RNA sequencing of the silenced gene CYTH4 in acute myeloid leukemia cell lines (human)

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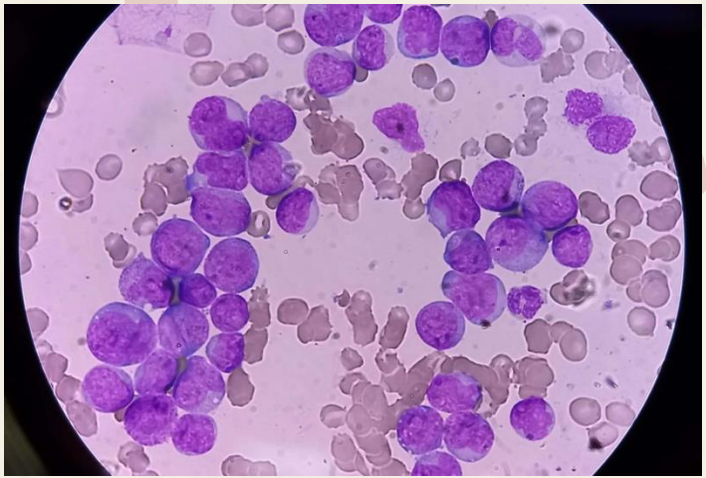
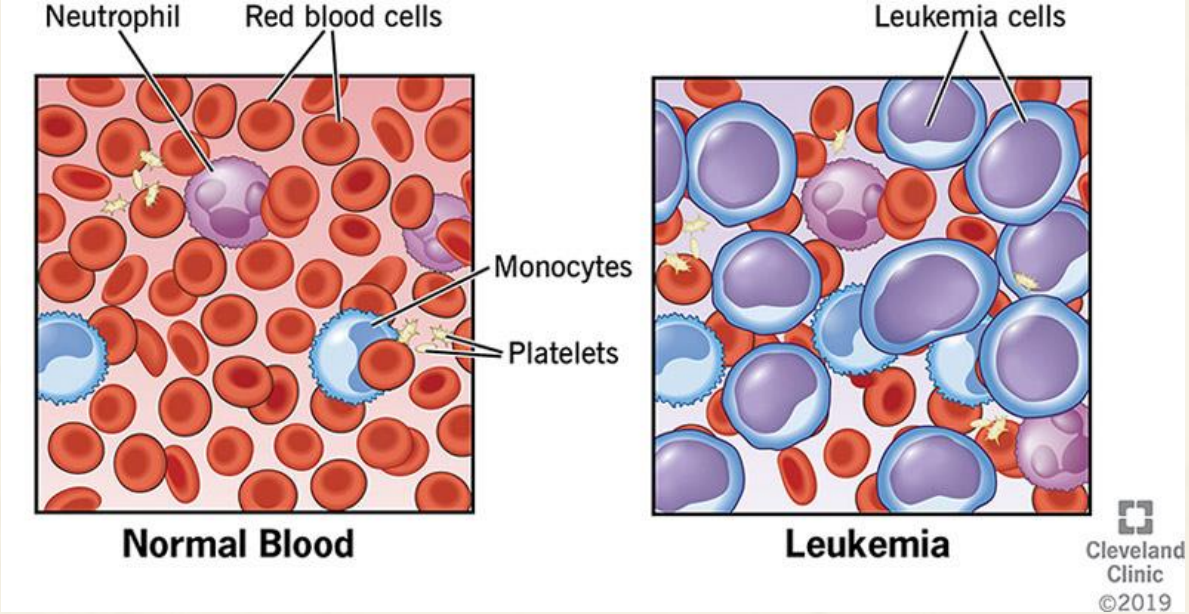
Introduction to Acute Myeloid Leukemia (AML)



Credit: Md Babul Hosen / Getty Images

- AML is a rare type of cancer that affects the blood and bone marrow in which the bone marrow produces a large number of abnormal blood cells.
- This type of cancer typically affects people age 60 and older but can also affect younger adult and children
- AML often spreads quickly from the bone marrow into the circulation.


Neutrophil
Red blood cells
Monocytes
Platelets
Normal Blood
Leukemia cells
Leukemia
Cleveland Clinic
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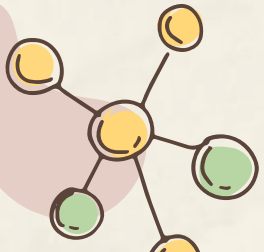
What is CYTH4?

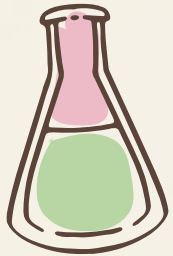
Credit: Gene Card The Human Gene Database

- CYTH4 is a protein coding gene
- It produces a protein that helps regulate ARF proteins, which send signals inside cells for important functions like cell growth and movement.
- Cancer cells often abuse signaling pathways to survive, grow and avoid the immune system

RNA sequencing of the silenced gene CYTH4 in acute myeloid leukemia cell lines

- This study aimed to explore the impact of the gene CYTH4 and its molecular functions. The focus was on CYTH4 because its expression was much higher than other cytohesin in AML
- THP-1 cell line was studied using in vitro validation because these are acute myeloid leukemia (AML) cell lines that exhibit high CYTH4 expression
    - People with higher levels of CYTH4 tended to have worse outcomes





study findings

01

This study looked at the gene called CYTH4, which was found to be much more active in people with AML

02

People with higher levels of CYTH4 tended to have worse outcomes

03

When the gene CYTH4 was turned off, it slowed the cancer's growth.

04

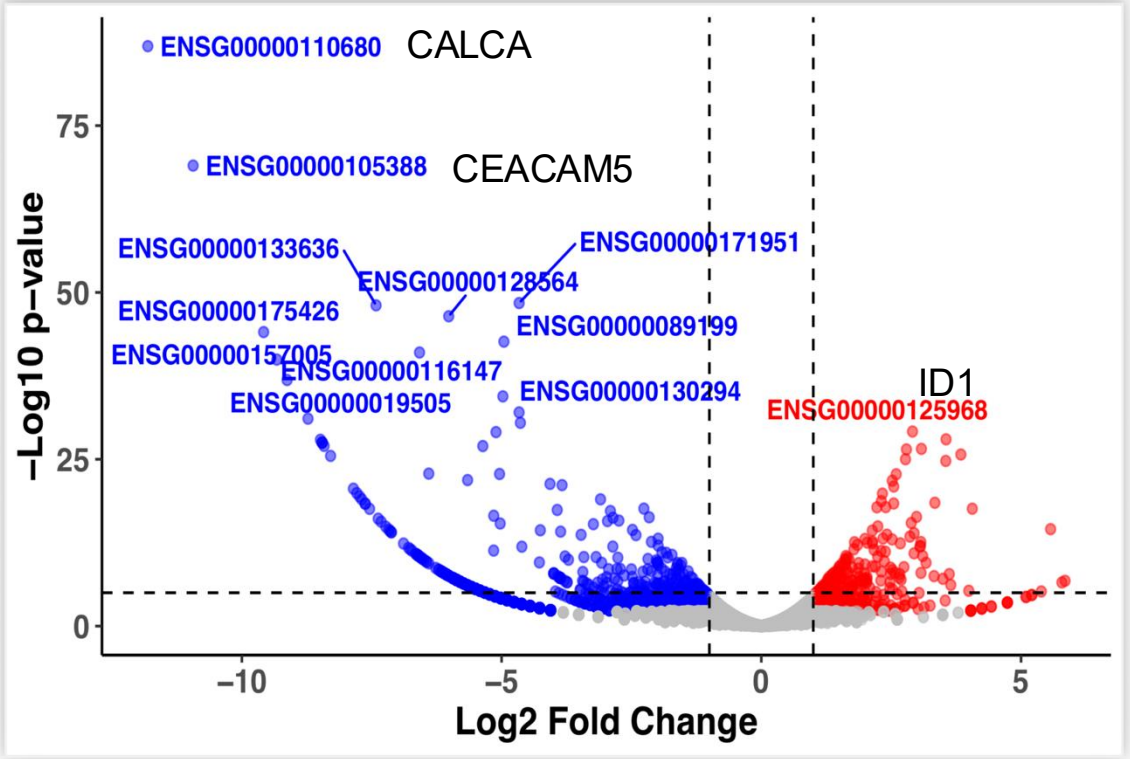
CYTH4 also affects the immune system

Hypothesis question:
What genes are affected when CYTH4 is silenced in AML cell lines (THP-1 specifically)?

Preprocessing

- STEP 1:
  - Obtain fastq files from NCBI database
    - Three control samples and three treatment samples were collected
- STEP 2:
  - Run initial quality control report on fastq files
- STEP 3:
  - Filter poor quality reads using cutadapt
- STEP 4
  - Align reads to reference genome using hisat2 to generate sam files
- STEP 5:
  - Compress, sort, align indexes from samtools
- STEP 6:
  - Generate count matrix for processing...
  - **About a 75% successfully alignment rate**

Differential Expression Volcano Plot



~1100 downregulated
~500 upregulated

ENSG00000110680

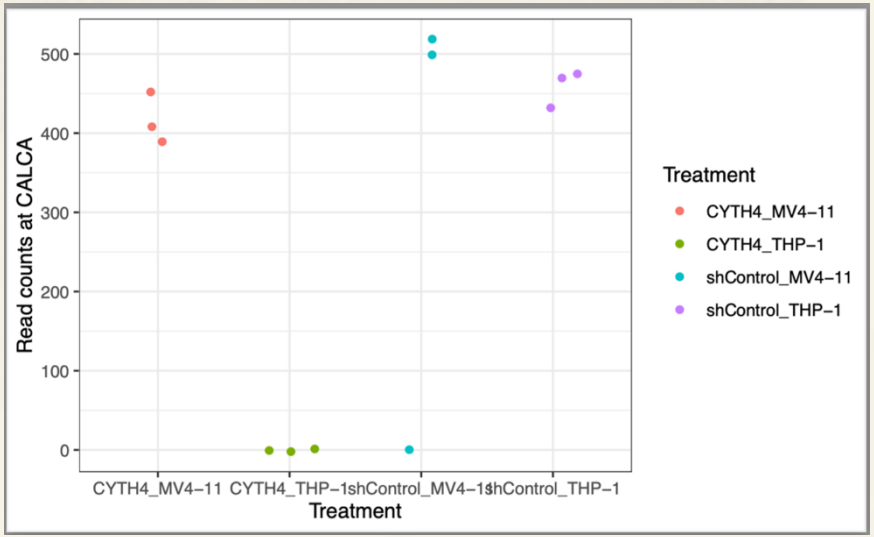
CALCA

Calcitonin related polypeptide alpha

Calca is a gene that encodes a protein

It produces calcitonin which helps regulate calcium levels in the body

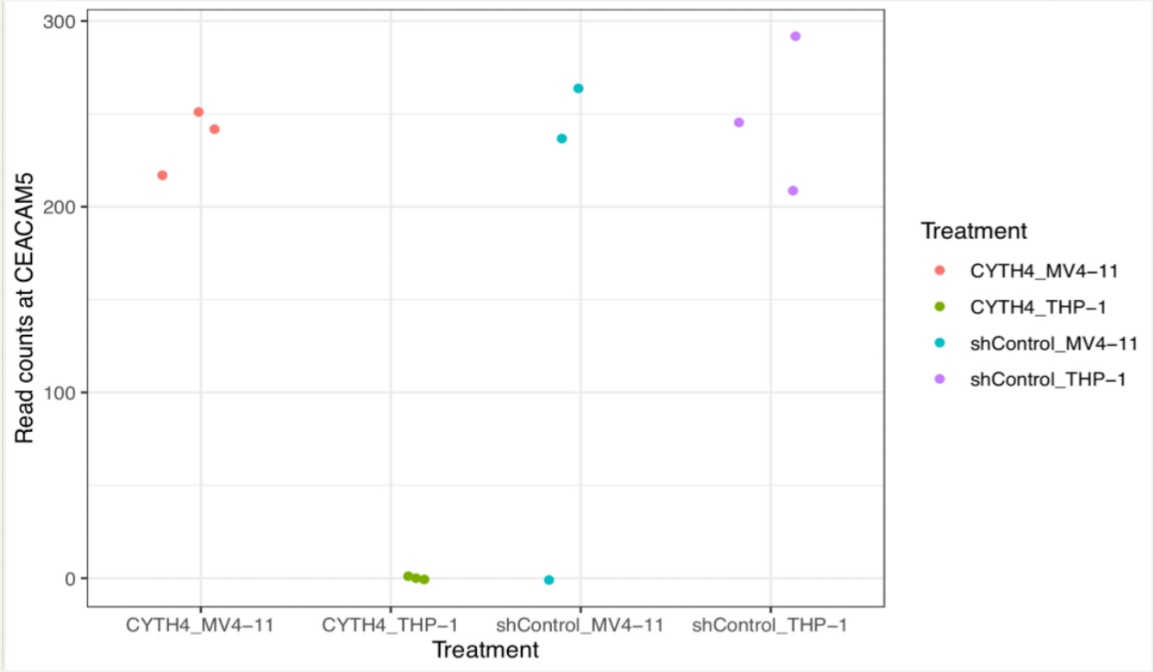
CALCA, through its encoded protein CGRP, can promote cancer growth by weakening the immune system and changing how cells use energy



CEACAM5

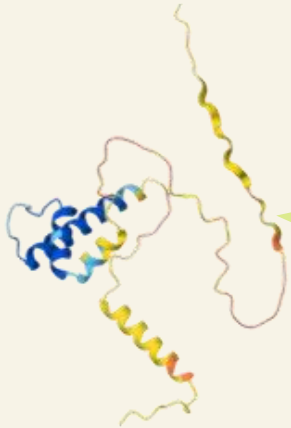
ENSG00000105388

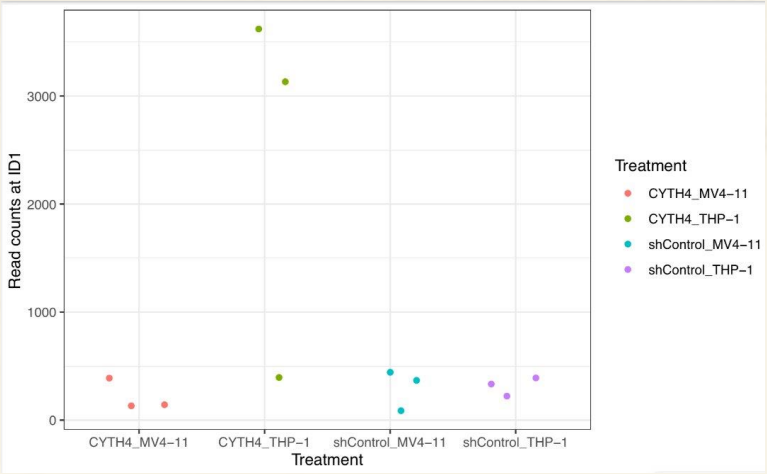
- CEACAM5 cell surface protein found on the surface of certain cells in the body.
- CEA (Carcinoembryonic Antigen) it is a protein that is regularly absent or low in healthy adults but is present in fetal growth.



ENSG00000125968

ID1

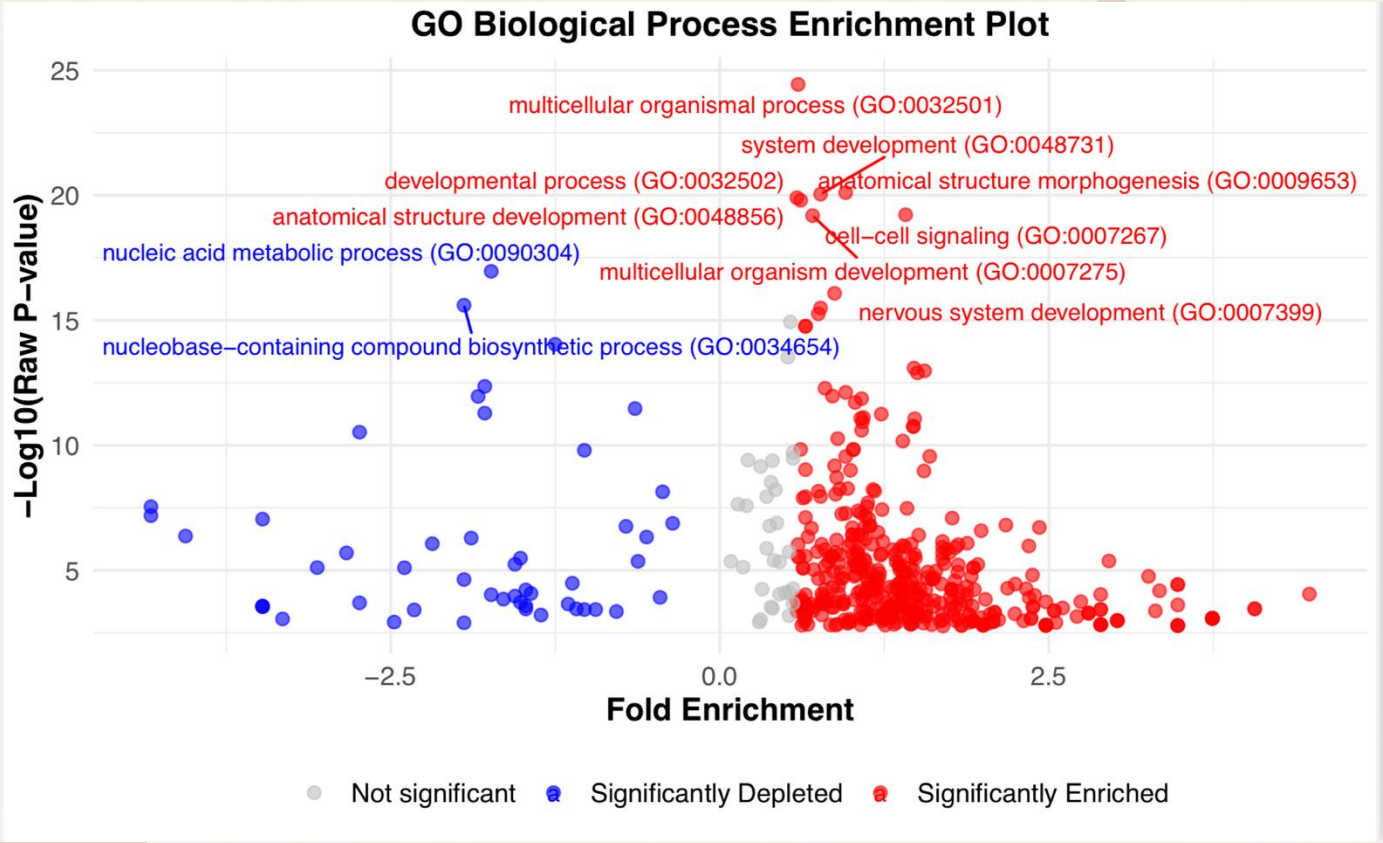




We can visualize that when CYTH4 is knocked down, in two of the three samples, the presence of ID1 is much higher in the THP-1 treatment group.

ID1 Upregulated When CYTH4 Knocked Down

- Do not directly interact with each other, but have similar impact
- ID1 is upregulated when CYTH4 is knocked down meaning it is more present when CYTH4 is less present (possible CYTH4 suppresses ID1)
- Both provide cancer-friendly environments
    - ID1: promotes cell growth, prevents differentiation (leading to less specialized cells) and prevents cell death (cell apoptosis)
    - CYTH4: Promotes cell growth, prevents cell death, particularly in leukemia



Plot helps visualize which biological functions are up and downregulated in experimental conditions

Finding clear connections between functions of CYTH4 and the genes that we studied was difficult.

References

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THANK YOU!