Putative prognostic epithelial-to-mesenchymal transition biomarkers for aggressive prostate cancer

Helen Whitelanda, Samantha Spencer-Hartyb, David Hywel Thomasb, Christine Daviesb, Claire Morgana, Howard Kynastond, Pradeep Bosec, Neil Fennc, Paul D. Lewisa, Owen Bodgera, Spencer Jenkins a,d, Shareen H. Doaka

a College of Medicine, Swansea University, Swansea SA2 8PP, Wales, UK
b Department of Urology, Morriston Hospital, Abertawe Bro Morgannwg University NHS Trust, Swansea SA6 6NL, Wales, UK
c Department of Cellular Pathology, Singleton Hospital, Abertawe Bro Morgannwg University NHS Trust, Swansea SA2 8QA, Wales, UK
d Department of Surgery, Cardiff School of Medicine, Heath Park, Cardiff CF14 4XW, Wales, UK

Article history:
Received 12 July 2013
Available online 6 August 2013

Keywords:
Prostate cancer
Epithelial-to-mesenchyme transition
E-Cadherin
Snail
Gleason score
Clinical stage

Prostate cancer is the second most frequently diagnosed cancer worldwide and is the sixth leading cause of cancer deaths in men, yet it varies greatly in its aggressiveness. Currently, it is not possible to adequately differentiate between patients whose tumors will remain indolent and those patients whose disease will progress, resulting in unnecessary aggressive treatment. Consequently, there is an urgent need to identify markers of prostate cancer progression, invasiveness and metastasis to more accurately predict prognosis. The aim of this study was to assess the ability of key epithelial-to-mesenchymal transition molecules in identifying prostate cancer patients who are likely to develop aggressive tumors. Using 215 archival patient tissue samples, immunohistochemistry was applied to examine the expression and sub-cellular localization of E-Cadherin, Snail, Slug, Twist, Vimentin, BMP-2 and BMP-7. Of the seven markers assessed, a significantly increased expression of Snail protein was observed within the nucleus of prostate cancer cells and was strongly associated with increasing Gleason score and clinical stage. In addition, loss of E-Cadherin expression at the cellular membrane of prostate cancer cells was also significantly associated with increasing Gleason score, clinical stage, and additionally, a reduction in survival.

Introduction

In 2008, prostate cancer was reported to account for 14% of all new male cancer cases and 6% of total cancer deaths per 100,000 men globally (Jemal et al., 2011). In the USA alone, an estimated 217,730 new cases of prostate cancer were reported with an estimated 32,050 prostate cancer deaths (Jemal et al., 2010). Since the widespread introduction of serum prostate specific antigen (PSA) testing, prostate cancer is being detected at an earlier stage. The main criteria used to determine risk of recurrence/progression following diagnosis of non-metastatic disease are serum PSA, clinical stage and prostate biopsy Gleason score (D’Amico et al., 1998) and these three factors help to direct clinical management. However, these three factors, even when taken together, do not adequately discriminate between those tumors that will remain indolent and those that will later progress to become metastatic. The main cause of death due to malignancy is metastasis, whereby the primary site tumor cells gain the ability to spread to a secondary site (Duffy et al., 2008). However, only a sub-population of tumor cells within the primary tumor will develop metastatic potential (Eccles and Welch, 2007) as these cells must accumulate several functional modifications such as the aversion of apoptosis and increased motility (Robson et al., 2006). Indeed, the process of metastasis is complex, involving a number of key stages. Firstly, primary cells must gain phenotypic alterations that allow them to dissociate away from the primary site becoming locally invasive. They then undergo modulations in adhesive properties to promote their invasavation into the vasculature system. Subsequently, the tumor cells must survive anoikis, leave the bloodstream by extravasation, and finally seed at the secondary site, all of which involve dramatic molecular adaptations.

Epithelial-to-mesenchyme transition (EMT) is increasingly thought to play a focal role in metastasis and is a process whereby immotile epithelial cells are converted into migratory mesenchyme cells via a complex signaling pathway (Mathias and Simpson, 2009). EMT induces a variety of cellular adaptations in morphology, cellular architecture, adhesion and migratory characteristics (Lee et al., 2006), and therefore involves an extensive network of signaling proteins. Thus, in carcinogenesis, evidence is accumulating that indicates EMT may drive the many phenotypic and functional alterations required for a cell to gain the properties that promote metastasis (Sethi et al., 2011; Tomaskovic-Crook et al., 2009).
Consequently, given the potential role of EMT in metastasis, in the present study we investigated the expression of several key EMT proteins, including Slug, Snail, Twist, E-Cadherin, Vimentin, BMP-2, and BMP-7, to determine whether they demonstrate prognostic value in identifying prostate cancer patients that are likely to progress to aggressive disease.

Materials and methods

Patient samples

Patient tissue for this study was formalin fixed paraffin embedded (FFPE) archival prostate cancer material. Ethical approval for this collection was obtained from the Swansea Local Research Ethics Committee (Ref NO: 07/WM02/59, approved in August 2007). Using the pathology archive database at Singleton hospital (Swansea, Wales, UK) and aided by the Secure Anonymised Information Linkage (SAIL) database (Swansea University (Ford et al., 2009), all patients diagnosed with prostate cancer from 2002 to 2010 were identified, amounting to 2158 patients in total. To ensure sufficient follow up data was available, patients diagnosed between the years 2002 and 2004 were studied; resulting in 320 identified prostate cancer samples. Some of these were multiple samples from the same patient or suspicious prostate cancer cases, reducing the number of suitable patient samples during the period to 286 cases. Adequate tumor involvement was assessed by a consultant pathologist (DHT) to ensure that sufficient material remained in the archive for future clinical need reducing the final total to 215 suitable patient samples.

The final sample set consisted of FFPE tissue from trans-rectal ultrasound (TRUS) guided biopsies (n = 145), radical prostatectomy and transurethral resection of the prostate (TURP) samples (n = 70). No patients in the study had undergone treatment prior to tissue collection, and the radical prostatectomy and TURP samples were obtained within 3 months of the initial diagnosis. Where available, data was collected on initial presenting PSA, clinical and pathological stage (TNM classification) (Sobin et al., 2009), age, Gleason score, and post-treatment PSA values. Patients were grouped into ‘progression status’ based on evidence of future relapse defined as a rising PSA or in those patients who had radical prostatectomy as a post-operative PSA level of >0.5 ng/mL on two separate occasions.

Sample preparation

TRUS biopsies were sectioned at 3 μm thickness using a tissue microtome (Microm HM325) and mounted onto a Superfrost plus glass slide (Thermo scientific, UK). Two patient samples were mounted per glass slide and then baked at 60 °C for 60 min. The TURP and radical prostatectomy samples were constructed into a tissue microarray (TMA). A hand core (0.6 mm diameter) was used to remove and transfer tumor areas into a donor block in which they were re-embedded. An H&E section was prepared from the donor block to ensure the presence of tumor in the selected cores. Three tissue cores per patient were then removed from the donor block and placed into a recipient block with 1 mm spacing between all the cores, using an automated Minicore® TMA machine (Alphelys, France). On average a total of 210 cores were prepared per TMA. A border of rat liver was constructed around the edge of the TMA for orientation. Three-micron thick sections were prepared from the TMA, with the first and last section used for H&E staining to confirm the presence of cancer cells throughout the selected cores.

Immunohistochemistry

Immunohistochemistry (IHC) was performed using the Benchmark XT automated staining system (Ventana, AZ) with iView™ DAB detection kit (Ventana, AZ) according to manufacturer’s instructions. Optimized antibody dilutions were: E-Cadherin 1:50 (Dako, UK), Snail 1:100 (abCam, UK), Vimentin 1:80 (Dako, UK), BMP-2 1:50 (Santa Cruz, CA), BMP-7 1:200 (abCam, UK), Twist 1:500 (abCam, UK), Slug 1:10 (abCam, UK). Slides were counterstained with Mayers haematoxylin and mounted with DPX.

Scoring of the resultant IHC slides was carried out by three trained analysts (HW, CM, SHD). A score was applied for the intensity of staining seen in the tumor: 0 (no staining), 1 (weak staining), 2 (moderate staining) and 3 (high staining). Additionally, a score for staining distribution was applied: 0 (no staining), 1 (<10% cellular involvement), 2 (10–60% cellular involvement), and 3 (>60% cellular involvement) (Fan et al., 2012). The sum score of intensity and distribution were determined for each of the markers examined (Jan et al., 2009). Samples with insufficient staining due to lack of tumor cells within the specimens were not evaluated.

Statistical analysis

Statistical analysis was performed using SPSS software version 16.0 for Windows. The Kruskal–Wallis test was used to assess differences between staining patterns of the EMT markers across the Gleason score, age, initial pre-operative PSA value, T stage, progression and cause of death. Differences between pairs of groups were assessed using the Mann–Whitney U-test. Finally, differences between biomarker expression and survival were analyzed using Fishers exact test. A p-value of <0.05 was considered significant.

Results

Using IHC, we investigated the expression of the EMT markers Snail, E-Cadherin, Vimentin, Slug, Twist, BMP-2 and BMP-7 within prostate cancer tissue samples from 215 patients of varying Gleason score. We aimed to assess the level of their expression within a number of patient clinical groupings and whether their expression patterns were correlated with those patients who later progressed to aggressive disease. Of the seven markers assessed in this study, Twist and Slug staining patterns were not substantially different between stromal, cancer and non-cancer cells and thus will not be discussed further as they did not serve as useful biomarkers.

Patient demographics

Full details on the patient cohort are provided in Table 1 and patient demographics in relation to age group in Table 2. For initial PSA value and T stage, data was not available for all patients. Data for follow-up PSA levels was only collected when two or more follow-up readings were available, resulting in a total of 127 patients with available PSA follow-up data indicating progression, and 89 patients were excluded from the follow-up PSA analysis due to no further records or less than two follow-up readings being available. Patients’ age ranged from 50 to 94 years (median, 74 years). Initial pre-operative serum PSA ranged from 1.0 to 2221 ng/mL (median, 21.1 ng/mL). PSA is currently used as the gold standard for indicating the possible presence of prostate cancer, while PSA relapse is an indicator of disease progression. Within this study, patients who presented with initial pre-operative PSA levels of >10 ng/mL (median 31.3 ng/mL) had 82% of these patients with a Gleason score of ≥7 compared with 65% of those patients who had initial pre-operative PSA levels of <10 ng/mL. Thus higher pre-operative PSA is associated with high Gleason score.

When considering PSA relapse, those patients who demonstrated no PSA relapse had a range of Gleason scores between 5–10 (median 7) and an initial PSA value of 1.9–260.5 ng/mL (median 14.2 ng/mL). Whilst patients that did suffer PSA relapse had very similar demographics, with wide ranging Gleason scores (median Gleason score 7) and initial PSA values of 1–930 ng/mL (median 23 ng/mL). However, although not statistically significant, there was a slight increase in patients’ initial PSA
readings in association with high Gleason score at first presentation (Fig. 1).

Table 1
Detailed patient demographics and biomarker associations.

<table>
<thead>
<tr>
<th>Group</th>
<th>Sub-group</th>
<th>Patient numbers</th>
<th>n (%)</th>
<th>Total number of patients</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gleason score</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5–6</td>
<td></td>
<td>47</td>
<td></td>
<td>22</td>
<td>0.039*</td>
</tr>
<tr>
<td>7</td>
<td></td>
<td>70</td>
<td></td>
<td>33</td>
<td>0.251</td>
</tr>
<tr>
<td>8–10</td>
<td></td>
<td>98</td>
<td></td>
<td>46</td>
<td>0.061</td>
</tr>
<tr>
<td>&lt;10</td>
<td></td>
<td>49</td>
<td></td>
<td>24</td>
<td>0.009**</td>
</tr>
<tr>
<td>10–20</td>
<td></td>
<td>52</td>
<td></td>
<td>26</td>
<td>0.287</td>
</tr>
<tr>
<td>20.1–100</td>
<td></td>
<td>75</td>
<td></td>
<td>37</td>
<td></td>
</tr>
<tr>
<td>100+</td>
<td></td>
<td>27</td>
<td></td>
<td>13</td>
<td></td>
</tr>
<tr>
<td><strong>Initial PSA value (ng/mL)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.01</td>
<td></td>
<td>48</td>
<td></td>
<td>22</td>
<td>0.002**</td>
</tr>
<tr>
<td>0.3</td>
<td></td>
<td>84</td>
<td></td>
<td>39</td>
<td>0.039</td>
</tr>
<tr>
<td>1.0</td>
<td></td>
<td>48</td>
<td></td>
<td>22</td>
<td>0.001**</td>
</tr>
<tr>
<td>8.1</td>
<td></td>
<td>37</td>
<td></td>
<td>17</td>
<td>0.002**</td>
</tr>
<tr>
<td><strong>T stage</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1/T2 a–c</td>
<td></td>
<td>19</td>
<td></td>
<td>30</td>
<td>0.754</td>
</tr>
<tr>
<td>T3 a–c</td>
<td></td>
<td>31</td>
<td></td>
<td>49</td>
<td>0.001***</td>
</tr>
<tr>
<td>T4</td>
<td></td>
<td>13</td>
<td></td>
<td>27</td>
<td>0.487</td>
</tr>
<tr>
<td>&lt;60</td>
<td></td>
<td>17</td>
<td></td>
<td>8</td>
<td>0.211</td>
</tr>
<tr>
<td>60–70</td>
<td></td>
<td>66</td>
<td></td>
<td>31</td>
<td>0.235</td>
</tr>
<tr>
<td>71–80</td>
<td></td>
<td>84</td>
<td></td>
<td>39</td>
<td>0.487</td>
</tr>
<tr>
<td>81+</td>
<td></td>
<td>48</td>
<td></td>
<td>22</td>
<td></td>
</tr>
<tr>
<td><strong>Survival</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCA†</td>
<td></td>
<td>37</td>
<td></td>
<td>17</td>
<td>0.039</td>
</tr>
<tr>
<td>Other‡</td>
<td></td>
<td>178</td>
<td></td>
<td>83</td>
<td>0.691</td>
</tr>
<tr>
<td><strong>Progression status</strong> (Indicated by PSA increase)**</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Relapse</td>
<td></td>
<td>98</td>
<td></td>
<td>53</td>
<td>0.281</td>
</tr>
<tr>
<td>No-relapse</td>
<td></td>
<td>87</td>
<td></td>
<td>47</td>
<td>0.720</td>
</tr>
</tbody>
</table>
| **a Death as a cause of prostate cancer.**
| **b Patients are still alive or died of other causes.**
| **c Due to rounding, n (%) may not be equivalent to 100.**

An increase in nuclear Snail expression correlates with high Gleason score and pathological stage

Snail expression was seen in all normal prostate tissue samples within the cytoplasm. However, tumors with poorer prognosis demonstrated dramatically increased Snail expression within the nuclei. An increase in nuclear Snail expression was significantly associated with both increased Gleason score (p = 0.009) and T stage (p = 0.001). Snail expression was significantly increased in Gleason score 7 (p = 0.016) and Gleason scores 8–10 (p = 0.003) compared to Gleason score 5–6, suggesting that this change was associated with worsening tumor grade (Fig. 2 G–I). When comparing Snail expression with T stage, there was a significant difference between T1/2 compared to T3 tumors (p = 0.001), and when comparing T3 to T4 tumors (p = 0.012; Fig. 2 J–K; Table 3). Thus, increased nuclear Snail expression was clearly associated with poorer prognosis prostate cancer. However, despite this correlation with histological parameters, Snail did not demonstrate a significant association with PSA recurrence or prostate cancer survival.

Elevated vimentin expression was associated with higher Gleason score

The normal tissue localization of Vimentin involved heavy staining in the stroma but there was a lack of staining within cells of the malignant glands. However, in a sub-set of tumors, cells of the malignant glands demonstrated elevated Vimentin expression in the cytoplasm (Fig. 3). Thus, for samples assigned a sum score of zero there was heavy stromal and non-cancerous cell staining (Fig. 3), which was observed in a total of 88.5% patient tissue samples of varying Gleason score. While 11.5% patients stained positively for the marker (elevated expression in the malignant glands), of these 60% were of Gleason score 6 or greater.

Table 2
Patient demographics in relation to age group.

<table>
<thead>
<tr>
<th>Age group</th>
<th>Median age (years)</th>
<th>Median Gleason score</th>
<th>Median initial PSA (ng/mL)</th>
<th>% Patients with PSA relapse</th>
<th>% Patients died of the disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;60</td>
<td>53.5 (Range 50–59)</td>
<td>6.5 (Range 5–10)</td>
<td>11.1 (Range 3.8–71 ng/mL)</td>
<td>7.7%</td>
<td>5.0%</td>
</tr>
<tr>
<td>60–70</td>
<td>67 (Range 60–70)</td>
<td>7 (Range 5–10)</td>
<td>19.2 (Range 1.9–1000 ng/mL)</td>
<td>48.1%</td>
<td>18.1%</td>
</tr>
<tr>
<td>71–80</td>
<td>76 (Range 71–80)</td>
<td>7 (Range 5–10)</td>
<td>22.1 (Range 4.2–930 ng/mL)</td>
<td>65.9%</td>
<td>23.5%</td>
</tr>
<tr>
<td>81+</td>
<td>85 (Range 81–9)</td>
<td>7 (Range 5–10)</td>
<td>27.8 (Range 2.7–1267 ng/mL)</td>
<td>50%</td>
<td>10.4%</td>
</tr>
</tbody>
</table>
EMT signaling cascade with a view to determining their potential for aiding the identification of prostate cancer patients likely to develop progression.

EMT is a process crucial in not only embryogenesis but also potentially in tumor progression. In order for cancer cells to metastasize from the primary tumor to a secondary site they must first undergo EMT to gain the ability to migrate (Mathias and Simpson, 2009). It is believed that Twist allows movement of tumor cells from the primary tumor site into the circulatory system. Nonetheless, in our study, Twist expression was indifferent in staining between the stroma, non-cancerous and cancerous cells and increasing Gleason scores. There is one study in prostate cancer patients that found Twist expression, while not detected in benign prostatic hyperplasia (BPH) cases, was elevated within malignant prostate cancer cases with increased staining in higher Gleason scores (Kwok et al., 2005). However, this report only analyzed a small number of patients (46 in total) using specimens from Chinese patients, thus patient demographics may explain the discrepancy between our study and the data reported by Kwok and colleagues.

Of the seven molecules analyzed, E-Cadherin was the only marker within the initial diagnostic tissue samples that was associated with patients that later died of the disease (p = 0.002). E-Cadherin role in tumorigenesis has been well documented and thus, a loss of this protein or reduced expression at the membrane of neoplastic cells is often associated with worsening histological grade and clinical stage and thus poor prognosis in a variety of cancers including prostate (Junior et al., 2010), gastric (Uchikado et al., 2011) and breast (Onder et al., 2008) adenocarcinomas. Snail is a repressor of E-Cadherin and it has been noted that an increase of Snail expression correlated with an increased risk of tumor relapse and poor survival rates within breast cancer cases (Moody et al., 2005), and with the progression of colorectal cancer (Fan et al., 2012). It has also been shown that Snail is an important and essential regulator in the up-regulation of matrix metalloproteinases (MMPs) to drive progression of cancer (Jin et al., 2010). Snail is a phosphoprotein that when phosphorylated is unable to accumulate within the nucleus and thus activate EMT (Domínguez et al., 2003). Phosphorylation regulates the subcellular location and activity of the Snail transcriptional repressor, and it has been observed within tumor cell lines that a lack of phosphorylation of Snail resulted in a loss of E-Cadherin production and an EMT-like morphological change (Zhou et al., 2004). Snail directly suppresses the expression of E-Cadherin by binding to the E-box of its promoter (Olmeda et al., 2006) resulting in the break-up of the E-Cadherin-dependent intercellular junctions (Cano et al., 2000).

Within this study, elevated nuclear Snail expression was significantly associated with increased Gleason score and more invasive tumors. There has been very little investigation into the role of Snail in prostate carcinogenesis, but one other study has also reported a correlation between increased Snail expression and more aggressive prostate cancer cases (Heeboll et al., 2009). Epithelial genes that are down regulated when Snail expression increases include desmoplakin, muc-1, cytokeratin 18, ocludins and claudins (Nieto, 2002; Olmeda et al., 2006; Thiery, 2003), and Snail will also induce the expression of mesenchymal markers such as Vimentin, fibronectin and MMPs (Nieto, 2002; Olmeda et al., 2006). Snail regulates and is responsible for altered gene expression profiles associated with EMT, and also indirectly regulates critical steps of EMT. An indirect pathway that Snail regulates involves the activation of β-Catenin (Przybyle and Radisky, 2007). Under normal conditions β-Catenin is suppressed by E-Cadherin at the membrane, but a loss of E-Cadherin expression as a result of Snail expression releases β-Catenin into the cytoplasm where it is due to undergo phosphorylation and proteosome-mediated degradation by glycoprotein synthase kinase 3 (GSK3). The activation of the canonical WNT pathway suppresses GSK3 allowing the translocation of β-Catenin into the nucleus where it is able to associate with T cell factor/Lymphocyte factor complex to alter the expression of genes (Thiery and Sleeman, 2006). Snail has also been shown to induce progression via the control of proteolytic activities of MMPs which are known to contribute to phenotypic changes seen in EMT and...
invasion (Przybilo and Radisky, 2007). MMP-1, -2, and -7 have been shown to be induced by Snail within liver and squamous cell carcinoma cell lines (Miyoshi et al., 2004) and within ovarian carcinoma a knockdown of Snail in vitro resulted in a reduction of MMP-2 mRNA levels and in vivo inhibited the catalytic activity of MMP-2, suggesting that Snail is an important and essential regulator in the up-regulation of MMPs to drive progression of cancer (Jin et al., 2010).

Vimentin is the major intermediate filament protein of mesenchymal cells and thus is highly expressed in this cell type, but absent in epithelial cells and consequently is a marker of EMT. In the present study, the majority of prostate cancer samples did not demonstrate variation in either intensity or distribution of Vimentin as compared to normal tissue. Moderate to high staining was observed in a small sub-set of patient tissues, but interestingly, of these patients, 60% were Gleason score

Fig. 2. IHC images of E-Cadherin and Snail within the clinical groupings. Complete E-Cadherin staining is illustrated in Gleason scores 5 tissue (A), followed by a progressive loss in Gleason 7 (B) and 8 (C) tissues. Progressive E-cadherin loss is also demonstrated with increasing tumor T stage: T2 (D), T3 (E) and T4 (F). Snail expression was largely cytoplasmic in lower grade prostate cancers (Gleason score 5 (G)), but was then more heavily localized in the nuclei with increased Gleason score (Gleason 7 (H) and 9 (I)); and T stage T2 (J) and T3 (K).
8 and above, with the remainder largely being Gleason 7, suggesting an association with higher grade.

There have been a few studies that have assessed the role of BMPs within prostate carcinogenesis, but a previous report has demonstrated an increased copy number of the gene loci for BMP2, BMP5 and BMP7 in prostate cancer (Doak et al., 2007). Additionally, the expression of BMP-7, when compared to normal prostate tissue, decreases in organ confined prostate cancer (Bobinac et al., 2010). Conversely, while BMP-7 expression appears to decrease with higher Gleason score, its expression has been reported to be enhanced in bone metastatic lesions derived from prostate cancer, suggesting that BMP-7 has a function in prostate cancer associated osteoblastic metastasis and osteoclastic lesions (Masuda et al., 2003). There is therefore some conflict in the literature, which may be related to study size (e.g. 42–74 patient samples; Bobinac et al., 2010; Masuda et al., 2004). The present study, involves a larger patient cohort (215 patient tissue samples) and demonstrated that the BMP-7 expression slightly increased within the higher Gleason scores. Although this change in expression pattern failed to reach significance, it did correlate with our previous findings (Doak et al., 2007), suggesting increased gene copy number for BMP-7 may be responsible for the observed increase in protein levels within this patient cohort.

Information on the treatments administered to each patient in this study was not available and is therefore a known caveat as it could potentially influence protein expression profiles observed. However, it is important to emphasize that this study focused on assessing the initial diagnostic tissue sample from each patient obtained when they were under investigation for prostate cancer. The TRUS biopsy tissue samples utilized represented the first diagnostic tissues obtained from the patient cohort, while the prostatectomy samples were obtained within three months of the patient’s initial biopsy (as in these cases the TRUS biopsy was not available) and the patient had not undergone any treatment within this time period. Thus, the resultant protein profiles observed in this study are believed to be representative of the neoplasms under investigation.

<table>
<thead>
<tr>
<th>Group</th>
<th>Sub-group</th>
<th>E-Cadherin</th>
<th>Snail</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gleason score</strong></td>
<td>5–6 vs 7</td>
<td>0.984</td>
<td>0.016*</td>
</tr>
<tr>
<td></td>
<td>7 vs 8–10</td>
<td>0.031*</td>
<td>0.644</td>
</tr>
<tr>
<td><strong>T classification</strong></td>
<td>T1/2 vs T3</td>
<td>0.420</td>
<td>0.001***</td>
</tr>
<tr>
<td></td>
<td>T3 vs T4</td>
<td>0.006**</td>
<td>0.012**</td>
</tr>
</tbody>
</table>

* p-Value ≤ 0.05.
** p-Value ≤ 0.01.
*** p-Value ≤ 0.001.

**Table 3**
Correlation between E-Cadherin and Snail expression with Gleason score or tumor T classification.

**Fig. 3.** IHC images of Vimentin, BMP-2, and -7 within the clinical groupings. Examples of prostate cancer tissue with elevated Vimentin expression (A) and basal level staining (B); increased BMP-2 staining within Gleason score 9 (C) as compared to normal levels in Gleason score 6 tissue (D) is illustrated; while positive BMP-7 staining in Gleason score 9 tissue (E) compared to the minimal expression levels observed in Gleason score 6 tissue (F) is provided.
In summary, there are limited investigations assessing the interaction between E-Cadherin and Snail in prostate cancer and their potential role in identifying patients with poor prognosis. However, in the present study, we observed a loss of E-Cadherin together with elevated nuclear Snail protein expression levels with increasing Gleason score and T stage. Nonetheless, reduced E-Cadherin protein expression was the only EMT marker that was associated with death from prostate cancer. This is the first investigation to our knowledge to associate an accumulation of Snail nuclear expression with increased tumor T stage, pointing to its potential role in the later stages of prostate cancer progression. It would therefore be pertinent to further study the underlying mechanisms of increased nuclear Snail expression and the downstream consequences in the molecular progression of prostate cancer. These potential biomarkers would not be sufficiently powerful alone in identifying patients who progress. However, together they may be utilized with a larger panel of biomarkers to enable a more detailed understanding of patient outcome, to better identify those patients who will likely progress to aggressive disease and therefore require radical treatment or earlier intervention.

Conflicts of interest statement

The authors declare that there are no conflicts of interest.

Acknowledgments

Funding for this work was kindly provided by grants to SHD from the National Institute for Social Care and Health Research (NISCHR) and the Swansea Prostate Cancer Research Fund.

References


