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研究課題名（和文）Immunological Mechanisms of Synergistic Anti-cancer Activities by Activation of TLR9 and STING

研究課題名（英文）Immunological Mechanisms of Synergistic Anti-cancer Activities by Activation of TLR9 and STING

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研究成果の概要（和文）：Toll様受容体9（TLR9）とインターフェロン遺伝子刺激因子（STING）アゴニストは、抗がん剤やワクチンアジュバントとして治療適用される。しかし、臨床的に利用可能なTLR9アゴニストはインターフェロン誘導が弱く、STINGアゴニストは好ましくないII型免疫応答を誘導するため、臨床応用が制限される。。今回はさらに、インターロイキン-12（IL-12）とI型インターフェロンの相互作用に関するメカニズムを通して、局所的なコンビネーション治療が、すい臓がんモデルにおける強力かつ長期的効果の見込める抗腫瘍免疫応答を賦活化することを示した。

研究成果の学術的意義や社会的意義
Identifying the mechanisms mediating synergistic as well as anti-tumor effect of combination of TLR9 and STING agonists is important. Because this may aid in development of novel treatment strategies based on combinatorial use of such adjuvants for cancer immunotherapy.

研究成果の概要（英文）：TLR9 and STING agonists offer therapeutic applications both as antitumor agents and vaccine adjuvants. Yet, clinically available TLR9 agonist is a weak IFN inducer and STING agonists induce undesired type 2 immunity, restricting their clinical applications. We previously showed that TLR9 and STING agonists synergized for induction of innate and adaptive IFNγ and became an advantageous type 1 adjuvant and robust antitumor agent. Here,we further show that local combination treatment promoted strong long term antitumor immunity in pancreatic cancer model via the mechanisms involving cooperative action of IL-12 and type I IFNs. Mechanistically, combination synergistically induce IL-12 and type I IFNs in APCs. By focusing on the Th1-inducing cytokine IL-12, we found that synergistic effect of the combination on IL-12p40 is transcriptionally regulated and observed on protein,mRNA and promoter activation levels. Thus,our combination may offer therapeutic applications as a potent Th1-inducer.

研究分野：Cancer immunotherapy

キーワード：TLR9　STING　Synergy　Antitumor　Combination　IL-12

１．研究開始当初の背景

STING is a key molecule required for several cytosolic nucleic acid-sensing pathways, such as cyclic guanosine monophosphate-adenosine monophosphate (cGAMP) synthase (cGAS) pathway, which can detect tumor-derived, microbial or even host DNA, and produce a secondary signaling molecule, 2'3' cGAMP that can directly bind to STING and activate TBK1-IRF3 axis to induce production of type I IFNs, therefore acting as an adjuvant capable of inducing antigen-specific B and T cell responses (Zhang et al., 2013 and Sun et al., 2013). On the other hand, agonists of TLR9 that can detect endosomal microbial DNA act as type 1 adjuvants (Krieg, 2006). Yet, as type 1 immune responses and IFNs are advantageous for fighting against intracellular pathogens and tumors, therapeutic applications of both STING and TLR9 agonists are limited due to induction of type 2 immunity by the STING agonists and weak IFN induction by the clinically available TLR9 agonist (Klinman, 2004 and Tang et al., 2013). Thus, by taking the advantage of combinatorial use of these two adjuvants, our previous study indicated that synergistic effect of TLR9 and STING agonists for innate as well as adaptive IFNγ induction makes this combination a potent type 1 adjuvant capable of suppressing type 2 immunity in addition to becoming a strong anti-tumor agent when used as a monotherapeutic agent in explanted tumor models of melanoma and thymoma (Temizoz et al., 2015). Furthermore, intracellular mechanisms of action of the combination has been revealed as summarized in Figure 1.

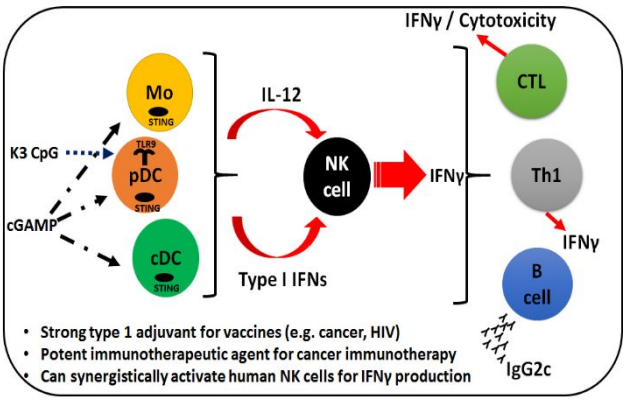

Fig. 1. Mechanisms of action of combination of TLR9 and STING agonists. K3 CpG can only stimulate pDCs for type I IFN production while cGAMP can activate many types of cells including monocytes/macrophages, pDCs and cDCs to induce high amounts of type I IFNs and IL-12 production. Together IL-12 and type I IFNs act on their receptors on NK cells to synergistically induce IFNγ production. These responses culminate in development of robust antigen-specific CTL, Th1 type T cell and B cell responses that are crucial for adjuvant as well as anti-tumor effect of the combination (Temizoz et al., 2015).

<u>According to our findings summarized above, key questions remained to be answered are:</u>

1- What are the intracellular/molecular mechanisms mediating the synergistic effect of combination of TLR9 and STING agonists especially for Th1-/type 1 immunity-related cytokine induction?

2- What is the breadth of anti-tumor immune responses induced by this combination?

3- What are the immunological mechanisms mediating strong anti-tumor effect of the combination?

Our preliminary data showed that synergistic effect of TLR9 and STING agonists for the induction of Th1-inducing cytokines IL-12 and type I IFN from dendritic cells (DCs) is mediated via some intracellular mechanisms, which remained to be clarified, requiring the simultaneous activation of TLR9 and STING within the same cells (Fig. 2).

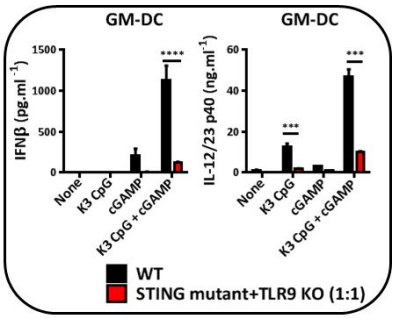

Fig. 2. Combination of TLR9 and STING agonists synergistically induce production of Th1-inducing cytokines IL-12 and type I IFN in DCs via the mechanisms requiring TLR9 and STING signaling pathway activation within the same cells. Stimulation of mixture of TLR9 KO and STING KO DCs significantly decreases the synergistic effect of the combination (unpublished).

In vivo, by using a more aggressive and clinically relevant pancreatic cancer model, we found that our combination also exerts a potent anti-tumor effect in Pan02 peritoneal dissemination model to result in 100% survival rates. More importantly, our re-challenge studies using these long term survivor mice suggested that combination can induce anti-tumor memory responses that can protect the mice from a secondary tumor re-challenge, emphasizing the potency of this combination (Fig. 3).

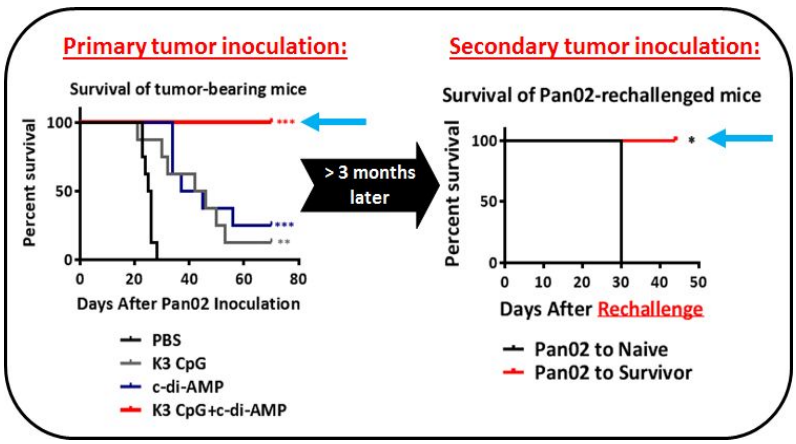

Fig. 3. Treatment of the Pan02-bearing mice with combination of K3 CpG and c-di-AMP provided 100% protection to mice. Moreover these long term survivors are also protected from a secondary Pan02 re-challenge suggesting the development of protective anti-tumor memory responses induced by the combination treatment (unpublished).

２．研究の目的

Aim 1: Elucidation of the molecular mechanisms of synergistic effect of TLR9 and STING agonist combinations on cytokine production (especially Th1-inducing cytokines, such as IL-12) in innate immune cells.

Aim 2: Identification of the immunological mechanisms mediating in vivo anti-tumor effects of combination of TLR9 and STING agonists (which will be used as a monotherapeutic agent without antigen injection) in murine tumor models of pancreatic cancer with a focus on both the primary and secondary (memory) anti-tumor responses.

３．研究の方法

Molecular mechanisms of cytokine synergy mediated by the combination of TLR9 and STING agonists will be investigated by using several cell lines, including J774 dual reporter cells and RAW264.7 cells stably expressing IL-12p40 promoter reporter constructs in order to elucidate the levels of combination-mediated synergy (e.g. at promoter activation, mRNA and protein production levels).

Mechanisms of anti-tumor effect of the combination of TLR9 and STING agonists will be analyzed by using mainly Pan02 peritoneal dissemination model. In particular, several knockout (KO) mice, such as the mice lacking the genes for IL-12p40 and type I IFN receptor, will be used. In addition, several depletion or adoptive transfer of the specific cell types will be performed in tumor-bearing mice for identification of the cell types involved in the mechanisms mediating both primary and secondary (memory phase) anti-tumor effects of the combination. Also specificity of the memory responses will be evaluated by re-challenging the survivor mice with irrelevant tumors like B16 F10 or EG-7. Moreover, several injection routes and schedules will be tested for optimizing/maximizing the anti-tumor effect of the combination in Pan02 peritoneal dissemination model.

４．研究成果

TLR9 and STING agonists offer therapeutic applications both as anti-tumor agents and vaccine adjuvants. Yet, clinically available TLR9 agonist is a weak IFN inducer and STING agonists induce undesired type 2 immunity, restricting their clinical applications. Our previous study, taking the advantage of combinatorial use of these adjuvants, showed that TLR9 and STING agonists synergized for induction of innate and adaptive IFNγ and became an advantageous type 1 adjuvant, suppressing type 2 immunity, in addition to exerting robust anti-tumor activities when used as a monotherapeutic agent for cancer immunotherapy. Here, we further show that local combination treatment promoted strong anti-tumor immunity in a clinically relevant murine tumor model via the mechanisms involving co-operative action of certain cytokines. Moreover, rechallenge studies in the long term survivors suggested the elicitation of tumor-specific memory responses that provide protection against secondary tumor challenge. Mechanistically, we found that combination of TLR9 and STING agonists synergistically induce certain cytokines, which are important for the antitumor effect of the combination, from APCs. By focusing on that certain Th1-inducing cytokine, we found that synergistic effect of the TLR9 and STING agonists on that cytokine are observed on protein, mRNA and promoter activation levels and the synergy is transcriptionally regulated by a region upstream of that certain cytokine. Thus, elucidation of the mechanisms mediating synergistic anti-tumor activities of TLR9 and STING agonists may open new doors for the development of combinatorial intervention strategies for immunotherapy of cancer.

５．主な発表論文等

〔雑誌論文〕　計1件（うち査読付論文　1件／うち国際共著　0件／うちオープンアクセス　1件）

１．著者名 Temizoz Burcu、Hioki Kou、Kobari Shingo、Jounai Nao、Kusakabe Takato、Lee Michelle S J、Coban Cevayir、Kuroda Etsushi、Ishii Ken J	４．巻 34
２．論文標題 Anti-tumor immunity by transcriptional synergy between TLR9 and STING activation	５．発行年 2022年
３．雑誌名 International Immunology	６．最初と最後の頁 353～364
掲載論文のDOI（デジタルオブジェクト識別子） 10.1093/intimm/dxac012	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 －

〔学会発表〕　計4件（うち招待講演　2件／うち国際学会　2件）

１．発表者名 Temizoz B.
２．発表標題 Combination and inducible adjuvants targeting nucleic acid sensors
３．学会等名 Gakuyukai Seminar at The Institute of Medical Science The University of Tokyo (IMSUT), Tokyo, Japan（招待講演）
４．発表年 2019年

１．発表者名 Temizoz, B., Hioki, K., Takayuki, S., Kobari, S., Jounai, N., Kusakabe, Lee, MSJ., T., Coban, C., Kuroda, E., and Ishii, K. J
２．発表標題 Immunological mechanism of synergistic anti-cancer activities by activation of TLR9 and STING
３．学会等名 13th Vaccine Congress, Bangkok, Thailand（国際学会）
４．発表年 2019年

１．発表者名 Temizoz, B., Hioki, K., Takayuki, S., Kobari, S., Jounai, N., Kusakabe, Lee, MSJ., T., Coban, C., Kuroda, E., and Ishii, K. J.
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１．発表者名 Temizoz B.
２．発表標題 DMXAA derivatives as potential immunotherapeutics for autoinflammation
３．学会等名 Young Investigators Symposium at The Institute of Medical Science The University of Tokyo (IMSUT), Tokyo, Japan（招待講演）
４．発表年 2020年

〔図書〕　計0件

〔出願〕　計1件

産業財産権の名称	発明者	権利者
TH1-inducing adjuvant comprising combination of different nucleic acid adjuvants, and use of same.	Ishii K., Kuroda E., Temizoz B.	同左
産業財産権の種類、番号	出願年	国内・外国の別
特許、11058758	2021年	外国

〔取得〕　計0件

〔その他〕

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６．研究組織

	氏名 （ローマ字氏名） （研究者番号）	所属研究機関・部局・職 （機関番号）	備考

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