Comparative Analysis of Cellular and Molecular Responses of Cancer and Normal Cells towards High and Low LET Radiation

WP8. Radiobiology-KI(B5)

Chitralekha Mohanty

Objectives

The proposed research works aims to ***in vitro,*** in human tumour and normal cells with different origin and gene-status investigate and compare

- the sensitivity of **low linear energy transfer (LET)** radiation exposure on clonogenic cell survival and different types of cell death, e.g. apoptosis, necrosis, mitotic catastrophe, autophagy and senescence.
- differences in **cell cycle alterations** and **molecular responses** after exposures to low LET photon and **high LET** protons, carbon and nitrogen ions.
- differences in cellular and molecular responses after exposures to some of the **lighter ions** e.g. helium, lithium, beryllium and boron.
- the **intra cellular targets** for specific types of cell death and cell cycle regulation; micro-beam studies on carbon and nitrogen ions.

Materials and Methods

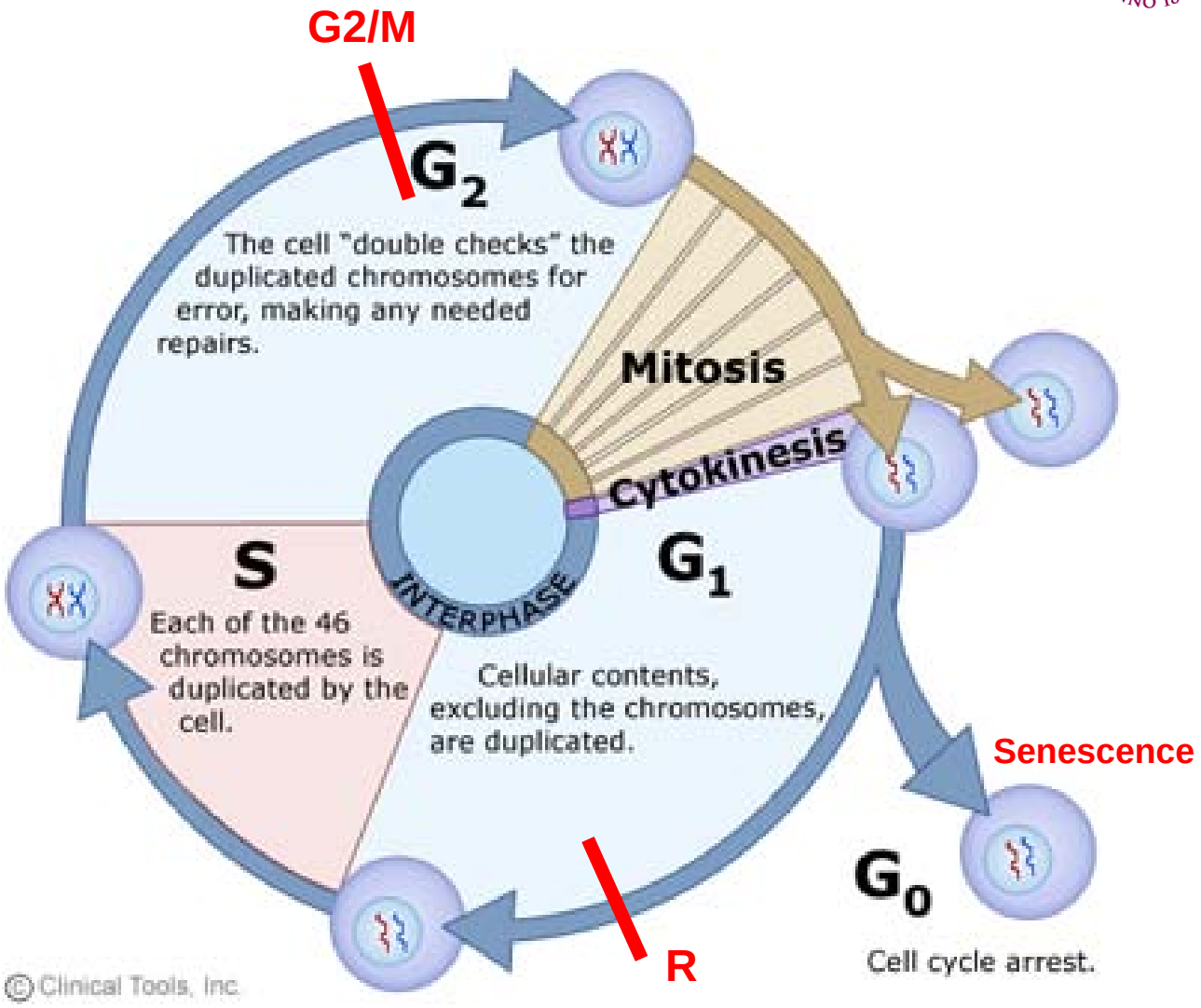
- **Cellular System** will consist of tumour cells and normal cells of human origin. Tumor cell lines with different gene status, derived from organs relevant for high LET radiotherapy will be used.

- **Irradiation experiments** will be performed at
  - The CCK Stockholm, Sweden (low LET γ-rays , ^{60}Co-source)
  - The TSL Uppsala, Sweden (protons, carbon and nitrogen ions)
  - The GSI Darmstadt, Germany (carbons)

Cellular responses

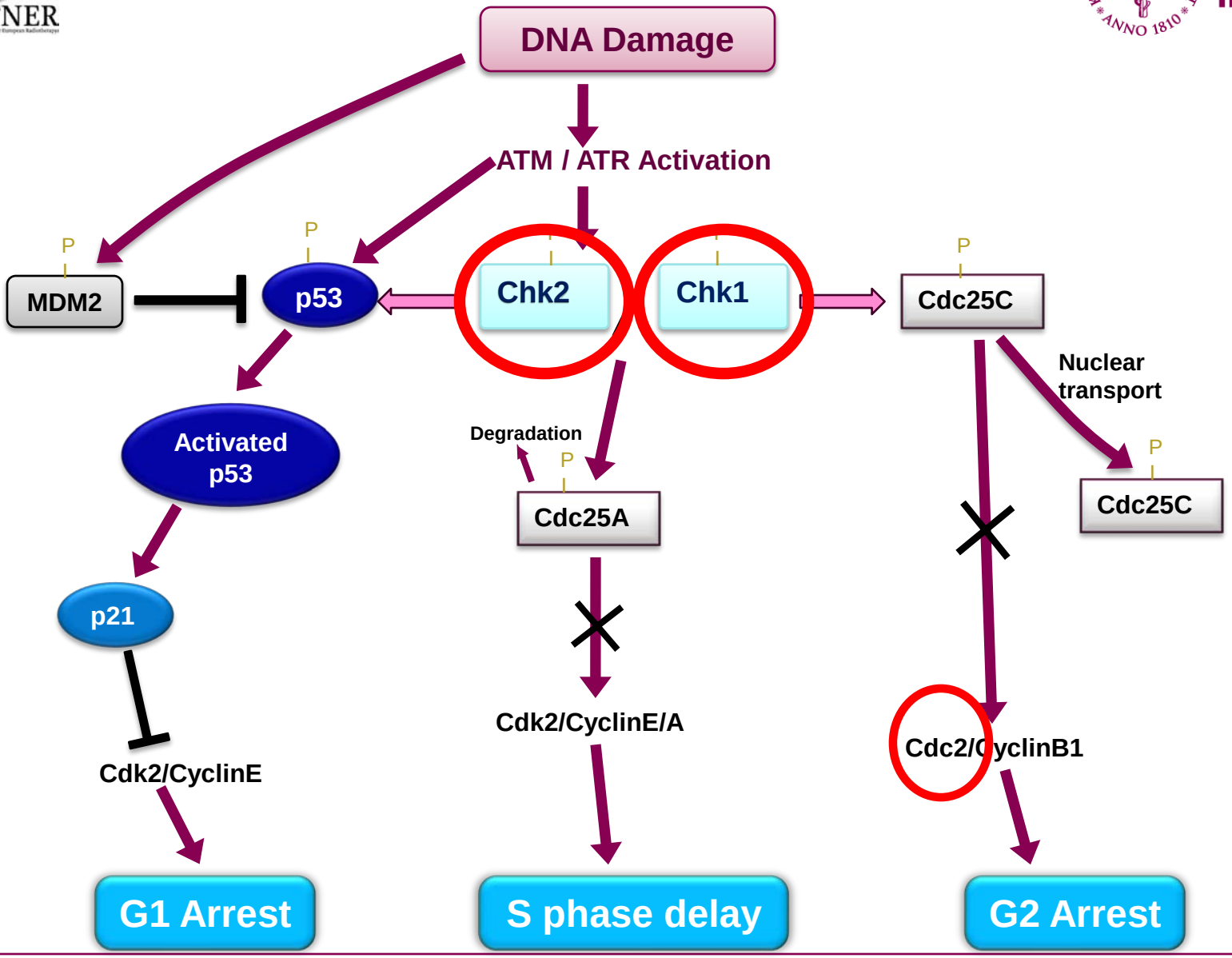
- Flourescence microscopy (morphology).
- Flowcytometry (different types of cell death).
- Gel electrophoresis (DNA ladders).
- Annexin-V staining (phosphatidyl serine switch).
- BrdU labelling (Cell progression and Senescence).

Cell cycle alterations will be studied using flow cytometry.


G2/M
G2
The cell "double checks" the duplicated chromosomes for error, making any needed repairs.
Mitosis
Cytokinesis
G1
INTERPHASE
S
Each of the 46 chromosomes is duplicated by the cell.
Cellular contents, excluding the chromosomes, are duplicated.
Senescence
G0
Cell cycle arrest.
R
© Clinical Tools, Inc.

Molecular responses

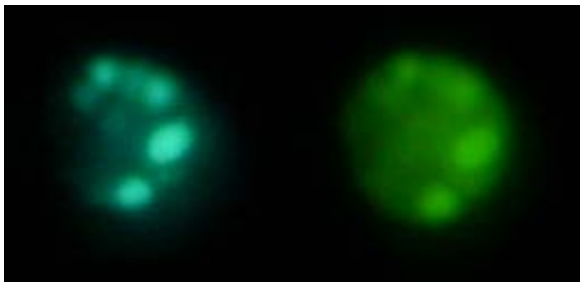
- The global gene expression will be studied using microarray technology – oligonuceotide arrays.
- Gene expression verified by RQ-PCR.
- Expression of specific proteins verified by ELISA and Western blot.


DNA Damage
ATM / ATR Activation
P
MDM2
P
p53
Chk2
Chk1
P
Cdc25C
Nuclear transport
P
Cdc25C
Activated p53
Degradation
P
Cdc25A
p21
Cdk2/CyclinE
Cdk2/CyclinE/A
Cdc2/CyclinB1
G1 Arrest
S phase delay
G2 Arrest

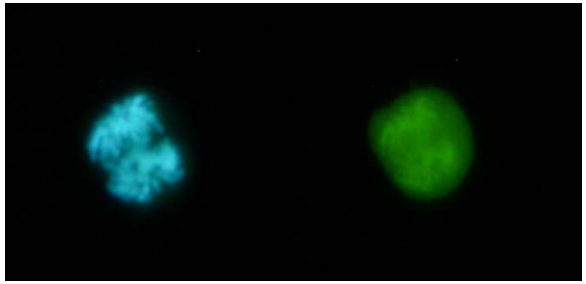
Time Plan

Goal	Year
Radiosensitivity of tumour cells towards Low LET	2009
Radiation induced cell cycle alterations after low LET and high LET ion exposures	2009- 2010
Molecular response of tumour cells towards high and low LET radiation	2009-2010
Response of tumour cells towards light ions	Later part of 2010
Molecular responses after micro-beam irradiation on different cellular targets	2011

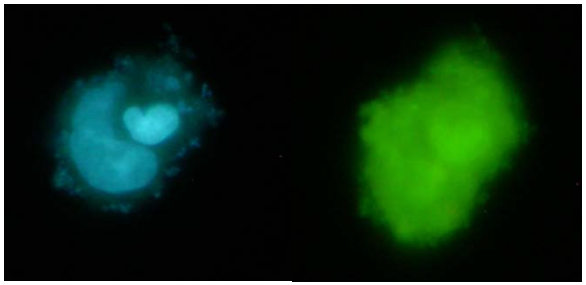
Normal tumour cells



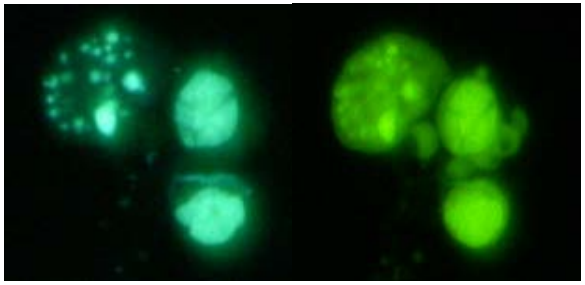
Apoptosis



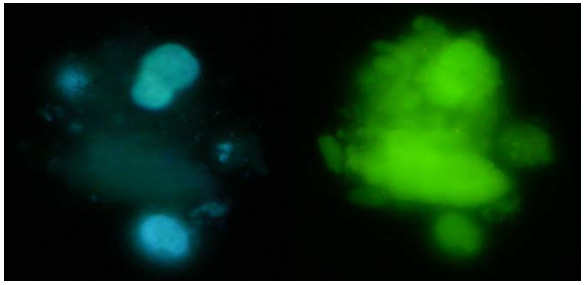
Mitosis



Engulfment/Phagocytosis



Apoptosis



Necrosis

Significance

- Indepth study of the biological response of normal and different tumor cells towards low and high LET radiation.
- Compare and analyse the response of tumour cells towards different light ions.
- Deeper understanding of the molecular pathways leading to cell cycle alterations and various types of cellular damage caused by high and low LET radiation.
- Identify intracellular targets involved in molecular pathways after High LET radiation.
- Increasing the efficiency and optimise radiation therapy for cancer patients


Thank you for attention