Comparison of metabolomic profiles of androgen-dependent and refractory prostate cancer cells using liquid chromatography-mass spectrometry (LC-MS)

ASMS 2010

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Introduction

Prostate cancer (PC) is the second most prevalent cancer among American men which is primarily treated by androgen ablation therapy. Although a number of patients respond to this regimen, a significant subset fail and the tumor invariably progresses into a hormone refractory metastatic state, which is lethal. Notably, clinicians lack tools that would allow them to select patients who respond to androgen ablation. Earlier we had reported the first unbiased metabolomic signature for localized prostate cancer. Advancing further, we attempt to delineate the subset of metabolome in prostate cancer which is regulated by androgen-action. In this study we examine global metabolomic alteration regulated by androgen in prostate derived cell lines using a validated unbiased LC-MS platform. In addition to revealing potential prognostic markers, these metabolomic profiles highlight the biochemical machinery altered in prostate cancer by androgen action.

Experimental

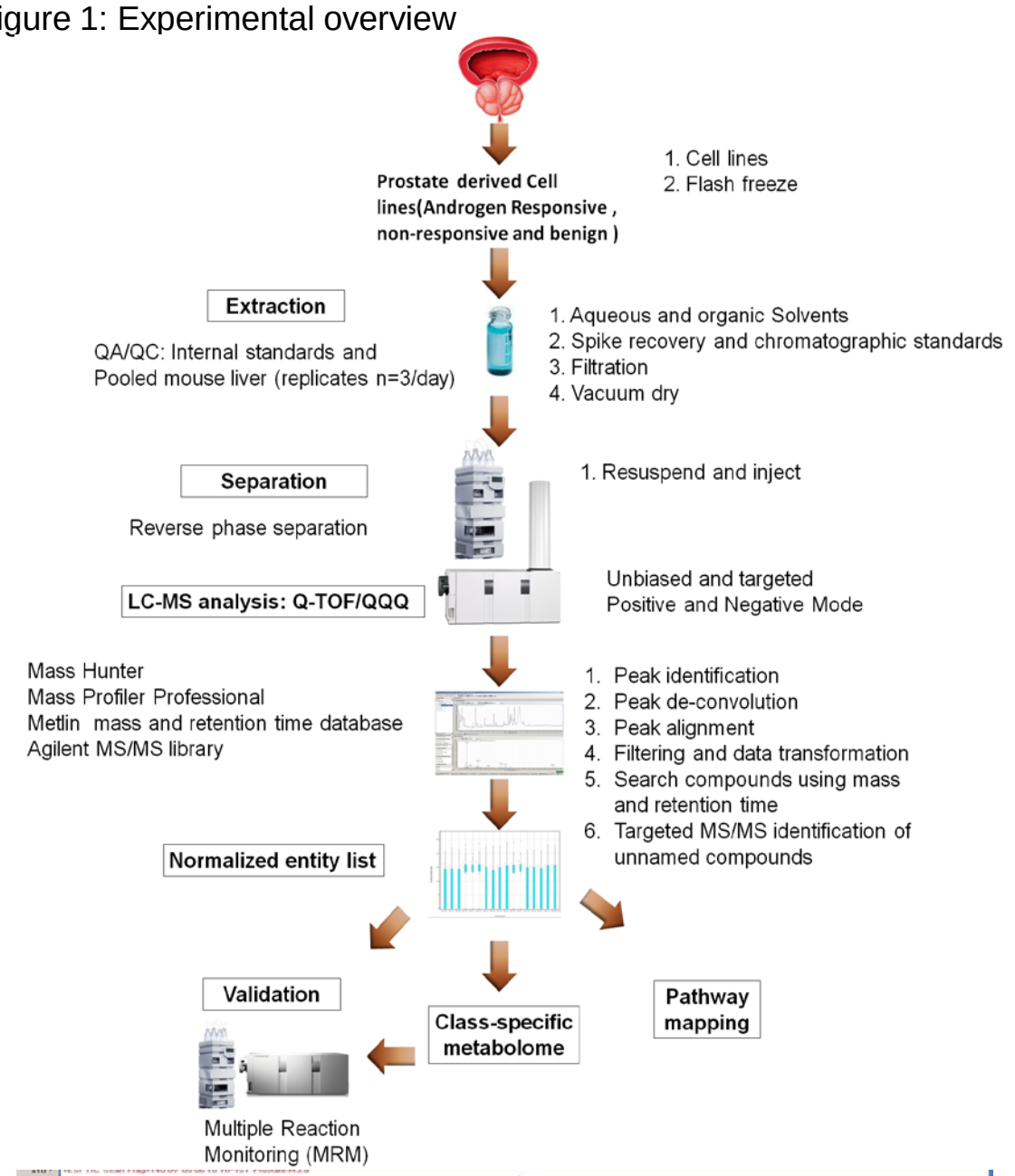
Instrumentation: An Agilent 1200 SL HPLC system with binary pump and degasser, well plate auto-sampler with thermostat controlled column compartment, and an Agilent 6520 Accurate-Mass QTOF mass spectrometer with dual ESI source operated in the positive and negative ionization modes. Dynamic mass axis calibration was achieved by continuous infusion of a reference mass solution using an isocratic pump connected to a dual sprayer feeding into an electrospray ionization source.

HPLC Method: Flow rate 0.6 ml/min, column temperature 60°C. Solvent A is Water + 0.2% Acetic Acid, solvent B is Methanol + 0.2% Acetic Acid. A 13 minute gradient from 2% B to 98% B and a 6 minute hold at 98% B with a stop time at 19 minutes, followed by 5 minutes post-run column re-equilibration. An Agilent Zorbax SB-Aq (2.1 X 50mm, 1.8 um) analytical column and an Agilent Zorbax-SB-C8 Rapid Resolution Cartridge (2.1X 30mm, 3.5 um) guard column were used for separation of the metabolome.

Experimental

Global Metabolomic Profiling of Prostate Cancer

Figure 1: Experimental overview



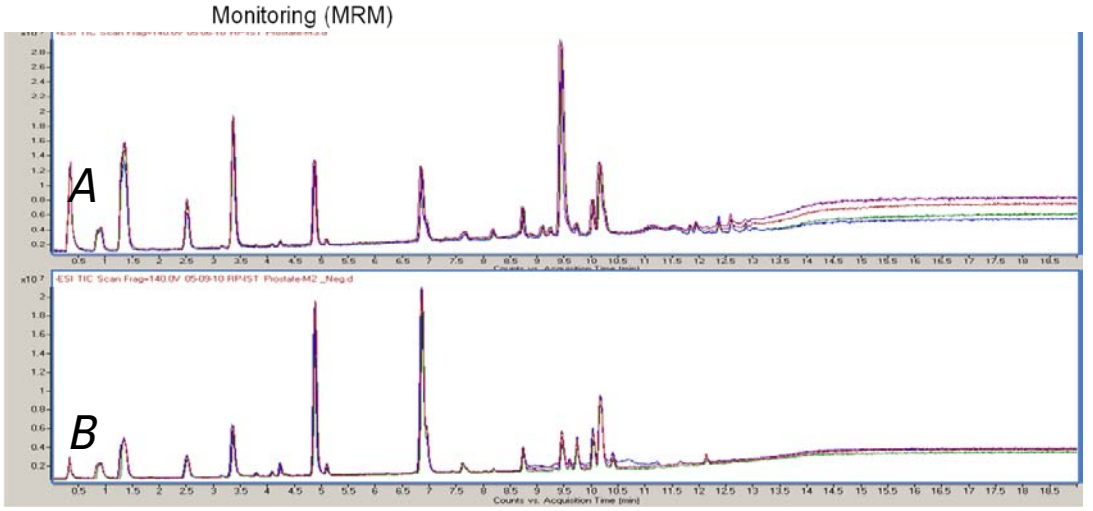


Figure 2: Reproducibility of profiling platform for spiked standards (n=12 compounds) across 7 independent replicates A) positive ionization mode B) negative ionization mode

LC-MS was used to profile metabolites in prostate derived cell lines

- *22Rv1, LNCaP and VCaP: androgen responsive (prostate cancer),*
- *DU145 and PC3: androgen non-responsive (prostate cancer)*
- *RWPE (benign).*

Using reverse phase positive and negative mode ionizations 6747 and 2288 metabolites respectively, were detected across the prostate-derived cell lines

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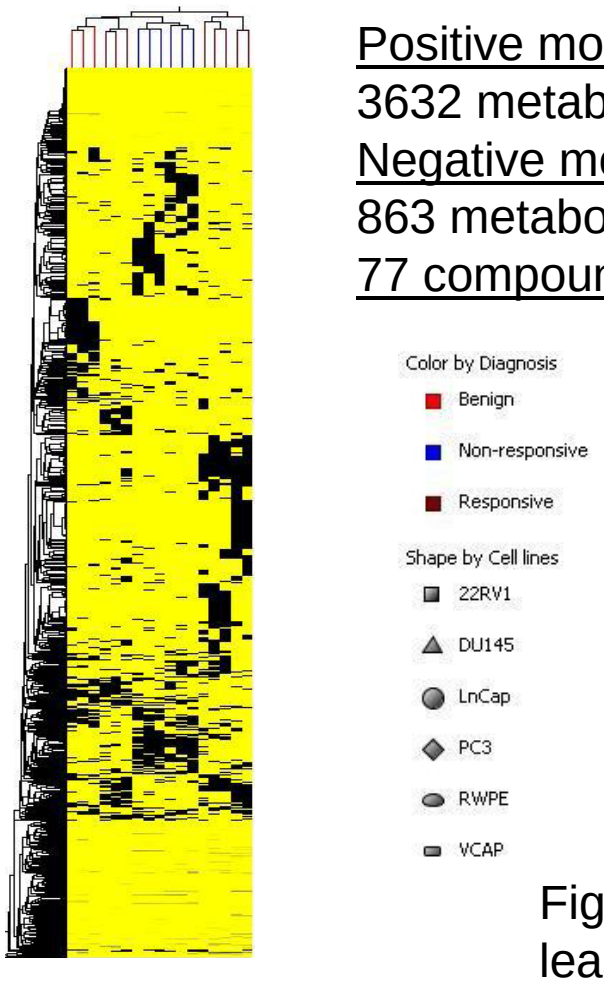
Results and Discussion

Figure 3: Heat map of compounds detected in at least 25% of cell lines examined (n= 17 including replicates)



Positive mode:
3632 metabolites
Negative mode:
863 metabolites
77 compounds were named using metabolomic library (Metlin, Agilent)

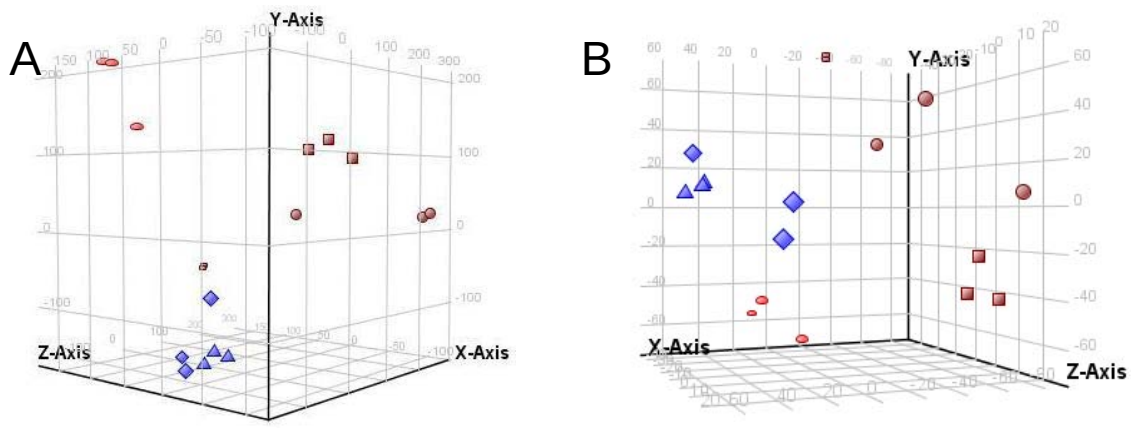


Figure 4: Principal component analysis using all compounds detected in at least 25 % of cell lines examined, A) Positive mode (B) Negative mode

Volcano plot was used to delineate class-specific signatures, namely benign, androgen-responsive and non-responsive

A

Positive mode

Result Summary

	P all	P < 0.05	P < 0.02	P < 0.01	P < 0.0050	P < 0.0010
FC all	3632	277	127	125	118	42
FC > 1.1	3390	277	127	125	118	42
FC > 1.5	3210	273	126	124	117	42
FC > 2.0	3054	258	118	117	112	40
FC > 3.0	2852	251	113	113	110	38
Expected by chance		13	2	1	0	0

Negative mode

Result Summary

	P all	P < 0.05	P < 0.02	P < 0.01	P < 0.0050	P < 0.0010
FC all	863	242	148	107	97	88
FC > 1.1	818	242	148	107	97	88
FC > 1.5	699	222	141	104	96	88
FC > 2.0	630	201	127	96	90	86
FC > 3.0	582	194	121	91	87	84
Expected by chance		12	2	1	0	0

B

Positive mode

Result Summary

	P all	P < 0.05	P < 0.02	P < 0.01	P < 0.0050	P < 0.0010
FC all	3632	220	199	191	191	186
FC > 1.1	2652	220	199	191	191	186
FC > 1.5	2452	208	192	188	188	186
FC > 2.0	2364	201	190	187	187	185
FC > 3.0	2322	188	184	183	183	182
Expected by chance		11	3	1	0	0

Negative mode

Result Summary

	P all	P < 0.05	P < 0.02	P < 0.01	P < 0.0050	P < 0.0010
FC all	863	114	103	101	92	87
FC > 1.1	734	114	103	101	92	87
FC > 1.5	601	111	102	100	92	87
FC > 2.0	566	106	98	97	91	86
FC > 3.0	546	96	91	91	88	84
Expected b...		5	2	1	0	0

C

Positive mode

Result Summary

	P all	P < 0.05	P < 0.02	P < 0.01	P < 0.0050	P < 0.0010
FC all	3632	420	137	134	133	132
FC > 1.1	3170	420	137	134	133	132
FC > 1.5	3033	418	137	134	133	132
FC > 2.0	2911	410	136	134	133	132
FC > 3.0	2785	406	133	132	132	132
Expected by chance		21	2	1	0	0

Negative mode

Result Summary

	P all	P < 0.05	P < 0.02	P < 0.01	P < 0.0050	P < 0.0010
FC all	863	106	56	50	46	44
FC > 1.1	778	106	56	50	46	44
FC > 1.5	683	106	56	50	46	44
FC > 2.0	640	98	52	48	46	44
FC > 3.0	595	91	50	46	45	43
Expected by...		5	1	0	0	0

Figure 5: A) 202 metabolites were altered between androgen non-responsive vs responsive cells B) 278 metabolites were altered between benign vs androgen non-responsive cells C) 179 metabolites were altered between benign vs androgen non-responsive cells ($p<0.005$, FC=2%).

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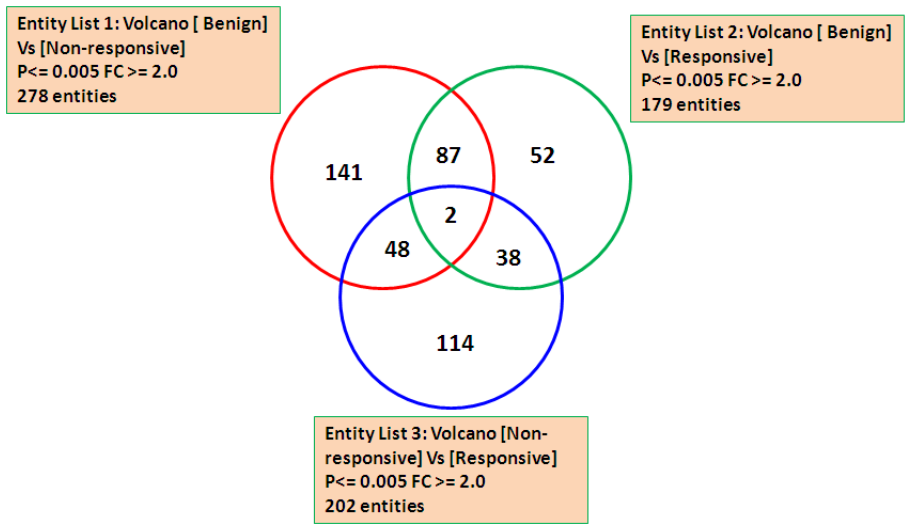


Figure 6: Distribution of altered metabolites across the three sample classes

204 metabolites were found only in the prostate cancer cell lines, of which 52 were specific to androgen responsive cells and 114 were detected solely in androgen refractory cells. 141 metabolites were seen in benign cells only.

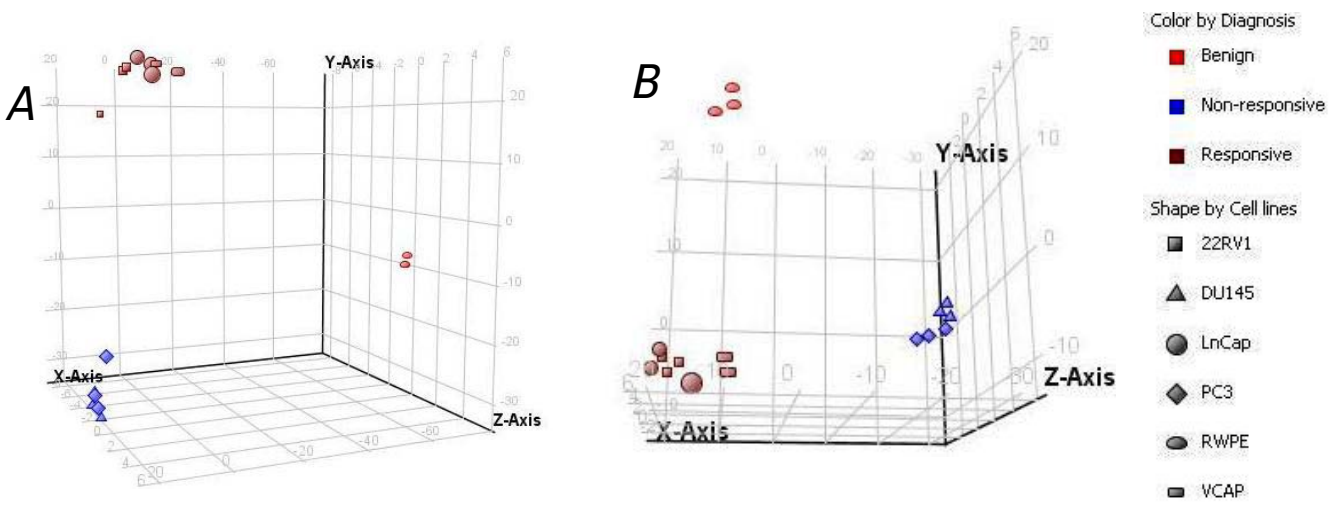
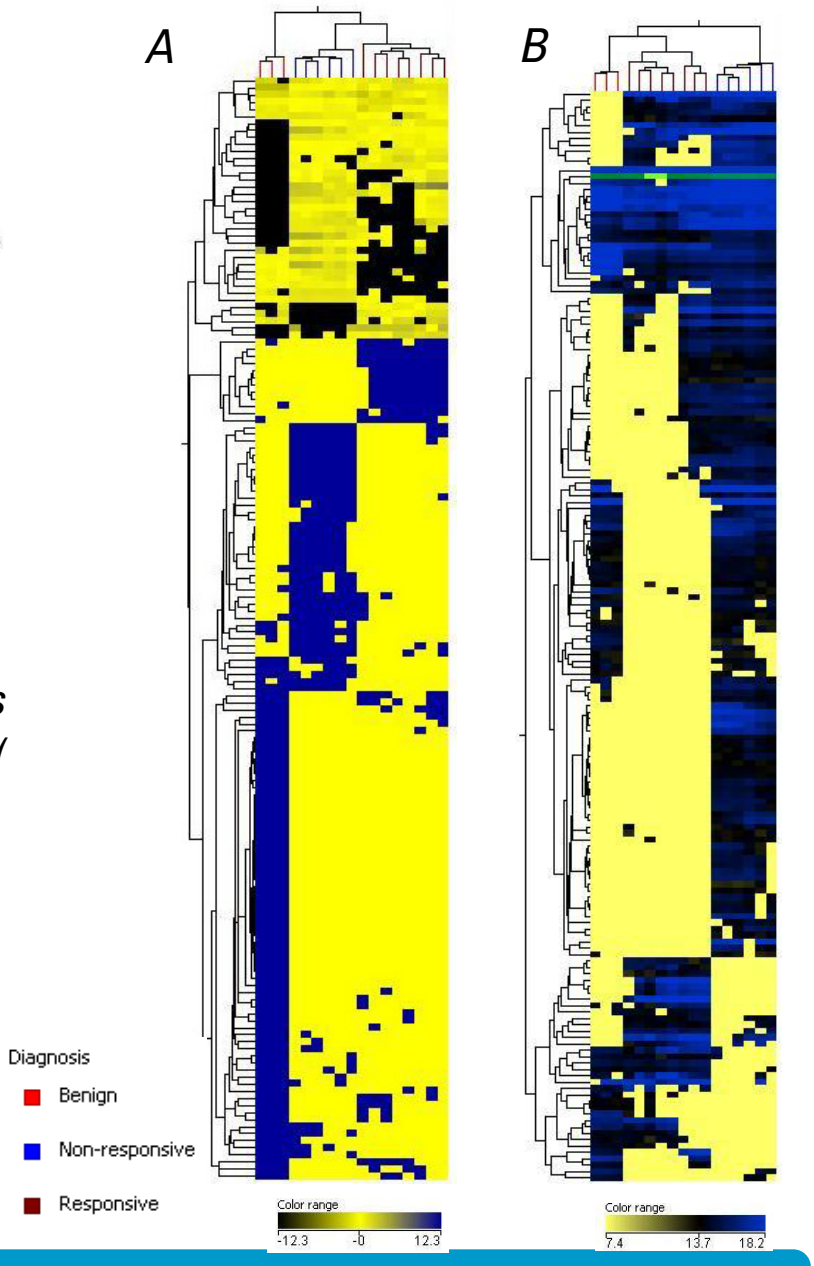


Figure 7: Principal component analysis using compounds significantly altered across the three classes A) 311 positive mode (n=311) and B) negative mode (n=171)

Figure 8: Heat map representation of compounds significantly altered across the three classes A) positive mode (n=311) and B) negative mode (n=171). In total 482 were significantly altered between benign, androgen-responsive and refractory cells.



List of named compounds significantly (p<0.005, FC>2) altered in prostate cancer by androgen action

1. *Kynurinine*
2. *Guanine*
3. *Cartisone*
4. *Carnitine*
5. *Thymidine*
6. *Hippuric acid*
7. *DL-2-Indole-3-Lacticacid*
8. *DL-3 Phenyllactic acid*
9. *3-Hydroxy-3-Methylvalerate*

Ref: Sreekumar, A et al. Metabolomic profiles delineate potential role for sarcosine in prostate cancer progression. Nature. 2009 Feb 12;457(7231):910-4.

Future Work:

Validate androgen regulated metabolome in prostate cancer tissue specimens and delineate their mechanistic role in prostate cancer progression. The latter will be examined using Oncomine Concept Maps (OCM) and their associated genes using the Kyoto Encyclopedia of Genes and Genomes (KEGG, www.kegg.com).

Funding: 1RO1CA133458-01 (AS), 1 R03 CA139489-01 (AS) and RCA145444A (AS and GM), Georgia Cancer Coalition (AS)