**Online Supplemental 1**

**Prediction of radio-resistant prostate cancer based on differentially expressed proteins**

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## Immunohistochemistry

For tissue microarrays (TMAs) H&E slides of the prostatectomy specimens of each patient were screened, tumor areas were marked. Gleason score was assigned and areas with different Gleason patterns were identified. TMA were constructed with tissue cylinders, each with a diameter of 1.2mm, punched from selected tumor tissue blocks and embedded in recipient paraffin blocks. In PRP 6 cores from different tumor areas were punched out, in SRP 3 cores were selected. For immunostaining, 3-μm sections of the resulting TMA blocks were transferred to an adhesive coated slide system (Surgipath X-tra Adhesive, Leica Biosystems).

Immunohistochemistry was performed for AKR1C3 [[1](#_ENREF_1)], ALDOA [[2](#_ENREF_2)], AR [[3-5](#_ENREF_3)], BCL-2 [[6](#_ENREF_6)], BIRC5 [[7](#_ENREF_7)], CAV-1 [[8](#_ENREF_8)], CLU [[9](#_ENREF_9)], CXCR4 [[10](#_ENREF_10)], Cyclin D1 [[11](#_ENREF_11)], DAB2IP [[12](#_ENREF_12)], HBP1 [[13](#_ENREF_13)], HIF-1a [[14](#_ENREF_14)], LDH Isoenzyme V [[15](#_ENREF_15)], PIK3R1 [[16](#_ENREF_16)], and PTEN [[17](#_ENREF_17)] using an automated staining system (Lab Vision Autostainer 480 S, Thermo Scientific, or Bond-Max, Leica Microsystems; **Table 1 suppl.**) according to the manufacturer's protocols. Primary and secondary antibodies are listed in **Table 1 supll.** including dilution, pretreatment, company and catalogue number.

The prerequisite for inclusion in the analysis was a homogenous staining intensity. Semiquantitative assessment was applied for cytoplasmic staining considering 0 (negative, <25%); 1 (weak, 25–50%); 2 (moderate, 50–70%); 3 (strong, >75%). Nuclear staining was distinguished as negative=0 or positive=1. In general, the highest staining intensity was reported. Evaluation of tissue staining was performed by light microscopy (DM2500, Leica, Germany) by two experienced pathologists (SS, MW) who were blinded concerning prior treatment. If scoring discrepancies occurred the cases were discussed for consensus. In a second analysis the SRP group was compared to the PRP group only considering the highest Gleason pattern. For publication slides were scanned (NanoZommer S360, Hamamatsu, Hamamatsu City, Japan) and processed using NDP.viewer 2 (NanoZommer S360, Hamamatsu, Hamamatsu City, Japan).

## ELISA in serum samples

For enzyme-linked immunosorbent assay (ELISA) serum samples of patients immediately prior PRP (low-risk n=11, high-risk n=24), SRP (n=24) and controls (n=7) without malignant disease were identified. Blood samples were collected in serum separation tubes (S-Monovette® 7.5ml Z-Gel, Sarstedt AG, Nürnbrecht, Germany) that were kept at room temperature for approximately 60 min to allow for complete coagulation after blood aspiration. The samples were then centrifuged for 10 min at 2,500 x g to separate serum and aliquots were frozen at -80°C.

96 well plates were washed twice with phosphate buffered saline (PBS) and 50µL of patient serum was added and incubated for 1 hour at room temperature. The washing step was repeated 3x with PBS followed by an incubation step with the primary antibody (AR, AKR1C3; 1:500) for 1 hour at room temperature. After washing twice the secondary antibody (anti-mouse IgG; 1:5000) was added and incubated for 1 hour at room temperature. Finally the ELISA was washed twice with PBS, 50µL substrate solution (Invitrogen Corporation) was added and the reaction was stopped exactly after 10min with a stopping solution (Bethyl Laboratories, Inc., Montgomery, TX, USA). For ELISA analysis the reader was used at 450nm.

## Statistical analysis

Values were expressed as median plus interquartile range. Continuous data were compared using the two-tailed Mann–Whitney U test while chi-squared test was used to compare categorical parameters. One-way ANOVA and Bonferroni correction were used to analyze the different ELISA groups.

Kruskal-Wallis test was used for multiple group comparison in order to identify radio-recurrent PCA patients with similar protein expression as patients in the subgroup of primary Gleason high-risk PCA. The test statistics were adjusted for ties. Dunn-Bonferroni-Test was used as post hoc test to analyze all pairs of each analysis to detect intergroup differences. Cohen’s d was used to calculate the intergroup effect size [[18](#_ENREF_18)]. Correlations of ≤0.10, 0.20, and ≥0.30 were considered as relatively small, typical, and relatively large [[19](#_ENREF_19)]. Values <0.1 indicate group equalities, values >0.3 indicate differences between the groups. All tests were 2-sided. A p-value of <0.05 was considered significant. Statistical calculations were performed using SPSS 24.0 (SPSS) and Prism 7 (GraphPad Software).

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