Journal of Theoretical Biology 394 (2016) 93–101

Contents lists available at ScienceDirect

Journal of Theoretical Biology

journal homepage: www.elsevier.com/locate/yjtbi

Mathematical models of radiation action on living cells: From the target theory to the modern approaches. A historical and critical review

Larry Bodgi [a,b,f], Aurélien Canet [a,c], Laurent Pujo-Menjouet [c], Annick Lesne [d,e,f], Jean-Marc Victor [d,e,f], Nicolas Foray [a,f,*]

[a] Institut National de la Santé et de la Recherche Médicale (INSERM), UMR 1052, Radiobiology Group, Cancer Research Centre of Lyon, 69008 Lyon, France
[b] Université Saint-Joseph, Faculté des sciences, 1107-2050 Beyrouth, Liban
[c] Université Claude Bernard-Lyon 1, Institut Camille Jordan, 69622, Villeurbanne, France
[d] Centre National de la Recherche Scientifique (CNRS), UMR 7600, Laboratoire de Physique Théorique de la Matière Condensée, Université Pierre et Marie Curie-Paris 6, Sorbonne Universités, 75252 Paris, France
[e] Centre National de la Recherche Scientifique (CNRS), UMR 5535, Institut de Génétique Moléculaire de Montpellier, Université de Montpellier, 34293 Montpellier, France
[f] Centre National de la Recherche Scientifique (CNRS), GDR 3536, Nuclear Architecture and Dynamics, Université Pierre et Marie Curie-Paris 6, 75252 Paris, France

HIGHLIGHTS

- Several mathematical models were proposed to describe the survival curves of irradiated cells.
- The Linear-Quadratic model is the most used but its biological meaning is unknown.
- We revisit literature by providing clues for resolving the Linear-Quadratic model.

ARTICLE INFO

Article history:
Received 26 July 2015
Received in revised form
9 January 2016
Accepted 12 January 2016
Available online 22 January 2016

Keywords:
DSB repair
Ionising radiation
Models of radiation action
Cell survival
Radiosensitivity

ABSTRACT

Cell survival is conventionally defined as the capability of irradiated cells to produce colonies. It is quantified by the clonogenic assays that consist in determining the number of colonies resulting from a known number of irradiated cells. Several mathematical models were proposed to describe the survival curves, notably from the target theory. The Linear-Quadratic (LQ) model, which is to date the most frequently used model in radiobiology and radiotherapy, dominates all the other models by its robustness and simplicity. Its usefulness is particularly important because the ratio of the values of the adjustable parameters, α and β, on which it is based, predicts the occurrence of post-irradiation tissue reactions. However, the biological interpretation of these parameters is still unknown.

Throughout this review, we revisit and discuss historically, mathematically and biologically, the different models of the radiation action by providing clues for resolving the enigma of the LQ model.

1. Introduction

Cellular radiosensitivity was conventionally defined as the inability of irradiated cells to produce daughter cells (i.e. colonies). It is quantified by the clonogenic assays that consist in determining the number of colonies resulting from a given number of cells irradiated at a given dose. The survival fraction obeys a decreasing exponential-like law (it is generally plotted on a semi-log scale) with or without shoulder (Puck and Marcus, 1956). Several mathematical models, detailed below, were proposed to describe cell survival curves (Curtis, 1991). Interestingly, the hypotheses on which they are based reflect the conceptual advances in our understanding of the radiation response (Fig. 1):

- between the 1920s and the 1950s, the most extensively used cell survival models were directly derived from the target theory, such as the single-target single-hit, n-targets single-hit and n-hits n-targets models, including the so-called (n, D_0) model (Elkind and Whitmore, 1967).
- between the 1950s and the 1980s, the (n, D_0) model was used intensely. However, the evidence that the initial slope of the

* Corresponding author at: INSERM UMR 1052, Centre de Recherche en Cancérologie de Lyon, Groupe de Radiobiologie, 28 Rue Laennec, 69008 Lyon, France.

http://dx.doi.org/10.1016/j.jtbi.2016.01.018
0022-5193/

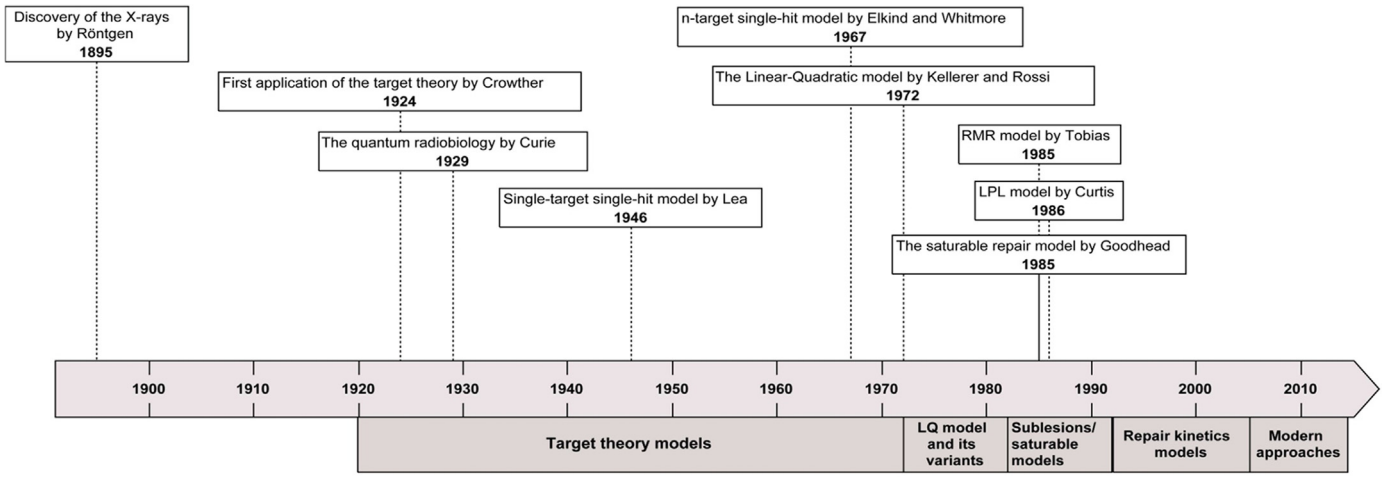

Fig. 1. Historical synopsis related to the cell survival models and their variants.

survival curve was not nil has significantly decreased its interest. In the early 1980s, the linear-quadratic (LQ) model was preferred because of its very good fitting qualities, but the empiric nature of its parameters, α and β, encouraged the authors to develop other models (Chadwick and Leenhouts, 1973).

- between the 80s and the 90s, more sophisticated models were proposed by introducing the notion of DNA damage repair but without leading to formulas simpler than the LQ model, nor providing a clear mechanistic explanation to the radiation-induced phenomena. It is notably the case of the Repair–MisRepair (RMR) (Tobias, 1985), the Lethal-Potentially Lethal (LPL) (Curtis, 1986), and the saturated repair models (Goodhead, 1985).
- since the 90s, while there is a lower infatuation towards the biostatistical models describing cell survival, radiobiologists started focusing on the description of the DNA damage repair kinetics linked to cell survival (Bodgi et al., 2013; Cucinotta et al., 2000; Foray et al., 2005; Gastaldo et al., 2008; Iliakis, 1991; Neumaier et al., 2012; Radivoyevitch et al., 1998; Sontag, 1997).

2. The target theory and its major related cell survival models

2.1. The genesis of the target theory

Funded by physicists, the target theory is based on two major principles:

- 1) *"radiation is considered to be a sequence of random projectiles;*
- 2) *the components of the cell are considered as the targets bombarded by these projectiles"* (Summers, 2011).

The target theory was first applied by Crowther in 1924 through an analysis of data from an experiment on chick embryo cells exposed to soft X-rays that was performed by Strangeways and Oakley in 1923 (Crowther, 1924; Strangeways and Oakley, 1923). In this case, the sensitive targets were hypothesized to be mitotic cells (Crowther, 1924). In 1929, Holweck[1] and Lacassagne obtained survival curves from bacillus irradiated by UV, X-rays or alpha-particles (Holweck, 1929; Lacassagne, 1929). Marie Curie analyzed the data and all these authors proposed the basis of the so-called *quantum radiobiology*: "*to destroy a bacillus it is necessary that its sensitive zone absorbs a minimal number s of quantas*" (Curie, 1929). From all these pioneering applications of the target theory, three important comments concerning *hits* and *targets* must be done:

- 1) The probability density function that was systematically applied to describe hits into sensitive cellular targets was a Poisson law.
- 2) The actual nature of the sensitive cellular targets was not consensual: they can be sub-populations of certain cells or some part of the nucleus.
- 3) The survival of irradiated cells was considered as the result of the absence of any hit on sensitive cells.

2.2. The basic ballistic models

2.2.1. The single-target single-hit model

It is the simplest application of the target theory. Directly derived from the hypotheses of Crowther and Curie, it was highlighted by Lea[2] at the end of 50's throughout his book *"Actions of radiation action on living cells"* (Lea, 1946). The single-target single-hit model dominates with both its simplicity and robustness all the approaches leading to cell survival. It is based on the hypothesis that a single impact in the sensitive part is enough to kill the cell. By considering the Poisson probability to hit k times a target:

$$P(k) = \frac{m^k}{k!} e^{-m} \tag{1}$$

The probability of no impact is therefore:

$$P(0) = e^{-m} \tag{2}$$

[1] Born in 1889, Fernand Holweck became assistant of Marie Curie in 1912. During the First World War, he helped Paul Langevin in his works for detecting submarines by ultrasonic waves. Holweck developed a number of instruments like the most powerful vacuum-producer, a gravimetric pendulum, the first X-ray tube with successive stages of acceleration. Through his collaboration with Dr. Antoine Lacassagne, Holweck rediscovered, independently of the previous work by James Arnold Crowther, the quantic interpretation of the biological action of radiation on microorganisms. During the Second World War, he was actively engaged in defense (*footnote continued*) work but was arrested, tortured and murdered by the Gestapo on December 14th, 1941, in a Paris prison.

[2] Douglas E. Lea (MA, PhD) was born in 1910 in Great Britain. During his career, he worked as a physicist at England's Strangeways Laboratory and as a reader in the Department of Radiobiology in the Department of Radiotherapeutics at Cambridge University. The majority of his work dealt with the effects of radiation on cells. Lea died in an accident in Cambridge, England, on June 16, 1947.

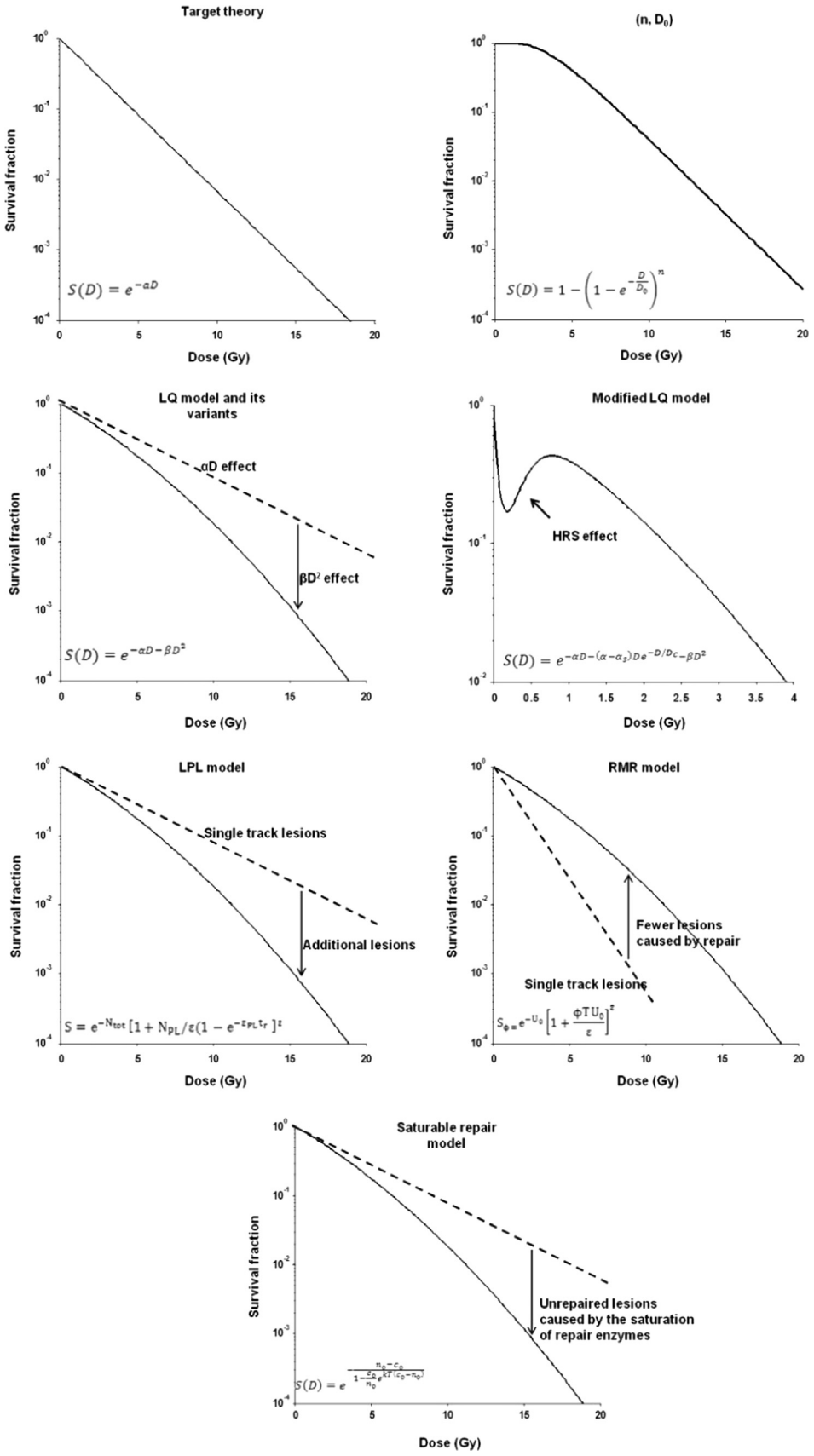

Fig. 2. Summary of the major cellular models describing cell survival curves with the corresponding mathematical formulas linking clonogenic cell survival and radiation dose.

We consider that m is proportional to the dose. Hence, if the survival is directly linked to the probability of no impact, we have:

$$S(D) = e^{-aD} \text{ or } S(D) = e^{-\frac{D}{D_0}} \quad (3a, b)$$

where D_0 is the mean lethal dose for which the mean number of lethal events per cell is equal to 1. At a dose D_0, the fraction of cell survival is equal to 1/e or 37% (0.367879 exactly) (Fig. 2A).

2.2.2. The n-target single-hit model

This model was based on the hypothesis that one cell contains n identical targets. The inactivation of one target was considered to be a sublethal event, and the accumulation of these sublethal events leads to cell death once the n targets are hit. Hence, by considering the probabilities of the single-target single-hit model, the probability that one target is hit once obeys the Poisson law:

$$p(1) = 1 - e^{-\frac{D}{D_0}} \quad (4a)$$

Therefore, the probability that n targets from the same cell are hit once is:

$$p(n) = \left(1 - e^{-\frac{D}{D_0}}\right)^n \quad (4b)$$

Thus, the survival fraction is given by the following formula:

$$S(D) = 1 - \left(1 - e^{-\frac{D}{D_0}}\right)^n \quad (4c)$$

The curve is described by the final slope D_0 and an additional parameter that defines the width of the shoulder n. The n-target single-hit model was therefore called the (n, D_0) model (Elkind and Whitmore, 1967) (Fig. 2B).

2.2.3. Criticisms of the ballistic models

The great majority of cell survival curves obtained from mammalian cells obey neither the single-target single-hit nor the (n, D_0) models: to the notable exception of the hyper-radiosensitive cells that show exponential curves, all the other human cases are characterized by a shoulder and an initial part that is not nil (Fig. 2A and B). These last observations are in clear discrepancy with the description showed by the (n, D_0) model. To overcome this problem, the two-component model was proposed by considering an additional single-target component, which allows the initial slope value to be fixed at a given dose D_1. The resulting survival equation becomes:

$$S(D) = e^{\frac{-D}{D_1}}\left(1 - \left(1 - e^{-D\left(\frac{1}{D_0} - \frac{1}{D_1}\right)}\right)^n\right) \quad (5)$$

However, although the two-component model is able to predict in an acceptable way the cell survival at low doses, it still has the flaw that the decrease in cell survival for a dose between 0 and D_q occurs linearly, which was never demonstrated experimentally. Even though the use of a multi-target instead of a single-target component would be able to solve this drawback, the general survival formula would become too complicated, and therefore not really useful to compare survival curves and explain the radiation response mechanisms (Steel, 1993).

To date, despite all the efforts in introducing some modifications and in addition to the problems evoked above, the models directly derived from the target theory appear to be unable to describe the phenomenon of hypersensitivity to low-dose that is characterized by a V-shaped part in the 1–400 mGy range of the survival curves, in clear discrepancy with the target theory (Marples and Collis, 2008) (Fig. 2D)

2.3. The linear-quadratic model and its variants

2.3.1. The linear-quadratic model

In 1972, Kellerer and Rossi introduced the linear-quadratic (LQ) model in which a lethal event is supposed to be caused by one hit due to one particle track (the linear component αD) or to two particle tracks (the quadratic component βD^2) (Kellerer and Rossi, 1972; Kellerer and Rossi, 1978).

$$S(D) = e^{-\alpha D - \beta D^2} \quad (6)$$

However, the probability that two particles tracks overlap is nil at biologically-relevant doses (Goodhead, 1989). In 1973, Chadwick and Leenhouts proposed that αD reflects nonrepairable (i.e. directly lethal) DNA double-strand-breaks (DSB) and βD^2 reflects the combination of two sublethal DNA single-strand breaks (Chadwick and Leenhouts, 1973). Again, at biologically-relevant doses, radiation-induced SSB are not close enough to produce DSB (Goodhead, 1989). To date, while the LQ model still generates numerous debates, inherent bio-molecular mechanisms remain misknown (Brenner and Herbert, 1997; Brenner et al., 2012). In spite of its empirical nature, the LQ model is considered as the best-fitting model to describe survival (Fig. 2C) (Fertil et al., 1994), and of great interest in radiation oncology through the link existing between the α/β ratio and the nature of radiotherapy-induced tissue (early or late) reactions (Barendsen, 1982; Brenner et al., 2012; Dale, 1985; Williams et al., 1985).

2.3.2. The variant LQ models to describe high-dose effects

While the LQ model provides good fit for survival curves at biologically relevant doses, the accuracy of the survival description was found to be limited for higher or repeated doses. Douglas and Fowler therefore proposed **the three-lambda model** that consisted in a superimposition of three exponential terms (Douglas and Fowler, 1976). In their model the survival equation was:

$$S(D) = e^{-\lambda_3\left(1 - e^{\lambda_1 D}\left(1 - \left(1 - e^{-(\lambda_2 - \lambda_1)D}\right)^2\right)\right)} \quad (7)$$

The linear-quadratic-cubic model was also proposed to describe the response to higher doses by adding another cubic term to the polynomial function of the LQ model (Joiner, 1993; Tobias, 1985):

$$S(D) = e^{-\alpha D - \beta D^2 + \gamma D^3} \quad (8)$$

2.3.3. Criticisms of the LQ models and its variants

While the usefulness of the three-lambda and the linear-quadratic-cubic models is quite relative since very high single doses are not really relevant for radiobiologists, the paradox of the LQ model is that it displays an actual robustness for fitting data while it remains an empirical model (Fertil et al., 1994).

3. The models based on the sublesions hypothesis and their variants

3.1. The repair–misrepair model

Proposed by Tobias in 1985, the repair–misrepair (RMR) model describes the evolution of the function, $U(t)$, that reflects the mean number of lesions before any repair activation (Tobias, 1985). The yield of the initially induced lesions, U_0, was considered proportional to the dose D:

$$U_0 = \delta D \quad (9)$$

The repair substates R are defined as being the results of the U lesions after the repair process. The author considered that the

evolution of the U lesions could be described by the following differential equation:

$$\frac{dU}{dt} = -\lambda U(t) - \kappa(t) \tag{10}$$

With λ the linear *self-repair coefficient*, considered to be the good repair pathway, and κ the *coefficient for cooperative repair*, involving the interaction of pairs of U lesions, which the author considered to be the misrepair pathway. By integrating the equation we have:

$$U(t) = \frac{U_0 e^{-\lambda t}}{1 + \frac{U_0(1-e^{-\lambda t})}{\frac{\lambda}{k}}} \tag{11}$$

Two *R*-states were therefore defined and quantified: $R_L(t)$, the total number of *self-repairs*, which are the non-lethal lesions, and $R_Q(t)$, the total number of *quadratic misrepair*s, which are considered to be the lethal lesions:

$$R_L = \int_0^t \lambda U dt \tag{12a}$$

$$R_Q = \int_0^t \kappa U^2 dt \tag{12b}$$

By considering that no new lesion is created during the repair process, we have:

$$U_0 = U(t) + R_L(t) + R_Q(t) \tag{13}$$

When $t \to \infty$,assuming that no lesion remains unrepaired Eq. (13) becomes:

$$R_Q(t \to \infty) = U_0 - R_L(t \to \infty) \tag{14}$$

If we consider the *repair ratio* $\varepsilon = \lambda/\kappa$, and by applying Poisson distribution, the survival equation becomes:

$$S = e^{-R_Q(t\to\infty)} = e^{-U_0}\left[1 + \frac{U_0}{\epsilon}\right]^{\epsilon} \tag{15}$$

By considering that the *linear repair* is not a perfect process, the author introduced the parameter ϕ that defines the probability that *self-repair* steps are all perfect "eurepairs" (or good repair). Furthermore, by considering that the repair time is limited at a time T, the survival equation becomes (Fig. 2E):

$$S_{\varphi} = e^{-U_0}\left[1 + \frac{U_0(1-e^{-\lambda T})}{\epsilon}\right]^{\varphi\epsilon} \tag{16}$$

3.2. The lethal-potentially lethal model

Curtis developed in 1986 the Lethal-Potentially Lethal model (LPL) model that takes the repair process into account (Curtis, 1986). He proposed a classification of the radio-induced lesions: lesions "that are unrepairable and are therefore lethal", and "potentially lethal lesions for which the repair process is activated". Thus, two differential equations are necessary to describe the repair kinetics

$$\frac{dn_{PL}}{dt} = -\epsilon_{PL} n_{PL}(t) - \epsilon_{2PL} n_{PL}(t)^2 \tag{17a}$$

$$\frac{dn_L}{dt} = \epsilon_{2PL} n_{PL}(t)^2 \tag{17b}$$

with n_{PL} the number of potentially lethal lesions, n_L the number of lethal lesions, ε_{PL} the constant per unit of time repair rate and ε_{2PL} the constant per unit of time rate of interaction between two potentially lethal lesions, a process that Curtis called the *binary misrepair* (Fig. 2F). The solutions to Eq. (16) are:

$$n_{PL}(t) = \frac{N_{PL} e^{-\epsilon_{PL} t_r}}{\left[1 + (N_{PL}/\epsilon)(1 - e^{-\epsilon_{PL} t_r})\right]} \tag{18a}$$

$$n_L(t) = N_L + \frac{N_{PL}\left(1 + \frac{N_{PL}}{\epsilon}\right)(1 - e^{-\epsilon_{PL} t_r})}{\left[1 + (N_{PL}/\epsilon)(1 - e^{-\epsilon t_r})\right] - \epsilon \ln\left[1 + (N_{PL}/\epsilon)(1 - e^{-\epsilon_{PL} t_r})\right]} \tag{18b}$$

where $N_{PL} = n_{PL}(T)$, $N_L = n_L(T)$, $\epsilon = \epsilon_{PL}/\epsilon_{2PL}$, T is the irradiation duration and t_r is the available repair time (the definition of the variables of this model are not the same as those of the above model). In order to predict the survival at a time $t = T + t_r$, time after which the repair process is ineffective, Curtis considered that the total number of lesions per cell is the sum of lethal and potentially lethal lesions. In other words, he hypothesizes that after a certain time, all the potentially lethal lesions become lethal. Hence, the total number of lesions n_{tot} is

$$n_{tot}(T + t_r) = n_L(T + t_r) + n_{PL}(T + t_r) \tag{19}$$

The survival equation becomes:

$$S = e^{-n_{tot}(T+t_r)} = e^{-N_{tot}}\left[1 + N_{PL}/\epsilon\left(1 - e^{-\epsilon_{PL} t_r}\right)\right]^{\epsilon} \tag{20}$$

with $N_{TOT} = N_L + N_{PL}$ and $\epsilon = \epsilon_{PL}/\epsilon_{2PL}$

3.3. The saturable repair model

In 1985, Goodhead proposed a new model, the saturable repair model that was based on the hypothesis that the efficiency of the repair system decreases with the dose, and that this decrease is caused by the saturation of the repair kinetics (Goodhead, 1985). He therefore considered the following repair rate for the induced lesions:

$$\frac{dn}{dt} = -kcn \tag{21}$$

where $n(t)$ is the number of unrepaired lesions, $c(t)$ the number of repair molecules or enzymes and k is a proportionality coefficient. By considering that $dc = dn$ and that T is the time available for repair, the residual number of lesions after repair becomes:

$$n_T = \frac{n_0 - c_0}{1 - \frac{c_0}{n_0} e^{kT(c_0 - n_0)}} \tag{22}$$

Hence the survival equation:

$$S(D) = e^{-\frac{n_0 - c_0}{1 - \frac{c_0}{n_0} e^{kT(c_0 - n_0)}}} \tag{23}$$

By considering the repair process as saturable, this model does not require the notion of sublesions like the lethal and potentially lethal/sublethal ones (Fig. 2G). It was therefore presented as an alternative to the RMR and LPL models (Goodhead, 1985).

3.4. Criticisms of the sublesion and saturable models

The notion of the lethal and potentially lethal lesions is directly derived from the attempts to interpret the LQ model. Particularly, as evoked above, the quadratic component was systematically explained by a duality of tracks, single-strand breaks or cooperative lesions. However, the actual nature of the sublesions still remains undefined. Besides, this idea is related to the direct/indirect effect hypothesis that suggests that some damages are directly induced by the impact of the physical particles and some others by the chemical radicals produced by such impact. In fact, it has been clearly shown that DNA damages are induced simultaneously and that radicals attack was not a 2-time-phase phenomenon (Foray et al., 1996, 1998; Kysela et al., 1993). Furthermore, both RMR and LPL models considered the misrepaired lesions as lethal lesions, which is in clear discrepancy with cytogenetics and new advances in radiobiology. Indeed, it is accepted

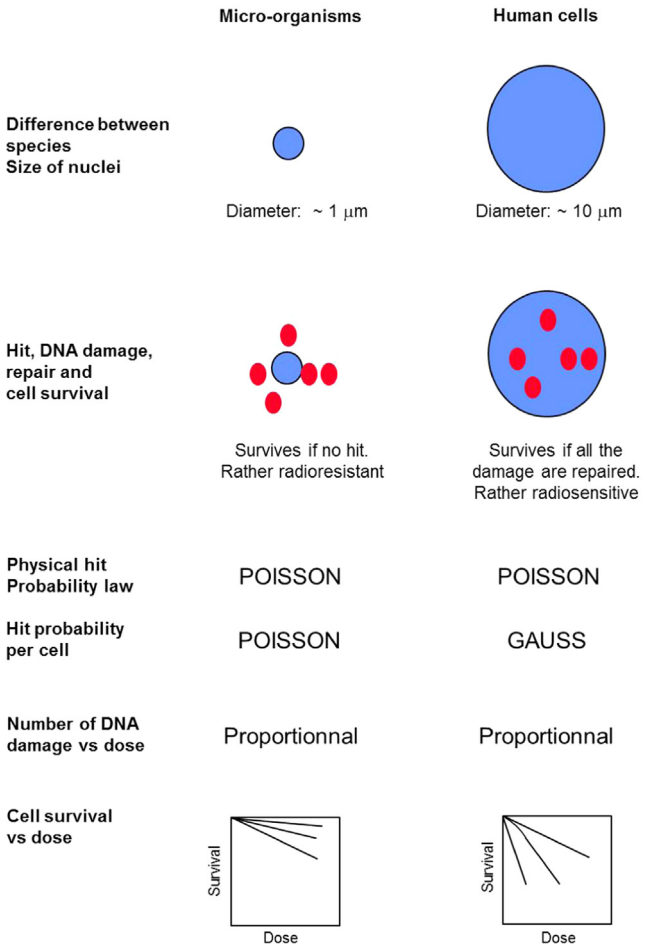

Fig. 3. Schematic illustration of the influence of the target size on the radiobiological endpoints.

that misrepaired lesions are more likely involved in genomic instability and cell transformation rather than cell death and radiosensitivity (Jeggo and Lobrich, 2007).

Unlike the models that are directly derived from the target theory, the sublesions (LPL and RMR) models are based on the notion of DNA damage repair. Although it is suggested that these lesions might be DSB, they are not formally identified as the damage of interest. Furthermore, their repair rate per unit of time was hypothesized to be constant in the LPL and RMR models. Such an assumption is in discrepancy with the multiphasic shape of the DSB repair kinetics, which would reflect the existence of a continuous spectrum of DSB of differing reparabilities rather than a limited number of DSB subcategories (Foray et al., 1996, 2005, 1998). An experimental proof of the continuous nature of DSB repair kinetics is given by the shape of the DSB repair curves obtained after a given dose followed by a period of time (some minutes to some hours): in these conditions, DSB repair curves are never mono- or bi-phasic but systematically continuously decreasing (Foray et al., 1996, 2005, 1998) which contradicts the hypothesis of the LPL and RMR models.

With regard to the saturable repair model, the major assumption is the saturation of the repair enzymes pool. Up to date, such an hypothesis was not verified. While some tens of DSB are induced per Gy, the average yield of each protein ranges from 10^4 to 10^8 molecules, it appears unlikely that the repair enzymes pool can be saturated. Some radiosensitive syndromes are not necessarily caused by a decreased of DSB repair kinetics, inasmuch as these syndromes are caused by mutations of cytoplasmic proteins like Huntington's disease, neurofibromatosis or Usher's syndrome (Deschavanne and Fertil, 1996; Ferlazzo et al., 2014).

Altogether, like the models derived from the target theory, the RMR, LPL and saturable repair models do not solve two important radiobiological questions, at least:

- the very documented hypersensitivity to low doses (Joiner et al., 2001)
- the fact that some radiosensitive syndromes are not necessarily associated with DNA damage repair defect (Deschavanne and Fertil, 1996; Ferlazzo et al., 2014).

4. Modern approaches

4.1. The target theory must be reconsidered to explain radiosensitivity of mammalians

The models deriving from the target theory are generally based on the following principles:

- (1) physical hits obey a Poisson distribution;
- (2) cell survival is due to the absence of hits in the sensitive areas of the irradiated cells;
- (3) since all the number of hits is proportional to the dose and since all the hits are lethal, survival is a simple exponential function of the dose.

Such hypotheses are relevant for micro-organisms but are not for mammalian cells. Indeed, by considering that the relevance of the above hypothesis (1) and that about 40 DSB are produced per human cell per Gy, the probability of an absence of impact is lower than 10^{-17}. Conversely, different DSB assays like pulsed-field gel electrophoresis and γH2AX immunofluorescence show that the yield of induced DSB per mammalian cell does not obey a Poisson but rather a Gauss distribution (Noda et al., 2012; Rothkamm and Lobrich, 2003). Hence, the hypotheses (2) and (3) deriving from the target theory must therefore be reconsidered for mammalian cells: cells likely survive *because all their DNA damage are repaired rather than because cells are not targeted by IR* (Lea, 1946; Sutherland, 2006) (Fig. 3). Furthermore, it must be reminded that, in addition to the 40 DSB induced per cells, X- and gamma-rays also induce simultaneously 1000 single-strand breaks and 10,000 base damage per Gy (these numbers of are divided by more than 100 in the case of the bacteria or yeasts): it is therefore surprising that from the pioneer works of Crowther and Curie (i.e. the 1930s), only few radiobiologists (Lea was one of them (Lea, 1946)) discussed about the relevance of the target theory for other species than micro-organisms.

4.2. The moderate radiosensitivity must be considered when testing survival models

The great majority of mammalian cells show a non-negligible initial slope and a shoulder when cell survival is plotted against dose, which is in clear discrepancy with target theory and especially (n, D_0) model (Malaise et al., 1987). The only cell lines that can show exponential survival curve are the most hyperradiosensitive ones such as those mutated for the ATM, LIG4 or DNA-PK proteins (Iliakis, 1991; Joubert et al., 2008). In the frame of the LQ model, the maximal shoulder is obtained by the cells that show a maximal β LQ parameter, which corresponds to a moderate radiosensitivity (average α) (Fig. 4). The cell lines showing the most intermediate radiosensitivity are therefore a good tool to exclude a number of irrelevant cell survival models. Interestingly, as evoked above, some genetic syndromes associated with

moderate sensitivity are not caused by mutations of proteins directly involved in DSB repair but are rather cytoplasmic and have no function in DNA damage repair. This is notably the case of the Huntington's disease, neurofibromatosis or Usher's syndrome (Deschavanne and Fertil, 1996; Ferlazzo et al., 2014). In complete contradiction with the target theory, such syndromes provide clues that cytoplasm proteins may impact on radiation response and that considering both nuclear targets and DNA repair is not sufficient to explain all the range of human radiosensitivity. Hence, the current paradigm that consists in considering only DNA repair deficiencies to explain radiosensitivity is not relevant for describing the moderate but significant radiosensitivity of some genetic syndromes.

4.3. *The radiation-induced nucleo-shuttling of ATM: a solution to some enigmas?*

The ATM protein is known to phosphorylate the γH2AX variant histone that is considered as the recognition step of DSB by the preponderant DSB repair pathway in humans, the non-homologous end-joining (NHEJ). In the frame of our collection of skin biopsies from radiotherapy patients showing adverse tissue reactions, we have accumulated from 2003 some hundreds fibroblast cell lines whose radiation response has been investigated with the major DSB repair biomarkers (Granzotto et al., in press). Interestingly, it appeared that the phosphorylated forms of the ATM protein translocate from cytoplasm to nucleus in response to radiation. For the patients showing hyper-radiosensitivity, the DSB repair defect is obvious. For the patients showing moderate radiosensitivity, the radiation-induced nucleo-shuttling of the ATM forms was delayed, because of some abundant mutated proteins that sequestrate them in cytoplasm (Bodgi and Foray, in press). This is notably the case of mutated huntingtin in cells providing from patients suffering from Huntington Disease (Ferlazzo et al., 2014).

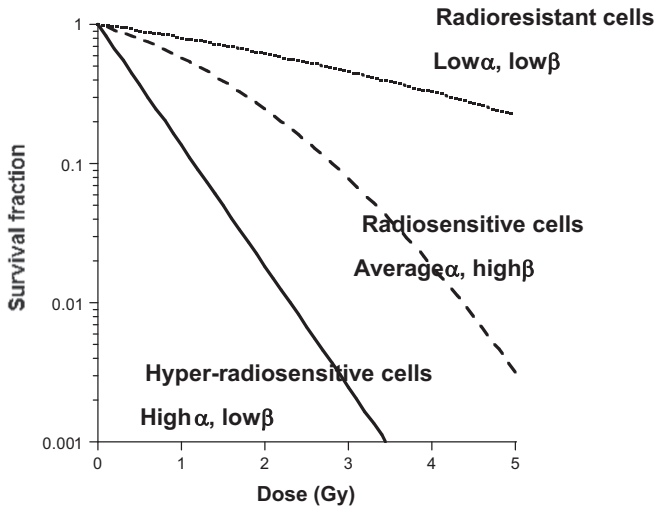

Fig. 4. Representative survival curves of human cells with radioresistance, moderate radiosensitivity and hyper-radiosensitivity. The α and β values of the LQ model are indicated.

From all these observations, we proposed a model based on the radiation-induced nucleo-shuttling of ATM. Ionizing radiation produce DSB in nucleus but also the dimerization of ATM, notably in cytoplasm. The ATM monomers are able to diffuse in the nucleus, or re-associate to form dimers again or bind to some proteins (called X) that prevent their diffusion (Fig. 5). Once in nucleus, the ATM monomers can recognize DSB through the phosphorylation of H2AX histones, which triggers DSB repair via NHEJ. Consequently, two categories of lethal DSB can be defined: 1) the recognized but non-repaired DSB; 2) the non-recognized therefore non-repaired DSB. We have shown that these DSB categories called α-type and β-type, increase with dose or with the square of the dose, respectively (Bodgi and Foray, in press). Such findings support therefore that the LQ model, that provides the best cell survival data fits, provides also the best relevance with molecular mechanisms in response to radiation (Fig. 5).

It is also noteworthy that, unlike the models describe above, the theory of the nucleo-shuttling of ATM and the LQ model are in agreement with the hypersensitivity to low-dose phenomenon (Bodgi and Foray, in press; Colin et al., 2011; Joiner et al., 2001; Thomas et al., 2013). Indeed, at low dose, the number of ATM monomers is too low to insure the recognition of the low number of radiation-induced DSB by the NHEJ pathway. Consequently, the DSB are either non-repaired or mis-repaired, which increases cell death and mutagenesis. For higher doses, while the number of induced DSB is larger than in the former condition, the number of

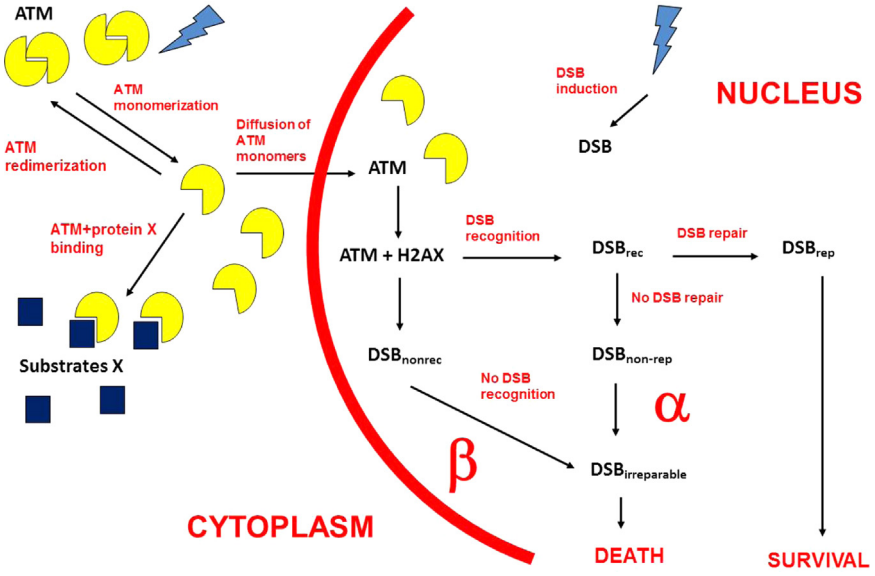

Fig. 5. Schematic illustration of our new model of the ATM nucleo-shuttling.

ATM monomers is larger and permits a better recognition of DSB by the NHEJ pathway: cell survival increases (Figs. 2 and 5).

5. Conclusions

To date, it appears that most of the biostatistical models of cell survival are not relevant to describe the radiation response of mammalians. The re-analysis of the princeps papers provides strong evidence that the general theory on which these models are based was built from micro-organisms data whose size and characteristics are clearly different from the mammalian case. It is therefore not surprising that the LQ model, based on a very permissive 2nd degree polynomial function provides the best cell survival data fits. Nevertheless, the biological interpretation of the LQ parameters, α and β, remained unsolved since the 1970s. Today, by taking into account the radiation-induced nucleo-shuttling of ATM that is very far from the target theory, coherent explanations of the descriptive power of the LQ model and of some misknown radiobiological phenomena can be proposed (Bodgi and Foray, in press). More sophisticated biomathematical models are therefore needed to consolidate this change of paradigms.

Conflict of interest

None.

Acknowledgments

We thank Madame Beaufrère for her assistance in editing English and all the staff of the Radiobiology Group. We are grateful to the Association Pour la Recherche sur l'Ataxie-Telangiectasie (APRAT), the Electricité de France (Comité de Radioprotection), the Plan Cancer/AVIESAN “Micromegas project, the Centre National d'Etudes Spatiales (CNES) and the Commissariat Général à l'Investissement (INDIRA project).

References

Barendsen, G.W., 1982. Dose fractionation, dose rate and iso-effect relationships for normal tissue responses. Int. J. Radiat. Oncol. Biol. Phys. 8, 1981–1997.
Bodgi, L., Foray, N., 2016. The nucleo-shuttling of the ATM protein as a basis for a novel theory of radiation response: resolution of the linear-quadratic model. Int. J. Radiat. Biol., in press
Bodgi, L., Granzotto, A., Devic, C., Vogin, G., Lesne, A., Bottollier-Depois, J.F., Victor, J. M., Maalouf, M., Fares, G., Foray, N., 2013. A single formula to describe radiation-induced protein relocalization: towards a mathematical definition of individual radiosensitivity. J. Theor. Biol. 333, 135–145.
Brenner, D.J., Herbert, D.E., 1997. The use of the linear-quadratic model in clinical radiation oncology can be defended on the basis of empirical evidence and theoretical argument. Med. Phys. 24, 1245–1248.
Brenner, D.J., Sachs, R.K., Peters, L.J., Withers, H.R., Hall, E.J., 2012. We forget at our peril the lessons built into the alpha/beta model. Int. J. Radiat. Oncol. Biol. Phys. 82, 1312–1314. http://dx.doi.org/10.1016/j.ijrobp.2011.12.045.
Chadwick, K.H., Leenhouts, H.P., 1973. A molecular theory of cell survival. Phys. Med. Biol. 13, 78–87.
Colin, C., Granzotto, A., Devic, C., Viau, M., Maalouf, M., Vogin, G., Joubert, A., Thomas, C., Foray, N., 2011. MRE11 and H2AX biomarkers in the response to low-dose exposure: balance between individual susceptibility to radiosensitivity and to genomic instability. Int. J. Low Radiat. 8, 96–106.
Crowther, J.A., 1924. Some considerations relative to the action of X-rays on tissue cells. Proc. R. Soc. Lond. B96, 207–211.
Cucinotta, F.A., Nikjoo, H., O'Neill, P., Goodhead, D.T., 2000. Kinetics of DSB rejoining and formation of simple chromosome exchange aberrations. Int. J. Radiat. Biol. 76, 1463–1474.
Curie, M., 1929. Sur l'étude des courbes de probabilité relatives à l'action des rayons X sur les bacilles. Comptes-Rendus de l'Acdémie des Sciences 188, 202–204.
Curtis, S.B., 1986. Lethal and potentially lethal lesions induced by radiation – a unified repair model. Radiat. Res. 106, 252–270.
Curtis, S.B., 1991. Mechanistic models. Basic Life Sci. 58, 382–386.
Dale, R.G., 1985. The application of the linear-quadratic dose-effect equation to fractionated and protracted radiotherapy. Br. J. Radiol. 58, 515–528.
Deschavanne, P.J., Fertil, B., 1996. A review of human cell radiosensitivity in vitro. Int. J. Radiat. Oncol. Biol. Phys. 34, 251–266.
Douglas, B.G., Fowler, J.F., 1976. The effect of multiple small doses of x rays on skin reactions in the mouse and a basic interpretation. Radiat. Res. 66, 401–426.
Elkind, M.M., Whitmore, G.F., 1967. The Radiobiology of Cultured Mammalian Cells. Gordon and Breach, New York.
Ferlazzo, M.L., Sonzogni, L., Granzotto, A., Bodgi, L., Lartin, O., Devic, C., Vogin, G., Pereira, S., Foray, N., 2014. Mutations of the Huntington's disease protein impact on the ATM-dependent signaling and repair pathways of the radiation-induced DNA double-strand breaks: corrective effect of statins and bisphosphonates. Mol. Neurobiol. 49, 1200–1211. http://dx.doi.org/10.1007/s12035-013-8591-7.
Fertil, B., Reydellet, I., Deschavanne, P.J., 1994. A benchmark of cell survival models using survival curves for human cells after completion of repair of potentially lethal damage. Radiat. Res. 138, 61–69.
Foray, N., Badie, C., Alsbeih, G., Fertil, B., Malaise, E.P., 1996. A new model describing the curves for repair of both DNA double-strand breaks and chromosome damage. Radiat. Res. 146, 53–60.
Foray, N., Charvet, A.M., Duchemin, D., Favaudon, V., Lavalette, D., 2005. The repair rate of radiation-induced DNA damage: a stochastic interpretation based on the gamma function. J Theor Biol 236, 448–458.
Foray, N., Monroco, C., Marples, B., Hendry, J.H., Fertil, B., Goodhead, D.T., Arlett, C.F., Malaise, E.P., 1998. Repair of radiation-induced DNA double-strand breaks in human fibroblasts is consistent with a continuous spectrum of repair probability. Int. J. Radiat. Biol. 74, 551–560.
Gastaldo, J., Viau, M., Bouchot, M., Joubert, A., Charvet, A.M., Foray, N., 2008. Induction and repair rate of DNA damage: a unified model for describing effects of external and internal irradiation and contamination with heavy metals. J. Theor. Biol. 251, 68–81.
Goodhead, D.T., 1985. Saturable repair models of radiation action in mammalian cells. Radiat. Res. Suppl. 8, S58–S67.
Goodhead, D.T., 1989. The initial physical damage produced by ionizing radiations. Int. J. Radiat. Biol. 56, 623–634.
Granzotto, A., Benadjaoud, M.A., Vogin, G., Foray, N., 2016. Influence of the nucleo-shuttling of the ATM protein in the healthy tissues response to radiotherapy: towards a molecular classification of human radiosensitivity. Int. J. Radiat. Oncol. Biol. Phys., in press
Holweck, F., 1929. Production de rayons X monochromatiques de grande longueur d'onde. Action quantique sur les microbes. Comptes-Rendus de l'Académie des Sciences 188, 197–199.
Iliakis, G., 1991. The role of DNA double strand breaks in ionizing radiation-induced killing of eukaryotic cells. Bioessays 13, 641–648.
Jeggo, P.A., Lobrich, M., 2007. DNA double-strand breaks: their cellular and clinical impact? Oncogene 26, 7717–7719.
Joiner, M.C., 1993. Models of radiation cell killing. In: Steel, G.G. (Ed.), Basic Clinical Radiobiology. Edward Arnold, London.
Joiner, M.C., Marples, B., Lambin, P., Short, S.C., Turesson, I., 2001. Low-dose hypersensitivity: current status and possible mechanisms. Int. J. Radiat. Oncol. Biol. Phys. 49, 379–389.
Joubert, A., Gamo, K., Bencokova, Z., Gastaldo, J., Rénier, W., Chavaudra, N., Favaudon, V., Arlett, C., Foray, N., 2008. DNA double-strand break repair defects in syndromes associated with acute radiation response: at least two different assays to predict intrinsic radiosensitivity? Int. J. Radiat. Biol. 84, 1–19.
Kellerer, A.M., Rossi, H.H., 1972. The theory of dual radiation action. Curr. Top. Radiat. Res. 8, 85–158.
Kellerer, A.M., Rossi, H.H., 1978. A generalized formulation of dual radiation action. Radiat. Res. 75, 471–488.
Kysela, B.P., Michael, B.D., Arrand, J.E., 1993. Relative contributions of levels of initial DNA damage and repair of double strand breaks to the ionizing radiation-sensitive phenotype of the Chinese hamster cell mutant, XR-V15B. Part I. X-rays. Int. J. Radiat. Biol. 63, 609–616.
Lacassagne, A., 1929. Action des rayons X de grande longueur d'onde sur les microbes. Etablissement de statistiques précises de la mortalité des bactéries irradiées. Comptes-Rendus de l'Académie des Sciences 188, 200–202.
Lea, D.E., 1946. Actions of Radiations on Living Cells. Cambridge University Press, London.
Malaise, E.P., Fertil, B., Deschavanne, P.J., Chavaudra, N., Brock, W.A., 1987. Initial slope of radiation survival curves is characteristic of the origin of primary and established cultures of human tumor cells and fibroblasts. Radiat. Res. 111, 319–333.
Marples, B., Collis, S.J., 2008. Low-dose hyper-radiosensitivity: past, present, and future. Int. J. Radiat. Oncol. Biol. Phys. 70, 1310–1318.
Neumaier, T., Swenson, J., Pham, C., Polyzos, A., Lo, A.T., Yang, P., Dyball, J., Asaithamby, A., Chen, D.J., Bissell, M.J., Thalhammer, S., Costes, S.V., 2012. Evidence for formation of DNA repair centers and dose-response nonlinearity in human cells. Proc. Natl. Acad. Sci. USA 109, 443–448.
Noda, A., Hirai, Y., Hamasaki, K., Mitani, H., Nakamura, N., Kodama, Y., 2012. Unrepairable DNA double-strand breaks that are generated by ionising radiation determine the fate of normal human cells. J. Cell Sci. 125, 5280–5287. http://dx.doi.org/10.1242/jcs.101006.
Puck, T.T., Marcus, P.I., 1956. Action of x-rays on mammalian cells. J. Exp. Med. 103, 653–666.

Radivoyevitch, T., Hoel, D.G., Hahnfeldt, P.J., Rydberg, B., Sachs, R.K., 1998. Recent data obtained by pulsed-field gel electrophoresis suggest two types of double-strand breaks. Radiat. Res. 149, 52–58.
Rothkamm, K., Lobrich, M., 2003. Evidence for a lack of DNA double-strand break repair in human cells exposed to very low x-ray doses. Proc. Natl. Acad. Sci. USA 100, 5057–5062.
Sontag, W., 1997. A discrete cell survival model including repair after high dose-rate of ionizing radiation. Int. J. Radiat. Biol. 71, 129–144.
Steel, G., 1993. Basic Clinical Radiobiology. Arnold Publishers, London.
Strangeways, T.S.P., Oakley, H.E.H., 1923. The immediate changes observed in tissue cells after exposure to soft X-rays while growing in vitro. Proc. R. Soc. Lond. B 95, 373–381.
Summers, W.C., 2011. Physics and genes: from Einstein to Delbrück. In: Sloan, P.R., Fogel, B. (Eds.), Creating a Physical Biology: The Three-Man Paper and Early Molecular Biology. The University of Chicago Press, Chicago, USA, pp. 39–68.
Sutherland, J.C., 2006. Repair dependent radiation survival: a stochastic model with Euler gamma function solutions. Phys. Med. Biol. 51, 4883–4901.
Thomas, C., Martin, J., Devic, C., Diserbo, M., Thariat, J., Foray, N., 2013. Impact of dose-rate on the low-dose hyper-radiosensitivity and induced radioresistance (HRS/IRR) response. Int. J. Radiat. Biol. 89, 813–822.
Tobias, C.A., 1985. The repair–misrepair model in radiobiology: comparison to other models. Radiat. Res. Suppl. 8, S77–S95.
Williams, M.V., Denekamp, J., Fowler, J.F., 1985. A review of alpha/beta ratios for experimental tumors: implications for clinical studies of altered fractionation. Int. J. Radiat. Oncol. Biol. Phys. 11, 87–96.