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Functional characterization of lncRNA implicated in lung cancer aggressiveness

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My project consists in functionally characterizing a set of long non-coding RNA (lncRNA) implicated in lung adenocarcinoma aggressiveness. Lung cancer is the leading cause of cancer death worldwide. Lung cancer aggressiveness is correlated with a low level of oxygen (hypoxia) in the tumor. Hypoxia triggers the activation of several signaling pathways inducing a complex transcriptomic response with multiple consequences on cell survival, migration, invasion and metabolic reprogramming. Hypoxia can induce the expression of epigenetic factors, mRNA and non-coding RNAs. Currently, a large set of miRNAs has been reported as crucial molecular regulators of the hypoxic response but the precise functional characterization of lncRNAs is still in its infancy. LncRNAs are transcripts longer than 200bp which can act by the protein partners recruitment at different levels in the cell.

My first aim was to characterize the whole set of hypoxia-regulated lncRNAs in lung adenocarcinoma. Using a combination of experimental profiling approaches in patient samples and exploring in silico TCGA datasets, we identified a validated signature of 28 lncRNAs correlated to the hypoxic status of tumors and the overall survival of patients.

My second aim consisted in molecular characterization of few candidates using RNA-Seq, northern blot and single molecule RNA FISH (Fluorescent In Situ Hybridization). We pointed to 2 candidates with different subcellular localization patterns: i) NLUCAT1, a large nuclear transcript composed of 6 exons and ii) LINC01116, a short cytosolic transcript composed of 3 exons.

My third aim was to functionally characterize this lncRNA candidates by loss of function with CRISPR/Cas9-mediated invalidation or RNA interference (ASOs and siRNA) combined with transcriptomic analyses and *in vitro* experiments (adhesion, migration, invasion, proliferation, spheroid formation…). Our publication concerning NLUCAT1 showed it's a regulator of the NRF2-mediated antioxidant response with an impact on cisplatin resistance. LINC01116 was mostly associated to the regulation of cell-to-cell contact and cell invasion.

Finally, my fourth aim consisted in determining the LINC01116 mode of action by the identification of its protein partners using RNA pulldown approach and the validation by RNA Immunoprecipitation (RIP). We've determined that LINC01116 interacts with ILF3 (Interleukin Like factor 3) implicated in stress granules formation. We've developed in our lab the transcriptional invalidation of the whole set of Hypoxia-regulated LncRNA by CRISPRi to determine concomitant function of 26 other lncRNA.

Key words: RNA, CRISPR/Cas9, RNA pulldown and smRNA FISH.