through the use of recombinant eIL23A, we demonstrated that eIL23A acts to strongly augment canonical IL-23 in primary mouse peritoneal exudate cells (PEC) to induce IL-17 in an innate manner without the need for activating T cell receptor (TCR). Accordingly, the target cells appear to be IL23R-positive CD5+ cell population. This ex vivo cell model will prove invaluable to future studies on

the molecular mechanism of eIL23A-IL-23 synergism;

3) We showed in two separate syngeneic cancer transplantation models, using CT-26 or primary intestinal cancer organoids, that ectopic IL-23 elicits potent anti-tumor activities. Of note, the anti-tumor activity is activated after tumor formation and in many cases is curative, i.e. the tumors are completely eradicated. Importantly, we showed that eIL23A further augments the anti-tumor activity elicited by IL-23 and significantly improves the survival of transplanted mice. Our analysis also showed that the strong correlation between $IL23R^{+}CD5^{+}$ leukocytes and IL-17 production, and their likely role in IL-23/eIL23A induced anti-tumor activities. We believe this observation provides an important basis for future studies to exploit this function in the development of future immunotherapy.

５．主な発表論文等

〔雑誌論文〕　計2件（うち査読付論文　2件／うち国際共著　2件／うちオープンアクセス　0件）

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２．論文標題 Inflammatory and mitogenic signals drive interleukin 23 subunit alpha (IL23A) secretion independent of IL12B in intestinal epithelial cells	５．発行年 2020年
３．雑誌名 Journal of Biological Chemistry	６．最初と最後の頁 6387～6400
掲載論文のDOI（デジタルオブジェクト識別子） 10.1074/jbc.RA120.012943	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 該当する

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オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 該当する

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〔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

共同研究相手国	相手方研究機関			
シンガポール	Cancer Science Institute of Singapore	National University of Singapore		