Press Release

Epirubicin-Loaded Nanomedicines Beat Immune Checkpoint Blockade Resistance in Glioblastoma

Summary:
A nanomedicine-based strategy for chemo-immunotherapy (CIT) of glioblastoma (GBM), which has the worst prognosis among brain tumors, was successfully developed. *In vivo* experiments demonstrated that the combined use of epirubicin-encapsulating nano-micelles (Epi/m) with immune checkpoint inhibitors (ICI) eradicated PTEN-negative GBM, which is highly resistant to ICI alone. Due to the synergistic effects of Epi/m plus ICI combination, the number of tumor-infiltrating T cells (TIL) and other antitumor immune cells significantly increased to kill cancer cells effectively. On the other hand, intratumoral bone marrow-derived immunosuppressive cells (MDSC), which interfere with the immune response, were significantly reduced. The CIT also provided robust immunological memory effects against the tumors, which effectively rejected newly implanted PTEN-negative GBM cells in the brain. While free epirubicin can cause damage to organs, including hematopoietic organs especially, our nanomedicine strategy significantly reduced these side effects, improving the immune response. Epi/m has already advanced into clinical trials for other cancer types, and this CIT strategy could be expected to be translated to clinical evaluation in the future. These results has been published in ACS Nano (Impact Factor = 14.588 in 2019) issued on August 6 by the American Chemical Society.

The Innovation Center of Nanomedicine ((Director: Prof. Kazunori Kataoka, Location: Kawasaki-City, Abbreviation: iCONM) announced that a new therapeutic option for glioblastoma (GBM) was demonstrated in mice, in a collaboration study with the Department of Bioengineering, Graduate School of Engineering, The University of Tokyo. GBM is a brain tumor with extremely rapid progression and poor prognosis (5-year survival rate: 10.1%). Although several compounds are being evaluated in clinical studies, there is no therapeutic option to significantly improve the survival period. In particular, patients with abnormalities in the PTEN gene (Note 1), one of the cancer suppressor genes, are highly resistant to currently available therapies and have high medical needs. In general, immune checkpoint inhibitors (ICIs) (Note 2) are considered to ineffective against GBM, as GBM is immunosuppressive with low T cell infiltration. In the method presented in this paper,

iCONM's nano-drug delivery technology allows selective tumor accumulation of epirubicin, which causes immunogenic cell death (ICD) (Note 3), to tumor tissues, thereby, causing ICD locally for synergizing with ICI. As a result, this nanomedicine-based chemo-immunotherapy (CIT) (Note 4) was effective in mice transplanted with GBM in the brain (hereinafter referred to as mouse GBM model), and succeeded in significantly prolonging mice survival. The combination of the epirubicin-loaded nano-micelles treated mice showed high infiltration of cytotoxic T cells (TIL) (Note 5) and decreased bone marrow-derived immunosuppressive cells (MDSC) (Note 6). Eventually suppression of the immune checkpoint function was observed.

Mutations in the PTEN gene occur frequently in GBM, resulting in immunosuppressive pathways that promote the resistance to ICIs. Thus, while ICIs eradicated 40% of tumors in a mouse GBM model in which the PTEN gene is normal, in a model in which the PTEN gene was knocked-out, ICIs were unable to extend mice survival. At the cellular level, it was found that PTEN-deficient cells (CT2A-luc) expressed approximately 5-fold more PDL1 than that of normal cells, which is probably connected to the therapeutic resistance with ICI. As epirubicin have shown the ability to suppress PDL1 expression in tumors, such as breast cancer (Note 7), it would be possible to decrease PDL1 levels of GBM if sufficient amount of epirubicin can be delivered into GBM lesions. Thus, CIT using nanomicelles containing epirubicin (Epi/m) in combination of ICI (Note 8) were used for enhancing the antitumor efficacy against GBM.

In a GBM model with normal PTEN expression (GL261-luc), Epi/m (5 mg/kg on Epi basis) plus anti-PD1 antibodies (5 mg/kg) resulted in the survival of all mice for more than 70 days, with a remarkable extension of survival time. In this model, PBS-treated mice died within 30 days, mice treated with anti-PD1 antibodies alone (5 mg/kg) allowed 40% of mice to survive for at least 70 days, and Epi/m (5 mg/kg of Epi basis) resulted 80% of mice survival for more than 70 days. In contrast, in the PTEN-deficient model (CT2A-luc), Epi/m (5 mg/kg on Epi basis) plus anti-PD1 antibodies (5 mg/kg) resulted in only 30% of mice survival for more than 70 days, and no clear survival effect could be confirmed for the other control groups. When the dose was increased to 15 mg/kg of Epi/m (in Epi basis) and combined with anti-PD1 antibodies (5 mg/kg), 90% of mice were able to survive for more than 70 days, remarkably prolonging mice survival.

The contents of this research will be published in *ACS Nano* (Note 9), which is will be issued by the American Chemical Society (ACS) on August 6.

Concerned Paper: H. Kinoh, S. Quader, H. Shibasaki, X. Liu, A. R. Maity, T. Yamasoba, H. Cabral and K. Kataoka, “Translational Nanomedicine Boosts Anti-PD1 Therapy to Eradicate Orthotopic PTEN-Negative Glioblastoma” ACS Nano, 2020, in press.

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(Note 1) PTEN (Phosphatase and Tensin homologue deleted on chromosome 10) gene: A tumor suppressor gene that negatively regulates the PI3K/Akt signaling pathway that promotes cell proliferation. Abnormal expression of this gene, such as mutation, frequently promotes carcinogenesis and creates an environment in which disease progression easily occurs in cancer tissues. In the present study, the therapeutic effect of GMB was evaluated in both a mouse having a normal PTEN gene and a mouse having a PTEN gene knockout.

(Note 2) Immune checkpoint inhibitor: When T cells infiltrating cancer tissues come close to the cancer cells, a ligand called PDL1 is expressed on the surface of the cancer cells. It is known that when PDL1 binds to the PD1 receptor on the surface of T cells, the T cells lose their role to attack cancer cells. Drugs that inhibit the binding of PDL1-PD1 are called "immune checkpoint inhibitors." In this study, mouse anti-PD1 antibody was used.

(Note 3) Immune-induced cell death (ICD): Some anticancer drugs, such as epirubicin, kill cancer cells in a manner that elicits an immune response more easily than normal necrosis and apoptosis. This cell death is called immunogenic cell death, and several mechanisms have been reported, such as exposure of molecules that are targets of immune attack to the cell surface.

(Note 4) Chemo-immunotherapy (CIT): A combined use of chemotherapy and immunotherapy to increase the aggressiveness of T cells that attack cancer cells, and also weaken the defense of cancer that attempts to weaken their aggressiveness to treat cancer.

(Note 5) Tumor infiltrating T cells (TIL): A type of lymphocyte, T cells need to invade and contact cancer cells in order to recognize and attack specific cancer cells. In refractory cancer, there is a mechanism to prevent T cell infiltration. In order for immune checkpoint inhibitors to be effective, T cell activation is required, and for this purpose a sufficient amount of TIL is essential.

(Note 6) Bone marrow-derived immunosuppressive cells (MDSC): Cancer cells secrete immunosuppressive substances and induce the production of immunosuppressive cells in the bone marrow. As a result, cells that attack cancer cells, such as T cells and dendritic cells, are inactivated, and the cancer cells can escape from immunity. For immune checkpoint inhibitors to be effective, T cell activation is required, which requires reducing the number of MDSCs.

(Note 7) H. Ghebeh et al. Breast Cancer Research, 2010, 12, R48., E. M. Rom-Jurek et al. Int. J. Mol. Sci. 2018, 19.

(Note 8) Nanomicelle: A spherical or rod-shaped molecular assembly with a size of several tens of nm formed by associating amphiphilic polymers with various functional molecules in water. 1 nm is one billionth of a meter. If one compares the height of a person to the diameter of the earth, the size of one cell is the size of a football stadium, and the size of a nanomicelle is the size of a soccer ball.
H. Cabral, K. Miyata, K. Osada, K. Kataoka, “Block copolymer micelles in nanomedicine applications” Chem. Rev. 2018, 118 6844-6892. (DOI: 10.1021/acs.chemrev.8b00199)

(Note 9) ACS Nano: An academic journal published by the American Chemical Society (ACS) that provides global-level scholarly communication on nanoscience and nanotechnology research in the areas of chemistry, biology, materials science, physics, and engineering. The Impact Factor (2019), which shows the impact of academic journals, is 14.588, and is one of the most prestigious scientific journals in the world.

Innovation Center of NanoMedicine (iCONM):
The Innovation Center of NanoMedicine (iCONM) is a leading facility of King Skyfront, that is a biotech and healthtech innovation cluster in Kawasaki City. iCONM started the operation in April 2015 with Kawasaki Institute of Industrial Promotion in order to drive "Center of Open Innovation Network for Smart Health (COINS)" as a part of Japanese governmental research program "Center of Innovation (COI) Stream". Designed for the purpose of promoting "open innovation" through industry-academia-government and medicine-engineering collaborations with state-of-the-art facilities and experimental equipment capable of conducting the R&D from organic synthesis and micro-processing to preclinical studies. This is a very unique research center that is hardly found in the world.
https://iconm.kawasaki-net.ne.jp/en/index.html

Department of Bioengineering, Graduate School of Engineering, The University of Tokyo:
In a society where the population ages and the birth rate declines with the sustainable development being longed for, the Department of Bioengineering aims to contribute to the promotion of health and well-being of the humanity. To achieve this goal, we promote the education and research of bioengineering, which is the multidisciplinary academic field integrating the existing disciplines of engineering and those of life sciences at their interface. The key features of bioengineering are to establish its theoretical basis by understanding and clarifying the interactions of materials and systems with living bodies, and to develop fundamental technologies that control these interactions based on the theory. The control of the interactions with living bodies renders materials and systems far more useful and compatible, promising the birth of groundbreaking medical technologies.
http://www.bioeng.t.u-tokyo.ac.jp/en/overview/index.html