Elevated expression of Ki-67 identifies aggressive prostate cancers but does not distinguish *BRCA1* or *BRCA2* mutation carriers

A.V. MITRA[1], C. JAMESON[2], Y. BARBACHANO[2], N. SODHA[1], Z. KOTE-JARAI[1], A. JAVED[2], E. BANCROFT[2], A. FLETCHER[1], C. COOPER[1], S. PEOCK[3], THE IMPACT and EMBRACE COLLABORATORS[**], D. EASTON[3], R. EELES[1,2*] and C.S. FOSTER[4*]

[1]Translational Cancer Genetics Team, The Institute of Cancer Research, Surrey; [2]The Royal Marsden, NHS, Foundation Trust, London; [3]Strangeways Research Laboratories, Cambridge; [4]Division of Cellular Pathology and Molecular Genetics, School of Cancer Studies, University of Liverpool, Liverpool, UK

Received September 17, 2009; Accepted October 20, 2009

DOI: 10.3892/or_00000636

Abstract. Prostate cancers in men with germline *BRCA1* and *BRCA2* mutations are more aggressive than morphologically similar cancers in men without these mutations. This study was performed to test the hypothesis that enhanced expression of Ki-67, as a surrogate of cell proliferation, is a characteristic feature of prostate cancers occurring in *BRCA1* or *BRCA2* mutation carriers. The study cohort comprised 20 cases of prostate cancer in mutation carriers and 126 control sporadic prostate cancers. Of the combined sample cohort, 65.7% stained only within malignant tissues while 0.7% stained in both malignant and benign tissues ($p<0.001$). Significantly increased expression of Ki-67 occurred in prostate cancers with higher Gleason score ($p<0.001$). Elevated Ki-67 expression was identified in 71% of prostate cancers in *BRCA1* or *BRCA2* mutation carriers and in 67% of the sporadic controls ($p>0.5$). Similar results were obtained when the data were analysed using a threshold set at 3.5 and 7.1%. This study shows that elevated expression of Ki-67 is associated both with aggressive prostate cancers and with high Gleason score irrespective of whether their occurrence is against a background of *BRCA1* or *BRCA2* mutations or as sporadic disease. The data suggest that, since elevated Ki-67 does not distinguish prostate cancers occurring in *BRCA1* or *BRCA2* mutation carriers from sporadic prostatic malignancies, the effects of these genetic mutations are probably independent. While all prostate cancers occurring in the presence of BRCA germline mutations are clinically aggressive, their potentially different phenotypes consistently involve maximal rates of cell proliferation.

Correspondence to: Professor Christopher S. Foster, Division of Cellular Pathology and Molecular Genetics, School of Cancer Studies, University of Liverpool, Sixth Floor, Duncan building, Daulby Street, Liverpool L69 3GA, UK
E-mail: csfoster@liv.ac.uk

[*]Joint senior authors
[**]List available on request

Key words: BRCA germline mutations, aggressive prostate cancer, cell proliferation

Introduction

We recently reported that prostate cancers occurring in *BRCA1* and *BRCA2* mutation carriers are aggressive and carry a worse prognosis than morphologically similar sporadic prostate cancers (1). There is controversy as to whether *BRCA1* mutations are associated with an increased incidence of prostate cancer since no mutations are found in some series (2). Although meta-analyses suggest an increased risk before the age of 65 years, for *BRCA2* there is greater evidence of an association; germline mutations in *BRCA2* being present in some 2% of men with early-onset prostate cancer (3). Mutations in *BRCA2* confer a significantly worse clinical prognosis (4,5). Hitherto, the molecular genetic basis of the aggressive phenotype promoted by *BRCA1* and *BRCA2* mutations has not been elucidated. However, in sporadic prostate cancers, elevated cell proliferation identified by increased expression of Ki-67 protein is associated with a more malignant phenotype and higher Gleason grade (6-8). Morphologically, there is no pathognomonic feature that distinguishes BRCA mutation-related prostate cancers (9). Since both genes are large and exhibit a low incidence of mutation carriers in the general population (10) genetic testing for *BRCA1* and *BRCA2* germline mutations is currently expensive and time-consuming. Thus, identification of phenotypic features that reliably characterise prostate cancers arising in *BRCA1* or *BRCA2* mutation carriers would be of value in targeting genetic testing to those men and possibly of developing biologically specific therapies (4).

Ki-67 is a large and highly basic protein (3256 amino acids, 358.7 kDa and pI 9.49) encoded by the gene *MKI-67* on human chromosome 10q25. Throughout evolution, the gene is highly conserved and, despite its size, contains surprisingly few splice variants. The gene contains many nuclear localisation sequences while the protein contains multiple sites of potential phosphorylation. Despite detailed knowledge of the sequence and structure of Ki-67, it exhibits minimal homology to other known proteins and little is known about its specific function or mode of action. However, the protein

is intimately involved in cell proliferation (11) through binding to chromobox homology proteins thus regulating chromatin assembly (12). Its activity is modulated by cycles of phosphorylation/de-phosphorylation during cell division (13,14). Regulated by kinase and proteolytic pathways, Ki-67 is expressed in proliferating cells during G_1 and increases through S and G_2, peaking at the M phase of the cell cycle before being degraded to become undetectable at G_0 and the beginning of the G_1 phase (11,15,16). The half-life of Ki-67 is tightly regulated at between 60-90 min, thus controlling the amount of the protein present within a cell (13,17).

Using the MIB-1 antibody to detect Ki-67 protein in histopathological tissues, we have previously analysed the relationship between tumour cell proliferation and the behaviour of individual prostate cancers (6-8). Other studies have investigated the expression of Ki-67 in a variety of malignancies and have confirmed a relationship with tumour aggression. Unfortunately, despite the frequency of its use in analysing histopathological tissues, interpretation of Ki-67 immunohistochemistry remains hampered by a lack of reliable systems to quantify signal intensity. To address this problem Philip *et al* employed digital image analysis to count the number of malignant cells staining in a unit area of prostate cancer, thus providing objective quantification of Ki-67 expression and showed a high correlation with Gleason grade (18). Previously, Pollack *et al* reported Ki-67 protein expression in prostate cancers treated in the RTOG 92-02 study and compared two cut-off thresholds (>3.5 and >7.1%) for positivity (19). Since this current study employs conventional immunohistochemistry interpreted visually, the same criteria, including these two thresholds for positivity, have been applied to these data.

Despite the aggressive nature of prostate cancers arising in *BRCA1* or *BRCA2* mutation carriers, Ki-67 expression has not been previously reported in these malignancies. Furthermore, it was considered that increased tumour cell proliferation might enhance the aggressiveness of prostate cancers occurring in poor prognosis *BRCA1* or *BRCA2* carriers. This is the first study to report expression of Ki-67 protein in a series of well-characterised prostate cancers arising in *BRCA1* or *BRCA2* mutation carriers. The study was performed to test the hypothesis that prostate cancers occurring in association with *BRCA1* or *BRCA2* mutations exhibit increased cell proliferation, identified by elevated levels of Ki-67, and that these levels are likely to be significantly higher than those occurring in a control group of morphologically similar sporadic prostate cancers without BRCA mutations. Thus, elevated Ki-67 expression in prostate cancer might be part of the phenotypic profile that identifies men likely to have germline *BRCA1* or *BRCA2* mutations.

Materials and methods

Study patients. Prostate tumour tissues were originally collected from 20 men referred throughout the UK with germline mutations in *BRCA1* or *BRCA2* (see below) and a control group of 126 men from throughout the UK who had sporadic prostate cancer with a low probability of *BRCA* mutation and who had not received any treatment. After morphological assessment, these tissues were stained immunohistochemically for Ki-67 protein expression. The prostate cancer cases from men with germline *BRCA1* or *BRCA2* mutations were identified from four sources described below:

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 H&E stained slides were not sent, new ones were cut at The Institute of Cancer Research from the blocks provided. Twelve cases were obtained 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, http://impact-study.co.uk) is an international prostate cancer screening study for men unaffected by cancer but with a known *BRCA1* or *BRCA2* germline mutation (and therefore believed to be at increased risk of developing prostate cancer). One man 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 at 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 *BRCA2* mutation analysis using conformational sensitive capillary electrophoresis (CSCE) which was confirmed on sequencing. Prostate tissues from the six men found to have deleterious *BRCA2* mutations were incorporated into the current study.

The summary of the characteristics of the germline *BRCA1* and *BRCA2* mutation carriers are shown in Table I. The control group prostate cancers were obtained from two TMA series. One series originated from young age of onset prostate cancers diagnosed in men between the ages of 38-55 years (median 51 years) as previously described (20). The PSA ranged from 0.9-1422 ng/ml, TNM stage ranged from T1a to M1. The second series was derived from men who had developed prostate cancer within England and were treated at the Royal Marsden NHS Foundation Trust 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 ICR/RMH. The 2 TMA series provided 126 control samples that were sufficient to produce reliable data.

Table I. Clinical characteristics and staining of Ki-67 in the *BRCA1/2* mutation carriers and controls.

	Mutation carriers	Control group 1	Control group 2
Number successfully analysed	17	41	85
Age range (years)	44-70	38-55	43-85
Median age	53	51	67
PSA (ng/ml)	<1-227	0.9-1422	Unknown
Year of presentation	1971-2006	1990-1998	1992-2002
Stage (AJCC 2002)	T1a-T4-M1	T1a-T4-M1	T1a-T4-M1
Ki-67 % staining (PCI)	1-60%	0-67.5%	0-77%
Mutations	*BRCA1* c.68_69delAG (n=2) c.3756_3759delGTCT c.1175_1214del40 *BRCA2* c.6486_6489delACAA c.6859_6863delAGAAA c.5946delT c.5682C>G c.7543dupA c.6275_6276delTT (n=2) c.3158T>G (n=2) c.2330dupA c.7545dupA c.7977-1G>C c.5303_5304delTT c.8167G>C c.8297delC c.6591_6592delTG		

Morphological assessment. Formalin-fixed and paraffin wax embedded tissue sections were cut at 4 μm and taken to water through xylene and graded alcohols. Following H&E staining, all tissues were reviewed independently by CJ to confirm the diagnosis and to ensure consistency in morphological assessment. Each case was independently graded (CSF) using conventional Gleason criteria (21,22).

Immunohistochemical analysis. Conditions (dilution, temperature and pH) for staining with the primary antibody were optimised by staining benign tissue from an appendix at the Histopathology Laboratories at the Royal Marsden NHS Foundation Trust (RMH). Immunohistochemical staining for Ki-67 was the fully-automated Ventana Benchmark XT™ immunohistochemistry platform which uses a labelled streptavidin-biotin system. Formalin fixed and paraffin-embedded tissues were dewaxed in xylene and taken to water through graded alcohols. Antigen retrieval was achieved by addition of CC1 (Ventana), a Tris-based buffer (pH 8.6). The CC1 buffer was added at 95˚C, for 30 min. The mouse primary monoclonal antibody (MIB-1 dilution 1 in 100, Dako Cytomation™, Cambridge, UK) was incubated for 40 min at room temperature. After washing in Tris buffered saline (pH 7.6) the slides were incubated with the secondary antibody (biotin-labelled rabbit anti-mouse) for 25 min at room temperature. A streptavidin-peroxidase complex was added for a further 25 min at room temperature. All steps in the staining process were performed automatically on a Benchmark XT platform, driven by a barcode containing all the protocol information affixed to each of the slides. On completion of staining, all sections were incubated with 3,3'-diaminobenzidine (DAB) in two stages, each taking 2.5 min at room temperature, before being clearing in xylene, counterstained with haematoxylin and mounted in DPX.

Immunohistochemical assessment. The percentage of nuclei in the malignant and benign components of each of the tissues staining with Ki-67 was counted. For each patient, more than one section of tumour and benign tissue was available. Where there was a discrepancy in Gleason score, the highest grade was used, as is undertaken in the clinical setting when diagnosing individual cases of prostate cancer. In those cases

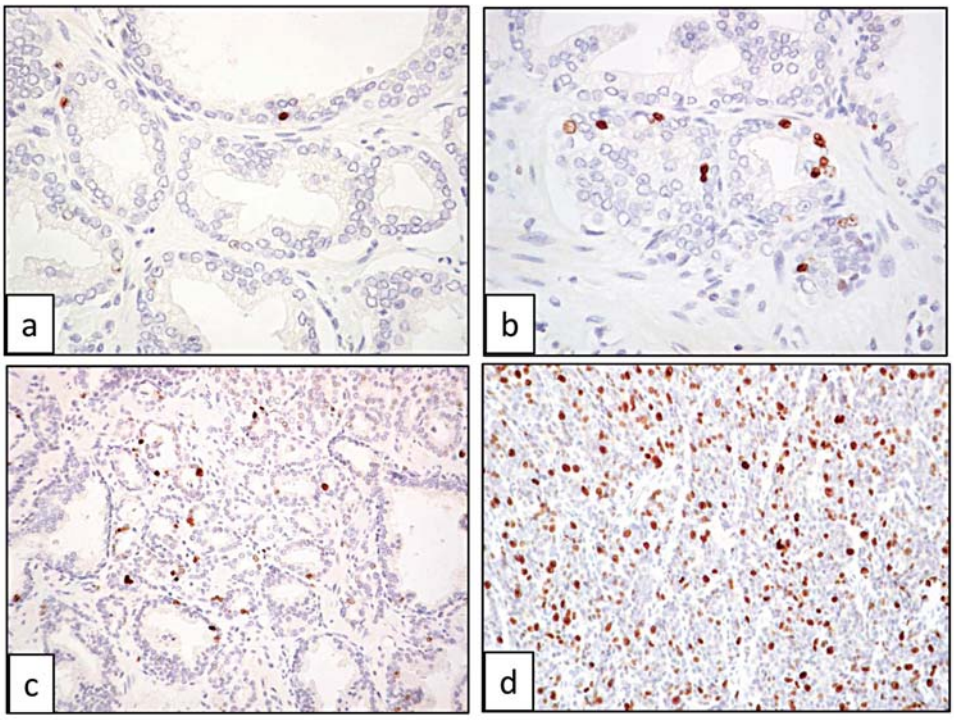

Figure 1. (a) The basal level of Ki-67 expression is low in the non-malignant prostatic epithelium of patients with sporadic prostate cancer not associated with BRCA genetic mutations (x400). (b) The basal level of Ki-67 expression in the non-malignant prostatic epithelium of patients with BRCA germline mutations is elevated in comparison to the equivalent prostatic glands of patients with sporadic prostate cancer (BRCA1, x400). (c) Ki-67 expression moderately elevated in sporadic prostate cancer (Gleason 3+3). The adjacent non-malignant prostatic glandular epithelium contains few cells expressing Ki-67 (x250). (d) Ki-67 expression significantly elevated in prostate cancer (Gleason 5+5) occurring in association with BRCA germline mutations (BRCA2, x250).

Table II. Ki-67 staining in cases and controls with a positive staining threshold >3.5% (n=17) (a=126).

Staining result	Gleason score		P-value
>3.5%	≤7	>7	Fisher's exact test
BRCA1/2 positive Ki-67 stains	2/6 (33.4%)	10/11 (91.0%)	0.028
Control positive Ki-67 stains	39/71 (55.0%)	46/55 (84.0%)	0.001

where there was a discrepancy in the percentage of cells stained (tumour or benign tissue), an average of malignant and non-malignant tissue was taken. The immunohistochemical staining was analysed using the concept of Positive Cell Index (PCI), the proportion of positively stained tumour cells (23). The PCI ranged from 0-90%. A sample was classified as positive if >3.5% of the cells (malignant or benign) were stained with the MIB-1 antibody. The data were then re-analysed with a threshold for positivity set at >7.1%. These cut-off thresholds allowed comparisons to be made between prostate cancers in mutation carriers with those in the control group to assess whether *BRCA1* or *BRCA2* mutation carriers were more likely to express the Ki-67 protein. The 3.5 and 7.1% cut-off levels have been used by other groups (19,24).

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 (≤7) and high Gleason (>7) groups were similarly compared. 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 at $p<0.05$.

Results

Of the original cohort of 20 cases of *BRCA1* or *2* mutation-associated prostate cancers, three were rejected because insufficient material was available to provide reliable information. Therefore, the data derived from these cases were omitted from the final analysis. The characteristics of Ki-67 expression in the remaining 17 cases and 126 controls are shown in Table I.

There were 143 combined cases and controls (17+126). At a 3.5% threshold for Ki-67 staining, a significant difference was identified between the malignant and benign tissues with 94 of 143 (65.7%) malignant tissues stained when compared with only one of the 143 (0.7%) benign tissues ($p<0.001$, sign test). Further analysis of these data according

Table III. Ki-67 protein staining in cases and controls with a threshold for positive staining threshold >7.1% (n=17) (a=126).

Staining result	Gleason score		P-value
>7.1%	≤7	>7	Fisher's exact test
BRCA1/2 positive Ki-67 stains	2/6 (33.4%)	8/11 (73.0%)	0.162
Control positive Ki-67 stains	19/71 (27.1%)	44/55 (80.0%)	<0.001

to Gleason score revealed 56 of 66 (85%) of cases with Gleason scores >7 and 41 of 77 (53%) of those with Gleason score ≤7 stained for Ki-67. This difference was statistically significant at p<0.001 (Fisher's exact test). When the *BRCA1* or *BRCA2* mutation carriers and the control group were tested individually, this statistical difference was maintained with respect to cancer vs. non-malignant tissue (Table II). Of the *BRCA1* or *BRCA2* mutation carrier cases, 12 of 17 (71%) expressed Ki-67 while 85 of the 126 controls (67%) were positive. This difference was not significant (Fisher's exact test, p=1.0). Fig. 1a-d demonstrates Ki-67 protein staining.

The analysis was repeated with a staining threshold of >7.1%. In the combined *BRCA1* and *-2* mutation carriers and the controls, a significantly higher proportion of Gleason >7 prostate cancers stained for Ki-67. At this threshold, 52 of 66 (79%) prostate cancers with Gleason score >7 stained for Ki-67 when compared with 21 of 77 (27%) cancers Gleason score ≤7. The percentage staining was also significantly higher in those with a Gleason score >7 in the controls alone, although this difference was no longer significant in the carriers alone (Table III). When comparing the *BRCA1* and *BRCA2* mutation carriers with the controls, 63 of the 126 controls (50%) stained for Ki-67 regardless of Gleason score while 10 of 17 cases (69%) stained. This difference was not statistically significant (p=0.608 Fisher's exact test).

Discussion

This study has demonstrated a strong relationship between aggressive prostate cancer and Ki-67 expression, irrespective of whether an individual malignancy is sporadic or occurs in association with a *BRCA1* or *-2* germline mutation. However, Ki-67 expression did not discriminate the BRCA-associated cancers as an independent cohort. In a number of different studies, Ki-67 expression predicts prognosis and clinical progression in breast (15,25) and prostate cancers (6,8,26). Although the precise biological function of Ki-67 protein is not understood, Ki-67 protein expression is an informative prognostic biomarker in the majority of malignant diseases (27). In breast cancer, where it is used to clinically stratify good and poor prognosis categories, the rate of tumour cell proliferation, measured by Ki-67 expression is related to tumour grade but not to tumour volume or lymph node status. Poor prognosis breast cancers are defined as expressing >20% Ki-67 positive nuclei, and this information may be used to influence a decision to use adjuvant chemotherapy. Shaaban *et al* showed that in early dysplastic, but not in malignant or in non-atypical hyperplastic lesions of the breast, the level of Ki-67 relative to ER-α accurately identified (p<0.001) lesions that would progress to breast cancer (28).

Current literature suggests that the levels of Ki-67 protein are related to Gleason or WHO-scoring of prostate cancer (19,29,30) although data often suggest these two parameters to be independent (26,31). Other studies have demonstrated the role of Ki-67 in predicting clinical outcome of individual prostate cancers following radiotherapy or radical prostatectomy and its association with biochemical and clinical failure (24,32,33). While there is promise for this proliferation marker to be employed routinely in the pathological assessment of prostate cancer, staining thresholds and cut-offs have been arbitrary and not universally standardised. Use of percent of positively stained nuclei has been described and used in some of these studies (23). Nevertheless, the threshold of Ki-67 staining considered positive varies in the percentages accepted both within a malignancy and also between different malignancies. Thus, in breast cancer, some authors have used Ki-67 levels as high as 20-25% (34,35). However, in prostate cancer, the level of cell proliferation appears to be much lower. Hence, the difference in Ki-67 expression used to identify tumours considered positive would be different. Recommended thresholds in prostate cancer have been in the region of >3.5 and >7.1% (19,24). A threshold level of Ki-67 >7.1% was a strong independent predictor of distant metastasis and mortality for men with prostate cancer treated with radiotherapy plus androgen deprivation therapy (19). It was concluded that, although the 7.1% cut-off point was related to metastases, the optimal threshold for Ki-67 expression in prostate cancer had not been established.

Inconsistencies in cut-off values make difficult comparisons of data reported by different groups. Hence, the cohort of men in this study were analysed using the two cut-points of >3.5 and >7.1%. Positive cell index was used to define positive staining. Both thresholds showed a significant difference in staining between high and low Gleason scores (high grade and low grade disease). Neither cut-off was associated with a difference in Ki-67 staining between mutation carriers and controls. The carriers alone did not show a significant difference between high and low grade disease at a cut-point of 7.1% although there was a significant difference at the threshold of >3.5%. When the statistical analyses were repeated for a Gleason score >6 compared ≤6, the results were similar to those reported with Gleason score >7 except that, at both thresholds (3.5 and 7.1%), there was a significant difference with positive staining, being more frequent in *BRCA1* or *BRCA2* mutations carriers alone with Gleason score >6 when compared with Gleason score ≤6.

Currently, few prospective studies have determined the prevalence of *BRCA1* or *BRCA2* germline mutations in a general population. Typically, the relationship between malignancy and BRCA mutation has been retrospective, the

frequency of mutation being estimated only after a disease cohort has been selected. By this definition, all studies using this approach are selective and hence biased. Nevertheless, sufficient information is now available to show that, while the prevalence of BRCA mutations is low, the precise level in an unselected population does not vary appreciably according to individual race and ethnicity (36). As a paradigm of human malignancies, in a group of Ashkenazi Jewish female carriers of *BRCA1* and *BRCA2* founder mutations who had a prophylactic oophorectomy, no significant difference was identified in Ki-67 protein expression between benign ovarian tissue in the carrier group and a control group. However, there was a significant difference between the benign and malignant ovarian tumours in the control population, with higher levels of Ki-67 expression reported in the malignant group (37). In this first series of *BRCA1* or *BRCA2* mutation carriers with prostate cancers analysed for Ki-67 expression, there is a highly significant difference in Ki-67 staining between malignant and benign prostate tissue. Only one (5%) benign prostate sample stained positively and this was found in the control group. Ki-67 protein expression was significantly associated with Gleason grade; a Gleason score >7 had significantly higher levels of the protein expression compared with Gleason score ≤7. However, even after adjusting for Gleason score, binary logistic regression confirms that there is no significant difference in staining between carriers and controls.

The findings of this study indicate that, in prostate cancer Ki-67 protein expression is an independent variable associated with poor prognosis but does not explain why *BRCA1* or *BRCA2* mutation carriers have worse prognosis disease. It appears that proliferation of the tumours developing in *BRCA1* or *-2* carriers is phenotypically similar to the sporadic form of this malignancy, possibly at a rate that is maximal for this type of epithelial cells. Thus, other biological factors, yet to be identified, are likely to be responsible for the significantly greater aggressiveness of prostate cancers that develop in *BRCA1* or *BRCA2* mutation-carriers (1).

Acknowledgements

We are pleased to acknowledge our benefactors: The Ronald and Rita McAulay Foundation, Cancer Research UK (grant C5047/A7357) and the North West Cancer Research Fund (grant CR760) whose continued support has enabled this work to be performed. We also acknowledge the support of the NIHR Biomedical Research Centre at The Institute of Cancer Research and Royal Marsden NHS Foundation Trust. We are grateful for the substantial assistance of Mrs. Jill Gosney in the preparation and submission of this manuscript.

References

1. Mitra A, Fisher G, Foster CS, *et al*: Prostate cancer in male BRCA1 and BRCA2 mutation carriers has a more aggressive phenotype. Br J Cancer 98: 502-507, 2008.
2. Agalliu I, Gern R, Leanza S and Burk RD: Associations of high-grade prostate cancer with BRCA1 and BRCA2 founder mutations. Clin Cancer Res 15: 1112-1120, 2009.
3. Edwards SM, Kote-Jarai Z, Meitz J, *et al*: Two percent of men with early-onset prostate cancer harbor germline mutations in the BRCA2 gene. Am J Hum Genet 72: 1-12, 2003.
4. Narod SA, Neuhausen S, Vichodez G, *et al*: Rapid progression of prostate cancer in men with a BRCA2 mutation. Br J Cancer 99: 371-374, 2008.
5. Tryggvadottir L, Vidarsdottir L, Thorgeirsson T, *et al*: Prostate cancer progression and survival in BRCA2 mutation carriers. J Natl Cancer Inst 99: 929-935, 2007.
6. Berney DM, Gopalan A, Kudahetti S, *et al*: Ki-67 and outcome in clinically localised prostate cancer: Analysis of conservatively treated prostate cancer patients from the Trans-Atlantic Prostate Group study. Br J Cancer 100: 888-893, 2009.
7. Foster CS, McLoughlin J, Bashir I and Abel PD: Markers of the metastatic phenotype in prostate cancer. Hum Path 23: 381-394, 1992.
8. McLoughlin J, Foster CS, Price P, WIlliams G and Abel PD: Evaluation of Ki-67 monoclonal antibody as prognostic indicator for prostatic carcinoma. Br J Urol 72: 92-97, 1993.
9. Giusti RM, Rutter JL, Duray PH, *et al*: A twofold increase in BRCA mutation related prostate cancer among Ashkenazi Israelis is not associated with distinctive histopathology. J Med Genet 40: 787-792, 2003.
10. Ford D, Easton DF, Stratton M, *et al*: Genetic heterogeneity and penetrance analysis of the BRCA1 and BRCA2 genes in breast cancer families. The breast cancer linkage consortium. Am J Hum Genet 62: 676-689, 1998.
11. Schluter C, Duchrow M, Wohlenberg C, *et al*: The cell proliferation-associated antigen of antobidy Ki-67: a very large, ubiquitous nuclear protein with numerous repeated elements, representing a new kind of cell cycle-maintaining proteins. J Cell Biol 123: 513-522, 1993.
12. Vincenz C and Kerppola TK: Different polycomb group CBX family proteins associate with distinct regions of chromatin using nonhomologous protein sequences. Proc Nat Acad Sci USA 105: 16572-16577, 2008.
13. Endl E and Gerdes J: The Ki-67 protein: fascinating forms and an unknown function. Exp Cell Res 257: 231-237, 2000.
14. MacCallum DE and Hall PA: Biochemical characterization of pKi67 with the identification of a mitotic-specific form associated with hyperphosphorylation and altered DNA binding. Exp Cell Res 252: 186-198, 1999.
15. Fitzgibbons PL, Page DL, Weaver D, *et al*: Prognostic factors in breast cancer. College of American Pathologists Consensus Statement 1999. Arch Pathol Lab Med 124: 966-978, 2000.
16. Gerdes J, Lemke H, Baisch H, Walcker H, Schwab U and Stein H: Cell cycle analysis of a cell proliferation-associated human nuclear antigen defined by the monoclonal antibody Ki-67. J Immunol 133: 1710-1715, 1984.
17. Scholzen T and Gerdes J: The Ki-67 protein: from the known and the unknown. J Cell Physiol 182: 311-322, 2000.
18. Philip J, Willmott S, Cornford P, *et al*: Analysis of potential biomarkers to enhance Gleason grading of prostate cancer. Br J Cancer (In press).
19. Pollack A, DeSilvio M, Khor LY, *et al*: Ki-67 staining is a strong predictor of distant metastasis and mortality for men with prostate cancer treated with radiotherapy plus androgen deprivation - Radiation Therapy Oncology Group Trial. J Clin Oncol 22: 2133-2140, 2004.
20. Jhavar S, Corbishley CM, Dearnaley D, *et al*: Construction of tissue microarrays from prostate needle biopsy specimens. Br J Cancer 93: 478-482, 2005.
21. Berney DM, Fisher G, Kattan MW, *et al*: Major shifts in the treatment and prognosis of prostate cancer due to changes in pathological diagnosis and grading. BJU Int 100: 1240-1244, 2007.
22. Deshmukh N and Foster CS: Grading prostate cancer. In: Pathology of the Prostate. Foster CS and Bostwick DG (eds). WB Saunders, Philadelphia, pp191-227, 1997.
23. Remmele W and Stegner HE: Recommendation for uniform definition of an immunoreactive score (IRS) for immunohisto-chemical estrogen receptor detection (ER-ICA) in breast cancer tissue. Der Pathologe 8: 138-140, 1987.
24. Khoo VS, Pollack A, Cowen D, *et al*: Relationship of Ki-67 labelling index to DNA-ploidy, S-phase fraction, and outcome in prostate cancer treated with radiotherapy. Prostate 41: 166-172, 1999.
25. Hall PA and Going JJ: Predicting the future: a critical appraisal of cancer prognosis studies. Histopathology 35: 489-494, 1999.
26. Feneley MR, Young MPA, Chinyama C, Kirby RS and Parkinson MC: Ki-67 expression in early prostate cancer and associated pathological lesions. J Clin Pathol 49: 741-748, 1996.
27. Brown DC and Gatter KC: Ki67 protein: the immaculate deception? Histopathology 40: 2-11, 2002.

28. Shaaban AM, Sloane JP, West CR and Foster CS: Breast cancer risk in usual ductal hyperplasia is defined by estrogen receptor-alpha and Ki-67 expression. Am J Pathol 160: 597-604, 2002.
29. Halvorsen OJ, Haukaas S, Hoisaeter PA and Akslen LA: Maximum Ki-67 staining in prostate cancer provides independent prognostic information after radical prostatectomy. Anticancer Res 21: 4071-4076, 2001.
30. Mirtti T, Kallajoki M, Aaltonen M and Alanen K: Cyclin A and Ki-67 with DNA content in benign and malignant prostatic epithelial lesions. Anal Quant Cytol Histol 23: 229-237, 2001.
31. Dunsmuir WD, Gillett CE, Meyer LC, *et al*: Molecular markers for predicting prostate cancer stage and survival. Br J Urol Int 86: 869-878, 2000.
32. Cowen D, Troncoso P, Khoo VS, *et al*: Ki-67 staining is an independent correlate of biochemical failure in prostate cancer treated with radiotherapy. Clin Cancer Res 8: 1148-1154, 2002.
33. Pollack A, Cowen D, Troncoso P, *et al*: Molecular markers of outcome after radiotherapy in patients with prostate carcinoma: Ki-67, bcl-2, bax and bcl-x. Cancer 97: 1630-1638, 2003.
34. Petit T, Wilt M, Velten M, *et al*: Comparative value of tumour grade, hormonal receptors, Ki-67, HER-2 and topoisomerase II alpha status as predictive markers in breast cancer patients treated with neoadjuvant anthracycline-based chemotherapy. Eur J Cancer 40: 205-211, 2004.
35. Spyratos F, Ferrero-Pous M, Trassard M, *et al*: Correlation between MIB-1 and other proliferation markers: clinical implications of the MIB-1 cut-off value. Cancer 94: 2151-2159, 2002.
36. Hall MJ, Reid JE, Burbidge LA, *et al*: BRCA1 and BRCA2 mutations in women of different ethnicities undergoing testing for hereditary breast-ovarian cancer. Cancer (In Press).
37. Kerner R, Sabo E, Gershoni-Baruch R, Beck D and Ben-Izhak O: Expression of cell cycle regulatory proteins in ovaries prophylactically removed from Jewish Ashkenazi BRCA1 and BRCA2 mutation carriers: correlation with histopathology. Gynecol Oncol 99: 367-375, 2005.