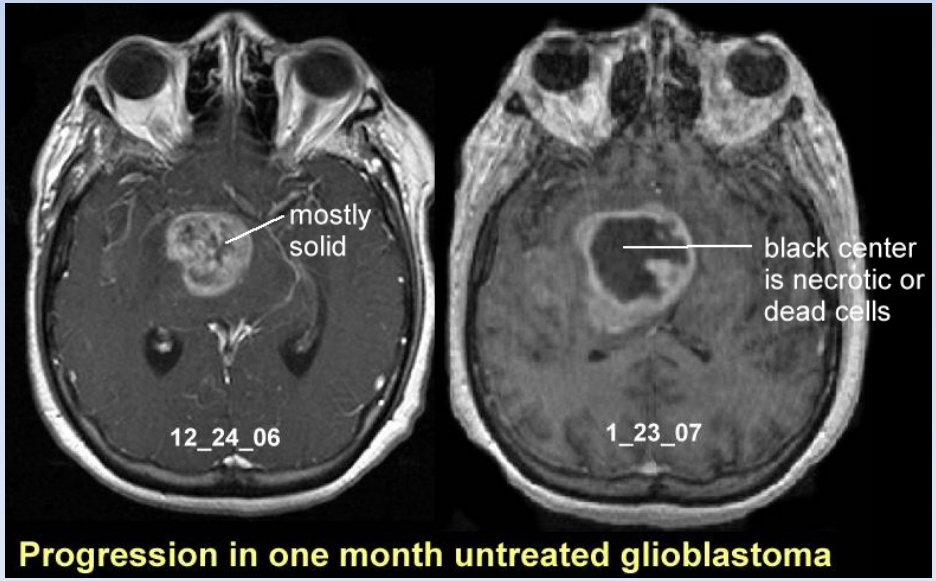
Global Profiling of Metabolic Adaptation to Hypoxic Stress in Human Glioblastoma Cells

Kucharzewska P, Christianson HC, Belting M (2015) Global Profiling of Metabolic Adaptation to Hypoxic Stress in Human Glioblastoma Cells. PLoS ONE10(1): e0116740. doi: 10.1371/journal.pone.0116740

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

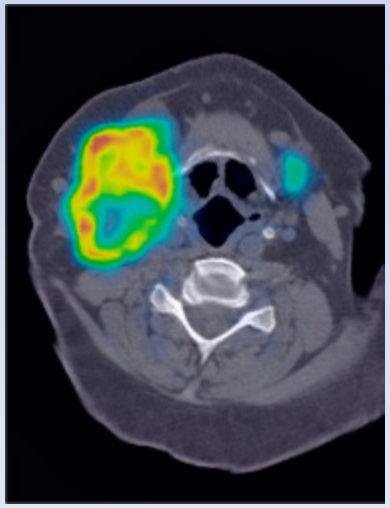
- Hypoxic microenvironment associate with
  - Invasion
  - Metastasis
  - Tumor recurrence
  - Decreased survival
  - Resistance to chemoradiotherapy

Hypoxia & Tumors

- Tumor metabolism changes under hypoxia
  - Shift from oxidative phosphorylation to anaerobic glycolysis
  - Increased synthesis of glycogen, lipids and phosphorylated lipid metabolites

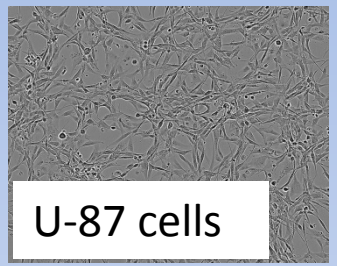
Goal of the study

- 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



http://www.med.wisc.edu

Methods - Cells



U-87 cells

InVitro$_2$ Hypoxia Work station

Hypoxia (1% O_2)

Normoxia (21% O_2)

6h

24 h

48 h

- Trypsinized, centrifuged, flash frozen

- Media and cells

Methods - Metabolites

6 samples
Each O_2
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-siyl-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

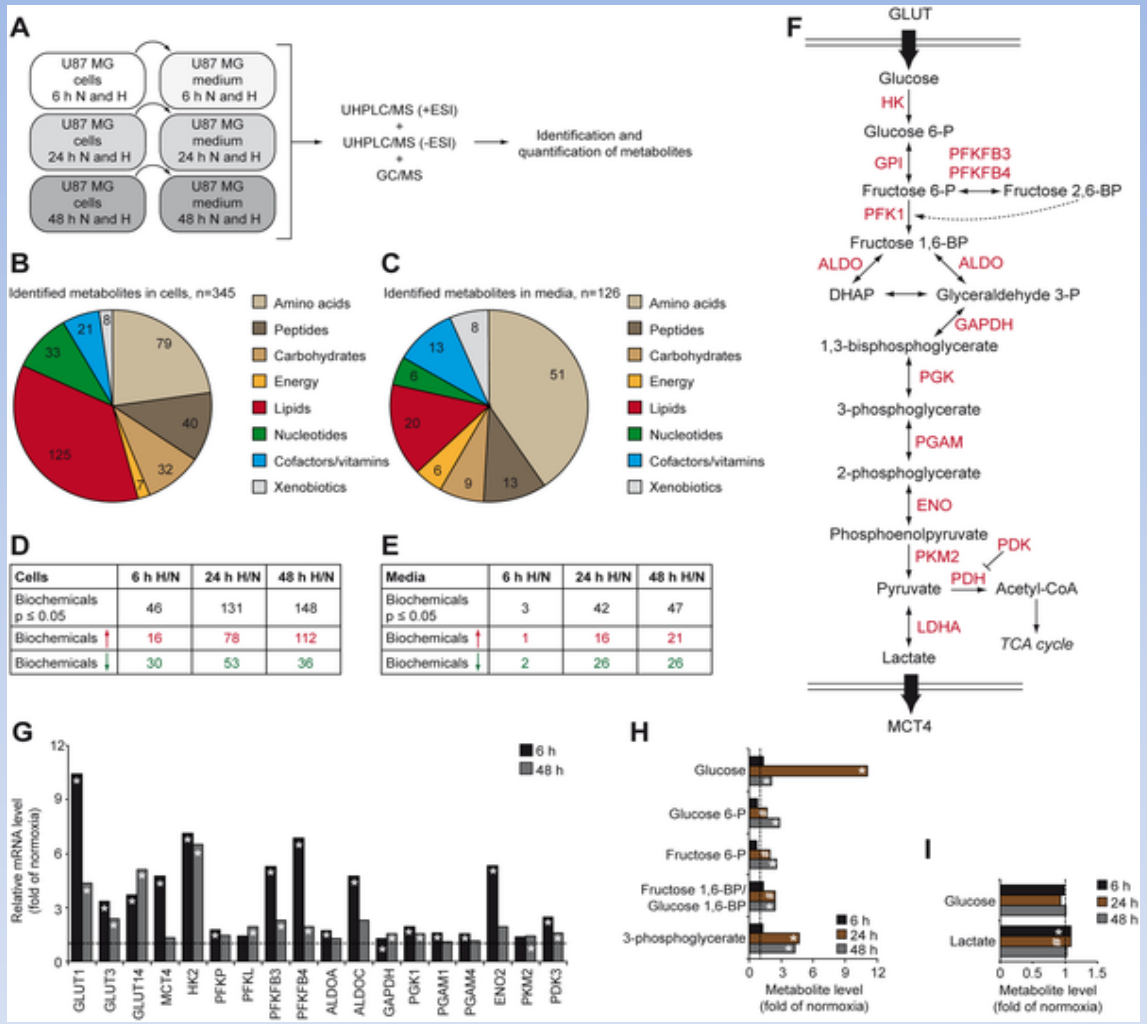
- Data extraction – peaks ID using Metabolon peak integration software
- Compound ID – compared to LIMS library
- Stats – used R, log-transformed, performed Welch 2-sample T-test, used FDR q-values

Gene expresssion

- 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

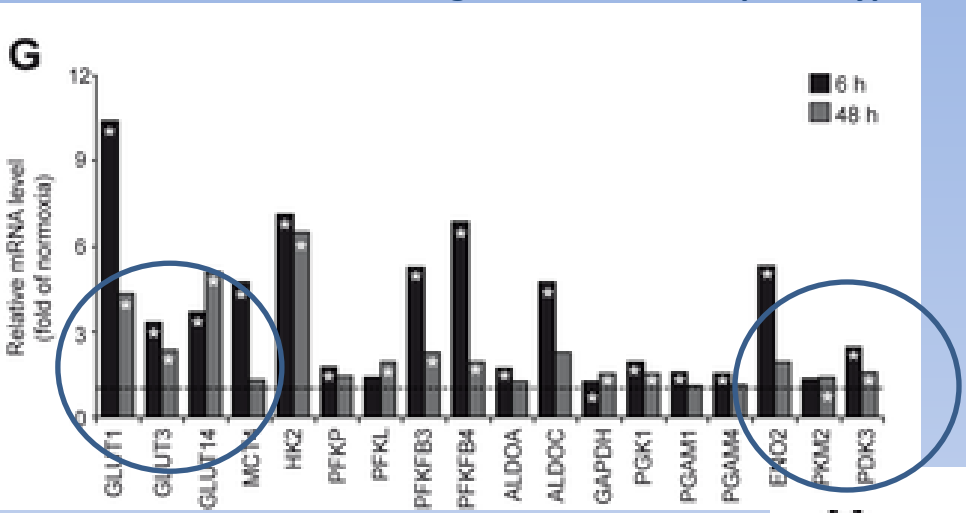
Results & Discussion

Fig 1. The metabolic phenotype of hypoxic glioblastoma cells.



Kucharzewska P, Christianson HC, Belting M (2015) Global Profiling of Metabolic Adaptation to Hypoxic Stress in Human Glioblastoma Cells. PLoS ONE 10(1): e0116740. doi:10.1371/journal.pone.0116740
http://127.0.0.1:8081/plosone/article?id=info:doi/10.1371/journal.pone.0116740

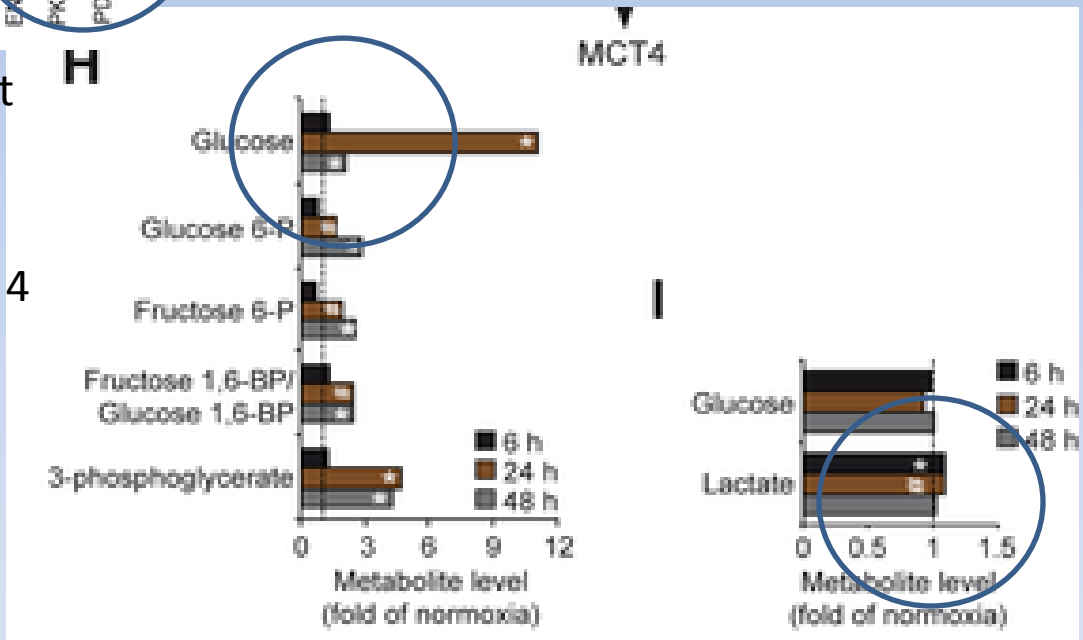
Fig 1. The metabolic phenotype of hypoxic glioblastoma cells.



PDK3 = pyruvate kinase dehydrogenase enzyme 3

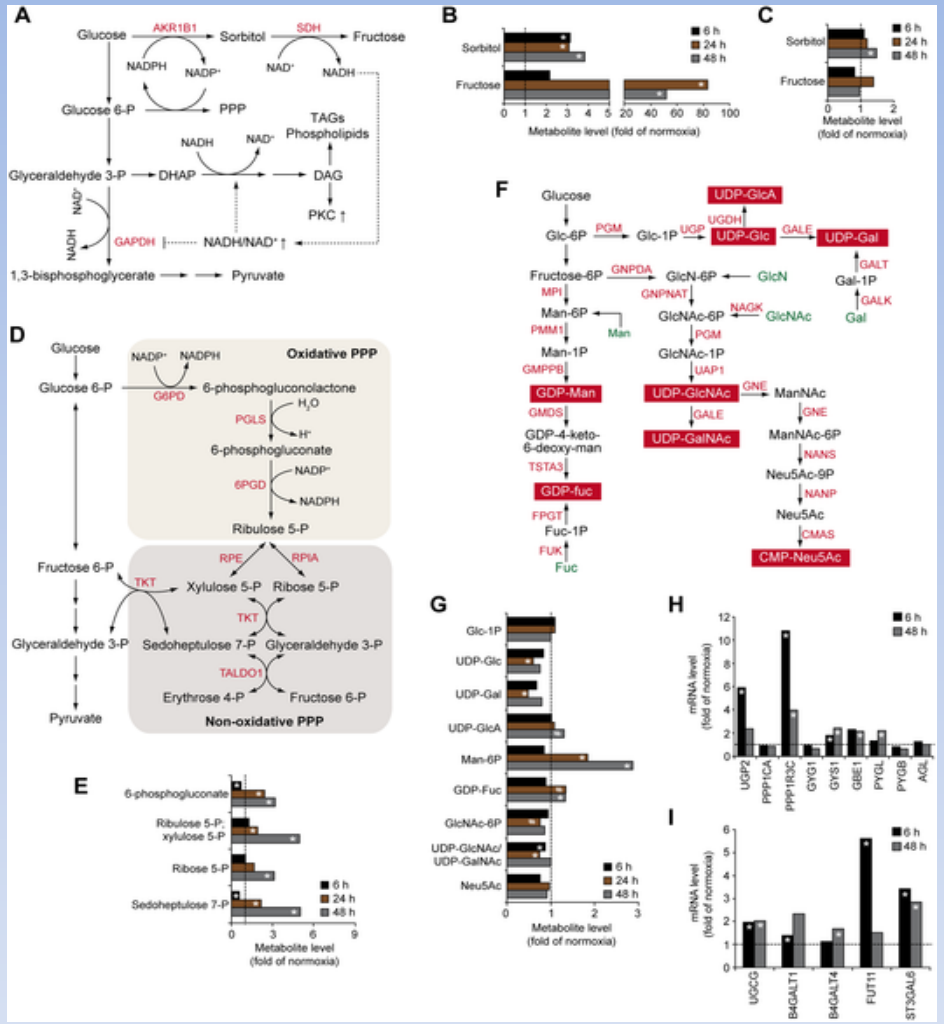
GLUT = Glucose transporter, transport glucose over plasma membrane

MCT4 - Monocarboxylate transporter 4 solute carrier



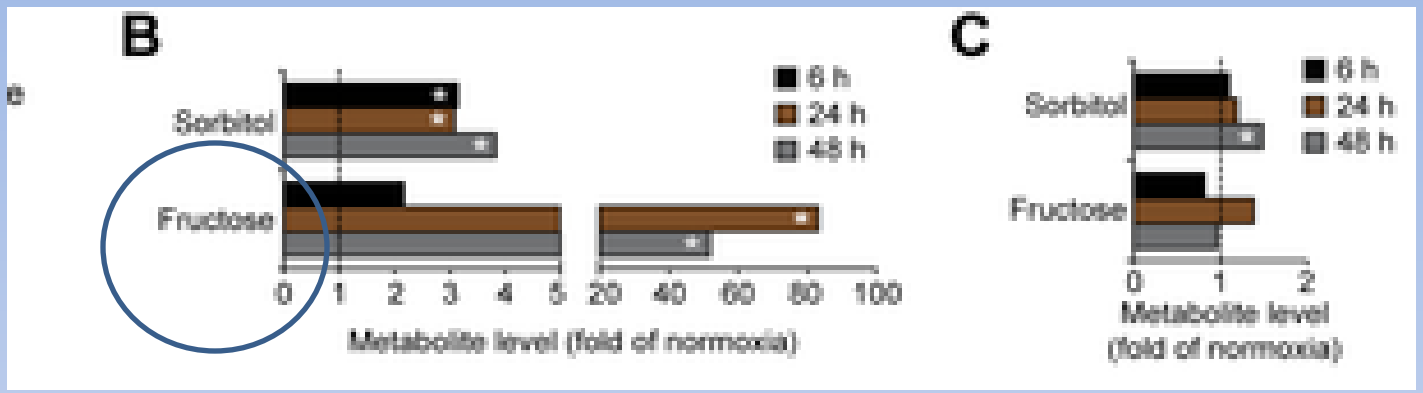
Kucharzewska P, Christianson HC, Belting M (2015) Global Profiling of Metabolic Adaptation to Hypoxic Stress in Human Glioblastoma Cells. PLoS ONE 10(1): e0116740. doi:10.1371/journal.pone.0116740
http://127.0.0.1:8081/plosone/article?id=info:doi/10.1371/journal.pone.0116740

Fig 2. Altered glucose shunting in hypoxic GBM cells.

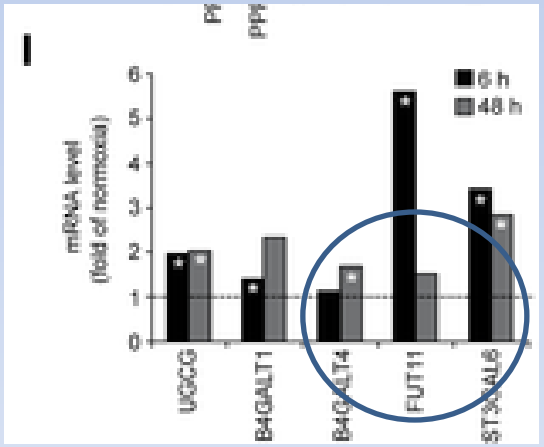


Kucharzewska P, Christianson HC, Belting M (2015) Global Profiling of Metabolic Adaptation to Hypoxic Stress in Human Glioblastoma Cells. PLoS ONE 10(1): e0116740. doi:10.1371/journal.pone.0116740
http://127.0.0.1:8081/plosone/article?id=info:doi/10.1371/journal.pone.0116740

Fig 2. Altered glucose shunting in hypoxic GBM cells.



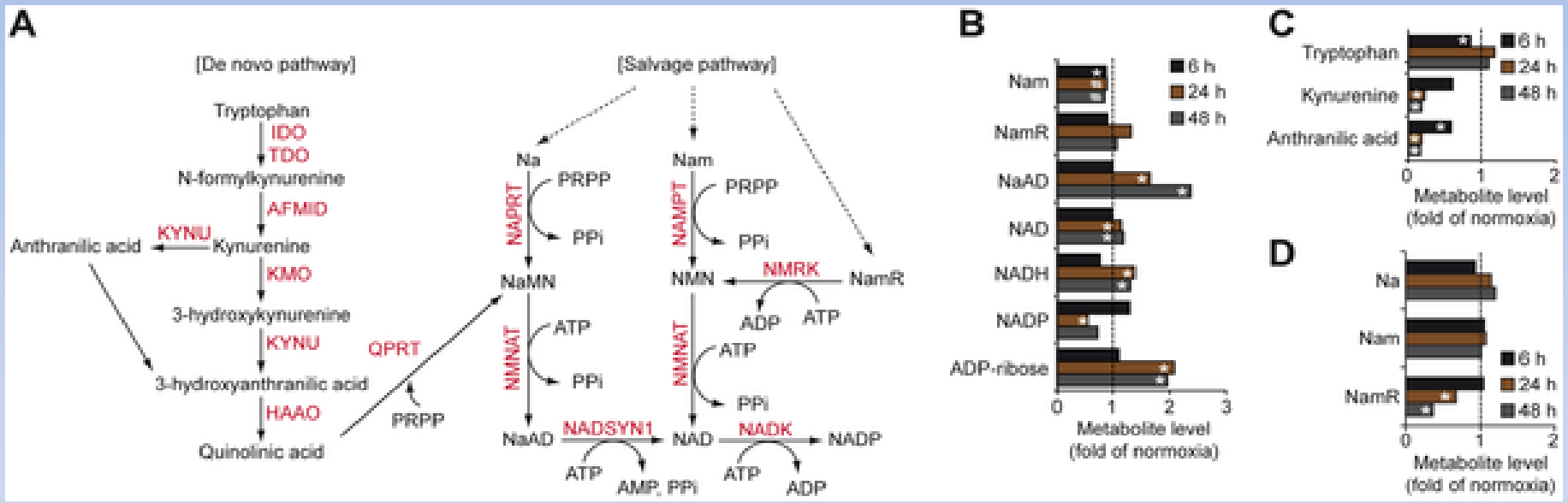
Alternative pathway to glucose – polyol pathway



FUT11 - Fucosyltransferase 11
Implicated in HIF pathway

Kucharzewska P, Christianson HC, Belting M (2015) Global Profiling of Metabolic Adaptation to Hypoxic Stress in Human Glioblastoma Cells. PLoS ONE 10(1): e0116740. doi:10.1371/journal.pone.0116740
http://127.0.0.1:8081/plosone/article?id=info:doi/10.1371/journal.pone.0116740

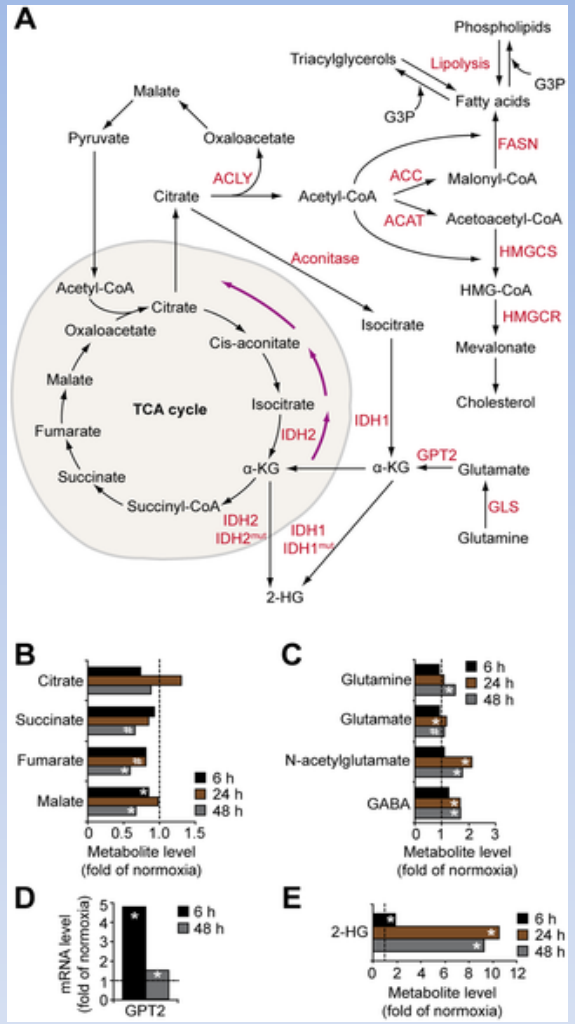
Fig 3. Hypoxic effects on the levels of nucleotide cofactors NAD and NADP.



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

Kucharzewska P, Christianson HC, Belting M (2015) Global Profiling of Metabolic Adaptation to Hypoxic Stress in Human Glioblastoma Cells. PLoS ONE 10(1): e0116740. doi:10.1371/journal.pone.0116740
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.



Kucharzewska P, Christianson HC, Belting M (2015) Global Profiling of Metabolic Adaptation to Hypoxic Stress in Human Glioblastoma Cells. PLoS ONE 10(1): e0116740. doi:10.1371/journal.pone.0116740
http://127.0.0.1:8081/plosone/article?id=info:doi/10.1371/journal.pone.0116740

- Less TCA intermediates with prolonged hypoxia

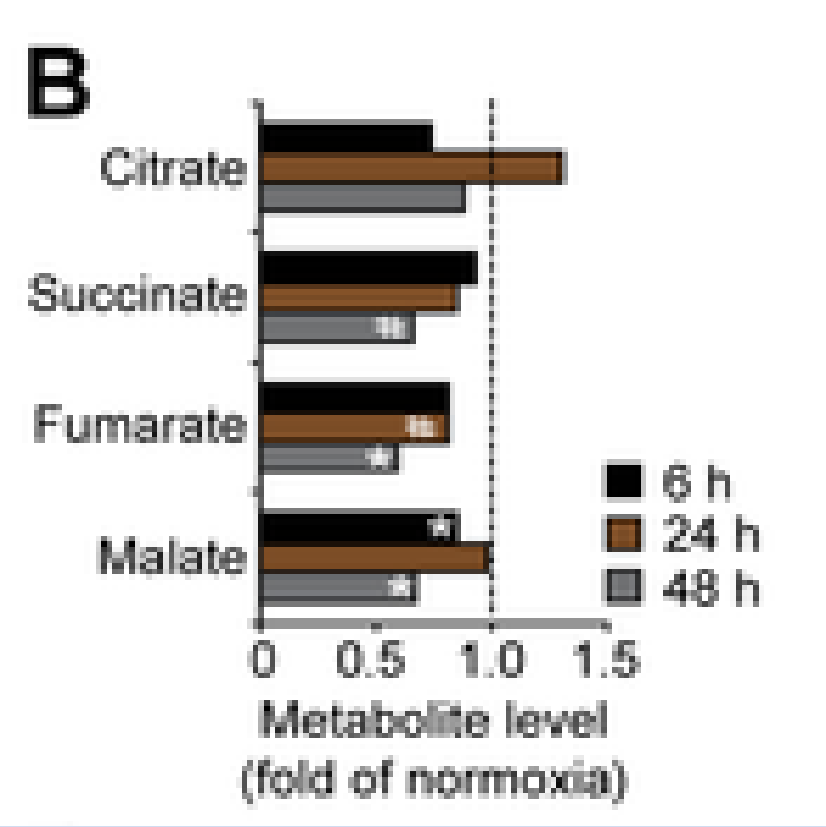
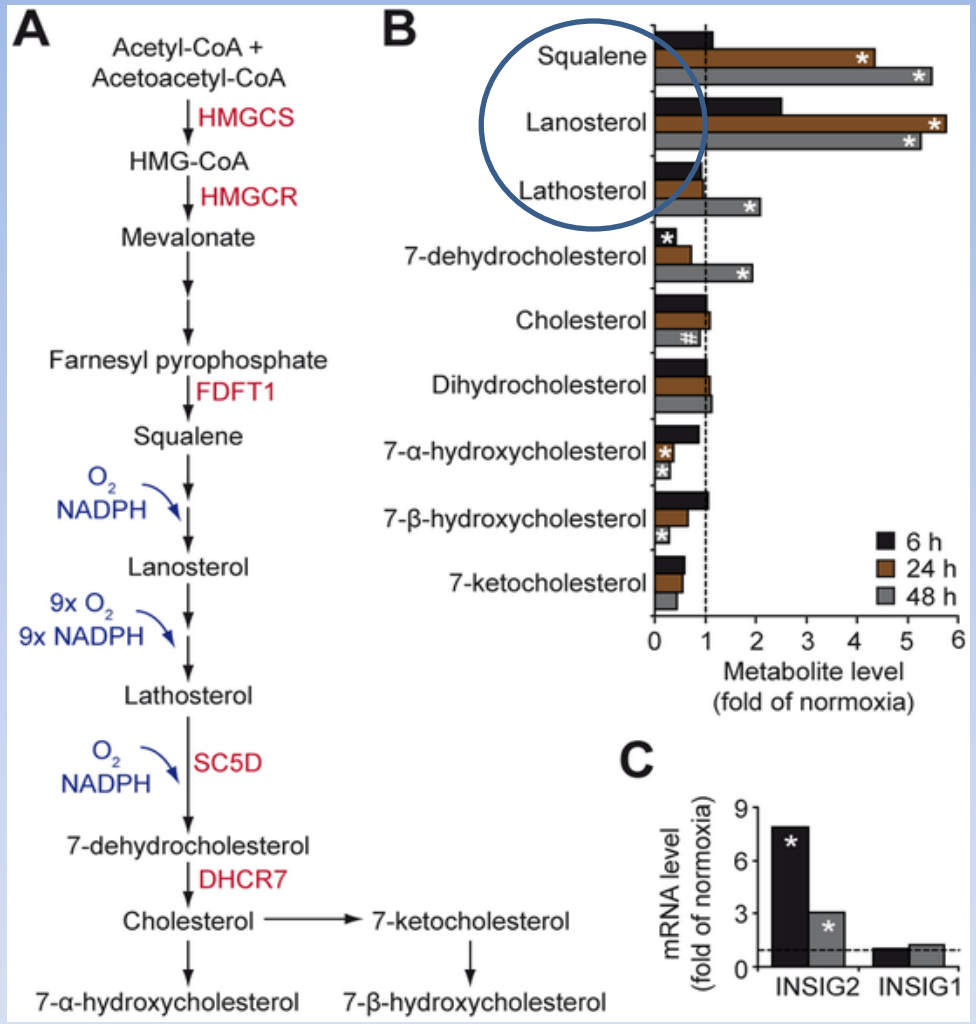


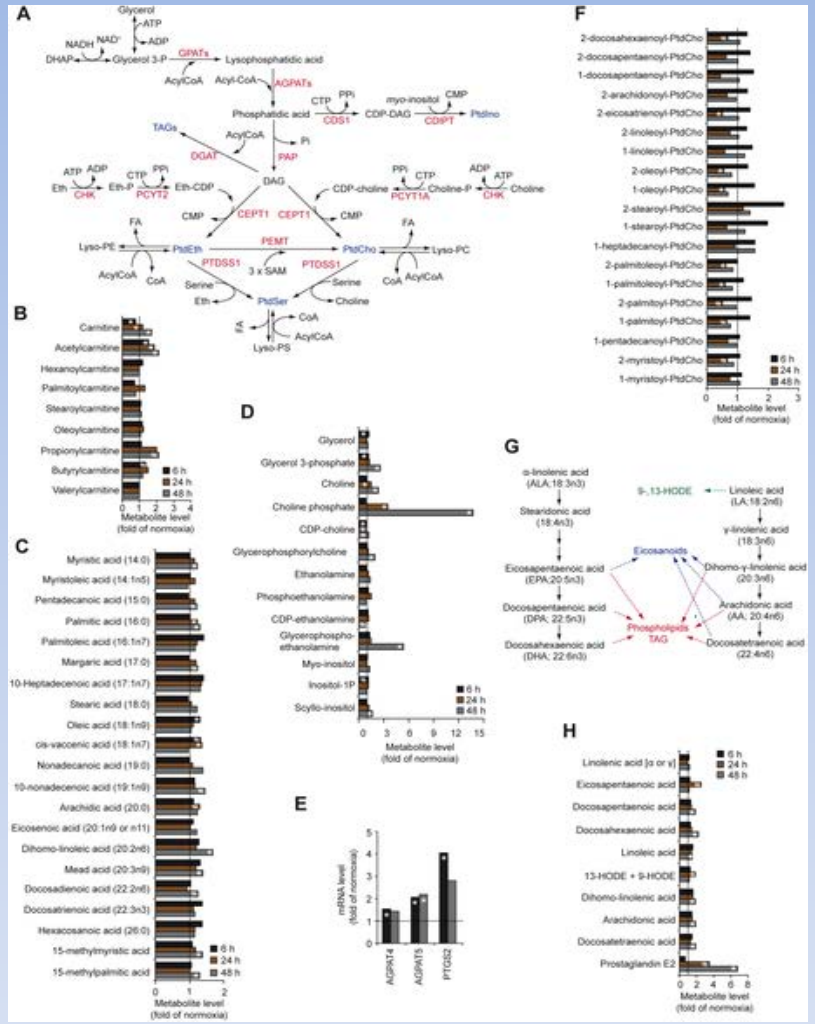
Fig 5. Hypoxic accumulation of cholesterol precursors in GBM cells.



- Conversion to cholesterol requires oxygen

Kucharzewska P, Christianson HC, Belting M (2015) Global Profiling of Metabolic Adaptation to Hypoxic Stress in Human Glioblastoma Cells. PLoS ONE 10(1): e0116740. doi:10.1371/journal.pone.0116740
http://127.0.0.1:8081/plosone/article?id=info:doi/10.1371/journal.pone.0116740

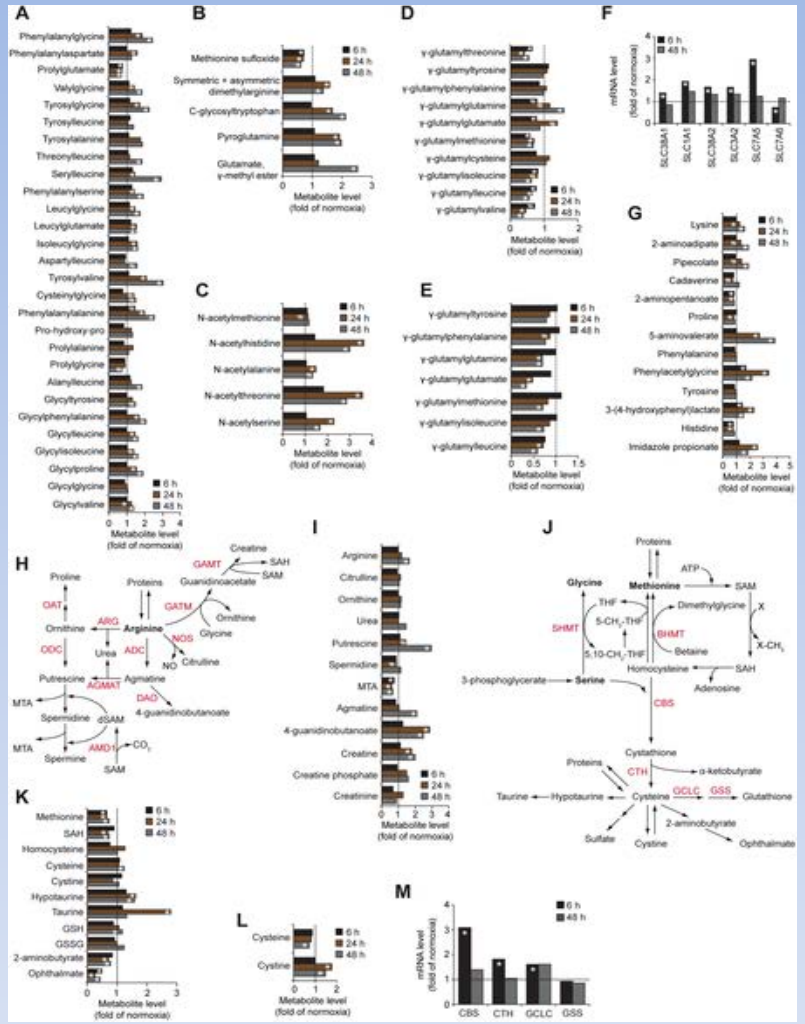
Fig 6. Effects of hypoxia on glycerolipid metabolism in GBM cells.



Kucharzewska P, Christianson HC, Belting M (2015) Global Profiling of Metabolic Adaptation to Hypoxic Stress in Human Glioblastoma Cells. PLoS ONE 10(1): e0116740. doi:10.1371/journal.pone.0116740
http://127.0.0.1:8081/plosone/article?id=info:doi/10.1371/journal.pone.0116740

Fig 7. Protein and amino acid metabolism in hypoxic GBM cells.

- Hypoxia results in accumulation of dipeptides and amino acids with post-translational modifications



Kucharzewska P, Christianson HC, Belting M (2015) Global Profiling of Metabolic Adaptation to Hypoxic Stress in Human Glioblastoma Cells. PLoS ONE 10(1): e0116740. doi:10.1371/journal.pone.0116740
http://127.0.0.1:8081/plosone/article?id=info:doi/10.1371/journal.pone.0116740

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