学位論文内容の要旨

博士の専攻分野の名称　博士（医　学）　氏 名　Muhammad Baghdadi

学 位 論 文 題 名

TIM-4 blockade augments therapeutic efficacy of Cancer therapy by immune-mediated mechanisms

（TIM-4 阻害は免疫介在性メカニズムによって癌治療効果を増強する）

【Background】

Antigen-presenting cells (APCs) play a central role in regulating both innate and adaptive immunity and have a major role in the initiations of immune responses against tumor. However, it is now clear that APCs within tumor microenvironment are characterized by functional deficiencies associated with impaired antitumor response. Thus, it is critically important to understand the mechanisms by which APCs interact with the tumor microenvironment in order to improve the clinical output of cancer therapy.

The T-cell immunoglobulin domain and mucin domain (*TIM*) family of genes consists of Type I cell surface molecules with a unique structure and important broad immune functions that include the regulation of allergy and asthma, and also the regulation of autoimmunity, transplantation immunity, and tumor immunity. Recent studies have revealed TIM-3 as a negative regulator of antitumor immunity, which blockade enhances immune responses against tumor.

TIM-4 is expressed exclusively in APCs, and regulates of engulfment of apoptotic cells by APCs through interaction with phosphatidylserine on apoptotic cells. Recent analysis of TIM-4-deficient mice has demonstrated that TIM-4 serves as a negative regulator of inflammation and autoimmunity. However, the role of TIM-4 in regulating antitumor immunity remains unknown.

【Purpose】

In this study we evaluate the role of TIM-4 in regulating APCs-mediated antitumor immunity. We also examine the impact of TIM-4 blockade on the therapeutic effects of cancer therapy. Finally, we evaluate the therapeutic effects of the combined blockade of TIM-3 and TIM-4 on enhancing antitumor immunity to produce durable clinical responses.

【Methods】

To explore a potential role for TIM-4 in regulating antitumor responses of APCs at the tumor microenvironment, we examined the expression of TIM-4 in dendritic cells and macrophages isolated from murine tumors or cancer patients. To evaluate the role of TIM-4 on the key functions

of APCs, we utilized a monoclonal antibody (RMT4-53) which targets and blocks the functional IgV domain of TIM-4, and examined the impact of TIM-4 blockade on cytokine production (IL-12, IL-10) and tumor-derived antigen presentation to $CD8^+$ effector T cells in $TIM\text{-}4^+$ DCs or macrophages. We also evaluate the impact of TIM-4 blockade on the therapeutic effects of cancer therapy through the combination between anti-TIM-4 mAbs and anticancer drugs like cisplatin, doxorubicin or dacarbazine, or through the combination with cancer vaccines like irradiated Flt3L secreting B16 tumor cells (FVAX), and DNA vaccines which encodes tumor-associated antigens sequences against established tumors. Finally, we asked if the combined blockade of TIM-3 and TIM-4 could further enhance antitumor immunity to produce durable clinical responses.

【Results】

TIM-4 was found to be highly induced in tumor-infiltrating DCs and macrophages in the tumor microenvironment by tumor-derived immunosuppressive factors such as IL-10 and VEGF-A. The expression of TIM-4 in tumor-infiltrating DCs and macrophages was found to induce the production of anti-inflammatory cytokine IL-10, while it suppresses the production of pro-inflammatory cytokine IL-12. TIM-4 expressing APCs were also shown to have impaired presentation of tumor-derived antigens to $CD8^+$ effector T cells.

The blockade of TIM-4 with a monoclonal antibody was found to enhance the production of IL-12, inhibit IL-10 production, and increase the capacity of $TIM\text{-}4^+$ DCs or macrophages to induce tumor-specific T cells immune response. The treatment with anti-TIM-4 mAb synergizes with cytotoxic chemotherapy or cancer vaccine to suppress the *in vivo* growth of established tumors by triggering effective cross-priming of antigen-specific CTLs.

Additionally, we found that the combined blockade of TIM-3 and TIM-4 mAbs has markedly increased vaccine-induced antitumor responses against established B16 melanoma. TIM-3 blockade mainly stimulated antitumor effector activities via NK cell-dependent mechanisms, while $CD8^+$ T cells served as the main effectors induced by anti-TIM-4 mAbs.

【Discussion & Conclusions】

In this study we identified TIM-4 as a critical factor for repressing antitumor immune responses mediated by antigen-presenting cells upon treatment with cytotoxic chemotherapy or cancer vaccines. TIM-4 blockade synergizes with cytotoxic chemotherapy or cancer vaccines to suppress the *in vivo* growth of established tumors by $CD8^+$ T cells-mediated mechanisms.

TIM-3 blockade was also found to enhance the therapeutic efficacies of cancer vaccines via NK cell-dependent mechanisms. Furthermore, the combination between anti-TIM-3 and anti-TIM-4 has markedly increased vaccine-induced antitumor responses against established B16 melanoma.

In summary, we have unveiled distinct roles for TIM-3 and TIM-4 in the regulation of innate and adaptive antitumor immunity. The molecular targeting of TIM-3 and TIM-4 provides a new therapeutic strategy to augment antitumor efficacy of immunotherapy and eradicate therapy-difficult tumors through the coordinated activation of innate and adaptive antitumor immune responses in tumor microenvironments.