# Heavy Ion Irradiation of Human Colon Adenocarcinoma Cells in Multilayer Culture

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**Introduction -** The major goal of this project is to study the effect of heavy ion irradiation on radioresistant human tumour cells which are grown in a specific tissue-like culture model.

**Materials and Methods -** WiDr colon adenocarcinoma cells were cultured in either conventional monolayers or cellular multilayers in a specific culture chamber [1]. Cell cultures were irradiated with 250 kV X-rays, with a 100 MeV/u, 200 MeV/u or 400 MeV/u $^{12}$C-beam (plateau region), or with an extended Bragg-peak. During irradiation the plane of the mono- and multilayers was positioned perpendicular to the direction of the radiation beam. After radiation, single cell suspensions were derived from the cultures by trypsinisation and appropriate dilution, and these suspensions were used for standardised clonogenic assays. The plating efficiency was determined as the percentage of colonies (>50 cells) in relation to the number of seeded cells, and relative cell survival was determined as a function of radiation dose. Cell cycle analysis was performed with standardised propidium iodide and BrdU labelling techniques and flowcytometry. Apoptoses were detected with a standardised TUNEL labelling using a commercial test kit.

**Results -** The fraction of apoptotic cells was very low, i. e., around 1 % in untreated cultures and could not be induced by heavy ion irradiation. There was a dose-dependent $G_2$/M-arrest which was more significant with heavy ions than with x-rays, and which resulted in a $G_2$-block in 91 % of the cells treated with 6 Gy in the extended Bragg peak. Cell survival rates were lower in monolayer than in multilayer cultures; this phenomenon was most pronounced in the Bragg peak compared to all other radiation conditions, as exemplified in Fig. 1.

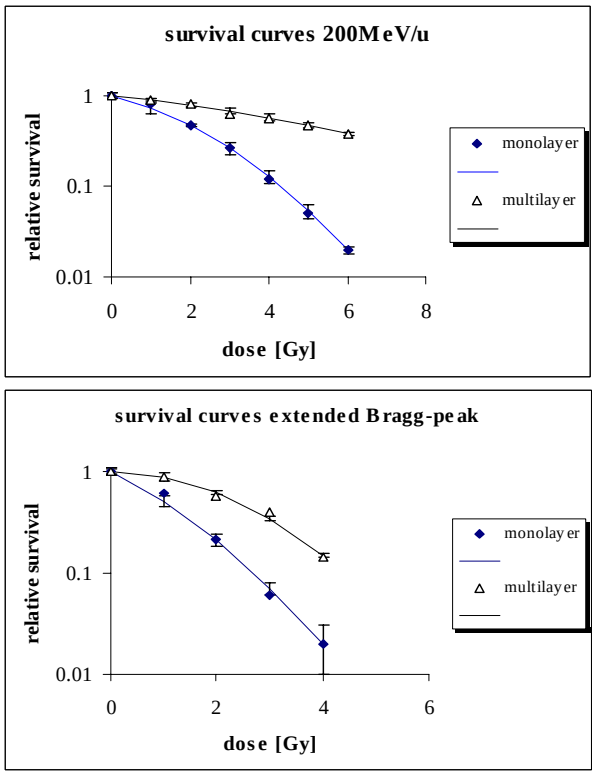


Fig. 1: Relative cell survival as a function of heavy Ion radiation dose in the plateau region (left panel) and in the extended Bragg peak (right panel) for monolayer (diamonds) and multilayer (triangles) cultures.

**Discussion –** The high radioresistance of WiDr cells compared to most other tumour cell lines can be partially explained by a pronounced $G_2$/M-arrest following irradiation. This intrinsic resistance is even enhanced by multicellular resistance when cells grow in a in a three-dimensional tissue-like arrangement. The molecular basis of these phenomena need to be investigated.

[1] Minchinton A. I., Wendt K. R. Clow K. A., and Fryer K. H., Acta Oncologica **36**, 13-16 (1997)