Cancer Functional Genomics Laboratory

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Introduction

CHROMOSOMAL ABBERATIONS CHR-4P

Triple-negative breast cancer (TNBC) is a complex and heterogeneous disease characterized by the absence of immunohistochemical detection of the ER (estrogen receptor), PR (progesterone receptor) and HER2 (human epidermal receptor 2) biomarkers. TNBC represents approximately 15-20% of all the breast tumors and is correlated with a worse prognosis compared to other subtypes, hence the need for the development of a targeted therapeutic strategy.

Large chromosomal alterations are common in cancer. Alterations show preferential gain or loss across many cancer types indicating their selective advantage. Specifically, TNBC displays consistent loss of large chromosomal regions. Around 60% of TNBC patients have deleted regions on 4p chromosome. Deleting overexpressing genes in this region in a cancer cell line region can lead to a proliferation defect. In this project, we are testing directly if the deletion of this region in a normal cell line would increase its growth rate.

IMMUNOTHERAPY – B7-H4

Immunotherapy was also proposed as a therapeutic strategy for TNBC due to the fact that they present with higher immune content compared to other breast cancer subtypes. Studies also showed that better prognosis was associated with a higher amount of cytotoxic immune cells interspersed within the tumor body.

Immunotherapy is a field of cancer research aimed at utilizing an organism's immune response to target the cancer itself. An immune response in an organism is tightly regulated by the interactions between the antigen-presenting cells (APCs) and immune cells. Several immune checkpoints are responsible for immune cell activation and further differentiation which leads to an increased or a decreased level of an immune response. Negative immune checkpoints in cancer therapy have gained relevance in recent years as they are the ones that attenuate an immune response and can be harnessed by the cancer for its own protection. The inhibition of such checkpoints can boost the activation of immune cells and, consequently, increase the immune reaction against the cancer, such is the power of immunotherapy.

In a subset of TNBC tumors, an immune checkpoint, B7-H4, was observed to coincide with poor immune infiltration. It was hypothesized that B7-H4 expression could be linked to a blockade to immune infiltration, and thus a marker of poor prognosis in patients. As this immune checkpoint is poorly studied, a genome-wide CRISPR screen was conducted on a TNBC breast cancer cell line to elucidate mechanisms of its expression and regulation. The study led to two pertinent regulators of B7-H4 expression, B3GNT2 and AMD1, as downstream validation targets.

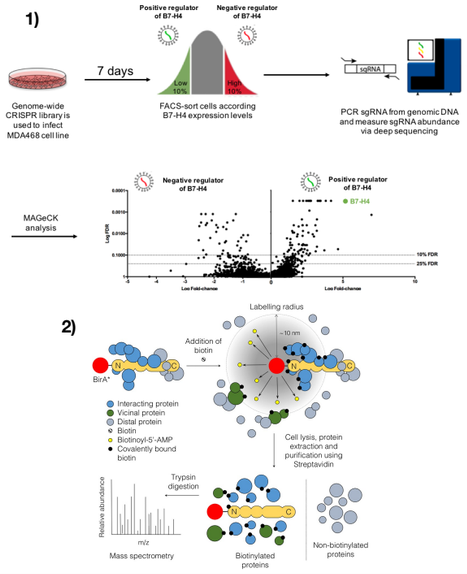
KIDNEY CANCER AND BIO-ID

Our work was focused on mechanisms of magnesium transport in kidney cancer cells. The re-absorption of divalent cations takes place in the kidneys. The availability of magnesium is important for proliferation of cancers since it is involved in multiple bioenergetic pathways as a cofactor. We designed sgRNAs to knock out two target proteins - CNNM2, which is a modulator of magnesium transport, and RPN1 - a subunit of an oligosaccharyltransferase complex (OST), involved in N-linked glycosylation. CNNM2 is a transmembrane protein that is glycosylated, which is required for proper plasma membrane localization. Aberrant glycosylation is often observed in cancers. A potential interaction between these proteins was identified using BioID approach.

Methodology

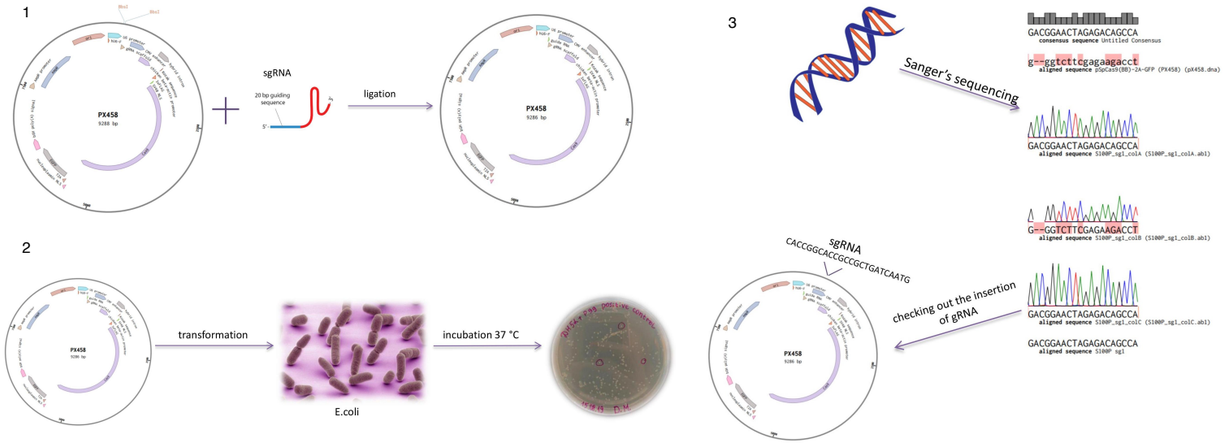
1) A genome-wide CRISPR screen is used to knock-out various genes and define if a particular gene affects the gene expression of another, in our case, B7-H4. This allows us to screen a lot of genes all at once, making the process less time-consuming and more convenient for researchers. It also allows us to do a broad survey of all the genes in the genome to get an idea of which pathways or protein complexes are involved in the expression and regulation of a particular protein.

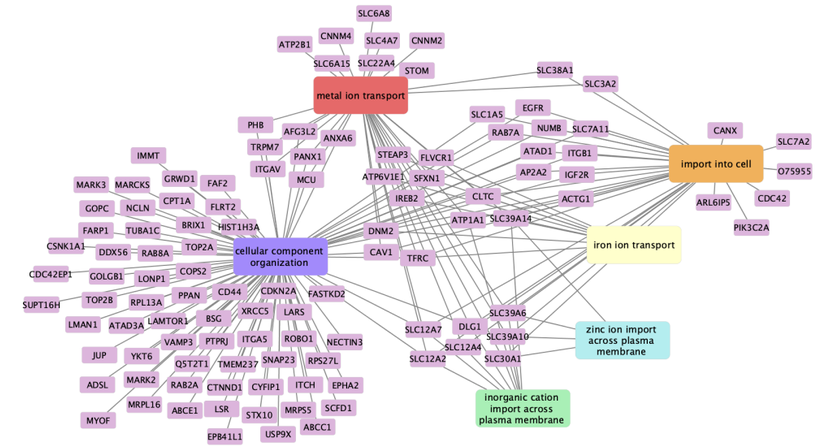
2) BioID works by biotinylating proteins located in the vicinity of the target protein. They are then precipitated from the lysate using streptavidin beads and identified using mass spec. Hits obtained from this study were used as



CRISPR-Cas9 system is a genome-editing tool widely used in the modern research and abundantly in genomic studies. The CRISPR system (Clustered Regularly Interspaced Short Palindromic Repeats) and associated Cas proteins (Cas = CRISPR associated) form the adaptive immune system of bacteria which enables the detection and destruction of foreign genetic material identified in viruses such as bacteriophages; this system also has the ability to form immunological memory. The particular system used in our research, the CRISPR-Cas9 system, is composed of the Cas9 endonuclease, which is guided to the recognition site by a single guide RNA (sgRNA) sequence. Then, the double-stranded break in the specific DNA sequence is induced by the nuclease enzyme, Cas9. The break is repaired by the error-prone procedure of non-homologous end-joining (NHEJ), which introduces mutations that often result in the knock-out of the gene of interest. Downstream applications of the system allow us to study consequences of the knock-out of genes of interest.

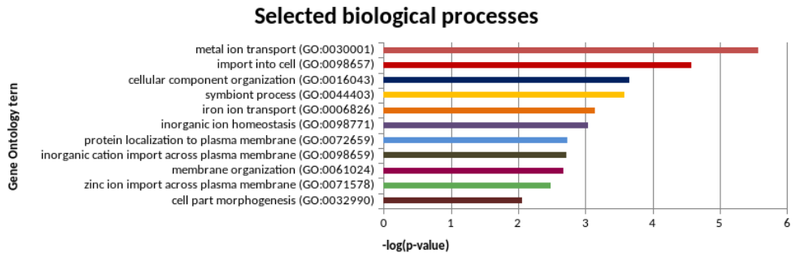
By creating various collections of knocked-out genes (i.e CRISPR genome-wide screens, over-expression library, etc.), one can use this system in a high-throughput manner to understand the role of each individual gene in the regulation of various cell processes.





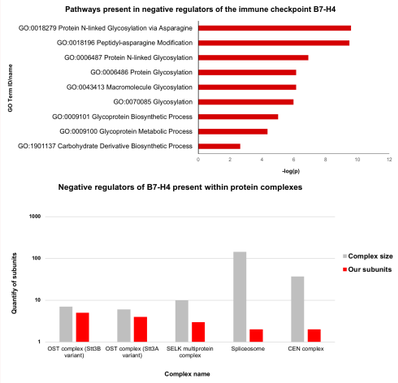
ITCH-FAM/USP9x complex- Interaction between Itch and FAM (USP9X) reverses Itch auto-ubiquitylation and protects the ligase from proteasomal degradation. ITCH plays an important role in protein degradation by adding ubiquitin to its target proteins.

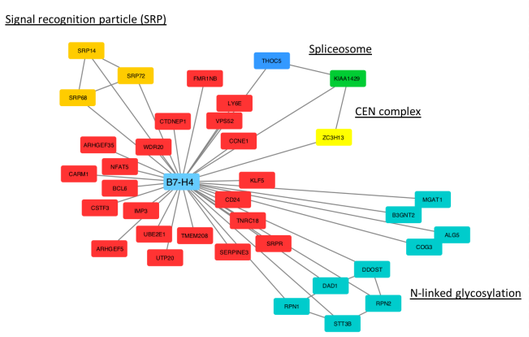
RAB9-TIP47-MPRI complex is composed of 3 proteins. TIP47 binds Rab9-GTP specifically. MPRI enhances Rab9-TIP47 interaction and Rab9 increases the affinity of TIP47 for the MPRI. The function of this complex is in regulating vesicular transport from the Golgi network.



Overrepresented biological processes identified through g:Profiler

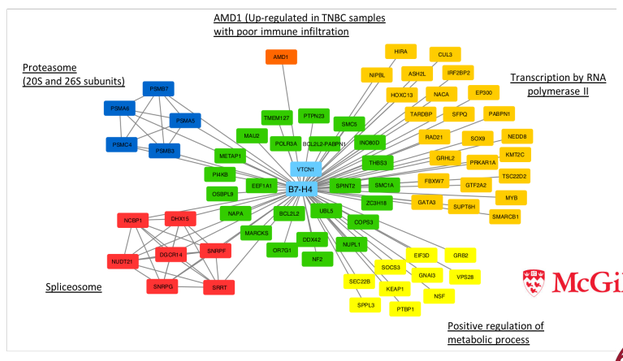
B7-H4 Negative regulators





Negative regulators of B7-H4 were observed to increase B7-H4 cell surface levels upon knock-out with CRISPR.
Glycosylation-related genes proved to be interesting targets, and B3GNT2 was chosen as a downstream validation target for individual CRISPR design.

B7-H4 Positive regulators



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ZIMIN FOUNDATION

Positive regulators of B7-H4 were observed to decrease B7-H4 cell surface levels upon knock-out with CRISPR.
Pathway analysis by CORUM and Gprofiler did not indicate pertinent pathways for study as a group, but AMD1 showed interest in individual analysis and was chosen as a validation target by individual CRISPR design.