Theories and Models for Cell Survival

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Models

- Target theory model
  - Single-hit model
  - Multitarget-single hit survival
  - Single-target-multihit model
- Molecular models
- Dual Radiation Action
- Repair-misrepair model
- Lethal-potentially lethal model
Molecular models for cell death

- Need for an alternative model
  - Target theory does not describe all experimental data

- Role of enzymatic repair
  - Target theory does not account for time or time-dependence enzymatic repair of DNA
  - Target theory does not define DNA as the susceptible target
  - Repair of DNA damage is central to the effectiveness of radiation in causing loss of clonogenic potential
DNA Molecule
Molecular Theory of radiation action

- Proposed by Chadwick and Leenhouts (1981)
- Assumptions
  - There are certain molecules in the cell, which is essential for the survival of the reproductive function of the cell
  - These molecules are the DNA
  - The action of radiation (direct or indirect) is the rupture of the DNA strand bonds (lesions)
Molecular theory of radiation action, *cont.*

- Assumptions, *cont.*
  - DNA lesions are capable of being repaired
  - Repair processes include:
    - Physicochemical recombinations
    - Charge transfer processes
    - Chemical restitution
    - Enzymatic repair
Development of Molecular model

- Let $N_0 =$ # of critical bonds per unit mass
- $N =$ number of bonds that remain intact after any dose
- $k =$ probability constant for rupture of a single bond per unit dose
- $D =$ dose

\[
\frac{dN}{dD} = kN \\
N = N_0 e^{-kD}
\]
Development of Molecular model – Single-Strand Break

- The number of bonds broken per unit mass per unit of dose is:
  \[ N_o - N = N_o - N_o e^{-kD} = N_o (1 - e^{-kD}) \]

- Let \( r \) be the proportion of damaged bonds that is repaired.
- And \( f = (1 - r) \) be the proportion that is not repaired, so:
  \[ N_o - N = N_o - N_o e^{-kD} = N_o (1 - e^{-kD}) \]

- Gives the # of effective DNA strand breaks – those that can lead to single-strand scission.
Two Mechanisms for DNA Damage

DNA can be envisioned as undergoing double-strand break as a result of 2 different mechanisms:

I. Both strands are broken by one event

II. Each strand is broken independently, and the breaks are close enough in time and space for the molecular rupture
Derivation for Double-Strand Break (DSB)

- $n_1$ = # of critical bonds on strand 1
- $n_2$ = # of critical bonds on strand ($n_1 = n_2$)
- $K$ = probability constant for bond rupture per bond per unit dose (analogous to $k$ – the same for each strand)
- $f_1, f_2$ = unrestored factions of bonds in strands 1 and 2
Derivation for Double-Strand Break (DSB)

- \( \Delta = \) fraction of dose \( D \) that acts thru mechanism (I), where both strands are broken in a single event
- \( (1 - \Delta) = \) the fraction of dose acting thru mechanism (II)
- The # of single strands broken on each of strands 1 and 2 by mechanism (II)
Derivation for Double-Strand Break (DSB)

- Then, the # of single strands broken on each of strands 1 and 2 by mechanism (II) can be developed as follows:
  - Let $q = \#$ of broken bonds cell
  - Broken bonds on strand 1 and 2 per cell will be, respectively:
    
    $$q_1 = f_1 n_1 [1 - e^{-K(1-\Delta)D}]$$
    
    $$q_2 = f_2 n_2 [1 - e^{-K(1-\Delta)D}]$$
Derivation for Double-Strand Break (DSB)

- For the two SSB’s to lead to a DSB they must be associated in both time and space
- **Effectiveness factor** is introduced, $E$
  - The likelihood of a rupture occurring from two SSB’s associated in time and space
  - An expression can be written for the mean # of DSB’s per cell that occur as result of mechanism (II) as the product of the # of SSB’s in strand 1, the # of SSB’s in strand 2, and the $E$. 
Derivation for Double-Strand Break (DSB)

- Let $Q = \#$ of DSB’s per cell
- Let $f_0 = \text{fraction of unrepaired DSB’s}$
- The $\#$ of unrepaired DSB’s formed by mechanism (II):

$$Q_{II} = E n_1 n_2 f_1 f_2 f_0 [1 - e^{-K(1-\Delta)D}]^2$$
Derivation for Double-Strand Break (DSB)

- \( n_0 \) = # of sites that can sustain a DSB
- \( K_0 \) = hit probability constant
- \( f_0 \) = unrestored fraction of DSB's
- The rupture of both strands of DNA at the same time by mechanism (I):

\[
Q_I = n_0 f_0 [1 - e^{-K_0 \Delta D}]
\]
Now, the mean # of DSB’s per cell as the result of mechanisms (I) and (II) is:

\[ Q = n_0 f_0 [1 - e^{-K_0 \Delta D}] + En_1 n_2 f_1 f_2 f_0 [1 - e^{-K(1-\Delta)D}]^2 \]
Derivation for Double-Strand Break (DSB)

- One more parameter has to be introduced
- \( \rho = \) proportionality constant between cell death and DSB’s

\[
Q_p = \rho \{ \chi [1 - e^{-K_0 \Delta D}] + \phi [1 - e^{-K(1-\Delta)D}^2] \}
\]

- Equation states the # of lethal DSB’s that occur as the result of dose \( D \) of radiation of quality characterized by \( \Delta \)
Derivation for Double-Strand Break (DSB)

- Equation does not state the fraction of cells killed or surviving.
- A cell is killed only once, and further action on the remaining cells is constrained to the smaller number of cells.
- This is a Poisson-type cell killing.
- So, the fraction of cells killed is:

\[ F_d = 1 - e^{-Q_p} \]
Linear Quadratic Formulation, LQ

- If $K$ and $K_0$ are very small (as in the target theory), the survival equal is:

$$S = e^{-p(\alpha D + \beta D^2)}$$

$$\alpha = (f_0, n_0, K_0, \Delta)$$

$$\beta = (f_0, E, n_1, n_2, f_1, f_2, K^2, (1-\Delta)^2)$$

- Note that $p$ has not be lumped in the constants – $p$ is a biological effectiveness factor for DSB’s and it can be examined experimentally and independently of the other constants.
Significance of the LQ Model

- Is the LQ model more useful biologically and a better fit mathematically?
- The fit of the data for survival of mammalian cells has been significantly improved.
- The LQ model is used widely by experimental radiobiologists.
- But, fundamental assumptions are not widely accepted.
Theory of Dual Radiation Action

- Proposed by Kellerer and Rossi (1971)
- Partially as an explanation for an empirical observation that the relative biological effectiveness (RBE) of neutron radiations increased as the dose was reduced
- Cell inactivation occurs thru the formation of lesions in critical sites
- At low doses, only a single neutron track would traverse a cell and lead to inactivation of the cell
The yield of lesions:

\[ \varepsilon = k_n D_n \]

\[ \frac{D_x}{D_n} = RBE = \sqrt{\frac{\lambda}{D_n}} \]

- \( \varepsilon \) = yield of lesions, \( k_n \) = proportionality constant for the neutron radiation used, \( D_n \) = dose; \( D_x \) = equivalent X-ray dose
Dual Radiation Action Model

- The yield of lesions is then:
  \[ \varepsilon = k D_x^2 \]
  \[ k = \frac{k_n}{\lambda} \]
- The general expression is:
  \[ \varepsilon = k(\lambda D + D^2) \]
Dual Radiation Action Model

- Assumptions
  - The exposure of a cell to radiation leads to *sublesions* in the cell, and their # is directly proportional to the dose
  - Lesion is formed thru the interaction of two sublesions
  - Once lesion is formed, there is a probability that the lesion will lead to a deleterious biological effect
Dual Radiation Action Model

- Assumptions, cont.
  - All pairs of sublesions within a specific distance of one another have equal probability of interaction
    - If the sublesions are further apart than the *sensitive* site dimension the probability of interaction is zero
Goodhead (1982) defined the mean # of lesions (not sublesions) as:

\[ E(D) = \int_0^\infty E(z) f(z, D) dz \]

\[ E(z) = kz^2 \]

\( f(z, D) dz \) = probability that for dose \( D \) the specific energy lies between \( z \) and \( z + dz \)

\( k \) = biological property of the system

\( z \) = specific energy; is the direct measure of the # of sublesions
The term $z$ is squared (sublesions are required to interact in pairs):

$$E(D) = \int_{0}^{\infty} E(z) f(z, D)dz E(D)$$

$$E(D) = \int_{0}^{\infty} k z^2 f(z, D)dz = k \bar{z}^2(D)$$

$$\bar{z}^2(D) = \frac{\bar{z}_1^2}{\bar{z}_1} D + D^2 = \int_{0}^{\infty} k z^2 f_1(z)dz$$

$$D + D^2 = \int_{0}^{\infty} z f_1(z)dz$$
Dual Radiation Action Model

So:

\[ \zeta = \frac{\int_{0}^{\infty} k z^2 f_1(z) \, dz}{\int_{0}^{\infty} z f_1(z) \, dz} \]

\[ E(D) = k (\zeta D + D^2) \]

\[ f_1(z) = \text{distribution of specific energies}, \]
\[ z_1 = \text{of each single radiation event} \]
Dual Radiation Action Model

- The survival equation is (Poisson conversion):
  \[ \frac{S}{S_0} = e^{-k(\zeta D + D^2)} \]

- The survival equation describes effect, much as the expression of the LQ model describes DSB’s of DNA
Significance of the DRA Model

- Both the DRA and LQ models have been criticized widely
- The empirical found relationship of neutron RBE to dose is inadequate to justify development of model
- Concept of a *site size* found to be experimentally untenable
Repair-Misrepair Model of Cell Survival

- Proposed by Tobias et al. (1980)
- Deals with DNA repair processes, instead of considering the geometric identity and location of lesion
Assumptions

- There is an initial process of physical energy transfer followed by migration of the deposited energy, and production of long-lived molecular species as a result of the radiation chemistry of the system.
- The radiation chemical stage is followed by biochemical processes: repair or damage or fixation.
- The cells, if survived, may express permanent alteration in their phenotype.
Repair-Misrepair Model of Cell Survival

- Describes the yield of relevant macromolecular lesions per cell as a function of dose ($D$)
- There is time ($t$) dependent transformation of these lesions
- And accompanying time- and dose-dependent probabilities of survival ($S$), lethality ($L$) and mutation ($M$)
- The model also postulates a class of lesions, ($U$) – uncommitted lesions –
- There are various repair stated ($R$) that are the result of transformation of ($U$) lesions
Repair-Misrepair Model of Cell Survival

Stable Macromolecular Lesion (10^{-3}s) \( U(t) \)

Linear process \( R_L(t) \)

Uncommitted \( U(t) \)

Quadratic process \( R_Q(t) \)

Lethality \( L \)

Cells with remnant lesions \( U \)

Survivors (S)

Viabile mutants (M)
Two repair (R) states are assumed:

- $R_L$ = yield per cell resulting from a monomolecular reaction that is linear on the concentration of $U$ lesions
- $R_Q$ = yield per cell of a repair process that is quadratic, where the rate is proportional to the square of the density of $U$ lesions

If $U$ lesions are homogeneously distributed in the reaction volume, then the rate, $R_Q$, is proportional to $U(U-1)=U^2$ (for $U \gg 1$)
Repair-Misrepair Model of Cell Survival

- Assuming the delivery of a dose of low LET radiation in a time that is brief compared to repair rates:
  \[
  \frac{dU}{dt} = -\lambda U(t) - kU^2(t)
  \]
  integrating
  \[
  U(0) - U(t) = \int_0^t \lambda U(t)\,dt + \int_0^t kU^2(t)\,dt
  \]
  where \(\lambda\) and \(k\) are the rate constants for linear and quadratic repair processes, respectively.
Repair-Misrepair Model of Cell Survival

- Defining $R_L$ and $R_Q$ as:

$$R_L = \int_{0}^{t} \lambda U(t)dt$$

$$R_Q = \int_{0}^{t} kU^2(t)dt$$

then

$$U(t) + R_L(t) + R_Q(t) = U(0)$$
If $U(0) = U_0$, $R_L(0)$ and $R_Q(0) = 0$, $U(\infty) = 0$ and $\epsilon = \lambda/k$:

$$U = \frac{U_0 e^{-\lambda t}}{1 + (U_0 / \epsilon)(1 - e^{-\lambda t})}$$

$$R_L(t) = \epsilon \ln \left[ 1 + \frac{U_0}{\epsilon} (1 - e^{-\lambda t}) \right]$$

$$R_Q(t) = \frac{U_0 (1 + U_0 / \epsilon)(1 - e^{-\lambda t})}{1 + (U_0 / \epsilon)(1 - e^{-\lambda t})} - \epsilon \ln \left[ 1 + \frac{U_0}{\epsilon} (1 - e^{-\lambda t}) \right]$$
Repair-Misrepair Model of Cell Survival

- Both the linear and quadratic repair process are assumed to be capable of:
  - Correct repair of the macromolecular damage, *eurepair*
  - Incorrect repair of the lisions, *misrepair*
- Two new parameters
  - $\phi =$ probability that the linear repair is correct (*eurepair*)
  - $\delta =$ probability that the quadratic repair is correct (*eurepair*)
  - $(1-\phi)$ and $(1-\delta) =$ probability of misrepair processes
- Number of lesions per cell repaired
  - By the linear process: $R_{LE}$ (*eurepair*) and $R_{LM}$ (*misrepair*)
  - By the quadratic process: $R_{QE}$ (*eurepair*) and $R_{QM}$ (*misrepair*)
Repair-Misrepair Model of Cell Survival

- **Case I: Linear Eurepair-Quadratic Misrepair**
- **Assumptions**
  - All linear repair is eurepair, \( \phi = 1 \)
  - All quadratic repair is misrepair and letha, \( \delta = 0 \)
  - Remnant \( U \) lesions that exist at time \( t \) are also lethal

\[
S(t) = e^{-U_0} \left[ 1 + \frac{U_0}{\epsilon} (1 - e^{-\lambda t}) \right]^\epsilon
\]

\[
U_0 = \alpha D; \quad U_0 = \alpha_1 D + \alpha_2 D^2
\]

constants
Repair-Misrepair Model of Cell Survival

- Case I: Linear Eurepair-Quadratic Misrepair
- An additional constraint:
  - $T = \text{related to the maximum time to repair}$

$$T = 1 - e^{-\lambda t_{\text{max}}}$$

$$S = e^{-\alpha D} \left[ 1 + \frac{\alpha DT}{\varepsilon} \right]^\varepsilon$$

$\lambda t_{\text{max}} \gg 1, \quad T \approx 1$

$$S = e^{-\alpha D} \left[ 1 + \frac{\alpha D}{\varepsilon} \right]^\varepsilon$$
Repair-Misrepair Model of Cell Survival

- Case II: Linear Repair is not always Eurepair

\[ S(t) = e^{-\alpha D} \left[ 1 + \frac{\alpha D}{\epsilon} \right]^{\epsilon \phi} \]

\[ \phi \neq 1 \]
Significance of the RMR Model

- It is a better fit than LQ model
- Very few assumptions are general and rigid
- The nature of the dose-response relationship for the production of $U$ lesions is not fixed (can be adjusted to fit experimental data)
Lethal-Potential Lethal Model

- Proposed by Curtis (1986)
- The number of $N_{PL}$ and $N_L$ lesions is linear related to dose by the proportionality constant $k_{PL}$ and $k_L$
- The $N_{PL}$ lesions are repaired by a 1st-order process to return them to undamaged $N$ state
- The alternative is that the $N_{PL}$ lesions are permanently converted to the lethal state $N_L$ by a 2nd-order process
Lethal-Potential Lethal Model

Assumptions

- Potentially lethal injuries (PL)
  - are spatially distributed and long-lived (minutes) potentially repairable injuries created in the microbial cell by low energy transfer (LET) radiation.
  - The injuries are repairable to return the PL injuries back to the undamaged state by an enzymatic process that is 1st order in the number of PL injuries.
  - If the lesions are not repaired, they will interact with each other to form lethal injuries that are irreparable and lethal.

- Lethal injuries (L)
  - can be formed at the time of irradiation on a very short time scale if they are created simultaneously and they are very close to each other.
  - The constant, $k_L$, describes the yield/dose for these formed lethal injuries.
Lethal-Potential Lethal Model

Assumptions

- The constants $k_{PL}$ and $k_{L}$ are the rate constants per unit absorbed dose for the production of both types of injuries.
- The constants $\varepsilon_{PL}$ and $\varepsilon_{L}$ are the rate constants for the formation of restored injuries per unit time and for the formation of irreparable injuries per unit of time, respectively.
- Since $\varepsilon_{L}$ is the result of a bimolecular interaction of potential lethal injuries, it will be a 2$^{nd}$-order reaction in the concentration of potential lethal injuries.
- The rate of repair of the injuries does not depend on the number of injuries; that is, there is no saturation of the repair process.
Lethal-Potential Lethal Model

During radiation

(1) \[ \frac{dN_{PL}(t)}{dt} = k_{pl} \dot{D} - \varepsilon_{PL} N_{PL}(t) - \varepsilon_{2PL} N_{PL}^2(t) \]

(2) \[ \frac{dN_{L}(t)}{dt} = k_{L} \dot{D} + \varepsilon_{2PL} N_{PL}^2(t) \]

I.C : \( N_{PL}(0) = N_{L}(0) = 0 \) (no injuries present at the start of irradiation).

The solution of the above equations are:

(3) \[ N_{PL}(t) = \frac{2k_{PL}(1 - e^{-\varepsilon_o t})}{\varepsilon_o + \varepsilon_{PL} + (\varepsilon_o + \varepsilon_{PL})e^{-\varepsilon_o t}}; \quad \varepsilon_o = \sqrt{\varepsilon_{PL}^2 + 4\varepsilon_{PL}k_{PL}\dot{D}} \text{ and} \]

(4) \[ N_{L}(t) = k_{L} D + \varepsilon \ln \left[ \frac{2\varepsilon_o}{\varepsilon_o + \varepsilon_{PL} + (\varepsilon_o - \varepsilon_{PL})e^{-\varepsilon_o t}} \right] + \frac{(\varepsilon_o - \varepsilon_{PL})^2 t}{4\varepsilon_{2PL}}; \quad \varepsilon = \frac{\varepsilon_{PL}}{\varepsilon_{2LP}} \]
Lethal-Potential Lethal Model

After irradiation is complete (at time $T$), there is no longer a source term for the production of new injuries but repair continues with the same rate constants for the repair of the remaining injuries:

\[ \frac{dN_{PL}(t)}{dt} = -\varepsilon_{PL} N_{PL}(t) - \varepsilon_{2PL} N_{PL}^2(t) \]  
\[ \frac{dN_{L}(t)}{dt} = \varepsilon_{2PL} N_{PL}^2(t) \]
Lethal-Potential Lethal Model

After irradiation is complete (at time $T$), there is no longer a source term for the production of new injuries but repair continues with the same rate constants for the repair of the remaining injuries:

$I.C's$ : $N_{PL}(T) = $ the value of result from Eq(3);

$N_{L}(T) = $ the value of result from Eq(4)

The solution of the above equations are :

\begin{align*}
(7) \quad N_{PL}(t) &= \frac{N_{PL}(T)e^{-\varepsilon_{PL}t_{r}}}{[1 + N_{PL}(T)/\varepsilon(1 - e^{-\varepsilon_{PL}t_{r}})]} ; \quad \text{and} \\
(8) \quad N_{L}(t) &= N_{L}(T) + N_{PL}(T)(1 + N_{PL}(T)/\varepsilon)/[1 + N_{PL}(T)/\varepsilon(1 - e^{-\varepsilon_{PL}t_{r}})] - \\
& \quad \varepsilon \ln[1 + N_{PL}(T)/\varepsilon(1 - e^{-\varepsilon_{PL}t_{r}})]
\end{align*}

where $\varepsilon = \frac{\varepsilon_{PL}}{\varepsilon_{2LP}}$ and $t_{r}$ = time available for repair after the end of irradiation
Lethal-Potential Lethal Model

- To calculate the survival time at time $t = T + t_r$, the time after which no more repair can occur and the fate the microbial cell has been determined, it is assumed that the total mean number of lethal injuries per cell is the sum of the lethal and potentially lethal injuries per cell ($N_{tot}(T + t_r) = N_{PL}(T + t_r) + N_L(T + t_r)$).

- Assuming that the distribution of lethal injuries per cell can be described by the Poisson distribution, then the survival probability that a cell has no lethal injury is:

$S = \exp(-N_{tot}(T + t_r)) = \exp[N_L(T + t_r) - N_{PL}(T + t_r)]$

$S(D, \dot{D}, t_r) = \exp(-N_{tot}(D / \dot{D})) \left[1 + \frac{N_{PL}(D / \dot{D})}{\varepsilon}(1 - \exp(-\varepsilon_{PL}t_r))\right]^\varepsilon$

where

$N_{tot}(D / \dot{D}) = N_{PL}(T) + N_L(T)$

$N_{PL}(D / \dot{D}) = N_{PL}(T)$