Radiation Induces Cathepsin S through ROS-IFN-γ Pathways: Involvement of Cellular Radioresistance

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1. Introduction

Ionizing radiation can elicit an activated phenotype that promotes rapid and persistent remodeling of the extracellular matrix (ECM) through the induction of proteases and growth factors, as well as in response to chronic production of reactive oxygen species (ROS). In addition, the results of previously conducted cDNA microarrays and real-time RT-PCR analysis (unpublished) suggest that radiation-induced mammary tumors were specifically induced by cathepsin S (CTSS), but that dimethylbenz(a)anthracene (DMBA)-induced mammary tumors were not.
CTSS is a lysosomal cystein protease that is synthesized as an inactive precursor (36kDa) and activated in the acidic environment of lysosomes by proteolytic cleavage of its propeptide. In this study, we further investigate the mechanism by which CTSS is induced by radiation as well as its function.

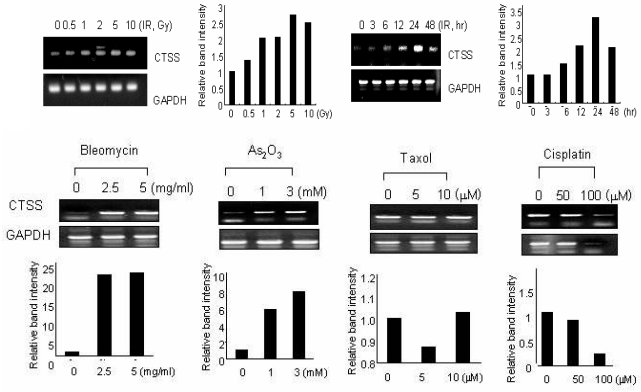
2. Materials and Methods

We performed RT-PCR to evaluate the mRNA expressions of CTSS and IFN-γ**,** and then confirmed the results by Western blotting. In addition, an EMSA assay was performed *in vitro* to measure the binding of IRF1 to the CTSS promoter region and a trypan blue dye exclusion assay, colony forming assay and PI staining were conducted to evaluate the radioresistance of CTSS.

3. Results

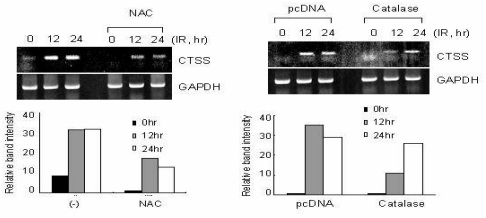
Radiation induced CTSS in a dose and time dependant manner
Previously, CTSS was found to be highly expressed in radiation-induced mammary tumors, but not in DMBA-induced tumors. Therefore, we treated MCF7 cells with radiation to determine if it directly induced CTSS. Radiation induced CTSS at both the mRNA and protein levels in a dose and time dependent manner. To determine if the induction of CTSS occurred specifically in response to radiation, cells were exposed to other stressors including bleomycin, As_2O_3, taxol and cisplatin, and the level of CTSS mRNA in the treated cells was then determined. As_2O_3 and bleomycin induced CTSS expression, however, taxol and cisplatin did not

Fig. 1. Radiation increased the CTSS expression level



Radiation-mediated ROS production was involved in CTSS expression
Because ROS producers such as radiation, bleomycin, and As_2O_3 induced CTSS gene expression, but other DNA damaging agents such as taxol and cisplatin did not, we evaluated ROS production after radiation treatment to determine if it was involved in CTSS gene expression. When MCF7 and MCF10A cells were pretreated with a ROS scavenger (NAC), the radiation induced CTSS mRNA expression was partially blocked. Moreover, transfection of cells with a retroviral vector that resulted in the production of catalase also inhibited radiation-induced CTSS mRNA expression

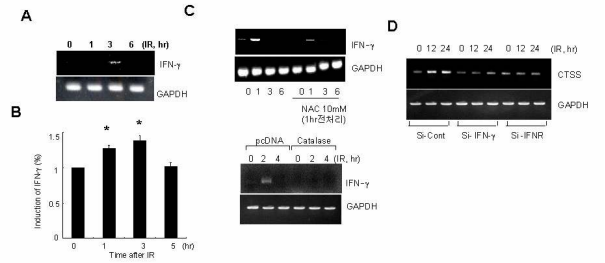
Fig. 2. Radiation-induced ROS regulates CTSS expression



IFN-γ production by radiation-induced ROS participated in CTSS expression
IFN-γ has been shown to be a potent inducer of CTSS and radiation is known to be involved in IFN-γ production, therefore, we examined radiation-induced IFN-γ to determine if it is responsible for CTSS gene expression. In the system evaluated in this study, radiation induced IFN-γ mRNA and protein were detected (Fig. 3A, 3B). Moreover, treatment with NAC

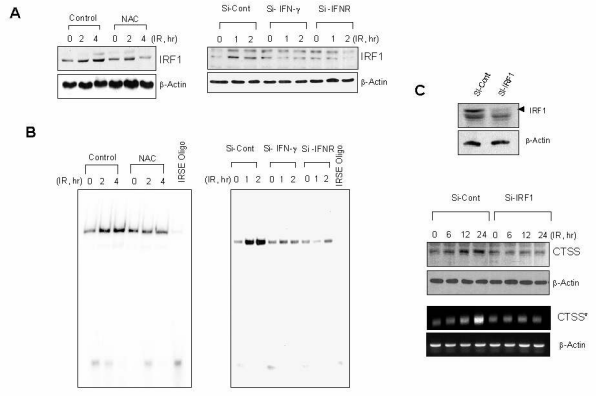
or catalase transfection inhibited this radiation-mediated IFN-γ production (Fig. 3C), which suggests that radiation-induced ROS may be responsible for radiation-induced IFN-γ production. Therefore, interference with the RNA of IFN-γ (Si- IFN-γ) and IFN-γ receptor (Si-IFNR) was evaluated to determine if IFN-γ production was important for radiation-induced CTSS gene expression in our system. Radiation-induced CTSS mRNA expression was blocked by these Si-RNAs (Fig. 3D), which indicates that radiation-induced CTSS mRNA was mediated by the ROS-IFN-γ pathway.

Fig. 3. Radiation and oxidative stress increases IFN-γ expression, which leads to transcriptional activation of CTSS.



IFN-γ dependent IRF1 regulated CTSS expression
Because IFN-γ dependent CTSS expression is mediated by IRF1 , we evaluated radiation treatment to determine if it could effect IRF1 expression. Radiation was found to induce IRF1 protein, however, this effect was inhibited by treatment with NAC, Si-IFN-γ and Si-IFNR (Fig. 4A). To demonstrate this, EMSA analysis using an IFN-stimulated response element (ISRE) oligo probe designed to bind to the CTSS promoter region was performed after radiation treatment. Increased DNA binding activity was found after radiation, however, this phenomenon was abolished in cells that were pre-treated with NAC, Si-IFN-γ or Si-IFNR (Fig. 4B). Finally, Si-RNA of IRF1 inhibited radiation induced CTSS mRNA and protein expression (Fig. 4C), suggesting that ROS-IFNγ-mediated IRF1 expression is involved in radiation-induced CTSS expression.

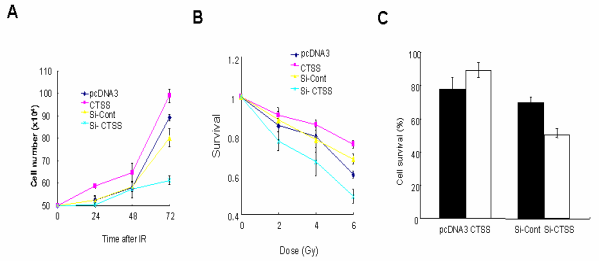
Fig. 4. IFN-γ dependent IRF1 regulated CTSS expression



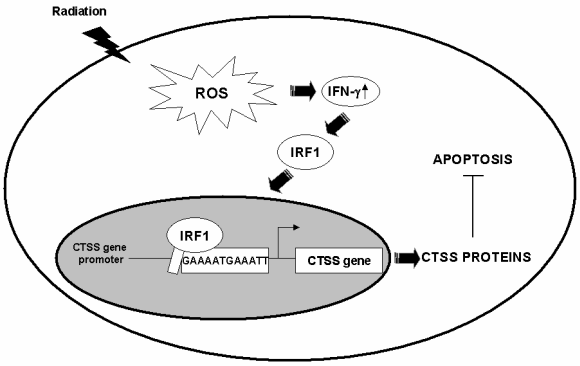
CTSS overexpression exhibited radioresistance

To examine the role that CTSS plays in the response to radiation, CTSS Si-RNA and CTSS overexpression was evaluated in MFC7 cells. Overexpression of CTSS significantly induced radioresistance, however, cells that were transfected with CTSS Si-RNA showed decreased radioresistance, when detected by trypan blue dye exclusion assay, clonogenic survivial assay, and PI staining (Figs 5A-5C).

Fig. 5. CTSS overexpression is involved in radioresistance



4. Conclusion



The results of this study indicate that radiation induced CTSS expression via ROS- IFN-γ pathways, and that this increased expression may be involved in radioresistance.

References

[1] Storm van's Gravesande K, Layne MD, Ye Q, *et al.* IFN regulatory factor-1 regulates IFN-gamma-dependent cathepsin S expression. *J Immunol* 2002;168:4488-4494.

[2] Wang B, Sun J, Kitamoto S, *et al.* Cathepsin S controls angiogenesis and tumor growth via matrix-derived angiogenic factors. *J Biol Chem* 2006;281:6020-6029.

[3] Gocheva V, Zeng W, Ke D, *et al.* Distinct roles for cysteine cathepsin genes in multistage tumorigenesis. *Genes Dev* 2006;20:543-556.