Identification of novel tumor biomarkers for sensitivity to radio- and chemotherapy of lung cancer based on genomic analyses

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ABSTRACT

Lung cancer (LC) is one of the most common human cancers and the leading cause of cancer-related deaths worldwide. Treatment of LC is by surgery if the tumor is resectable, otherwise chemo- and/or radiotherapy (CT/RT) is given. However, development of resistance to CT/RT is very common which makes the prognosis of LC very poor with median overall survival of only about one year. This calls for discovery of novel diagnostic and/or therapeutic modalities. Biomarkers predicting response of LC to conventional CT have been identified based on some signaling alterations for a subset of NSCLC patients. The molecular aberrations for the rest of LC remain largely unclear. The main aims of this thesis were to investigate the potential of miRNAs as novel prognostic or therapeutic biomarkers of LC and to understand if some miRNA could drive RT resistance.

In Paper I we found that miRNAs are widely expressed in LC cell lines and we found a link between miRNA expression and RT sensitivity. We reported that miRNA-214 and miRNA-324-5p are exclusively higher expressed in the radioresistant NSCLC and SCLC cell lines, respectively, as compared to the radiosensitive counterparts. Interestingly, we found that ablation of miRNA-214 in the NSCLC cells reversed RT resistance and induced senescence concomitant with up regulation of p27Kip1. In line with this, we observed that overexpression of miRNA-214 in RT sensitive NSCLC cells blocked caspase-3-mediated apoptosis concomitant with activation of p38MAPK and phosphorylation of FoxO4. In conclusion we show that miRNA-214 confers resistance of NSCLC to RT.

In Paper II we showed that miRNA-214 is modulating invasiveness of NSCLC cells. We demonstrated that ablation of miRNA-214 enhanced while overexpression of the miRNA reduced invasiveness of NSCLC cells. Through gene expression and bioinformatics analyses, we found that 18 genes out of the predicted miRNA-214 targets were regulated in the NSCLC cells. Among these, we focused on four genes, PAPP-A, ALPK2, CDK6 and TNFAIP3, which were previously reported to be regulating metastasis in a mouse lung cancer model. Through argonaute 2 (Ago2) immunoprecipitation, we found that only ALPK2 is directly regulated by miRNA-214 whereas PAPP-A, CDK6 and TNFAIP3 are indirect targets of this miRNA. Moreover, we performed immunohistochemical analysis of these targets in tissue microarrays of about 600 NSCLC tumors to reveal their expression pattern and to examine if a correlation could be made to metastatic potential of the tumors or overall survival (OS) of the patients. We showed that NSCLC tumors express these proteins at a moderate to high level although with no correlation to OS or clinical records of metastasis. In summary, we demonstrate that miRNA-214 regulates invasiveness of NSCLC yet further studies are required to delineate the molecular components involved.

In Paper III the aim was to find biomarkers of cisplatin resistance in NSCLC cells. We showed that long-term NSCLC clones surviving cisplatin treatment had a heterogeneous gene expression pattern. We found DKK1, XRCC2 and LGALS9 to be linked to cisplatin resistance of NSCLC cells. Accordingly, we showed that knockdown of DKK1 sensitized NSCLC cells to cisplatin. Through Ingenuity pathway analysis we identified TCF4, EZH2, DNAJB6 and HDAC2 as altered upstream regulators of DKK1 and GSK3B as a possible downstream signaling molecule. Further work is required to demonstrate their signaling role in cisplatin resistance of NSCLC cells.

In summary, we show in this thesis that genomics techniques in combination with bioinformatics can be used to identify biomarkers of LC which could be used for prediction of prognosis or treatment response but also to reveal novel RT or CT sensitizing targets.