

### 3 - 72 Lack of Evidence for Serum Serotonin Levels Determining Radiation Induced Bystander Effects in V79 and HepG2 Cells

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Radiation induced bystander effect (RIBE) has been defined as a biological response to radiations resulting in damage to cells that had not themselves been traversed by a track of the ionizing radiations. It has been shown that the transfer of irradiated cell conditioned medium is able to kill cells that are not directly irradiated. But interlaboratory variability in the production of bystander effects using medium transfer protocols is a well known and frustrating problem. Even within the same laboratory, the effect can fail to be manifested. The study conducted by Mothersill et al. showed that serum serotonin levels determine the magnitude and type of bystander effects<sup>[1]</sup>.

To investigate how serum serotonin levels affect the toxicity of the medium harvested from irradiated cells, we examined the radiation induced bystander effect with different serotonin levels, using a standard medium transfer colony-forming assay and irradiation of 0.5 Gy high-LET carbon ions (30 keV/ $\mu\text{m}$ ).

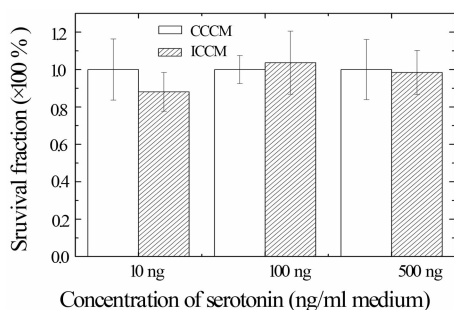


Fig. 1 Survival fractions of V79 cells cultivated with CCCM and ICCM at different serotonin concentrations.

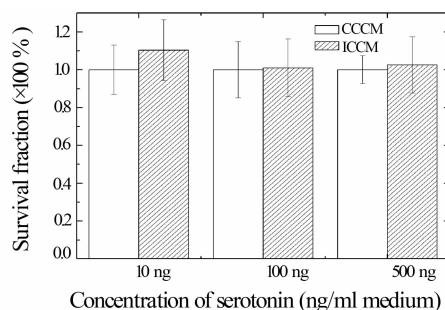


Fig. 2 Survival fractions of HepG2 cells cultivated with CCCM and ICCM at various serotonin concentrations.

Chinese hamster V79 lung fibroblast and human hepatoma HepG2 cell lines were used in our study. T25 flasks destined to donate the media were plated with cells of approximately  $1 \times 10^6$ . Cells were either sham-irradiated (0 Gy) or directly irradiated with the carbon ions. 18 h later, bystander effects were investigated by medium transfer experiments as described by Mothersill and Seymour<sup>[2]</sup>. Medium was removed from each donor flask 2 h postirradiation and passed through a 0.22- $\mu\text{m}$  sterile filter gently to eliminate cells or debris from the transferred medium, which termed irradiated cell conditioned medium (ICCM). The medium from control cells plated at the same density is termed control-cell conditioned medium (CCCM). 5 ml of the ICCM or CCCM was directly filtered to unirradiated cells in recipient flasks. The recipient flasks were then incubated for 9 d to allow colonies formation. Serotonin (5-hydroxytryptamine) as the creatinine sulphate salt, which was made up as a concentrated aqueous solution and then serially diluted so that the desired concentration of the drug could be added to the 5 ml of medium in the culture flasks.

Under these conditions, we did not detect a significant bystander effect using the medium transfer technique. The results contradict those in the published reports, where bystander effects have been reported for the similar end points. A possibility to explain the contradiction is that the bystander effects may occur only in specific cell types which have not been understood fully.

#### References

- [1] C. Mothersill, R. Saroya, et al., *Radiation Research*, 174, 1(2010)119.
- [2] C. Mothersill, and C. Seymour, *International Journal of Radiation Biology*, 71, 4(1997)421.