Supplementary Material

Glut1 promotes cell proliferation, migration and invasion by regulating epidermal growth factor receptor and integrin signaling in triple-negative breast cancer cells

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Running Title: Glut1 regulates tumorigenesis of breast cancer

Supplementary MATERIALS AND METHODS

Glucose uptake

Cells were seeded at 5×10^3 cells/well in 96-well plates. After 48 h, the levels of glucose uptake were measured with a Glucose Uptake Cell-Based Assay Kit (Cayman Chemical, Ann Arbor, MI, USA) according to the manufacturer's protocol. Relative fluorescence units were determined at 485-535 nm using a Varioskan Flash (Thermo, Waltham, MA, USA).

ATP assays

Cellular levels of ATP were determined with a CellTiter-Glo Luminescent Cell Viability Assay (Promega). Cells were seeded at 5×10^3 cells/well in 96-well plates. After 24, 48, or 72 h, 100 µl of CellTiter-Glo® Reagent was added to each well without removing the cell culture medium and incubated for 15 min at room temperature. Cellular ATP levels were measured by luminescence using a Varioskan Flash.

Lactate production

Cells were seeded in 100-mm dishes at a density of 1×10^6 cells/dish and the levels of lactate production were examined with a Lactate Assay Kit (Biovision, Milpitas, CA, USA). After 24 h, the culture medium was replaced with FBS-free DMEM. After further incubation for 8 h, cultured media were harvested and lactate levels in the media were analyzed by colorimetric assay. Optical density was measured at 590 nm using a Multiskan EX (Thermo).

LDH zymography

Proteins were prepared from each cell line and 30-µg samples were subjected to native gel electrophoresis. After electrophoresis, the gels were stained in 10 ml of solution containing

lithium lactate, NAD, Tris-HCl (pH 8.0), NaCl, $MgCl_2$, phenazine methosulfate (PMS), and nitroblue tetrazolium (NBT) for 10 min at room temperature.

Supplementary Figure legend

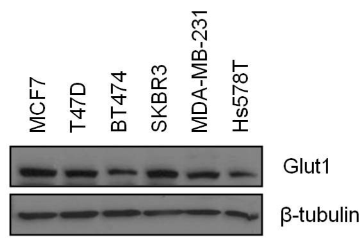
Supplementary Figure S1. Expression of Glut1 according to breast cancer subtype

Total cell lysates were prepared from MCF7, T47D, BT474, SKBR3, MDA-MB-231 and Hs578T cells and subjected to Western blot analyses with Gut1 and β-tubulin antibodies.

Supplementary Figure S2. Effects of Glut1 silencing on proliferation of MCF7 and T47D cells

Control shRNA cells (MCF7 Cont sh and T47D Cont sh) and Glut1 shRNA cells (MCF7 Glut1 sh and T47D Glut1 sh) were seeded at 1×10^4 cells/well in 12-well plates and counted with a hemocytometer over 4 days ($*p<0.05$).

Supplementary Fig.S1



Supplementary Fig.S2