Over-expression of RAD51 occurs in aggressive prostate cancer

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ABSTRACT

Aims: This study has tested the hypothesis that, in a matched series of prostate cancers, either with or without BRCA1 or BRCA2 mutations, RAD51 protein expression is enhanced in association with BRCA mutation genotypes.

Methods and Results: RAD51 expression identified immunohistochemically was compared between prostate cancers occurring in BRCA1 or BRCA2 mutation-carriers and controls. RAD51 protein expression in the cytoplasm and nuclei of the benign tissues was significantly less than in the malignant tissues (p<0.001). In all cancers, cytoplasmic expression of RAD51 was more prevalent and associated with higher Gleason score (p < 0.05) irrespective of BRCA mutation status, than its expression in benign tissues (p < 0.001). Although nuclear staining was not observed in BRCA-associated cancers with Gleason score ≤ 7, it was significantly increased in all other groups of prostate cancers when compared to benign tissues (p < 0.001).

Conclusions: RAD51 protein is strongly expressed in high-grade prostate cancers, whether sporadic or associated with BRCA germline mutations. Distinct localisation of RAD51 between cytoplasm and nucleus, particularly in cancers of Gleason score ≤ 7, reflects distinct levels of RAD51 regulatory activity, from transcription to DNA repair. This biomarker may be of value in identifying patients requiring urgent treatment at diagnosis as well as in analysing biological mechanisms underlying aggressive phenotype of human prostate cancer.

Key words
Prostate cancer, BRCA1 and BRCA1 mutation carriers, RAD51 immunohistochemistry
INTRODUCTION

Prostate cancer is the second most common cancer to affect males worldwide. In the USA, in 2008, 186,320 new cases of prostate cancer and 28,660 deaths from this disease were reported. Equivalent figures from the United Kingdom reveal that in 2006, the last year for which complete data are available, 35,515 new cases were diagnosed and that 10,239 men died of prostate cancer in 2007. Globally, prostate cancer is currently the 5th commonest malignancy and the most common in men, with more than 679,000 new cases estimated to have occurred in 2002. Despite its ubiquity, prostate cancer exhibits a diverse spectrum of behaviour so that management of the disease is controversial. Unlike some human malignancies, the aetiology of prostate cancer does not appear to be associated with a specific genetic susceptibility but with multiple gene loci each independently conferring a low but cumulative risk. Within the overall male population, men who carry a mutation in the BRCA2 gene, and to a lesser extent in the BRCA1 gene, have an increased risk of prostate cancer, particularly of the disease occurring below the age of 65 years. Prostate cancers containing BRCA1 or BRCA2 germine mutations are more aggressive than morphologically identical sporadic cancers. For BRCA2 mutation carriers, this relative risk of developing prostate cancer may be from 7 - 23 times that in the general population while in BRCA1 mutation-carriers the risk of prostate cancer is 1.8 times. Previously, it has been shown that prostate cancers in carriers of BRCA2 mutations have a significantly higher Gleason score, lower mean age at diagnosis, more advanced stage and shorter median survival when compared with a control group. Thus, identifying men who harbour a BRCA2 or BRCA1 gene mutation might define a population likely to develop aggressive prostate cancer, in addition to identifying close family members at increased risk of other malignancies such as breast and ovarian cancer also associated with a BRCA1 or BRCA2 gene mutation. However, genetic testing for BRCA1 and BRCA2 germ line mutations is expensive and time-consuming since both genes are large and there is a low percentage of mutations in the general population. Therefore, finding pathological features using immunohistochemical analysis characteristic of prostate cancer specific for BRCA1 or BRCA2 mutation-carriers could suggest or exclude a phenotype that would be of value in targeting genetic testing.

RAD51 is an evolutionarily conserved enzyme encoded by the RAD51 gene located on human chromosome 15q15.1 and plays a critical role in homologous recombination (HR) repair of double-strand DNA breaks (DSBs). RAD51 protein co-localises with BRCA2 protein in nuclear foci (discrete sub-nuclear structures) in mitotic cells. These foci are observed to contain BRCA1 together with the BRCA1 binding protein BARD1, both before and after DNA damage. RAD51 foci appear during S-phase and are required to initiate stalled or broken DNA replication forks. The RAD51 recombinase directly associates with BRCA2, an essential interaction for normal recombination and genome stability. Interaction between BRCA2 and RAD51 is essential for error-free HR to take place in response to DSBs. While BRCA2 is directly involved in RAD51-mediated repair, BRCA1 acts upstream from these pathways. BRCA1 is thought to be required for the transport of RAD51 from the cytoplasm into the nucleus and to the sites of DNA damage. There, BRCA2 contains nuclear localisation signals not found in RAD51, supporting the notion that BRCA2 also facilitates RAD51 transport into the nucleus. However, the direct interaction of RAD51 with BRCA1 is not yet fully
elucidated although gene expression profiling and network modelling have revealed a complex heterogeneity in the mechanisms of BRCA1 involvement in tumorigenesis \(^\text{17}\).

Since RAD51 protein normally co-localises with, and regulates, BRCA2 in a complex with BRCA1, we have compared the expression of RAD51 protein in prostate cancers from \textit{BRCA1} and \textit{BRCA2} gene mutation-carriers with a control group of sporadic prostate cancers. The objective of this study was to test the hypothesis that enhanced expression of RAD51 protein is particularly associated with \textit{BRCA1/2} mutation-carriers or with the aggressive phenotype of sporadic prostate cancer.
MATERIALS AND METHODS

Study patients
Prostate tumour tissues were collected from 20 men referred throughout the UK with germline mutations in \textit{BRCA1} or \textit{BRCA2} and a control group of men who had a low probability of mutation prior to treatment from throughout the UK. These tissues were immunohistochemical histochemically stained for RAD51 protein.

The prostate cancer cases from men with germline \textit{BRCA1} or \textit{BRCA2} mutations were identified from four sources (described below) and summarised in Table 1:

1. The EMBRACE study
Men with prostate cancer 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 haemotoxylin 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 \textit{BRCA1/2} Mutation-carriers and Controls, http://impact-study.co.uk) is an international Prostate cancer screening study for men unaffected by cancer with a known \textit{BRCA1} or \textit{BRCA2} mutation (and therefore believed to be at increased risk of developing Prostate cancer). One man who was diagnosed with prostate cancer was recruited from the IMPACT study.

3. Cancer Genetics Outpatient Clinic
One individual was recruited from the Cancer Genetics outpatient clinic in the Royal Marsden Hospital NHS Foundation Trust (RMH).

4. Young Onset Prostate Cancers
From a study at The Institute of Cancer Research, (www.icr.ac.uk/research/research_sections/cancer_genetics/uk_prostate_study_group), a combination of prostatectomies, trans-urethral resection of prostates (TURPs) and prostate biopsies individually mounted on slides were used. A series of 263 men who had prostate cancer diagnosed under the age of 55 years had previously undergone retrospective \textit{BRCA2} mutation analysis using conformational sensitive capillary electrophoresis (CSCE) which was confirmed on sequencing. Prostate tissues from the six men found to have deleterious \textit{BRCA2} mutations were incorporated into the current study.\(^8\)
A summary of the characteristics of the BRCA1 and BRCA2 germline mutation-carriers is presented in Table 2.

The control group of prostate cancers was obtained from two tissue microarray (TMA) series. One series originated from young age of onset prostate cancers diagnosed in men between the ages of 38-55 years with a median of 51 years. The PSA ranged from 0.9-1422ng/ml, TNM stage from T1a -T4 to M1, with all stages in between. The method of the creation of this series of patients is described elsewhere 18. The second series came from men who had developed prostate cancer 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 group patients via the UK Genetic Prostate Cancer Study (UKGPCS) currently being conducted at The Institute of Cancer Research/ The Royal Marsden NHS Foundation Trust. The two TMA series provided 119 control samples that were sufficient to produce reliable data.

Morphological assessment
Formalin-fixed and paraffin wax embedded tissue sections were cut at 4µm and taken to water through xylene and graded alcohols. Following H&E staining, all tissues were reviewed independently by CJ and CSF to confirm the diagnosis and to ensure consistency in morphological assessment. Each case was graded using conventional Gleason criteria 19.

Immunohistochemical analysis
Antibody conditions were optimised by staining control tissues, benign tonsillar tissue and infiltrating ductal breast carcinoma with a mouse monoclonal antibody to RAD51. The temperature, pH and dilution had been adjusted to produce the best control staining at the Centro Nacional de Investigaciones, Oncologicas laboratories. Immunohistochemical staining was performed using the EnVision system (DAKO, Copenhagen, Denmark) with a heat-induced, antigen retrieval step. Formalin fixed and paraffin wax-embedded tissues were dewaxed in xylene, graded alcohols and water. Antigen retrieval was performed with the addition of EDTA (1mM at pH 8) and 2 minutes of pressure cooking 20. The primary antibody (51RAD01-Neomarkers™) diluted 1 : 25 was incubated on the tissue sections for 40 minutes. After washing, the secondary antibody conjugated to horseradish peroxidase (Dako™) was incubated over 25 minutes. Staining was revealed by incubation of sections with 3-3'-diaminobenzidine (DAB) for 10 minutes before washing, taken through graded alcohols, clearing in xylene and counter-staining with haematoxylin before mounting in DPX.

Immunohistochemical assessment
Assessment was made of the percentage and intensity of cells stained within the nucleus and the cytoplasm of the malignant cells and of the benign cells. Immunohistochemical staining was analysed for the proportion of positively stained tumour cells using the concept of Positive Cell Index (PCI) 21. The PCI ranged from 5-90%. A sample was classified as positive if more than 10% of the cells (malignant or benign) were stained with the RAD51 antibody with any intensity (greater than 0). The 10% cut-off enabled us to easily compare the staining levels in prostate cancer from BRCA1/2
mutation-carriers versus non-carriers’ prostate cancer. The 10% cut-off is identical to that used by other groups.\textsuperscript{22, 23}

Controls
For the immunohistochemistry, tonsil control specimens confirmed nuclear RAD51 protein staining in >80% of the lymphocytes and cytoplasmic staining in >75% of the squamous epithelium. In the breast cancer control tissues, nuclear staining occurred in >75% of the cells and cytoplasmic staining in >75%.

Only the \textit{BRCA1} or \textit{BRCA2} mutation-carriers were genotyped in this study, but not the sporadic cases of prostate cancer. In this series, the number of controls was large enough to justify not testing the mutation status of all of them. \textit{BRCA1} and \textit{BRCA2} mutation-carriers are not common in the UK, occurring in only 0.12% and 0.20% of the population respectively.\textsuperscript{24} Therefore, the chance that a mutation carrier would contaminate the control group is extremely low.

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 Gleason (score $\leq 7$) 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 $p < 0.05$. 

\textit{RAD51 protein expression of prostate cancer in BRCA1 & 2 mutation carriers}
RESULTS

Prostate cancer tumour samples were assembled from 20 BRCA1 or BRCA2 germline mutation carriers. The location of each of the mutations is recorded in Table 2. When the histopathology of each malignancy was reviewed, three of the BRCA2 mutation carrier specimens were insufficient to provide reliable data and were omitted from further analysis. 119 control group samples were analysed. Immunohistochemical staining of the breast and tonsil controls confirmed both the nuclear and cytoplasmic RAD51 protein to be demonstrated adequately and hence the technique to be robust. Figure 1 shows the RAD51 protein staining in a range of prostate cancers occurring in patients with BRCA1/2 germ-line mutations.

The data comparing the expression of RAD51 in the BRCA1/2-associated cancers and the controls with Gleason scores are summarised in Table 3. A total of 136 (17 + 119) cases of prostate cancer were examined. Immunohistochemical staining scores for RAD51 protein were significantly higher in both the cytoplasm and the nuclei of the prostate cancers than in the benign prostatic tissues. Of the benign tissues, only one (0.74%) of the combined cases and control samples contained cytoplasmic staining when compared with 44 (32.5%) cases in the malignant tissue (sign test, p < 0.001). Only one (0.74%) of the benign tissue samples stained for RAD51 within the nuclei when compared with 18 (13.3%) of the malignant areas (sign test, p<0.001). Nuclear expression of RAD51 protein was not identified in the BRCA1/2 group of prostate cancers with Gleason score ≤ 7. However, in the controls alone and in the sum of the controls and BRCA1/2 mutation carrier cases (Table 4), there was a significant difference in cytoplasmic staining of RAD51 between prostate cancers with a low and a high Gleason pattern (≤ 7 vs. 8, 9 and 10). Cancers with high Gleason scores expressed significantly more staining than those with low Gleason scores. In the sum of cases and controls, RAD51 immunohistochemical staining was significantly higher in the cytoplasm of those with Gleason score >7 (Fisher’s exact test p=0.017).

When the control data alone, the BRCA2 mutation-carriers alone and the BRCA1/2 mutation-carriers were analysed together, a significant difference was found between the cytoplasmic and nuclear immunohistochemical staining in the controls. Cytoplasmic staining was significantly higher than the nuclear (p = 0.003, sign test). This finding was not seen in the BRCA2 mutation-carriers alone (p = 0.289, sign test) or in the combined BRCA1/2 mutation-carriers (p = 0.109). No other significant differences were seen in the expression of RAD51 protein between BRCA1/2 mutation-carriers and the control group.
RAD51 protein expression of prostate cancer in BRCA1 & 2 mutation carriers

**DISCUSSION**

This is the first report of RAD51 protein expression in prostate cancer specimens, including prostate tissues from BRCA1/2 mutation carriers. This study demonstrates that prostate cancers express RAD51 protein more frequently than benign prostatic tissues (p < 0.001) and that expression is particularly enhanced in aggressive prostate cancers (Gleason > 7). Within the total cohort of prostate cancers, a significant difference between the BRCA1/2 group and the controls was the absence of RAD51 expression from the nuclei of the better-differentiated (Gleason ≤ 7) prostate cancers. No significant difference in this feature was seen in the control group.

Previously, immunohistochemical expression of RAD51 protein has been reported in other human cancers. Its presence correlated with decreased survival in head and neck cancers, in non-small cell lung cancers and with poorly differentiated breast cancer. In a study to detect BRCA1 and RAD51 proteins in the same breast cancer tissues, the less differentiated the cancer, the lower the level of BRCA1 protein expression. In these specimens, RAD51 protein was simultaneously expressed at a higher level than in the poorly differentiated tumours. Although no correlation was identified between RAD51 and BRCA1 expression, loss of BRCA1 and increased expression of RAD51 appeared to confer an advantage in favour of tumour cell survival during cancer progression. In our recent study, prostate cancers occurring in BRCA1/2 mutation-carriers comprised a poorly differentiated cell type and were more aggressive, supporting an observation of decreased survival in BRCA2 founder mutation-carriers with prostate cancer.

At the outset of this study, it was hypothesised that RAD51 expression and/or localisation would be different in the BRCA1/2 mutation-carriers when compared with non-carrier controls and further that RAD51 expression levels should be higher in poorly differentiated prostate cancers. Our current experimental evidence supports both of these hypotheses. A significant difference (p < 0.001) was seen in nuclear RAD51 protein expression between the mutation-carrying prostate cancers and controls as well as between prostate cancers with low and high Gleason scores (< 7 vs >7). In the total patient group, the differences in cytoplasmic expression were significant and were maintained when the statistical analyses were repeated for Gleason scores ≤ 6 vs > 6 to compare well-differentiated prostate cancers. Since RAD51 is a DNA repair protein, its action was originally expected to occur only within the nucleus of a cell. In sporadic prostate cancers, nuclear expression of RAD51 was detected in only 13% of cases. In BRCA1/2 mutation carriers, nuclear staining was identified in 18% of prostate cancers. However, when the cases and controls were examined together, cytoplasmic expression of RAD51 was significantly greater in those with Gleason score >7 (Fisher’s exact test p=0.017) and cytoplasmic staining was significantly higher than nuclear (p = 0.003, sign test). These are important observations since RAD51 is a dynamic protein that translocates between cytoplasmic and nuclear compartments where it participates in distinct regulatory processes. Although identified in the nucleus and involved in the repair of DNA double-strand breaks by homologous recombination, RAD51 also transits the cytoplasm where it associates with extramitochondrial DNA, undergoes differential phosphorylation at Thr-309 and Tyr-315 that regulate...
binding to transcription factors \(^{36}\) and finally undergoes degradation via the ubiquitin-mediated proteasome pathway \(^{37}\). In the cytoplasm, RAD51 competes with other proteins for binding to a highly promiscuous site on p53, thus regulating the latter’s activity \(^{38}\) while squelching the associated network of transcription factors \(^{39}\). Recently, we showed that elevated expression of p53 is a characteristic feature of the aggressive phenotype of prostate cancer \(^{40},^{41}\) and hence available to modulation by RAD51. While p53 mutations are very common in many human malignant diseases \(^{42}\), they are less common in primary prostate cancer, their frequency ranging from 3\% \(^{43}\) to 42\% \(^{44}\). Thus, while activated RAD51 is effective in the nucleus, much of its regulation and interaction with transcription factors occurs in the cytoplasm.

A striking finding of this study was that very few benign tissues exhibited RAD51 protein expression. This is in-keeping with observations in pancreatic adenocarcinoma \(^{26}\) where no mutations were identified in the RAD51 gene after sequencing 13 pancreatic cell lines and 12 pancreatic cancer specimens, suggesting the RAD51 protein identified by immunohistochemistry to be the wild-type. It is possible that RAD51 protein is only expressed in the S+G\(_2\) phase of the cell cycle and hence forms only a small percentage of the cells found in the wax-embedded tissues. Alternatively, the DNA repair complex may not be activated in benign prostatic tissues.

Comparison of RAD51 protein expression within the cytoplasm and nuclei of breast cancers occurring in BRCA2 gene mutation-carriers revealed RAD51 levels to be selectively elevated in the cytoplasm, supporting the hypothesis that nuclear levels would be low because the mutated BRCA-2 protein might not transfer the RAD51 protein into the nucleus of the cell \(^{45}\). Our examination of the nuclear and cytoplasmic localisation of RAD51 protein in prostate cancers of BRCA1/2 mutation-carriers found a significant difference in nuclear expression in the carcinomas of Gleason score \(\leq\) 7. One of the characteristic properties of BRCA2 gene-defective cell lines is their inability to form RAD51 foci after DNA damaging treatments \(^{46},^{47}\). In this instance, RAD51 protein levels might be expected to be less in the mutation-carriers when compared with the sporadic cases of prostate cancer. Unfortunately, prostate cancers containing a BRCA1 mutation are relatively infrequent. Therefore, the number of BRCA1 gene mutation-carriers in this series is too small to comment on this group alone. However, the mutated protein produced may only exhibit defective function when the involved tissue is exposed to a DNA-damaging agent such as ionising radiation.

In summary, this is the first series in which RAD51 protein has been assessed immunohistochemically in the human prostate. Expression of RAD51 protein clearly distinguishes between benign and malignant prostatic tissue (p < 0.001). However, RAD51 expression did not differentiate prostate cancers occurring in BRCA1/2 mutation-carriers from control sporadic prostate cancers. While enhanced cytoplasmic and nuclear expression of RAD51 protein was identified in all poorly differentiated prostate cancers, irrespective of BRCA1/2 mutation status, lack of nuclear RAD51 expression in BRCA1/2 cancers of Gleason score \(\leq\) 7 was significant, suggesting this group may be biologically distinct from that in which RAD51 is expressed within the cytoplasm. Contemporary reports provide compelling evidence that networks interacting at the RNA level are biologically
important in the regulation of prostate cancer phenotypes\textsuperscript{48}. To strengthen our current studies, high-throughput scanning and semi-automated scoring would by making the data more objective. Although we are not able to perform such analyses at this time, RAD51 is a potential biomarker involved in repairing multiple DS breaks in DNA. However, following RAD51 over-expression, fidelity of the original structures is not maintained, resulting in genome instability, aneuploidy and multiple chromosomal rearrangements\textsuperscript{49}. Thus, in common with mutations in mismatch repair genes\textsuperscript{50} elevated expression of RAD51 might have diagnostic value in identifying patients requiring urgent treatment as well as in analysing the biological basis of the aggressive phenotypes of human prostate cancer.

ACKNOWLEDGEMENTS

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Figure 1

[A] BRCA2: In-situ and invasive prostatic carcinoma in which the in-situ components express high levels of nuclear RAD51 staining (++).

[B] BRCA1: Invasive prostatic carcinoma expressing high levels of nuclear RAD51 in the nuclei of invasive carcinoma cells. The in situ component is predominantly unstained or the cells are expressing RAD51 within the cytoplasm (+).

[C] BRCA2: Nests of poorly-differentiated prostatic carcinoma (Gleason 4+4) carcinoma expressing nuclear RAD51 (+++) in approximately 40% of nuclei.

[D] BRCA2: Poorly-differentiated (Gleason 5+5) invasive prostatic adenocarcinoma expressing RAD51 (++) within the nuclei of approximately 40% of nuclei in this field. Low levels of cytoplasmic staining are also identified.

[E] BRCA2: Nests and cords of poorly-differentiated (Gleason 5+5) prostatic carcinoma expressing high levels of RAD51 (+++) in the cytoplasm of the invasive carcinoma cells. No nuclear staining is identified.

[F] BRCA1: Cribriforming prostatic adenocarcinoma (Gleason 4+4) expressing moderate levels of RAD51 (++) within the cytoplasm of the malignant cells. No nuclear staining is evident.
REFERENCES

21. Remmele W, Stegner HE. [Recommendation for uniform definition of an immunoreactive score (IRS) for immunohistochemical estrogen receptor detection (ER-ICA) in breast cancer tissue]. Der Pathologe 1987;8;138-140.


Table 1:

<table>
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<th>Age (years)</th>
<th>PSA (ng/ml)</th>
<th>TNM stage</th>
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<td>2004</td>
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<td>107</td>
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<td>2004</td>
<td>Unknown</td>
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<td>Screened</td>
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<td>Negative bone scan, clinically localised</td>
<td>1998</td>
<td>Screened</td>
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<tr>
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<td>4.5</td>
<td>M1- ext iliac lymph node</td>
<td>2004</td>
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<tr>
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<td>4.7</td>
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<td>Symptomatic</td>
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<td>2006</td>
<td>Screened</td>
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Clinical features of the series of BRCA1/2 mutation carriers whose prostate tissue was stained for RAD51 expression.
### Table 2:

<table>
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<th>Mutation Status</th>
<th>Mutation</th>
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<tr>
<td>BRCA1</td>
<td>c.3756_3759delGTCT</td>
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<td>BRCA1</td>
<td>c.1175_1214del40</td>
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<td>BRCA1</td>
<td>c.68_69delAG</td>
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<td>BRCA1</td>
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<tr>
<td>BRCA2</td>
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<td>BRCA2</td>
<td>c.5682C&gt;G</td>
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<td>BRCA2</td>
<td>c.7543dupA</td>
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<tr>
<td>BRCA2</td>
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<td>BRCA2</td>
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<td>c.8167G&gt;C</td>
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<td>BRCA2</td>
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Genotype of the **BRCA1** and **BRCA2** mutation carriers
Table 3:

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<th>BRCA1/2 cases (combined)</th>
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<td>Gleason ≤ 7</td>
<td>0/6 (0)</td>
<td>3/6 (50%)</td>
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<tr>
<td>Gleason &gt; 7</td>
<td>3/11 (27%)</td>
<td>6/11 (55%)</td>
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<tr>
<td>p-value</td>
<td>0.515</td>
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Nuclear and cytoplasmic distribution of RAD51 in prostate cancers with respect to Gleason score
Table 4:

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<th>Cellular distribution</th>
<th>BRCA2 carriers</th>
<th>Controls</th>
<th>p-value*</th>
<th>BRCA1/2 carriers</th>
<th>Controls</th>
<th>p-value*</th>
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</thead>
<tbody>
<tr>
<td>Nuclear</td>
<td>3/13 (23%)</td>
<td>15/119 (13%)</td>
<td>p = 0.386</td>
<td>3/17 (18%)</td>
<td>15/119 (13%)</td>
<td>p = 0.700</td>
</tr>
<tr>
<td>Cytoplasm</td>
<td>7/13 (54%)</td>
<td>36 /119 (30%)</td>
<td>p = 0.118</td>
<td>9/17 (53%)</td>
<td>36/119 (30%)</td>
<td>p = 0.096</td>
</tr>
</tbody>
</table>

* The p-values refer to the difference between the controls and cases

RAD51 protein immunohistochemical staining of prostate cancers in the BRCA2 mutation carriers alone and in BRCA1/2 carriers compared with controls