Abstract

Purpose: To establish xenograft mouse models of metastatic and nonmetastatic human prostate cancer and to apply these models to the search for aberrant glycosylation patterns associated with tumor progression in vivo and in patients.

Experimental Design: Prostate cancer cells (LNCaP, PC-3, LuCaP 23.1, and DU-145) were xenografted subcutaneously into immunodeficient pfp−/−rag2−/− mice. Tumor growth and metastasis formation were quantified and as altered glycosylation patterns have been associated with metastasis formation in several other malignancies, prostate cancer cells were profiled by a quantitative real-time PCR (qRT-PCR) glycosylation array and compared with normal human prostate cells. The activity of upregulated glycosyltransferases was analyzed by their sugar residues end products using lectin histochemistry on primary tumors and metastases in the animal experiments and on 2,085 clinical samples.

Results: PC-3 cells produced the largest number of spontaneous lung metastases, followed by LNCaP and LuCaP 23.1, whereas DU-145 was nonmetastatic. qRT-PCR revealed an upregulation of β1,6-N-acetylgalactosaminyltransferase-5b (Mgat5b) in all prostate cancer cell lines. Mgat5b products [β(1,6)-branched oligosaccharides] were predominantly detectable in metastatic xenografts as shown by increased binding of Phaseolus vulgaris leukoagglutinin (PHA-L). The percentage of prostate cancer patients who were PHA-L positive was 86.5. PHA-L intensity correlated with serum prostate-specific antigen and a cytoplasmic staining negatively affected disease-free survival.

Conclusion: We show a novel xenograft mouse model for human prostate cancer respecting the complete metastatic cascade. Specific glycosylation patterns reveal Mgat5b products as relevant markers of both metastatic competence in mice and disease-free survival in patients. This is the first description of Mgat5b in prostate cancer indicating a significant biologic importance of β(1,6)-branched oligosaccharides for prostate cancer progression.

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Translational Relevance

This is the first study investigating the prognostic effect of β(1,6)-branched oligosaccharides recognized by their specific lectin Phaseolus vulgaris leucoagglutinin (PHA-L) binding in human prostate cancer. The potential relevance of these glycan structures was identified in newly established spontaneous metastasis xenograft models of prostate cancer indicating PHA-L as a marker of metastatic competence. To translate these findings into the clinic, PHA-L binding was analyzed in tissue spots of 2,085 prostate cancer patients. As PHA-L was detectable in the vast majority of the patients, it can be suggested as a potent marker of prostate cancer. Moreover, PHA-L binding significantly correlated with preoperative prostate-specific antigen (PSA) and adversely affected PSA recurrence-free survival, which supports our in vivo findings and indicates the clinical relevance of our model. Although these results show a significant biologic importance of β(1,6)-branched oligosaccharides for prostate cancer progression, the prognostic effect of PHA-L is not strong enough to add independent information to the established clinicopathologic variables.

cancer cell lines (such as LNCaP, PC-3, and DU-145) have been used in subcutaneous, renal, and orthotopic xenograft models. The latter ones are in so far limited, as orthotopic implantations of human tumor cells into the mouse prostate might bear the risk of an artificial route of metastasis delivery (5), although previous subcutaneous xenograft models rarely showed spontaneous metastatic spread (6). Other xenograft approaches should rather be designated as "dissemination models" as they use intravenous injections of tumor cells (7) or "metastasis microenvironment models" as they implant prostate cancer cells directly into the mouse bone marrow as the site of metastasis (8), both of which do not reflect the entire metastatic cascade. Consequently, most of these previous attempts are the subject of debate with regard to their clinical and pathophysiologic relevance (4).

The clinical relevance of xenograft mouse models was previously proven using the binding ability of specific lectins toward defined carbohydrate structures on glycoproteins or proteoglycans. Aberrant glycosylation patterns, as detected by lectin histochemistry, have been shown to be common events and hallmarks of cancer progression for several tumor entities (9). In particular, the binding of the lectin Helix pomatia agglutinin (HPA) was proven to predict metastasis formation both in clinical and xenograft mouse studies of human breast and colorectal cancer, therefore indicating the clinical relevance of the models (10, 11).

The aim of this study was to develop novel xenograft mouse models for spontaneous metastatic human prostate cancer and to furthermore provide evidence for its clinical relevance by identifying a potential new prognostic glycosylation marker based on these models. By using subcutaneous xenograft models, we aimed to mimic the entire metastatic cascade in a reproducible and easy-to-handle mouse xenograft model.

Materials and Methods

Cell lines and culture conditions

The LNCaP, PC-3, and DU-145 cell lines (all obtained from Deutsche Sammlung von Mikroorganismen und Zellkulturen in 2007) and primary prostate epithelial cells (PPEC, obtained from American Type Culture Collection in 2010) were cultured according to the manufacturer’s protocol. For maintenance of LuCaP 23.1 cells (12), the primary tumors were resected when exceeding 2 cm³ or ulcerating the mouse skin, cut into small pieces and treated with 0.05% Trypsin/EDTA (Gibco) at 37°C for 20 minutes. Cell viability was determined by trypan blue counting, and only preparations with more than 95% viability were used for in vivo injections (see below).

Subcutaneous xenograft mouse model

Pfp<sup>−/−</sup>rag2<sup>−/−</sup> double knock out mice were generated by targeted mutation in C57Bl/6 mice (B6.129S6-Pfp<sup>tm1ClrkRag2<sup>tm1Fwa</sup></sup>N12) and were obtained from Taconic. All mice were males aged 8 to 12 weeks and with a body weight between 20 and 25 g. Due to their immunodeficiency, the animals were maintained under pathogen-free conditions in individually ventilated cages and fed with sterile standard food and water ad libitum. A total of 1 × 10⁶ vital LNCaP, PC-3, or DU-145 cells were resuspended in 200 μl medium without supplements and were subcutaneously injected between the scapulae of narcotized (O₂/CO₂) pfp<sup>−/−</sup>/rag2<sup>−/−</sup> mice (10 mice per cell line). The LuCaP 23.1 cell suspension of one primary tumor was transferred to up to 3 pfp<sup>−/−</sup>/rag2<sup>−/−</sup> mice using a Strauss cannule. All animal experiments were approved by the local animal experiment approval committee and assigned to the project number G08/75.

Evaluation of tumor growth and metastasis formation

When primary tumors exceeded 2 cm³ or ulcerated the mouse skin, the mice were terminally anesthetized followed by cervical dislocation. Primary tumors were excised, weighed, and processed for histologic examinations as described before (13, 14). Lungs were excised en bloc and fixed in formalin. After fixation, lungs were cut in 1-mm thick slices using razor blades, and the lung sections were embedded in agar and transferred to paraffin like conventional tissue blocks. The embedded lungs were cut into 5-μm thick sections, every tenth section was retained and 10 sections from the middle of the block were stained with hematoxylin and eosin (H&E). Subsequently, pulmonary metastases in these sections were counted using a light microscope, and the total number of lung metastases was quantified as described before (15). We furthermore determined circulating and disseminated tumor cells in a “proof-of-principle experiment” with LNCaP-injected mice: after
terminal anesthesia, murine blood and bone marrow were collected by cardiocentesis and by flushing the femora diaphyses with NaCl 0.09%, respectively. DNA isolation kits were used to prepare genomic DNA from 200 μL mouse blood and bone marrow suspension (QIAamp DNA Blood Mini Kit; Qiagen) and concentrations of DNA were quantified using a NanoDrop spectrophotometer (Pqlab). Human tumor cells were quantified by real-time PCR using established primers specific for human Alu sequences as previously described (16). Numerical data were determined against a standard curve established using murine blood and bone marrow DNA containing log-fold dilutions of DNA from 1 × 10^6 cell culture grown LNCaP cells (extracted using the QIAamp DNA Mini Kit; Qiagen). Negative controls included tissues obtained from noninjected mice. For each sample, analyzes were done in duplicates and as independent experiments at least twice.

**Immunohistochemistry and lectin histochemistry**

To confirm the human origin of metastatic deposits, parallel sections to H&E-stained sections, in which at least one metastasis was observed, were immunostained with human-specific monoclonal antibodies directed against prostate-specific membrane antigen (Dako#M3620, Clone: 3E6), a 65-kDa nonglycosylated protein component of mitochondria exclusively found in human cells (Millipore#MAB1273, Clone: 113-1) and HLA-1 (eBioscience#14-9958, Clone: A4) by a standard procedure (14). Lectin histochemistry was done using biotinylated Phaseolus vulgaris leukoagglutinin (PHA-L; Vector Labs) in a concentration of 2 μg/mL as described before (17).

**Quantitative real-time PCR glycosylation array**

RNA was extracted from cell culture grown LNCaP, PC-3, DU-145, and PPEC using a RNA isolation kit (RNaseasy Mini Kit; Qiagen). LuCaP 23.1 xenograft tumors were not included, as they not only consist of human prostate cancer cells but also of murine blood vessels, blood cells, connective tissue, etc. RNA concentrations were quantified using a NanoDrop spectrophotometer and 1 μg was processed to cDNA with RT^2 First Strand Kit (SA Biosciences). Quantitative PCR analysis of 84 human glycosylation key genes was assessed with the Human Glycosylation RT2 Profiler PCR Array (SA Biosciences) and RT^2 SYBR Green qPCR master mix (SA Bioscience) in a LightCycler 480 (Roche). Five housekeeping genes and internal controls for genomic DNA contamination, RNA quality, and general PCR performance were also included. The array was repeated twice with independently isolated RNA to generate statistically relevant data.

**Tissue microarray**

Prostate cancer specimens were available from 2,411 patients, who underwent radical prostatectomy at the Department of Urology at the University Medical Center Hamburg-Eppendorf, Germany, between 1992 and 2005. None of the patients received neoadjuvant endocrine therapy. Additional (salvage) therapy was initiated in case of a biochemical relapse (BCR). In all patients, PSA values were measured quarterly in the first year, followed by biannual measurements in the second and annual measurements after the third year following surgery. Recurrence was defined as a postoperative PSA of 0.2 ng/mL and rising thereafter; patients without evidence of recurrence were censored at last follow-up. All prostatectomy specimens were analyzed according to a standard procedure. All prostates were completely paraffin embedded, including whole-mount sections as previously described (18). All H&E-stained histologic sections from all prostatectomy specimens were reviewed for the purpose of this study and one 0.6-mm thick tissue core was punched out from a representative cancer area and transferred onto a tissue microarray (TMA) format as described (19–22). For lectin histochemistry, freshly cut TMA sections were transferred to xylol over night, deparaffinized and pretreated with 0.1% trypsin in lectin buffer (0.05 mol/L Tris-buffered saline, pH 7.6, with 1 mmol/L CaCl₂ and MgCl₂ added) for 15 minutes at 37°C. Afterwards, sections were incubated with biotinylated PHA-L for 1 hour at room temperature. Slides were then incubated with an avidin–alkaline phosphate complex (Vectastain, Vector, ABC Kit) for 30 minutes and lectin binding was visualized as described before (17). PHA-L binding was examined with regard to intensity (scaled from 1–3) and localization (cytoplasmic vs. apical/membranous).

**Statistical analyses**

Statistical evaluations of the in vivo experiments were done with GraphPad Prism software version 4. Glycosylation array data were analyzed based on the ΔCt method using an Excel-based data analysis template (SA Bioscience) and were represented as x-fold up- or downregulation. The PHA-L staining patterns on the TMA were statistically evaluated using SPSS 19 software package (SPSS Inc.). Kaplan–Meier curves were plotted for PSA recurrence analyses and compared using log-rank tests in a subset of patients with completed follow-up. Multivariate Cox proportional hazard regression models were used for examination of competing risk factors. Patient C, Fisher exact, Kruskal–Wallis, and Mann Whitney U tests were done as appropriate to assess associations between variables. Statistical significance was assigned at 2-tailed P values less than 0.05.

**Results**

**Spontaneous metastasis xenograft model**

Primary tumor growth was observed in at least 90% of injected mice (Fig. 1A) and the termination criteria were reached after a mean growth period of 72 (LNCaP), 47 (PC-3), 56 (LuCaP 23.1), and 77 (DU-145) days (Fig. 1B). The growth period of PC-3 xenografts was significantly shorter than that of LNCaP and DU-145 xenografts (P < 0.05). At necropsy, the average tumor weight was 1.48, 1.38, 1.29, and 0.87 g, respectively, without significant differences between the cell lines (Fig. 1C). Pulmonary metastases were found in 100% of LNCaP- and PC-3–injected mice, in 50% of LuCaP 23.1 xenograft mice and
were not detectable in mice engrafted with DU-145 cells (Fig. 1D). The average total numbers of metastases were 548 ± 503 (LNCaP), 1,387 ± 1,171 (PC-3), and 74 ± 1 (LuCaP 23.1) per lung (Fig. 1E), so that the metastatic potential of the cell lines was classified as strong/moderate versus weak/negative in the order PC-3/LNCaP versus LuCaP 23.1/DU-145. According to statistical analyses, PC-3 xenograft mice developed significantly more lung metastases than LuCaP 23.1 and DU-145 xenograft mice (P < 0.05). The human origin of lung metastases was confirmed by immunohistochemical staining of human prostate–specific membrane antigen, mitochondria, and HLA-1 (Fig. 1). According to Alu-PCR analyses, which were restricted to LNCaP-injected mice, DNA of 266 circulating tumor cells (CTC) per mL blood and of 35 disseminated tumor cells (DTC) per mL bone marrow was detectable (Fig. 1F).

Mgat5b expression and PHA-L binding

On the basis of glycosylation quantitative real-time PCR (qRT-PCR) array data, the gene for β1,6-N-
acetylglucosaminyltransferase-5B (Mgat5b, also reported as GnT-Vb or GnT-IX) was the single glycosyltransferase gene consistently overexpressed in all 3 prostate cancer cell lines compared with PPEC (Fig. 2). In detail, Mgat5b expression was upregulated 8.5-fold in LNCaP cells, 18-fold in DU-145 cells, and 26-fold in PC-3 cells ($P < 0.05$). As the products of the corresponding protein Mgat5b, that is, N-linked β(1,6)-branched glycans, specifically bind PHA-L (23, 24), PHA-L lectin histochemistry was used to evaluate the Mgat5b activity in normal human prostate, xenograft primary tumors, and lung metastases. PHA-L binding was closely related to the metastatic potential of the xenografts with the strongest intensity in LNCaP and PC-3 primary tumors (and their lung metastases) and the weakest intensity in LuCaP 23.1 and DU-145 primary tumors (Fig. 2), suggesting PHA-L as a helpful tool for metastasis prediction in our in vivo model. Moreover, we detected a differential translation of Mgat5b transcripts or differential regulation of Mgat5b activity in DU-145 cells and xenografts. PPEC and normal human prostate revealed almost absent Mgat5b expression levels and PHA-L binding, respectively.

### Table: Gene Expression Analysis

<table>
<thead>
<tr>
<th>Gene name</th>
<th>Average $C_t$</th>
<th>Fold up- or downregulation</th>
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<tbody>
<tr>
<td></td>
<td>PPEC</td>
<td>LNCaP</td>
</tr>
<tr>
<td>MGAT5B</td>
<td>33.85</td>
<td>8.56</td>
</tr>
<tr>
<td>FUT8</td>
<td>26.08</td>
<td>3.42</td>
</tr>
<tr>
<td>FUT11</td>
<td>26.29</td>
<td>1.7</td>
</tr>
<tr>
<td>GALNT13</td>
<td>34.95</td>
<td>31.6</td>
</tr>
<tr>
<td>GALNT14</td>
<td>27.63</td>
<td>-84.74</td>
</tr>
<tr>
<td>EDEM1</td>
<td>24.06</td>
<td>-1.64</td>
</tr>
<tr>
<td>ST8SIA6</td>
<td>31.55</td>
<td>-6.57</td>
</tr>
<tr>
<td>GCNT4</td>
<td>27.97</td>
<td>-20.09*</td>
</tr>
<tr>
<td>HEXA</td>
<td>28.17</td>
<td>-1.24</td>
</tr>
</tbody>
</table>

* $P < 0.05$; ** $P < 0.001$.
As PHA-L binding was associated with the metastatic potential in our mouse model, we evaluated whether it was also associated with diverging clinical courses of prostate cancer patients using a TMA. Within our study population of 2,411 patients, 2,085 (86.5%) were PHA-L positive and were subjected to further statistical analyses (clinicopathologic findings are summarized in Table 1). PHA-L binding intensity was significantly correlated with the preoperative PSA values (available for 1,950 patients, Fig. 3A, Table 2, $P = 0.003$, Kruskal–Wallis test). In addition, cytoplasmic staining of PHA-L was significantly associated with a higher preoperative PSA compared with apical/membranous staining (Fig. 3A, Table 2, $P = 0.017$, Mann–Whitney $U$ test).

However, PHA-L intensity or localization did not correlate with the Gleason score or tumor stage (data not shown). In addition, the small subset of PHA-L-negative patients tended to lower PSA values, but these patients were statistically not different with regard to their PSA recurrence-free survival (data not shown). We furthermore examined, whether the localization of PHA-L was important for the clinical course of the PHA-L–positive patients as measured by postoperative PSA recurrences. Therefore, 639 patients had to be excluded due to incomplete follow-up, resulting in a total of 1,446 spots (Table 1). The mean recurrence-free survival was 95.8 (90.6–101.0; 95% CI) months. Most interestingly, a cytoplasmic binding of PHA-L adversely affected PSA recurrence-free survival compared with apical/membranous binding (Fig. 3B, Table 2, $P = 0.027$, log-rank test). Unlike PHA-L localization, intensity had no significant effect on PSA recurrence (Table 2). According to multivariate analysis including all statistically relevant biochemical and clinical parameters (PSA, pT, pN, surgical margin status, Gleason, and age), PHA-L intensity and PHA-L localization were not independent predictors of PSA recurrence.

### Table 1. Clinicopathologic features of the study populations

<table>
<thead>
<tr>
<th></th>
<th>PHA-L–positive patients ($n = 2,085$)</th>
<th>PHA-L–positive patients with completed follow-up ($n = 1,446$)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Patient age (y)</strong></td>
<td>Mean (median) 62.0 (62.0)</td>
<td>62.13 (62.0)</td>
</tr>
<tr>
<td></td>
<td>Range 6.0–77.0</td>
<td>22.0–77.0</td>
</tr>
<tr>
<td><strong>Follow-up (mo)</strong></td>
<td>Mean (median) 30.96 (26.17)</td>
<td>30.96 (26.17)</td>
</tr>
<tr>
<td></td>
<td>Range 1.0–140.0</td>
<td>1.0–140.0</td>
</tr>
<tr>
<td><strong>PSA (ng/mL)</strong></td>
<td>Mean (median) 9.4 (6.8)</td>
<td>10.1 (7.1)</td>
</tr>
<tr>
<td></td>
<td>Range 0.0–102.8</td>
<td>0.0–102.8</td>
</tr>
<tr>
<td></td>
<td>Missing data 135 pat. (6.5%)</td>
<td>21 pat. (1.5%)</td>
</tr>
<tr>
<td><strong>pT stage</strong></td>
<td></td>
<td>No. of patients (percentage)</td>
</tr>
<tr>
<td>pT2</td>
<td>1,339 (64.2%)</td>
<td>912 (63.2%)</td>
</tr>
<tr>
<td>pT3a</td>
<td>373 (17.9%)</td>
<td>308 (21.3%)</td>
</tr>
<tr>
<td>pT3b</td>
<td>218 (10.5%)</td>
<td>184 (12.7%)</td>
</tr>
<tr>
<td>pT3c</td>
<td>25 (1.2%)</td>
<td>22 (1.5%)</td>
</tr>
<tr>
<td>pT4</td>
<td>18 (0.8%)</td>
<td>17 (1.2%)</td>
</tr>
<tr>
<td>pTx</td>
<td>112 (5.4%)</td>
<td>2 (0.1%)</td>
</tr>
<tr>
<td><strong>Prostatectomy Gleason score</strong></td>
<td></td>
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<tr>
<td>≤6</td>
<td>906 (43.4%)</td>
<td>607 (42.0%)</td>
</tr>
<tr>
<td>3+4</td>
<td>853 (40.9%)</td>
<td>658 (45.5%)</td>
</tr>
<tr>
<td>4+3</td>
<td>189 (9.1%)</td>
<td>159 (11.0%)</td>
</tr>
<tr>
<td>≥8</td>
<td>27 (1.3%)</td>
<td>22 (1.5%)</td>
</tr>
<tr>
<td>Missing data</td>
<td>110 (5.3%)</td>
<td>0</td>
</tr>
<tr>
<td><strong>pN stage</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pN0</td>
<td>862 (41.3%)</td>
<td>784 (54.2%)</td>
</tr>
<tr>
<td>pN1–3</td>
<td>56 (2.7%)</td>
<td>52 (3.6%)</td>
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<tr>
<td>pNx</td>
<td>1,167 (56.0%)</td>
<td>610 (42.2%)</td>
</tr>
<tr>
<td><strong>Surgical margin status</strong></td>
<td></td>
<td></td>
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<tr>
<td>R0</td>
<td>1,567 (75.2%)</td>
<td>1,115 (77.1%)</td>
</tr>
<tr>
<td>R1</td>
<td>407 (19.5%)</td>
<td>330 (22.9%)</td>
</tr>
<tr>
<td>Rx</td>
<td>111 (5.3%)</td>
<td>1 (0.07%)</td>
</tr>
<tr>
<td><strong>Overall biochemical recurrences</strong></td>
<td>318 (15.3%)</td>
<td>309 (21.4%)</td>
</tr>
</tbody>
</table>

**Lectin histochemistry on a prostate cancer TMA**

As PHA-L binding was associated with the metastatic potential in our mouse model, we evaluated whether it was also associated with diverging clinical courses of prostate cancer patients using a TMA. Within our study population of 2,411 patients, 2,085 (86.5%) were PHA-L positive and were subjected to further statistical analyses (clinicopathologic findings are summarized in Table 1). PHA-L binding intensity was significantly correlated with the preoperative PSA values (available for 1,950 patients, Fig. 3A, Table 2, $P = 0.003$, Kruskal–Wallis test). In addition, cytoplasmic staining of PHA-L was significantly associated with a higher preoperative PSA compared with apical/membranous staining (Fig. 3A, Table 2, $P = 0.017$, Mann–Whitney $U$ test). However, PHA-L intensity or localization did not correlate with the Gleason score or tumor stage (data not shown). In addition, the small subset of PHA-L–negative patients tended to lower PSA values, but these patients were statistically not different with regard to their PSA recurrence-free survival (data not shown). We furthermore examined, whether the localization of PHA-L was important for the clinical course of the PHA-L–positive patients as measured by postoperative PSA recurrences. Therefore, 639 patients had to be excluded due to incomplete follow-up, resulting in a total of 1,446 spots (Table 1). The mean recurrence-free survival was 95.8 (90.6–101.0; 95% CI) months. Most interestingly, a cytoplasmic binding of PHA-L adversely affected PSA recurrence-free survival compared with apical/membranous binding (Fig. 3B, Table 2, $P = 0.027$, log-rank test). Unlike PHA-L localization, intensity had no significant effect on PSA recurrence (Table 2). According to multivariate analysis including all statistically relevant biochemical and clinical parameters (PSA, pT, pN, surgical margin status, Gleason, and age), PHA-L intensity and PHA-L localization were not independent predictors of PSA recurrence.
Discussion

As in practically all other solid neoplasms, formation of distant metastases makes prostate cancer largely incurable (25). To study metastasis formation, xenografted human tumor cells in immunodeficient mice proved to be valuable tools to analyze mechanisms of metastasis in different tumor entities in general (13, 26). Yet, the prostate cancer field is critically lacking a metastasis xenograft model, in which human tumor cells can be implanted in vivo and all the steps of the metastatic cascade are mimicked adequately (27). Most of previous attempts used severe combined immunodeficient (SCID) mice and cumbersome procedures (6, 28, 29), bearing the risk of an artificial route of metastasis delivery in some of these models (5). In contrast, subcutaneous injections offer a valuable alternative with a lower risk of artificial dissemination. However, this approach was previously hindered by rare metastatic events in SCID-beige mice and difficult detection of the commonly micrometastatic lesions (6). Moreover, previous attempts (also including genetically “engineered” metastases) often missed evidence for their clinical relevance (4). To overcome these problems, we chose the pfp−/−/rag2−/− mouse as the carrier of the human prostate cancer cells and showed a considerable spontaneous pulmonary metastatic spread after subcutaneous injection of prostate cancer cells. We additionally showed a multimodal dissemination of tumor cells into murine blood and bone marrow and confirmed the human origin of lung metastases by immunohistochemical analyses. In contrast to previously used “leaky” SCID mice, pfp−/−/rag2−/− mice are completely devoid of functional T- and B-lymphocytes as well as natural killer (NK) cells (30, 31). As NK cells are potent defenders against circulating tumor cells (14), the pfp−/−/rag2−/− mouse strain is of particular interest in metastasis research.

As aberrant glycosylation patterns of cancer cells have been shown to be relevant for tumor progression in different tumor entities (9), we investigated the glycosylation profile of prostate cancer cells used in the present mouse model. We surprisingly identified an overexpression of Mgat5b in all prostate cancer cell lines compared with

![Figure 3. PHA-L binding in prostate cancer patients. A, PHA-L binding intensity was significantly correlated with the preoperative PSA values (n = 1,950, *P = 0.023, **P = 0.003). In addition, cytoplasmic staining of PHA-L was significantly associated with a higher preoperative PSA compared with apical/membranous staining (†, P = 0.017). B, Kaplan-Meier curves showing PSA recurrence-free survival significantly declining from cytoplasmic to apical/membranous binding of PHA-L (n = 1,446). Photomicrographs showing cytoplasmic versus apical/membranous staining patterns of PHA-L in representative prostate cancer tissue microarray spots.](image-url)
ports the pathophysiologic relevance of neuroendocrine patients with adverse prognostic markers in our study sup-

Mgat5b in metastatic xenografts and in prostate cancer of predominance of the neuron-specific glycosyltransferase human advanced prostate cancer (35). In this context, the epithelium has already been noticed as a special feature of that the neuroendocrine transdifferentiation of prostate 

oligosaccharides in prostate cancer progression.

Mgat5 (GnT-V). Both enzymes synthesize the 

as GnT-Vb or GnT-IX) was characterized as an isozyme of normal prostate epithelium. The corresponding protein, 

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N-acetylglucosaminyltransferase-Vb (also reported as GnT-Vb or GnT-IX) was characterized as an isozyme of Mgat5 (GnT-V). Both enzymes synthesize the 

b

(1,6)-branched oligosaccharides, were predomi-

nantly restricted to metastatic prostate cancer xenografts (PC-3, LNCaP; Fig. 2). In accordance with these findings, we showed 

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(1,6)-branched oligosaccharides as markers of tumor progression in prostate cancer patients (Fig. 3). However, PHA-L intensity and localization were indeed no independent predictors of recurrence-free survival (and might be therefore not useful in clinical practice), but raise the question of a mechanistic relevance of 

b

(1,6)-branched oligosaccharides in prostate cancer progression.

With regard to this question, it is of paramount interest that the neuroendocrine transdifferentiation of prostate epithelium has already been noticed as a special feature of human advanced prostate cancer (35). In this context, the predominance of the neuron-specific glycosyltransferase Mgat5b in metastatic xenografts and in prostate cancer of patients with adverse prognostic markers in our study supports the pathophysiologic relevance of neuroendocrine transdifferentiation during malignant progression of prostate cancer. In addition to prostate cancer, increased amounts of 

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(1,6)-branched oligosaccharides and corresponding PHA-L binding are well-known predictors of poor prognosis and decreased survival time in breast and colorectal carcinomas (33, 36). Our results therefore widen the significance of 

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(1,6)-branched oligosaccharides as markers of tumor progression in human cancer. However, there are only few studies available about the underlying mechanisms, by which 

b

(1,6)-branched oligosaccharides might lead to tumor progression and metastasis formation in prostate cancer. It is evident that increased 

b

(1,6) branching of oligosaccharides on 

b

ß1 integrin stimulate the migration of human fibrosarcoma cells in vitro (38), thus contributing to a more metastatic phenotype. In addition, different expression levels of Mgat5b potently regulate integrin and laminin-dependent adhesion and migration of human neuroblasto
ta cells in vitro (39).

There are, however, different pathways by which Mgat5 and Mgat5b determine the malignant phenotype of cancer cells by 

ß1,6 branching of oligosaccharides. Mgat5b glyco-

sylates the neuron-specific receptor protein tyrosine phosphatase 

ß by and by this causes higher levels of phosphorylated 

ß-catenin, subsequently leading to decreased cell–cell adhe-

sion and increased migration (40). In contrast to Mgat5b, Mgat5 interacts with PTEN in an opposing manner and regulates growth factor signaling and cell polarity via PI3K/ Akt, leading to a more invasive malignant cell phenotype (41). It has further been shown that cancer growth and

<table>
<thead>
<tr>
<th>Table 2. PHA-L staining results on prostate cancer TMA and univariate analysis</th>
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<tbody>
<tr>
<td>PHA-L staining patterns and preoperative PSA-values (n = 1,950)</td>
</tr>
<tr>
<td><strong>Intensity</strong></td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
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<th>PHA-L staining patterns and PSA recurrence-free survival (n = 1,446)</th>
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<td><strong>Intensity</strong></td>
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metastasis formation of a spontaneous tumor model of breast cancer (induced by the polyomavirus middle T oncogene) are reduced in mgtat5–/– mice (42). As expression of Mgtat5 in humans is restricted to the CNS and testes, which are protected by a special blood–tissue barrier system, this enzyme represents a very interesting target for inhibitory treatment approaches (small molecules, miRNA, etc.), as prostate cancer cells are beyond this barrier and thus directly and selectively targetable (43).

In summary, this study describes a novel spontaneous metastasis mouse model of human prostate cancer and by this identifies PHA-L as a marker of metastatic competence in vivo and of tumor progression in prostate cancer patients. We therefore suggest subcutaneous xenografts in pfp−/−/trag2−/− mice as a suitable model of human prostate cancer, as we (i) can indicate its clinical relevance and (ii) show a considerable amount of metastatic events that (iii) reliably occur through the spontaneous metastatic cascade. Moreover, this is the first description of Mgtat5b expression and Mgtat5b products in human prostate cancer.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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Human Prostate Cancer in a Clinically Relevant Xenograft Mouse Model: Identification of β(1,6)-Branched Oligosaccharides as a Marker of Tumor Progression

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