Expression of ATM Gene in Nasopharyneal Carcinoma Cell lines with Different Radiosensitivity–Studied with Laser Scanning Confocal Microscope*

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Abstract **Purpose** To determine the expression of ATM gene in Nasopharyngeal carcinoma (NPC) cell lines with different radiosensitivity. **Methods** Two NPC cell lines, CNE1 and CNE2, with different radiosensitivity were establised. The localization and quantity of ATM protein were analyzed by fluorescence immunohistochemical method and laser scanning confocal microscope(LSCM). **Results** The fluorescence intensity of ATM protein was stronger in karyon of CNE1 than that in CNE2. **Conclution** The expression level of ATM gene differs in NPC cell lines. The variance may be a potential factor, which was linked to their different radiosensitivity.

Key Words ATM protein; nasopharyngeal carcinoma; radiosensitivity; LSCM

The opening reading frame of ATM (ataxia telangiectasia mutant) cDNA coding for 3056 amino acids gave rise to a 350kDa protein, that is, ATM protein. ATM protein was extensively distributed in many kinds of human cells. It´s reported that the variant expression levels of ATM protein were related to cellular radiosensitivities [1]. In primary glioblastoma cells, ATM protein expression correlated with their radioresistance[2]. To date, there are no studies on the relation between ATM protein expression and cellular radiosensitivities of nasopharyngeal carcinoma (NPC).

Our previous data suggested that human NPC cell line CNE2 is more radiosensitive to ionizing radiation (IR) than another NPC cell line CNE1[3]. To investigate the the relation between ATM protein expression and the variant radiosensivities, we analyzed the localization and quantity of ATM protein in the two cell lines by fluorescence immunohistochemical method and laser scanning confocal microscope (LSCM). The results were expected to be a laboratory basis for understanding the radiobiological characteristics of NPC cells.

MATERIALS AND METHODS

Cell lines and Cell culture

Two NPC cell lines CNE1 and CNE2, which donated by the Cancer Center of Sun Yat–Sen University of Medical Science, were cultured at 37℃ in a humidified atmosphere of 5% CO_2 and 95% air in RMPI1640 medium containing 10% calf serum with 1% penicillin/streptomycin.

Immunofluorescence staining

Monolayer–cell slides were rinsed by 0.1M PBS, then fixed by acetone overnight at 4℃. The fixed cells were stained with polyclonal rabbit anti–ATM antibody (Ab–3: oncogene. Cambridge MA, 1:200). After incubation with the primary antibody, secondary anti–rabbit antibody labeled FITC was applied. Finally, glycerol buffer without fluorescence was added to envelope the slides.

LSCM analysis

The expression of ATM protein was examined by Leica TCS/CP LSCM system controlled by TCS/NT system. The emitting wave length of FITC was 488nm and the number area was 1.09. The image was amplified 400 times, and saved by 512*512 pels type. The type of image collecting microscope was Leica–HC, and the scanning stimulation value was 3%.

RESULTS

Expression of ATM protein in CNE1

The very intense green fluorescence of ATM protein labeled with FITC was detected in CNE1,

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and mostly in karyon (Fig.1).

Expression of ATM protein in CNE2

The green fluorescence of ATM protein labeled with FITC was visualized in CNE2 (Fig.2).

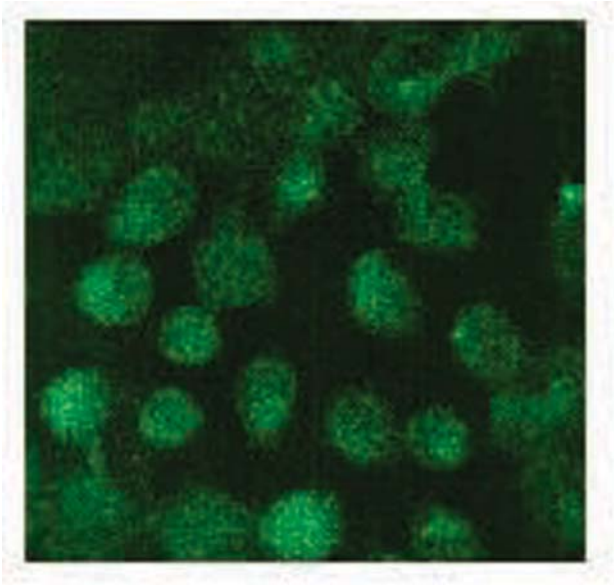

Fig.1 Expression of ATM protein in CNE1
LSCM: FITC marked IgG(×400)

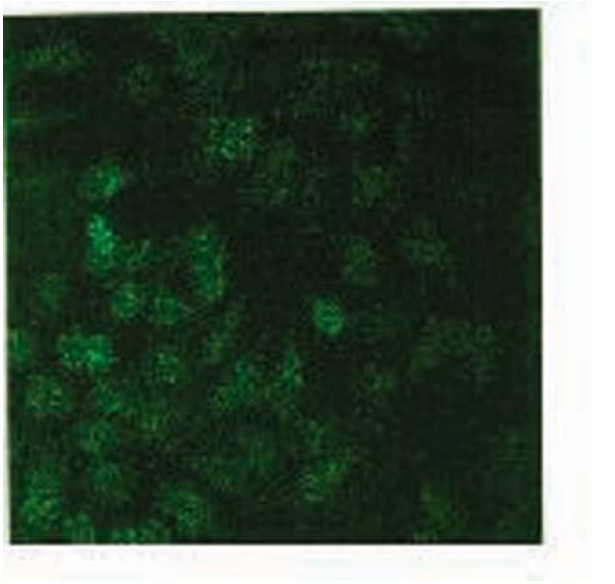

Fig.2 Expression of ATM protein in CNE2
LSCM: FITC marked IgG(×400)

DISCUSSION

As all know, NPC was one of the nine high-frequent malignant tumors in China. To date, radiotherapy is still the major modality for the treatment of NPC. Although the majority of NPCs completely regress following radiotherapy, a substantial proportion of it subsequently recurs. According to the analysis of long curative effect, almost 50% NPC were radioresistant[4]. The mechanisms of radioresistance or radiosensitivity were still the hot topic of most radiobiologists´ investigation. It´s reported that the radiosensitivities of NPC cell were related with bcl-2 protein expression, p53 gene deletion and BHRF1 gene expression, etc [5,6]. To examine the possible involvement of other elements in NPC cellular radiosensitivities, we employed ATM protein expression in two NPC cell lines, which were differently radiosensitive to ionizing radiation (IR), with LSCM technique. Our data showed that ATM protein was located in nuclei and cytoplasm of CNE1 and CNE2, and nuclei have a more intense fluorescence than the cytoplasm in most CNE1, which was in keeping with other data[7] . The distribution was much consistent with ATM functions, such as recognizing DNA damage, participating in nuclear phosphorylation, etc. Our findings also showed that the fluorescence intensity of ATM protein was stronger in CNE1 than that in CNE2. It indicated that the variance of ATM protein expression may be a potential factor, which was linked to their different radiosensitivities.

Sequence homology searches indicate that ATM protein is a member of a family of large protein as sharing significant homology to the catalytic domain of phosphatidylinositol-3(PI) kinase including TEL1, MEI-41, RAD3, DNA-PK, etc. These sequence homologies appear to reflect functional homology because many of the PI3-kinase-related proteins are, like ATM protein, involved in DNA repair, cell cycle control, and recombination [9,10]. Previous studies on the mechanism of the relation between ATM protein expression and radiosensitivities suggested that AT cells and fibroblasts derived from the atm-/- mouse are hypersensitive to IR and defective in cell cycle checkpoint control, radiation-induced activation of the tumor suppressor gene product p53. The result was that the p53 signal transduction pathway including p21/Gadd 45/MDM2 and cyclin-dependent kinase activity will in turn be defective. Emerging evidence supports that ATM protein plays a central role in radiation-induced DNA damage, DNA repair and cell cycle control. Other proteins interacting with ATM protein include c-Ab1, a protein tyrosin kinase. Activation of c-Ab1 is ATM-dependent, as are its down stream targets SAPK/JNK [8]. AT patients and Atm-null mice are

extremely radiosensitive, while cells with the heterozygous genotype demonstrate an intermediate radiosensitivity[1] . In this report, we demonstrate that the level of ATM protein expression in CNE1, which is more radioresistant to IR than CNE2, is much higher than CNE2. It indicated that ATM protein expression is consistent to cellular radiosensitivities [2]. We proposed that attenuation of ATM protein expression in NPC cells would increase their sensitivity to IR, which has been validated in other cancer cells[11-13] . So our data will support a practicable evidence for a new radiosensitization approach to NPC by weakening ATM protein expression.

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