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Radiosensitivity of Human Cell Lines to Small Doses. Are There Some Clinical Implications?

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The concept of intrinsic radiosensitivity is now strongly associated with the linear-quadratic (LQ) model which is currently the best and the most reliable method to fit the first three decades of a survival curve for both human fibroblast and human tumor cell lines. This approach has led to the major conclusions that it is the initial part, and not the distal part, of the survival curve which truly characterizes intrinsic cellular radiosensitivity and there is a correlation between the parameters describing mainly the initial part of the survival curve (α, SF_2, $\overline{D}$) and the clinical radioresponsiveness. More accurate analysis with flow cytometry or a dynamic microscopic image processing scanner (DMIPS) has allowed further study of the survival curve which has shown two sorts of substructure. On one hand, the overall survival curve of exponentially growing cells is described by two or more sets of α, β parameters (heterogeneity in radiosensitivity due to the cell cycle). On the other hand, hypersensitivity at very low doses (<0.5 Gy) followed by an increase of the radioresistance of the whole population at higher doses has also been observed. This phenomenon is not described by the conventional LQ model and has been interpreted as an induced radioresistance which seems to be *negatively* correlated with intrinsic radiosensitivity. In clinical radiotherapy, there are two sorts of response of normal tissues: (1) the early and late damage and (2) the carcinogenesis. Concerning the first point, the clinically detectable radiation damage appears at doses usually around 20 Gy (in 2-Gy fractions) with the exception of the hemopoietic and the lymphatic tissues. Therefore, the small doses delivered at the edges or in the penumbrae of treatment fields in routine radiotherapy cannot create detectable damage, despite a potentially much higher effect per unit dose, because the total doses are still very small. However, it may be important to bear in mind the possible extra effect of low doses outside the target volume if regions in the vicinity are subsequently retreated. Concerning clinical radiation-induced carcinogenesis, three studies described a higher relative risk associated with small doses per fraction or very low dose rate. The results and the interpretation of these studies are discussed.

HISTORICAL OVERVIEW

Twenty-five years elapsed between the first description of the survival curve of a mammalian cell line (*1*) and the time when it was realized that the survival curves of human cell lines were not all the same (*2*), and that these differences were reflected in the clinical responsiveness of tumors treated with ionizing radiations (*3*, *4*). We believe that this lengthy delay in the careful study of human cell survival curves is largely due to the almost universal use of an inappropriate statistical model (the single-hit multitarget model); the two parameters n and D_0 are generally obtained from the distal part of the survival curve (often the second and third decades), and the curve is described as a shoulder followed by an exponential. Fitting the survival curve with n and D_0, almost always leads to a major underestimation of the killing effect in the initial part of the survival curve. But it is now well established that it is precisely this initial part of the survival curve, and this alone, that characterizes intrinsic radiosensitivity (*5*).

The first step was to find a mathematical model that adequately described the whole survival curve. The linear-quadratic (LQ) model (or the so-called α-β model) was tested on six human cell lines, and found to be better than three models based on target theory, including the model with an initial linear component (*2*). The LQ model has now been used to analyze several dozens of published survival curves. These studies have shown that the linear component (characterized by α), or more simply, the survival at 2 Gy (SF_2), is a biological determinant of local tumor control by radiotherapy (*3*, *4*, *6*). This is the reason why the concept of intrinsic radiosensitivity is closely associated with the LQ model. Although this is an empirical model, it fits reliably the initial two or three decades of survival curves for both human fibroblasts and human tumor cell lines (*5*).

Both the α and β components of the LQ model can depend on repair phenomena. However, the practical demonstration of repair phenomena is based on experimental protocols that differ depending on whether these phenomena involve α or β. Split-dose recovery (as well as low-dose-rate recovery) is directly associated with parameter β. Peacock *et al.* (*7*) have suggested a method of evaluating repair based on this relationship; it uses split doses rather than single doses.

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However, two types of experiments also emphazise the contribution of repair to the *linear* component: repair inhibitors increase the initial slope and thus α (*8*), while delayed plating of plateau-phase cells always leads to a reduction in survival levels. This reduction occurs mainly in the initial part of the curve, and thus produces a drop in the coefficient α (*9*).

SURVIVAL CURVES: EVIDENCE OF SUBSTRUCTURE

Two sophisticated techniques have been used to improve the accuracy in the determination of analysis of survival curves: flow cytometry (FCM) and the dynamic microscopic image processing scanner (DMIPS). These two very different techniques have a common feature: they both provide an accurate measure of the exact number of cells plated out. They thus considerably improve the precision with which the surviving fraction is estimated. Their use has revealed the presence of two substructures in survival curves for human cells (*10*). As predicted by the theory, a population of human cells growing exponentially is radiobiologically heterogeneous. Description of the survival curve with a single α and a single β is thus, in fact, a compromise. Standard methods have never been capable of detecting this heterogeneity in a nonsynchronized population of exponentially growing cells, but Skarsgard, Harisson and Durand have used FCM to describe it in several human cell lines. This heterogeneity results in a curve with two α and two β. It is lost when the same experiments are carried out on synchronized cell populations.[1] Similarly, Lambin *et al.* (*11*) have used DMIPS to describe another category of substructure. The sensitivity of cells to doses lower than 0.5 Gy is much greater than predicted by the LQ model by extrapolation from the survival curve at doses above 1 Gy. This hypersensitivity to low doses was particularly evident in cell lines that are not very sensitive to the usual doses of radiation (*12*). This phenomenon has also been demonstrated using FCM (*13*). Low-dose hypersensitivity has been observed with several human tumor cell lines; it is interpreted as being associated with an induced radioresistance that seems to be *negatively* correlated with intrinsic radiosensitivity.

CLINICAL RADIORESPONSIVENESS

There are several types of response in clinical radiotherapy. The tumors themselves (mainly primary tumors and satellite lymph node areas) receive total doses that are generally of 50–70 Gy. The dose per session has generally been about 2 Gy, although in the last few years, certain hyperfractionated protocols have been used with doses per session typically at or above 0.8 Gy. As the tumor lies within the target volume, it usually receives near the full prescribed dose and the phenomenon of hypersensitivity to doses per session below 0.5 Gy/dose is not relevant in this case. However, normal tissues pose quite a different problem. With the single exception of the band of normal tissues that has apparently not been invaded, but immediately surrounds the tumor itself, all radiotherapy protocols attempt to keep the irradiation of normal tissues as low as possible. However, with external beam radiotherapy, even using the best techniques, the volume of normal tissue that receives doses of 0.1–0.2 Gy per session may not be negligible. Let us consider a four-field external irradiation protocol to treat uterine cancers (25 MV X rays, Institut Gustave-Roussy). Our calculations show that the volume of normal tissue receiving doses per session of 0.1–0.2 Gy is 2 to 3.10^3 cm^3. If there is a total of 35 sessions, the total dose administered to such a volume is 3.5–7 Gy over 7 weeks. Such a total dose, especially spread over this treatment time, is highly unlikely to have detectable acute effects on most of the normal tissues likely to show early reactions during external-beam radiotherapy, such as the mucosa and skin, even in cases of hypersensitivity. You will recall that the initial acute reactions are usually observed with total doses of 20 Gy given over 2 weeks, and these reactions are seen only in the irradiation fields. Late damage is known to appear in submucosal and subcutaneous tissue only after quite high doses; scleroses when they occur, are seen after doses of 60–70 Gy with fractions of 2 Gy given over a standard overall treatment time (5–7 weeks). It is understandable that, under these conditions and with these criteria, hypersensitivity to low doses is not seen; the total dose administered with the low doses is at least 5- to 10-fold smaller than the dose that could cause late damage. Thus it does not seem that the acute and late damage to normal tissues that occurs in patients treated by radiotherapy could reflect the hypersensitivity described in mice. Nevertheless, there is no clear evidence that this hypersensitivity does not exist in the clinic.

Rodent cells have been found to be hypersensitive not only to low doses per fraction, but to *very low dose rates* (4.5–29 mGy h^{-1}) (*14*), if the criterion used is the induction of mutations (resistance to 6-thioguanine). Although the mutation level per surviving cell drops between acute dose rates to low dose rates (0.20 Gy h^{-1}), it increases again at *very* low dose rates. A comparable phenomenon cannot be studied in humans, but a similar approach could be used. This would involve cancers in excess that develop in irradiated patients. Epidemiological studies of X-ray-induced cancers in patients were carried out some time

[1] L. D. Skarsgard, Predictive assays: survival at low doses and the complications of substructure. Presented at the Forty-First Annual Meeting of the Radiation Research Society, Dallas, TX, March 1993.

ago. The concept of the dose that doubles the leukemia incidence being about 1 Gy has also been established for some time. More recently, a study of 150,000 women treated for cancer of the uterus was published (*15*). A total of 195 patients suffered from leukemia during the years after treatment. The relative risk was calculated as a function of the dose, and was found to be greatest (2.5), when the bone marrow received a total dose of 4 Gy, and gradually fell thereafter. Such a bell-shaped curve was interpreted assuming that the lethal effect was greater than the cancer-causing effect above 4 Gy. But, there may be another explanation. If the irradiation had been given by brachytherapy, the maximal risk dose would probably be that of a dose rate in the region of 20 mGy h^{-1}. This is the dose rate at which the mutation rate is high in rodents (*14*). Fijuth *et al.* (*16*) monitored 525 patients with head and neck carcinomas that were treated with ^{192}Ir alone, or combined with external beam radiotherapy. Of the total, 19% developed a second cancer, and of these, 7.5% developed a cancer in an area other than the head and neck. A multivariate study showed that brachytherapy played a key role in the development of these second primary cancers. Although, there was no information given on either the dose rate or the dose received by the cancer-forming tissues, the dose rate and the doses delivered to these tissues were probably low. Lastly, Chmelevsky *et al.* (*17*) published a study of 50 bone sarcomas that occurred among 800 patients who had been given ^{224}Ra to treat bone tuberculosis or ankylosing spondylitis. In addition to the dose–effect relationship, they found that time played an important role. When the dose was given over 15 months, the cancer incidence rate was twice that found when the dose was given over 5 months. Thus they observe an *inverse* dose rate effect with regard to the risk of cancer.

Clearly, no definitive conclusion can be drawn from these three studies. Nevertheless, they suggest that very low dose rates can be more dangerous than standard low dose rates. This may be a particular feature of hypersensitivity to very low dose rates in human.

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