TMPRSS2:ERG gene fusion and ERG overexpression in human prostate cancer are associated with changed metabolism

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Introduction: TMPRSS2:ERG gene fusion has been reported to be the most common gene rearrangement in prostate cancer and is found in about 50 % of prostate cancer patients[1]. It is shown to be associated with increased epithelial-to-mesenchymal potential, cell invasion and cell proliferation [2], although its clinical and prognostic significance is not fully understood. TMPRSS2:ERG is the most important contributory factor in overexpression of ERG [3] and recent research indicates a link between overexpression of ERG and cancer metabolism [4]. However, the mechanisms linking metabolism and ERG overexpression are yet to be solved and further insight into cancer metabolism and ERG expression is needed. The main aim of this study was to investigate the potential metabolic alterations associated with the overexpression of ERG or the presence of TMPRSS2:ERG and thereby increase the understanding of the biological mechanisms behind fusion positive and negative patients.

Methods: A 2 mm transversal prostate tissue slice was collected from 41 patients with no previous treatment for prostate cancer, using a standardized harvesting method [5]. From each tissue slice, several samples (median: 3, range: 1 to 6 samples per slice, depending on tumor size) were collected from cancerous areas assessed by histopathological evaluation of cryosections from the tissue slices. In total 95 cancerous samples were collected. A second cohort of prostate cancer patients was included for validation and included 40 needle biopsy samples with cancerous tissue obtained after radical prostatectomy from 40 patients. ^{1}H HRMAS MRS was performed on a Bruker Avance DRX600 (14.1 T) Spectrometer (Bruker Biospin, Germany) with a dual 1H/13C MAS probe. Each tissue sample (mean weight: 12.7 mg, range: 3.0 to 21.9 mg (tissue slices), 12.3 mg, range: 6.7 to 21.9 mg (needle biopsies)) was transferred to disposable HRMAS inserts together with 3 µl deutermium oxide (D_2O) solution containing 25mM formate and placed into zirconium rotors (4 mm). Proton spectra were acquired with a 1D NOESY pulse sequence (noesygppr1d, Bruker Biopsin, Germany) at 5 ºC with a spin rate of 5 kHz. Samples were hematoxylin, eosin and saffron (HES) stained for histopathological evaluation of Gleason grading and assessment of cancer and stromal content. Gene expression profiles were measured with Illumina Human HT-12 v4 Expression BeadChip (Illumina, USA) on the 95 tissue slice samples. Gene sets related to prostate cancer were downloaded from Markert et al.[6] and Gene Set Enrichment Analysis (GSEA) was performed for detection of specific enrichment of the ERG-fusion gene set, as previously described [7]. Samples were regarded to show an overexpression of ERG if the GSEA score for the ERG-signature was increased two-fold compared to the mean GSEA-score for the ERG-signature of the 95 tissue slice samples. Results were validated in a cohort of 40 needle biopsy samples using a three-probe detection fluorescence in situ hybridization (FISH) kit for indirect detection of TMPRSS2:ERG (Kreatech, Germany). For each sample, 25 non-overlapping nuclei in cancerous areas were evaluated for deletion of the TMPRSS2 (21q22) gene region associated with TMPRSS2:ERG and regarded as fusion-positive if the deletion was found in >0.8 of the evaluated nuclei. LCModel was used to quantify 25 metabolites against the formate standard. Comparison of metabolite concentrations between ERG high (>2 fold ERG score) and ERG low (<2 fold ERG score) samples, were analyzed by linear mixed models, while metabolite differences between TMPRSS2:ERG positive and negative samples were compared using Student t-test. Metabolite concentrations were normalized prior to analysis. The statistical analysis was performed using Stata 13 (StataCorp, 2013) and SPSS 21 (IBM, 2012).

Results: In total 34 (35.8 %) of the 95 tissue slice samples were found to show an overexpression of ERG. In total, high ERG expression were found in 20 (48.8 %) of the 41 patients. TMPRSS2:ERG were detected in 7 (17.5 %) of the 40 needle biopsy samples. Comparing the ERG-high samples with the ERG-low samples, significant differences were found for citrate, spermine, glutamate, glycine and isoleucine, $p<0.05$. Citrate and spermine concentrations were lower, while the concentration of glutamate, glycine and isoleucine were higher in samples with a high ERG-score compared to samples with lower ERG-scores. Results were also adjusted for cancer and stromal content, but did not prove to have any significant impact on the obtained p-values (results not shown). In the TMPRSS2:ERG positive samples, significant lower citrate and spermine concentrations were found, compared to the TMPRSS2:ERG negative samples. No difference in lactate concentration or Gleason grading could be detected between the two groups in both cohorts (results not shown). Significant metabolites for the tissue slice samples are shown in table 1 and corresponding results for the same metabolites in the needle biopsy samples are shown in table 2.

Table 1: Significant differences in several metabolites were found comparing samples with high ERG score with samples with lower ERG score.

Metabolites	<2 fold ERG score n=61 Concentrations mmoles/kg wet weight (IQR)	>2 fold ERG score n=34 Concentrations mmoles/kg wet weight (IQR)	p-value
Citrate	7.45 (4.42 to 11.50)	3.91 (2.59 to 7.20)	**0.010**
Spermine	1.51 (0.87 to 2.42)	0.89 (0.45 to 1.40)	**0.008**
Glutamate	4.70 (3.82 to 6.47)	5.98 (4.26 to 7.79)	**0.037**
Glycine	2.43 (1.74 to 2.93)	2.90 (1.93 to 3.65)	**0.018**
Isoleucine	0.16 (0.06 to 0.25)	0.19 (0.11 to 0.31)	**0.028**

Table 2: Significant lower concentrations of citrate and spermine were found in samples positive for TMPRSS2:ERG compared to samples negative for TMPRSS2:ERG.

Metabolites	TMPRSS2:ERG negative n=33 Concentrations mmoles/kg wet weight (IQR)	TMPRSS2:ERG positive n=7 Concentrations mmoles/kg wet weight (IQR)	p-value
Citrate	6.74 (3.88 to 8.74)	3.05 (1.25 to 4.69)	**0.013**
Spermine	0.69 (0.38 to 0.81)	0.31 (0.10 to 0.52)	**0.021**
Glutamate	4.08 (3.12 to 4.62)	3.94 (3.45 to 4.64)	0.832
Glycine	2.37 (1.92 to 2.86)	2.41 (1.54 to 2.64)	0.857
Isoleucine	0.17 (0.09 to 0.25)	0.13 (0.08 to 0.16)	0.413

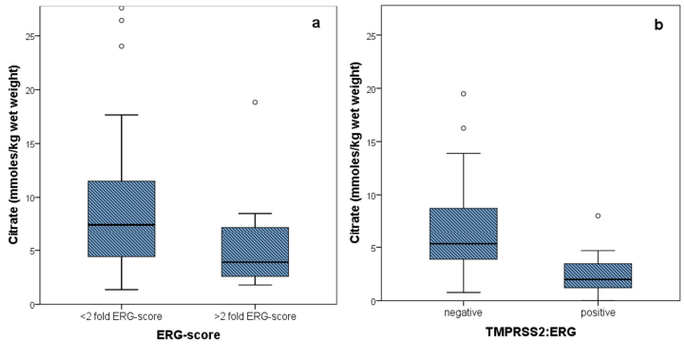

Figure 1: Significant lower concentrations of citrate were found both in samples with high ERG-score (figure 1a) and TMPRSS2:ERG positive samples (figure 1b) compared to samples with lower ERG expression and samples negative for TMPRSS2:ERG.

Discussion: Significant differences were found for citrate, spermine, glutamate, glycine, and isoleucine comparing ERG-high samples and ERG-low samples. However, a low prevalence of the fusion gene in the needle biopsy samples only allowed a validation of the findings of citrate and spermine (figure 1), metabolites previously shown to distinguish between an aggressive and low-risk phenotype of prostate cancer [8]. Interestingly, there could not be found any differences in Gleason score or lactate levels between the two groups in both cohorts. These initial results suggest that overexpression of ERG or presence of TMPRSS2:ERG is associated with lower citrate and spermine levels, but further research is needed to clarify the effect of ERG overexpression/TMPRSS2:ERG upon cancer metabolism.

References: 1. Esgueva R et al. *Mod Pathol* 2010;23(4):539-46. **2.** FitzGerald L et al. *BMC Cancer* 2008;8(1):230. **3.** Tomlins SA et al. *Science* 2005;310(5748):644-48. **4.** Massoner P et al. *PLoS ONE* 2013;8(2) **5.** Bertilsson H et al. *The Prostate* 2011;71(5):461-69. **6.** Markert EK et al. *PNAS* 2011;108(52):21276-81. **7.** Rye M et al. *BMC Medical Genomics* 2014;7(1):50. **8.** Giskeødegård GF et al. *PLoS ONE* 2013;8(4).

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