

Neoplastic transformation induced by carbon ions

Daniela Bettega¹, Francesca Botta¹, Paola Calzolari¹, Petra Hessel², and Wilma Weyrather²
¹Dept.Physics, Univ. of Milan, Italy; ²GSI Darmstadt, Germany

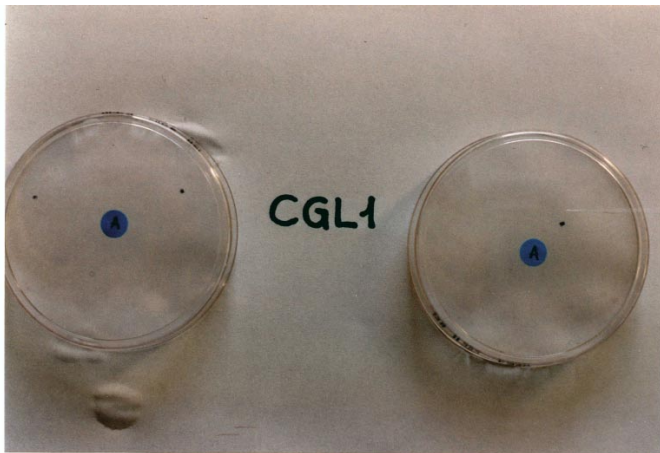


Figure 1: The figure shows two petri dishes with CGL1 cell cultures. The little blue points are WB-stained IAP-positive foci, indicating a colony of transformed cells.

The good results of the tumorconform irradiation with carbon ions with a high local control and low toxicity [1] makes this method a probable candidate for the irradiation of pediatric tumors. In this case the induction of secondary tumors in the low-dose margins would play a larger role than in the treatment of adult people primarily because of their longer life span [2] Animal experiments in respect to late effects and tumor induction are very time consuming and expensive because of the high number of treated animals to get a sufficient statistic and the long time necessary for observation. Therefore a suitable cell system for reliable predictions has to be found.

Oncogenic transformation in vitro has proved to be a valuable tool for providing data of pragmatic usefulness in the field of radiological protection and in human risk estimation. The first widely used quantitative system was the C3H10T1/2 rodent cell line [3].

In the last two decades much effort has been devoted on developing quantitative human systems for transformation studies. The human hybrid (HelaXskin fibroblast) cell line, designated CGL1, has been developed by Stanbridge et al [4] and Redpath et al [5], for quantitative studies of radiation induced neoplastic transformation in vitro. These cells are non tumorigenic when injected into nude mice, whereas transformed cells form carcinomas [5]. The expression of tumorigenicity is associated with the expression of a cell surface protein which has been identified as an intestinal alkaline phosphatase IAP [6]. By staining with the alkaline phosphatase chromogenic substrate Western Blue which allows direct detection of cells expressing IAP [7], the radiation induced transformed cells can be detected (Fig.1).

In a first experiment CGL1 cells were irradiated with a 270 MeV/u carbon beam (266.4 MeV/u on target, LET = 13.7 keV/ μ m). After the irradiation the cells were plated for transformation assay at 50 survivors/ cm^2 , with a total

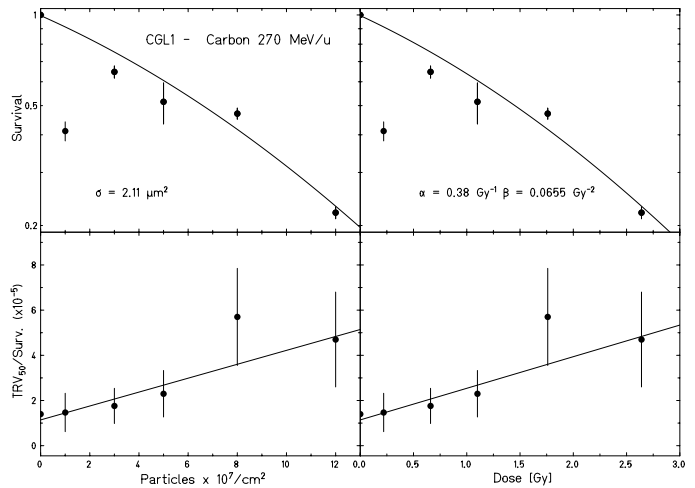


Figure 2: Survival and transformation frequency for CGL1 cells irradiated with a 270 MeV/u carbon beam. The cross section σ for cell inactivation is calculated for a survival level of 0.05.

number of survivors between 10^5 and 2×10^5 depending in the dose value. Survival has been determined in parallel. After plating cells have been incubated for 10 days for clonogenic survival and for 21 days for the assay for neoplastic transformation, with the growth medium changed once a week. Then the cultures were fixed with 2% paraformaldehyde/PBS and stained with Western Blue following the standard protocol for this system [6],[7]. Blue foci were scored against a white background. Simply counting the number of foci may give an overestimation of transformation frequency due to the possibility that satellite foci may form in flasks already containing one transformed colony. Therefore the 'null method' suggested by Han and Elkind [8] was adopted and the mean number of transformants per flask was calculated as $-\ln P(0)$ where $P(0)$ is the fraction of flasks without any foci. First results are shown in fig.2. For comparison an X-ray experiment, performed in the same way, is now in progress for evaluating the RBE.

References

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