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SIGNALOMICS: UNSUPERVISED AND GLOBAL STRATEGIES TO EXPLORE MECHANISMS OF RADIORESISTANCE IN LUNG CANCER CELLS

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ABSTRACT

To combat lung cancer (LC) which is the number one cancer associated killer, a combination of surgery, chemotherapy, radiotherapy and targeted agents are currently used. The main subtype of LC, Non small cell lung cancer (NSCLC) unfortunately responds poorly to conventional radiotherapy (RT) for reasons yet only partly understood. Searching for the underlying mechanisms of RT-resistance has the potential to indentify signaling targets which can be used for radiosensitizing purposes. The overall aim of this thesis aims was to set up and use unsupervised global methods to gain novel insight of cellular signalling events responsible for the mechanisms controlling radioresistance in NSCLC cells and potentially find radiosensitizing targets. In **paper I** we studied differences in NSCLC cellular response between irradiation of conventional low Linear Energy Transfer (LET) photons and high LET accelerated particles, which in the latter case induced an apoptotic response. A shotgun MS-based proteomics approach was used to analyze global protein expression after low and high LET ionizing radiation (IR). By applying the novel pathway search engine (PSE) on protein expression data, a JNK-pathway was suggested as a key event in apoptotic response to high-LET IR in these cells. Both JNK as well as additional molecules of the JNK-pathway i.e. MKK4 and 14-3-3α, were proven to be regulated. This study demonstrates that, in contrast to low LET IR, high LET IR can trigger activation of the JNK pathway, which in turn is critical for induction of apoptosis in these cells. In **paper II** genomic profiling was used to identify putative low LET IR sensitizing targets used by the staurosporin analogue PKC 412 in RT sensitization of NSCLC cells. Out of a several altered genes, suppressed *Ephrin B3* expression was selected and further studied. We found that siRNA-mediated silencing of *Ephrin B3* resulted in increased senescence, apoptosis and mitotic catastrophe and combination with IR also decreased IR-mediated G_2-arrest. In **paper III** and **IV** a phosphoproteomic method consisting of strong cat ion exchange (SCX) and TiO2-based fractionation followed by nano-LC mass spectrometry analysis was set up and used to further study the role of Ephrin B3 (**Paper III)** and reveal signaling events which may further explain how high LET IR can overcome low LET IR resistance (**Paper IV**). In **paper III,** this method in combination to network analysis found and verified that silencing of Ephrin B3 in NSCLC cells results in loss of a specific phosphorylation on Ser 897 on the erythropoietin-producing hepatocellular receptortyrosine kinase class A2 (EphA2), a Eph kinase member shown to be upregulated in NSCLC patient tumor material. Moreover, inhibition of Ephrin B3 expression blocked phosphorylation on Ser 129 of Akt1, a kinase previously implicated in regulation of Ser 897 on EphA2. Our data also support a process in which a Heat shock protein 90 isoform (HSP90AA1) acts as a protector of EphA2, thereby saving it from degradation. In **paper IV** the phosphoproteomic profiling of low and high LET irradiated U-1810 cells and subsequent pathway analysis suggested that in addition to JNK, down regulation of Akt-mediated survival signaling as well as impaired t-RNA synthesis and eIF-mediated protein translation may contribute to high LET IR induced apoptosis. Hopefully, when further validated these findings may have the potential of becoming clinically useful for RT sensitizing strategies of NSCLC.

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