論　文　概　要

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Administration of dendritic cells and anti-PD-1 antibody following X-ray irradiation enhances both local control and abscopal effect in B16/BL6 murine tumor models
（エックス線照射と樹状細胞および抗 PD−1 抗体の併用投与による B16/BL6 マウス皮下腫瘍の局所制御とアブスコパル効果の増強）

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Purpose:

The purpose of this study is to investigate whether the combination of anti-PD-1 antibody (aPD1-ab), a checkpoint inhibitor, and bone marrow-derived dendritic cells (BM-DCs) following X-ray radiotherapy improves local tumor control and whether this combination therapy enhances systemic antitumor immunity and promotes abscopal effects in a murine B16/BL6 model.

Materials and methods:

B16/BL6 cells were irradiated with 0, 2, 4, 8, 12, or 16 Gy X-rays and incubated further. After 72 h of incubation, the cells were collected and analyzed using flow cytometer.

For mouse model, B16/BL6 cells were implanted subcutaneously in the left thigh of syngeneic C57BL/6 mice [B6(Cg)Tyrc-2J/J] as primary tumors. BM-DCs were induced from bone marrow cells using GM-CSF and IL-4. The primary tumors were treated with 8 Gy X-ray, followed by simultaneous intratumoral injection of BM-DCs and intraperitoneal injection of aPD1-ab. To examine the abscopal effect, B16/BL6 cells were also inoculated in the right thighs as metastatic tumors 4 or 26 days after the left side primary tumor inoculation, and only the primary tumors were treated with the same protocols. *In vivo* analyses of tumor growth and survival rates as well as *in vitro* analyses of splenic T-cell proliferation and interferon-γ (INF-γ) release were performed.

Results:

The PD-L1 expression decreased with increase in irradiation dose, especially in dying cells. The expression of H-2kd was low before irradiation, it increased significantly after X-ray irradiation in a dose-dependent manner, especially in dying cells.

The induced BM-DCs were approximately 80% of the collected cells. The harvested BM-DCs were positive for all markers (CD40, CD80, CD86, I-A/I-E) and the induced BM-DCs have a good function to endocytose antigens.

In the mouse model, the triple combination treatment of X-ray irradiation with BM-DCs and aPD1-ab administration inhibited primary tumor growth and significantly extended the survival time. In addition, this triple combination treatment significantly inhibited the growth of metastatic tumors.

Discussion:

In this study, we aimed to improve the local control and prevent or cure distant metastases of cancers using tumor-specific immune response. Although radiotherapy of tumors may act as "*in situ* vaccine", this effect depends on the balance between promotion and inhibition of immune response in the tumor microenvironment and also depends on radiation dose, dose rate, and the number of surviving immune cells.

We induced DCs from syngeneic bone marrow cells and the harvested cells contain approximately 15% macrophages. The contaminated macrophages may suppress the anti-tumor immune response and promote tumor cell metastasis. Also, it is difficult to predict the number of injected immature DCs that will transform into regulatory DCs that suppress the immune response which needs to be solved in the future study.

In addition, certain risks are associated with intratumoral injection of DCs. Intratumoral injection may be technically difficult and risky for deep-seated tumors. Other possible risks associated with DC and aPD1-ab treatment may include induction of a cytokine storm and induction of autoimmune disorders because of checkpoint inhibition.

Vanpouille-Box et al. reported that TGF-β was a major negative regulator of in situ tumor vaccination induced by irradiation. Although the triple combination of RT, DC, and aPD1-ab acted synergistically in this study, the optimal combinations with local radiation should be determined from a view point of safety, efficacy, and feasibility for clinical translation.

Conclusions:

The combination treatment of intratumoral BM-DC injection and intraperitoneal administration of aPD1-ab after localized X-ray irradiation significantly inhibited local tumor growth and extended the survival time of mice bearing subcutaneous tumors. In addition, this combination treatment can convert the tumors to *in situ* vaccines, inducing an effective systemic antitumor immunoreaction that may be used for treating or preventing metastatic tumors.