論　文　内　容　要　旨

論文題目　**Effects of Irradiation on Bone Metastasis of Breast Cancer Cells**

（放射線による乳がんの転移抑制効果の研究）

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Background

Radiotherapy is a widely-used treatment option in cancer. However, it has some associated problems such as increased bone metastasis after irradiation and side effects to the surrounding normal bone. Overexpression of periostin (POSTN) has been observed in bone metastatic cancer. Many studies have indicated that POSTN plays an important role in bone metastasis. However, the role of POSTN in the microenvironment of bone invaded by destructive cancer cells remains unclear. There have been few studies of the interactions between bone-invading cancer cells and bone matrix-forming cells after irradiation. It has been reported that expression of POSTN is significantly enhanced in bone tissues requiring reconstruction. High-LET radiation therapy induces bone hyperplasia and calcification in the irradiated area. POSTN is suspected to play an important role in the microenvironment of cancer bone invasion and bone tissue remodeling.

The purpose of this study was to clarify the side effects of radiation to bone, especially the changes in POSTN and bone metabolism-related cytokines.

Materials and Methods

FM3A/R breast cancer cells were exposed to 0, 5, 10, or 15 Gy of gamma-ray or carbon-ion beam irradiation.

1. POSTN expression in irradiated and non-irradiated control FM3A/R cells was measured by RT-PCR at 3 days after irradiation. MC3T3-E1 osteoblast cells were cocultured with irradiated and non-irradiated FM3A/R cells. The expression of receptor activator of nuclear factor kappa-B ligand (RANKL) and vascular endothelial growth factor (VEGF) in MC3T3-E1 cells was evaluated by western blotting to confirm the invasive potential of the tumor cells. To confirm the effect of POSTN only, MC3T3-E1 cells were cultured with recombinant POSTN-supplemented medium. In addition, siRNA transfection for POSTN knockdown was performed to investigate the influence of decreased POSTN. The expression of RANKL and VEGF in MC3T3-E1 cells was also measured in cells with recombinant POSTN and siRNA transfection.

2. The time course of POSTN expression in irradiated and non-irradiated control FM3A/R cells was measured by RT-PCR until 7 days after irradiation. MC3T3-E1 osteoblast cells were cocultured with irradiated and non-irradiated control FM3A/R cells. The expression of bone metabolism factors osteopontin (OPN) and osterix (OSX) was evaluated by western blotting to evaluate the calcification potential in the microenvironment.

Results

1. POSTN expression increased after gamma-ray and carbon-ion beam irradiation. Carbon-ion beam-irradiated cells expressed less POSTN than gamma-ray-irradiated cells. Higher doses of radiation were associated with rapid increases in POSTN expression. POSTN expression in irradiated cells increased to higher levels than those in non-irradiated cells. Moreover, cells with gamma-ray irradiation expressed higher levels of POSTN than cells with carbon-ion beam irradiation. The expression levels of RANKL and VEGF in osteoblast cells cocultured with carbon-ion beam-irradiated breast cancer cells were lower than those in cells cocultured with gamma-ray-irradiated cells. The cytokines influencing bone invasion levels increased in tandem with the increase in POSTN expression. The levels of both RANKL and VEGF increased with the recombinant POSTN level, and the levels were similar to those in cocultures with irradiated breast cancer cells. POSTN synthesis was suppressed by siRNA transfection that inhibited the expression of RANKL and VEGF in MC3T3-E1 cells.

2. POSTN expression increased during the time course after gamma-ray and carbon-ion beam irradiation. Gamma-ray-irradiated cells expressed higher levels of POSTN than carbon-ion beam-irradiated cells in a dose-dependent manner. Expression of OPN and OSX in MC3T3-E1 cells cocultured with carbon-ion beam-irradiated FM3A/R cells increased in a dose-dependent manner until the end of the experimental period, while the expression decreased in cells cocultured with gamma-ray-irradiated cells.

Discussion

Carbon-ion beam irradiation was slightly superior to gamma-ray irradiation in terms of killing FM3A/R breast cancer cells capable of invading bone, but induced lower levels of POSTN synthesis than gamma-ray irradiation. Thus, carbon-ion beam irradiation may reduce the production of bone-destroying cytokines and vascularization factors by osteoblasts in the

microenvironment of cancer invasion in bone. Moreover, carbon-ion beam irradiation could be a more effective therapy for cancers capable of invading bone, by suppressing the development of distant metastases. However, metastases after carbon-ion beam irradiation are still found clinically. To suppress the expression of POSTN in breast cancer cells after irradiation, combined treatment with both carbon-ion beam irradiation and POSTN inhibitor administration might become a treatment of choice.

It has been inferred that POSTN in the microenvironment of bone metastatic cancer was produced by FM3A/R cells. High expression of POSTN after gamma-ray irradiation in FM3A/R cells might be a cause of metastasis and bone destruction in the bone microenvironment. In the case of carbon-ion beam irradiation, bone destruction and metastasis were milder compared with conventional gamma-ray irradiation, and it can thus be expected that the expression of calcification factors may increase. Further studies are required to clarify the differences in POSTN expression in the microenvironment arising from differences in radiation quality.

Conclusion

To suppress the expression of POSTN in breast cancer cells after radiation therapy, combined treatment with a POSTN inhibitor may become a treatment of choice to improve the bone microenvironment.