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Radiation Response Protein, Sialyltransferase (ST6Gal I)

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Recently we identified ß-galactoside α(2, 6)-sialyltransferase (ST6Gal I) as a candidate biomarker for ionizing radiation. The expression of ST6Gal I and the level of protein sialylation increased following radiation exposure in a dose-dependent manner. We also found that radiation induced ST6Gal I cleavage and the cleaved form of ST6Gal I was soluble and secreted. Sialylation of integrin ß1, a glycosylated cell surface protein, was stimulated by irradiation and this increased its protein stability. Overexpression of ST6Gal I in SW480 colon cancer cells that initially showed a low enzyme activity of ST6Gal I increased the sialylation of integrin ß1 and also increased the stability of the protein. Inhibition of sialylation by transfection with neuramidase or by treatment with short interfering (si) RNA targeting ST6Gal I (Si-ST6Gal I) reversed the effects of ST6Gal I expression. In addition, ST6Gal I overexpression increased clonogenic survival following radiation exposure and reduced radiation-induced cell death and caspase 3 activation. In conclusion, we suggest that exposure to ionizing radiation was found to increase sialylation of glycoproteins such as integrin ß1 by inducing the expression of ST6 Gal I, and finally protein sialylation contributed to cellular radiation resistance.

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Ionizing radiation increases ST6Gal I expression

Sialic acids, nine carbon acidic sugars bearing negatively charged at physiological pH, are known to be ubiquitously expressed on the non-reducing ends of the sugar chains of glycoproteins and glycolipids in tissues. They are known to be key determinants for a large variety of biological processes, including cell-cell communication, immune defense, tumor cell metastasis and inflammation.[1] The ß-galactoside α(2, 6)-sialyltransferase (ST6Gal I) has been identified as being able to catalyze the α(2, 6)-sialylation of N-acetyllactosamine. Since our previous study indicated that mRNA expression levels of ST6Gal I in the mouse spleen and intestine were increased by whole body irradiation, we confirmed these findings in the mouse spleen system. Induction of ST6Gal I at both the mRNA and protein levels was observed following whole body radiation with a dose of 1 Gy.[2] Additionally, induction of other sialyltransferase mRNAs such as ST8Sia I, ST3Gal I, ST3Gal II, ST3Gal III, and ST3Gal IV were also observed in the spleen following irradiation of the mice, suggesting that radiation exposure increased the expression of a variety of sialyltransferase genes.

Sialylation of integrin ß1 by irradiation

From variety of reports, ST6Gal I was expressed particularly in human malignancies.[8,9] The upregulation of ST6Gal I is probably the basis for the increased α(2, 6)-sialylation seen in cancer cell.[3,4] Several clinical and experimental studies suggest a positive correlation between high ST6Gal I levels and the invasive behavior of cancer cells, but other studies have reported opposite conclusions.[5,6] Furthermore, there have been no reports of ST6Gal I expression-induced sialylation of glycoproteins in response to radiation exposure. To elucidate whether increased expression of ST6Gal I following radiation exposure affects protein sialylation, FACS analysis using a fluorescent lectin from *Sambucus negra* agglutinin (SNA), which is an α2,6 sialylation-specific lectin or *Maackia amurensis* agglutinin (MAA), which is specific for α2,3 sialylation, was performed. A total body irradiation dose of 1 Gy resulted in increased binding of SNA and MAA to splenocytes, suggesting that radiation exposure increased sialylation of cellular proteins. When we examined these phenomena in the colon cancer cell line (SW480), similar effects were observed. Radiation-induced SNA binding was inhibited by either Si-ST6Gal I treatment on SW480 cells. Then, we examined total protein sialylation patterns using lectin blot assay, proteins of

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approximately between 100 and 150 kDa molecular weight showed increased sialylation following radiation exposure. Interestingly, there have been reports that integrin ß1 (120kDa) is sialylated proteins.[10] Indeed, integrin ß1 was sialylated following radiation exposure either *in vivo* or *in vitro* with a slight increase in their protein levels. Flow cytometry analysis also indicated that radiation increased integrin ß1 protein expression in spleen and SW480 colon cancer cells, suggesting that radiation exposure increased levels of integrin ß1 in both an *in vitro* and *in vivo* system.

Integrin ß1 proteins, recently reported as having increased levels in patients involved in radiation accidents, are substrates of several glycosyltransferases including ß1,6, N-acetylglucosaminyltransferase V, sialyltransferase ST6GalNAc I, and ST6Gal I.[7-10] Various phenotypic changes have been shown to be the direct result of altered glycosylation of integrin molecules, but in some cases, they have also been related to the differential glycosylation of integrin-associated cell surface molecules. In our system, integrin ß1 was sialylated following radiation exposure and sialylated integrin ß1 exhibited increased protein stability. Moreover, neuraminidase treatment inhibited the radiation-induced increase in protein stability, indicating that sialylation of integrin ß1 was directly related to the stability of the protein. Several studies support a role for integrin carbohydrate groups in regulating the association between integrins and ligands. Akiyama *et al.* reported that the treatment of human foreskin fibroblasts with glycosylation inhibitors blocked cell adhesion to fibronectin.[11] Similarly, Zheng *et al.* demonstrated that ligand binding was altered when N-linked carbohydrates were enzymatically cleaved from cell surface α5ß1 integrins, suggesting that the presence of sialic acids can directly modulate ligand/receptor interactions.[8] We do not know exactly why sialylation of integrin ß1 affected its protein stability. However, one possibility is that adhesion to a solid substrate can stabilize integrin ß1 on the cell surface through a ST6Gal I-dependent mechanism.[12] From these results we suggests that the radiation-induced increase in sialylation of glycoproteins was dependent on ST6Gal I activity.

Overexpression of ST6Gal I induces radiation resistance

To examine the role of ST6Gal I in the radiation response, we performed the clonogenic survival assay and PI staining using SW480 colon cancer cells stably transfected with ST6 Gal I. SW480 cell clones stably overexpressing ST6Gal I (#6 and #22) also showed increased clonogenic survival following radiation and reduced radiation-induced cell. Caspase-3 activation and PARP cleavage following exposure to a dose of 10 Gy gamma radiation were also inhibited by ST6Gal I overexpression. Furthermore, the additional transfection of ST6Gal I overexpression clones with Si-ST6Gal I inhibited ST6Gal I-mediated. To elucidate whether the radioresistance by ST6Gal I was related to sialylation by ST6Gal I, cells were co-transfected with Neu2 and cell death was examined. Following co-transfection with Neu2, inhibition of radiation-induced cell death and caspase 3 activation by ST6Gal I were reversed, suggesting that the ST6Gal I-induced radioresistance was mediated by protein sialylation. ST6Gal I has been suggested to have an important role in oncogenic transformation and metastasis. Increased expression of ST6Gal I has been observed in colorectal cancer, breast cancer, cervical cancer, and choriocarcinoma. However, elevated ST6Gal I inhibited the formation of a glioma *in vivo*.[6] Therefore, expression of ST6Gal I may have different effects in different cancer types. However, an altered radiation response by ST6Gal I was never suggested. In this study, ST6Gal I induced radioresistance and when Si-ST6Gal I or neuramidase 2 was co-transfected, the increased radioresistance was abolished, suggesting that ST6Gal I-mediated protein sialylation is involved in the radiation resistance response and protein sialylation enables the cell to resist radiation-induced damage through the inhibition of apoptosis. Ionizing radiation causes cancer and metastasis.[13,14] Therefore, we are now examining the effects of radiation-induced increases in protein sialylation on adhesion and metastasis using sialylation site mutants of integrin ß1. Finally, radiation-induced expression of sialyltransferases includes ST6Gal I. Protein sialylation by ST6Gal I has been frequently shown to be higher in cancer cells, is involved in the protein stability of integrin ß1, and provides cellular radioresistance, suggesting that protein sialylation might be a novel target to overcome radioresistance in radiation therapy.

Conclusion

In the present study, we observed that radiation exposure increased the expression of ST6Gal I. Our previous study suggested that mRNA expression of ST6Gal I was induced by exposure to a low dose of radiation, specifically in the spleen and intestine, both radiation-sensitive organs.[23] Here, we elucidated the mechanisms of ST6Gal I in the radiation response. From these results, we found that radiation induced expression of sialyltransferases including ST6Gal I. Protein sialylation by ST6Gal I has been frequently shown to be higher in cancer cells, is involved in the protein stability of integrin ß1, and provides cellular radioresistance, suggesting that protein sialylation might be a novel target to overcome radioresistance in radiation therapy.

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