Overexpression of TP53 is Associated with Aggressive Prostate Cancer but does not Distinguish Disease in BRCA1 or BRCA2 Mutation Carriers from a Control Group

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Abstract: We have shown that prostate cancer occurring in men with germline BRCA1 and BRCA2 mutations is more aggressive. In an attempt to identify an associated immunohistochemical phenotype, we have studied TP53 immunostaining in prostate cancers in mutation carriers versus prostate cancers occurring in a control group of men.

There was a significantly higher expression of TP53 protein in prostate cancer with a higher Gleason score (p<0.001). Twenty four per cent of prostate cancer occurring in BRCA1/2 mutation carriers and 19% of those from controls stained positively for the TP53 protein; this difference was not significant.

Cases and controls were combined and matched for benign and malignant disease within the same individual. There were 152 men who had a sample of each within the tissue samples. Thirty one (20%) stained positively within the malignant tissue alone; none had positive staining in benign tissue, p=0.001.

Over expression of TP53 cannot distinguish prostate cancer on a background of BRCA1/2 mutation, but it is associated with prostate cancer malignant tissue per se, in particular aggressive disease.

INTRODUCTION

Prostate cancer (PrCa) is one of the most common male cancers in the UK. It has a wide spectrum of aggressiveness and so its management is controversial. Men who carry a mutation in the BRCA2 gene and to a lesser extent BRCA1 gene have an increased relative risk of PrCa, particularly of disease which occurs at a younger age of onset (< 65 years). This relative risk may be as high as 7-23 times the general population risk in BRCA2 mutation carriers and 1.8 times in BRCA1 mutation carriers by 65 years [1, 2]. In addition, men who are carriers of BRCA2 mutations have a significantly higher Gleason score, lower mean age of diagnosis, more advanced stage and shorter median survival when compared with a control group [3, 4]. Identifying those who harbour a BRCA1 or BRCA2 gene mutation would enable us to easily highlight a group of men who are more likely to develop PrCa that has an aggressive natural history. It would also enable the identification of their families as being at increased risk of other BRCA1/2 gene mutation associated cancers.

Genetic testing for BRCA1 and BRCA2 germ line mutations is expensive and time consuming because of the large size of both the genes and the low percentage of mutations in the population. Finding pathological features characteristic of PrCa specifically from BRCA1/2 mutation carriers would be of value in targeting genetic testing.

Levels of TP53 protein detected by immuno-histochemistry are increased in BRCA1 gene mutation carriers with breast cancer compared with sporadic disease. This difference remains when the mutation carriers and sporadic breast cancers are matched for grade [5, 6].

TP53 protein is one of the major regulators of the cell cycle preventing inappropriate cell proliferation and ensuring maintenance of the genome following cellular stress. Malignant tumours developing in BRCA1 mutation carriers have increased levels of TP53 protein as detected by immunohistochemistry. BRCA1 and BRCA2 proteins interact with RAD51 protein, which is involved in the recombination and repair of DNA double stranded breaks [7, 8]. A cell that lacks functional BRCA1 or 2 proteins may have a decreased ability to repair DNA damage resulting in increased genomic instability and ultimately leading to TP53 mediated cell cycle arrest and/or apoptosis. In support of this hypothesis, researchers have observed that brca1 gene deficient mice die early in embryogenesis, exhibiting reduced cellular proliferation and increased expression of the p21 tumour suppressor gene [9, 10]. In addition, brca1 deficient mice can be partially rescued from early embryonic lethality by the presence of p53 or p21 null mutations [10]. This finding suggests that loss of p53 checkpoint control may be obligatory for the malignant transformation in cells with a brca1/2 gene mutation and that it must occur prior to the ‘second hit’ in the brca1 gene, in order for the brca1 -/- (null) cells to overcome cell cycle arrest. In this case, the p53 gene would be mutated producing an abnormal protein. Loss of p53 checkpoint control in such a clonal population would enable the accumula-
tion of the further somatic genetic abnormalities necessary for tumourigenesis.

Mutant p53 protein is resistant to degradation and therefore has a prolonged half life allowing it to be detected by immuno-histochemical staining. Carcinomas in patients who carry BRCA1 gene mutations might therefore be expected to exhibit a high rate of somatic TP53 gene mutations. This may also be expected in the development of PrCa in BRCA1/2 gene mutation carriers. Indeed, TP53 gene mutations have been sequenced in BRCA1 gene mutation carriers with breast cancer [5, 11].

Protein levels of TP53 in sporadic prostate cancer have been studied by several groups in a variety of specimen types (primary tumours and metastases). These studies have revealed a relationship between raised TP53 protein levels and high tumour grade [12-17] and advanced stage [13, 14, 18, 19]. They suggest that TP53 protein expression is associated with poorer prognosis PrCa and in some cases is an independently poor prognostic factor [20]. There are no data in the literature however regarding TP53 protein levels in BRCA1/2 gene mutation carriers with PrCa. This paper presents findings on immunohistochemical staining for TP53 protein in prostate tumours from BRCA2/1 mutation carriers.

MATERIALS AND METHODS

Study Patients

Prostate tumour tissue was collected from men prior to treatment with germline mutations in BRCA1 or BRCA2 and a control group. The controls had a low probability of BRCA1/2 mutation. The samples were collected from throughout the UK. These tissues were immunohistochemically stained for TP53 protein.

The PrCa cases from men with germline BRCA1 or BRCA2 mutations were identified from four sources described below:

1. The EMBRACE Study

Men with PrCa enrolled in the Epidemiological Study of Familial Breast Cancer (EMBRACE www.srl.cam.ac.uk/genepi/embrace/embrace) had consented to the use of their prostate tissue samples for further research. The hospitals where these men had undergone prostate biopsy, prostatectomy or transurethral resection of the prostate (TURP) sent blocks/slides containing prostate tissue to AM. This material was coded anonymously with a unique study number. Where original haematoxylin and eosin slides were not sent, new ones were cut at The Institute of Cancer Research from the blocks provided. Twelve cases were obtained in this manner from England, Ireland and Scotland.

2. The IMPACT Study

IMPACT, Identification of Men with a Genetic Predisposition to Prostate Cancer: Targeted Screening in BRCA1/2 Mutation Carriers and Controls (www.impact-study.co.uk) is an international PrCa screening study for men unaffected by cancer with a known BRCA1 or BRCA2 mutation. One man who was diagnosed with PrCa was recruited from the IMPACT study.

3. A Cancer Genetics Out Patient Clinic

One individual was recruited from the Cancer Genetics outpatient clinic in the Royal Marsden Hospital NHS Foundation Trust (RMH) via the UK Genetic Prostate Cancer Study (UKGPS http://www.icr.ac.uk/research/research_sections/cancer_genetics/uk_prostate_study_group).

4. A series of Young Onset PrCas from the Institute of Cancer Research

A mixture of prostatectomies, trans-urethral resection of prostates (TURPs) and prostate biopsies mounted individually on slides were used. A series of 263 men who had prostate cancer diagnosed under the age of 55 years had previously undergone retrospective BRCA2 mutation analysis using conformational sensitive capillary electrophoresis (CSCE), which was then confirmed on sequencing. Prostate tissues from the six men found to have deleterious BRCA2 mutations were incorporated into the current study [2].

The clinical features are outlined in Table 1.

Controls were obtained from 2 sources. 52 cases were obtained from prostate tissue micro-arrays (TMAs) formed from transurethral resection of the prostate (TURPs) and prostatectomy samples from men with young onset PrCa diagnosed between the ages of 38-55 years with a median of 51 years, diagnosed between 1990 and 1998. The PSA ranged from 0.9-1422ng/ml, TNM stage from T1a to T4 (2002 classification). The majority of men presented with symptomatic disease. These men were diagnosed with PrCa from throughout the UK between the years of 1990-1998. The method of the creation of this series of patients is described elsewhere [21]. A further ninety one controls were obtained from TMAs from TURP and prostatectomy samples from men who had developed PrCa within England and were treated at the Royal Marsden NHS Foundation Trust (RMH) from 1992. Their ages ranged from 43-85 years with a median of 67 years. Written consent was obtained from the control patients via the UK Genetic Prostate Cancer Study (UKGPCS) study currently being conducted at the ICR/RMH.

Immunohistochemical Analysis for TP53

The antibody conditions were optimised by choosing a control of colonic tissue which stains well for TP53. The temperature, pH and dilution had been adjusted at the Royal Marsden NHS Foundation Trust histopathology laboratories.

Immunohistochemical staining for TP53 was performed by the Ventana Benchmark XT™ immunohistochemistry platform which uses a labelled streptavidin-biotin system. Paraffin was removed by heating, adding detergent and vortex mixing. Antigen retrieval was achieved by the addition of CC1, a tris based buffer added at 95°C, for 60 minutes. The primary antibody was a rabbit anti-p53 purified polyclonal antibody (Novocasra™ added at room temperature over a 40 minute period. The dilution was 1 in 50. The secondary antibody (anti-mouse antibody), labelled with biotin was added at room temperature over a 25 minute period. The streptavidin-peroxidase complex was added at room temperature over 25 minutes. Diaminobenzidine (DAB) was added at room temperature and a haematoxylin counter-stain...
was added before the slide was dehydrated in alcohol and cleaned in xylene.

Morphological Studies and Immuno-Histochemical Staining

The histopathology slides were cut at a thickness of 3-4 microns. Two weeks elapsed between cutting the sections of the paraffin blocks and staining them for the TP53 protein. A single histopathologist (Charles Jameson) and AM reviewed all the tissue microarray (TMA) controls and case samples. Each TMA and each conventionally prepared case was stained with TP53 and a haemotoxylin and eosin (H and E) stain. The H and E stain was taken to give an accurate representation of the Gleason score at the layer as close as possible to the section where the tissue was stained with TP53 antibody.

Each case was scored for Gleason pattern and the percentage of the tumour cells and benign cells that had stained for the TP53 antibody. Note was made of whether the nucleus or cytoplasm had stained in each specimen. For each individual there was more than one section of tumour and benign tissue. Where there was a discrepancy in Gleason score, the highest grade was taken as is undertaken in the clinical setting when diagnosing individual cases of PrCa. In cases where there was a discrepancy in the percentage of cells stained (tumour or benign tissue), an average was taken. The immuno-histochemical staining was analysed using the concept of Positive Cell Index (PCI), the proportion of positively stained tumour cells [22]. The PCI ranged from 0-95%. A sample was classified as positive for TP53 immuno-staining if more than 20% of the cells were stained. Figs. (1 and 2) show examples of TP53 protein staining in prostate tissue samples used in this study.

Statistical Analysis

Fisher’s exact test was used to compare the proportion of subjects staining positive in the carrier and control groups. The proportions with positive staining in the low/intermediate Gleason score (≤7) and high Gleason score (>7) groups were compared in the same way. To test whether there was a difference in staining between the benign and malignant tissue of the same patient a sign test was used. Differences were considered significant if the p-value was <0.05.

RESULTS

When the histopathology of each malignancy was reviewed, 3 of the cases of BRCA2 mutation carrier specimens were insufficient to provide reliable data and were omitted from the analysis. Of the controls, 135 of the 143 samples provided sufficient material for analysis.

There was a significantly higher expression of TP53 protein in PrCa with a higher Gleason score i.e. more poorly differentiated disease (p< 0.001, Fisher’s exact test). This was seen in the combined cases and controls (Table 2) and
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also in the controls alone. There was a highly significant difference between TP53 protein staining seen in malignant and benign tissue. None of the benign tissue but 20% of the malignant tissue stained for TP53 (p < 0.001 sign test). There was no significant difference between the TP53 protein staining seen in BRCA1/2 mutation carriers and controls (Table 2).

Fig. (1). PrCa Gleason 3 + 5 tissue in a BRCA1 mutation carrier; 75% of the nuclei stain with TP53 antibody.

Fig. (2). PrCa Gleason 4 + 4 tissue in a BRCA2 mutation carrier; 33% of the nuclei stain with TP53.
The Gleason pattern ranged from 3+3 to 5+5. All tumours were prostate adenocarcinomas. The PCI within the nuclei of the PrCa ranged from 0 to 75%. The cytoplasm did not stain in any of the cases. Table 1 summarises the mutation carriers’ characteristics.

The Gleason scores in the young onset PrCa controls ranged from 3+3 to 5+5. Twelve of these controls stained within the nucleus of the tumour, percentage staining ranging from 0 to 80% of the cancerous cells. None of the benign tissues stained either in the cytoplasm or the nucleus. In the 91 cases of PrCa of onset in all age ranges, the Gleason scores ranged from 3+3 to 5+5. Twelve of these controls stained for TP53 at a level greater than 5% of the cancerous cells. This was not a significant difference (p = 0.770). The latter data are not shown.

### DISCUSSION

This is the first UK series of PrCa from BRCA1/2 gene mutation carriers that has been stained for TP53 protein. These PrCa did not express significantly higher levels of nuclear TP53 protein compared with the PrCa in the control group. TP53 immuno-staining was seen in cases and controls in the nuclei of the cancerous cells but none were seen in the cytoplasm or within benign tissue. As TP53 is present in the nucleus this confirms that the optimisation and immuno-histochemical technique was appropriate.

Immu-no-histochemical staining of TP53 protein was in accordance with other reports in the literature in PrCa. The presence of positive staining for TP53 protein has been found to vary between tumours and is found in 18-25% of sporadic PrCa [17, 23, 24]. In the study reported here, twenty four per cent of the PrCa cases who were carriers of BRCA1/2 gene mutations and 18.5% of the PrCa controls (non-mutation carriers) stained for a level of TP53 expression less than 20% did not show any prognostic significance in any situation. Shurbaji et al. (1995) also found TP53 expression was an independent prognostic indicator for time to progression for PrCa but any degree of immuno-reactivity was prognostically significant, in contrast to the 20% cut-off used by the Visakorpi paper [17]. The variations in TP53 positivity between papers make them difficult to compare. A cut-off of 20% to define ‘high-intensity’ TP53 expression was used in this study. When Visakorpi (1992) used this level it was significantly associated with a poor outcome and this has been confirmed in other studies investigating PrCa and in other organ systems [26, 30]. Grignon et al. (1997) point out that studies of breast cancer and PrCa from their laboratories show that a 20% level of TP53 expression correlates with the presence of a mutation in more than 90% of cases [31, 32]. There was still no difference in this study between the BRCA1/2 cases and controls in TP53 expression at the threshold of 5%.

The BRCA1/2 mutation carriers were genotyped in this study, but not the sporadic patient cases. The number of controls was large enough to justify not testing the mutation

### Table 2. Positive Staining (Defined as >20% PCI) for TP53 Protein in Mutation Carriers and Controls

<table>
<thead>
<tr>
<th>Comparison Groups</th>
<th>Subjects</th>
<th>TP53 Positive Staining Result Numbers (%)</th>
<th>p-Value Fisher’s Exact Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mutation carriers vs controls</td>
<td>BRCA1/2 mutation carriers</td>
<td>4/17 (23.5%)</td>
<td>p = 0.52</td>
</tr>
<tr>
<td></td>
<td>Controls</td>
<td>24/135 (18.5%)</td>
<td>-</td>
</tr>
<tr>
<td>Combined cases and controls</td>
<td>Gleason &gt; 7</td>
<td>21/64 (32.8%)</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>Gleason ≤ 7</td>
<td>7/88 (7.9%)</td>
<td>-</td>
</tr>
</tbody>
</table>
status in all of them. BRCA1 and BRCA2 mutation carriers are not common in the UK, occurring in 0.12% and 0.20% of the population respectively [33]. The chance that a BRCA1/2 mutation carrier would contaminate the control group is therefore very low.

The small numbers of cases limit the power of this study. The comparison of whole tissue sections in the cases compared with TMAs in the control group is also a limitation. The uptake of the TP53 protein can be patchy and comparing differently cut sections may introduce bias in the staining of the tissue. However, further investigation of TP53 immunostaining of PrCa in BRCA1 and BRCA2 gene mutation carriers is warranted.

CONCLUSION

This is the first time immuno-histochemical staining for TP53 protein has been undertaken in a series of BRCA1/2 mutation carriers with PrCa. This series is strengthened by the fact that the mutation carriers are a heterogeneous group and do not consist of only founder mutations and this group of men is therefore representative of the mutation carriers within the population of the UK. Unlike BRCA1 gene mutation carriers with breast cancer, there is not an increased TP53 expression in PrCa tissue from BRCA1/2 mutation carriers so although TP53 has been associated with a higher Gleason score, our results indicate that this protein is not a useful immuno-histochemical marker in distinguishing PrCa in BRCA1/2 mutation carriers from non carriers in the UK population.

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ABBREVIATIONS

PrCa = Prostate cancer
RMH = Royal Marsden NHS Foundation Trust
ICR = Institute of Cancer Research

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**REFERENCES**


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