Discovering vulnerabilities associated to pathogenic chromosome amplifications in human cancers: the role of the long non-coding RNA *MINCR*

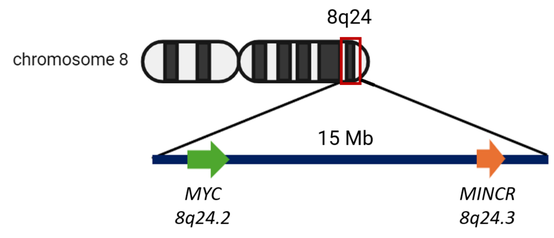
Ilaria Cassotti, Cecilia Pandini, Alessandro Macchia, Giulia Pagani, Paolo Gandellini

Dipartimento di Bioscienze

Background

8q24 chromosomal locus constitutes a fragile site for genetic aberrations and hosts multiple risk loci for cancer and other diseases.

Specifically, **amplifications** of the 8q24 locus are frequently found in **prostate cancer (PRAD) patients**, especially in cases of most severe disease, where it seems to be associated with bad prognosis. One of the few protein-coding genes that can be **found in the locus is c-*MYC*,** while the remainder of the region is abundant in sequences coding for lncRNAs (Long Non-Coding RNAs).

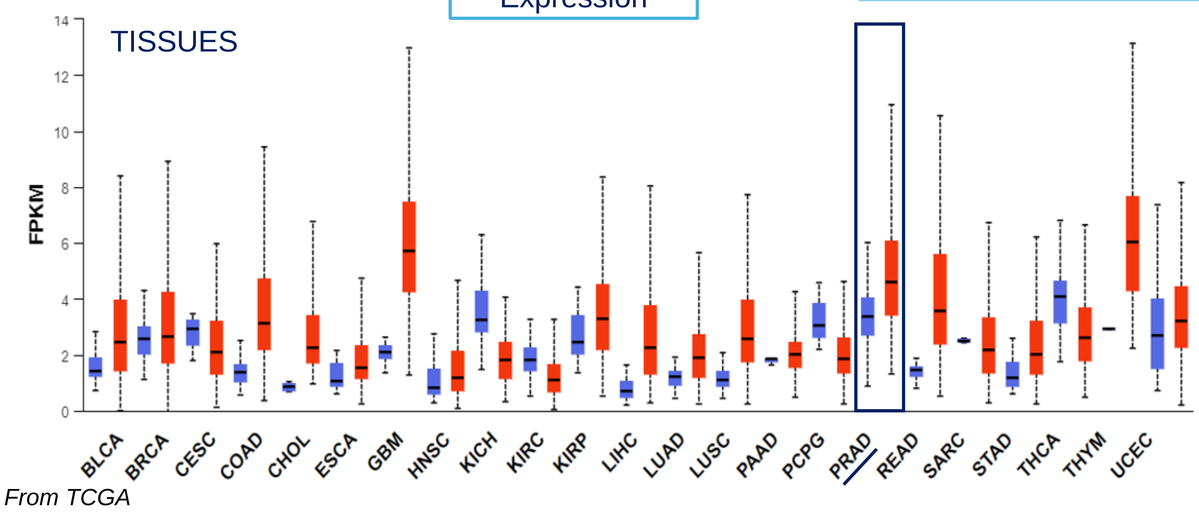


One of such is ***MINCR* (MYC-induced Long Non-Coding RNA)**, which has been described to exert an oncogenic role in different types of cancer. However, a possible involvement in PRAD, especially considering the relevance of 8q24 amplifications in this disease, has not been yet explored.

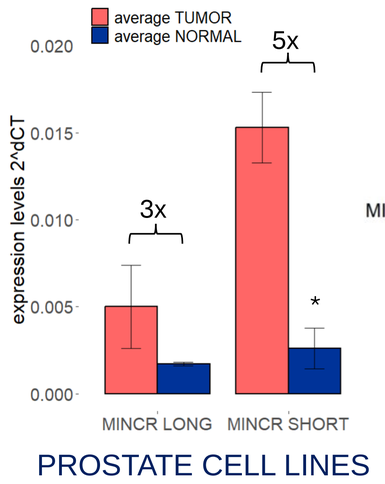
AIM: Functionally **characterize *MINCR* in prostate cancer**, focusing on investigating the **roles of its different isoforms**.

1. *MINCR* is upmodulated in prostate cancer samples

Expression

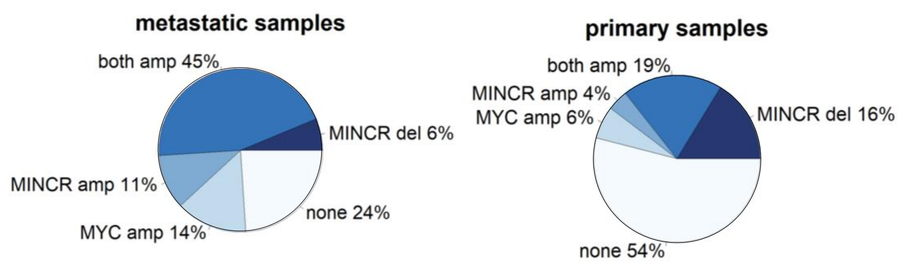


From TCGA



PROSTATE CELL LINES

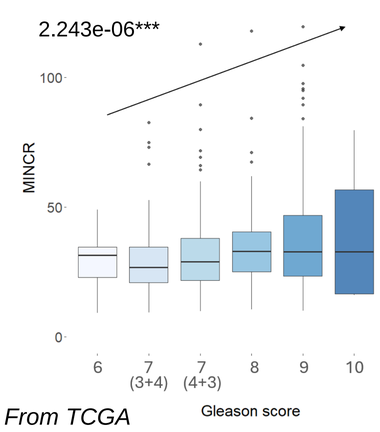
Copy Number Variations



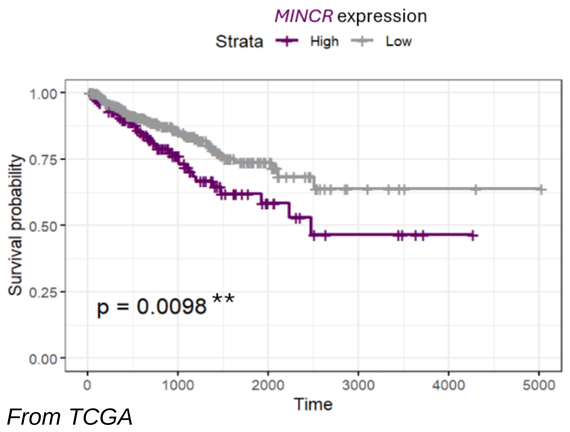
***MINCR* amplification** is especially relevant in **metastatic prostate cancer**.

Data from *Prostate Adenocarcinoma (MSK/DFCI, Nature Genetics 2018), available on cBioPortal*

2. *MINCR* correlates with clinical features



From TCGA



From TCGA

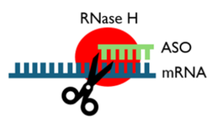
3. Investigating *MINCR* role in the cell: approach

MINCR TRANSCRIPT → MODULATION OF *MINCR* EXPRESSION → ASSESS *MINCR* ROLE IN THE CELL

Which **isoforms** are really expressed in prostate cancer cells and **where they locate.**

SILENCING

Gapmers

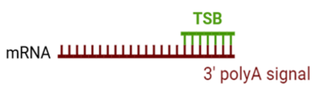


OVEREXPRESSION

Isoform-specific-carrying vectors

ISOFORM-SPECIFIC modulation

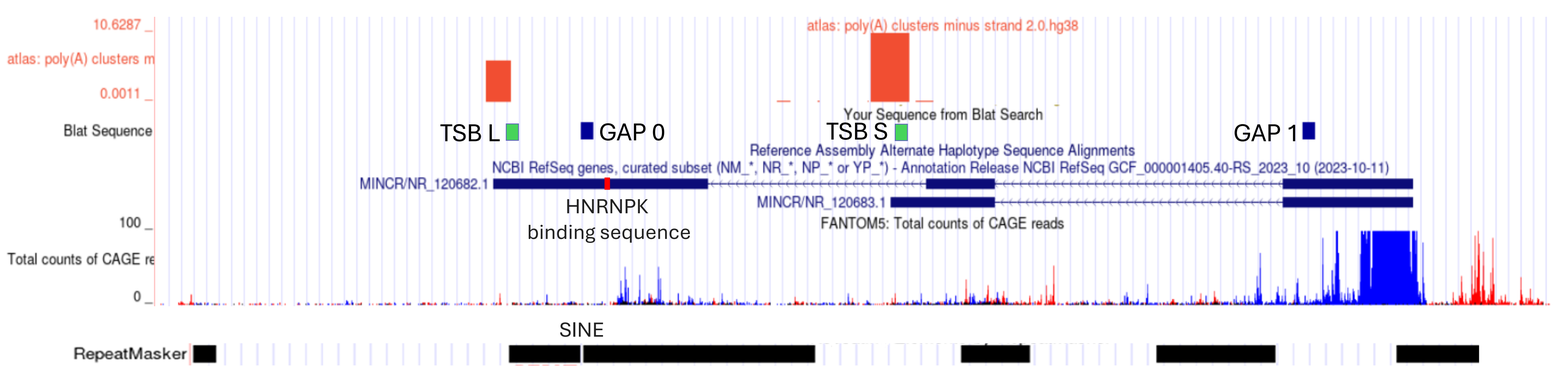
Target Site Blockers

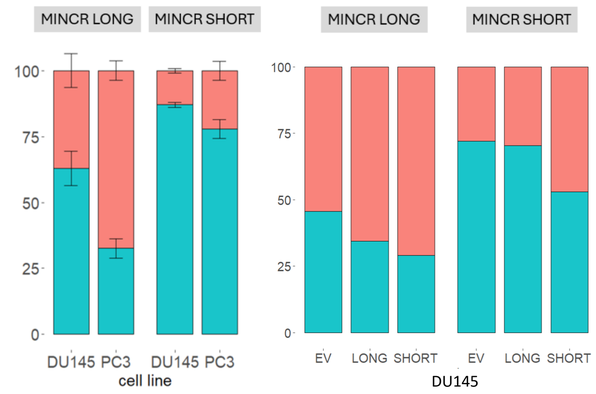


Which **cellular pathways** *MINCR* is involved in. Through:

- **RNAseq** of *MINCR* modulated cells
- **Cell-based assays**

4. *MINCR* transcript





endogenous transcript

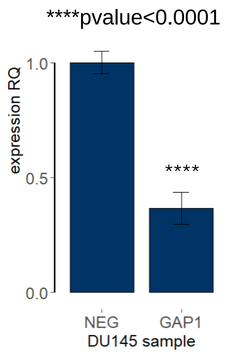
exogenous transcript

***MINCR* isoforms** show a differential **subcellular localization:**

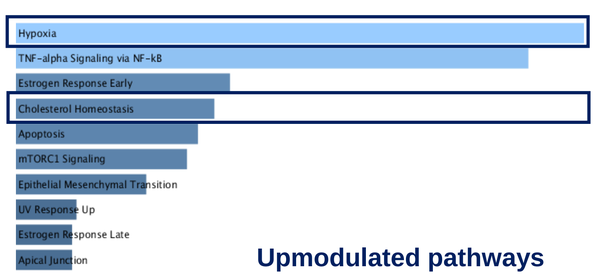
- **MINCR LONG** → NUCLEUS
- **MINCR SHORT** → CYTOPLASM

5. *MINCR* modulation impacts cell proliferation

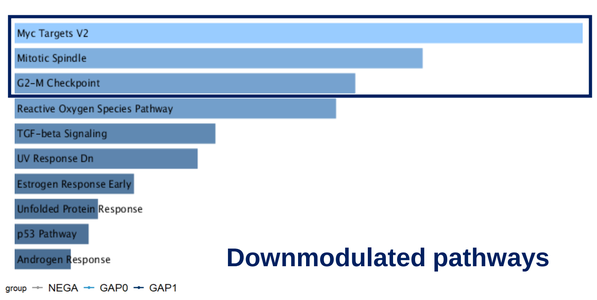
Silencing



EnrichR analisys: MSigDB Hallmark 2020



Upmodulated pathways

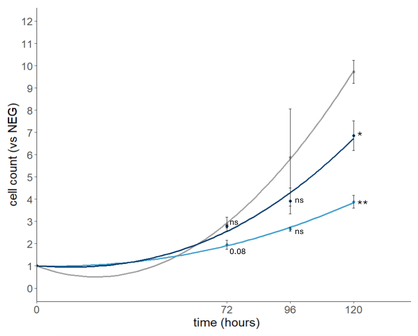


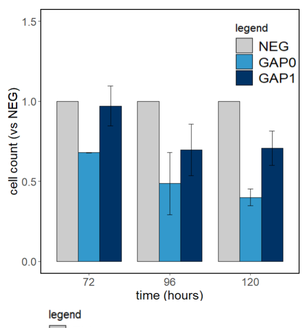
Downmodulated pathways

RNA sequencing of gapmer-mediated DU145 cells carrying *MINCR* knockdown.

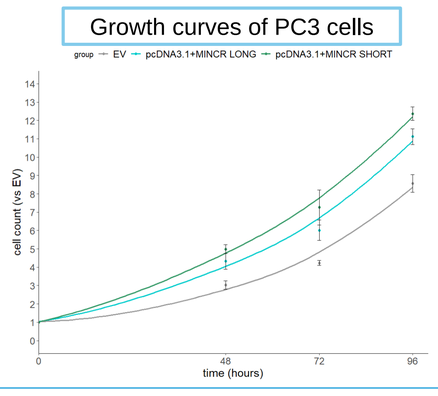
Pathways **upmodulated** upon *MINCR* silencing: **metabolism-related**
While for the pathways enriched in **downmodulated** genes: **cell cycle-related.**

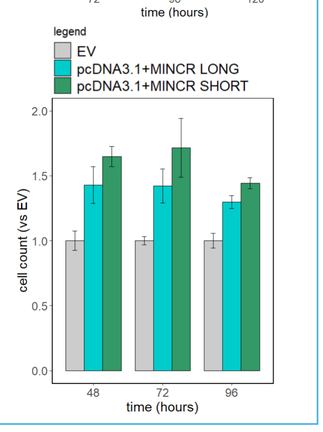
Observed **decreased cell proliferation** upon *MINCR* **silencing**.



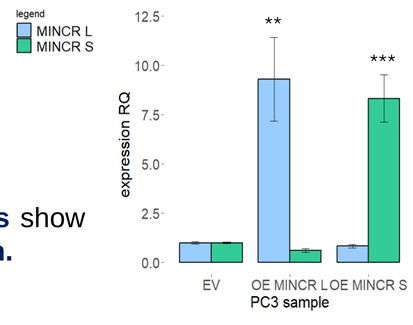


Growth curves of PC3 cells





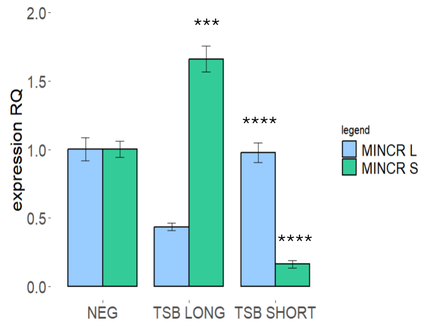
Overexpression



Isoform-specific **overexpressing clones** show **increased proliferation.**

In progress

1. Replicate **growth curves** and perform **MitoTracker** and **SeaHorse** assays to validate *MINCR* roles in the cell.
2. Try **Target Site Blockers** designed to **mask** *MINCR* alternative **PolyA signals** in order to induce cells to produce **only one isoform.**



3. Perform **RNAseq analysis** on ***MINCR*-overexpressing** cell populations and on **TSB-silenced** PC3 cells to have an isoform-specific view of *MINCR* role in the cell.

Conclusions

1. *MINCR* can be overexpressed **without** concurrent **amplification of *MYC*.**
2. There is a positive relationship between *MINCR* upregulation and an **increased severity** of prostate cancer.
3. *MINCR* exists in different **isoforms that locate differently** in prostate cancer cells suggesting **different roles** and functions within the cell.
4. The presence of an **Alu-sequence** and **HNRNPK** binding motif on *MINCR* LONG transcript may drive its nuclear localization.
5. **RNAseq analysis** of *MINCR*-knocked down prostate cancer cells and **growth curves** of both knocked down and overexpressing cells suggest a **pro-proliferative** role of this lncRNA.

Department of Biosciences
University of Milan

Funding: Italian Association for Cancer Research

ilaria.cassotti@unimi.it
paolo.gandellini@unimi.it