

Lentivirus-mediated RNA Interference of Ku70 to Enhance Radiosensitivity of Human Mammary Epithelial Cells.

V. Vandersickel^a, M. Mancini^b, E. Marras^b, G. Perletti^b, H. Thierens^c and A. Vral^c

^a*Ghent University, De Pintelaan 185, 9000 Gent, Belgium;* ^b*Università degli Studi dell'Insubria, via A. da Giussano, 12, 21052 Busto A, Italy;* ^c*Dept Basic Medical Sciences, University Hospital Ghent, Building B3, De Pintelaan 185, 9000 Gent, Belgium*
anne.vral@ugent.be

Breast cancer patients are characterised by an enhanced chromosomal radiosensitivity pointing to a defect in the repair of DNA double strand breaks (dsb). In mammalian cells, radiation induced dsb are mainly repaired by the non homologous end-joining (NHEJ) repair pathway, a pathway in which the Ku70/Ku80 heterodimer plays a key role as it binds to the broken DNA ends. In this study we wanted to investigate the radiosensitizing effect of Ku70/80 knockdown, by lentivirus-mediated RNA interference, in a spontaneous immortalised human mammary epithelial cell line (MCF10A). Several endpoints for measuring radiosensitivity were taken into account: micronucleus formation (chromosomal radiosensitivity), cell survival, apoptosis and senescence. For all endpoints, MCF10A cells were infected with lentiviral vectors for RNAi of Ku70 (pLVTHM/shKu70/GFP). Western blot analysis showed that the Ku70 lentiviral vector was effective in silencing the expression of both Ku70 and Ku80. When a satisfactory knockdown was obtained (70-90% vs. mock-infected (pLVTHM/GFP) cells), the cells were used to perform radiation experiments. For the in vitro MN assay, cells were irradiated with doses of 2 and 4 Gy ⁶⁰Co gamma-rays. A significantly higher radiation-induced MN yield was obtained in the Ku70/80 knock down cell line compared to the mock-infected cell line, pointing to an increased chromosomal radiosensitivity. This increased chromosomal radiosensitivity demonstrates that the repair genes, Ku70 and Ku80, are involved in the repair of DNA double strand breaks, which are the main DNA lesions resulting in chromosomal aberrations such as micronuclei. Besides chromosomal radiosensitivity we also investigated radiosensitivity at the cellular level by cell survival experiments. Cells were irradiated with doses ranging between 0 and 8 Gy and cultured for 5 days before being analysed. The results of the cell survival assay show that Ku70/80 knockdown cells have a lower survival yield after irradiation compared to mock-infected cells, pointing to an enhanced cellular radiosensitivity. Analysis of the cell death pattern showed that MCF10 cells (Ku70/80 knockdown and mock-infected) do not undergo apoptosis but go into cellular senescence. In conclusion, we can state that knockdown of Ku70 and Ku80 by RNAi of Ku70 resulted in an increased chromosomal and cellular radiosensitivity in an immortalised MCF10 human mammary cell line after irradiation with low LET ⁶⁰Co gamma-rays. These results may further support the role of DNA dsb repair genes in breast cancer.