Prostate Cancer

Hedgehog Signalling in Androgen Independent Prostate Cancer

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Abstract

Objectives: Androgen-deprivation therapy effectively shrinks hormone-naïve prostate cancer, both in the prostate and at sites of distant metastasis. However prolonged androgen deprivation generally results in relapse and androgen-independent tumour growth, which is inevitably fatal. The molecular events that enable prostate cancer cells to proliferate in reduced androgen conditions are poorly understood. Here we investigate the role of Hedgehog signalling in androgen-independent prostate cancer (AIPC).

Methods: Activity of the Hedgehog signalling pathway was analysed in cultured prostate cancer cells, and circulating prostate tumour cells were isolated from blood samples of patients with AIPC.

Results: AIPC cells were derived through prolonged culture in reduced androgen conditions, modelling hormone therapy in patients, and expressed increased levels of Hedgehog signalling proteins. Exposure of cultured AIPC cells to cyclopamine, which inhibits Hedgehog signalling, resulted in inhibition of cancer cell growth. The expression of the Hedgehog receptor PTCH and the highly prostate cancer–specific gene DD3PCA3 was significantly higher in circulating prostate cancer cells, and circulating prostate tumour cells were isolated from blood samples of patients with AIPC. There was an association between PTCH and DD3PCA3 expression and the length of androgen-ablation therapy.

Conclusions: Our data are consistent with reports implicating overactivity of Hedgehog signalling in prostate cancer and suggest that Hedgehog signalling contributes to the androgen-independent growth of prostate cancer cells. As systemic anti-Hedgehog medicines are developed, the Hedgehog pathway will become a potential new therapeutic target in advanced prostate cancer.

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1. Introduction

Androgen-deprivation therapy can be used to shrink androgen-dependent tumours. However, prolonged androgen deprivation often results in relapse and androgen-independent prostate cancer (AIPC), which progresses and undergoes metastasis [1]. Once the cancer becomes androgen-independent, the median survival is 20–24 mo [2,3]. AIPC may arise as a consequence of amplification or mutations affecting the activity of the androgen receptor (AR) or cell signalling pathways, which enable prostate cancer cells to proliferate in reduced androgen conditions [4]. Developing diagnostic and therapeutic approaches that target AIPC, therefore, has significant potential for improving survival and quality of life for prostate cancer patients.

Hedgehog pathway activity is important in organogenesis; the plant steroidal alkaloid cyclopamine, a Hedgehog pathway inhibitor, is a profound teratogen [5]. The Hedgehog pathway has recently been implicated in prostate cancer development and metastasis [6]. Patched (PTCH) is the receptor for vertebrate Hedgehog proteins. In the absence of Hedgehog, PTCH inhibits Smoothened (SMO), a G protein–coupled-like receptor. When Hedgehog binds to PTCH, SMO is disinhibited and initiates a signalling cascade that results in activation of GLI transcription factors and increased expression of target genes (including PTCH and GLI1) [7,8]. Hedgehog pathway overactivity has been shown to render prostate epithelial cells tumorigenic, whereas inhibition of the Hedgehog pathway induces apoptosis and decreases invasiveness of prostate cancer cells [6]. Recent studies have shown a high prevalence of Hedgehog activity in high-grade or metastatic prostate cancers [6,9,10], but the contribution of Hedgehog signalling to AIPC is unclear.

To investigate the role of Hedgehog signalling in AIPC, we used a cell line model to demonstrate that Hedgehog signalling is active and required for the growth of AIPC cells. To correlate our findings with clinical samples we used a published protocol to isolate prostate cancer circulating tumour cells (CTCs) from peripheral blood samples of patients with AIPC. CTCs are cells that have become detached from the primary prostate tumour and are migrating via the circulation. Their detection has been associated with poor prognosis in breast and prostate cancer patients [11,12]. Our results suggest that the Hedgehog signalling pathway represents a potential new therapeutic target in AIPC.

2. Methods

2.1. Cell and tissue culture

Androgen-dependent LNCaP cells were from the American Type Culture Collection (LGC Promochem, Teddington, UK) and androgen-independent LNCaP C4-2 and C4-2B were from Viromed Laboratories (Minnetonka, MN, USA). LNCaP AR2 cells were generated through prolonged (9-mo) culture of LNCaP cells in androgen-free media. All cells were grown at 37 °C in Rose Park Memorial Institute (RPMI) medium without phenol red (Sigma-Aldrich, Gillingham, UK), supplemented with 10% (v/v) fetal bovine serum (FBS) (Invitrogen, Paisely, UK) or 10% (v/v) charcoal stripped FBS (Hyclone, Logan, USA), 2.4 mmol/l glutamine (Sigma-Aldrich), 1% (v/v) pyruvate (Sigma-Aldrich), penicillin, and streptomycin (50 U and 50 μg/ml) (Invitrogen). Cells were treated with cyclopamine (Sigma-Aldrich) as detailed.

2.2. MTT assay for cell proliferation

LNCaP cells were seeded onto 96-well plates at 2 × 10^4 per well in 200 μl media, and synchronised in the G0 (quiescent) phase of the growth cycle by culturing in serum-free medium. After 12 h, this serum-free medium was replaced with RPMI containing 10% serum and cyclopamine. After 48 h, 20 μl of the 5 mg/ml MTT substrate (Sigma-Aldrich) was added to each well, incubated for 1 h, washed, and absorbance measured spectrophotometrically. All experiments were performed in triplicate.

2.3. Isolation of circulating tumour cells

Peripheral blood samples were obtained from 15 patients (named according to the format ABC 12) and 5 normal male subjects (named NORM 1–5, all under 40 yr of age with no significant intercurrent illness). Two 10-ml peripheral blood samples were collected in EDTA-coated tubes (Greiner Bio-One, Stonehouse, UK) and processed through Oncoquick (Greiner Bio-One) tubes according to the manufacturer’s instructions. As further purification of the CTCs, the Oncoquick-enriched samples were depleted of CD45-positive cells and positively selected for EpCAM-expressing cells with the use of AutoMACS (Miltenyi Biotec GmbH, Bergisch Gladbach, Germany).

The study was fully approved by the local research ethics committee (reference number 05/Q0602/48).

2.4. RNA isolation, amplification, and quantitative polymerase chain reaction

RNA was isolated with the use of the RNeasy Mini kit (Qiagen, Crawley, UK). The MessageAmp II RNA amplification kit (Ambion, Foster City, CA, USA) was used to amplify and prepare CTC complementary DNA (cDNA). LNCaP cDNA was synthesised after DNase treatment by reverse transcription with the use of Superscript III (Invitrogen) and oligo-dT priming. Quantitative polymerase chain reaction (QPCR) was performed in triplicate on CTC and LNCaP cDNA with the use of Platinum SYBRGreen qPCR Supermix-UDG (Invitrogen) on
an Opticon DNA Engine 2 (Bio-rad Laboratories, Hemel Hemstead, UK). The primer sequences used to determine the relative expression of PTCH, GLI1, AR, prostate-specific antigen (PSA), DD3PCA3, and housekeeping gene β2-microglobulin were PTCH-f, CTCCCAAGCATAATGACGAGCA; PTCH-r, TGAGTTGAGTTCTGTGCGGACAC; GLI1-f, GAAGACTCTCAGCCTTGGG; GLI1-r, GGGCAGCAATGAGCAGAG; AR-f, GACGCTTCTACGGAGTTCTGTGCGACAC; AR-r, GAAAGGATCTTGGGCACTTG; PSA-f, GGTGGGAAGGACCTGATGATAG; DD3PCA3-f, GGTGGGAAGGACCTGATGATAG; DD3PCA3-r, GGGACACATGAGCAGAG; GLI1-r, GGCTGACAGTATAGGCAGAG; AR-f, GACGCTTCTACGGAGTTCTGTGCGACAC; GLI1-f, GAAGACCTCTCCAGCTTGGA; PTCH-f, CTCCCAAGCAAATGTACGAGCA; PTCH-r, TGAGTGAGTTCTGTGCGGACAC. All standard curves for each primer set using LNCaP C4-2B–derived genomic DNA. To validate primer specificity, we generated observed in the cDNA samples, indicating an absence of across an intron/exon boundary. No PCR products were observed in the cDNA samples, indicating an absence of genomic DNA. To validate primer specificity, we generated standard curves for each primer set using LNCaP C4-2B-derived cDNA and detected appropriately sized single products. All cDNA samples expressed the housekeeping gene β2-microglobulin, which was used for normalisation.

2.5. Antibodies and immunostaining

Primary antibodies used were as follows: PTCH (#sc-6149; Santa Cruz Biotechnology, Santa Cruz, CA, USA), GAPDH (#9484; Abcam, Cambridge, UK), and EpCAM (#ab20160; Abcam) at concentrations recommended by the manufacturers. For western blot analysis, primary antibodies were incubated overnight at 4 °C in blocking buffer, and visualised with the use of an appropriate horse radish peroxidase-conjugated secondary antibody and enhanced chemiluminescence (GE Healthcare UK Ltd, Little Chalfont, UK). For immunofluorescence staining, primary antibodies were incubated overnight in blocking buffer and detected with the use of appropriate secondary antibodies. Nuclear counterstaining was performed with the use of DAPI (4',6-diamidino-2-phenylindole). Stained preparations were analysed on a Zeiss 510 confocal microscope (Carl Zeiss Ltd, Welwyn Garden City, UK).

2.6. Statistics

Statistical analysis was performed with the Student t test and Pearson correlation coefficient. p values < 0.05 were considered significant.

3. Results

Androgens are essential for optimal growth of the prostate cancer cell line LNCaP, despite expression of a promiscuous gain-of-function mutant AR [13]. We generated the LNCaP subline AR2 after 9 mo of culturing the parental LNCaP cells in androgen-free media (AFM). We also used androgen-independent sublines LNCaP C4-2 and C4-2B, which were originally isolated and characterised after their growth in castrated athymic mice from the site of grafting (C4-2) or bony metastases (C4-2B) [14]. The derived cell lines showed a continuum of increasing growth rates in androgen-free conditions (Table 1). To investigate the contribution of Hedgehog signalling to androgen-independent cell growth, we determined the messenger RNA (mRNA) expression levels of components of this pathway in LNCaP (androgen-dependent LNCaP cells maintained in androgen-containing medium), LNCaP-A (androgen-dependent LNCaP cells maintained in AFM for 5 d), and LNCaP AR2, LNCaP C4-2, and LNCaP C4-2B cells (maintained in AFM). We performed QPCR to determine the expression level of PTCH, GLI1, AR, and PSA mRNA. There was significant up-regulation of PTCH, GLI1, and AR expression in LNCaP AR2, LNCaP C4-2, and LNCaP C4-2B cells (Fig. 1A), consistent with previous reports of increased AR expression during the transition of LNCaP cells to androgen independence [15]. Androgen-independent LNCaP C4-2B cells expressed similar levels of PSA as androgen-dependent LNCaP cells (Fig. 1A). The PTCH RNA changes also correlated with increased expression of PTCH protein (Fig. 1B), indicative of active Hedgehog signalling [8]. To verify the importance of the Hedgehog pathway to AIPC cell growth, we used the SMO inhibitor cyclopamine, which blocks Hedgehog signalling [16]. PTCH and GLI1 are both constituents of the Hedgehog pathway and targets that are also up-regulated as a consequence of Hedgehog signalling. Application of cyclopamine for 48 h to androgen-independent LNCaP C4-2B cells resulted in a significant decrease in expression of PTCH and GLI1 (Fig. 1C), consistent with inhibition of Hedgehog signalling activity. The growth of androgen-independent LNCaP C4-2B cells, but not androgen-dependent LNCaP cells, was affected by cyclopamine in 10% FBS. Because LNCaP cell growth can also be inhibited by cyclopamine at lower FBS concentrations, this finding suggests that androgen-independent LNCaP C4-2B cells, like LNCaP C81 cells, are more sensitive to Hedgehog inhibition [4]. Significant LNCaP C4-2B growth inhibition was observed with 100 nmol/l cyclopamine (p < 0.05), and the mean inhibitory concentration (IC50), 11 μmol/l, was similar to reported values in other systems (Fig. 1D) [4,5].

Table 1 – Androgen-independent growth of LNCaP sublines

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Androgen-independent growth</th>
</tr>
</thead>
<tbody>
<tr>
<td>LNCaP</td>
<td>0.3</td>
</tr>
<tr>
<td>LNCaP AR2</td>
<td>0.7</td>
</tr>
<tr>
<td>LNCaP C4-2</td>
<td>1.0</td>
</tr>
<tr>
<td>LNCaP C4-2B</td>
<td>1.2</td>
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</table>

* Growth after 5 d in androgen-free medium/growth in regular medium.
Please cite this article in press as: Shaw G, et al., Hedgehog Signalling in Androgen Independent Prostate Cancer, Eur Urol (2008), doi:10.1016/j.eururo.2008.01.070
To investigate whether the Hedgehog pathway contributes to clinical cases of AIPC, we isolated CTCs from 20-ml peripheral blood samples of patients with advanced prostate cancer who (with the exception of SUY25) are on second-line treatment, having failed primary hormone therapy (with a rising PSA in the face of castrate serum testosterone levels) and are therefore androgen-independent. The patients’ clinical characteristics are age, 53–89 yr (median, 76); last PSA, 12–667 ng/ml (median, 64); testosterone less than 0.7 nmol/l; and duration of hormone therapy, 1.2–13.9 yr, (median, 4.4), as detailed in Table 2. The use of CTCs is advantageous because biopsies are rarely performed on patients at this cancer stage, and obtaining CTCs is a low-risk, minimally invasive technique compared with biopsy. Healthy male subjects have been widely used as controls and typically have between 0–2 CTCs per 7 ml of blood, whereas 2–10 CTCs and 4–500 CTCs per 7 ml of blood are reported in localised prostate cancer and advanced prostate cancer, respectively [17–19].

Our immunofluorescence analysis showed that each sample from an AIPC patient contained more than five nucleated, EpCAM-positive CTCs per 7 ml of blood, which has also been associated with poor prognosis [12]. No CTCs were observed in the normal controls.

PTCH expression was detectable by immunofluorescence (Fig. 2) and QPCR (Fig. 3) in the androgen-independent CTC samples. We observed very good discrimination between the normal subjects and the cancer patients for expression of PTCH RNA and the prostate cancer–specific transcript DD3PCA3 (Fig. 3) [20,21], although a very low level of DD3PCA3 expression was detected in one of the controls (reflecting the sensitivity of QPCR). Heterogeneity of expression was observed within the cancer patient samples; this result has also been previously reported for other markers using PCR profiling of CTCs [19]. Expression of the androgen-responsive prostate cancer marker DD3PCA3 in CTCs isolated from patients receiving androgen-ablation therapy and having low levels of serum testosterone is consistent with the presence of AIPC cells in the CTC samples. PTCH showed the most discriminatory power between prostate cancer patients and normal subjects owing to the absence of detectable expression in normal subjects. No correlation was

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**Table 2 – Clinical characteristics of AIPC prostate cancer patients**

<table>
<thead>
<tr>
<th>ID</th>
<th>Age</th>
<th>Radiological metastasis</th>
<th>Previous treatment</th>
<th>Years of androgen deprivation</th>
<th>Type of medication</th>
<th>PSA velocity</th>
<th>Last PSA</th>
<th>Castrate testosterone level</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAP</td>
<td>87.08</td>
<td>N</td>
<td>—</td>
<td>7.7</td>
<td>MAB</td>
<td>0.22</td>
<td>56.3</td>
<td>Y</td>
</tr>
<tr>
<td>PEJ</td>
<td>89.72</td>
<td>Y</td>
<td>—</td>
<td>2.8</td>
<td>MAB</td>
<td>0.23</td>
<td>159.3</td>
<td>Y</td>
</tr>
<tr>
<td>BLR</td>
<td>53.26</td>
<td>Y</td>
<td>—</td>
<td>1.6</td>
<td>MAB</td>
<td>ND</td>
<td>19.0</td>
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</tr>
<tr>
<td>BRL</td>
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<td>Y</td>
<td>RT</td>
<td>7.9</td>
<td>MAB</td>
<td>0.60</td>
<td>199.6</td>
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<tr>
<td>RYR</td>
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<td>Y</td>
<td>—</td>
<td>4.4</td>
<td>MAB</td>
<td>0.02</td>
<td>23.2</td>
<td>Y</td>
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<tr>
<td>FLR</td>
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<td>RP</td>
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<td>MAB</td>
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</tr>
<tr>
<td>ROA</td>
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<td>Y</td>
<td>—</td>
<td>2.4</td>
<td>DAS</td>
<td>3.73</td>
<td>471.0</td>
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<tr>
<td>LAD</td>
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<td>Y</td>
<td>—</td>
<td>2.5</td>
<td>DA</td>
<td>0.25</td>
<td>152.5</td>
<td>Y</td>
</tr>
<tr>
<td>SUY</td>
<td>55.27</td>
<td>N</td>
<td>RT</td>
<td>1.8</td>
<td>LHRH</td>
<td>0.03</td>
<td>43.6</td>
<td>N</td>
</tr>
<tr>
<td>GIP</td>
<td>63.38</td>
<td>Y</td>
<td>RT</td>
<td>12.1</td>
<td>LHRH</td>
<td>26.60</td>
<td>666.9</td>
<td>Y</td>
</tr>
<tr>
<td>MUH</td>
<td>86.67</td>
<td>Y</td>
<td>—</td>
<td>10.3</td>
<td>MAB</td>
<td>ND</td>
<td>164.1</td>
<td>Y</td>
</tr>
<tr>
<td>TUK</td>
<td>71.36</td>
<td>Y</td>
<td>—</td>
<td>3.2</td>
<td>MAB</td>
<td>3.86</td>
<td>153.4</td>
<td>Y</td>
</tr>
<tr>
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<td>76.72</td>
<td>Y</td>
<td>—</td>
<td>1.2</td>
<td>MAB</td>
<td>–0.11</td>
<td>11.8</td>
<td>Y</td>
</tr>
<tr>
<td>HOD</td>
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<td>—</td>
<td>5.1</td>
<td>LHRH</td>
<td>0.16</td>
<td>63.9</td>
<td>Y</td>
</tr>
</tbody>
</table>

AIPC, androgen-independent prostate cancer; PSA, prostate-specific antigen; ND, not determined; RT, radiotherapy; RP, radical prostatectomy; MAB, maximum androgen blockade; DA, dexamethasone and aspirin; CLE, cisplatin, lomustine, etoposide; LHRH, leutenising hormone-releasing hormone therapy; Orchid, castration by bilateral subcapsular orchidectomy.

Samples were anonymised according to the format ABC 12.

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**Fig. 1** – (A) QPCR showing relative expression of PTCH, GLI, androgen receptor, and PSA by LNCaP cells cultured in RPMI (LNCaP); LNCaP cells cultured in AFM for 5 d (LNCaP-A) and LNCaP AR2, C4-2, and C4-2B cultured in AFM. (B) Immunoblot showing expression of PTCH in LNCaP cells and sublines. (C) Cyclopamine treatment (14 μmol/l) inhibits expression of PTCH and GLI1 RNA in AIPC LNCaP C4-2B cells: Control cells (lanes 1 and 3); 48-h cyclopamine treatment (lanes 2 and 4). (D) Concentration-effect curve showing that cyclopamine treatment inhibits growth of AIPC LNCaP C4-2B cells. The means ± SD are shown. (A, C) *** Indicates significant difference in expression (p < 0.05). QPCR, quantitative polymerase chain reaction; PTCH, Patched; PSA, prostate-specific antigen; RPMI, Rose Park Memorial Institute [medium]; AFM, androgen-free medium; AIPC, androgen-independent prostate cancer; SD, standard deviation.
Fig. 3 – QPCR showing relative expression of DD3\(^{\text{PCA3}}\) and PTCH in control (Norm1-5) and prostate cancer patient (ABC12) CTC samples. The expression of DD3\(^{\text{PCA3}}\) and PTCH is significantly higher \((p < 0.05)\) in the prostate cancer patient group. Patient samples are shown sorted left to right in order of last serum PSA measurement (FOJ33 lowest PSA-HOD34 highest PSA). No association exists between PSA measurement and expression of DD3\(^{\text{PCA3}}\) \((\text{Pearson} \ r, 0.318, p > 0.05)\) or PTCH \((\text{Pearson} \ r, 0.191, p > 0.05)\). QPCR, quantitative polymerase chain reaction; PTCH, Patched; CTC, circulating tumor cell; PSA, prostate-specific antigen.

Fig. 2 – Representative immunofluorescence of CTCs from patient < 4-yr ADT (BLR22: ADT 1.6 yr) or > 4-yr ADT (HOD34: HT 13.9 yr) with PTCH and EpCAM antibodies. CTCs, circulating tumor cells; ADT, androgen-deprivation therapy; PTCH, Patched.
found between PTCH or DD3\textsuperscript{PCA3} expression levels and the last PSA serum level (Fig. 3), age (data not shown), or PSA velocity (calculated by gradient of best fit with at least three readings in the past 18 mo; data not shown). PATCH and DD3\textsuperscript{PCA3} expression was very low in two patients (SUY25 and BAP20) who do not show radiologically detectable metastasis.

There was a strong correlation between the CTC expression levels of PTCH and DD3\textsuperscript{PCA3} (Fig. 4). Interestingly, we also observed that expression of PTCH protein tended to be highest in CTCs isolated from patients who had received androgen-ablation therapy for the longest periods of time (Fig. 2), and a highly significant increase in the CTC expression levels of DD3\textsuperscript{PCA3} and PTCH RNA was found when the patients were dichotomised about the median duration of androgen deprivation (Fig. 5). In our cancer patient group, there is 85% survival 12 mo
after CTC analysis, although there is no difference in survival between high or low expression of PTCH. Further follow-up will be required to determine long-term survival affects.

4. Discussion

Hedgehog signalling is required for normal prostate development. Several studies have recently reported that active Hedgehog signalling occurs in localised and metastatic prostate cancer [6,9,10,22]. PCR analysis has shown that, although Hedgehog ligands are expressed abundantly in localised and metastatic prostate cancer, in metastatic lesions the expression of PTCH and GLI1 is dramatically increased; in addition, treatment of an aggressive prostate xenograft model with cyclopamine prevented metastasis to the lungs, whereas overexpression of GLI1 caused lung metastasis [6]. This finding is consistent with our observation that PATCH expression was very low in two patients (SUY25 and BAP20) who do not show radiologically detectable metastasis. On this basis, inhibiting Hedgehog activity has been described as having “tremendous promise for controlling advanced prostate cancer” [23]. However, whether Hedgehog activity is also of importance in the development and progression of AIPC is unclear. In this study, we show that Hedgehog signalling is active in AIPC. We observed increased expression of components of the Hedgehog signalling pathway in androgen-independent LNCaP sublines and in CTCs isolated from patients with AIPC. In addition, targeting Hedgehog activity also inhibited growth of AIPC cells, consistent with the antiproliferative results recently reported after cyclopamine treatment of a spontaneously arising, separate androgen-independent LNCaP C81 subline [4].

We found significant expression in CTCs isolated from patients with AIPC of PTCH, consistent with active Hedgehog signalling; this finding correlated with expression of the prostate cancer–specific marker DD3PCA3. This is the first reported expression of PTCH and DD3PCA3 RNA in prostate cancer CTCs. Interestingly, there was heterogeneity in the expression of PTCH in CTCs isolated from AIPC patients, similar to the relative expression reported for other markers using PCR profiling of CTCs [19]. We also found a significant association between the duration of patient androgen-deprivation therapy and the level of PTCH expressed. Patients who had received androgen-deprivation therapy for longer periods of time expressed higher levels of PTCH. Increased Hedgehog signalling could further promote the growth or metastasis of AIPC cells in these patients. However, because there is 85% survival 12 mo after CTC analysis in our prostate cancer patient group, additional follow-up will be necessary to determine if Hedgehog signalling affects long-term survival.

Profiling of CTCs has the potential to identify prostate patients in whom the Hedgehog signalling pathway is active, thus enabling targeted treatments in prostate cancer, similar to breast cancer in which Herceptin therapy is targeted to the 25% of advanced or metastatic breast cancers that are positive for ErbB2 (HER2). Although topically applied cyclopamine showed efficacy against basal cell carcinoma of the skin in humans [24], there is at present no clinically available systemic treatment that specifically targets the Hedgehog signalling pathway. The SMO inhibitor cyclopamine, which we show can be used to inhibit AIPC cell proliferation, along with other Hedgehog signalling targeting compounds are currently being developed (Curis, Cambridge, USA), and a phase 1 clinical trial of a systemically administered, small-molecule Hedgehog antagonist for testing in advanced cancer has been initiated (Genentech, San Francisco, USA). A combination of epidermal growth factor (EGF) receptor gefitinib and cyclopamine has also recently been shown to improve the cytotoxic effects of docetaxel on cultured metastatic prostate cancer cells [25]. In addition, we have found strong synergistic effects on AIPC cell growth from combination treatments of cyclopamine with the ErbB signalling inhibitors gefitinib or lapatinib (unpublished data 2007, Shaw and Prowse). The synergy may occur through a direct effect of EGF signalling selectively enhancing Hedgehog activity [26]. The use of small-molecule Hedgehog antagonists as adjuvant therapies may therefore improve the survival rate observed with current regimens that are ineffective against AIPC and may prove to be a useful addition to the armamentarium.

5. Conclusion

The Hedgehog signalling pathway is active in AIPC, and PTCH-positive CTCs can be identified in patients with metastatic AIPC. As systemic anti-Hedgehog medicines are developed, targeting the Hedgehog pathway has the potential to become a new therapeutic strategy in treatment of advanced prostate cancer.

Financial disclosures: The authors have none to disclose.

Acknowledgement statement: We would like to thank the Orchid Cancer Appeal for study funding, and Drs Powles and Shamash for their clinical support.
References


[10] Sheng T, Li C, Zhang X, et al. Activation of the Hedgehog pathway is driven by a family of peptide ligands (referred to as hedgehogs) that engage a transmembrane receptor from the gene family. Binding of the Hedgehog ligand to a Ptc receptor de-represses the associated Smoothened (Smo) protein and this action ultimately increases the intracellular stability and activity of Gli transcription factors at the end point of the pathway [1]. Hedgehog signaling pathway is driven by a family of peptide ligands (referred to as hedgehogs) that engage a transmembrane receptor from the Patched (Ptc) gene family. Binding of the Hedgehog ligand to a Ptc receptor de-represses the associated Smoothened (Smo) protein and this action ultimately increases the intracellular stability and activity of Gli transcription factors at the end point of the pathway [1].

Editorial Comment on: Hedgehog Signalling in Androgen Independent Prostate Cancer

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Hedgehog signaling is an area of relatively high interest for contemporary cancer research. This
growth and morphogenetic processes associated with normal development. Indeed, the name of this signaling pathway was derived from Drosophila mutants that produce short and stubby embryos with an abnormal distribution of denticles that give the appearance of hairiness. With regard to the prostate, standard mouse genetic models to study the effects of Hedgehog inactivation in the prostate are relatively difficult to interpret because the loss of any single Hedgehog ligand or gli family gene appears to be compensated by increased prostatic expression of another member from these families so prostate development proceeds in these models in a manner that suggests it is independent of Hedgehog signaling [2]. Despite this complication, evidence suggests an active Hedgehog-driven paracrine process during prostate development affects ductal patterning in this tissue [3].

The Gli transcription factors that are engaged by Hedgehog signaling autoregulate some key molecular players within the Hedgehog pathway (including Gli expression itself) as well as a number of other gene products that are involved in the control of cell division or cellular differentiation. Therefore, it should not be surprising that dysregulated Gli activity is involved in human carcinogenesis [4]. Hedgehog signaling is also thought to be important for stem cell self-renewal [5] and this role also contributes to the remarkable interest in this pathway for cancer. Particular efforts have explored the role of Hedgehog signaling in prostate cancer. Here, the key findings have been sporadic reports that over-expression of Hedgehog ligands can transform prostate epithelial cells in vitro and in vivo [6,7] and that expression of Hedgehog-regulated proteins are elevated in aggressive (metastatic) and lethal prostate tumors [7]. This latter finding may be relevant to the work reported here by Shaw et al who describe an eclectic series of experimental analyses in which the basal expression of two Hedgehog inducible genes (Ptc and Gli) was examined in a collection of androgen-sensitive and -insensitive human prostate cancer cell lines, even those that appear to be completely refractory to stimuli of canonical Hedgehog signaling [8,9]. Whereas the reported work in this article is insufficient to establish a role for Hedgehog in androgen-independent cancer, at the least it should stimulate a focused and more expansive effort to understand whether the Hedgehog signaling pathway contributes to the development of hormone-independent disease [10]. Furthermore, the knowledge that reliable Hedgehog signaling inhibitors are readily available and that at least one of these drugs (cyclopamine) has reproducibly shown activity against both hormone-sensitive and -insensitive prostate cancer cell lines, even those that appear to be completely refractory to stimuli of canonical Hedgehog signaling [8,9].

References


DOI: 10.1016/j.eururo.2008.01.071

DOI of original article: 10.1016/j.eururo.2008.01.070

Please cite this article in press as: Shaw G, et al., Hedgehog Signalling in Androgen Independent Prostate Cancer, Eur Urol (2008), doi:10.1016/j.eururo.2008.01.071