PrECISE

D2.3

Targeted profiling of prospective cohort

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Abstract:	This report will provide profiles of selected biopsies and will be used to inform sample and assay selection in WP6
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Executive Summary

In D2.3 we report the status of the targeted profiling efforts of the ProCOC and ZTMA76/80 cohorts.

To identify driving mutations and characterize intra-tumor heterogeneity, that is arising due to evolution of several co-localized clones, we have conducted in-depth genomic and proteomic analysis of multiple biopsies of 3 representative patients from ProCOC cohort, reported previously as Prostate Cancer Variance (PCV) study. Using SWATH-MS and targeted extraction of peptide to spectrum-matches, we have characterized the heterogeneity of proteome in prostate cancer samples (Chapter 2).

To suppresses growth of prostate cancer cells, patients are often treated with hormones. However, certain cancer clones can develop resistance to castration therapy leading to relapse of cancer. To understand mechanisms of castration resistance, we have acquired DIA proteomic data (DIA is a non-commercial variation of SWATH-MS technology) for the ZTMA76 cohort of castration-resistant prostate cancer patients. Based on the quantitative proteomics data from the analysis of ZTMA76 we found two significantly regulated proteins: DCD und POLR2B.

This study thus suggested proteins that can be validated by immuno-histochemical (IHC) and tissue microarray assays (TMA). We have done IHC of ZTMA76 and 80 with the corresponding antibodies. The IHC for the TMA evaluation using these antibodies are currently being established within WP6.

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Chapter 1 Introduction

Clonal development of cancer leads to intra-tumor heterogeneity, which makes it hard to diagnose, as high intra-tissue variance may prevent reliable difference detection between patients. Applying treatments, suppressing growth of cancer cells, exhibits selective pressure on cancer cells, until a clone, resistant to the treatment, develops within the cancer tissue. Thus, characterization of heterogeneity and castration-specific proteomes is essential for diagnosis and treatment of prostate cancer.

In this deliverable, we describe the work done to address both problems. First, we describe the heterogeneity study, described in previous reports as PCV study. This study operates with ProCOC – prospective cohort of prostate cancer patients. We profiled this cohort with SWATH-MS – technology, that allows targeted re-extraction of peptide spectra of interest. Using SWATH-MS, we characterize intra- and inter-tissue heterogeneity of prostate cancer.

Second, we describe preliminary results on differential expression in castration-resistant prostate cancer, which was done in ZTMA76/80 cohorts. The motivation to quantify the proteome of ZTMA76 was to understand the mechanism of castration resistance (multiple clones). The samples, used in this study, have been described in D6.1 and D6.2, and the proteomic data acquisition has been described in D6.3 "Generate SWATH proteome profiles from sample punches prepared in D6.2".

Our efforts to map out tumor clones in these cohorts included genetic analyses that identified driver mutations, initiating subclones, resistant subclones, and subclones whose emergence coincided with changes in Gleason Scores. These finding will be reported in upcoming deliverables in WP1 and WP2.

The motivation to quantify the proteome of ZTMA76 and 80 was to understand the mechanism of castration resistance (multiple clones).

In D6.3 we reported the ZTMA76 and we have the analysis of this data now. Targeted profiling of two regulated proteins in prospective cohorts. Based on the quantitative proteomics data from the analysis of ZTMA76 we found two significantly regulated proteins: DCD und POLR2B. We will schedule the IHC validation assay of ZTMA76 and 80 with the corresponding antibodies and will report the outcome in WP6. The IHC for the TMA evaluation using these antibodies are currently being established.

Chapter 2 Multi-region analysis of proteome

During the development of cancer, multiple clones can evolve from an ancestor cancer stem cell. These clones, however, are still spatially co-localized, yet they differ in gene expression and protein abundance patterns, that can lead to intra-tumor heterogeneity. To understand, whether this hypothesis holds true also for prostate cancer, we have conducted Prostate Cancer Variance (PCV) study using 30 samples of 3 patients from ProCOC cohort (Figure 1) [6]. Using SWATH-MS, we've profiled multiple punches from the same region of the tumor and corresponding benign tissue.

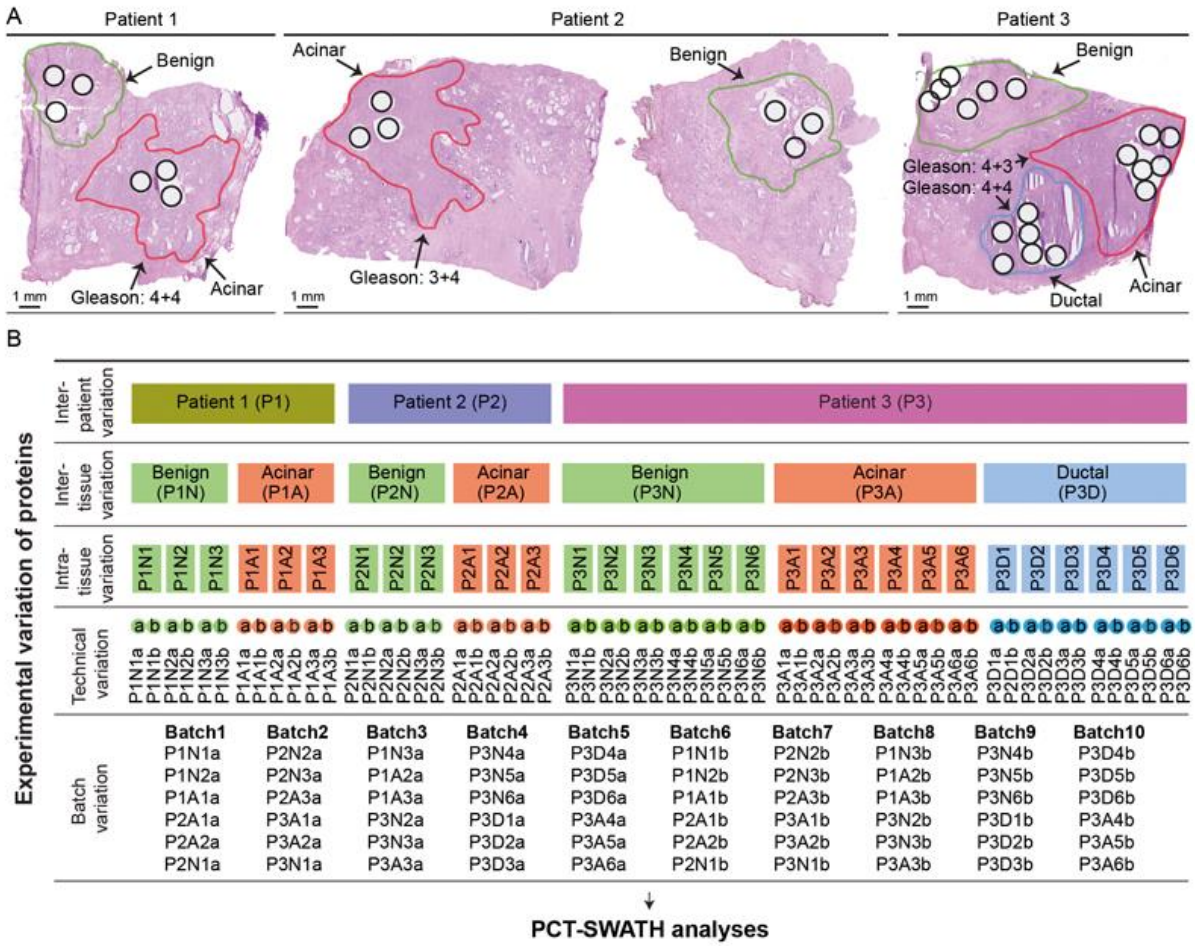

Figure 1: Prostate Cancer Variance study design

Intra-tissue heterogeneity (ITH) was quantified for 3517 proteins. We compared both intra-tissue and inter-tissue (benign vs. malignant) tissue variability. This analysis revealed strong dependence of the protein on the tissue type. Remarkably, prostate specific antigen (PSA) which is currently the most common biomarker for prostate cancer, was among top-3 most variable proteins in benign tissue, while high degree of variability has been observed for DNA repair pathways in tumor regions only, which is likely to contribute to genomic heterogeneity of tumorous tissues.

The data acquired in this study indicate that the variation of some protein levels between patients is similar in magnitude to the variation within a single tissue region. Thus, such proteins are not suitable for usage as biomarkers and means that studies of similar design are critical to establish intra-tissue variability of a candidate biomarkers.

To validate the findings, tissue microarray assays (TMA) and immune-histochemical assays have been developed. These assays are going to be applied within WP6.

Chapter 3 Protein quantitation

Similar to the results reported in task 2.3 protein maps of cohort samples have been acquired using mass spectrometry and next generation proteomics data analysis tool for the targeted analysis of data independent measurements [4,5]. This software enables maximal proteome coverage and data completeness by utilizing the power of Hybrid Libraries – a combination of core proteome libraries (project or resource libraries) with sample-specific libraries (from PC samples).

Statistical analysis considering the composition of the data sets [1,2,3] have been performed by IBM in order to detect significant differences in protein expression. The resulting list comprises roughly 150 proteins that exhibit variations in the expression patterns in both directions, for example: DCD (Dermcidin) is overexpressed in Hormone-Resistant Prostate Cancer (HRPC), while ACTN2 (Actinin Alpha 2) is overexpressed in Adenocarcinoma (ADCA) with Radical Prostatectomy (RPE) (see Figure 2).

The antibody-based validation of such significantly regulated and interesting candidates by IHC is currently being established and the outcome will be reported in WP6.

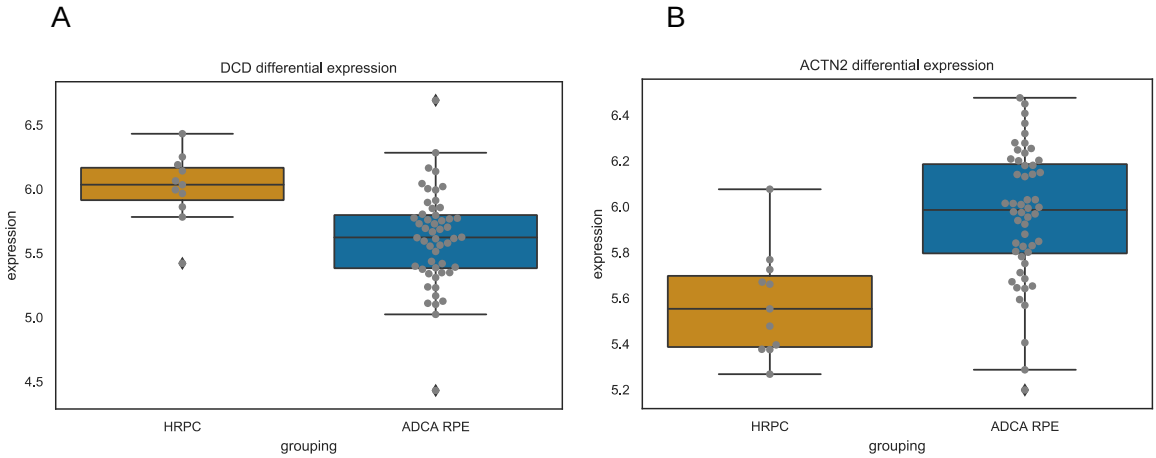

Figure 2: A. Box plot for differential expression of DCD. In HRPC (orange) we observe a consistent and significant over expression of the protein. Points in grey are the expression values observed in the two groups. B. Box plot for differential expression of ACTN2. In ADCA RPE (blue) we observe a consistent and significant over expression of the protein. Points in grey are the expression values observed in the two groups

Chapter 4 Summary and Conclusion

For this deliverable we generated protein profiles of a patient cohort by applying mass spectrometric analysis.

In D6.3 we reported preliminary data on ZTMA76 and here we have the analysis of prepare now for the validation. Our here presented findings deliver candidates and assays that will be applied to further validate the protein expression results.

As a next step, will correlate the results of proteomic analysis with characterization of clonality of genomic level based on phylogeny inferences of multiple regions and time points. This analysis is likely to shed light on mechanisms, underlying development of prostate cancer and its sensitivity to medical treatments. This will then facilitate the development of biomarker panels and also guide the choice of drug treatment for prostate cancer

Chapter 5 List of Abbreviations

IHC	Immunohistochemistry
CRPC	castration resistant prostate cancer
LC	Liquid Chromatography
DDA	Data dependent acquisition
MS	Mass Spectrometry
SWATH-MS	Sequential Windowed Acquisition of All Theoretical Fragment Ion Mass Spectra
TMA	Tissue microarray
PC	Prostate cancer
ITH	Intra-tissue heterogeneity
FFPE	Formalin fixed paraffin embedded
RPE	Radical prostatectomy

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