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Optimizing experimental radioimmunotherapy

-Investigating the different mechanisms behind radiation induced cell deaths

Theres Lindgren

Akademisk avhandling

som med vederbörligt tillstånd av Rektor vid Umeå universitet för avläggande av filosofie/medicine doktorsexamen framläggs till offentligt försvar i sal E04, byggnad 6E, NUS
fredag den 4 oktober, kl. 09:00.
Avhandlingen kommer att försvaras på engelska.

Fakultetsopponent: Professor Bo Stenerlöw,
Institutionen för radiologi, onkologi och strålningsvetenskap, Enheten för biomedicinsk strålningsvetenskap/Uppsala Universitet, Sverige.

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Author
Theres Lindgren

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Abstract

Radiation therapy (RT) and radioimmunotherapy (RIT) induce DNA damage in tumor cells in order to kill them. RIT is successfully used to treat lymphoma, but treatment of solid tumors with either RT or with RIT is still difficult. In this thesis we elucidated the molecular and cellular events responsible for inducing the main responses to low dose radiation (apoptosis, mitotic catastrophe (MC), or senescence) in HPV infected HeLa Hep2 tumor cells, isogenic solid tumor cell lines HCT116 p53 +/+ and HCT116 p53 -/-, and finally the leukaemia cell line MOLT-4. Also, to identify the kinetics behind gene expression patterns, identify key signaling pathways in the cell lines, in response to irradiation, and correlate these to the molecular and cellular events. Illumina Bead chip arrays exploring the expression of 24500 transcripts and Metacore (Genego) pathway analysis software were utilized.

The major findings were: Irradiated p53 deficient cell lines (HeLa Hep2, HCT116 p53 -/-) exhibited a transient G2/M arrests followed by premature entry into mitosis due to checkpoint adaptation. Premature entry was initiated due to an accumulation of genes promoting mitosis entry as observed by gene expression analysis in both cell lines. Anaphase bridges, centrosome amplification, as well as deregulation of genes involved in maintaining the spindle assembly checkpoint (SAC), centrosome amplification and clustering was also observed. A prolonged SAC arrest has been shown to be important for execution of MC. We observed multipolar mitoses (both cell lines), multiple- and micronuclei (HeLa Hep2, paper I), and an increased frequency of tetraploid cells (both). A fraction of HeLa Hep2 cells also displayed apoptotic features, including caspase activation and DNA fragmentation (paper I). HCT116 p53 +/+ cells induced both G1 and G2 arrest following irradiation (paper III). There was a decreased expression of genes responsible for cell cycle progression (pronounced decrease compared to HeLa Hep2 and HCT116 p53 -/-), especially mitotic progression. The prolonged arrest transitioned into senescence. Several genes associated with SASP were upregulated in the same time frame as senescence was induced. We saw that MOLT-4, similar to HCT116 p53 +/+, induced both G1 and G2 arrest in response to irradiation (paper IV). Morphological studies revealed apoptotic features like shrunken cells with condensed DNA. Caspase assay showed increased activity of caspases -3, -8, and -9. Changed expression in genes involved in cell cycle checkpoints and their regulation, apoptosis induction and T-cell activation/proliferation was observed

In conclusion, this thesis shows the complexity behind MC, senescence and apoptosis, and we identify several important mechanisms that could be utilized in order to increase treatment efficacy. As an example, inhibitors targeting regulators of SAC, centrosome clustering or senescence maintenance, in combination with radiation therapy could be successful.

Keywords
Radiation, Mitotic catastrophe, Senescence, p53, Delayed apoptosis, Cell cycle checkpoint

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