Racial Disparity in Bladder Cancer and Identification of Altered Metabolism in African American Compared to European Bladder Cancer

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Introduction

Bladder cancer (BCa) incidence and mortality rates vary substantially among racial and ethnic groups. Most notably, European-Americans (EA) have a higher incidence of the disease, while African-Americans (AA) experience higher mortality rates and poorer survival. To date, a metabolomic analyses aimed at understanding of bladder cancer health disparity has not been reported. We have used an Agilent LC/TQ 6495 instrument to do the metabolic measurements and also used Biocrates, a targeted quantitative metabolomics kit p180. The LC-MS/MS based AbsoluteIDQ® p180 Kit is an easy-to-use research assay for quantifying up to 188 endogenous metabolites from 5 different compound classes (i.e. acylcarnitines, amino acids, hexoses, phospho- and sphingolipids and biogenic amines). The assay requires very small sample amounts (10 μL) and shows excellent reproducibility. Our results using AA and EA BCa tissues have confirmed elevated expression of enzymes involved in the metabolism of mitochondrial metabolites and lipids, specifically in AA BCa.

Experimental

Targeted metabolomics was performed using 6460 Triple Quadrupole LC/MS system. The experiments were carried out in both positive and negative ionization modes. Data normalization was achieved by using isotopically labelled compounds. Quantitative data analysis was conducted using Agilent MassHunter software. J82 (EA) and Scaberg (AA) cell lines were maintained and grown as per ATCC’s instructions. Western Blot and qPCR experiments were performed on these cell lines and patient tissues to confirm expression of GLS1, IDH2, ADHFE1, NAT8L and ASPA.

Figure 2: Agilent 6495 LC/TQ instrument

Figure 1. Altered pathways in AA BCa and formations of 2-HG, NAA and lipids.
Results and Discussion

Figure 3A: Heat map showing altered mitochondrial metabolites in A) AA from EA BCa tissues - highlighted in red are 2-HG.

Figure 3B: Altered Pathways in AA and EA BCa patients C) Box plot showing D-2HG in AA from EA BCa tissues and AA BCa patients having higher levels (uM) of D-2HG.

Figure 4: Heat map showing altered lipids in AA from EA BCa tissues

- African American BCa – Lower PC
- African American BCa – High lyso PC

Figure 5: Enzymes involved in conversion of PC to Lyso PC
Results and Discussion

Figure 6: A-C) Bar graphs showing higher expression of IDH2, GLS, ADHFE1, NAT8L, PLAT1, and LRAT in AA from EA BCa tissues and lower levels of ASPA2. D) Western blots showing higher expression of PLAT1, ADHFE1, PHGDH, IDH2 and GLS in AA from EA BCa tissues.

Conclusions

We conducted a metabolomics study on 15 AA, and 20 EA BCa. Using a targeted LC/MS approach we were able to measure the >200 metabolites. Among the metabolites elevated in AA BCa tumors compared to EA BCa tumors were intermediates of mitochondrial metabolism namely Glutamine, 2-hydroxyglutarate (2-HG), N-acetyl aspartate. We performed Western blot and qPCR experiments to estimate the enzymes involved in the D-2HG synthesis and NAA synthesis. It was observed that Glutaminase 1 (GLS1), isocitrate dehydrogenase 2 (IDH2), and iron-containing alcohol dehydrogenase 1 (ADHFE1), three enzymes involved in D-2HG synthesis, were significantly elevated at mRNA and protein levels in AA BCa compared with EA BCa tissues. N-Acetyltransferase 8-Like Protein (NAT8L), which is required for synthesis of NAA, was higher in AA BCa, but ASPA, which is needed for NAA breakdown, was significantly lower in AA BCa tissues.

Altered lipid metabolism between AA BCa from EA BCa show, Phospholipase A1 (PLA1A), Lecithin retinol acyltransferase (LRAT), two key enzymes required for conversion of (PC) into LPC were significantly elevated in AA BCa tissues. Consistent with this finding, global levels of PC were significantly lower in AA BCa tumors than EA BCa tissues.

In summary, these data show that mitochondrial and lipid metabolism are altered in AA BCa tissues, resulting in accumulation of key metabolites that could result in oncogenic transformation and/or disease progression.

References

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