Metabolic Reprogramming in Clear Cell Renal Cell Carcinoma From Long-term Tobacco Smokers

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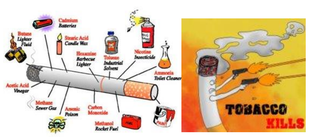
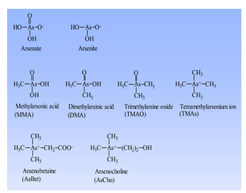
Introduction

Clear cell Renal cell carcinoma (ccRCC) is the most common type of renal cancer (~75%). Kidney cancer is common in males in North America, and tobacco smoking has been stablished as a risk-factor, but its mechanism is still unknown. Tobacco smoking (TS) has a profound negative effect on kidneys for life-time smokers (LTS 28+ years). Tobacco plant is a known metal hyper accumulator that can take up toxic heavy elements such as Arsenic, Cadmium, and Lead in higher concentration than the soil where it grows. These toxicants are eventually released into the smoke inhaled by smokers in the inorganic forms carried in nano-sized particles . Inorganic Arsenic (As(V), As(III), etc.) is methylated to be converted into organic Arsenic (MMA, DMA, etc.) which can then be excreted in the kidneys. ccRCC cells are known to reorganize their energy production from the aerobic TCA cycle towards inorganic glycolysis in order to preserve carbon for structure and metabolite production and to increase cell proliferation. Once the toxic metals enter the kidney cells in LTS they displace physiological metals, such as Manganese, Zinc, Copper, and Iron. These are important metals for targeting Reactive Oxygen Species and are used as co-factors for tumor suppressor genes. Since different chemical forms or species of metals have very different toxicity, and binding to protective proteins like metallothioneines can dramatically alter the toxicity of metals, the total amount is not enough to their study toxicity.



A single source, tobacco smoking can produce a complex and deadly combination of toxicants, metals are persistent. Cd and As end up in the kidneys to be excreted or accumulated.



Research Aims

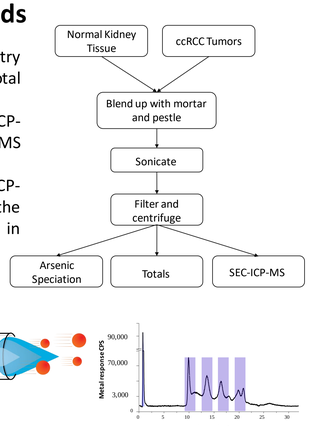
The main goal of my project was to analyze differences in metal accumulation and metal distribution in normal kidney (NKT) and ccRCC tissues from non-smokers vs life-time smokers and to correlate those differences with metabolic changes originated by TS

Specific aims:

- Quantify the total concentration of toxic metals in NKT vs ccRCC in both NS and LTS
- Separate by size exclusion and quantify the toxic metal concentration in High molecular weight, Metallothionein fraction, and Low molecular weight
- Quantify the total Arsenic speciation between inorganic and organic Arsenic
- Correlate the changes in metal content and distribution with available data from metabolomics profile in the same samples

Methods

- Inductively coupled plasma mass spectrometry (ICP-MS) was used to quantify metals as total content
- For arsenic speciation, HPLC was coupled to ICP-MS to separate the As species before ICP-MS quantification
- For size exclusion, HPLC was coupled to the ICP-MS to separate by size all molecules in the lysates and quantify the metal content in different molecular sizes



Results

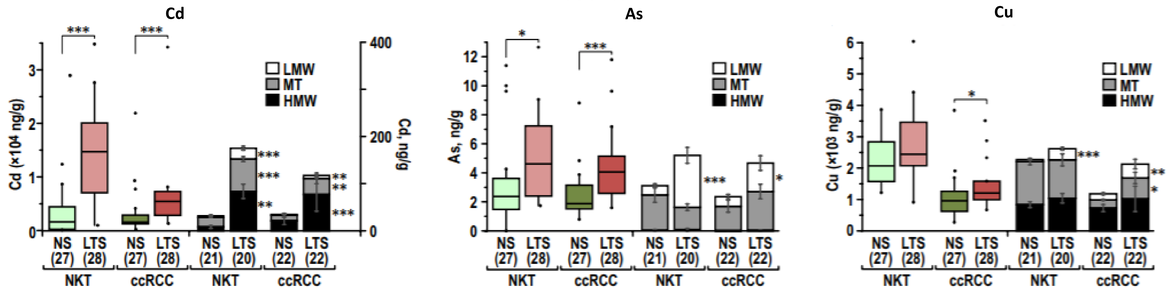


Figure 1: Box and whisker plot showing the concentration by totals and SEC-ICP-MS of Cadmium, Arsenic, and Copper. Boxes in color are totals, and black-and-white stacked bar graph, are SEC fractions, Low Molecular Weight, Metallothionein, and High molecular weight. Cadmium box and whisker plot shows that there is significantly more Cadmium in NKT and in tumors in LTS, the majority of the distribution is in the High molecular weight. Arsenic is more abundant in LTS than NS, especially in the NKT. The majority of Arsenic is in the Metallothionein and Low molecular weight molecules in the cells. Copper is significantly decreased in tumors than in NKT. Copper is predominantly in High molecular weight for LTS in tumors than the tumors in NS.

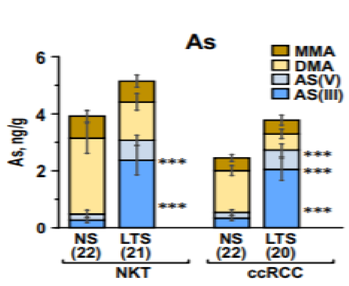


Figure 2: Stacked bar graph of Arsenic Speciation. Methylated Arsenic (organic) vs. non-methylated Arsenic (inorganic) in Kidney tissue and Tumors in both Non-smokers and Life-time smokers. More Arsenic is accumulated in NKT, especially in LTS. More methylated Arsenic in NS for both NKT and tumors. More inorganic Arsenic in LTS for both NKT and tumors.

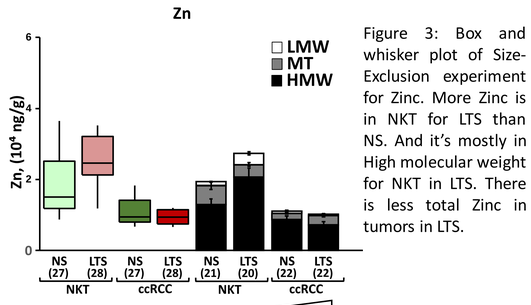


Figure 3: Box and whisker plot of Size-Exclusion experiment for Zinc. More Zinc is in NKT for LTS than NS. And it's mostly in High molecular weight for NKT in LTS. There is less total Zinc in tumors in LTS.

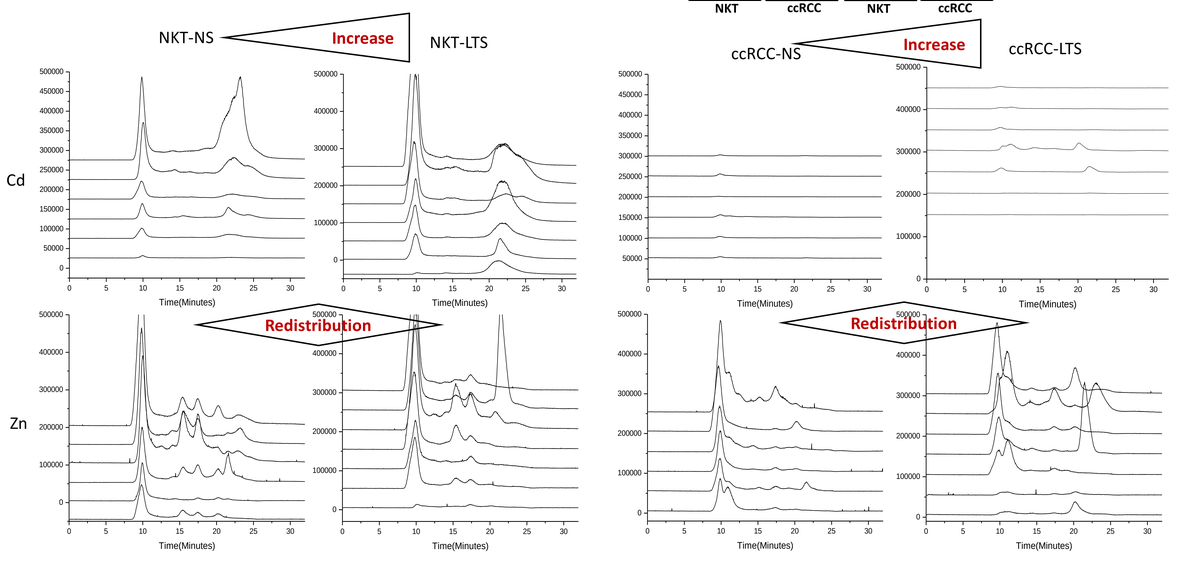


Figure 4: Size-Exclusion chromatograms of Cadmium and Zinc in Kidney tissue and Tumors in both Non-smokers and Life-time smokers. First column in Normal kidney tissue in Non-smokers shows that Zinc and Cadmium are accumulated in the High molecular weight, but Zinc is also in the Metallothioneins. There is very little Cadmium in NS tumors unlike Zinc which is still distributed in the High molecular weight. Huge peaks are shown for both Cadmium and Zinc in NKT for LTS. There are large peaks for Zinc in High molecular weight, and very little for Cadmium in tumors for LTS.

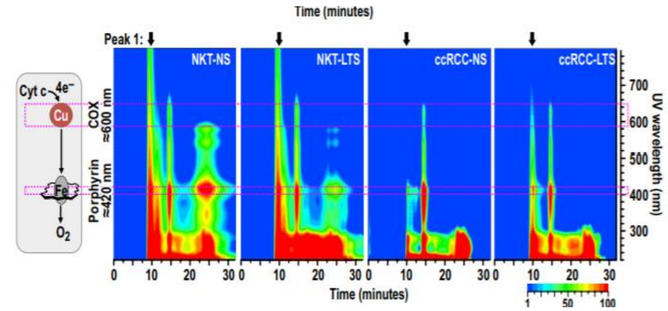


Figure 5: Isoabsorbance plots of SEC-UV-Vis to screen the UV profile of the peaks associated to the SEC-ICP-MS signal. Porphyrin rings absorbe at 420nm, Cytochrome C Oxidase (COX) Cu clusters at 600nm. COX is decreased in tumors, while it is still prevalent in NKT. Copper is increased in LTS in both NKT and tumors, while the distribution is shifted to accommodate the metalation of COX.

Discussion

Based on the Metabolomics information (data not shown), we observe a correlation between ATP, malate, and NADH in LTS tumors. Normally in ccRCC, Glycolysis is preferred for energy production and cell proliferation, but our Metabolomics results show that tumors from LTS have a decrease in Glycolysis intermediates, and an increase TCA cycle intermediates. Because of this correlation we hypothesize that the metals in tobacco smoke induce this reorganization of energy production metabolism. Because Cadmium has a higher toxicity than Arsenic, it is more likely to bind to Metallothioneins and therefore lead to an increase in "free" or LMW Arsenic and Copper, which is shown in Figure 1. A decrease in Arsenic methylation is shown in Figure 2 by the distribution of organic vs. inorganic Arsenic. Copper has more opportunities to bind COX and induce oxidative phosphorylation without the need for Glycolysis.

Conclusions

After the totals, size-exclusion, and arsenic speciation experiments, it can be concluded that Arsenic and Cadmium are more prevalent in LTS than in NS, but Arsenic has the capabilities to be methylated and therefore excreted. But these methylation mechanisms decrease in LTS and therefore more Arsenic can be accumulated in Metallothioneins and/or low molecular weight. Cadmium on the other hand, is found in high molecular weight in LTS and does not have the ability to be excreted. Copper is essential for Cytochrome C Oxidase, the last enzyme in the Electron Transport Chain. Copper is important for the production of ATP. This decrease would mean that the cells are investing more in Glycolysis rather than Oxidative Phosphorylation. Zinc is an essential metal for the cell and immune system, based on the chromatograms, the Zinc accumulation decreases in LTS tumors and it is redistributed in the cells, which can lead to an increase in cancer cell division and tumor growth suppression.

Acknowledgements

- The University of Cincinnati, Chemistry Department
- The University of Cincinnati, Cancer and Cell Biology Department
- Agilent Technologies
- Professor Julio Landero
- Professor Maria Czykzyk-Krzeska
- My Research Group

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