INVESTIGATING MECHANISMS OF TUMOR DEVELOPMENT USING 4T1 CANCER MODEL IN THE VIEW OF CANCER IMMUNITY AND ENERGY METABOLISM

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（4T1 移植モデルを用いたがん免疫およびエネルギー代謝機構の研究）

Title

区　　分　：Kou

Category

論 文 内 容 の 要 旨

Thesis Summary

Cancer treatment faces a number of challenges ranging from low efficacy to failure to benefit all cancer patients. Cancer cells grow at a fast rate primarily due to their ability to evade the immune surveillance. However, mechanisms by which tumors evade immune surveillance remain incompletely understood. This study had two aims: 1) to investigate the immunosuppressive activity of soluble factors secreted by 4T1 and CT26 tumor cells and 2) to investigate altered metabolism in cancer cells as a target for bongkrekic acid (BKA), a potential anti-cancer agent.

Chapter 1 introduces cancer immunity and some of the mechanism through which the tumor cells evade surveillance by the immune system. Mechanisms such as abnormalities in tumor antigen presentation, creation of a tolerant microenvironment by tumors, activation of negative regulatory checkpoints in the tumor microenvironment, recruitment of immunosuppressive cells and secretion of immunosuppressive cytokines and soluble inhibitory factors are elaborated. In this chapter, cancer metabolism is also introduced focusing on glycolysis which is one of the targeted pathways by some of antitumor agents. Since BKA is known as an inhibitor of adenine nucleotide translocase (ANT) inhibitor, the structure of the mitochondria and the ANT are introduced.

Chapter 2 focuses on the studying immunosuppressive factors secreted by the murine mammary tumor (4T1) cells. These cells have previously been reported to secrete immunosuppressive soluble factor(s), which was identified to be a 10-100 kDa protein and further analysis of 4T1-conditioned media showed that macrophage colony-stimulating factor (M-CSF) is one of the immunosuppressive factors present in the active fractions. Surprisingly, there are no reports on immunosuppressive activity of M-CSF. This chapter, therefore, details the methods that were used to study M-CSF activity and presents some of the key findings. Reduced levels of interferon gamma (IFN-γ) in the presence of M-CSF were observed in a dose-dependent manner indicating suppressive activity of M-CSF. M-CSF reduces the secretion of IFN-γ without affecting its intracellular protein expression. The activity was dependent on the length of days after tumor inoculation and this correlated with emergence of myeloid cells in the spleen. M-CSF activity is, therefore, thought to be mediated by the emerging myeloid cells during tumor progression.

In chapter 3, using another tumor cell line, CT26 colon cancer cells, the presence of secreted immunosuppressive is tested. Details on the methods used to fractionate the CT26 conditioned media are explained and the results are also shown. Although different approaches were used to try and isolate the active compounds, the results are still not satisfying. However, it is clear that, there are suppressive factors are secreted by these cells and better methods may enhance their isolation and identification.

Chapter 4 focuses on altered cancer cell metabolism, which is one of the hallmarks of cancer cells, and how this can be exploited as a target for cancer therapy. Altered metabolic activities including high rate of glycolysis

and reduced mitochondrial respiration. These alterations have attracted great attention and are explored as potential therapeutic targets. Here, activity BKA was investigated using 4T1 cancer cells and compared with NIH3T3 cells, which are normal cells. The experimental methods are explained and the key findings are presented. Reduced viability was associated with reduced ATP and NADH levels in 4T1 cells with no effect on NIH3T3 cells. Blockade of ANT resulted in reduced ATP in the cells and caused cells to rely solely on glycolysis. Tumor cells are more sensitive to BKA since they have a higher rate of glucose consumption compared with normal cells. Combining BKA with a glycolysis inhibitor such as 2-deoxyglucose (2-DG) enhanced the cytotoxic effect of BKA in 4T1 cells.

The final chapter 4 is the conclusion of the study. The results presented here have demonstrated that tumor-derived M-CSF is a significant contributor of immune suppression and strategies to block M-CSF activity in tumor cases may help reduce tumor-induced immune suppression, and consequently enhance tumor immune responses. On the other hand, BKA was shown to specifically reduce viability of tumor cells and its activity was enhanced in combination with glycolysis inhibitor. These results suggest that BKA is a potential anticancer agent. Taken together, enhancing tumor immune response by blocking tumor-mediated suppressive mechanisms combined with effective anti-cancer agents may be a more efficient way to eradicate cancer and enhance the survival rate of cancer patients.