Global Profiling of Metabolic Adaptation to Hypoxic Stress in Human Glioblastoma Cells

Glioblastoma

• Common brain tumor
• Hypoxia, vascular hyperproliferation & therapy resistance
• Even with surgery, radiation & chemotherapy – median lifespan is 15 months

http://www.aboutcancer.com/gbm_progression.jpg
Hypoxia & Tumors

• Hypoxic microenvironment associate with
  – Invasion
  – Metastasis
  – Tumor recurrence
  – Decreased survival
  – Resistance to chemoradiotherapy
Hypoxia & Tumors

• Tumor metabolism changes under hypoxia
  – Shift from oxidative phosphorylation to anaerobic glycolysis
  – Increased synthesis of glycogen, lipids and phosphorylated lipid metabolites
Goal of the study

• Use metabolomics to ID the metabolic “Achilles heel” of cancer cells
• Coupled metabolomic and gene profiling to investigate metabolic response to hypoxic stress in human Glioblastoma cells
Methods - Cells

U-87 cells

InVitro₂ Hypoxia Work station

Hypoxia (1% O₂)
- 6h
- 24 h
- 48 h
- Trypsinized, centrifuged, flash frozen

Normoxia (21% O₂)

• Media and cells
Methods - Metabolites

6 samples
Each O₂ treatment

- Thawed on ice, protein precipitated with methanol, recovery standards added, freeze dried

Non-targetted

- UHPLC/MS-MS2
  - Positive ion mode
  - 0.1% formic acid
- UHPLC/MS-MS2
  - Negative ion mode
  - 6.5 mM Ammonium bicarbonate pH 8

GC/MS
- Derivatized under N2 with bistrimethyl-silyl-trifluoroacetamide (MSTFA)
Methods – mass spectrometry

- UHPLC/MS
  - Waters Acquity UHPLC with an LTQ mass spec
  - Electrospray ionization (ESI) with linear ion-trap (LIT) mass analyzer
  - Gradient eluted over 11 minutes
  - Flow rate: 350 ul/min
  - MS – 900-1000 m/z
  - MS2 scans – data dependent using dynamic exclusion
Methods – mass spectrometry

• GC/MS
  – 5% phenyldimethyl silicone column
  – Helium carrier case
  – Temp ramp 40-300°C over 16 minutes
  – Analyzed on a Thermo-Finnigan Trace DSQ MS
  – 50-750 atomic mass unit scan range
Metabolite Analysis

• Data extraction – peaks ID using Metabolon peak integration software
• Compound ID – compared to LIMS library
• Stats – used R, log-transformed, performed Welch 2-sample T-test, used FDR q-values
Gene expression

- RNA extracted with TRIzol Reagent
- 3 samples of each treatment – BeadChip
- Data filtered & normalized with BASE2
- Analysis with R, p value < 0.01
- Looked at transcripts differentially expressed between hypoxic and normoxic
Results & Discussion
Fig 1. The metabolic phenotype of hypoxic glioblastoma cells.

http://127.0.0.1:8081/plosone/article?id=info:doi/10.1371/journal.pone.0116740
Fig 1. The metabolic phenotype of hypoxic glioblastoma cells.

PDK3 = pyruvate kinase dehydrogenase enzyme 3

GLUT = Glucose transporter, transport glucose over plasma membrane

MCT4 - Monocarboxylate transporter 4 solute carrier

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Fig 2. Altered glucose shunting in hypoxic GBM cells.

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Alternative pathway to glucose – polyol pathway

FUT11 - Fucosyltransferase 11
Implicated in HIF pathway

http://127.0.0.1:8081/plosone/article?id=info:doi/10.1371/journal.pone.0116740
Fig 3. Hypoxic effects on the levels of nucleotide cofactors NAD and NADP.

- Enhanced *de novo* synthesis of NAD in hypoxic cells


http://127.0.0.1:8081/plosone/article?id=info:doi/10.1371/journal.pone.0116740
Fig 4. Hypoxic effects on TCA cycle and glutamine metabolism in GBM cells.

http://127.0.0.1:8081/plosone/article?id=info:doi/10.1371/journal.pone.0116740
• Less TCA intermediates with prolonged hypoxia
Fig 5. Hypoxic accumulation of cholesterol precursors in GBM cells.

- Conversion to cholesterol requires oxygen

http://127.0.0.1:8081/plosone/article?id=info:doi/10.1371/journal.pone.0116740
Fig 6. Effects of hypoxia on glycerolipid metabolism in GBM cells.

http://127.0.0.1:8081/plosone/article?id=info:doi/10.1371/journal.pone.0116740
Hypoxia results in accumulation of dipeptides and amino acids with post-translational modifications.
Conclusions

• Tumor samples contain higher levels of enzymes and other compounds associated with hypoxic pathways

• Metabolic studies helpful for therapy
  – Couple with magnetic resonance & positron emission tomography

• Important for understanding cancer cell adaptation to microenvironment