**Acid Ceramidase in colon cancer**

Acid Ceramidase (ASAH1) is the enzyme which converts ceramide to sphingosine and might have a significant role in colorectal cancer (CRC). We used ASAH1 knockdown human and mouse colorectal cancer cell lines and specific inhibitors of ASAH1 for in vitro studies. Silencing ASAH1 expression inhibited the proliferation, invasion and migration of colorectal cancer lines. However we did not observe a synergetic activity between existing chemotherapeutic drugs and ASAH1 inhibitor. During the course of investigations we noticed that modulating the ASAH1 expression regulates the expression of check point inhibitors especially PD-1 and PD-L1. Currently we are about to start the in vivo studies using immunocompetent and immunodeficient mouse models of CRC.

We are interested in the following using computational analysis of existing dataset and TCGA

1. How ASAH1 expression in different stages, grade, lymph node metastasis of colorectal cancer using available data set of CRC.

Stage and grade data was retrieved from GEO database (<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE4045>) with gene ASAH1(210979\_at). Total 37 patients were analyzed, data was extracted with Feature Extraction software 10.7 (Agilent technologies, Santa Clara, CA, US). Raw data normalized by Quantile algorithm, limma packages in R. Following is a scatter plot comparing colorectal cancer stages, grade, and gender. None of the differences within each of these comparisons were significant (P>0.05) so there seems no difference for gene ASAH1 amongst different stages, grade and gender.

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1. Overall patient survival and DFS based on the expression level of ASAH1 expression?

This data is not available.

1. Does ASAH1 expression has any direct or inverse correlation with PD-1 and PD-L1

Looking at the Pearson correlation coefficient values, ASAH1 seems to have a negative correlation with PD-1 and PD-L1 expressions with correlation values of -0.57312836 and -0.845759801 respectively.

1. GSEA analysis for gene interactions with ASAH1

For GSEA analysis, normal and CRC patients expression was retrieved from GEO ([https://www.ncbi.nlm.nih.gov/gds/?term=GSE156355[ACCN]%20AND%20gsm[ETYP]](https://www.ncbi.nlm.nih.gov/gds/?term=GSE156355%5bACCN%5d%20AND%20gsm%5bETYP%5d)).

Three genes and probe ids were PD-L1:CD274: A\_23\_P338479 & A\_33\_P3381513, PD-1: PDCD1: A\_23\_P136405 and ASAH1: A\_33\_P3284463. 12 patients (6 cancer and 6 normal) were analyzed, and following is the plot comparing three genes. Again, P values were insignificant (P>0.05), so no significant difference in expression of three genes within cancer and normal patients was observed.

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Interactome analysis was performed by obtaining (String10 database) confidence values of protein ASAH1 with its first degree interactor, which were then inputted into Bingo plugin in Cytoscape for pathway analysis. The pathways associated are provided in sheet, Processes.xlsx. Following are the interactomes for first degree interactions of protein ASAH1 (Left) and processes (Right).

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