Göteborg, 2021

Pan-cancer study of transcriptional responses to oncogenic somatic mutations

Akademisk avhandling

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

Fredagen den **18 juni 2021**, klockan **09:00**

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Avhandlingen baseras på följande delarbeten

I. Pan-cancer transcriptomic analysis associates long non-coding RNAs with key mutational driver events.
Ashouri A, Sayin VI, Van den Eynden J, Singh SX, Papagiannakopoulos T, Larsson E.
Nat Commun. 2016 Oct 25;7:13197.

II. Genome-wide characterization of the NRF2 targetome.
Ashouri A, Larsson E.
Manuscript

III. Phosphoproteome and gene expression profiling of ALK inhibition in neuroblastoma cell lines reveals conserved oncogenic pathways.
Van den Eynden J, Umapathy G, **Ashouri A**, Cervantes-Madrid D, Szydzik J, Ruuth K, Koster J, Larsson E, Guan J, Palmer RH, Hallberg B.
Sci Signal. 2018 Nov 20;11(557)

SAHLGRENSKA AKADEMIN
INSTITUTIONEN FÖR BIOMEDICIN

GÖTEBORGS UNIVERSITET

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Abstract

Cancer cells typically carry acquired somatic mutations in key cancer driver genes, which can be identified on the basis of recurrence in cancer cohorts. Such mutations may cause aberrant protein activity and altered gene expression in the nucleus, driving the cell toward a cancerous phenotype. Understanding the transcriptional consequences of cancer driver mutations can guide us towards better diagnosis and prognosis.

While the phenotypic effects of driver mutations are typically mediated by protein-coding genes, recent studies also describe roles for long non-coding RNAs as effector molecules in oncogenic pathways. LncRNAs are long transcripts that are transcribed and processed similarly to coding messenger RNAs but lack coding capacity. However, a systematic exploration of lncRNAs as potential mediators of oncogenic mutational events in cancer has been missing. In the first study, we established a methodology to uncover associations between key driver mutations and transcriptional alterations in lncRNAs, using publicly available genomic data from thousands of tumours and 19 different cancer types from The Cancer Genome Atlas (TCGA). Based on this, we found many putative lncRNA effectors, some of which could be validated in terms of transcriptional responsiveness using previously published data. We also designed experiments to investigate the function of NRF2 (also known as nuclear factor erythroid 2–related factor 2 (NFE2L2)) responsive lncRNAs in cancer cell lines. NRF2 is a master regulator of the cellular oxidative stress response, and it is frequently mutated in several cancer types. Using small interfering RNAs (siRNAs) targeting *NRF2*, followed by deep RNA sequencing we validated the expression changes of these lncRNAs downstream of NRF2. Furthermore, using ChIP-PCR (chromatin immunoprecipitation followed by PCR) we could confirm direct binding of NRF2 to the promoter of selected lncRNAs identified as NRF2-responsive in our screen. In summary, this study provides a comprehensive overview of lncRNA transcriptional alterations in relation to key driver mutational events in human cancers.

In the second study, we have focused further on the transcription factor NRF2, which is a key driver gene in lung cancer and several other cancer types. Despite this, the target repertoire of NRF2 remains incompletely characterized. To solve this, we performed NRF2 ChIP-seq (ChIP followed by high-throughput sequencing) using two different antibodies against NRF2 in two lung cancer cell lines, as well as *NRF2* knock-down with siRNA transfections plus control siRNAs followed by deep RNA sequencing, to discover genes with expression alteration upon blocking *NRF2*. Integrative analysis of the ChIP and RNA sequencing data has resulted in the most detailed characterization of the NRF2 targetome to date. This analysis confirmed most known targets and also revealed several new targets of NRF2. The resulting dataset constitutes an important resource that can facilitate our understanding of NRF2 and its complex role in cancer.

The third study describes transcriptional and phosphoproteomic responses following treatment with marketed ALK tyrosine kinase inhibitors (TKIs). *ALK* is a major driver gene in neuroblastoma and other cancers with frequent somatic mutations both in primary and relapsed tumours. Mapping of signaling and transcriptional events downstream of ALK revealed relevant biomarkers and signaling networks, such as ETS family transcription factors and the MAPK phosphatase *DUSP4*, some of which are potential therapeutic targets.

In conclusion, this thesis links key driver events in human cancer to specific transcriptional effects, providing insights into oncogenic signaling and clues to new treatments.

Keywords: Cancer, driver mutations, lncRNAs, NRF2, transcriptome, ALK.

ISBN: 978-91-8009-318-7 (TRYCK) http://hdl.handle.net/2077/68071
ISBN: 978-91-8009-319-4 (PDF)