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microRNAs: Significance for Sensitivity/Resistance of Lung Cancer Cells to Treatment

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ABSTRACT

Intrinsic or acquired resistance of lung cancer (LC) to chemo- or radiotherapy (CT/RT) limits the treatment effectiveness, which, in turn contributes to tumor progression and ultimately increases the mortality rate of the LC patients. Although resistance to tumor treatment is a multifactorial event for which several factors were identified, still many critical features underlying the mechanisms of resistance remain elusive. One of the main mechanisms, evasion of apoptosis, was recently shown to be modulated by miRNAs, an emerging class of important regulators of various biological processes, including apoptotic cell death that essentially contributes in CT/RT response. Thus miRNAs, as oncomiRs, might regulate anti-apoptotic protein's expression or suppress a pro-apoptotic cellular response. In the current thesis we therefore, analyzed the role of miRNAs and the core proteins involved in their biogenesis in LC cells response to CT/RT.

The paramount question we addressed was to infer whether the expression of core proteins involved in miRNA biogenesis can be associated with LC resistance to RT and if so, can we sensitize resistant LC cells to RT upon silencing of these proteins. Detailed analysis of a panel of SCLC and NSCLC cell lines revealed that the major proteins of miRNA biogenesis machinery, Drosha and Dicer, were expressed at higher levels in RT resistant LC cells as compared to RT sensitive counterparts. However, knock-down of these proteins by siRNA appeared to be insufficient to sensitize for RT. Moreover, knock-down of downstream components of miRNA biogenesis pathway, Ago-2 and TSN, did not either enhance the sensitivity of NSCLC cells to ionizing radiation. These data suggest that RT resistance in LC cells cannot be reverted by modulation of a single component of the miRNA biogenesis machinery. Next, to find out whether miRNA expression can affect RT sensitivity of LC cells, a global miRNA profiling was performed using the same panel of SCLC and NSCLC cell lines with different RT sensitivity. We observed that miRNA-214 had a higher expression in radioresistant NSCLC cells as compared to their sensitive counterparts. Considering miRNA-214 as an important modifier of LC cells radioresistance capacity, expression of this miRNA was silenced in radioresistant and overexpressed in sensitive NSCLC cells, respectively. Indeed, knock-down of miRNA-214 in radioresistant NSCLC cells increased their RT sensitivity and these cells underwent senescence after irradiation. Importantly, overexpression of miRNA-214 in radiosensitive NSCLCs protected them from RT-induced apoptosis, an effect that in part was mediated by p38MAPK as downregulation of this kinase reversed the protective response of miRNA-214 overexpression.

Finally, to determine the key modifiers of LC CT resistance, we observed that downregulation of an evolutionally conserved multifunctional protein TSN increased the NSCLC cell death response either alone or in combination with CT drugs. A higher expression of TSN was detected in NSCLC cell lines than in normal lung fibroblast cells. Gene expression profiling upon silencing of TSN revealed that TSN likely contributes to NSCLC CT resistance by regulating expression of several tumor survival genes, such as S100A11, ATP6V1F, and MDC1, and simultaneously suppressing many of pro-apoptotic genes e.g., BNIP3, DRAM1, PDCD4, BCL2L13, and LAMP2 that eventually compromise tumor ability to undergo apoptosis. Altogether this suggests a potential contribution of high TSN expression towards LC malignancy and a CT resistant phenotype.

In conclusion, in this study, we demonstrate the role of some miRNAs and the regulators of their biogenesis in LC therapy response. It is anticipated that further understanding of their functional impacts on mechanism(s) of resistance of LC cells to the current treatment modalities will generate novel therapy approaches as well as biomarkers of treatment response of this tumor malignancy. **ISBN 978-91-7549-027-4**.