Short And Long Term Effects In Human Cells As A Consequence Of High LET Irradiation

J.Kool, B. Klein, M. van Eck-Smaling, A.J. van der Eb and C.Terleth.
Department of Radiation Genetics and Chemical Mutagenesis, Leiden University Medical Centre, Leiden, The Netherlands.

Introduction

In effort to mimick immortalization of human cells, a specific stage of the carcinogenic process *in vitro*, many experimental approaches have been used. Reproducible immortalization could be achieved by transforming human cells with DNA tumor viruses, such as for example SV40 and HPV. The oncogenes largeT and E6/E7 respectively, will modify the human fibroblast's regulatory systems in such a way that the cells will first bypass senescence. Subsequently, during the extended lifespan, the cells will accumulate chromosomal aberrations. Finally, genomic instability will be so massive that the transformed cells enter crisis, a stage defined by massive cell death. Occasionally, at a frequency of 10^{-7}, cells escape crisis and immortalize [1]. Alternatively, immortalization could be achieved by repeated treatments with DNA damaging agents such as 4-NQO and ^{60}Co rays. In these cases, cells would be treated 60-140 times at three day intervals [2]. Our initial interest in irradiating human cells with heavy ions originated from an observation by Dr. Laure Sabatier (CEA,F) that cells which had been exposed to a single high LET irradiation seemed to display an extended lifespan [3]. We wondered whether high LET would indeed lead to such an extension. The molecular basis for the processes discussed above is probably the length of the telomeres. Telomeres are specialized DNA structures at the end of the chromosomes which have a protective role. During the lifespan of the cell telomeres shorten until they reach a certain minimal size, which will trigger senescence. If the cells are forced past senescence, for example by viral oncogenes from DNA tumor viruses, the telomeres will shorten further. At this stage the telomeres become reactive leading to chromosomal instability. For a cell to escape the ensuing crisis, it is necessary to reactivate telomerase, a protein able to stabilize the ends of chromosomes. Indeed, in immortal tumor cells and in *in vitro* immortalized cells telomerase activity is commonly found (although alternative mechanisms of telomere lengthening (ALT) have also been described).

Long term effects

As extension of lifespan is an important step in tumorigenesis, we wanted to determine the effect of high LET irradiation on the lifespan of human cells. Thus we have irradiated normal fibroblasts as well as fibroblasts lacking key factors in installment of senescence (i.e. p16 and p53). In separate experiments, we have irradiated the cells with Ni or Ar, and with different fluences. Subsequently, the cell cultures were closely followed. Population doublings were calculated carefully, samples for RNA, DNA or protein isolations were taken at regular intervals, and chromosome analysis was performed. Results sofar show a gradual, and expected, decrease of telomeres in the cells as population doublings increase. No telomerase is activated and cells enter senescence eventually. In the irradiated cell cultures profound changes can be observed in the chromosomes as a consequence of irradiation, including a delayed genomic instability as has been described previously. The effect of high LET irradiation on lifespan is in this set of experiments very moderate, if significant, with an extension of 3 population doublings at most. However, in one experiment we included a fibroblast line derived from a patient with Nijmegen breakage syndrome (NBS). Interestingly, in these cells a single high LET irradiation has led to an extension of 10 population doublings, which is in the order of magnitude of the effects of the viral oncogenes E6 and E7 from HPV. Even more intriguing, the telomeres in the irradiated cells have increased in the absence of telomerase activity, suggesting activation of ALT. We have recently repeated this experiment including NBS cells from different patients, to see if this a reproducible phenomenon.

Short term effects

When human cells are exposed to irradiation many responses are induced. These responses include the induction of genes, which will eventually lead to processes such as cell cycle arrest, DNA repair or apoptosis. We have focussed in particular on the stress induced pathways involving the MAPkinases. Two kinases play an important role in these pathways; JNK which can activate transcription factors like c-Jun and ATF2, and p38 which can activate ATF-2 [4,5]. We examined activation of the kinases p38 and JNK after irradiation of human cells. Also activation of ATF2 and cJun was examined. In initial studies we have compared the effects of UV and X-rays. Whereas UV is a potent activator of both p38 and JNK, X-rays only weakly activate p38 but not JNK. We then decided to extend our studies to high LET as the RBE might be higher. Initial experiments were performed at GANIL (Caen, F) with Ar, and showed activation of p38 and ATF-2, but no JNK activation. However, our results at GSI for both Ar and C (UniLac and SIS) have been quite different. With Ar we did see clear activation of ATF2 and also cJun, but not of JNK and p38. With C irradiation with a high LET value (135keV/μm) minor activation of ATF2 and cJun was detected but no activation whatsoever could be observed in cells irradiated with C with a lower LET value (27.9keV/μm). Our hypothesis is that the differences seen in activation of the stress responses between irradiated cells are caused by the differences in LET values. It is known that cell survival after irradiation varies with different LET values. Our results suggest that the differences we find after irradiation with different LET values in short term responses, i.e. activation of stress-response pathways, could play a role in the differences seen on survival in the long term.

References

[1] Sedivy JM. (1998). *Proc. Natl. Acad. Sci.*, **95**, 9078-9081

[2] Tsuji T, Miyazaki M, Sakaguchi M, Inoue Y, Namba M. (2000) *Biochem. Biophys. Res. Commun.*, **268**, 20-24

[3] Martins MB, Sabatier L, Ricoul M, Pinton A, Dutrillaux B. (1993) *Mutat. Res.*, **285**, 229-23

[4] Tibbles LA, Woodgett JR. (1999) *Cell. Mol. Life. Sci.*, **55**, 1230-54

[5] Davis RJ. (2000) *Cell*, **103**, 239-252