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Epigenomic profilingof lung cancer: Molecular characterization of lung tumor types and lung cancer risk

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Recent advances in biomedical research have accelerated the discovery of molecular alterations in cancer. Lung cancer is highly heterogeneous in terms of histology, molecular alterations, and treatment response. Although genome-wide profiling has identified multiple genetic driver events that are regularly screened for in clinic, a major proportion of patients do not carry these mutations. Hence early intervention is neglected for such onco-negative patients, who do not carry these clinically screened mutations. Epigenetic aberrations are universal to cancers and have been identified as putative markers for tumor typing, cancer management, and risk assessment. Since relatively little is known about inter- and intra-patient epigenetic heterogeneity in lung cancer, this thesis aims to improve current tumor typing and risk prediction through: 1) Characterizing inter- and intra-tumor methylation heterogeneity in histological subtypes of lung adenocarcinoma (ADC), 2) Identifying miRNA-based prognostic molecular tumor types among onco-negative ADC patients and 3) Investigating smoking-induced methylation changes as risk markers for lung cancer.

To investigate methylation profiles that characterize the histological subtypes of ADC, methylome data from microdissected FFPE tissues from multiple cohorts (Discovery (n=35), Replication (n=29) and Intratumoral (n=14)) was generated on the Illumina 450k-Human bead chip array platform. Methylation profiling identified differentially methylated regions (DMRs) across histological subtypes of ADC, whose methylation patterns follow a continuous trend from non-invasive subtypes towards an invasive one. This trend indicates a gradual change in methylation that accumulates as the tumor progresses. These observations were replicated and further validated in an intratumoral cohort, where profound intratumoral methylation heterogeneity was identified. DMRs include key genes involved in tumor progression: *HOXB9, EMP4, HDAC4, TNSF18* and *miR-30e* that show subtype-specific patterns of methylation changes. This analysis identified a trend in methylation patterns across histological subtypes that may reflect the underlying molecular changes, which confer invasiveness to acinar and solid subtypes of ADC.

Although histology based classification prevails in ADC, current personalized treatment approaches are available to a section of patients who carry certain driver mutations. However, a large proportion of onco-negative ADC patients are not eligible for such personalized treatment options. Hence to identify prognostic molecular subtypes among the onco-negative patients has the potential to improve patient stratification and clinical management. miRNA profiling of this subset of onco-negative patients (Discovery cohort (n=29)) on the Agilent miRNA microarray platform identified prognostic molecular subtypes. This was replicated in an independent cohort (Replication cohort (n=13)). A myriad of miRNA deregulation was identified within the worst prognostic group. Functional characterization of the novel differentially expressed miRNA miR-1260a identified its tumor suppressor role in ADC. The pathway reporter assay on HEK293Tcells identified that inhibition of miR-1260a activates the FOS pathway that may contributes to the pathogenesis of ADC. Inhibition of miR-12560a in A549 cell lines upregulates members of the *NBPF* family and *KIAA1199* that are the *in-silico* predicted targets of miR-1260a. Ingenuity pathway analysis identified that these genes are among the network of genes involved in the FOS pathway. Such molecular profiling of patients has the potential to improve clinical management of onco-negative patients who are otherwise denied the benefits of the personalized treatment plan.

To further enhance clinical management, identification of high-risk individuals is required to allow screening with the aim of early detection of lung cancer. Towards this aim, blood based screening for risk markers, including certain methylation aberrations is warranted. Smoking-induced differentially methylated regions (sDMRs) from 43 pairs of monozygotic twins discordant for smoking using Medip-Seq (TwinsUK) were available. In order to identify these sDMRs as risk markers, a nested control study was designed within Heidelberg case-control cohort. These sDMRs were screened in blood samples (109 matched pairs, (Current smokers)) for differential methylation. MassARRAY quantification has identified 3 significant sDMRs (paired t-test, $p<0.05$) associated with *PBX1, ETS1* and *CNTNAP2* as blood based risk markers for lung cancer among current smokers. This might be useful in identifying high-risk individuals who can be prioritized for further screening.

In summary, this thesis highlights the prevalence of epigenetic heterogeneity in lung cancer and studies its implications in defining tumor types, tumor progression and clinical management of high-risk individuals.