Substance-P stimulates bone marrow recovery from radiation-induced damages

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1. Introduction

Radiation therapy causes various degrees of damage to the system. Particularly, bone marrow suppression is the primary cause of death after irradiation. Bone marrow includes two types of multipotent cells, hematopoietic stem cell and mesenchymal stem cells to support hematopoietic stem cells. Suppression of these cells by irradiation can affect to entirely immune system. Many patients exposed to radiotherapy exhibit long-term residual damage to bone marrow function by a defect in hematopoietic stem cell self-renewal. Bone marrow suppression is long lasting and shows little tendency for recovery. Therefore, a research for mechanism that can stimulate recovery of bone marrow population is essential. Substance-p (SP) is a neuropeptide known to control both of neuronal signal and immune response. SP is consisting of 11 amino acids and signal via NK-1receptor on cell surface. Our previous data showed that SP could enhance proliferation of mesenchymal stem cells *in vitro*. Therefore, it can be expected that SP can recover suppression of mesenchymal stem cell after irradiation. It also will can help recovery of hematopoietic stem cell and furthermore, regeneration of bone marrow population. Our experiments were performed to recover or protect bone marrow suppression after irradiation *in vivo* through stimulating mesenchymal stem cell by SP.

2. Methods and Results

2.1 Stimulated Effect of Substance-P on Bone Marrow stem cell population after Irradiation

In previous study, we showed SP induced proliferation of hMSC, next, we investigated whether SP increased the population of radiation-damaged cells. After 4Gy irradiation, SP was injected to mouse and the viable cell number was counted in the bone marrow. Using a different culture system, we distinguished mesenchymal stem cell (MSC) and hematopoietic stem cell (HSC) population. In both cell populations, SP stimulated cell proliferation. This result showed that SP enabled to recover from the radiation-induced cell damage.

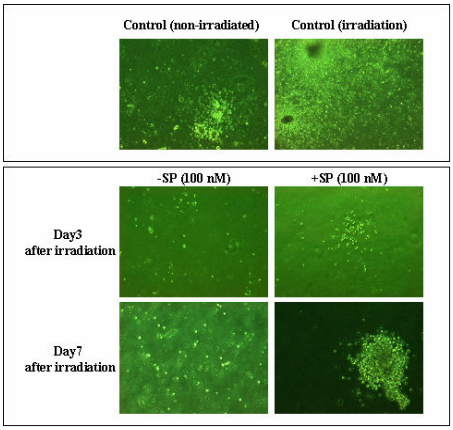

Fig. 3 Morphology of colonies formed in HSC-CFU
SP was injected into mouse right after irradiation and this injection was repeated 24h later. Bone marrow cells were isolated at day 3, 7 after irradiation and cultured in semi-solid medium. Morphology of colonies were observed.(100x)

2.2 Protective Effect of Substance-P on Bone Marrow Stem cells after Irradiation

We knew that SP had a capability to recover cell population from damages in the previous result, next we were interested in whether SP protects cells from radiation-induced damages. To observe protection effect of SP, SP was injected into mouse before 2 or 4Gy irradiation and colony-forming assay was done. As we expected, colony-forming unit of MSC and HSC was increased by pretreatment of SP. This suggested that SP also had a protection effects on bone marrow stem cells from radiation.

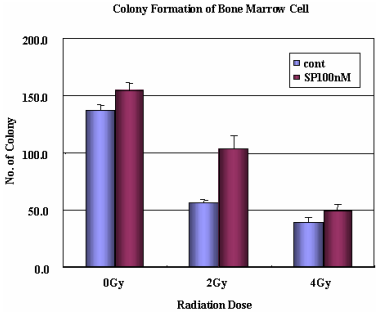

Fig.2 Colony-forming unit assay for bone marrow mesenchymal cells from irradiated mice
Mouse was injected with SP 24 h before irradiation. At day 3 after irradiation, bone marrow cells from mouse were cultured in MSCGM for 12 days. Observed colonies were counted. Five subjects were used for each group.

3. Conclusion

Substance-p can enhance proliferation of human mesenchymal stem cell *in vitro*.
In vivo, substance-P increased viability of bone marrow cells and this caused increase in formation of colonies in vitro.
Substance-P may play a critical role in bone marrow repopulation by enhancing the viability of cells and the protection of cells from radiation.

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