Mechanisms conferring sensitivity to low dose radiation exposure and consideration of potentially sensitive individuals.

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Health Effects of Ionising radiation: Bridging the Experimental and Epidemiological Divide.
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Radiation induces a range of DNA damage of which a DNA double strand break is the most biologically significant. The DSBs may be complex involving additional damages.
DNA damage response (DDR) processes

2 Strategies for defence:

Optimising recovery and triaging

DSB

REPAIR

NHEJ/HR

cell cycle checkpoint arrest

Optimises time for repair and prevents proliferation of damaged cells

SIGNAL TRANSDUCTION

ATM

apoptosis

Eliminates damaged cells
DSBs are the main lethal lesion induced by ionising radiation—hence cells lacking DSB repair proteins are very radiation sensitive. Radiosensitivity of cells lacking Artemis or DNA ligase IV.
The DDR processes aim to achieve:

1) Resistance to the killing effects of radiation damage – enhance survival

2) Limit the genomic instability caused by radiation damage – prevent carcinogenesis.
AIMS

• Will overview the damage response processes of relevance to DSB formation.

• Consider the known syndromes where patients have MARKED defects in these processes – RARE and extreme cases but useful to consider.

• Consider whether small or modest changes – which might be more common - might confer sensitivity to low dose/dose rate
Damage response to DNA double strand breaks

2 Strategies:

- DSB
- REPAIR
  - NHEJ/HR
- SIGNAL TRANSDUCTION
  - ATM
Non-Homologous End-Joining

1. DSB protection
2. DSB remodelling
3. DSB processing
4. DSB ligation
5. DSB resealed
Mutations in DNA ligase IV, XLF, DNA-PKcs and Artemis have been identified in patients – but they are RARE.

NHEJ also functions during immune development to rejoin enzyme induced DSBs – all the above patients show immunodeficiency.

NHEJ also function during development to repair endogenously induced DSBs – therefore the patients show developmental and growth delay – particularly microcephaly.

One patient was identified from his over response to radiotherapy (clinical radiosensitivity).
Patients defective in MRN (Mre11 and Nbs1), ATM and RNF168 have been identified.
Ataxia telangiectasia (A-T) - ATM
AT like disorder – Mre11
Nijmegen Breakage syndrome – Nbs1
Riddle Syndrome – RNF168.

All are associated with immunodeficiency
Growth and developmental delay
Clinical radiation sensitivity (when given radiotherapy)
CANCER PREDISPOSITION
A-T cells are defective in the repair of a subfraction of DSBs.

 Artemis and ATM are essential for a subset of DSB repair

•Exposure to low doses does not cause major sensitivity to MOST of the cell lines from these patients although some may accumulate unrepaired DSBs – but these patients are rare.
Slow repair of DSBs may have two consequences:

1) Some cell types may be activate apoptosis resulting in increased cell turnover – maybe important for certain stem cells.

2) Slow DSB repair may cause increased misrepair – this may be particularly important in increasing cancer predisposition.
LigIV\textsuperscript{m/m} mice have reduced lymphocytes in peripheral blood consistent with unpaired V(D)J recombination and growth retardation - identical to LIG4 patients.

The heads seem also small (microcephaly seen in patients).

**THIS IS BECAUSE THE EMBRYONIC NEURONAL STEM CELLS ARE VERY SENSITIVE TO UNREPAIRED DSBS**

See loss of Haematopoietic stem cell (HSC) numbers and function with age in LigIVm/m mice.

ie one sees age dependent stem cell depletion
What about Milder mutational changes in DDR proteins.

1) Heterozygotes – can be present at 1 % of populations where there are founder mutations.

2) Common (or less common) polymorphisms may be impacting.

3) Dominant negative mutational changes
Heterozygosity in mice, humans and cell lines confers repair defects and cancer predisposition

• Heterozygosity for DNA ligase IV in a tumour prone mouse strain, ink4a/art-/-, results in an elevated frequency of soft-tissue sarcomas with clonal amplifications, deletions and translocations (Sharpless, Mol Cell, 8, 1187-1196, 2001).

• In several studies, heterozygosity for NHEJ proteins confers elevated chromosome breaks or other phenotypes.

• NBS and A-T are prevalent in the Slavic and Ashkenazi Jewish populations, respectively. Epidemiological studies on these populations has shown increased cancer predisposition Nbs1 and ATM heterozygotes.


• One study showed sensitivity to low dose radiation exposure in AT heterozygotes.
AT heterozygotes show a DSB repair defect following low dose rate exposure.

Control fibroblasts

AT heterozygotes

AT cells

Plateau phase Fibroblasts exposed to 100 mg/h for 24 h

Data from Kato TA, Nagasawa H, MW Michael, JB Little and Joel Bedford

Radiation Research 166 443-453, 2006
Impact of mild mutational changes.

LigIV Syndrome represents defects in DNA ligase IV.

180BR was a mild patient with no observable clinical features but developed leukaemia and died from radiation morbidity.

411BR was a more severe patient – who had immunodeficiency, mild microcephaly and developmental delay.
They both had the same homozygous mutational change in DNA ligase IV but 411BR had additionally two linked polymorphic changes.
1) All of the immunodeficient patients had no detectable adenylation activity

ie clinical severity correlates with nature of mutational change.

2) N-terminal polymorphisms add to second mutational change
Mild polymorphisms or mutations can affect the function of a protein 2-3 fold (ie similar decrease in activity compared to a heterozygote)– this does not have a major impact on protein function – may not give a clear clinical phenotype but aspects of the function may be impaired.

Dominant negative mutations – are changes that impair the function of the wild type protein. They may also cause mild loss of protein function.

Of relevance:

• Mild reduced function may lead to enhanced stem cell turnover during chronic exposure.

• Mild reduced function may enhance the infidelity of repair and enhance carcinogenesis
Summary

• The DDR (DNA damage response) play a major role in maintaining resistance and genome stability following radiation exposure.

• Loss of DDR proteins confers major radiosensitivity and cancer predisposition.

• Loss of DDR proteins have been observed in disorders termed DNA damage response disorders.

• However, these patients are rare and have defined clinical features.

• Heterozygosity for these proteins also confers a detectable phenotype in mouse and cell models including sensitivity to low dose radiation. It also confers cancer predisposition in humans.

• Mild mutational changes or dominant negative mutational changes in these proteins have been described also – and some impact on protein function.

• It is likely, but not proven, that patients harbouring such mutational changes may show low dose radiation sensitivity – particularly to cancer induction.
The fidelity of DSB repair is VERY important.

DSB repair is a slow process, which allows for cell cycle progression to occur and also degradation of the DNA ends.

Replication in the presence of a DSB is difficult.

Mitosis with DSBs will result in loss of genetic material.

$G_0$ State. The cell is in a resting state and does not progress through the cell cycle. Terminally differentiated cells may become permanently arrested at this step.