

14-3-3 σ as a new important regulator of cancer glycolysis

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Abstract

Metabolic reprogramming is an important cancer hallmark and provides cancer cells with major advantages in proliferation, survival and drug resistance 1, 2, 3, 4.

In fact, enhanced glycolysis or Warburg effect is very common in many cancer cells and enhances their survival upon hypoxia 1, 2, 3, 4. Furthermore, glycolysis suppresses apoptosis, and strongly promotes proliferation, drug resistance, and metastasis 2, 3, 4. However, current understanding about cancer glycolysis regulation is still limited, preventing the development of effective cancer metabolism - targeted therapies.

In this study, our results show that 14-3-3 σ considerably suppresses cancer glycolysis by repressing glucose consumption, glucose uptake, and lactate production. 14-3-3 σ also significantly impairs energy production in cancer cells. Furthermore, re-expression of 14-3-3 σ causes sharp decline of ¹⁸Fluoro-deoxyglucose uptake in xenograft breast cancer mouse model.

Together, our findings demonstrate an important role of the tumor suppressor 14-3-3 σ in controlling glycolysis and energy production in cancer cells.

Results

14-3-3 σ reduces glucose consumption

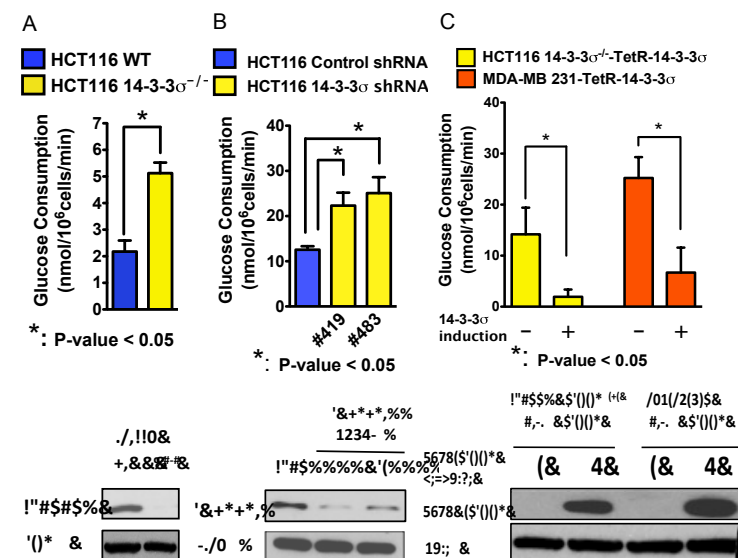


Fig 1. (A) Loss of 14-3-3 σ enhances glucose consumption. Glucose consumption of human colon carcinoma cells HCT116 and HCT116 14-3-3 σ ^{-/-} were measured using Freestyle Glucose Meter (Abbott) for 24 hours. **(B) Glucose consumption increases upon 14-3-3 σ knockdown.** 14-3-3 σ was knockdown in HCT116 cells using lentiviral shRNA (Sigma). Control cells were infected with Luciferase shRNA for comparison. **(C) Inducible re-expression of 14-3-3 σ diminishes glucose consumption.** 14-3-3 σ -deficient cells HCT116 14-3-3 σ ^{-/-} and MDA-MB-231 were infected with a retroviral Tet On Flag-14-3-3 σ system. 14-3-3 σ induction was done for 48 hours with 20 ng/ml Doxycycline. Non-induced cells were used as control. Each experiment has been independently repeated 6 times. Error bars represent 95% Confidence Intervals. *: P-value is less than 0.05.

14-3-3 σ suppresses glucose uptake

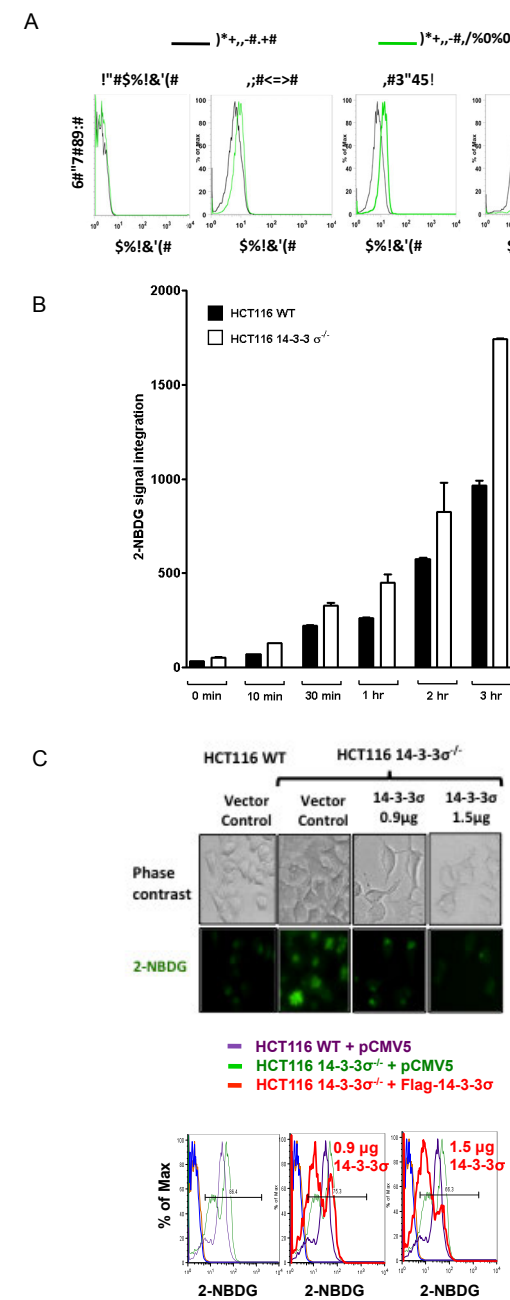


Fig 2. (A & B) Loss of 14-3-3 σ enhances glucose uptake. HCT116 and HCT116 14-3-3 σ ^{-/-} cells were incubated in glucose-free DMEM containing 120 μ M 2-NBDG for 0, 10 minutes, 1 hr, and 3 hr. 2-NBDG uptake was analyzed by fluorescence microscope (Olympus) and BD flow cytometer. **(C) Re-expression of 14-3-3 σ reduces glucose uptake in a dose dependent manner.** HCT116 14-3-3 σ ^{-/-} cells were transfected with increasing doses of Flag-14-3-3 σ . pCMV5 plasmid transfection served as control. All cells were incubated in glucose-free DMEM with 120 μ M 2-NBDG for glucose uptake measurement. 2-NBDG signals were measured by fluorescence microscope (Olympus) and flow cytometer (BD Bioscience). All error bars are 95% Confidence Intervals. Each experiment has been independently repeated 6 times.

14-3-3 σ causes lactate production decline

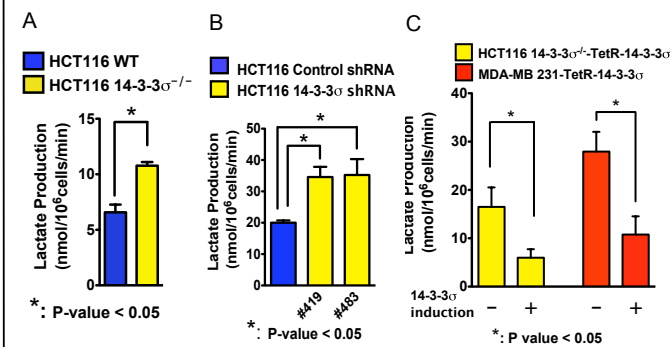


Fig 3. (A) Loss of 14-3-3 σ increases lactate production. (B) Lactate production increases upon 14-3-3 σ knockdown. 14-3-3 σ was knockdown using lentiviral shRNA (Sigma). Control cells were infected with Luciferase shRNA for comparison. **(C) Inducible re-expression of 14-3-3 σ diminishes lactate production.** 14-3-3 σ -deficient cells HCT116 14-3-3 σ ^{-/-} and MDA-MB-231 were infected with a retroviral Tet On Flag-14-3-3 σ system. 14-3-3 σ induction was done for 48 hours with 20 ng/ml Doxycycline. Non-induced cells were used as control. All error bars represent 95% Confidence Intervals. Each experiment has been independently repeated 6 times. *: P-value is less than 0.05.

14-3-3 σ decreases Extracellular Acidification Rate

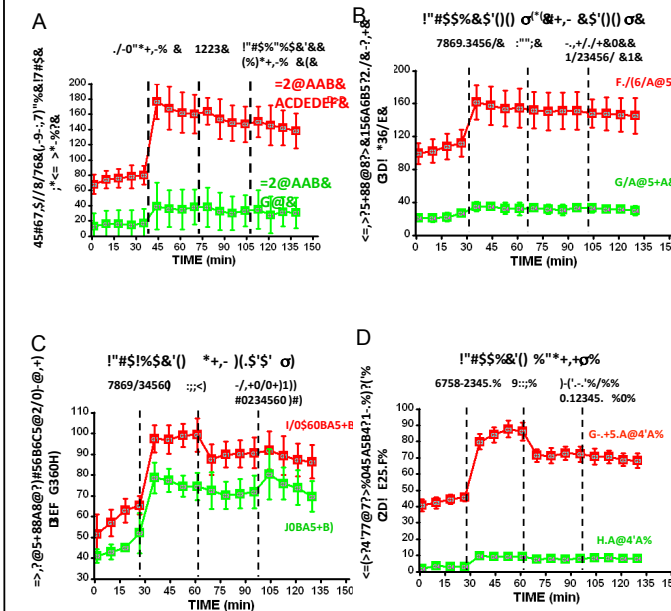


Fig 4. (A) Loss of 14-3-3 σ enhances Extracellular Acidification Rate. Extracellular Acidification Rate was measured using Seahorse Extracellular Flux Analyzer XF24 following Seahorse Bioscience's instructions. 5 μ M Oligomycin (inhibitor of F₀/F₁ ATP synthase), 1 μ M FCCP (ionophore), 5 μ M Rotenone (inhibitor of mitochondrial complex I) and 5 μ M Antimycin A (inhibitor of mitochondrial complex III) were sequentially added into culture medium to study the impact of 14-3-3 σ on cancer metabolism. **(B, C, D) Inducible re-expression of 14-3-3 σ in HCT116 14-3-3 σ ^{-/-} TetR 14-3-3 σ , MDA MB 231 TetR 14-3-3 σ and H1299 TetR 14-3-3 σ reduces Extracellular Acidification Rate.** HCT116 14-3-3 σ ^{-/-} TetR 14-3-3 σ , MDA MB 231 TetR 14-3-3 σ and H1299 TetR 14-3-3 σ cells that carried a retroviral Tet On Flag-14-3-3 σ system were treated with 20 ng/ml Doxycycline for 24 hours to induce Flag-14-3-3 σ expression. Non-induced cells were used as control. Extracellular Acidification Rate was measured as described in figure 4A. All error bars represent 95% Confidence Intervals. Each experiment has been independently repeated 5 times.

14-3-3 σ diminishes ATP level in cancer cells

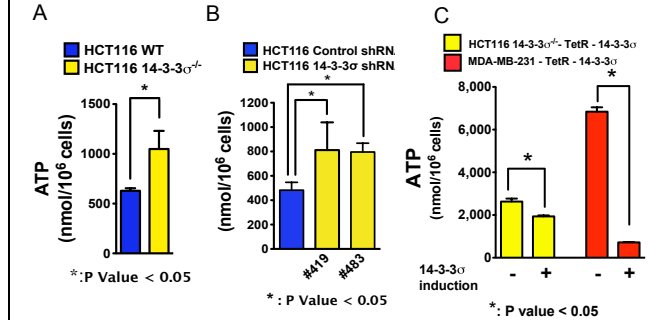


Fig 5. (A) Loss of 14-3-3 σ increases ATP concentration. ATP concentrations of HCT116 and HCT116 14-3-3 σ ^{-/-} cell lysates were measured with ATP Bioluminescence CLS II Kit (Roche). **(B) 14-3-3 σ knockdown increases ATP level.** **(C) Inducible re-expression of 14-3-3 σ significantly reduces ATP concentration.** 14-3-3 σ -deficient cells HCT116 14-3-3 σ ^{-/-} and MDA-MB-231 were infected with a retroviral Tet On Flag-14-3-3 σ system. 14-3-3 σ induction was done for 48 hours with 20 ng/ml Doxycycline. Non-induced cells were used as control. All error bars are 95% Confidence Intervals. Each experiment has been independently repeated 6 times. *: P-value is less than 0.05.

14-3-3 σ impairs glucose uptake *in vivo*

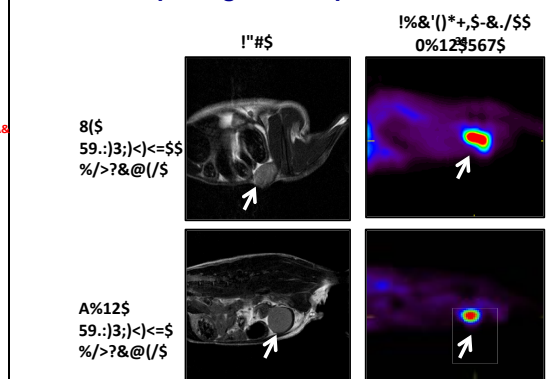


Fig 6. 14-3-3 σ reduces ¹⁸Fluoro-deoxyglucose (¹⁸FDG) uptake in xenograft breast cancer mouse model. 20 7-week-old female nude mice were injected with 1x10⁶ MDA-MB-231 TetR 14-3-3 σ (Tet On) breast cancer cells into mammary fat pad. After 4 weeks, mice were split into 2 groups. Experimental group was treated for 1 week with drinking water containing 200 μ g/ml Doxycycline and 5% sucrose to induce Flag 14-3-3 σ expression. Control group only received drinking water containing 5% sucrose. Tumors were visualized with Burker 7.0 Tesla MRI at Small Animal Imaging Facility (SAIF), M.D. Anderson Cancer Center. To measure *in vivo* glucose uptake assay, all mice were imaged with MicroPET Rodent -R4 Scanners 30 minutes post-injection with 400 μ Ci ¹⁸FDG according to imaging protocol from SAIF. Arrows indicate the tumor positions.

Conclusions

In summary, our data show that 14-3-3 σ has a strong suppressive impact on cancer glycolysis by repressing glucose uptake, glucose consumption, lactate production and impairs ATP production in cancer cells.

References

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