A CRISPRi screen to identify RNA Binding Proteins with oncogenic functions in colorectal cancer

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Abstract

RNA Binding Proteins (RBPs) are paramount for cell development and function, mainly via exerting post-transcriptional control of RNA processing and metabolism. Being critical effectors of gene expression, RBP misregulation underlies the basis of numerous diseases, including cancer. However, not much is known about their role in colorectal cancer (CRC). The aim of this study is to examine the function of RBPs in CRC by conducting and validating a CRIPSRi screen for RBPs using the CRC cell line HCT116. From the screen, the most significant protein effects were observed for *PELO*, *RBM22*, and *ZMAT2*. We found that *PELO* was expressed higher in CRC patients, in patient data. This evidence was also supported by data on DepMap showing that PELO is essential for cell survival. We are currently in the process of validating these results by using CRISPRi with three or four different guide RNAs to target each protein and cells expressing dCas9-ZIM3 under a doxycycline-inducible promoter. gRNA sequences were cloned into a lentiviral vector and packaged into a lentivirus. The cells were infected with the lentivirus to achieve stable expression of the gRNAs. Quantitative PCR and Western Blot will be utilized to confirm the successful knockdown of the proteins. The resulting phenotype of the cells will then be examined. We will investigate the effect of these genes on various properties of cancer cell lines (cell proliferation, apoptosis, cell migration, cell invasion). In the future, we plan to explore the effect of these RBPs on global post-transcriptional gene regulation and further examine their mechanism of action. This study will allow us to identify RBPs in the context of colorectal cancer, facilitating future research in designing therapeutic techniques.

A CRISPRi screen to identify RBPs important in CRC

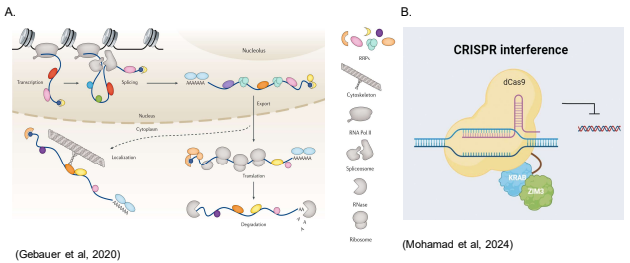
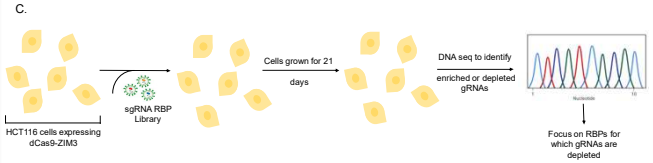


Figure 1. **(A)** Depiction of the importance of RBPs. RBPs contribute to multiple aspects of post transcriptional gene regulation. In the nucleus, RBPs are involved in transcription, splicing, and RNA export, while in the cytoplasm they are involved in RNA translation, localization, and degradation. **(B)** Illustration of CRISPRi. The inactive form of Cas9, dCas9, is fused to KRAB which mediates the transcriptional repression of the targeted gene through the protein domain ZIM3.



C. Diagram of CRISPRi screen. HCT116 cells with stable expression of dCas9-ZIM3 were transduced with a lentiviral gRNA library targeting RBPs. After 21 days, several cell populations were gaining an advantage whereas others grew less. The pool of cells were then subjected to DNA sequencing to identify enriched or depleted gRNAs targeting specific RBPs. Finally, we focused on RBPs for which gRNAs are depleted.

CRISPRi screen suggests several RBPs promote proliferation in CRC

A.

	1st replicate			2nd replicate		
Gene	P-value	Goodsgrna	log Fold Change	P-value	Goodsgrna	log Fold Change
PELO	6.32E-06	4	-2.4291	5.77E-06	4	-2.7633
RBM22	3.49E-05	3	-4.6639	0.000212	4	-4.8164
RBMX	5.52E-05	4	-3.9332	0.099426	3	-1.6223
RBM42	3.94E-04	3	-2.5928	0.008126	4	-2.0365
HNRNPC	8.74E-03	4	-1.6808	0.000277	4	-3.3168
ZMAT2	1.89E-02	4	-1.8826	0.00011	4	-2.8574

B.

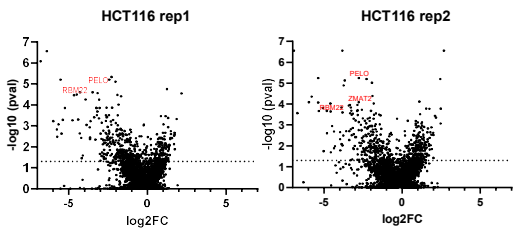


Figure 2. **(A)** Table showing RBPs promoting cell proliferation. In red are the RBPs that were picked for further analysis. Four gRNAs were used for each RBP and at least three of them showed an effect on cell proliferation to be considered a good candidate. **(B)** Volcano plots showing how RBPs regulate cell proliferation. The Y-axis shows the negative log base 10 of the p-value and the X-axis shows the log Fold Change. *PELO*, *ZMAT2*, AND *RBM22* points are indicated in red.

Generation of CRISPRi cells

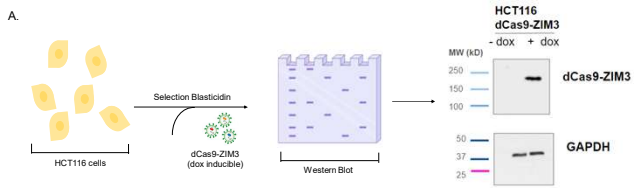
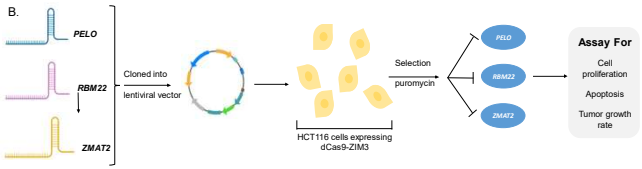


Figure 3. (A) Generation of dCas9-ZIM3 expressing cells**.** HCT116 cells were transduced with the lentivirus expressing dCas9-ZIM3 under a doxycycline (dox) inducible promoter. After Blastidin selection, the cells were treated with dox and a Western Blot was performed to confirm the expression of dCas9-ZIM3. GAPDH is used as a loading control.



B. gRNAs targeting *PELO, RBM22, ZMAT2* were cloned into a lentiviral vector, which is incorporated into the HCT116 cell line expressing dCas9-ZIM3. Puromycin is added to the cell line for selection. Assays will be carried out in the future to assess properties of cancer such as cell proliferation, apoptosis, and tumor growth rate.

PELO is upregulated in CRC

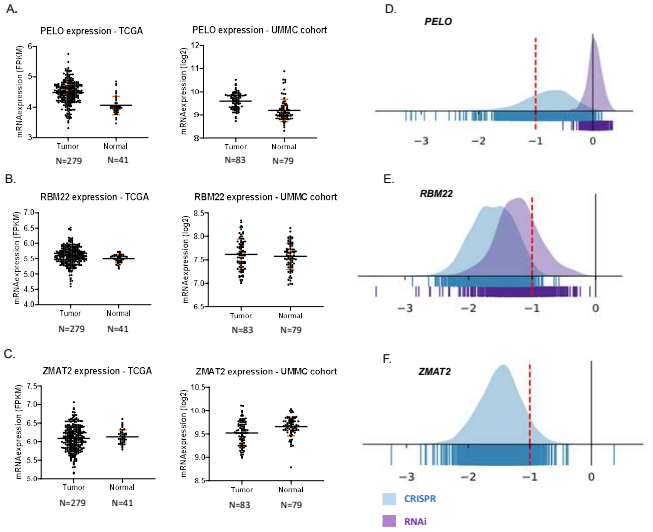


Figure 4. **(A-C)** Expression of *PELO* (A), *RBM22* (B), and *ZMAT2* (C) in tumor vs normal colorectal tissues. Left panels show patient data from the TCGA database. Right panels show data from the University of Maryland CRC patient cohort. *PELO* shows significantly higher expression in tumor samples. No significant difference is observed for *RBM22* and *ZMAT2*. **(D-F)** DepMap data showing *PELO* (D), *RBM22* (E), and *ZMAT2* (F) are required for cell survival. A negative score indicates the gene is more likely to be essential on a given cell line.

Summary and future directions

- This study allowed us to identify RBPs that have the potential to play a role in proliferation in the context of CRC
- CRISPRi of *PELO, RBM22,* and *ZMAT2* resulted in a decrease in cell proliferation
- *PELO* is upregulated in CRC
- These proteins were selected for further validation and their effect in the CRC cell line, HCT116 is being investigated
- Strong RBP candidates will be further tested for global post-transcriptional gene regulation, and we will examine their mechanism of action

References and acknowledgements

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