Development of a Multiplex Panel of Biomarkers to Assess Energy Metabolism in Cancer

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Abstract

Cancer cells have an altered glucose metabolism that not only permits them to survive under hypoxic conditions, but also confers a distinct growth advantage by sustaining higher proliferation rates, invasiveness, and subsequent distant metastasis. This unique difference in glucose metabolism between healthy and cancerous cells opens up a selective mechanism for killing cancer cells that bypasses normal healthy cells. Consequently, several enzymes in glucose metabolism are attractive targets for the development of first-in-class therapeutic agents. We describe the development of a multiplex panel comprising biomarkers involved in glucose metabolism to support drug development and to interrogate the glycolytic pathway in patients with cancer.

Results

Specific inhibitors of PKM2 and LDHA were in fractionated cell extracts from phycoreythrin capture recombinant HK2, LDHA, and PKM2 specific to HK2. Immunoassay Materials

Materials and Methods

Immunodetection and Antibodies. Antibodies specific to HK2, PKM2, and LDHA were coupled to MagPlex. Luminex beads using MiniG™ Coupling Reagent (Antec Diagnostics, Braintree, MA). Full-length recombinant HK2, LDHA, and PKM2 proteins were produced in both bacterial and HEK293 cells. Reporter antibodies specific to an epitope distinct from the capture antibodies were labeled with phycoerythrin using Physochrome Physico Kit (Hayward, CA) and used for multiplex immunoassay detection. The multiplex panel is designed to measure protein concentrations and protein-protein interactions from fresh-frozen tumor biopsies. Specific inhibitors of PKM2 and LDHA were used in vitro to demonstrate the utility of the multiplex panel.

Cell lines. Human cancer cell lines HepG2, HCT116, A431, ANTs7, MCF7, Jurkat, and SW620 were obtained from American Type Culture Collection (ATCC) or the National Cancer Institute (NCI). SW620 cells were obtained from the Culture Collection (A TCC) or the Division of Cancer Treatment & Diagnosis Repository, National Cancer Institute (NCI). All cancer cell lines were maintained in accordance with ATCC recommendations.

Tumor extracts preparation. Sample handling and preparation were developed to generate cytosolic and mitochondrial (+/−) fractions of the tissue that maintain the non-covalent association of different proteins during the assays. Assay optimization and analytical performance. Assay optimization and analytical validation are ongoing using tumor cell lysates, to address reproducibility, recovery, and sample stability.

PKM2 also functions as a transductional co-activator by translocating to the nucleus to regulate the transcription of downstream genes including cancer, NADP+, and MEK5. We interrogated the mechanism by measuring PKM2 levels in cells treated with an MEK inhibitor.12 NSC 772991 (compound 1, 100 μM) was used to show modulation of PKM2. (C) PKM2 i+ir, PKM2 i+ir, HCT116 cells treated with NSC 772991, were significantly lower compared to vehicle treated samples. (D) PKM2 i+ir, HCT116 cells treated with NSC 772991 were significantly lower compared to vehicle treated samples.

Nuclear Functions of PKM2

PKM2 functions as a transductional co-activator by translocating to the nucleus to regulate the transcription of downstream genes including cancer, NADP+, and MEK5. We interrogated the mechanism by measuring PKM2 levels in cells treated with an MEK inhibitor.12 NSC 772991 (compound 1, 100 μM) was used to show modulation of PKM2. (C) PKM2 i+ir, PKM2 i+ir, HCT116 cells treated with NSC 772991, were significantly lower compared to vehicle treated samples. (D) PKM2 i+ir, HCT116 cells treated with NSC 772991 were significantly lower compared to vehicle treated samples.

References