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Institut für Pathologie

Prof. Dr. med. Guido Sauter

Eine erhöhte Expression von Lysophophatidylcholin- Acyltransferase 1 erhöht das Rezidivrisiko in Prostatakarzinomen

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Stella Sanader

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High lysophosphatidylcholine acyltransferase 1 expression independently predicts high risk for biochemical recurrence in prostate cancers

Katharina Grupp[a,b,1], Stella Sanader[b,1], Hüseyin Sirma[b], Ronald Simon[b], Christina Koop[b], Kristina Prien[b], Claudia Hube-Magg[b], Georg Salomon[c], Markus Graefen[c], Hans Heinzer[c], Sarah Minner[b], Jakob R. Izbicki[a], Guido Sauter[b], Thorsten Schlomm[c,d], Maria Christina Tsourlakis[b,*]

[a]General, Visceral and Thoracic Surgery Department and Clinic, University Medical Center Hamburg-Eppendorf, Germany
[b]Institute of Pathology, University Medical Center Hamburg-Eppendorf, Germany
[c]Martini-Clinic, Prostate Cancer Center, University Medical Center Hamburg- Eppendorf, Germany
[d]Department of Urology, Section for translational Prostate Cancer Research, University Medical Center Hamburg-Eppendorf, Germany

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ABSTRACT

Lysophosphatidylcholine acyltransferase 1 (LPCAT1) has been suggested to play a role in cancer. To assess its role in prostate cancer, LPCAT1 expression was analyzed on a tissue microarray containing samples from 11,152 prostate cancer patients. In benign prostate glands, LPCAT1 immunostaining was absent or weak. In prostate cancer, LPCAT1 positivity was found in 73.8% of 8786 interpretable tumors including 29.2% with strong expression. Increased LPCAT1 expression was associated with advanced tumor stage (pT3b/T4) ($p < 0.0001$), high Gleason score (≥4 + 4) ($p < 0.0001$), positive nodal involvement ($p = 0.0002$), positive surgical margin ($p = 0.0005$), and early PSA recurrence ($p < 0.0001$). High LPCAT1 expression was strongly linked to ERG-fusion type prostate cancer. Strong LPCAT1 staining was detected in 45.3% of ERG positive but in only 16.7% of ERG negative tumors ($p < 0.0001$). Within ERG negative cancers, LPCAT1 staining was strongly increased within the subgroup of PTEN deleted cancers ($p < 0.0001$). Further subgroup analyses revealed that associations of high LPCAT1 expression with PSA recurrence and unfavorable tumor phenotype were largely driven by ERG negative cancers ($p < 0.0001$) while these effects were substantially mitigated in ERG positive cancers ($p = 0.0073$). The prognostic impact of LPCAT1 expression was independent of histological and clinical parameters. It is concluded, that LPCAT1 measurement, either alone or in combination, may be utilized for better clinical decision-making. These data also highlight the potentially important role of lipid metabolism in prostate cancer biology.

* Corresponding author. Tel.: +49 40 7410 55393; fax: +49 40 7410 55997.
E-mail addresses: k.grupp@uke.de (K. Grupp), stella.sanader@hotmail.de (S. Sanader), h.sirma@uke.de (H. Sirma), r.simon@uke.de (R. Simon), c.koop@uke.de (K. Koop), k.prien@uke.de (K. Prien), c.hube@uke.de (C. Hube-Magg), g.salomon@uke.de (G. Salomon), graefen@uke.de (H. Graefen), heinzer@uke.de (H. Heinzer), s.minner@uke.de (S. Minner), izbicki@uke.de (J.R. Izbicki), g.sauter@uke.de (T. Sauter), tschlomm@uke.de (T. Schlomm), mtsourlakis@uke.de (M.C. Tsourlakis).
[1] These authors contributed equally to this work.
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1. Introduction

Prostate cancer represents a major cause of cancer-related mortality and morbidity (Siegel et al., 2013). In consequence of the clinical and histological heterogeneity of prostate cancer, possible treatment options vary from active surveillance to surgical or radiation therapy. As the common preoperative prognostic parameters Gleason grade, tumor extent on biopsies, preoperative PSA and clinical parameters are not satisfactory for optimal individual treatment decisions, the identification of novel biomarkers reflecting the tumor aggressiveness are desperately needed.

Alterations in lipid metabolism affect numerous cellular processes that are relevant for cancer biology, including cell growth, proliferation, differentiation and motility (Santos and Schulze, 2012). Lysophosphatidylcholine acyltransferase 1 (LPCAT1) is a key enzyme of the Lands cycle that contributes to the synthesis of diverse individual phosphatidylcholine specimens, which are prominent constituents of eukaryotic membranes and major structural features of serum lipoproteins (Kent, 2005). LPCAT1 has physiological roles in the lung where it generates the component dipalmitoyl phosphatidylcholine of pulmonary surfactant (Bridges et al., 2010; Chen et al., 2006; Nakanishi et al., 2006), in non-inflammatory platelet-activation factor remodeling pathway (Harayama et al., 2008) and in retinal photoreceptor homeostasis (Cheng et al., 2009).

Only recently, overexpression of LPCAT1 has also been described in human malignancies, such as human colorectal cancer (Mansilla et al., 2009). Functional analyses in colon cancer cell lines had also demonstrated that overexpressed LPCAT1 results in a significant growth advantage (Mansilla et al., 2009).

In one recent study elevated LPCAT1 expression was also found in prostate cancer and an association of LPCAT1 expression levels with PSA recurrence was suggested in a cohort of 148 patients (Zhou et al., 2012).

To further evaluate the potential of LPCAT1 as a clinically relevant prognostic prostate cancer biomarker, and to search for possible associations with molecularly defined cancer subgroups, a preexisting tissue microarray (TMA) containing 11,152 prostate cancer specimens with follow-up data and attached molecular information was analyzed for LPCAT1 expression. Our data identify high LPCAT1 expression as a strong and independent prognostic biomarker of early PSA recurrence in prostate cancer.

2. Materials and methods

2.1. Patients

Radical prostatectomy specimens were available from 11,152 patients, undergoing surgery between 1992 and 2011 at the Department of Urology, and the Martini Clinics at the University Medical Center Hamburg-Eppendorf. Follow-up data were available of 9695 patients with a median follow-up of 36.8 months (range: 1–228 months; Table 1). Prostate specific antigen values were measured following surgery and recurrence was defined as a postoperative PSA of 0.2 ng/ml and rising. All prostate specimens were analyzed according to a standard procedure, including complete embedding of the entire prostate for histological analysis (Erbersdobler et al., 2002). The Gleason score had been diagnosed in the prostatectomy specimens. The TMA manufacturing process was described earlier in detail (Mirlacher and Simon, 2010). In short, one 0.6 mm core was taken from a representative tissue block from each patient. The tissues were distributed among 24 TMA blocks, each containing 144 to 522 tumor samples. Presence or absence of cancer tissue was validated by immunohistochemical AMACR and 34BE12 analysis on adjacent TMA sections. For internal controls, each TMA block also contained various control tissues, including normal prostate tissue. The molecular database attached to this TMA contained results on ERG expression in 9,628, ERG break apart fluorescence *in-situ* hybridization (FISH) analysis in 6106 (expanded from Minner et al. (2011a)), and deletion status of 5q21 in 3037 (Burkhardt et al., 2013), 6q15 in 3528 (extended from Kluth et al. (2013)), *PTEN* in 6130 (Krohn et al., 2012), and 3p13 in 1290 (unpublished data) tumors.

Table 1 – Composition of the prognostic tissue microarray containing 11,152 prostate cancer specimens.

	No. of patients	
	Study cohort on TMA ($n = 11,152$)	Biochemical relapse among categories (i=1824)
Follow-up (mo)		
Mean	53.4	–
Median	36.8	–
Age (y)		
<50	318	49
50–60	2.768	460
60–70	6.548	1.081
>70	1.439	232
Pretreatment PSA (ng/ml)		
<4	1.407	142
4–10	6735	827
10–20	2159	521
>20	720	309
pT category (AJCC, 2002)		
pT2	7.370	570
pT3a	2.409	587
pT3b	1.262	618
pT4	63	49
Gleason grade		
≤3 + 3	2.859	193
3 + 4	1.565	573
4 + 3	6.183	849
≥4 + 4	482	208
pN category		
pN0	6.117	1.126
pN+	561	291
Surgical margin		
Negative	8.984	1.146
Positive	1.970	642

Note: Numbers do not always add up to 11,152 in the different categories because of cases with missing data. Abbreviation: AJCC, American Joint Committee on Cancer.

2.2. Immunohistochemistry

Freshly cut TMA sections were immunostained in one day and in one experiment. Primary antibody specific for LPCAT1 (rabbit, ProteinTech; at 1/1350 dilution) was applied, slides were deparaffinized and exposed to heat-induced antigen retrieval for 5 min in an autoclave at 121 °C in pH 7.8 Tris-EDTA-Citrate buffer. Bound antibody was then visualized using the EnVision Kit (Dako). LPCAT1 staining was analyzed by one person (KG) experienced in immunohistochemisty. LPCAT1 staining was evaluated according to the following scoring system: The staining intensity (0, 1+, 2+, and 3+) and the fraction of positive tumor cells were recorded for each tissue spot. A final score was built from these two parameters according to the following established score, as previously described (Grupp et al., 2013; Minner et al., 2011b,c; Muller et al., 2013) Negative scores had absence of LPCAT1 staining, weak scores had staining intensity of 1+ in ≤70% of tumor cells or staining intensity of 2+ in ≤30% of tumor cells; moderate scores had staining intensity of 1+ in ≥70% of tumor cells, staining intensity of 2+ in >30% but in ≤70% of tumor cells or staining intensity of 3+ in ≤30% of tumor cells; strong scores had staining intensity of 2+ in >70% of tumor cells or staining intensity of 3+ in >30% of tumor cells.

2.3. Statistics

Statistical calculations were performed with JPM 9 software (SAS Institute Inc., NC, USA). Contingency tables and the chi^2-test were performed to search for associations between molecular parameters and tumor phenotype. Survival curves were calculated according to Kaplan–Meier. The Log–Rank test was applied to detect significant survival differences between groups. COX proportional hazards regression analysis was performed to test the statistical independence and significance between pathological, molecular and clinical variables.

3. Results

3.1. Technical issues

A total of 2366 of 11,152 (21.2%) tissue cores were non-informative for LPCAT1 immunohistochemistry due to the complete lack of tissue or absence of unequivocal cancer cells on individual TMA spots.

3.2. Immunohistochemistry of LPCAT1

LPCAT1 expression in epithelial cells was - if present - generally cytoplasmic and occasionally showed a granular pattern. In prostate cancers, 6482 of our 8786 interpretable tumors (73.8%) showed positive LPCAT1 immunostaining, which was considered weak in 17.7%, moderate in 26.9% and strong in 29.2% of cases. Representative images of LPCAT1 expression in benign and cancerous prostate tissue are given in Figure 1. Increased LPCAT1 expression was significantly linked to advanced pathological tumor stage (pT3b/T4) ($p < 0.0001$), high Gleason score (≥4 + 4) ($p < 0.0001$), positive nodal involvement ($p = 0.0002$) and positive surgical margin ($p = 0.0005$) if all tumors were analyzed (data not shown).

3.3. Relationship with fusion type in prostate cancer and ERG protein expression

To evaluate whether LPCAT1 expression is linked to fusion type in prostate cancer, our pre-existing database including data on ERG-fusion status obtained by FISH in 5414 patients and by IHC in 8526 tumors with available LPCAT1 expression data was used. Strong LPCAT1 expression was significantly associated with *TMPRSS2-ERG* fusion type ($p < 0.0001$). Strong LPCAT1 expression was seen in 1104 of 2494 (44.3%) cancers with ERG rearrangement detected by FISH but in only 471 of 2920 (16.1%) cancers without ERG rearrangement ($p < 0.0001$, Figure 2). Accordingly, strong LPCAT1 staining was detected in 1707 of 3765 (45.3%) tumors with ERG expression positivity but in only 794 of 4761 (16.7%) cancers with ERG expression negativity by IHC ($p < 0.0001$, Figure 2). The associations retained its significance in subset analysis of tumors with a Gleason grade ≤3 + 3, 3 + 4, 4 + 3, and ≥4 + 4. Furthermore, associations with tumor phenotype and clinical cancer features were separately analyzed in the subsets of ERG positive and negative prostate cancers (Tables 2 and 3). In 4761 ERG negative cancers, high LPCAT1 expression was significantly associated with advanced tumor stage, high Gleason grade, positive nodal involvement ($p < 0.0001$ each) and positive surgical margin ($p = 0.006$). LPCAT1 immunostaining was only significantly linked to advanced Gleason grade ($p < 0.0001$) in 3765 ERG positive prostate cancers, however.

3.4. Relationship with key genomic deletions associated with distinct subgroups of prostate cancers

Earlier studies had provided evidence for distinct molecular subgroups of prostate cancers defined by *TMPRSS2-ERG* fusions and several genomic deletions. We and others had described a strong link of *PTEN* and 3p13 deletion to ERG positivity and of 5q21 and 6q15 deletions to ERG negativity (Berger et al., 2011; Burkhardt et al., 2013; Kluth et al., 2013; Krohn et al., 2012; Lapointe et al., 2007; Taylor et al., 2010). To study, whether LPCAT1 expression might be associated with one of these genomic deletions, LPCAT1 IHC results were compared with preexisting findings on deletions of *PTEN*, 3p13, 6q15 and 5q21. In all cancers, deletions at *PTEN* and 3q13 were significantly linked to increase LPCAT1 expression ($p < 0.0001$, $p = 0.0028$) while deletions at 6q15 were associated with slightly reduced LPCAT1 expression ($p = 0.0395$, Figure 3). Based on the strong associations of both LPCAT1 and these deletions with *ERG* status, such associations were expected in mixed cohorts. It was therefore not surprising, that none of these associations was retained within ERG positive cancers (Figure 4). However, a strong positive association between LPCAT1 expression and *PTEN* deletions was retained in ERG negative cancers ($p < 0.0001$, Figure 5). In the same subgroup, statistically significant associations were also observed between LPCAT1 and 6q15 as well as 5q21 deletions ($p = 0.0005$ and $p = 0.0065$), although the differences in absolute numbers were small for these deletions. Additional analysis revealed that these associations mostly retained its significance in subset analysis of tumors with a Gleason grade ≤3 + 3, 3 + 4, 4 + 3, and ≥4 + 4 although the absolute number of analyzable tumors was rather small.

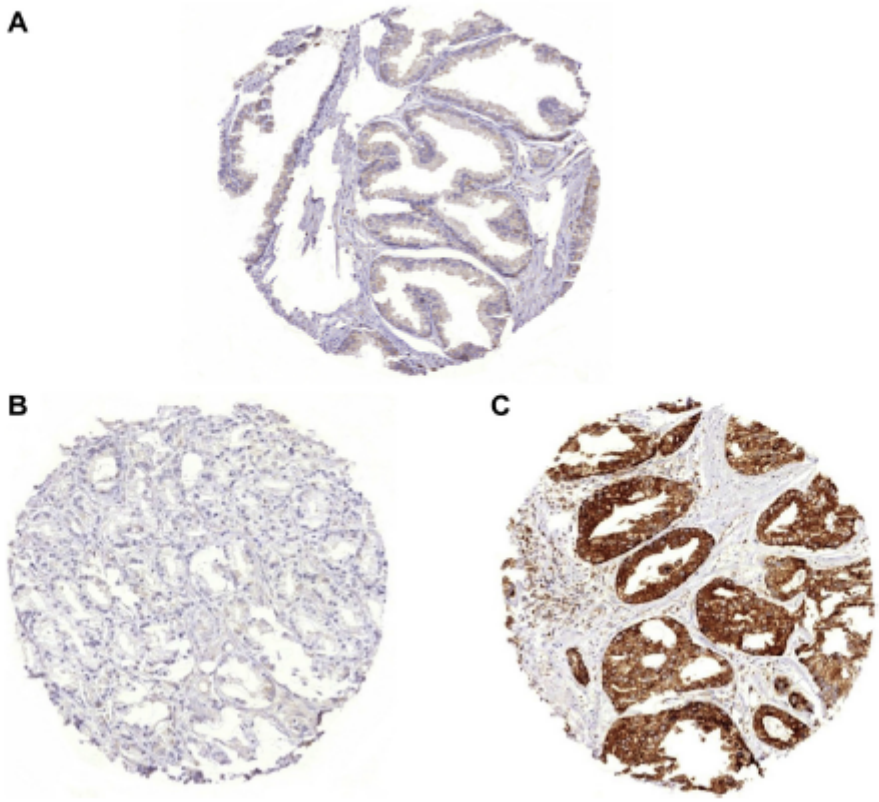

Figure 1 – Representative immunohistochemical pictures of (A) weak LPCAT1 staining in benign prostate, (B) negative LPCAT1 expression in prostate cancer and (C) strong LPCAT1 staining in prostate cancer.

3.5. Prognostic role of LPCAT1 expression

Follow-up data were available for 7620 patients with informative LPCAT1 data. The prognostic role of Gleason grade is given for this patient subset in order to demonstrate the overall validity of our follow-up data ($p < 0.0001$, Figure 6A). LPCAT1 was related to early biochemical recurrence in the analysis of all cancers ($p < 0.0001$, Figure 6B). This association was particularly evident in 4102 ERG negative cancers ($p < 0.0001$, Figure 6C) but was only marginal in 3283 ERG positive cancers ($p = 0.0073$, Figure 6D). Another analysis stratifying according to the *PTEN* deletion status revealed that LPCAT1 expression was significantly associated with prognosis in the subgroup of 3627 *PTEN* non-deleted cancers ($p = 0.0045$, Figure 6E) but not in the smaller subset of 846 *PTEN* deleted cancers ($p = 0.6603$, Figure 6F).

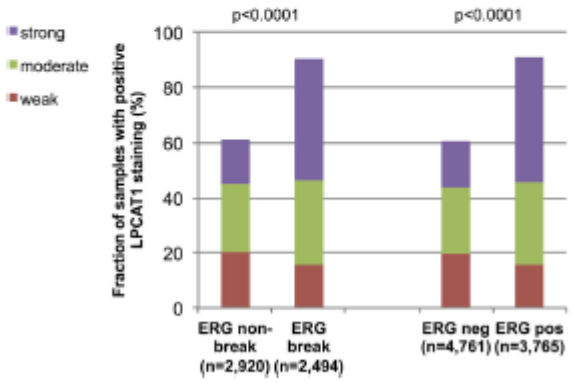

Figure 2 – Relationship of LPCAT1 expression with ERG-fusion probed by fluorescence in-situ hybridization analysis and immunohistochemistry in all prostate cancers.

3.6. Multivariate analysis

Four multivariate analyses were performed evaluating the clinical relevance of LPCAT1 expression in different scenarios (Table 4). No 1 was utilizing all post-operatively available parameters including pT, pN, margin status, pre-operative PSA value and Gleason grade obtained on the resected prostate. Scenario 2 included all postoperatively available parameters with the exception of the lymph node status. The rational for this approach was that lymphadenectomy is not a routine procedure in the surgical therapy of prostate cancer and that excluding the nodal status in multivariate analysis increases case numbers. The next two scenarios tried to better model the pre-operative situation. Scenario 3 included the LPCAT1

Table 2 – Associations between LPCAT1 expression results and ERG negative prostate cancer phenotype.

Parameter	n Evaluable	LPCAT1 IHC result				p value
		Negative (%)	Weak (%)	Moderate (%)	Strong (%)	
All cancers	4.761	39.36	19.3	24.66	16.68	
Tumor stage						
pT2	3.225	42.73	20.12	23.41	13.74	<0.0001
pT3a	958	36.12	18.16	25.47	20.25	
pT3b	532	25.56	16.17	30.45	27.82	
pT4	28	25	25	21.43	28.57	
Gleason grade						
≤3 + 3	1.112	52.16	19.15	17.9	10.79	<0.0001
3 + 4	2.646	38.7	20.26	24.87	16.18	
4 + 3	727	28.89	17.47	31.22	22.42	
≥4 + 4	252	20.24	15.48	32.94	31.35	
Lymph node metastasis						
N0	2.691	36.94	19.73	25.12	18.21	<0.0001
N+	236	20.76	17.37	36.44	25.42	
Surgical margin						
negative	3.819	40.35	19.46	24.38	15.82	0.006
positive	856	35.51	18.57	25.82	20.29	

expression, pre-operative PSA, clinical stage (cT) and the Gleason grade obtained on the prostatectomy specimen. However, the pre-operative determination of a tumors Gleason grade is subject to sampling errors and therefore results in under grading in more than one third of cases (Epstein et al., 2012). Because the post-operative Gleason grade thus varies from the pre-operative Gleason grade, another multivariate analysis was added as scenario 4. In this scenario, the pre-operative Gleason grade obtained on the original biopsy was combined with pre-operative PSA, clinical stage and LPCAT1 expression. If all tumors are analyzed, all four scenarios suggest a tendency towards LPCAT1 representing an independent predictor of prognosis, especially in scenarios 3 and 4 using preoperative parameters. Subgroup analyses further revealed that independent prognostic value was found in both ERG negative and positive cancers if preoperative parameters were used (Table 4b and c). In multivariate analysis including parameters that are available after surgery, independent prognostic relevance of LPCAT1 was found in ERG positive prostate cancers only (Table 4c).

4. Discussion

The results of our study identify LPCAT1 immunostaining as a strong predictor of an increased risk for biochemical recurrence in prostate cancer.

Our immunohistochemical analysis showed positive LPACT1 staining in 73.8% of the interpretable prostate cancers. The intensity of the immunohistochemical signal was

Table 3 – Associations between LPCAT1 expression results and ERG positive prostate cancer phenotype.

Parameter	n Evaluable	LPCAT1 IHC result				p value
		Negative (%)	Weak (%)	Moderate (%)	Strong (%)	
All cancers	3.765	9.0	15.62	30.04	45.34	
Tumor stage						
pT2	2.260	9.42	15.58	29.78	45.22	0.5767
pT3a	1.006	8.85	15.81	30.02	45.33	
pT3b	460	7.39	14.78	31.52	46.3	
pT4	21	0	9.52	33.33	57.14	
Gleason grade						
≤3 + 3	854	11.94	18.27	32.67	37.12	<0.0001
3 + 4	2.249	8.49	15.07	29.57	46.87	
4 + 3	521	6.91	13.44	28.6	51.06	
≥4 + 4	116	6.03	12.93	27.59	53.45	
Lymph node metastasis						
N0	2.092	7.17	15.58	29.54	47.71	0.57
N+	205	9.76	14.15	27.8	48.29	
Surgical margin						
Negative	2.975	9.31	15.73	30.29	44.67	0.2687
Positive	724	7.73	14.5	29.56	48.2	

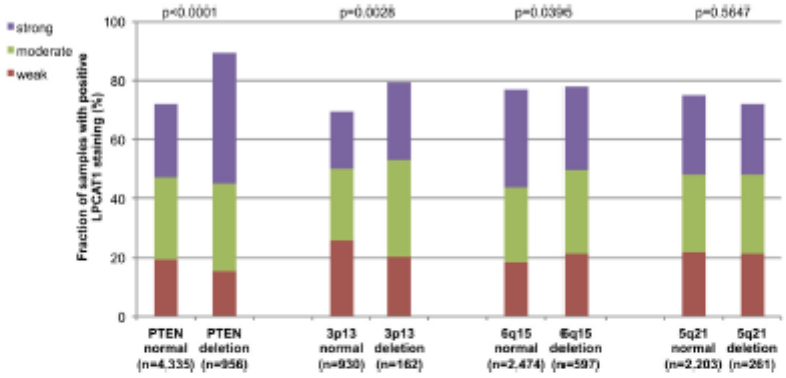

Figure 3 – LPCAT1 expression versus deletions at PTEN, 3p13, 6q15 and 5q21 probed by fluorescence in-situ hybridization analysis in all prostate cancers.

typically stronger in malignant than in benign prostate epithelium. These data are generally in line with one earlier study analyzing LPCAT1 expression on a TMA containing 251 samples from 148 patients who had undergone prostatectomy, or transurethral resection of the prostate (Zhou et al., 2012). In this study, LPCAT1 expression was assessed by a score defined by intensity and extent of LPCAT1 immunostaining with a maximum score of 9 (Zhou et al., 2012). In accordance with our study, the authors found that LPCAT1 staining was predominantly localized in the cytoplasm of the cells (Zhou et al., 2012). Increasing levels of LPCAT1 positivity were then described from benign prostatic changes (mean IHC score: 2.68) to high-grade prostatic intraepithelial neoplasia (mean IHC score: 2.72), non-metastatic (mean IHC score: 4.63) and metastatic (mean IHC score: 8.00) prostate cancers (Zhou et al., 2012).

The most striking finding in our study is the strong association of LPCAT1 expression with unfavorable histological phenotype and clinical outcome. The strong and independent prognostic value of LPCAT1 overexpression suggests a biologically relevant role in prostate cancer cells. This assumption is consistent with numerous previous studies implicating tumor relevant functional consequences of alterations in the lipid metabolism (Furuta et al., 2010; Menendez, 2010; Menendez and Lupu, 2007; Santos and Schulze, 2012; Suburu and Chen, 2012). For example, several studies have presented data suggesting that activation of lipid biosynthesis and lipid remodeling is a common feature of cancer cells, and that high expression of proteins involved in lipid metabolism, such as fatty acid-binding protein (C-FABP) (Morgan et al., 2008), fatty acid synthase (FASN) (Flavin et al., 2010; Shah et al., 2006), Caveolin-1 (Freeman et al., 2012) or fatty acid elongase 7 (ELOVL7) (Tamura et al., 2009) may be linked to progression and metastatic growth in prostate cancer. Our data, identifying LPCAT1 as a strong prognostic factor for prostate cancer, further support the emerging concept of an important role of lipid metabolism for the behavior of prostate cancer cells (Faas et al., 1996, 2001).

It was one major aim of this project to investigate for possible interactions of LPCAT1 with key genomic alterations in prostate cancer. About 50% of prostate cancers are characterized by gene fusions linking the androgen-regulated gene *TMPRSS2* with the transcription factor *ERG* of the *ETS* family (Tomlins et al., 2005). In consequence of this rearrangement,

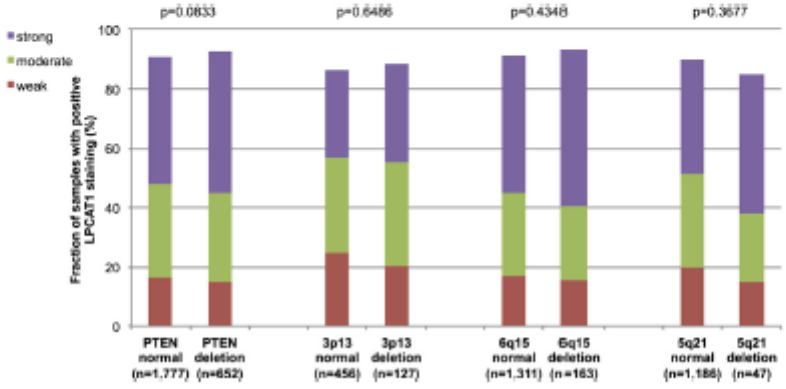

Figure 4 – LPCAT1 expression versus deletions at PTEN, 3p13, 6q15 and 5q21 probed by fluorescence in-situ hybridization analysis in ERG positive prostate cancers.

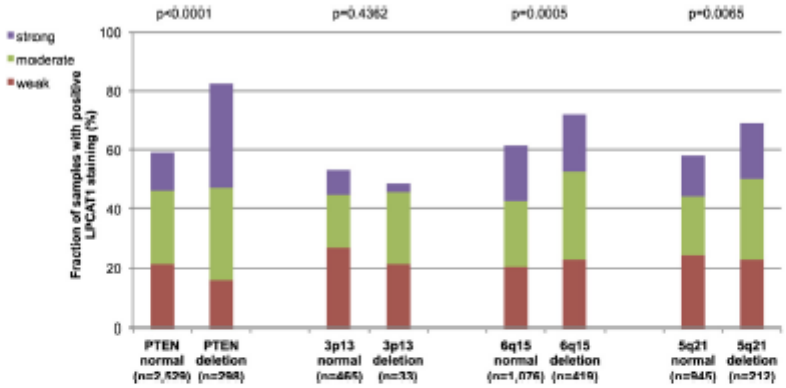

Figure 5 – LPCAT1 expression versus deletions at PTEN, 3p13, 6q15 and 5q21 probed by fluorescence in-situ hybridization analysis in ERG negative prostate cancers.

the ERG expression becomes massively overexpressed. Our data demonstrate that high level LPCAT1 expression is significantly associated with ERG fusion type prostate cancer. As this link was found by IHC and FISH, two independent approaches for ERG fusion detection, a false positive association due to inefficient immunostaining for both LPCAT1 and ERG in a subset of damaged non-reactive tissues can be largely excluded. Our finding of high LPCAT1 expression in fusion positive prostate cancers is coherent with earlier data showing that ERG dependent pathways target the lipid metabolism of cancer cells (Iljin et al., 2006; Vainio et al., 2011a). For example, ERG induces phospholipase PLA2G7, an important enzyme for cell growth, signaling and maintenance of membrane phospholipids (Dong et al., 2006; Iljin et al., 2006; Scott et al., 2010; Vainio et al., 2011a,b) required for the growth of ERG positive, but not of ERG negative prostate cancer cells (Vainio et al., 2011a). PLA2G7 releases lysophosphatidylcholine from the cell membrane, which in turn is a substrate for LPACT1. Upregulation of LPCAT1 in ERG positive cancer cells may thus be a result of PLA2G7 activation in these cells. It remains to be shown whether ERG is also directly involved in LPCAT1 regulation. Of note, ETS1, another member of the ETS family of transcription factors, induces LPCAT2, an isoform of LPCAT1, in ovarian cancer cells (Verschoor et al., 2010). Based on these data, it could be possible, that ERG increases the cellular phosphatidylcholine content by a dual action on both PLA2G7 and LPCAT1.

While LPCAT1 was generally high in ERG positive prostate cancer and independent of any of the key chromosomal deletions (5q, 6q, 3p, *PTEN*) in this subgroup, a comparable level of LPCAT1 expression could be observed in the *PTEN* deleted subset of ERG negative cancers. This observation may possibly be explained by the known role of PTEN to upregulate the LPCAT1-antagoists phospholipase D and C (Horie et al., 2004). *PTEN* inactivation may thus – through reduced activity of LPCAT1 antagonists - induce increased LPCAT1 expression to a similar level as achieved by ERG activation. At the same time our findings also demonstrate, that such a *PTEN* induced effect on LPCAT1 expression is no longer operational in ERG positive cancers where other mechanisms are obviously in place to induce high LPCAT1 expression. Alternatively, it cannot be completely excluded, that the striking association of *PTEN* deletions with LPCAT1 overexpression in ERG negative cancers is caused by the known link of both alterations to fusion type prostate cancer. As our classifier for fusion type prostate cancer only includes the most frequent ETS family member (ERG), it must be assumed that a small fraction of fusion type positive prostate cancers are missed by our approach. It is estimated, that about 5–10% of all fusion type prostate cancers do not have ERG activation (Rubin et al., 2011).

The large number of cancers analyzed in this study enabled us to determine, that associations of LPCAT1 expression with unfavorable tumor phenotype and PSA recurrence were largely driven by the subset of ERG negative cancers, while the impact of LPCAT1 expression on tumor phenotype and PSA recurrence was substantially less obvious in ERG positive cancers. These observations again, suggest a markedly different role of LPCAT1 in fusion type and non-fusion type prostate cancer. It is remarkable, that the prognostic effect of LPCAT1 expression predominantly occurs in the subset of ERG negative cancers with a markedly lower basic LPCAT1 expression than in ERG positive cancers. It is tempting to speculate, that the marked attenuation of the prognostic relevance of LPCAT1 in ERG positive cancers could be due to interference of ERG with other molecules involved in LPCAT1 action, thus rendering effects of LPCAT1 expression on tumor aggressiveness inactive. Candidate genes for such a role include the lipoxygenase ALOX15, the lipid transporter SORL1, the lipoprotein receptor VLDLR, and the phospholipases PLA1A and PLA2G2A, all of which have been reported to be differentially expressed in ERG negative and ERG positive prostate cancers (Brase et al., 2011; Jhavar et al., 2009) and all of which may have a potential impact on LPCAT function by either involvement in lipoprotein uptake (Klinger et al., 2011; Shen et al., 2012), production of lipid signaling mediators (Il Lee et al., 2011), glycerophospholipids and lysophosholipids (Kudo and Murakami, 2002). The mechanism by which LPCAT1 exerts a direct impact on cancer cell aggressiveness remains unclear. It appears possible that LPCAT1-mediated

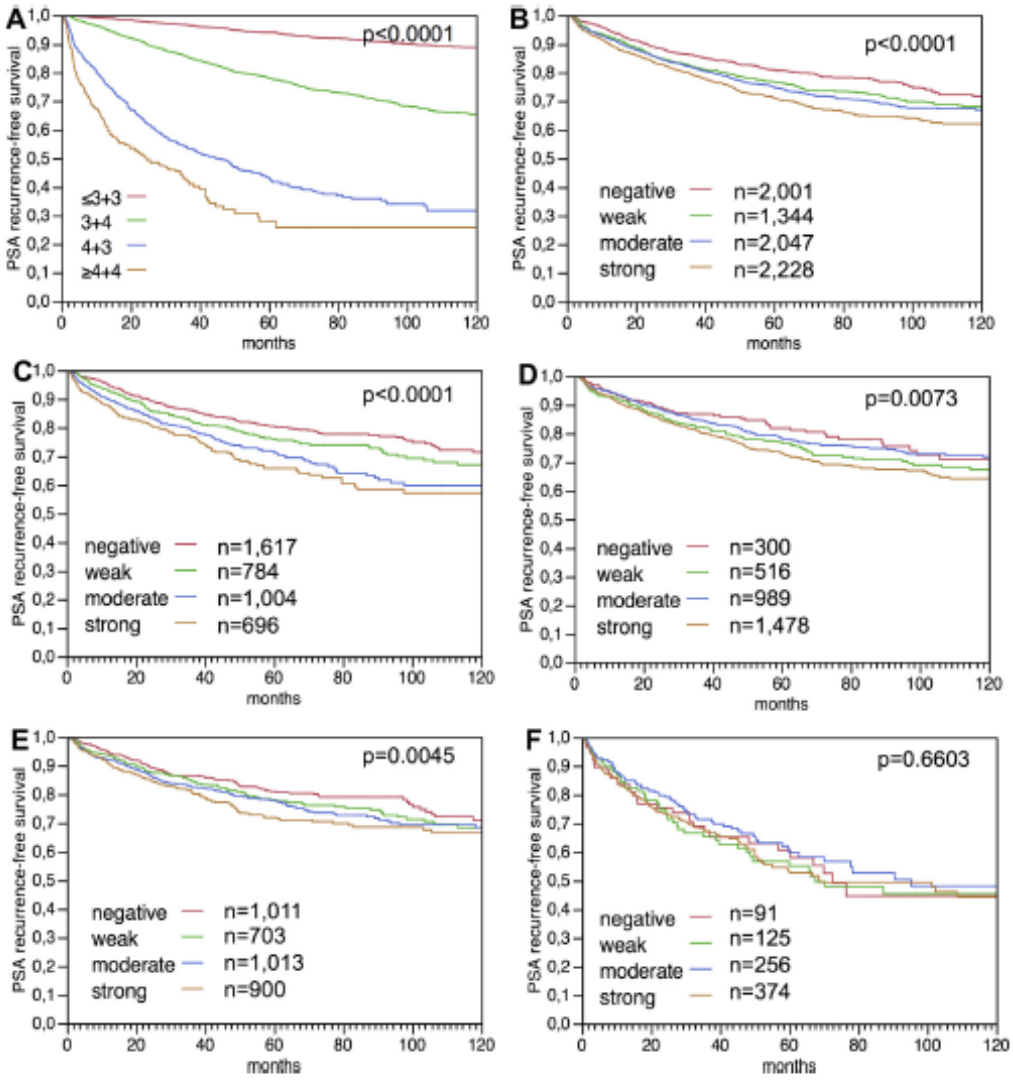

Figure 6 – Relationship of Gleason grade with biochemical recurrence (A). Association of LPCAT1 immunostaining intensity with biochemical recurrence (PSA) in the analysis of (B) all prostate cancers (n = 7620), (C) in the subset of ERG negative cancers (n = 4102) and (D) in the subset of ERG positive cancers (n = 3283). Figure E and F include the PTEN deletions status and demonstrate the relationship of LPCAT1 immunostaining intensity with biochemical recurrence in (E) prostate cancers lacking PTEN deletions (n = 3627), and (F) prostate cancers harboring PTEN deletions (n = 846).

phospholipid accumulation increases the membrane potential and membrane fluidity, important factors for cell proliferation, adhesion and motility that have been linked to tumor progression and metastasis before (Dobrzynska et al., 2005; Kohno et al., 1998; Monet et al., 2009; Zeisig et al., 2007).

Our TMA containing more than 10,000 prostate cancer specimens represents a suitable system for assessing potential prognostic markers. In earlier studies we had successfully validated all established prognostic biomarkers in prostate cancer such as nuclear p53 accumulation (Schlomm et al., 2008), *PTEN* inactivation (Krohn et al., 2012), and Ki67 labeling index (Zellweger et al., 2009) on even smaller prostate cancer TMAs, and identified several other prognostic biomarkers such as CRISP3 overexpression (Grupp et al., 2013) and deletions at 8p (El Gammal et al., 2010), 6q15 (Kluth et al., 2013), 5q21 (Burkhardt et al., 2013). It is noteworthy, that our approach of analyzing molecular features on one-minute tissue specimen per patient on a TMA measuring 0.6 mm in diameter represents a close model of molecularly analyzing core needle biopsies. Core needle biopsies enable the molecular analysis of comparable amounts of tissue as on a TMA. The optimal biomarker evaluation strategy would include the molecular analysis of the original needle biopsy of a patient and compare its prognostic value with preoperative Gleason grade

Table 4 – Multivariate analysis including LPCAT1 expression status in a) all cancers, b) ERG negative, and c) ERG positive prostate cancers.

Scenario (n)	p value							
	Preoperative PSA-level	pT stage	cT stage	Gleason grade prostatectomy	Biopsy Gleason grade	N status	R status	LPCAT1
a)								
1 (n – 4499)	<0.0001	<0.0001	–	<0.0001	–	<0.0001	<0.0001	0.1493
2 (n – 7445)	<0.0001	<0.0001	–	<0.0001	–	–	<0.0001	0.0983
3 (n – 7308)	<0.0001	–	<0.0001	<0.0001	–	–	–	0.0324
4 (n – 7185)	<0.0001	–	<0.0001	–	<0.0001	–	–	0.0001
b)								
1 (n – 2437)	0.0002	<0.0001	–	<0.0001	–	<0.0001	0.0191	0.4607
2 (n – 4008)	<0.0001	<0.0001	–	<0.0001	–	–	0.0003	0.214
3 (n – 3965)	<0.0001	–	<0.0001	<0.0001	–	–	–	0.0381
4 (n – 3912)	<0.0001	–	<0.0001	–	<0.0001	–	–	0.0001
c)								
1 (n – 1934)	0.0266	<0.0001	–	<0.0001	–	0.083	0.0004	0.0218
2 (n – 3205)	0.0009	<0.0001	–	<0.0001	–	–	<0.0001	0.0157
3 (n – 3120)	<0.0001	–	<0.0001	<0.0001	–	–	–	0.052
4 (n – 3068)	<0.0001	–	<0.0001	–	<0.0001	–	–	0.0218

obtained on the same biopsy as well as the preoperative PSA value. For practical purposes, this approach is not feasible because preoperative biopsies are typically distributed over many different centers and not available for studies. Moreover, even if available, these precious core needle biopsies would be exhausted after only few studies. A convoluted approach evaluating multiple different scenarios was thus utilized in this study and scenario 4 including the preoperative Gleason grade obtained on the original biopsy, the pre-operative PSA, clinical stage and LPCAT1 expression was used. Overall, these data suggest a strong independent prognostic relevance of LPCAT1 expression in the subset of ERG positive prostate cancer. The statistical significance becomes particularly high in scenario 4 where the biopsy Gleason grade is considered instead of the more representative Gleason grade obtained from the radical prostatectomy specimen. Although this scenario is somewhat biased since it overestimates the true prognostic value of LPCAT1, it strongly suggests that LPCAT1 might be a powerful marker in this clinically relevant scenario.

In summary, our data identify LPCAT1 expression as a potential biomarker with clinical utility in prostate cancer. It appears well possible, that LPCAT1 measurement, either alone or in combination, may be utilized for better clinical decision-making. The findings also highlight the potentially important role of lipid metabolism in prostate cancer biology.

Disclosure/Conflict of interest

The authors declare no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.molonc.2013.07.009.

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2 Darstellung der Publikation

2.1 Einleitung

Das Prostatakarzinom ist der häufigste bösartige Tumor, der bei Männern diagnostiziert wird. Es gehört mit dem Bronchial - und dem Kolorektalkarzinom zu den häufigsten krebsassoziierten Todesfällen (Siegel et al., 2013). Die Inzidenz des Prostatakarzinoms hat aufgrund des demographischen Wandels in den letzten Jahren immer weiter zugenommen, sodass der Diagnostik und Therapie immer mehr Aufmerksamkeit geschenkt werden muss. Im frühen Stadium der Erkrankung bleiben die meisten Patienten asymptomatisch. Heutzutage werden auch im Rahmen der empfohlenen Vorsorgeuntersuchungen häufiger Zufallsdiagnosen durch klinische Untersuchungen und/oder suspekte laborchemische Werte des prostata-spezifischen Antigens (PSA) gestellt. Treten bereits Symptome wie Miktionsstörungen auf, muss man bereits von einem ausgedehnteren Tumorwachstum ausgehen, auch die Metastasierungswahrscheinlichkeit erhöht sich. Zu den häufigsten Lokalisationen von Tochtergeschwülsten zählen zunächst die regionalen Lymphknoten, danach vorallem Skelett, Leber und Lunge.

Die meisten Karzinome werden allerdings in frühen, behandelbaren Tumorstadien entdeckt. Die Therapieoptionen variieren insbesondere bei „low risk“ Situationen vom aktiven Zuwarten über die operative Sanierung bis hin zur Strahlentherapie. Kenntnisse über das zu erwartende biologische Verhalten des individuellen Prostatakarzinoms wären bei der Therapiewahl äußerst hilfreich. Zu den wichtigsten präoperativen prognostischen Parametern zählen der Gleason Score, die Tumorausdehnung, der Differenzierungsgrad in Biopsien und die Höhe des PSA-Werts. Diese sind statistisch

aussagekräftig, im Einzelfall aber leider nicht immer hinreichend für eine individuelle Therapieentscheidung. Es wird deswegen gehofft, dass die Bestimmung von molekularen Tumormarkern zusätzlich zu den beschriebenen konventionell angewandten Prognosekriterien eine bessere individuelle Therapieplanung und eine genauere Verlaufseinschätzung ermöglichen könnte.

Die Identifikation von neuen potentiellen Biomarkern ist aktuell Gegenstand intensiver Forschung. Die Technologie der Mikroarray-Methoden spielt auf dem Forschungsgebiet der Karzinogenese eine wesentliche Rolle. Veränderungen im Lipidstoffwechsel sind in der Karzinogenese ein bereits bekannter beeinflussender Faktor des Zellwachstums und des Differenzierungsgrades (Santos und Schulze, 2012).

Die Lysophosphatidylcholin-Acyltransferase 1 (LPCAT1) ist ein wesentlicher Bestandteil des ‚Lands Zyklus', der bei der Synthese von unterschiedlichen Phosphatidylcholinen eine Rolle spielt. In der Lunge kommt es unter Einfluss der LPCAT1 zur Bildung und Regulation des Surfactant-Faktors (Goss et al., 2013; Bridges et al., 2010; Cheng et al., 2006; Nakanishi et al., 2006). Eine Überexpression von LPCAT1 in Malignomen wie dem Kolorektalkarzinom wurde 2009 bereits durch Mansilla et al. beschrieben. Hier wurde dadurch bereits eine signifikante Steigerung der Wachstumstendenz der LPCAT1 exprimierenden Tumoren bestätigt. Zhou et al. haben 2012 daraufhin Untersuchungen an 148 Patienten vorgenommen, die an einem Prostatakarzinom litten und eine Assoziation zwischen postoperativem Wiederauftreten von erhöhten PSA-Werten und einer erhöhten Expression von LPCAT1 beschrieben.

In unserer Studie untersuchten wir nun die Prävalenz der LPCAT1-Expression in Prostatakarzinomen und die klinische Signifikanz auch in Verbindung mit bereits

bekannten molekular definierten Subtypen des Prostatakarzinoms. Dieses wurde mithilfe eines Tissue Microarray (TMA), der 11 152 Präparate von Prostatakarzinomen der Jahre 1992 -2011 umfasste, umgesetzt.

Unsere Daten belegen, dass die erhöhte Expression von LPCAT1 in Prostatakarzinomen einen unabhängigen prognostischen Faktor für das Rezidiv-Risiko darstellt.

2.2 Material und Methoden

2.2.1 Patienten und Tissue Microarray (TMA)

Es wurden 11 125 Proben von Präparaten aus radikalen Prostatektomien der Jahre 1992 -2011 standardisiert untersucht. Zunächst erfolgte die Einbettung des Gesamtpräparates. Der Gleason-Score wurde aus den Präparaten bestimmt. Der Prozess der Untersuchung mittels Tissue Microarray (TMA) wurde bereits detailliert im zugrundeliegenden Paper (Seite 5 und 6) beschrieben.

2.2.2 Immunhistochemie und Fluoreszenz-in-situ-Hybridisierung

Das Verfahren der Immunhistochemie (ICH) beschreibt einen Prozess der gezielten Kenntlichmachung von im Tumor enthaltenen Proteinen. Dieser Nachweis wird durch das Prinzip der Antigen-Antikörper-Bindung ermöglicht. Der zum Gewebe hinzugefügte Antikörper ist aufgrund der Koppelung an ein bestimmtes Detektionssystem nach Bindung an das im Gewebe enthaltene Antigen nachweisbar. Die Fluoreszenz-in-situ-Hybridisierung (FISH) macht es möglich Chromosomenstrukturen zu markieren. Hierzu werden markierte einsträngige Nukleinsäuren (DNA oder RNA), die sogenannten Sonden, mit einer doppelsträngigen zu untersuchenden Patienten-DNA zusammengebracht. Nach Denaturierung kommt es zu dem Prinzip der Hybridisierung, bei dem sich die Sonde an die komplementären Sequenzen der Patienten-DNA anlagert. Die Chromosomenstrukturen werden dann durch Fluoreszenz-Signale der Sonden sichtbar.

2.2.3 Statistik

Die verwendeten statischen Verfahren sind im zugrundeliegenden Paper (Seite 6) ausführlich aufgeführt.

2.3 Resultate

LPCAT1 wurde in Epithelzellen generell zytoplasmatisch nachgewiesen und zeigte ein granuläres Muster. In gutartigem Prostatagewebe war LPCAT1 nicht detektierbar bzw. die Expression vernachlässigbar klein. In 73,8% Fällen unserer auswertbaren Karzinome konnte LPCAT1 nachgewiesen werden. Eine genauere Auswertung der Ergebnisse kann im Paper (Seite 6) nachgelesen werden. Es zeigte sich eine signifikant erhöhte LPCAT1-Expression in sowohl ERG-positiven- als auch TMPRSS2-ERG-positiven Fusionskarzinomen (siehe Seite 7, Figure 2). ERG-negatives Tumorgewebe ging nur in 16,7% mit stark erhöhter Expression von LPCAT1 einher. In Untersuchungen von ERG-positivem und -negativem Tumorgewebe konnte in ERG-negativen Karzinomen ein Zusammenhang zwischen erhöhter LPCAT1-Expression und fortgeschrittenem Tumorstadium ($p<0,0001$), hohem Gleason-Score ($p<0,0001$), postoperativem R1-Resektionsstatus ($p=0,0002$) und positivem Lymphknotenstatus ($p=0,0005$) nachgewiesen werden.

Da bereits ein bekannter Zusammenhang zwischen ERG-positiven Prostatakarzinomen und PTEN- sowie 3p13-Gendeletionen bzw. zwischen ERG-negativen und 5q21- oder 6q15-Deletionen bestand (Berger et al., 2011; Burkhardt et al., 2013; Kluth et al., 2013; Krohn et al., 2012; Lapointe et al., 2007; Taylor et al., 2010), untersuchten wir vergleichend die LPCAT1-Expression in diesen Subgruppen. Diese Ergebnisse können auf Seite 9 (Figures 3 und 4) nachgelesen werden. Deutlich wurde eine Assoziation von erhöhter LPCAT1-Expression in ERG-negativen Karzinomen mit PTEN-Deletionen ($p< 0,001$, siehe Seite 10, Figure 5).

Wichtig waren vor allem auch die Ergebnisse bezüglich der prognostischen Relevanz von LPCAT1. Hier konnte ein signifikanter Zusammenhang zwischen frühem Wiederauftreten von erhöhten PSA-Werten und überexprimierter LPCAT1 nachgewiesen werden ($p<0{,}001$, siehe Seite 11, Figure 6B). In der Durchführung multivarianter Verfahren inkl. 4 Szenarien, die im Paper (siehe Seite 7) beschrieben sind, wurde deutlich, dass LPCAT1 ein unabhängiger prognostischer Faktor zu sein scheint. Im Vergleich mit postoperativ zu Verfügung stehenden Parametern, ergab sich dieser Zusammenhang allerdings nur in ERG-positiven Karzinomen (siehe Seite 12, Table 4c).

2.4 Diskussion

Die Resultate unserer Studie belegen, dass LPCAT1 ein attraktiver Prognose-Faktor für ein erhöhtes Rezidivrisiko des Prostatakarzinoms darstellen könnte.

LPCAT1 konnte in 73,8% der interpretierbaren Tumoren in immunhistochemischen Untersuchungen nachgewiesen werden. Die Intensität des Färbesignals war typischerweise in malignen Epithelzellen stärker als in benignen. Diese Daten sind generell im Einklang mit einer früheren Studie, die die Expression von LPCAT1 auf einem TMA, der 251 Proben von 148 prostatektomierten Patienten bzw. Patienten nach transurethraler Prostataresektion, untersucht hat (Zhou et al., 2012). Die Autoren konnten LPCAT1 ebenso vornehmlich im Zytoplasma der neoplastischen Zellen nachweisen (Zhou et al., 2012). In benignem Prostataepithel fand sich mit dem in dieser Studie verwendeten Auswertungsverfahren ein IHC Score von 2,68. Der gleiche Score war in nicht-metastasierten Karzinomen 4,63. Ein Score von IHC 8 fand sich in metastasierten Karzinomen (Zhou et al., 2012).

Die starke Assoziation der LPCAT1-Expression mit einem histologisch ungünstigen Phänotyp und einem prognostisch ungünstigen klinischen Verlauf, war ein zentrales Ergebnis unserer Studie. Aufgrund des unabhängigen prognostischen Wertes einer Überexpression von LPCAT1, können wir von einer relevanten biologischen Rolle der LPCAT1 in Prostatakarzinomen ausgehen. Diese Annahme stimmt mit diversen vorrangegangenen Studien überein, die Veränderungen im Lipidstoffwechsel als einen wichtigen Faktor in der Tumorgenese beschrieben (Furuta et al., 2010; Menendez, 2010; Menendez and Lupu, 2007; Santos and Schulze, 2012; Suburu and Chen, 2012). Einige Studien konnten zeigen, dass Aktivierung und Remodelling, ebenso wie eine

vermehrte Expression von Proteinen, die am Lipidstoffwechsel beteiligt sind, typische Eigenschaften von Tumorzellen darstellen. Diese wurden z.B. für das fettsäurebindende Protein C-oder E-FABP (Morgan et al., 2008), die Fettsäuresynthase FASN (Flavin et al., 2010; Shah et al., 2006), Caveolin-a (Freeman et al., 2012) oder die Fettsäure-Elongase 7 (ELOVL7) (Tamura et al., 2009) gezeigt. Unsere Ergebnisse unterstützen die These, dass dem Lipidstoffwechsel in Prostatakarzinomzellen eine wichtige Funktion zukommt (Faas et al., 1996, 2001).

Es war ein Ziel unseres Projektes mögliche Interaktionen von LPCAT1 mit anderen wichtigen Veränderungen in Prostatatumoren zu untersuchen. Etwa 50% der Prostatakarzinome sind durch Genfusionen des androgen-regulierten TMPRSS2-Gens mit dem Transkriptionsfaktor ERG (aus der ETS-Familie) charakterisiert (Tomlins et al., 2005). Diese Fusion ist offenbar signifikant mit einer massiven Überexpression von LPCAT1 assoziiert. Da diese Assoziation durch zwei unabhängige molekularbiologische Verfahren bestätigt werden konnte, also sowohl durch IHC als auch durch FISH-Untersuchungen, kann eine falsch-positive Verknüpfung durch ineffiziente Färbungen in beschädigtem, unreaktivem Gewebe ausgeschlossen werden.

Die in unseren Ergebnissen gefundene hohe Expression von LPCAT1 in ERG-positiven Tumoren unterstützt die bereits vorhandenen Daten, die einen Einfluss der ERG-abhängigen Pathways auf den Lipidstoffwechsel von Karzinomzellen beschrieben haben (Iljin et al., 2006; Vainio et al., 2011a). Beispielsweise induziert ERG die Phospholipase PLA2G7, die ein wichtiges Enzym für Zellwachstum, Signalübertragung und Aufrechterhaltung von Phospholipidmembranen in ERG-positiven, nicht aber in ERG-negativen Prostatakarzinomzellen darstellt (Dong et al., 2006, Iljin et al., 2006; Scott et al., 2010; Vainio et al., 2011a,b).

PLA2G7 setzt Phophatidylcholin, ein Substrat des LPCAT1, aus der Zellmembran frei. Eine Hochregulation von LPCAT1 in ERG-positiven Karzinomen könnte also aus der Aktivierung von PLA2G7 in diesen Zellen resultieren. Es verbleibt zu untersuchen ob ERG direkt in die LPCAT1-Regulation involviert ist. ETS1, ein weiterer Transkriptionsfaktor und Mitglied der ETS-Familie induziert in Ovarialkarzinomzellen LPCAT2, eine Isoform des LPCAT1 (Verschoor et al., 2010). Basierend auf diesen Daten ist es möglich, dass ERG sowohl durch Interaktion mit PLA2G7 als auch mit LPCAT1 zu einer Erhöhung des zellulären Gehalts an Phosphatidylcholin führt.

Während LPCAT1 in ERG-positiven Karzinomen unabhängig von jeglichen Chromosomendeletionen (5q, 6q, 3p oder PTEN) erhöht war, konnte eine erhöhte LPCAT1-Expression in PTEN-deletierten ERG-negativen Prostatakarzinomen nachgewiesen werden. Diese Beobachtung kann möglicherweise durch die Rolle von PTEN als Stimulator der LPCAT1-Antagonist- Phospholipase D und C erklärt werden (Horie et al., 2004). Eine PTEN-Deletion oder -inaktivierung kann demnach, aufgrund einer reduzierten Expression von LPCAT1-Antagonisten, zu einer ähnlichen Aktivierung der LPCAT1 Expression führen, wie bei einer ERG-Aktivierung.

Es ist bemerkenswert, dass der prognostische Effekt der LPCAT1-Expression in der Teilmenge der ERG-negativen Karzinome wesentlich geringer ausfiel als in ERG-positiven Prostatatumoren. Diese Beobachtung unterstützt unsere Annahme, dass LPCAT1 in ERG-positiven und -negativen Geweben unterschiedliche Funktionen einnimmt. Es bleibt zu spekulieren, ob diese ausgeprägte verminderte prognostische Relevanz von LPCAT1 in ERG-positiven Karzinomzellen aus Interferenzen zwischen ERG und anderen Regulations-Molekülen der LPCAT1 resultiert und die Tumoraggressivität dadurch einschränken kann.

Mögliche Gene für solch eine Rolle beinhalten die Lipoxygenase ALOX15, den Lipidtransporter SORL1, den Lipoproteinrezeptor VLDLR und die Phospholipasen PLA1A und PLA2G2A, welche alle in ERG-positiven und -negativen Prostatatumoren unterschiedlich exprimiert werden (Brase et al., 2011; Jhavar et al., 2009). Jeder dieser Faktoren kann potenziell Einfluss auf die Funktion von LPCAT1 ausüben, da alle an der Lipoproteinaufnahme der Zellen (Klinger et al., 2011; Shen et al., 2012), der Produktion von Signalmediatoren (Il Lee et al., 2011) und Glycerophospholipiden sowie Lysophospholipiden (Kudo and Murakami, 2002) beteiligt zu sein scheinen. Durch welchen Mechanismus LPCAT1 direkten Einfluss auf die Aggressivität von Karzinomzellen ausüben kann, bleibt unklar. Es erscheint möglich, dass eine durch LPCAT vermittelte Akkumulation von Phospholipiden das Mempranpotential und die -fluidität erhöhen kann. Diese sind bekannte Einflussfaktoren auf die Zellproliferation, -adhäsion und -motilität und wurden bereits zuvor mit Tumorprogress und Metastasierung assoziiert (Dobrzynska et al., 2005; Kohno et al., 1998; Monet et al., 2009; Ziesig et al., 2007).

Unser TMA, der mehr als 10 000 Prostatagewebsproben umfasste, repräsentiert ein angemessenes System um mögliche Biomarker auch in Zukunft beurteilen zu können. In früheren Studien konnte unsere Arbeitsgruppe alle etablierten prognostischen Biomarker in Prostatakarzinomen wie z.B. die nukleäre Akkumulation von p53 (Schlomm et al., 2008), die PTEN-Inaktivierung (Krohn et al., 2012) und den Ki67-Labeling-Index (Zellweger et al., 2009) auf unseren TMAs erfolgreich bestätigen und zusätzlich weitere prognostisch relevante Biomarker wie die Überexpression von CRISP3 (Grupp et al., 2013), 8p-Deletionen (El Gammal et al., 2010) und 6q15-Deletionen (Kluth et al., 2013) identifizieren.

Es ist erwähnenswert, dass unsere Methode zur Analyse von molekularen Eigenschaften an 0,6 mm durchmessenden Gewebsproben, mengenmäßig in etwa dem Tumormaterial von Stanzbiopsien aus Tumorgewebe entspricht. Die optimale Strategie für eine Evaluation von klinisch relevanten Biomarkern wäre eine Untersuchung an echten Stanzbiopsien von Patienten mit bekanntem fortgeschrittenem klinischem Verlauf. Leider ist diese Vorgehensweise in der Realität nicht praktikabel, da die präoperativen Biopsien typischerweise durch viele verschiedenen Pathologen interpretiert werden, deshalb im ganzen Land verteilt sind und damit für Studien kaum verfügbar. Weiterhin wäre eine solche Sammlung an Stanzbiopsien bereits nach nur wenigen Studien aufgebraucht. Mit den multivariaten Analysen in dieser Untersuchung haben wir versucht, die präoperative Situation zu imitieren. Hierbei wurde der an präoperativen Stanzen ermittelte Gleason Grad zusammen mit dem präoperativen PSA-Wert, dem klinischen Stadium und der LPCAT1-Expression vergleichend evaluiert. Insgesamt deuten all diese Ergebnisse auf eine starke unabhängige prognostische Relevanz von LPCAT1- Expressionen in ERG-positiven Prostatakarzinomen hin und bestätigen zugleich die potentiell wichtige Rolle des Lipidstoffwechsels in der Prostatakarzinogenese.

Zusammenfassend konnte unsere Studie die Expression von LPCAT1 als einen potentiellen Biomarker mit klinisch hohem Nutzen identifizieren. Es ist gut möglich, dass die Bestimmung von LPCAT1 allein oder in Kombination zu einer besseren klinischen Therapieempfehlung verhelfen kann.

2.5 Zusammenfassung

Dass die Lysophosphatidylcholin-Acyltransferase 1 (LPCAT1) eine wichtige Rolle in Karzinomen einnimmt, wurde bereits zuvor in einigen Studien postuliert. Um die Rolle von LPCAT1 beim Prostatakarzinom zu bestimmen, wurden in unserer Studie 11152 Präparate von Prostatakarzinomen auf einem Tissue Microarray (TMA) auf die Expression von LPCAT1 untersucht. In benignen Prostatadrüsen ergab sich keine oder eine nur geringe Expression von LPCAT1. In Malignomen fand sich hingegen ein positives Ergebnis bei 73,8% der 8786 untersuchten Prostatakarzinom-Proben. Eine vermehrte Expression von LPCAT1 war mit einem fortgeschrittenen Tumorstadium (pT3b/pT4), einem hohen Gleason-Score, positivem Lymphknotenstatus, positiven Resektionsrändern und mit einem frühen Wiederauftreten von PSA verbunden (p jeweils <0,0001). Eine LPCAT1-Überexpression war zudem mit einem positiven ERG-Status assoziiert.

Eine starke Expression von LPCAT1 wurde in 45,3% der ERG-positiven Karzinome und nur in 16,7% der ERG negativen Karzinompräparate gemessen. In ERG-negativen Karzinomen war das Auftreten von LPCAT1 bei Prostatakarzinomen in der Subgruppe mit PTEN-Deletion allerdings ebenso deutlich erhöht. Weitere Subgruppen-Analysen deckten auf, dass in ERG-negativen Karzinomen eine hohe LPCAT1-Expression mit einem Wiederauftreten von PSA und einem ungünstigen histologischen Grading (Gleason Score) einherging. Diese Effekte waren in ERG-positiven Prostatakarzinomen abgeschwächt. Der prognostische Wert von LPCAT1 war jeweils unabhängig vom histologischen Bild und klinischen Parametern.

Als Schlussfolgerung kann allein die Bestimmung von LPCAT1 für eine individualisierte Therapieempfehlung und eine genauere Verlaufseinschätzung von Nutzen sein. Unsere Ergebnisse bestätigen weiterhin die relevante Rolle des Lipidstoffwechsels in der Prostatakarzinogenese.

2.5.1 Summary

Lysophosphatidylcholine acyltransferase 1 (LPCAT1) has already been suggested to play a role in cancer. To assess its role in prostate cancer, LPCAT1 expression was analyzed on a tissue microarray containing samples from 11.152 prostate cancer patients. In benign prostate glands, LPCAT1 immunostaining was absent or weak. In prostate cancer, LPCAT1 positivity was found in 73.8% of 8786 interpretable tumors including 29.2% with strong expression. Increased LPCAT1 expression was associated with advanced tumor stage (pT3b/T4) ($p<0.0001$), high Gleason score (4.4) ($p< 0.0001$), positive nodal involvement ($p=0.0002$), positive surgical margin ($p=0.0005$) and early PSA recurrence ($p<0.0001$). High LPCAT1 expression was strongly linked to ERG-fusion type prostate cancer. Strong LPCAT1 staining was detected in 45.3% of ERG positive but in only 16.7% of ERG negative tumors ($p< 0.0001$). Within ERG negative cancers, LPCAT1 staining was strongly increased within the subgroup of PTEN deleted cancers ($p<0.0001$). Further subgroup analyses revealed that associations of high LPCAT1 expression with PSA recurrence and unfavorable tumor phenotype were largely driven by ERG negative cancers ($p<0.0001$) while these effects were substantially mitigated in ERG positive cancers ($p=0.0073$). The prognostic impact of LPCAT1 expression was independent of histological and clinical parameters. It is concluded, that LPCAT1 measurement, either alone or in combination, may be utilized

for better clinical decision-making. These data also highlight the potentially important role of lipid metabolism in prostate cancer biology.

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3 Eigenanteil

Die Ergebnisse der Publikation gehen aus einer experimentellen Versuchsreiche hervor, die ich mithilfe der am Pathologischen Institut angestellten medizinisch-technischen Assistent-/innen und Ärzt-/innen durchführte. Die Projektskizze mit der Vorgehensweise erfolgte zuvor in gemeinsamer Besprechung mit Herrn Prof. Dr. Sauter. In meinem Aufgabenbereich lag es zunächst die Patientendaten zu sichten, diese bezüglich der unterschiedlichen klinischen Parameter zu selektieren und daraufhin die große Menge an Präparaten für die Versuchsreihe aus den Archiven herauszusuchen um die Objektschnitte mit dem entsprechendem paraffinierten Gewebe bereitzustellen. Auf den Objektträgern habe ich nach Einweisung der Pathologen mikroskopisch die Fläche der höchsten Tumorkonzentration und höchstem Gleason Score eingezeichnet. Ich habe pro Fall alle Schnitte mikroskopiert und am Ende selektiert. Weitere Doktoranden waren am Heraussuchen der Schnitte beteiligt.

Damit war gewährleistet, dass die daraufhin durchgeführten Stanzen ausschließlich Tumorgewebe eines möglichen hohen klinischen Tumorstadiums enthalten.

Mithilfe der medizinisch-technischen Assistent/innen konnte solch ein großer TMA aus 11 152 Präparaten entstehen und die experimentellen Versuche durchgeführt werden.

Die Ergebnisse wurden nach Eigenerarbeitung von Hintergrundwissen mit umfangreicher Fachlektüre gedeutet und mithilfe eines wissenschaftlichen Mitarbeiters statistisch analysiert. Wir konnten die Ergebnisse daraufhin publizieren.

4 Danksagung

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5 Eidesstattliche Erklärung

Ich versichere ausdrücklich, dass ich die Arbeit selbständig und ohne fremde Hilfe verfasst, andere als die von mir angegebenen Quellen und Hilfsmittel nicht benutzt und die aus den benutzten Werken wörtlich oder inhaltlich entnommenen Stellen einzeln nach Ausgabe (Auflage und Jahr des Erscheinens), Band und Seite des benutzten Werkes kenntlich gemacht habe.

Ferner versichere ich, dass ich die Dissertation bisher nicht einem Fachvertreter an einer anderen Hochschule zur Überprüfung vorgelegt oder mich anderweitig um Zulassung zur Promotion beworben habe.

Ich erkläre mich einverstanden, dass meine Dissertation vom Dekanat der Medizinischen Fakultät mit einer gängigen Software zur Erkennung von Plagiaten überprüft werden kann.

Unterschrift: .....