Proline metabolism in Triple Negative Breast Cancer

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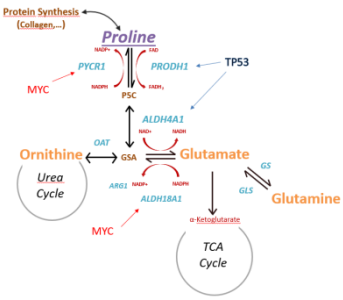
Background

Most cancer cells use large amounts of glutamine for nucleotides, lipids and glutamate biosynthesis. Other amino acids, such as proline, can play a role in this metabolic reprogramming as its degradation can generate glutamate through PRODH and ALDH4A1. Conversely ALDH18A1 and PYCR1, mediate the conversion of glutamate to proline. Finally, this Pro<->Glu cycle is also connected to others metabolic pathways important for cancer cells, such as TCA cycle through α-Ketoglutarate synthesis and Urea cycle through ornithine generation.

The regulation of these 4 enzymes reinforce the idea of metabolic reprogramming of proline pathway in cancer, since the synthesis enzymes ALDH18A1 and PYCR1 are transcriptionnal target of MYC, and the degradation enzymes PRODH and ALDH4A1 are transcriptional targets of TP53 (Fig.1). In agreement with this idea, lower levels of ALDH4A1 and PRODH were reported in a variety of TP53-mutated primary human tumors compared to normal tissues from the same patients.

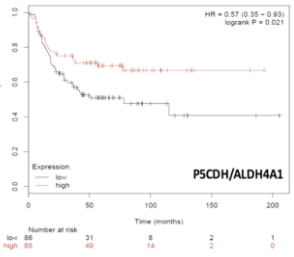
Fig.1 : Proline Pathway.



In addition, whereas ALDH18A1 and PYCR1 have been described as pro-tumoral genes, PRODH have anti-tumoral properties and no information about the role of ALDH4A1 in cancer have been published yet, but we suggest that ALDH4A1 could also have anti-tumoral properties since patients expressing high levels of this enzyme in several type of cancers including a subpopulation of basal-like and Triple Negative Breast Cancer (TNBC) tumor, show better survival overtime (Fig.2).

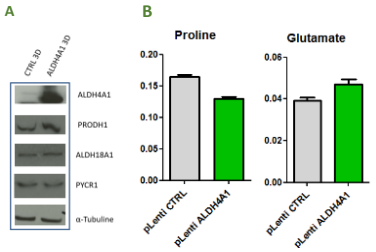
Altogether, these informations led us to test whether the ALDH4A1-dependent proline degradation pathway could act as a tumor suppressor in TNBC, a cancer with a high frequency of TP53 mutations and a poor outcome due to the lack of targeted therapies.

Fig.2 : Kmplot of a subpopulation of basal-like and TNBC.



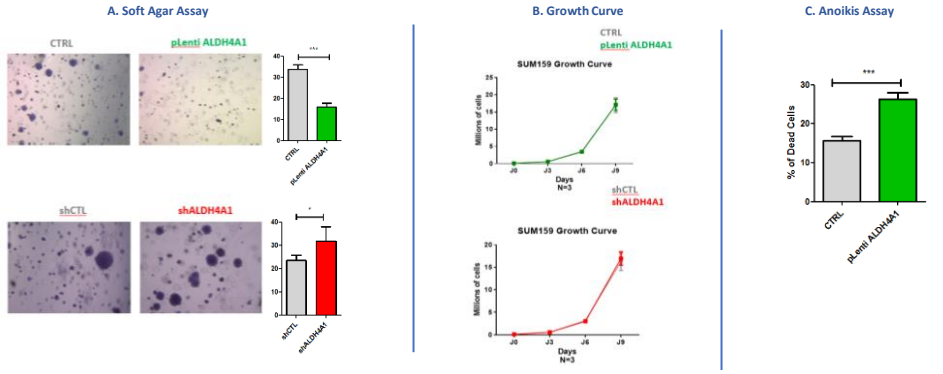
Results

Fig.3 : ALDH4A1 reexpression in TNBC cells increases proline degradation.



A. Expression of the proline pathway enzymes in SUM159 cells reexpressing ALDH4A1. B. Intracellular proline and glutamate levels of SUM159 cells reexpressing ALDH4A1.

Fig.4 : ALDH4A1 modulation impacts growth and viability of TNBC cells in 3D growth conditions but not in 2D.



A. SUM159 reexpressing or inhibiting ALDH4A1 were grown in Soft Agar. Graphs shows counting of big colonies that were grown after 3 weeks B. SUM159 reexpressing or inhibiting ALDH4A1 growth curve in 2D conditions. Cells were counted each 3 days. C. Cells were grown in 3D conditions using polyHEMA-treated plates. After 3 days, aggregates were dissociated and cell death was counted using trypan blue.

Fig.5 : RNASeq Analysis of TNBC cells reexpressing ALDH4A1 in 3D growth conditions show genes differentially expressed implicated in hypoxic stress response.

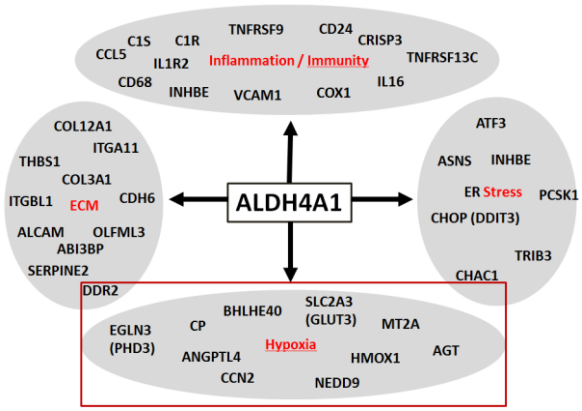
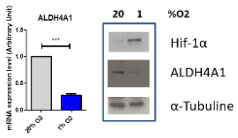
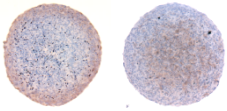


Fig.6 : Hypoxia decrease endogenous ALDH4A1 mRNA and protein levels of TNBC cells in 2D and 3D conditions.

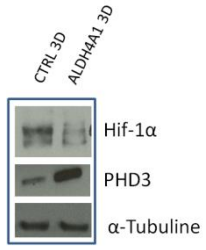


A. ALDH4A1 expression in SUM159 cells in hypoxic conditions.



B. IHC staining for ALDH4A1 and CA9 in spheroïds derived from SUM159 cells. CA9 is a marker of hypoxic regions.

Fig.7 : ALDH4A1 reexpression decreases HIF1-α and increases PHD3 protein levels of TNBC cells grown in 3D conditions.

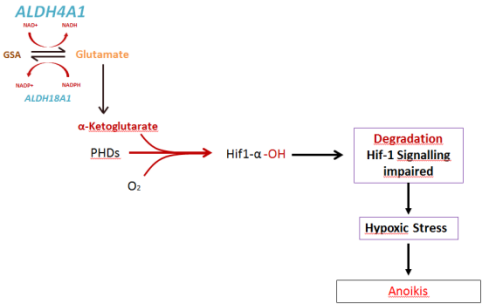


Perspectives

The reactivation of the proline degradation pathway in p53-deficient TNBC cells has an unexpected effect on their response to hypoxia as shown by the decrease of HIF1- α. This could explain the loss of resistance to hypoxia of cells grown in 3D conditions and the increase in anoïkis.

We hypothesize that the decrease in HIF1- α levels could be driven by PHD3, whose expression is increased in ALDH4-reexpressing cells.

The reexpression of ALDH4A1 could increase the expression and/or activity of PHD3 by the generation of α-Ketoglutarate from glutamate. This could in turn hydroxylate HIF1-α, leading to its degradation.



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