Expression of Ku correlates with radiation sensitivities in the head and neck cancer cell lines

Sang-wook Lee,[a] Eun Kyung Choi,[a] Eunsil Yu,[b] Seung Do Ahn,[a] Seong Soo Shin,[a] and Jong Hoon Kim[a]
aDepartments of Radiation Oncology, University of Ulsan, College of Medicine, Asan Medical Center, Seoul, Korea.
lsw@amc.seoul.kr
bDepartments of Pathology, University of Ulsan, College of Medicine, Asan Medical Center, Seoul, Korea.

1. Introduction

DNA-dependent protein kinase (DNA-PK) is a serine/threonine kinase consisting of a 470 kDa catalytic subunit (DNA-PKcs) and a heterodimeric regulatory complex, called Ku, which is composed of 70 kDa (Ku 70) and 86 kDa (Ku 80) proteins. The DNA-PK has been shown to play a pivotal role in rejoining DNA double-strand-breaks (dsb) in mammalian cells. DNA double-strand breaks (DSBs) are the most lethal form of DNA damage. The purpose of this study is to examine the relationship between the level of Ku expression and radiation sensitivity.

2. Methods and Materials

2.1 CellCulture

Nine human head and neck squamous cell carcinoma cell lines were established from primary head and neck squamous carcinoma specimens in the laboratory of Dr. Sang Yoon Kim. Cells were grown in Dulbecco's modified Eagle's medium (DMEM) containing glutamine, nonessential amino acids, and 10% fetal bovine serum in a humidified atmosphere of 5% CO_2 at 37℃.

2.2 Laboratory Experiment

Nine head and neck, cancer cell lines showed various intrinsic radiation sensitivities. Among the nine, AMC-HN-3 cell was the most sensitive for X-ray irradiation and AMC-HN-9 cell was the most resistance. The most sensitive and resistant cell lines were selected and the test sensitivity of radiation and expression of Ku were measured. Radiation sensitivity was obtained by colony forming assay and Ku protein expression using Western blot analysis. Apoptosis and cell cycle distributions were determined by flow cytometry after labeling with propidium iodide.

3. Results and Discussion

There was a correlation between Ku80 expression and radiation resistance. Ku80 was shown to play an important role in radiation damage response. The AMC-HN-3, and -9 cell lines were examined for Ku70 and Ku80 proteins expression using immunofluorescent stainning at 4 hrs later after radiation (12 Gy). Ku80 increased expression by radiation in AMC-HN-9, whereas Ku70 did not. (Fig. 1).

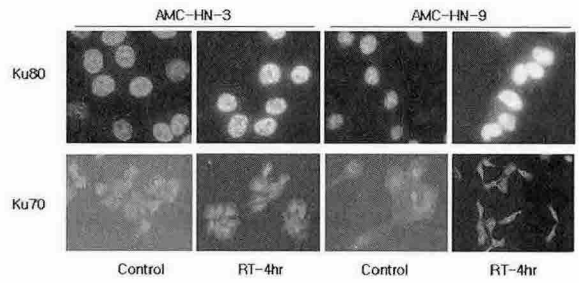

Fig. 1. Immunofluorescent staining was performed in Ku70 and Ku80 (x 250 magnification) after 12 Gy of ionizing radiation. Expression of Ku80 was increased in AMC-HN-9 cell line postirradiation.

The AMC-HN-3, and -9 cell lines were examined for Ku80 protein expression using Western blot analysis at 0.5 hr, 1 hr, 2 hrs, and 4 hrs later after radiation (8 Gy). Ku80 protein expression was increased after 0.5 hr. Overexpression of Ku80 protein increased radiation resistance in AMC-HN9 cell line (Fig. 2).

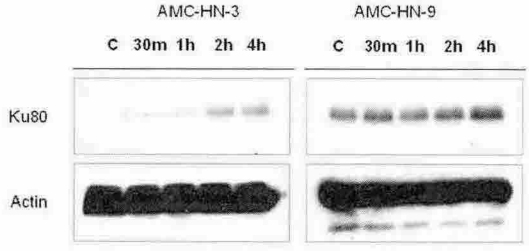

Fig. 2. Western blot showing the levels of Ku80 protein (30 minutes interval) in the AMC-HN-3 and AMC-HN-9 after 12 Gy of ionizing radiation. Expression of Ku80 was increased in AMC-HN-9 more than AMC-HN-3.

Flow cytometric measurements after labeling with propidium iodide revealed that in AMC-HN-3 and –9 cell line. The apoptosis was increased in AMC-HN-3. The fraction of G1-phase and G2-phase cells increased in AMC-HN-9 According to the FACS scan, radiation induced apoptosis were significantly increased in AMC-HN-3 than AMC-HN-9. G1 and G2 cell cycle arrest were increased in AMC-HN-9 (Fig. 3). We think that AMC-HN-9 has capability for DNA damage repair than AMC-HN-3.

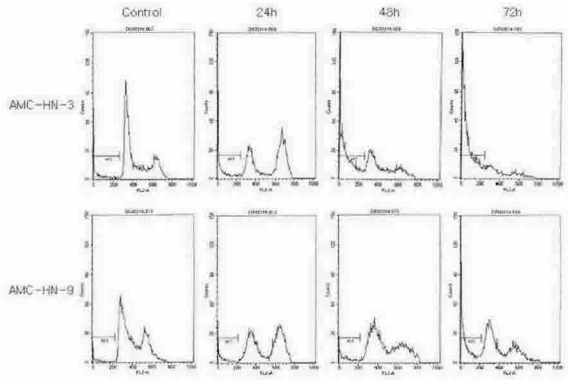

Fig. 3. Cell cycle analysis by flowcytometry in the AMC-HN-3 and AMC-HN-9 after 12 Gy of ionizing radiation

4. Conclusions

Induction of Ku80 expression had an important role in DNA damage repair by radiation. Ku80 expression may be an effective predictive assay of radiosensitivity on head and neck cancer.

REFERENCES

[1] O. Hammarsten, L. G. DeFazio, G. Chu, Activation of DNA-dependent protein kinase by single-stranded DNA ends, J Biol Chem, Vol. 275, pp. 1541-1550, 2000.
[2] M. R. Lieber, U. Grawunder, X. Wu, M. Yaneva, Tying loose ends: roles of Ku and DNA-dependent protein kinase in the repair of double-strand breaks, Curr Opin Genet Dev, Vol. 7, pp. 99-104, 1997.
[3] M. A. Plumb, G. C. Smith, S. M. Cunniffe, S. P. Jackson, P. O'Neill, DNA-PK activation by ionizing radiation-induced DNA single-strand breaks, Int J Radiat Biol, Vol. 75, pp. 553-561, 1999.
[4] D. T. Weaver, Regulation and repair of double-strand DNA breaks, Crit Rev Eukaryot Gene Expr, Vol. 6, pp. 345-375, 1996.
[5] 6. Doherty AJ, Jackson SP. DNA repair: how KU makes ends meet. Curr Biol 2001;11:R920–R924
[6] W. S. Dynan, S. Yoo, Interaction of ku protein and DNA-dependent protein kinase catalytic subunit with nucleic acids, Nucleic Acids Res, Vol. 26, pp. 1551–1559, 1998.
[7] T. Mimori, M. Akizuki, H. Yamagata, S. Inada, S. Yoshida, M. Homma, Characterization of a high molecular weight acidic nuclear protein recognized by autoantibodies in sera from patients with polymyositis-scleroderma overlap, J Clin Invest, Vol. 68, pp. 611–620, 1981.
[8] D. T. Weaver, Telomeres: moonlighting by DNA repair proteins, Curr Biol, Vol. 8, pp. R492-R494, 1998.
[9] J. E. Haber, Sir-Ku-itous routes to make ends meet, Cell, Vol. 97, pp. 829-832, 1999.
[10] G. C. Smith, S. P. Jackson, The DNA-dependent protein kinase, Genes Dev, Vol. 13, pp. 916-934, 1999.
[11] R. Tuteja, N. Tuteja, Ku autoantigen: a multifunctional DNA-binding protein, Crit Rev Biochem Mol Biol Vol. 35, pp. 1-33, 2000.