

The Intrinsic Radiosensitivity of Chinese Hamster Ovary Cells (CHO-K1) using the Clonogenic and Micronuclei Assays

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Many factors affect tumor response to radiotherapy and these include the inherent radiosensitivity of cells, repopulation, reoxygenation, repair, recruitment, resorption and redistribution in the cell cycle during treatment with ionising radiation (Crompton *et al.*, 1997)

To evaluate intrinsic radiosensitivity, single-cell plating techniques are considered to be the standard for evaluating cellular response and clonogenic cell survival to agents such as radiation (Rockwell and Kallman, 1973; Deschavanne and Fertil, 1996). Fertil and Malaise (1981), found significant variations in radiosensitivities of tumors evaluated within each histologic group and demonstrated that radiosensitivity may vary with the DNA characteristics of the tumor cells. A correlation of the intrinsic radiosensitivity of tumor cell lines with the radiocurability of related tumor groups was reported. A predictive assay for intrinsic radiosensitivity was established by correlating the *in vitro* radiosensitivity of cell lines developed from human tumors with the clinical responsiveness of tumors of the same histological classification (West *et al.*, 1998).

Also called the colony assay, the clonogenic assay determines the cells ability to retain reproductive integrity in the response to exposure to radiation or other genotoxic agents. The assay necessitates a cell to pass through a number of post-treatment mitoses proceeding to form a visible, viable colony of 50 or more cells. A cell that has attained this has proved its clonogenic ability and is scored as a survivor. The systematic loss of this ability as a function of radiation is described by means of the dose-survival curve.

To overcome the fact that cells taken from fresh specimens do not maintain their reproductive integrity for more than a few weeks, established cell lines are used extensively in experimental radiobiology. Cell lines are developed from cells taken from biopsies, grown in culture and repeatedly reseeded. The first *in vitro* survival curve for mammalian cells irradiated with x-rays was of HeLa cells reported by Puck and Marcus (1956).

Survival curves have contributed greatly to the understanding of radiobiology. Deschavanne and Fertil (1996) reviewed the data documenting the radiosensitivity of about 700 cell lines *in vitro* and described a scale of radiosensitivity for human cells.

In this study, CHO-K1 cells were exposed to gamma (⁶⁰Co) irradiation at doses of between 0 - 12 Gy and their response measured by means of the clonogenic and micronuclei assays. The purpose was to establish what type of correlation exists between the intrinsic radiosensitivity of a cell line, defined in terms of survival, and the frequency of micronuclei formation in the first wave of post mitotic cells.

Materials and Methods

Clonogenic Assay

CHO-K1 cells were taken from growing stock cultures and prepared as single cell suspensions by trypsinisation, which caused cells to round up and detach from the surface of the culture flask. Serial dilutions of known cell numbers were made and plated according to expected survival, thus to ensure that about 200 colonies per petri dish could be counted. Alpha minimal essential medium (α-MEM), completed with 10% foetal calf serum, was added to stop the trypsinisation. An estimate of cell numbers per unit volume was quantified using a haemocytometer. An hour after plating cells were exposed to doses of 0, 2, 4, 8 and 12 Gy ⁶⁰Co (γ-irradiation and incubated for 7 days.

Following incubation, colonies were fixed with glacial acetic acid, methanol and water in a ration of 1:1:8 (v/v/v), stained with a solution of 0.01% Amido Black and those containing more than 50 cells enumerated. The plating efficiency (PE) is defined as the fraction of colonies to the number of cells plated in the control samples. Surviving fraction is defined as the number of colonies counted divided by the number of cells seeded multiplied by the PE.

Micronucleus Assay

Around 5×10^4 cells were plated into a 30mm petri dish containing a coverslip. Following irradiations, Cytochalasin-B was added to each sample at a final concentration of 2(μg/ml). Cytochalasin-B interferes with mitosis and yields bi-nucleated cells. Cells were incubated for 24 hours and fixed using 3:1 methanol:acetic acid. The cells adherent to the coverslips were stained using Acridine Orange, mounted in buffer (pH 6.8) and visualized using a fluorescent microscope equipped with FITC filter set. The number of micronuclei in about 500 bi-nucleated cells was counted.

Results and Discussion

The clonogenic photon survival curve generated for CHO-K1 cells is presented in Figure 1. The graph displays an initial shoulder, which is followed by a straightening part that represents the exponential decline in surviving fractions as a function of dose. Colonies consisting of 50 or more cells were enumerated and mean cell survival per dose point was calculated corrected for plating efficiency.

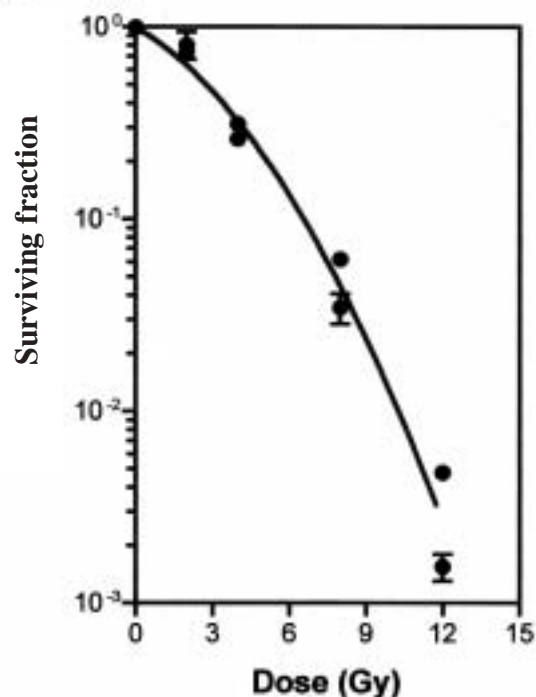


Figure 1: Cell survival for CHO-K1 cells exposed to 2-12 Gy ⁶⁰Co (γ-irradiation). Data plotted on a log-linear scale and fitted to a linear-quadratic model.

Survival data were fitted to the linear quadratic equation $S = \exp(-\alpha D + \beta D^2)$, where S is the survival fraction, following a dose D and α and β are coefficients. The radiobiological characteristics of CHO-K1 cells calculated from the respective experiments are depicted in Table 1. The mean inactivation dose or D₀ values represent the area under the curve and was 3.25 ± 0.44 and 3.23 ± 0.77 respectively. By comparing these data with that of Slabbert *et al.*, 2000, who reported a mean inactivation dose in the range of 1.65 - 4.35 for the numerous cell lines studied, CHO-K1 cells fall within the 75 percentile of this range. From this, relative radioresistance of the CHO-K1 cells is demonstrated.

Table I: The inactivation parameters for CHO-K1 cells exposed to ^{60}Co γ -irradiation where α = lethal damage; β =reparable damage and \bar{D} = mean inactivation dose

Experiment	$\alpha \pm \text{SE}$ (Gy ⁻¹)	$\beta \pm \text{SE}$ (Gy ⁻²)	$\bar{D} \gamma$	SF ₂
1	0.1996 ± 0.027	0.0203 ± 0.0025	3.25 ± 0.44	0.7420
2	0.150 ± 0.036	0.0328 ± 0.0034	3.23 ± 0.77	0.8064
Mean	0.175 ± 0.032	0.027 ± 0.0029	3.24 ± 0.61	0.7742

The mean inactivation dose is a one-dimensional parameter reflecting the average cell response and thus may underestimate the differences in radiosensitivity between cell lines. α -Values that quantify the initial slope of the curve are a more sensitive parameter for indicating differences in radiosensitivity.

Comparing the α -values ranging from 0.09 - 0.55 presented by Slabbert *et al.* 2000, and that of 0.176 calculated for the CHO-K1 cells, it is interesting to note that the smaller the α -coefficient, the greater the relative radioresistance displayed by the cell to gamma irradiation.

Within each experiment the parameters display little variation although a significant variation is evident on repetition as indicated in Table I. In order to reduce variation within an experiment, a single dilution route is followed with replicates plated from one dilution. This does not reflect the uncertainties associated with the experiment, which is addressed when the experiment is repeated and as a result small error bars are observed (Fig.1). In order to achieve the most quantitative results four dose points and a control are set up in triplicate. In an attempt to address counting variation as well as to better quantify the response to radiation the dilution is performed on untreated cells.

An important observation is that the α -coefficient, indicating lethal damage and the β -coefficient, representing reparable damage, are co-variant. As the α -value increases the corresponding β -value decreases. As a result of this the \bar{D} values remain similar as observed by Steel *et al.*, (1989) who reported that radioresistance does not correlate with the lack of reparable damage.

The frequency of micronuclei formations in bi-nucleated CHO-K1 cells increase in a smooth manner ($R^2 = 0.997$) with radiation dose (Fig. 2). A linear-quadratic expression fitted to the experimental observations yield an α -value of 76.1 ± 4.7 and a β -value of 1.1 ± 1.1 . As the reparable component of radiation damage is not reflected in a statistical significant manner by the micronuclei data it is concluded that a simple linear relationship exists between physical dose and cell damage using this endpoint. Within the range of doses used, the rate of micronuclei induction per unit dose appears to be constant and as such reflect in full cellular damage by ionizing radiation.

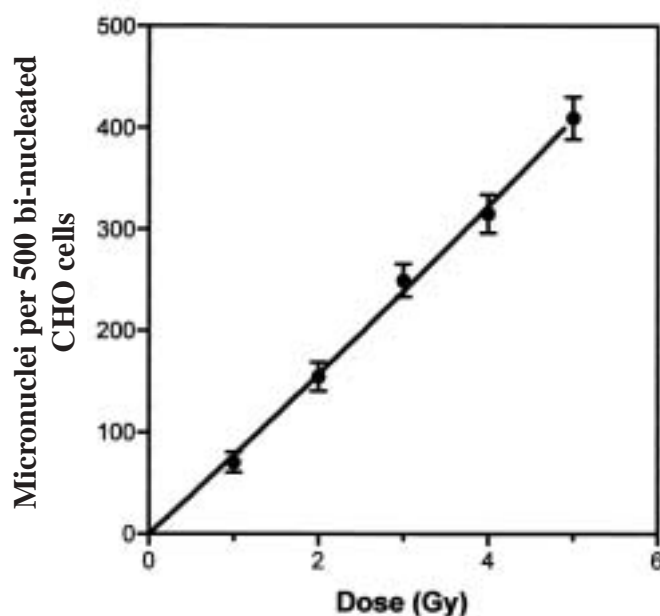


Figure 2: Micronuclei formations in bi-nucleated CHO-K1 cells following exposures to different doses of Gy ^{60}Co γ irradiation.

The relation between micronuclei and surviving fraction is shown in Fig.3. For gamma doses of between 1 and 5 Gy used in this study a linear inverse relationship is evident between micronuclei frequency and single cell survival.

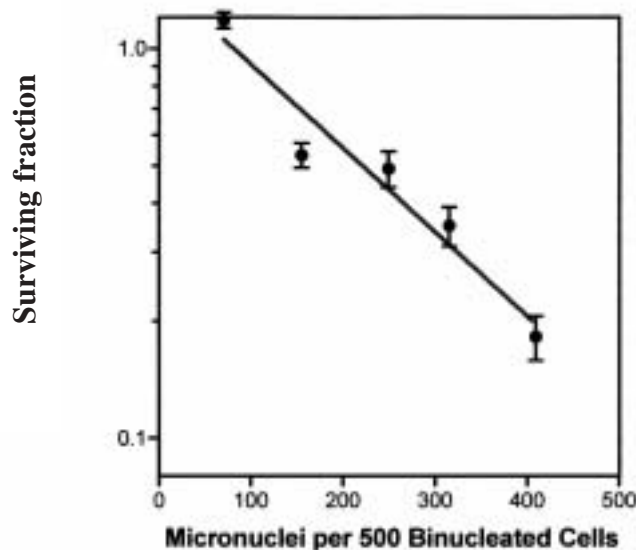


Figure 3: Micronuclei formations in relation to the clonogenic survival of CHO-K1 cells exposed to graded doses of 1 - 5 Gy ^{60}Co γ irradiation.

It is concluded that the sensitivity of CHO-K1 cells to ionizing radiation can be estimated using both clonogenic survival and micronuclei formations. Both endpoints yield measurable data over a useful dose range and cellular damage can be quantified with a reasonable precision. With this in mind it would be most useful to follow the influence of a variation in radiosensitivity using these endpoints.

References

1. Crompton, N.E.A., Ozsahin, M., Schweizer, P., Larsson, B. and Luetolf, U.M. (1997). Theory and practice of Predictive Assays in Radiation Therapy *Strahlenther.Onkol.* 173 (2), 58-67
2. Deschavanne PJ and Fertil B. (1996). A Review of Human Cell Radiosensitivity *in vitro.* *Int.J.Rad.Oncol.Biol.Phys.* 34 (1), 251-266
3. Fertil B and Malaise E-P. (1981). Inherent cellular radiosensitivity as a basic concept for human tumor radiotherapy. *Int.J.Rad.Oncol.Biol.Phys.* 7:621-629
4. Puck, TT and Marcus PI. (1956) Action of X-rays on mammalian cells. *J.Exp.Med.* 103, 653-666
5. Rockwell, S.C. and Kallman, R.F. (1973) Cellular radiosensitivity and tumor radiation response in the EMT6 tumor cell system. *Radiat. Res.* 53, 281-284
6. Slabbert, J.P., Theron, T., Zölzer, F., Streffer, C. and Böhm, L. (2000). A comparison of the potential therapeutic gain of p(66)/Be neutrons and d(14)/Be neutrons. *Int.J.Rad.Oncol.Biol.Phys.* 47(4), 1059-1065
7. Steel G.G. and Peacock J.H. (1989). Why are some human tumors more radiosensitive than others? *Radiother. Oncol.* 15, 63 - 72
8. West, C.M.L., Davidson, S.E., Roberts, S.A. and Hunter, R.D. (1993) Intrinsic radiosensitivity and prediction of patient response to radiotherapy for carcinoma of the cervix. *Br.J.Cancer.* 68, 819-823