Multiscale Modeling of Radiation Response

Effects of Radiation Quality and Hypoxia

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Outline

As is sometimes done in Hollywood, the “Story” will be told from the end to beginning…

- Literature Citations
- Conclusions
- Results
- Methods
- Future Direction
- Acknowledgements

*Presenter has no conflicts of interest to disclose*
Literature Citations

Conclusions

- We have successfully developed a multiscale system of *Monte Carlo* and *deterministic* models to link absorbed dose to reproductive cell death
- Captures many of the quantitative and qualitative features of cell survival curves
  - Relative biological effectiveness (RBE), acute hypoxia, dose, dose rate and fractionation effects included (*cellular and sub-cellular effects*)
  - Bystander effects, adaptive responses, and many other (*larger-scale, multi-cellular and tissue*) effects neglected in current simulations

All models are incomplete (*wrong*) but some are useful...
Physics → Chemistry → Biology → Clinic

Absorbed Dose

Radiation

10^{-18} to 10^{-10} \text{s}

Ionization Excitation

10^{-3} \text{s}

Chemical Repair

O_2 fixation

1 Gy ~ 1 in 10^6

Correct Repair

10^{-6} \text{s}

Enzymatic Repair

(BER, NER, NHEJ, …)

10^2 \text{s} \uparrow 10^4 \text{s}

Chronic hypoxia

(> 1-2 h)

Incorrect or Incomplete Repair

10^3 \text{s}

DNA damage

10^5 \text{s}

Cell Death

Early Effects

(erythema, …)

Loss of Function and Remodeling

10^6 \text{s}

Angiogenesis and Vasculogenesis

Inflammatory Responses

Self renewal and Differentiation

Heritable Effects

10^7 \text{s}

Germline

Genome Instability

Somatic cells

Small- and large-scale mutations

(point mutations and chromosomal aberrations)

Late Effects

(fibrosis, …)

Clonal Expansion

10^8 \text{s}

Neoplastic Transformation

2nd Cancer

10^8 \text{s}

Late Effects

(fibrosis, …)

Clonal Expansion

10^8 \text{s}

Neoplastic Transformation

2nd Cancer

10^8 \text{s}

Late Effects

(fibrosis, …)

Clonal Expansion

10^8 \text{s}

Neoplastic Transformation

2nd Cancer

10^8 \text{s}
Advantages of a Multiscale Approach

- Modeled mechanisms at different levels in biological hierarchy can be independently tested against measured *in vitro* and *in vivo* data
  - Exploits idea that different biological endpoints are observable (*measurable*) on very different time scales after irradiation

- Proposed system of models ultimately has just two *critical* cell- or tissue specific adjustable parameters
  - 2 parameters related to biological processing of DNA damage (*critical*)
  - 1 parameter related to dose rate effects (*repair half-time*) and one related to the microdosimetric deposition of energy within the cell nucleus (*most important for short-range, high LET radiations*)
  - All four adjustable parameters are independent of dose, dose rate, radiation quality (LET), and oxygen concentration
Why Might this be Useful?

- Critical biological parameters can be estimated from survival data for low LET radiations
  - Use parameters unchanged in simulations for higher LET radiations under the same or different oxygen concentrations

- Easy to incorporate RBE and O$_2$ information from simulations into isoeffect (BED, EUD, …) and outcome (TCP and NTCP) calculations

- Hypothesis
  - For the endpoint of reproductive death, *in vitro* and *in vivo* radiation sensitivity mainly differ because of differences in the way cells *in vitro* and *in vivo* process and/or express the initial sub-cellular damage
Results-1 Human Kidney T1 Cells (aerobic)

In vitro irradiation of T1 cells by selected ions (Barendsen circa 1960-1966). Simulation parameters: $\theta = 3.07 \times 10^{-2}$ Gbp/DSB, $\kappa = 7.05 \times 10^{-4}$ Gbp/DSB, $\tau = 2$ h, ndia=3.5 $\mu$m. Equivalent x-ray LQ parameters: $\alpha_X=0.265$ Gy$^{-1}$, $\alpha/\beta_X=10.1$ Gy.
Results-1 Comparison to LQ fits (aerobic)

In vitro irradiation of T1 cells by selected ions (Barendsen circa 1960-1966). Simulation parameters: $\theta = 3.07 \times 10^{-2}$ Gbp/DSB, $\kappa = 7.05 \times 10^{-4}$ Gbp/DSB, $\tau = 2$ h, ndia=3.5 $\mu$m.
**Results-1a Human Kidney T1 Cells (hypoxic)**

In vitro irradiation of T1 cells by selected ions (Barendsen circa 1960-1966). Simulation parameters: $\theta = 3.07 \times 10^{-2}$ Gbp/DSB, $\kappa = 7.05 \times 10^{-4}$ Gbp/DSB, $\tau = 2$ h, ndia=3.5 μm. Equivalent x-ray LQ parameters: $\alpha = 0.265$ Gy$^{-1}$, $\alpha/\beta = 10.1$ Gy (aerobic)
Results 1 - Some Observations

- Direct LQ fits to survival data are comparable to fits obtained from the multiscale model. But...
  - LQ fits: 15 x 2 parameters = 30 adjustable parameters (*one set for each particle type, energy and oxygen condition*).
  - Multiscale model: 2 adjustable parameters (\(\theta\) and \(\kappa\)), which can be estimated from x-ray cell survival data for aerobic conditions.

- For short-range (*higher LET*) particles, direct LQ fits to cell survival data are sensitive to
  - Uncertainties in dosimetry
  - Experimental artifacts in the measured data (e.g., “floaters”)

- Global (*simultaneous*) fits of the multiscale model are possible but produce modest improvements in quality of fit.
Results-2 V79 Cells (protons and $^4$He$^{2+}$)

In vitro irradiation of V79 cells under aerobic and hypoxic conditions by selected ions (Prise et al. IJRB 58, 261-277 1990). Simulation parameters: $\theta = 3.71 \times 10^{-2}$ Gbp/DSB, $\kappa = 2.32 \times 10^{-4}$ Gbp/DSB, $\tau = 2$ h, ndia = 4 $\mu$m.
Results 2 - Some Observations

- Can perform simultaneous fits to aerobic and hypoxic data for one or more particle types
  - For the smaller, often noisy, datasets common for higher LET radiations, simultaneous fits to multiple particle types provide more accurate estimate of parameters than fits to data for a single particle type.

- Anecdotal testing of the model suggests that estimates of $\kappa$ parameter (related to $\beta$ in the LQ) are more accurate when survival data for low LET radiations available
  - Dosimetry more accurate for low LET radiations than for high LET radiations
  - Low LET radiations not so sensitive to “floaters” and other experimental artifacts
  - Datasets usually larger for low LET radiations than high LET radiations
Results 3a - Dose Rate and Hypoxia (low LET)

In vitro irradiation of V79 cells by 10 MV x-rays or \(^{137}\)Cs \(\gamma\)-rays under aerobic and hypoxic conditions by selected ions (Spiro et al. BJR 58, 357-363 1985). Simulation parameters: \(\theta = 1.48 \times 10^{-2}\) Gbp/DSB, \(\kappa = 5.42 \times 10^{-4}\) Gbp/DSB, \(\tau = 0.523\) h, ndia = 3.5 \(\mu\)m.
Results 3b - Dose Rate and Hypoxia (low LET)

In vitro irradiation of V79 cells by 10 MV x-rays or $^{137}$Cs $\gamma$-rays under aerobic and hypoxic conditions by selected ions (Spiro et al. BJR 58, 357-363 1985). Simulation parameters: $\theta = 1.48 \times 10^{-2}$ Gbp/DSB, $\kappa = 5.42 \times 10^{-4}$ Gbp/DSB, $\tau = 0.523$ h, ndia = 3.5 $\mu$m.
Results 3c - Dose Rate and Hypoxia (low LET)

Poor agreement for extreme hypoxia may be due to a slowing of repair process (acute vs chronic hypoxia) or modulation of cell death modes not related to DNA damage induction (plating efficiency substantially reduced under extreme hypoxia).

In vitro irradiation of V79 cells by 10 MV x-rays or $^{137}$Cs $\gamma$-rays under aerobic and hypoxic conditions by selected ions (Spiro et al. BJR 58, 357-363 1985). Simulation parameters: $\theta = 1.48 \times 10^{-2}$ Gbp/DSB, $\kappa = 5.42 \times 10^{-4}$ Gbp/DSB, $\tau = 0.523$ h, ndia = 3.5 $\mu$m.
Overall Summary of Results

- Model appears to have substantial predictive power
  - Fit model to low LET survival data under aerobic conditions
  - Predict cell survival for other types of radiation and oxygen conditions

- Accuracy of model predictions can be improved somewhat by fitting the model to data for multiple particle types and/or $O_2$ conditions
  - Such datasets are available in vitro but scarce for in vivo models (including humans ;)

- May need to incorporate a model for the effects of reduced oxygen on the rate of damage repair and/or modulation of cell death modes (e.g., apoptosis vs mitotic death)
Methods

- **Monte Carlo Damage Simulation (MCDS)**
  - Effects of LET and Oxygen on DNA damage induction
  - Microdosimetry (lineal energy, frequency-mean specific energy, CSDA range)

- **Repair-Misrepair-Fixation (RMF) Model**
  - Motivated by the breakage and reunion theory of chromosomal aberrations
  - DNA damage induction linked to cell killing through a couple system of deterministic non-linear differential equations
    - Cell survival curves are LQ for low doses and become linear at high dose
  - RMR (CA Tobias) and LPL (S Curtis) models (circa 1985) are special cases of the RMF
    - Compound Poisson distribution for damage induction (RMF) instead of Poisson
  - As with the RMR and LPL, the linear-quadratic (LQ) is a low dose or low dose rate approximation for the RMF.
Clustered DNA lesions

Groups of several DNA lesions within one or two turns of the DNA are termed *clustered DNA lesions*.

* Clustered DNA lesions are also referred to as *locally multiply damaged sites* (LMDS), *multiply damaged sites* (MDS) or just “clusters”.

*Interesting Trivia:* Over $10^{12}$ (!) possible types of clustered DNA lesion, i.e., the number of possible ways a 10 bp segment of DNA (20 nucleotides) can be damaged is on the order of $4^{20} = 10^{12}$ possible types of cluster. Most of the DNA clusters formed by ionizing radiation, including single- and double-strand breaks, are composed of 3 or more individual lesions.
MCDS – General Features and Capabilities (1)

Developed to simulate number and small-scale spatial distribution of lesions forming clusters ("nucleotide-level maps")

- Individual particles or arbitrary mixtures of charged particles up to and including $^{56}$Fe (new in 2011)
  - Simulate damage from neutral particles using the distribution of secondary charged particles (e.g., see Hsaio and Stewart, PMB 53, 233-244, 2008)

Additional Information and Software Available at
http://faculty.washington.edu/trawets/mcds/

“trawets” = “stewart” backwards
MCDS – General Features and Capabilities (2)

- Simulates the effects on cluster formation of O₂ fixation and chemical repair (new in 2011) – “oxygen effects”

First and only (at present) MC simulation to account for the effects of oxygen concentration on clusters
MCDS – General Features and Capabilities (3)

- Particle and Dosimetric Information (*new in 2011*)
  - Stopping power in water, CSDA range, absorbed dose per unit fluence, mean specific energy, energy imparted per radiation event, and lineal energy

<table>
<thead>
<tr>
<th>Particle Type</th>
<th>Kinetic Energy</th>
<th>S - $S_{rad}$ (keV/μm)</th>
<th>CSDA Range (μm)</th>
<th>$\bar{z}_F$ (Gy)</th>
<th>MCDS</th>
<th>Analytic</th>
</tr>
</thead>
<tbody>
<tr>
<td>$e^-$</td>
<td>2.56 x 10^{-5}</td>
<td>21.13</td>
<td>2 x 10^{-3}</td>
<td>$&lt; 10^{-11}$</td>
<td>0.17</td>
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<tr>
<td>$^1$H</td>
<td>6.47 x 10^{-3}</td>
<td>6.47 x 10^{-3}</td>
<td>0.28</td>
<td>$&lt; 10^{-4}$</td>
<td>0.29</td>
<td></td>
</tr>
<tr>
<td>$^4$He $^{2+}$</td>
<td>0.294</td>
<td>7.35 x 10^{-2}</td>
<td>2.70</td>
<td>0.14</td>
<td>1.53</td>
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<tr>
<td>$^{12}$C $^{6+}$</td>
<td>14.8</td>
<td>1.23</td>
<td>21.13</td>
<td>5.32</td>
<td>5.08</td>
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<tr>
<td>$^{16}$O $^{8+}$</td>
<td>38.1</td>
<td>2.38</td>
<td>42.03</td>
<td>6.01</td>
<td>5.86</td>
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<tr>
<td>$^{20}$Ne $^{10+}$</td>
<td>78.4</td>
<td>3.92</td>
<td>73.14</td>
<td>6.60</td>
<td>6.50</td>
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<tr>
<td>$^{56}$Fe $^{26+}$</td>
<td>1750</td>
<td>31.3</td>
<td>963.7</td>
<td>9.35</td>
<td>9.34</td>
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</tbody>
</table>

Analytic Formula: $\bar{z}_F = 0.204 \left[ S - S_{rad} \right] / \rho d^2$
Chemical Basis of the Oxygen Effect

Competition between oxygen fixation and chemical repair modeled in the MCDS using a scheme that mimics the pathways suggested by von Sonntag (2006)

(1) DNA + ionizing radiation → DNA lesion (*biochemical repair required*)

(2) DNA + ionizing radiation → DNA⋅ (various)

Lesions and DNA radicals formed through direct and indirect interaction mechanisms

____________________

(3) DNA⋅ + O₂ → DNA-O₂ ("oxygen fixation" – *biochemical repair required*)

(4) DNA⋅ + RSH → DNA ("chemical repair" – restoration of the DNA*)

(5) DNA⋅ → DNA lesion (*biochemical repair required*)

* Von Sonntag notes that donation of a proton to a DNA radical may or may not restore the original chemical structure of the DNA. But, the chemical repair process evidently converts the DNA radical (or cluster of radicals?) into a form that is more amenable to biochemical repair and reduces the number of strand breaks.

**RBE and HRF (induction of DNA damage)**

**Relative Biological Effectiveness (RBE)** for the \(i^{th}\) type of cluster

\[
RBE_i(q) = \frac{\Sigma_i(q)}{\Sigma_i(q_0)}
\]

\(\Sigma_i = \) Measured or MC simulated number of the \(i^{th}\) type of cluster Gy\(^{-1}\) Gbp\(^{-1}\) (or per cell),
\(q\) denotes radiation quality of the particle of interest (e.g., a proton or neutron), and \(q_0\) denotes the radiation quality of the reference radiation (typically high-energy x-rays or \(\gamma\)-rays from \(^{60}\)Co or \(^{137}\)Cs)

**Hypoxia Reduction Factor (HRF)** for the \(i^{th}\) type of cluster

\[
HRF_i([O_2]) = \frac{\Sigma_i(100\% O_2)}{\Sigma_i([O_2])}
\]

In general, the RBE and HRF varies with endpoint (cell killing vs DNA damage) and even the type of damage (e.g., SSB vs DSB). For DNA damage induction up to doses of at least a few hundred Gy, little if any good evidence that the RBE or HRF depend on dose or dose rate.
DSB induction in human skin fibroblasts

Pulsed-field gel electrophoresis (PFGE) and DNA fragmentation analysis

DNA Damage and Hypoxia

Presence of oxygen within a cell substantially enhances the initial yield of DNA damage

100% O₂

2.91 DSB Gbp⁻¹ Gy⁻¹

100% N₂

1 DSB Gbp⁻¹ Gy⁻¹

DSB induction is proportional to dose up to at least 2400 Gy under aerobic and hypoxic conditions

Initial yield ∝ dose

Figure adapted from Frankenberg D, Frankenberg-Schwager M, Blocher D, Harbich R. Evidence for DNA double-strand breaks as the critical lesions in yeast cells irradiated with sparsely or densely ionizing radiation under oxic or anoxic conditions. Radiat Res. 88(3), 524-532 (1981)
Many of the published experimental studies (symbols, right panel) detect a subset of the total number of DSB because not all DNA fragments counted.
DNA Fragmentation Analysis

Agarose plug containing irradiated DNA isolated from cells

Fragments migrate out of the gel because of the negative charge carried by the sugar-phosphate backbone of DNA

Short fragments quickly migrate
Larger fragments slowly migrate

Quantify number and sizes of fragments

<table>
<thead>
<tr>
<th>Size in Kb</th>
<th>Markers</th>
<th>Non Rad</th>
<th>Rad 5 Gy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P/LR</td>
<td>W</td>
<td>C</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>+</td>
<td>-</td>
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<td></td>
</tr>
<tr>
<td>3300</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2200</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1600</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1125</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>194</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.1</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Courtesy A. Georgakilas (ECU)
Fragment size distributions

Small fragments usually not counted in most experiments (DSB yield underestimated)

Fragment size distributions

**HRF for DSB Induction**

For low LET radiations, DSB induction is about 3-fold lower under maximally hypoxic conditions than in well oxygenated cells (i.e., $HRF \approx 3$).

$HRF$ decreases towards unity (O$_2$ concentration has no effect) as particle LET increases.

Filled *symbols* are data from PFGE experiments. *Solid line* is the MCDS predicted $HRF$ for a range of particle types and energies.
**HRF for Cell Survival and DSB Induction**

**Solid Black Line:** HRF for DSB induction predicted by the MCDS (0% O₂ concentration)

**Symbols:** HRF derived from published clonogenic survival data (negligible O₂ concentration)

\[
\alpha_H = \frac{\alpha_A}{HRF_\alpha}
\]

\[
(\alpha / \beta)_H = (\alpha / \beta)_A \cdot HRF_{\alpha/\beta}
\]

\[
HRF_\alpha \cong HRF_{\alpha/\beta}
\]
Effect of Oxygen Concentration on the HRF

Symbols: HRF derived from published clonogenic survival data

Solid, dotted and dashed black lines: HRF for DSB induction predicted by the MCDS for selected particle types
Low Dose Approximation to the RMF

Trends in DSB induction with radiation quality and oxygen concentration are closely related and predictive of general trends in Linear-Quadratic (LQ) survival parameters $\alpha$ and $\alpha/\beta$ (e.g., Carlson et al. 2008)

$$\alpha = \theta \Sigma + \kappa \bar{z}_F \Sigma^2$$

$$\beta = \frac{\kappa}{2} \Sigma^2$$

$$\frac{\alpha}{\beta} = \frac{2}{\Sigma} \left( \theta / \kappa \right) + 2 \bar{z}_F$$

$\theta, \kappa$ are adjustable cell- or tissue-specific parameters related to biological processing of DNA damage (independent of LET and $O_2$ concentration)

$\Sigma$ is the number of DSB Gy$^{-1}$ Gbp$^{-1}$ (or per cell) and is estimated using the MCDS (strong function of LET and $O_2$ concentration)

$\bar{z}_F$ is the frequency-mean specific energy (in Gy) for the cell nucleus (strong function of LET but independent of $O_2$ concentration) – estimate with the MCDS or other Monte Carlo code(s)

Strategies to estimate $\theta$ and $\kappa$ measured data

1. Non-linear regression analysis of LQ to survival data for particles of one or more radiation quality and one or more $O_2$ concentrations

$$S(D) = \exp\left\{ -\alpha D - \beta GD^2 \right\} = \exp\left\{ -\left( \theta \Sigma + \kappa \bar{z}_f \Sigma^2 \right) D - \frac{\kappa}{2} \Sigma^2 GD^2 \right\}$$

Estimate $\Sigma$ and $\bar{z}_f$, $a$ priori using MCDS or other methods. Potentially most accurate. BUT... can be sensitive to uncertainties in microdosimetry for short-range (high $LET$) radiations

2. Use analytical formulas to estimate $\theta$ and $\kappa$ from published LQ parameters for a reference radiation ($clinical$, $animal$, $in$ $vitro$)

$$\theta = \frac{1}{\Sigma} \left( \alpha - \kappa \bar{z}_f \Sigma^2 \right) \cong \frac{\alpha}{\Sigma} \quad \kappa = \frac{2\beta}{\Sigma^2} = \frac{2\alpha / \Sigma^2}{(\alpha / \beta)}$$

Estimate $\Sigma$ and $\bar{z}_f$, $a$ priori using MCDS or other methods. In practice, insensitive to uncertainties in microdosimetry because for most low LET radiations

$$\frac{\alpha}{\Sigma} \gg \frac{\kappa \bar{z}_f \Sigma^2}{\Sigma}$$
Importance of Good Dosimetry (high LET)

LQ fits to data for each particle type relatively insensitive to systematic dosimetry errors – it’s the product of $\alpha D$ and $\beta D^2$ that matters!

But when attempting to predict effects for high LET radiations from lower LET radiations, good absolute dosimetry is important – rejoice physicists!

\[
\alpha D = \left( \theta \Sigma + \kappa \bar{Z}_F \Sigma^2 \right) D
\]

macrodosimetry

microdosimetry
Cellular and Sub-cellular Dosimetry

<table>
<thead>
<tr>
<th>Particle</th>
<th>Range (μm)</th>
<th>Entrance</th>
<th>Exit</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.9 MeV p</td>
<td>67.90</td>
<td>26.90</td>
<td>27.70</td>
<td>27.20</td>
</tr>
<tr>
<td>1.15 MeV p</td>
<td>30.00</td>
<td>39.00</td>
<td>41.70</td>
<td>40.00</td>
</tr>
<tr>
<td>0.76 MeV p</td>
<td>15.90</td>
<td>52.90</td>
<td>59.40</td>
<td>54.60</td>
</tr>
<tr>
<td>3.8 MeV α</td>
<td>25.30</td>
<td>174.20</td>
<td>189.30</td>
<td>179.50</td>
</tr>
</tbody>
</table>

< 2-3% difference in entrance vs exit dose for G1 phase cell

G1 phase  M phase  “floater”

14% larger  60% larger

25-35 μm
Microdosimetry → \( \alpha D = (\theta \Sigma + \kappa \bar{z}_F \Sigma^2)D \)

Analytic formula neglects changes in stopping power while particle passes through target. Also assumes particle passes all the way through target. MCDS accounts for “stoppers” and changes in stopping power within target.
(1) Contrary to conventional wisdom, RMF predicts that $\beta$ tends to increase with increasing LET. (2) Multiple combinations of LQ parameters fit measured data about equally.
Future Direction

- **Additional testing and refinement of MCDS+RMF**
  - Numerical solution of the RMF system of differential equations instead of low dose LQ approximation
  - Effects of chronic hypoxia (?)

- **Combine MCDS+RMF model with MCNPX radiation transport code**
  - RBE and O₂ effects in pristine and spreadout proton and carbon ion Bragg peaks (see also Frese et al. IJROBP, 83, 442-450. 2012).
  - RBE and O₂ effects in the fast neutron beamline at UWMC
    - Estimate neutron tolerance doses from first principles
    - Compare to clinical experience and RBE estimates from in vitro experiments
  - Dosimetry and biological modeling support for small animal proton irradiation facility under development at UWMC
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