Sugar Awakens Cancer Cells: Ras between Survivability & Apoptosis

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INTRODUCTION

Breast cancer (BC) is the most frequent cancer diagnosed, and the first cause of death among females in Egypt. Altered metabolism is a characteristic feature in BC that link glycolysis to tumor growth mediated by the activation of RAS genes. In BC, mutations in RAS gene are not common, however indirect activation of Ras or Ras-mediated signaling pathways are involved in BC development. Metabolic reprogramming of tumor cells and the conservative activation of Ras is similar to fermentation of yeast.

AIM

Owing to this genetic & metabolic similarities, a functional yeast survival screen was performed exploiting a genetically well-defined yeast system to screen a cDNA library for novel inhibitors of Ras in overactive glycolytic flux to hinder tumorigenesis &/or resolve therapy resistance.

MATERIALS & METHODS

1- Yeast Screen: an unbiased screen was executed, where the human cDNA library encoding the whole human genome was transformed in *Δtps1* mutant yeast cells, the hit that will rescue these mutant cells from these lethal conditions is expected to inhibit Ras activity.

2- Spot test: The strains were allowed to grow on SC-X 100mM glucose or galactose in the presence of 3% ethanol, the cells were incubated at 30^{0}C for 2d. Ten-fold serial dilutions were spotted.

3- DAPI & Annexin/PI staining: Wild type, mutant and ALDOB expressing cells were stained with DAPI and analyzed for their morphologies using fluorescence microscopy. The occurrence of glucose induced cell death in *tps1Δ* cells was characterized by markers of both apoptosis and necrosis. Assessment of cell death was executed by FITC-coupled annexinV and PI staining. The cells were treated with 100mM glucose for 120 minutes at 30°C, before being processed for determination of phosphatidylserine externalization and membrane integrity by fluorescence microscopy.

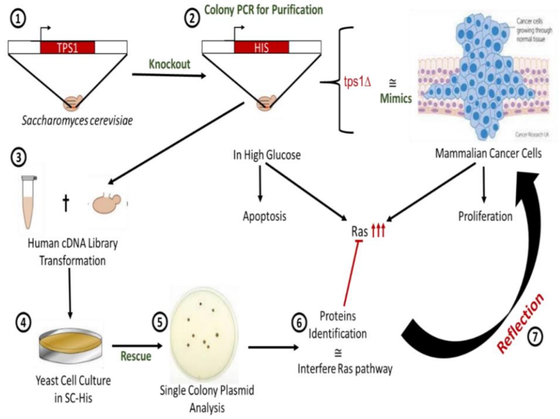
4- ROS identification: wild type, Mutant, and *tps1Δ* cells expressing ALDOB exponentially growing cells were treated with 100mM of glucose for 120 minutes at 30°C. ROS accumulation was assayed using the dye dihydrorhodamine 123 (DHR123) by fluorescence microscopy. The % of cells accumulating ROS was quantified using ImageJ software. The means of 3 independent experiments with standard deviations are reported.

5- Statistical Methods: All statistics were performed using Unpaired t-test using Graphpad prism 5.0, the results are considered significant ($p<0.05$) .

RESULTS

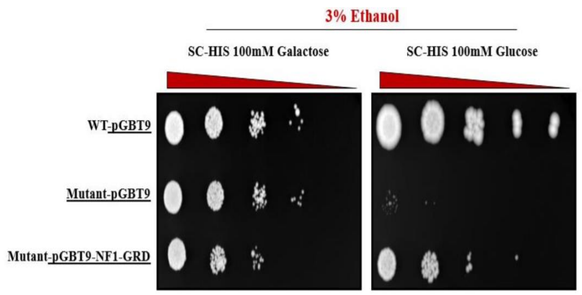
Metabolic screen using *tps1Δ* strain:

1-Modelling the *tps1* knockout in yeast cells mimicking the Warburg effect in cancer cells & it can be used as a model to identify novel players that can inhibit the Ras activation under these conditions. 2- Validation of the knockout by colony PCR and apoptosis verification on hyperglycemic media as these mutant yeast cells were sensitive to the minimal glucose concentration due to hyperactivity of Ras pathway unlike cancer cells that proliferate rapidly in presence of glucose. 3-Transformation of yeast cells with the human cDNA library. 4- & 5- Screening of the rescued colonies. 6-Identification of the protein players that interfered with Ras signaling pathway. 7-Reflection upon the mammalian cancer cells.



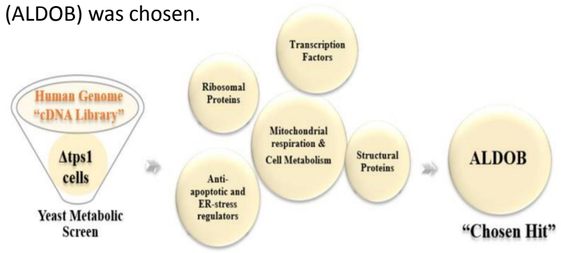
NF1 as a positive control to validate tps1Δ Metabolic Screen

A proof-of-principle experiments were also performed to evaluate a known Ras inhibitors as NF1. A drop test was performed by 3 strains to examine the rescue. As shown, mutant strain transformed with pGBT9-NF1-GRD rescued the death of the mutant yeast cell *(tps1Δ)* on 100mM glucose concentration and showed similar phenotype to the strains spotted on the control Gal plates. All spotted at starting OD_{600} of 0.06.

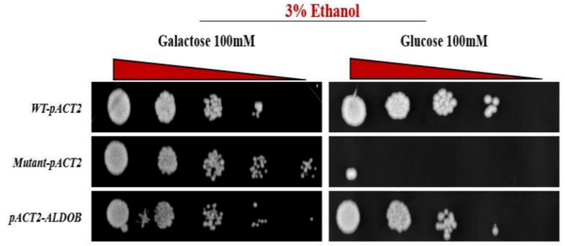


Modifiers of Ras signaling pathway in yeast cells

The 14 genes identified in 3 screens were categorized into 5 classes encompassing ribosomal proteins, transcription factors, anti-apoptotic proteins, structural proteins & mitochondrial respiration & cell metabolism. 3 genes were known to inhibit Ras, therefore validating the screen system. Of the isolated genes, the human Aldolase B (ALDOB) was chosen.

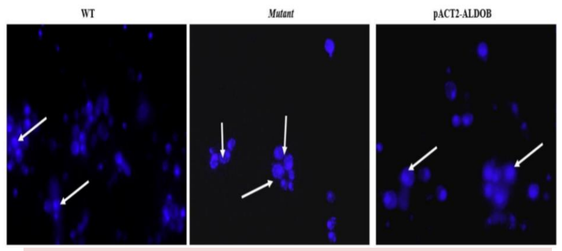


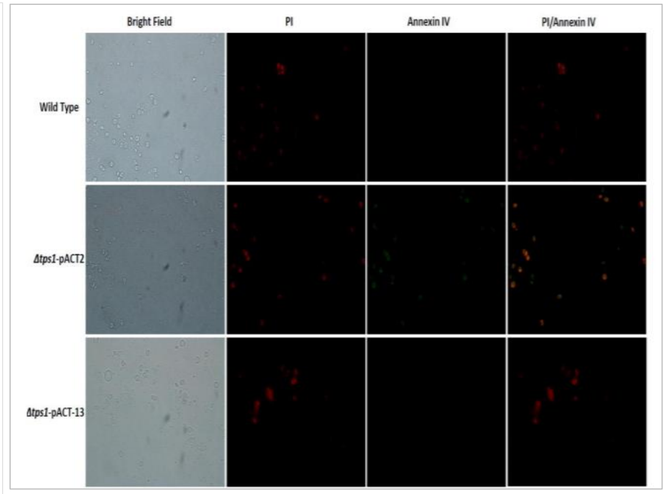
ALDOB expressing plasmid was isolated & its expression was confirmed by blotting against HA tag. Then, ALDOB rescue was confirmed by viability test.



ALDOB repressed apoptosis and necrosis

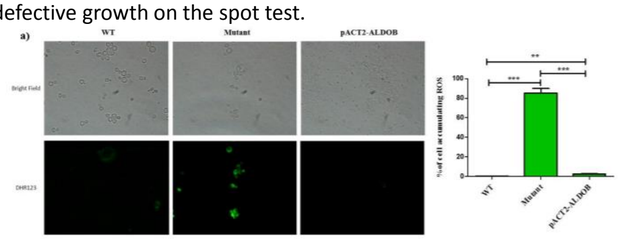
tps1Δ cells showed nuclear fragmentation & condensation in the presence of high glucose. But, in the mutant strain expressing ALDOB a repression of apoptosis was observed & the nucleus was round that might indicate that it regained its integrity as assessed by DAPI & Annexin/PI staining.

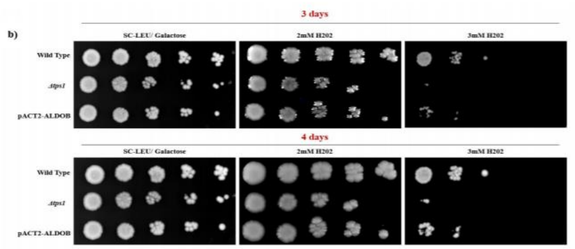




ALDOB suppressed ROS accumulation resulting from mitochondrial dysfunction

By the DHR123 ROS probe & fluorescence microscopy, ALDOB was also shown to suppress ROS accumulation in mutant yeast cells. Upon, growing ALDOB expressing cells on high concentration of hydrogen peroxide, this strain was able to rescue the cells behaving similar to wild type & different from mutant cells that showed defective growth on the spot test.





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CONCLUSION

Our results suggest that ALDOB might be a Ras signaling inhibitor as shown by regulating the defects hyperactive Ras caused in yeast cells. These findings open avenues for understanding the metabolic cues of tumor cells that are activated or inactivated in response to glucose to identify accurate treatment approaches of metabolic disease-associated cancer.