学 位 論 文 内 容 の 要 旨

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学 位 論 文 題 名

Study of novel adjuvant treatments to improve radiotherapy by targeting radiation-induced

invasiveness in cancer cells

（放射線照射後に生き残ったがん細胞の浸潤能亢進を抑制するための

新規アジュバント療法に関する研究）

Radiotherapy is a standard and efficacious treatment that uses ionizing radiation (IR) to treat various cancers. Although the therapeutic efficacy of radiotherapy has significantly improved, several patients suffer from local recurrence and distant metastasis after treatment. IR-induced invasiveness in IR-surviving cells is believed to be a factor that contributes to distant metastasis in radiotherapy patients. To overcome this problem, finding new adjuvant therapies that could suppress IR-induced invasiveness while increasing radiosensitization is a promising strategy. To further improve the therapeutic efficacy of radiotherapy by enhancing cancer cell death and suppressing IR-induced invasiveness, we studied two adjuvants, RGD peptide-conjugated gold nanoparticles (AuNPs) and λ-carrageenan (CGN), to improve radiotherapy.

Chapter 1. RGD peptide-conjugated gold nanoparticles suppress IR-induced invasiveness in cancer cells

【Background】Gold nanoparticles have recently attracted attention as radiosensitizers that can increase the therapeutic efficacy of radiotherapy. We therefore produced arginine–glycine–aspartic acid (RGD)-conjugated AuNPs to specifically target integrin-expressing cancer cells. RGD is a peptide that specifically binds to integrin, a cell surface receptor that is overexpressed in highly invasive cancer cells. In this chapter, we describe how we validated the therapeutic efficacy of RGD-conjugated AuNPs by determining the extent of radiosensitization and invasiveness after RGD-conjugated AuNPs treatment in the targeted cancer cells.

【Methods & Results】
(1) We produced polyethylene-glycolylated AuNPs (P-AuNPs) to stabilize the AuNPs and then conjugated them with RGD peptides to produce RGD/P-AuNPs. RGD/P-AuNPs were then characterized in terms of size variations, hydrodynamic diameter, and zeta potential.
(2) The expression levels of RGD-binding integrins in different breast cancer cell lines were measured. RGD/P-AuNPs were efficiently internalized by integrin-overexpressing cell lines but not by low integrin-overexpressing cell lines.
(3) By immunofluorescent imaging, RGD/P-AuNPs were found to colocalize with integrins in the late endosomes and lysosomes in these cells.
(4) Cell viability was measured by Cell Counting Kit-8 (CCK-8). DNA damage was measured by counting γ-H2AX foci. A combination of RGD/P-AuNPs and IR treatment reduced cell viability and increased DNA damage compared to IR alone in the targeted cells.
(5) The invasiveness of cancer cells was measured by the Matrigel chemoinvasion assay. The IR-induced invasiveness of the breast cancer cell lines was significantly inhibited by RGD/P-AuNP treatment.
(6) We performed gene expression microarray analysis to elucidate molecular mechanism underlying the abovementioned effects. Our analysis revealed that the expression levels of fibronectin (FN) and its downstream signaling, the extracellular signal-regulated kinase (ERK) pathway, in irradiated cells were suppressed by RGD/P-AuNPs.

【Discussion】In this chapter, we reveal that RGD/P-AuNPs could improve the therapeutic

efficacy of radiotherapy by increasing the cytotoxic effect of IR and suppressing IR-induced invasiveness in integrin-overexpressing cancer cells.
(1) RGD/P-AuNPs showed specific targeting to integrin-overexpressing cancer cells. Further evaluation of the therapeutic efficacy of RGD/P-AuNPs and IR in animal models should be performed.
(2) We found that RGD/P-AuNPs suppressed IR-induced invasiveness, which may be caused by the suppression of the FN-ERK pathway. Further research on the relationship between this pathway and IR-induced invasiveness is recommended.

Chapter 2. Adjuvant of Lambda-carrageenan suppresses radiation-induced invasiveness in cancer cells through Racgap1

【Background】Lambda-carrageenan, a sulfated polysaccharide used as a daily food additive, has been recently found to exhibit anti-tumorigenic activities. In this chapter, we describe the evaluation of the therapeutic benefit of CGN in enhancing the efficacy of IR treatment and investigate its underlying molecular mechanism.

【Methods & Results】
(1) We determined the therapeutic efficacy of IR coupled with CGN treatment in human breast cancer cell line, head and neck cancer cell line, and pancreatic cancer cell line. We found that CGN treatment increases cytotoxic efficacy and apoptosis in irradiated cancer cells.
(2) We measured the change in several biological responses to further evaluate the mechanism behind the cytotoxic effect of this treatment. CGN treatment after IR significantly increased reactive oxygen species (ROS) accumulation, caspase activities, and polyploid formation in the cancer cells.
(3) To measure cell invasiveness, Matrigel chemoinvasion assay was performed. CGN treatment was found to suppress IR-induced invasiveness.
(4) CGN treatment after IR suppressed invasive growth, as observed by the 3D lrECM culture method.
(5) We screened target molecules by performing microarray analysis and focused on Rac GTPase-activating protein 1 (RacGAP1). We found that RacGAP1 was upregulated in several cancer cell lines after IR treatment. Knockdown of RacGAP1 increased the cytotoxic effect of IR.
(6) CGN treatment significantly suppressed RacGAP1 expression in breast cancer cells. RacGAP1 overexpression partially rescued CGN cytotoxicity.
(7) In a mouse xenograft model, IR followed by CGN treatment significantly decreased tumor growth and lung metastasis compared to either treatment alone.

【Discussion】In this chapter, we describe how CGN enhances IR treatment by significantly increasing cytotoxicity, suppressing IR-induced invasiveness, and distant metastasis by downregulating RacGAP1 expression.
(1) CGN and IR have been reported to induce specific immune responses in cells. Further research is needed to determine the immune response elicited after the combination treatment of CGN and IR.
(2) CGN was found to suppress RacGAP1 expression significantly. Apart from RacGAP1, other genes were also found to be suppressed by CGN treatment. These genes may be involved in radioresistance or IR-induced invasiveness. The roles of these molecules and related mechanisms could be investigated in the future.

Conclusion

In the first chapter, we describe how we produced integrin-targeting RGD/P-AuNPs to increase the therapeutic efficacy of IR treatment. Our results showed that RGD/P-AuNPs effectively target integrin-overexpressing cancer cells. Furthermore, RGD/P-AuNPs suppress IR-induced invasiveness in addition to increasing radiosensitization. With regard to the molecular mechanism underlying these observations, the expression of FN and activation of ERK, key modulators of cancer cell invasion, were found to be suppressed by RGD/P-AuNPs used in combination with IR. In the second chapter, we describe how we used CGN as an adjuvant to improve radiotherapy. CGN treatment increased the therapeutic efficacy of IR, suppressing both IR-induced invasiveness and distal metastasis by downregulating RacGAP1 expression.

These results have provided us novel strategies for improving radiotherapy by targeting IR induced invasiveness in cancer cells. By understanding the molecular mechanisms of these treatments, we can also obtain new knowledge of how cancer cells respond to IR.