Modelling of Biological Processes

WHAT HAPPENS AFTER EARLY MOLECULAR DAMAGE?

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3rd August 2016

Do we need biology?

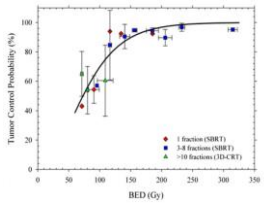
The Linear-quadratic relationship dominates traditional clinical radiobiology.

$$S = e^{-\alpha D - \beta D^2}$$

Extremely simple, but (surprisingly?) effective.

Do we need to understand more biology to optimise clinical treatment?

Right: Tumour Control Probability model for Stage I lung cancer, treated with different schedules, compared using the LQ model. From: Brown et al, IJROBP, 85:5, 2013



Interpreting the LQ model

LQ parameters are not directly associated with any biological process.

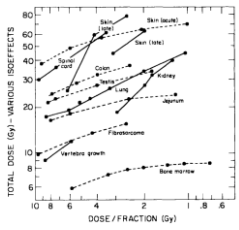
In clinical practice, it is often said:

- High α/β tissues are 'early responding' and not sensitive to fraction size;
- Low α/β are 'late responding' and more sensitive to fraction size

Tumours are typically assumed to be high α/β structures, but this is increasingly challenged.

What drives these differences?

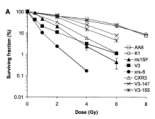
Right: Isoeffect curves for different endpoints in a range of tissues. Late (solid) and early (dashed) responding tissues have different dependencies on fraction size. Withers, Cancer 55, 1985

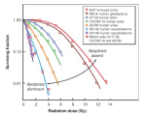


Intrinsic drivers of radiation response

DNA Repair

Rothkamm et al, Molecular and Cellular Biology, 23, 2003



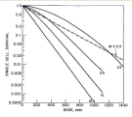


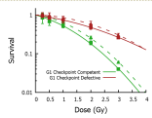
Apoptosis

Hall & Giaccia, Radiobiology for the Radiologist, 7th ed

Cell Cycle Distribution

Sinclair, Radiation Research 33, 1968





Cell Cycle Defects

Adapted from McIlwrath et al, Cancer Research, 54(14), 1994

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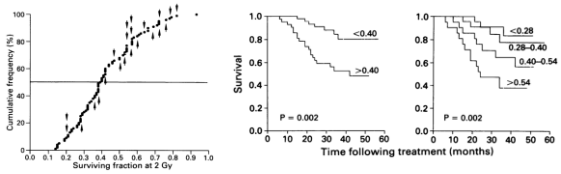
Interpatient heterogeneity



Left: Distribution of experimental SF2 values for 88 cervical cancer samples, showing wide range of responses.
Right: Stratification of patient survival by median SF2 or by quartiles, showing impact of intrinsic sensitivity.

West et al, British Journal of Cancer, 68, 1993

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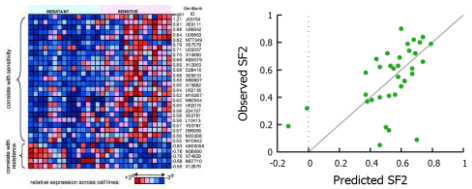
Predicting radiosensitivity



'Omics' approaches seek to unpick markers of sensitivity from complex datasets.
Illustration of radiosensitivity gene signature, adapted from: Torres-Roca, Cancer Research, 65:16, 2005

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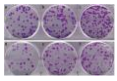
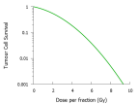
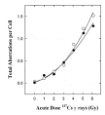
Not just survival data

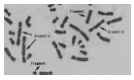
Cell Survival



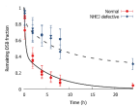


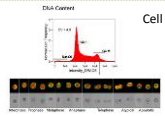


Chromosome Aberrations



DNA Repair





Cell Cycle Distributions

DNA Repair

Cells deal very well with DNA damage

DNA Double Strand Breaks (DSB) are most dangerous type of DNA lesion, but only a small fraction are lethal.

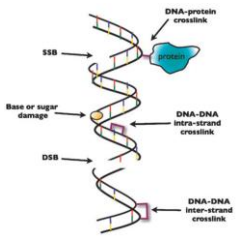
Endogenous stresses cause ~10 DSB/cell/day, and going through a cell cycle incudes up to 50 DSB[1].

We have had to develop sophisticated repair processes to cope with these effects, which also confer resistance to ionising radiation, meaning 1% or less of DSBs lead to lethal events.

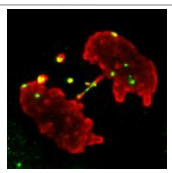
But even small disruptions in these processes have serious consequences.

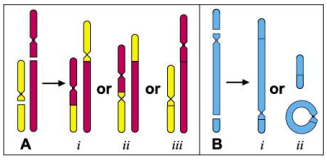
[1] *Vilenchik & Knudson, PNAS, 100:22, 2003*

Kavanagh, Antioxidants & Redox Signaling, 18:18, 2013



Consequences of misrepair





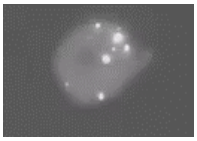
Unrepaired DSBs lead to significant replication stress and loss of large amounts of genetic material during cell division (left, red nuclei, green DSB)

Joining of incorrect ends can lead to formation of aberrant chromosomes and loss of genetic material.

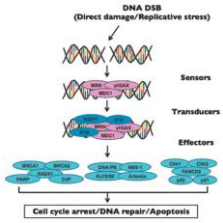
Hlatky, Bioessays, 24(8), 2002; Löbrich, TU Darmstadt

Sensing DNA damage

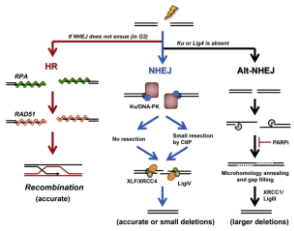
DNA damage rapidly reacts with specific binding proteins which stabilise the break, and drive subsequent cellular responses, based on cellular state and break characteristics.



Georgescu et al, PLoS ONE, 10(6), 2015



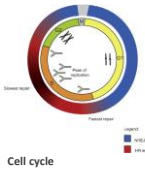
Repairing DNA double strand breaks



Cells have three key mechanisms to repair DSBs:

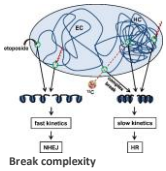
- Nonhomologous End Joining (NHEJ) is a fast, relatively accurate process which is available throughout the cell cycle;
- Homologous Recombination (HR) is much more accurate but is slower and depends on the availability of a sister chromatid, meaning it is only available late in the cell cycle;
- Alternative- or Backup-End Joining is a fall-back process which is typically extremely slow and error-prone.

Choosing a repair process



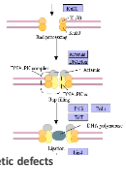
Cell cycle

- HR requires replicated chromatid, and so is only available in S or G2; NHEJ is always available



Break complexity

- NHEJ works rapidly on simple breaks, while more complex damage is often repaired by HR



Genetic defects

- All pathways depend on a series of key genes. Disrupting these genes can lead to repair failure

Karanam, Molecular Cell, 47(2), 2012
Jeggo, Radiotherapy and Oncology, 101(1), 2011

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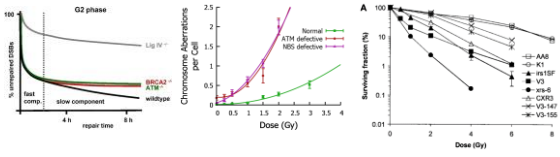
Impact of repair pathway choice



Availability of different DNA repair pathways is a major factor in a range of key radiobiological endpoints, including DNA repair times, chromosome aberration formation, and cell survival.

George, Radiation Research, 171, 2009
Jeggo, Radiotherapy and Oncology, 101(1), 2011

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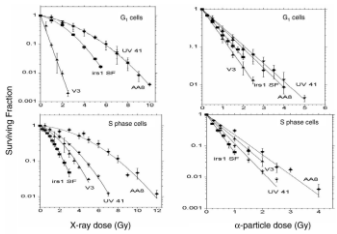
Impact of repair pathway choice



Impact of DNA repair depends on other environmental factors, as seen by comparing impact of high LET radiation on cells with different repair defects and cell cycle phases

(AA8 = Normal)

(V3 = NHEJ defect)

(irs1SF = HR defect)

Frankenberg-Schwager, Radiation Research, 171, 2009

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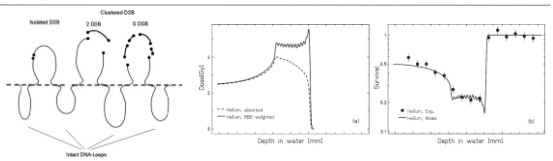
Mechanistic models of DSB interaction



DSB proximity effects are an extensively modelled aspect of DNA repair, due to close relation to RBE and physics. However, most models still rely on empirical fitting parameters to characterize individual cell lines.

- *Local Effect Model: Elsässer, IJROBP, 78(4), 2010*
- *MCDS, Semenenko, Phys. Med. Biol, 51(7), 2006*
- *PARTRAC, Friedland, Mutation Research, 711(1-2), 2011*
- *GLOBLE, Friedrich, Radiation Research, 178(5), 2012*

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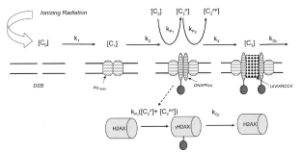
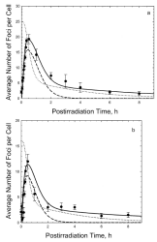
Modelling DNA repair processes





Kinetics of DNA repair can be explicitly modelled – while still challenging, this could allow us to put empirical response parameters on a mechanistic foundation

- *Cucinotta, Radiation Research, 169, 2008*
- *Taleei, Radiation Research, 179, 2013*
- *Friedland, Rad Prot Dosim, 143(2-4), 2011*

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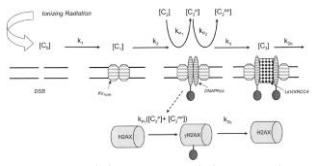
Individualising repair models

Parameter	Process
α, Gy^{-1}	DSB Induction
$k_{y'}$ h^{-1}	Ku70/80 binding
$\kappa_{y'}$ h^{-1}	Lig-IV binding
$k_{Py'}$ h^{-1}	DNA-PK$_{cs}$ Phosphorylation
κ_{Py}, h^{-1}	H2AX Phosphorylation
k_{Dsr}, h^{-1}	Enzyme Release
k_{Dy}, h^{-1}	H2AX Dephosporylation
k_{res}	Repair Failure



Mechanistic models let us directly link parameters to underlying processes, which in turn can be linked to measurable genetic parameters. Great untapped potential to link fundamental models of radiation-induced DNA damage and mechanistic, genetically-informed descriptions of biology.

Adapted from Cucinotta, Radiation Research, 169, 2008 & Torres-Roca, Cancer Research, 65:16, 2005

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Summary

DNA repair is a crucial mediator between physics and biological outcomes

- DNA repair drives many variations in radiation sensitivity
- Mutations in DNA repair genes can dramatically affect radiation sensitivity

We know a lot about these processes

- There has been extensive research on DNA repair kinetics, mechanisms and results
- Much of this research has been applied directly to radiation exposure scenarios

This knowledge can be used to improve our response models

- Survival alone is hard to relate to fundamental processes
- Integrating mechanistic information may offer a more natural link to the growing genomic understanding of cancer and cellular biology to deliver individualised predictions

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Cell Death

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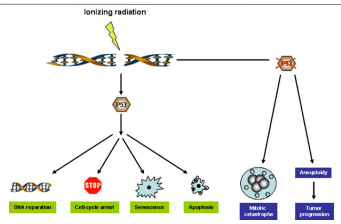
"How does radiation kill cells?"[1]

Surprisingly complex question – while genomic stress is root cause, how and why a cell dies depends on a range of factors.

Cells can die through apoptosis or mitotic catastrophe, as well as suffer long-term cell cycle arrest, senescence, or a number of other 'programmed' death pathways.



Eriksson, Tumor Biology, 31(4), 2010
Cohen-Jonathan, Current Opinion in Chemical Biology, 3(1), 1999

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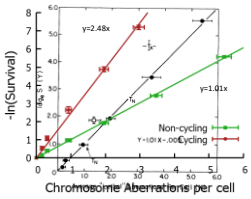
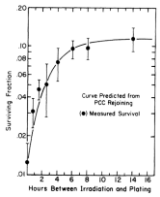
Active death responses

Some death is linked to 'lethal' aberrations, which remove cell's ability to proliferate. But there is a broad class of 'potentially lethal' damage which may also contribute to cell death through active cellular responses.





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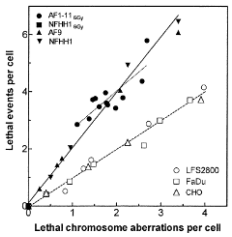
Regulation of death response

Some cell death processes are active responses, and so like DNA repair depend on the presence of key genes.

Knockout of p53, an important cell cycle gene, can eliminate much of the sensitivity to 'potentially lethal' damage by preventing detection of DNA DSBs and necessary cell cycle arrests.

Borgmann et al, International Journal of Radiation Oncology, Biology, Physics, 58(2), 2004



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Summary

DNA repair is not the whole story

- While some aberrations are lethal, they do not necessarily represent all cell death
- 'Programmed' or active death pathways can be dominant contribution

Genetic alterations remain a key factor

- Active pathways are dependent on a range of key genes
- Inhibition or deletion of these genes can significantly increase cell survival

These processes remain poorly modelled, despite their importance

- Cell cycle and death pathways are frequently mutated in cancer, making this an important driver of radiation sensitivity

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Beyond the cell

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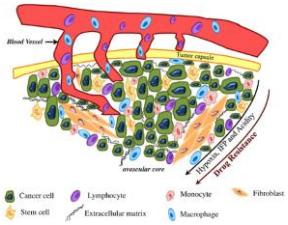
No cell is an island

When translating from preclinical work to clinical models, it is important to remember that *in vivo*, cells are part of a complex system.

Shift to three-dimensional structures, interactions with tumour and stromal cells, and availability of oxygen and nutrients can all impact on tumour responses.

Mechanistic models can help us understand some of these changes, but it's often as important to understand what is *not* modelled, as it is to understand what is.



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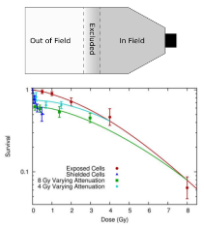
Intra-tumour systemic effects

Cell survival can thus depend not only on the dose seen by a cell, but also that seen by its neighbours.

Numerous proposed mechanisms, including, reoxygenation, vascular collapse and intercellular signalling impact responses both *in vitro* and *in vivo*.

This highlights the need to understand the response of the whole tumour *in vivo*, rather than simply a collection of independent cells.



Right: Illustration of modulated field impacts on DU145 cells *in vitro*, where survival varies strongly with dose delivered to in-field cells. *McMahon et al, PLoS ONE, 8(1), 2013*

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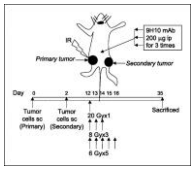
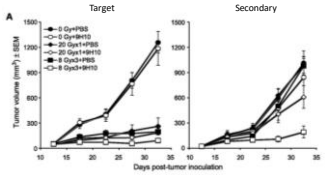
Distant systemic effects





"Abscopal" effects, where tumours not targeted by radiation show regression has long been anecdotally reported, but new studies suggest this was early evidence of effects driven by immune system.

Dewan, Clinical Cancer Research, 15(17), 2009

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Summary

Single cell response models are useful, but not everything

- Tumours respond as integrated structures, not groups of individuals

Tumour responses evolve over time

- Changes in tumour size, oxygenation, signalling availability and more throughout treatment change the impact of a given dose, and potentially how best to deliver it

Systemic effects can be significant

- Long-distance immune responses were an occasional curiosity, but as immunotherapy becomes an increasingly large part of radiotherapy they may become more important

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Conclusions

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Conclusions

Biology is hard!

- Compared to most problems in medical physics, radiobiology is difficult to quantify and assess;
- Dozens of known mutations and environmental factors can impact on cellular responses to radiation, along with a number we probably do not yet appreciate.

But we have plenty of data, and lots of tools

- Huge amounts of data are available on mechanisms of radiation response;
- Modelling techniques exist which let us understand many of these processes, and address some of the challenges in directly analysing clinical data.

There is a great opportunity to improve outcomes

- Treatment personalisation is a key goal of modern medical research, and better models of mechanisms radiation responses can play a key role here;
- Provide a natural complement to data-mining approaches, as they can inform one another and provide a more robust understanding.