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Biological effect of high single doses of irradiation on tumor an normal cells in vitro

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With high single doses and very large dose fractions becoming more widely applied in clinical radiotherapy, the biological effect of HD irradiation has to be investigated. The purpose of the present work was to determine the relative biological effectiveness (RBE) of 10 MeV electrons radiation as used in intra-operative radiotherapy (IORT) relative to 6 MV X rays radiation, and to study DNA damage induction and repair up to high dose levels. During these investigations an increase in the number of seeded cells resulted in a reduction in clonogenic survival. The involvement of a high dose bystander effect and its mechanism was additionally investigated.

The clonogenic survival curve for a breast cancer cell line (MCF7) and normal tissue cells (HUVEC) was successfully extended up to HD levels and was used for determination of the RBE of 10 MeV electrons versus 6 MV X rays for both cell types. The result revealed a significant reduction of RBE (RBE: 0.90 at SF= 2*10-3; P= 0.02) in normal human umbilical vein endothelial cells (HUVEC) in the high dose range (6-8 Gy), but did not reveal any significant changes in the breast cancer (MCF7) cells over the low or high dose region. This observation confirmed and extended preliminary previous data from our lab for the lower doses.

Studying the DNA double strand break (DSB) induction and repair kinetics using γ-H2AX foci quantification in MCF7 tumor cells and HUVEC normal tissue cells up to HD level revealed for both cell types a fast induction of DSB with maximum at 0.5 h. However, the higher dose (12 Gy) did not produce a proportionally increased foci number relative to lower dose (6 Gy). After induction, a decrease in foci number within 6-8 hours post irradiation (6 h in MCF7, 8 h in HUVEC) was observed. Thereafter the decay of the residual foci induced by high dose was slower relative to low dose induced foci number.

Above all, it suggests that the DNA repair system might become gradually saturated at the HD level.

During the study of the biological effect at high dose, it was observed that increasing the number of seeded cells in order to obtain more colonies for scoring at higher doses unexpectedly resulted in a reduction in clonogenic survival. This observation was further investigated. An inverse correlation ($R= 0.53$, $P<0.0001$) between the number of colonies obtained in a clonogenic cell assay and the cell number of seeded cells could be shown in the HD region. It could also be shown that this effect correlated with the dose ($R= 0.5$, $P= 0.022$) and was, in part, transferable to un-irradiated cells using irradiated cells conditioned-medium (ICCM) transfer. These observations were performed and confirmed using two breast cancer cell lines (MCF7, MDA-MB-231) and normal tissue HUVEC cells. An investigation of the underlying mechanism and the potential component responsible for this effect was performed. Apoptosis and cell cycle analysis did not reveal any differences in the use of CM from irradiated and un-irradiated cells. Similarly, the addition of TGF-β1, a cytokine found involved in low dose bystander effects had no effect either. Screening of additional cytokines using a cytokine array also did not reveal any differences between the two types of CM. Testing the gene expression patterns of bystander recipient cells treated with the different CM also did not show any significant differences. However, addition of ICCM from 15 Gy irradiated cells resulted in a 2.25 fold increase ($P= 0.002$) in γ-H2AX foci number compared to 0 Gy CM, while irradiated medium had no effect on the number of foci. These data suggest that the observed HD bystander effect functions, at least in part, by induction of DNA DSB damage and has to be further investigated.

All the data obtained from the present work support a difference in the biological effects of high-dose compared to low-dose irradiation.