Metabolomics Analysis Reveals Distinct Profiles of Non-Muscle Invasive and Muscle-Invasive Bladder Cancer

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Bladder cancer

• Affects >400,000 people every year worldwide
  — Male : female ratio steady at 3:1

• **Risk factors** include smoking, chronic inflammation, radiation, aromatic amines, arsenic, genetics, low fluid intake

• Due to risk of multifocality and recurrence, requires lifelong patient follow-up
  • In the US, is considered one of the most expensive diseases

• Treatment is strongly influenced by grade and stage of the disease
  • Bacillus Calmette-Guérin indicated for high-grade NMIBC
  • Radical cystectomy with or without neoadjuvant therapy for MIBC
  • Metastatic disease treated with standard chemotherapy (cisplatin, MVAC), immunotherapy
Normal urothelium

FGFR3
KRAS
PIK3CA

Early alterations
(UPUMP, dysplasia)

9q-/9p-
TP53
RB1
Other

10-15%

Frequent recurrence (70%)

40-60%
Metabolomics in pathway activity assessment

• Comprehensive metabolite signature as indicator of biological activity in cells or tissue

• Has been applied to many cancer types to identify enzymes that could be potential therapeutic targets
  • May or may not reflect gene changes, but can be mapped back onto genomic and mRNA alterations

• Two prior studies in bladder cancer tissue have compared benign to cancer

• Additional studies have been performed on urine and serum

• We have focused on a comparison between normal, non-muscle invasive high-grade urothelial ca and muscle-invasive high-grade urothelial ca, as these represent major cut points for type of therapy offered and patient outcomes
Methods

• Data was obtained from a combined cohort of patients from two institutions (CCF, UTSW) – matched demographics

• Limited analysis to patients with pure urothelial carcinoma at radical cystectomy
  • 72 patients
  • 24 of these patients had matched normal controls

• Use of gas chromatography-mass spectrometry (GC/MS) and liquid chromatography-mass spectrometry (LC/MS) to quantify metabolites in snap-frozen, annotated tissue through collaboration with Metabolon

• Used Wilcoxon rank sum test and Student’s t-test comparisons to compare metabolite levels; $P<0.05$ considered significant

• Comparison was performed between:
  • Normal versus cancer
  • Non-muscle invasive versus muscle-invasive cancer
Global changes identified in multiple key pathways

• Metabolomics analysis detected 1146 biochemicals, including 613 named and 533 unnamed LC compounds

• Benign and tumor tissue were compared using matched pairs from and 513 metabolites were found to be significantly altered (matched pair t-test, $P \leq 0.05$)

• We subsequently used the second cohort of samples to validate results, as well as to compare NMIBC (pTa-T1) and MIBC (pT2-T4)

• Differential findings between both cohorts largely held true

• Most key metabolic pathways in carcinoma progression affected
Glucose metabolism shows a shift to glycolysis and glycogen synthesis via sorbitol pathway activity

- Most cancers generate energy via aerobic glycolysis with glucose utilized for glycolysis rather than oxidative phosphorylation (Warburg effect)
- G6P intermediate also appears to shunt into pentose phosphate pathway as well

Glucose $\rightarrow$ sorbitol $\rightarrow$ fructose $\rightarrow$ fructose-1-phosphate $\rightarrow$ G3P $\rightarrow$ **Glycolysis**
TCA cycle intermediates show increased late stage intermediates that may mean anaplerotic activity in cancer.
Lipid metabolism shows preferential formation of fatty acids, glycerophospholipids and sphingolipids.

**Sphingolipid Pathway**

- Sphingosine
- Sphingosine-1-P
- P-ethanolamine
- Ceramide
- Sphingomyelin
- Sphinganine

**Cell membranes**

**Lipid Signaling**

- Scaled Intensity
- (stearoyl form)
Additional metabolic differences evident in benign versus neoplastic categories

- Amino acid metabolism is elevated, further supporting a potential anaplerotic mechanism in bladder cancer and association with p53 function
  - Most amino acids were elevated
  - May reflect increased protein breakdown OR uptake

- Purine/pyrimidine metabolism displays enhanced production of deoxy-nucleotides and derivation of purine from de novo synthesis and catabolism
  - Both purine and pyrimidine metabolites were upregulated
  - Both de novo synthesis and synthesis from catabolic processes occurs
Energy status in bladder cancer

• Glycolysis is enhanced in cancer and increases glucose uptake, lactate production, increased citrate for the TCA cycle, and increased activity of the pentose phosphate pathway
  • This provides energy, fatty acids, nucleotide biosynthesis, and NADPH generation in the context of cancer growth
  • Many components of the glucose metabolomics pathway can be regulated by activity of p53

• Anaplerosis likely also replenishes TCA cycle intermediates
  • Process by which TCA cycle intermediates are formed from substrates such as amino acids and fatty acids
  • May be a complementary mechanism to enhance the TCA cycle

• Higher proliferative capacity in malignant cells is likely fueled through glucose and TCA cycle intermediate catabolism
Lipid metabolism in bladder cancer

• Elevation of virtually every form of lipid within malignant cells
  • Can be utilized for membrane components, energy sources, biochemical precursors, and signaling molecules in cancer cells
  • One possible explanation of elevated fatty acid levels in bladder cancer is that increased biosynthesis of fatty acids may be derived from citrate, which is elevated in bladder cancer
  • A second explanation may be increased rates of lipid membrane turnover and membrane remodeling in bladder cancer cells caused by increased proliferation or inflammation may produce higher fatty acid levels

• Decreased palmitoyl and stearoyl sphingomyelin could result from an increase in microvesicle formation
  • Reported to play critical roles in tumor biology such as carrying RNA, proteins and signaling molecules
Increases in a subset of prostaglandin and thromboxane subtypes occur in MIBC

- linoleate → linolenate → dihomo-linolenate → PGE\textsubscript{1}
- 5-HETE
- 15-HETE
- arachidonate
- PGH\textsubscript{2}
- Prostacyclin (PGI\textsubscript{2})
- PGE\textsubscript{1} → PGD\textsubscript{2}
- Thromboxane B2
- PGE\textsubscript{2}
Two additional major pathways uniquely altered between NMIBC and MIBC

• Muscle-invasive bladder cancer derives NAD+ from tryptophan rather than through the salvage pathway
  • Inflammatory cells may influence this pathway and are the subject of future study (most patients BCG naïve)
  • Prior studies have shown that cancers are highly dependent upon NAD+ for both energy (Warburg metabolism) and for DNA repair by functioning as a cofactor for the DNA repair enzyme PARP

• Hemoglobin catabolites are increased in muscle-invasive bladder cancer
  • Hemoglobin catabolites bilirubin and biliverdin were significantly higher in muscle-invasive tumors
  • Heme oxygenase-1 is upregulated in cancer and mediates oncogenic factors such as MMPs, VEGF-A, COX2 and can play an important role in cell proliferation and angiogenesis
Conclusions

• Metabolomics may be useful to identify biological changes in cancer and in progressive disease

• Energy and lipid metabolism are commonly altered in bladder cancer, with additional changes in cyclooxygenase and lipoxygenase metabolites with MIBC

• Gene alterations, when present, reflect the directional change of the metabolite-generating enzyme

• Our data supports that of other early studies in bladder cancer metabolomics and expands the spectrum of findings

• Additional research to identify the stability of these changes in samples and the pervasiveness of these changes with stage are critical to apply this data to potential targeted therapeutics
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