Functional characterization of gamma radiation induced nuclease, *AIN*

Jihyun Lee, Mi-jin Yoon, Eunhee Kim, Chul-Koo Cho, Su-Jae Lee, Yun-Sil Lee and Sangwoo Bae*
Korea Institute of Radiological and Medical Sciences (KIRAMS)
215-4 Gongneung-Dong, Nowon-Gu, Seoul, Korea
*Correspondence: swbae@kcch.re.kr

1. Introduction

In order to screen ionizing radiation induced early-response genes, we employed subtractive hybridization method and isolated nuclease domain-encoding gene *ain* (Apoptosis Inducing Nuclease). *ain* gene expression was very sensitive to ionizing radiation as revealed by a rapid induction of its messenger RNA by low and high doses of gamma radiation. Analysis of amino acid sequence of *ain*–coding gene showed homology to various nucleases with the highest similarity to exonuclease III. Consistent with its putative function as a nuclease, *AIN* protein localized in nucleus as revealed by immunofluorescence microscopy. Gamma-irradiation of *AIN*-expressing cells showed no differece in the protein localization compared with unirradiated cells. Functional characterization of *AIN* was carried out by *in vitro* nuclease assay with various DNA substrates. It was shown that *AIN* has 3'→5' exonuclease activity and that its overexpression in the presence or absence of ionizing radiation increased apoptotic cell death. Collectively, the results suggest that ionizing radiation-induced expression of *ain* might result in potentiation of DNA digestion activity and lead to enhanced apoptotic cell death.

2. Material and Methods

2.1 Subtractive hybridization and Gene expression .

For subtractive hybridization analysis of radiation-induced genes, we used Clontech (USA) kit and manual that accompanied the kit. *ain* gene expression was monitored by RT-PCR analysis with beta-actin or GAPDH as control.

2.2AIN localization and *in vitro* nuclease assay.

Cellular localization of *AIN* was examined by immunofluorescence analysis of *AIN*-overexpressing H-460 cell line. Nuclease activity of *AIN* protein was determined by incubating the specified DNA substrates with *AIN* immunoprecipitates from AIN transfected cells.

2.3 Apoptosis assay

SubG1 fraction of FACS analysis was used to determine apoptotic cell death before and after ionizing radiation in the presence or absence of *AIN* overexpression

3. Results and Discussion

As an initial characterization of radiation-induced genes, RT-PCR analysis of RNA samples from gamma radiation-exposed H-460 cells was carried out (Fig. 1).

ABCD

Fig. 1 RT-PCR analysis. Each panel of sample A,B,C, and D contains unirradiated, 1cGy, 1Gy, 10Gy irradiated and (-) RT control. A is *ain* gene, B and C are two of unidentified genes D is beta-actin as a control. Control gene shows consistent mRNA level regardless of gamma irradiation.

Ain gene expression showed dose-dependency with 1Gy producing highest level at 2-hr post IR. Other IR responsive genes B and C showed similar induction pattern albeit with different expression level.

Since amino acid sequence of *AIN* revealed similarity to nuclease, especially to exoIII, we wanted to verify the nuclease function by protein localization (Fig. 2) and by *in vitro* nuclease assay (Fig 3). Since nuclease is supposed to function in nucleus, we determined *AIN* protein localization in ain transfected H-460 lung cancer cell line.

Immunofluoprescence detection of *AIN* protein showed nuclear localization with no distinct subnuclear structure (fig. 2). Irradiation of *AIN* expressing cells resulted in modest change in localization with mixed localization in nucleoplasmic and perinuclear region. The cause or significance of perinuclear localization of *AIN* is not clear.

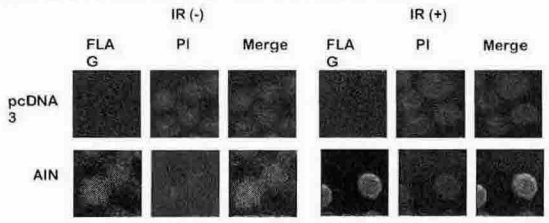

Fig. 2 Localization of *AIN* protein by immunofluorescence analysis. *AIN* protein was detected with tag antibody. Localization in (+) or (-) IR sample was determined. Nucleus was visualized after staining cells with PI.

We verified nuclease activity of *AIN* protein by incubating various oligonucleotide substrates with *AIN* immunoprecipitates. Because production of recombinant *AIN* protein was met with technical difficulty, we decided to overexpress *AIN* by transfecting human cells and immunoprecipitated *AIN* using specific tag antibody.

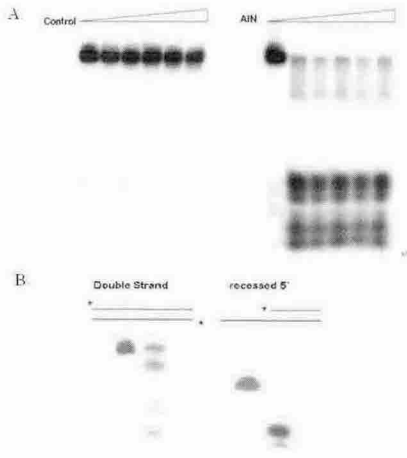

Fig. 3 Nuclease assay. A. Enzyme reaction with full length *AIN* immunoprecipitates. B. Enzyme reaction with two different substrates to determine the direction of oligonucleotide digestion..

AIN immunoprecipitates contained nuclease activity (Fig. 3). Increased nuclease activity was obvious with the immunoprecipitates whereas control immunoprecipitates was not (Fig. 3A). Full length *AIN* degraded double stranded DNA oligonucleotide substrate (Fig. 3A). To determine the direction of the nuclease enzyme reaction, we used double stranded blunt-ended on both sides and 5' recessed 3' blunt-ended oligonucleotide substrate (Fig. 3A). Both substrates were labeled at 5' end. We verified degradation of both substrates in 3' to 5' direction (Fig. 3B) since we could not observe 5'-end digestion product, free isotope. If the direction of DNA degradation were 5' to 3', we would have observed only labeled free isotope on the autoradiogram.

Functional significance of *AIN* in cellular radiation response was investigated by transfecting human cells with ain cDNA and observing apoptotic cell death by FACS analysis. *AIN* expression alone increased apoptotic cells as seen in increased subG1 fraction (Fig. 4). *AIN* expression in combination with IR further increased apoptotic cell population with almost three fold. This result suggests that nuclease activity of *AIN* is involved in potentiation of radiation induced apoptotic cell death.

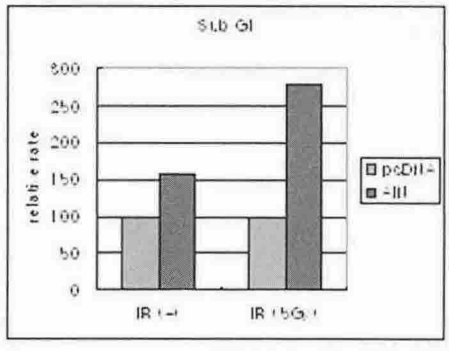

Fig. 4 Enhancement of radiation induced apoptosis by AIN overexpression.. Control values were set as 100%.

Taken together, we can envision the following model in which 1) ionizing radiation induces early responsive gene *Ain*, 2) exonuclease activity of *AIN* digests IR-damaged DNA, and 3) *AIN* cooperates with apoptotic DNA degradation and increases apoptosis. We still need a clear picture of how AIN expression is repressed during normal unstressed cellular life and how it is induced by ionizing radiation. Considering role of many nucleases in DNA repair, recombination, replication and other nucleic acid metabolism, it is of interest to determine whether *AIN* is involved in those processes other than apoptotic DNA digestion.