RIP140 inhibits glycolysis-dependent proliferation of breast cancer cells by regulating GLUT3 expression through transcriptional crosstalk between hypoxia induced factor and p53

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RIP140/NRIP1

The transcriptional co-regulator RIP140 represses the activity of transcription factors that drive cell proliferation and metabolism and plays a role in mammary tumorigenesis. **Does it play a role in cancer cell metabolism?**

RIP140 inhibits cell proliferation and tumor growth

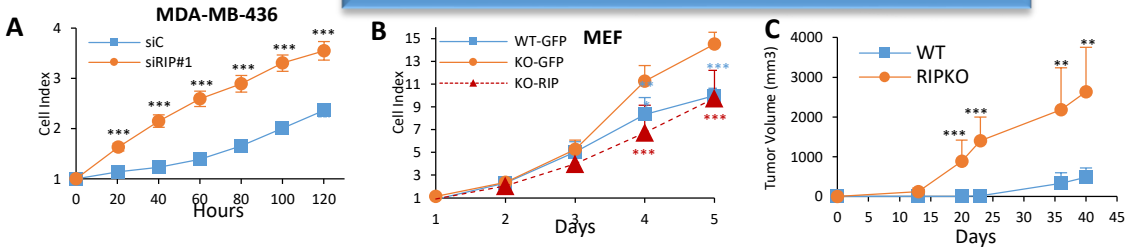

Fig. 1. A-B) Cell proliferation monitored by xCELLigence of breast cancer cells MDA-MB-436 after silencing RIP140 by siRNA (A), MEF stably overexpressing GFP or RIP140 (B). C) Tumor volume after xenograft of transformed MEF into Nude mice (n=6).

RIP140-deficient cells are more sensitive to glycolysis inhibition

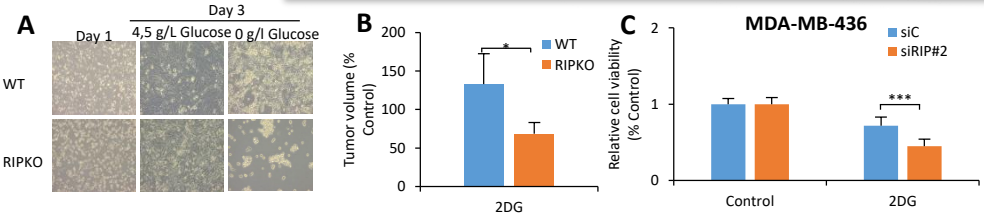

Fig. 2. A) Representative pictures of MEF #1 under glucose starvation. B) Tumor volume after xenograft of transformed MEF into Nude mice after eighteen days of 2DG (20mg/g) administrated intra-peritoneally every other day. C) Cell viability assessed by crystal violet staining of MDA-MB-436 after RIP140 silencing by siRNA at day seven and represented as percent of control after 2DG (5mM) treatment

RIP140-deficient cells are glycolytic

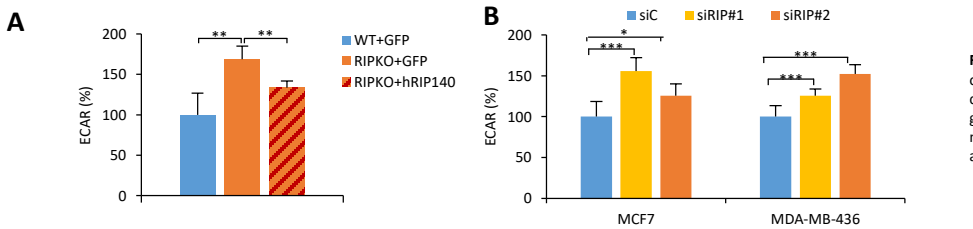

Fig. 3. A) Glycolysis measured by Seahore Analyzer in MEF overexpressing or not hRIP140 and represented by ΔECAR calculated as the difference between basal ECAR and ECAR after-glucose injection and normalized by that in MEF WT. B) Glycolysis measured in breast cancer cell lines MCF7 and MDA-MB-436 after RIP140 silencing by siRNA and represented as in A.

GLUT3 is essential for the growth advantage of RIP140-deficient cells

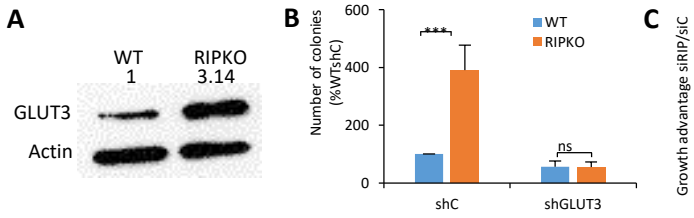

Fig. 4. A) Protein expression level of GLUT3 and Actin quantified by western blot in MEF. B) Number of colonies of transformed MEF #1 stably expressing shGLUT3 and expressed as percent of WT shControl (shC). C) Cell proliferation measured by MTT assay of MEF #1 stably expressing an shRNA Control (shC) or a specific shRNA targeting Glut3 (shGLUT3) and normalized by that of MEF WT at day 1 for each shRNA.

RIP140 and p53 inhibit the expression of GLUT3 induced by HIF-2α

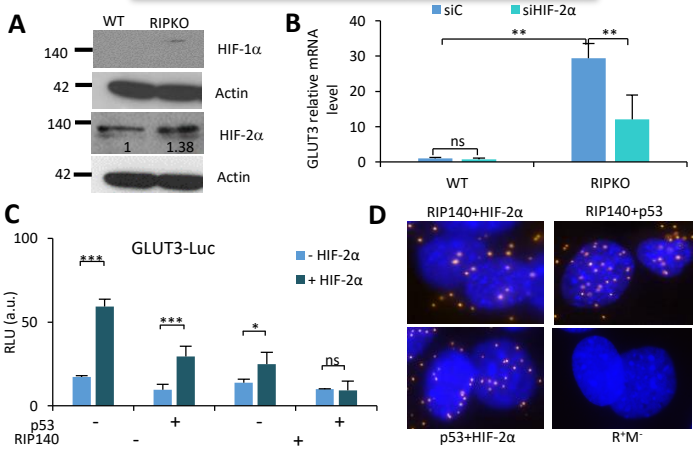

Fig. 5. A) HIF-1α and HIF-2α protein level quantified by western blot in MEF in normoxia (21% O2). B) *GLUT3* mRNA level quantified by RT-qPCR in MEF #1 48h after HIF-2α silencing by siRNA. C) Luciferase assay in MEF KO transiently transfected with TK-Renilla, GLUT3-Luc reporter plasmids, HIF-2α and p53 expression plasmids in combination with c-myc-RIP140 vector. Luciferase values were normalized to the renilla luciferase control. D) Proximity ligation assay in MEF WT between RIP140 and HIF-2α, RIP140 and p53 and p53 and HIF-2α to detect endogenous proteins.

The prognostic value of RIP140 is correlated with the levels of GLUT3 expression

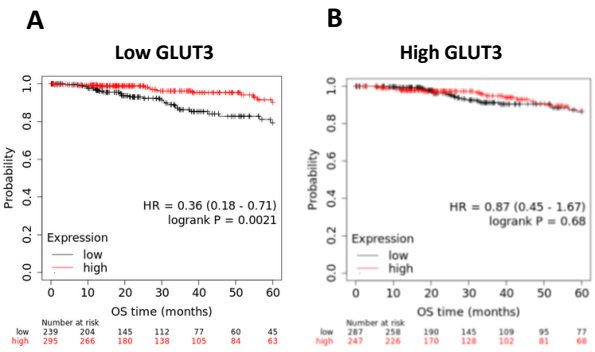

Fig. 6. A) GLUT3 expression groups have been defined on the basis of the median GLUT3 expression. Kaplan-Meier curves showed better overall survival rates for breast cancer patients with high RIP140 expression in low GLUT3 expression group (P=0.0021). B) On the contrary, RIP140 prognostic value was not significant in high GLUT3 expression group (P=0.68)