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Cell Cycle Regulation in Cancer: A noncoding perspective

Akademisk avhandling

Som för avläggande av medicine doktorsexamen vid Sahlgrenska akademin, Göteborgs universitet kommer att offentligen försvaras i **Arvid Carlsson**, **Academicum**, **Medicinaregatan 3**, **Göteborg**

Tisdagen den **17 December 2019**, klockan **13:00**

av **Mohamad Ali**

Fakultetsopponent: **Dr. Paul Hofman, Professor**

Institute for Research on Cancer and Aging, CNRS, Nice, France

Avhandlingen baseras på följande delarbeten

I. Meryet-Figuiere M, Alaei-Mahabadi B, **Ali MM**, Mitra M, Subhash S, Pandey GK, Larsson E, and Kanduri C. "Temporal separation of replication and transcription during S-phase progression" 2014, Cell Cycle, 13: 3241-8.

II. **Ali MM***, Akhade VS*, Kosalai ST*, Subhash S*, Statello L, Meryet-Figuiere M, Abrahamsson J, Mondal T and Kanduri C. "PAN-cancer analysis of S-phase enriched lncRNAs identifies oncogenic drivers and biomarkers" 2018, Nature Communications, 9: 883. * Authors contributed equally

III. Statello L, **Ali MM**, Reischl S, Kosalai ST, Akhade VS, and Kanduri C. "*SCAT7* lncRNA regulates TOP1 turnover and DNA homology-directed repair in lung cancer" (Manuscript)

IV. **Ali MM**, Mahale S, Marco M, Kosalai ST, Mishra K, Statello L, Umpathy G, Hallberg B, and Kanduri C. "*LY6K-AS* lncRNA regulates mitotic progression and chemoresistance in lung adenocarcinoma cells" (Manuscript)

SAHLGRENSKA AKADEMIN
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Abstract

The cell cycle progression is tightly regulated to ensure error-free cell replication. The complexity of the transcriptional machinery aids to function in a spatiotemporal pattern across different phases and genomic loci. However, the cell cycle regulation has always been associated with a "protein-centric" view that implicates an intricate network of closely related proteins and transcription factors. This view neglects the fact that only 2−2.3% of the human genome codes for proteins. On the other hand, more than 70% of the human genome undergoes pervasive transcription of, most likely, regulatory non-coding RNA (ncRNA) counterparts. Thus, the interrogation of the intimate functional relationship of ncRNAs to cell cycle progression and tumor homeostasis in different cancer types is indispensable. To this end, in the first study of the current thesis, we optimized a nascent RNA capture assay coupled with high throughput sequencing that enables high-resolution mapping of ongoing RNA transcriptional events. The study revealed the temporal separation between DNA replication and RNA transcription, where replication timing has an inverse correlation with transcription.

Given that the DNA replication is the most critical process during cellular division, the regulatory elements governing the S phase progression would be of great importance for cell survival. Thus, in the second study, we utilized our optimized nascent RNA capture assay to identify the long noncoding RNAs (lncRNAs), which are enriched in different compartments of the S phase in HeLa cells. Then, we analyzed the expression patterns of the identified lncRNAs across the cancer genome atlas (TCGA) datasets and determined their clinical relevance in different types of cancer. We uncoupled the function of an uncharacterized lncRNA, termed as *SCAT7*, which harbored oncogenic properties that promote cell cycle progression. Transcriptome-wide analysis of cells depleted of *SCAT7* demonstrated its role in activating FGF/FGFR signaling and the downstream PI3K-AKT pathway in different cancer models, including lung adenocarcinoma (LUAD) and renal cell carcinoma. The *SCAT7*-mediated activation of PI3K/AKT signaling depends on the lncRNA interaction with a protein complex comprising hnRNPK and YBX1 proteins. Therefore, the therapeutic targeting of *SCAT7* in mouse xenografts and PDX models reduced tumors progression significantly.

In the third study, we uncoupled the DNA replication-related functions of *SCAT7*. Using a combination of immuno-precipitation, immuno-fluorescence, and DNA combing assays, we report that *SCAT7* physically interacts and regulates the topoisomerase I (TOP1) turnover via protein ubiquitination. The depletion of *SCAT7* induces accumulation of TOP1 that creates replication stress and double-stranded breaks. However, *SCAT7* abrogation also interferes with DNA homology-directed repair and inhibits the phosphorylation of ATM protein. Subsequently, the TOP1-induced DNA damage persists, causing further replication stress and cellular death. We also uncover the potential implication of *SCAT7* silencing in circumventing cisplatin resistance in LUAD cells.

In the last study, we identified *LY6K-AS* lncRNA, which has elevated expression levels in LUAD tissues compared to healthy counterparts. *LY6K-AS* acts as an independent prognostic biomarker of survival for LUAD patients. The silencing of *LY6K-AS* induces chromosomal abnormalities and interferes with the mitotic progression of LUAD cells. Mechanistically, it interacts with 14-3-3 proteins to modulate the transcriptional programs of several factors involved in spindle assembly checkpoint. The silencing of *LY6K-AS* in cisplatin-resistant and crizotinib-resistant cells reduces their proliferation significantly. *In vivo* experiments indicated that *LY6K-AS* is a potential therapeutic target against naive and chemoresistant tumors.

Collectively, the presented studies in the current thesis establish novel functions for lncRNAs in regulating cell cycle progression in different cancer models.

Keywords: Long Noncoding RNA, lncRNA, Cell Cycle, S phase, Mitosis, Checkpoints, Cancer, SCAT7, LY6K-AS

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