

Integrated analysis of prostate cancer progression

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Prostate cancer (PCA) is the most prevalent tumor in males in developed countries and a major cause of death due to malignancy. This problem will be aggravating with increasing lifetime expectancy in the future since the frequency of PCA is rampant in elderly men. Because specific early diagnosis and decision on appropriate individual therapy is hampered by the lack of markers, novel diagnostic and prognostic tools for PCA management are urgently needed [1-3].

To detect such markers, we are using an integrated genomic, proteomic, and metabolomic analysis of biological samples from prostate cancer patients. First, we focused on the analysis of **gene expression** differences between aggressive (Gleason score (GS) 8-10) and non-aggressive (GS6) tumors. To this end, 65 tissue samples (25 GS6, 27 GS8-10; and 13 normal) from PCA patients were microdissected and used for microarray analysis on 37,500 spotted human cDNA clones. Using statistical tests, we identified 687 genes that were differentially expressed between GS8-10 and GS6

tumors. We developed a method detecting alterations of entire pathways in two experimental conditions. This procedure is based on the translation of pathway annotation (e.g. KEGG) into a two-dimensional statistical test problem. It involves a Wilcoxon's signed rank sum test to compute a Z-score quantifying the degree of alterations across different experimental conditions [4,5]. We also performed gene enrichment analysis based on multiple categories from public databases in order to identify functional groups that are over-represented in our list of candidate genes. By applying these approaches to the comparison of GS8-10 and GS6 tumors, we identified biological pathways associated with PCA progression.

In a second approach, we performed quantitative targeted MS/MS analysis [6] of **serum metabolites** from the same 65 patients. Samples were directly extracted or derivatized and analyzed either by FIA-MS/MS or LC-MS/MS combined with MRM, precursor and NL scans using a 4000 Q TRAP® system equipped with an electrospray source. Concentrations were calculated from the raw MS spectra by reference to a wide range of appropriate internal standards. Wilcoxon's signed rank sum tests adjusted for FDR and PCA were performed to reveal deregulated biological processes in tumors and healthy controls, followed by biochemical pathway mapping and interpretation of the pre-annotated targeted metabolites. These were incorporated into public gene sets ("MSigDB"[7]) using KEGG-based identifiers and annotated links between genes, enzymes, reactions and compounds.

Finally, we applied modified carriers materials for specific binding of **serum peptides and proteins**, followed by matrix-assisted laser desorption/ionization (MALDI) mass spectrometry. This approach is referred to as material-enhanced laser desorption/ionization (MELDI) and allows for a fast and sensitive detection of tumor marker signatures. A list of the *m/z* signals, which are associated with Gleason scores, has been worked out and is still under investigation.

The integrated analysis of several types of molecular data from the same patient cohort reduces the bias inherent to each individual technique and improves the identification of biological processes and markers associated with prostate cancer progression.

References

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“Systems Biology of Prostate Cancer” is an international research consortium consisting of four Austrian and two German partner institutes with strong expertises in prostate cancer, molecular (gene, protein and metabolite) profiling, and systems biology. The common aim is to perform a comprehensive analysis of PCA progression to enable an enhanced clinical management of PCA in the future. The consortium is funded by the Austrian Nationalstiftung and the Austria Wirtschaftsservice GmbH in the framework of the IMGUS research program (Institute for Medical Genome Research and Systems Biology, Wien).

