

ONLINE MULTIDIMENSIONAL LC-LC-nESI-QqTOF-MS-MS ANALYSIS FOR NOVEL PROTEIN BIOMARKERS IN PROSTATE CANCER USING MULTI-PHASIC CAPILLARY CHROMATOGRAPHIC CHEMISTRIES

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1. Prostate Cancer Biomarkers

BACKGROUND AND OVERVIEW:

- 2nd most frequent occurring cancer
- Ageing related disease – environmental factors may also contribute
- Lifetime risk is gradually increasing
- Existing screening methods involving the use of PSA and DRE have proven to be ineffective

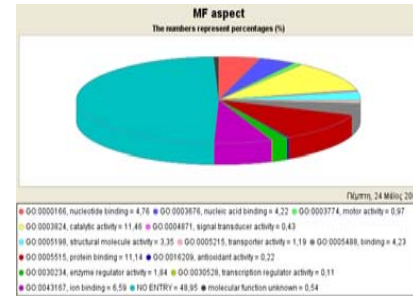
PROJECT OBJECTIVES:

- Characterization of proteomic profiles of clinically traceable normal and cancerous tissue specimens
- Determination of novel prognostic and diagnostic biological markers
- Determination of biomarker suitability with *ex vivo* models and pharmacologic intervention studies with known drug targets
- Develop targeted proteomic analysis strategies in plasma

4. PCa Patient Baseline Characteristics

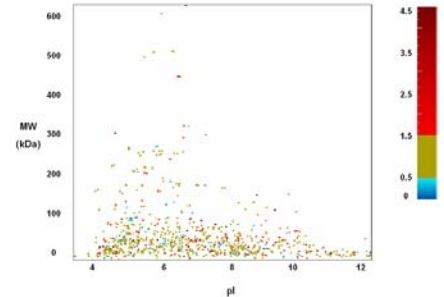
GROUP A Prostate Cancer					
No. patient	Age (years)	PSA (ng/mL)	Stage	Gleason score	% prostate cancer procurement
1	70	5.0	pT3aNoMx	3+3	70% left lobe
2	69	9.7	pT3bNxMx	4+5	100% right lobe
3	63	14.5	pT3aNoMx	3+5	80% left lobe
4	69	3.2	pT3bNoMx	3+4	100% left lobe
5	59	8.7	pT3aNxMx	3+4	80% right lobe
6	61	9.0	pT3aNoMx	3+4	60% right lobe
7	63	11.0	pT3bNxMx	3+4	60% right lobe
8	63	6.8	pT3aNxMx	3+4	70% left lobe
9	68	11.0	pT3aNoMx	3+5	80% right lobe
10	71	7.0	pT3aNxMx	3+4	100% right lobe
11	60	19.0	pT3NoMx	4+5	80% left lobe
12	68	7.6	pT3aNoMx	3+4	70% right lobe

7. Protein Distribution by Molecular Function (MF)

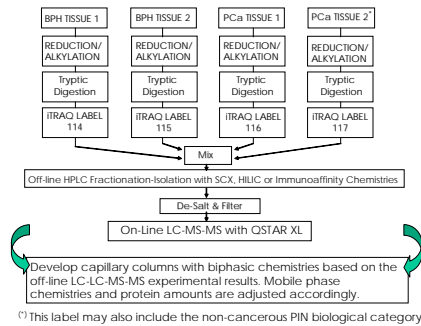


NOTE: The «NO ENTRY» category includes hypothetical proteins, predicted proteins, unnamed proteins and novel proteins. Its sub-categorization is currently under investigation.

10. LC-MS Virtual Expression Map of 838 Proteins (≥ 95 conf.)



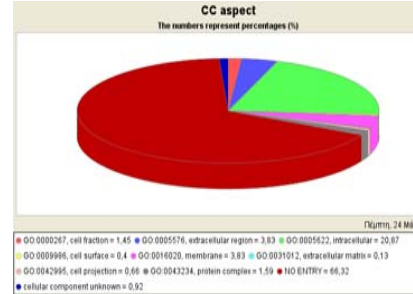
2. iTRAQ LC-LC-MS-MS Experimental Design



5. BPH Patient Baseline Characteristics

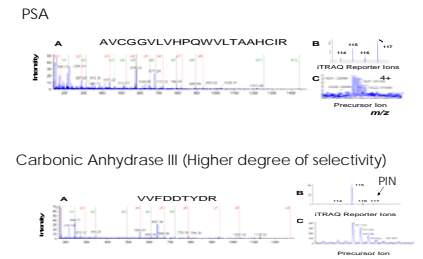
GROUP B Benign Prostate Hyperplasia		
No. patient	Age (years)	PSA (ng/mL)
1	75	3.2
2	49	4.1
3	68	2.1
4	69	5.5
5	75	1.7
6	68	2.8
7	76	5.7
8	76	3.7
9	78	8.0
10	66	3.8

8. Protein Distribution by Cellular Component (CC)



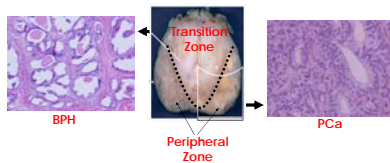
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11. Product Ion MS-MS Spectra of Diagnostic Peptides



3. Tissue Procurement and Patient Characteristics

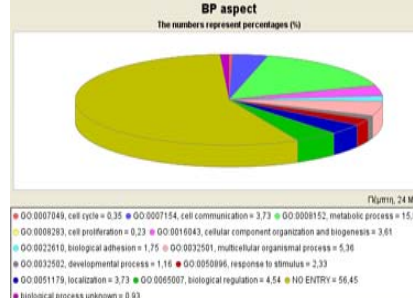
- Well defined inclusion/exclusion criteria were used for both clinical groups (staging, Gleason Score ≥ 6, no androgen blockade, etc.)
- 10 Patients BPH / 12 PCa Cases
- Cancer specimens were pathology report confirmed, occupying more than 60% of the hematoxylin-eosin section's total plan
- Cancer specimen stage distribution was pT3
- PSA levels for both categories (BPH and pCa) varied extensively



6. Preliminary Results (N=12 PCa, 10 BPH)

- A total of **838 non-redundant** proteins identified
- Approx **50 proteins** exhibited a **significant differential expression** between the 2 biological states: BPH, pCa
- Uniquely occurring proteins** identified in each biological state
- Approx. **25% of proteins identified are novel to the prostate tissue proteome** – including membrane bound or membrane associated proteins
- Approx. 40% of the known proteins identified have been assessed through **biochemical assay or chip array** experiments
- Most differentially or uniquely occurring proteins appear to have **mechanistic relevance** to known pathways associated with prostate carcinogenesis
- Inflammation, bone metastasis, cellular motility** seem to play a central role in the expression of pCa
- A significant number of **gene products identified (>30%) with unknown molecular function, cellular distribution and biological function**

9. Protein Distribution by Biological Process (BP)



NOTE: The «NO ENTRY» category includes hypothetical proteins, predicted proteins, unnamed proteins and novel proteins. Its sub-categorization is currently under investigation.

12. Future Plans

- Analyze more tissue specimens
 - Develop methods for the targeted analysis of protein biomarkers in plasma using **immunoprecipitation – LC-MS-MS techniques**
 - Analyze samples derived from **Laser Capture Microdissection** techniques
 - In order to complement the existing LC-MS infrastructure, a high capacity ion trap system (Agilent 6330 model) is currently being installed
- In collaboration with the Pharmacology Department:
- Verify proteomic findings with **absolute quantification methods** based on the use of a **hybrid triple quadrupole – linear ion trap LC-MS-MS system** (i.e. iMRM techniques)
 - Develop/implement **immunoprecipitation-IRMS** based methods for the targeted analysis of protein biomarkers in plasma for **clinical validation and subsequent use**

References

- Constantinides, C., et al. Quantitative Proteomic Determination of Potential Cancer Biomarker in Prostate Tissue Derived from a Human Pilot Clinical Study. *Manuscript in preparation*.
- Hatsibaskaki, M., et al. *Ex vivo* Pharmacoproteomic Evaluation of a Novel Benzofuran Isolated from *Onobrychis ebeoides* Using DU-145 Cells. *Manuscript in preparation*.

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