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ORIGINAL ARTICLE

A Variant Epidermal Growth Factor Receptor Protein is Similarly Expressed in Benign Hyperplastic and Carcinomatous Prostatic Tissues in Black and White Men.

Une Variante de Protéine Ré ceptrice Facteur de Croissance Epidermique Exprimée par des Tissus carcinomes et Hyperplastique Bénignes chez des Hommes Noirs et Blancs.

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ABSTRACT

BACKGROUND AND OBJECTIVE: **Anti-epidermal growth factor receptor strategies are now established in cancer treatment. We have recently described the presence of EGFRvIII (a variant EGFR) in prostatic tumours from UK white men and this is now a target for anti-prostate cancer treatments. However, there has been no report on the expression of this abnormal protein in black men.**

MATERIALS AND METHODS: **We determined EGFRvIII expression in sections of normal, benign hyperplastic (BPH) and carcinomatous (CaP) prostatic archival tissues from Nigerian men and UK white men using streptavidin immunohistochemical techniques. The EGFRvIII immunoreactivity was scored visually using a semi-quantitative method and the results compared statistically.**

Results: **EGFRvIII expression increased with increasing malignancy in both study populations (CaP > BPH > Normal p, <0.0001). Furthermore, EGFRvIII expression was similar in both BPH and CaP tissues in black and white men (p, 0.86 and 0.31 respectively).**

CONCLUSION: **These results demonstrate that EGFRvIII immunoreactivity in prostatic tumours in black men is similar to that in white men. Anti-cancer treatments directed at the EGFRvIII should be equally effective in men from both subpopulations. WAJM 2007; 26(1): 42 – 47.**

Keywords: Cancer, Prostate, Immunohistochemistry, Growth factors, Black and White Men, EGFR, EGFRvIII.

RESUMÉ

Contexte et Objectif: *Les récepteurs des facteurs de croissance anti-épidermiques (RFCE) sont à présent établis dans le traitement du cancer. Nous avons récemment décrit la présence de RFCEvIII (une variante du RFCE) dans les tumeurs prostatiques des hommes blancs du Royaume- Unis et ceci est à présent la cible des traitements du cancer anti-prostatiques. Cependant, aucun document rapportant l'expression de cette protéine anormale chez les hommes noirs est disponible.*

Matériels et méthodes: *Nous avons determiné l'expression du RFCEvIII dans les sections des hyperplastiques bénignes normales (HPB) et les tissues archivaux prostatiques carcinomes (PCa) des hommes Nigérians et chez des hommes blancs du Royaume Unis en utilisant les techniques immunohistochimiques streptavidines. L'immunoréactivité du RFCEvIII a été visuellement notée en utilisant une méthode semi-quantitative et les résultats analysés statistiquement.*

Résultats: *L'expression du RFCEvIII croit avec l'augmentation de malignance chey les deux groupes en etude (PCa>HPB>Normale p, <0.0001). Par ailleurs, l'expression du RFCEvIII était identique dans les deux tissus PCa et HPB chez les hommes noirs et blancs (p, 0.86 et 0.31 respectivement).*

Conclusion: *Ces résultats démontrent que l'immunoréactivité du RFCEvIII dans les tumeurs prostatiques chez les hommes noirs est identique à ceux des hommes blancs. Les traitements anti-cancereux à l'égard du RFCEvIII devrait egalement être aussi effectif chez les hommes noirs que chez les hommes blancs. WAJM 2007; 26(1): 42 – 47.*

Mots Clés: Cancer, Prostate, Immunohistochimie Facteurs de croissance, Hommes blancs et noirs EGFR, RFCE, RFCEvIII.

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Abbreviations: BPH, Benign prostatic hyperplasia; CaP, carcinoma of the prostate; PSA, prostate specific antigen; EGFR, epidermal growth factor receptor.

INTRODUCTION

Although carcinoma of the prostate (CaP) is now the most commonly diagnosed male cancer worldwide[1-3], black men have been shown to have the highest incidence and mortality of the disease[4,5]. Furthermore, black men with CaP present with a higher prostate specific antigen (PSA) , worse Gleason score, more advanced stage, and higher recurrence rate than Caucasian men[6]. Accordingly, it has been suggested that CaP may have more aggressive biology of prostate cancer in this subpopulation as compared to their Caucasian counterparts and several basic science studies have provided evidence of molecular bases for subpopulation differences in disease phenotype[7-9]. However, other researchers have found that race is not an independent prognostic factor in CaP especially when the disease is detected and treated early [10, 11].

The epidermal growth factor receptor (EGFR) is a tyrosine kinase receptor that plays an important role in the differentiation and proliferation of epithelial cells[12,13]. Over-expression of this receptor has been detected in many malignancies[14]. Several mutations of the receptor have also been described in human cancers of which the EGFRvIII is the most common[15]. This variant EGFR results from the deletion of exons 2-7 which leads to an 801-bp in-frame deletion mutation of the external domain of the normal receptor[16]. The re-arrangement deletion preserves the reading frame of the receptor message resulting in a mutant protein that is active independent of ligand-binding (i.e. constitutively active). We have recently described the presence of the EGFRvIII, in benign and malignant prostatic tumours in UK (white) men and correlated the level of its expression with CaP phenotype[17]. This variant protein is now the target for anti-cancer strategies currently being developed[18]. Detection of the EGFRvIII in protatic tumours from black men would confirm that these anti-EGFRvIII strategies would be applicable to men of this subpopulation with CaP. However, until now there were no reports of studies investigating the expression of this variant protein in prostatic tissues from black men. We now report on a study that determined and compared EGFRvIII immunoreactivity in archival sections of normal, benign hyperplastic (BPH) and CaP prostatic tissues from black and white men.

MATERIALS AND METHODS

EGFRVIII ANTIBODY

The antibody used in this study was a rabbit polyclonal antibody to EGFRvIII produced by DKM and AJW. This antibody was raised against pep EGFRvIII (LEEKKGNYVVTDHC) and affinity purified as previously described[19]. The sensitivity and specificity of this antibody for the EGFRvIII in prostatic tissues has been confirmed in our earlier reports[17, 20].

ARCHIVAL MATERIAL

Black African men: 4-μm sections from archival prostatic tissues from 19 patients with BPH, and 36 age-matched patients with newly diagnosed CaP were supplied by the Department of Pathology University College Hospital (UCH), Ibadan.

White men: 4-μm sections from archival prostatic specimens from 19 patients with BPH, and 36 age-matched patients with hormone naïve CaP, were obtained from the Pathology Department of Leicester General Hospital (LGH).

Confirmation of Suitability of Selected Prostatic Tissues for Comparison

There have been no previous reports on the comparison of EGFRvIII expression in prostatic tissues from black and white men from different centers. The following steps were therefore taken to ensure suitability of our test tissues for comparison: 1) Representative sections from all blocks of test tissues from both centers were graded histologically (using the Gleason score[21]) by a single experienced pathologist (EHM) at the LGH using routine haematoxylin and eosin staining. 2) The tissues from both centers were obtained from age-matched patients. 3) CaP tissues were from patients diagnosed when neither country had established PSA screening programs for CaP. Thus, all patients in both groups were symptomatic at the time of diagnosis. 4) The effect of disease progression was excluded by selecting CaP specimens from men with primary disease who had not been treated with hormone ablation (i.e. hormone sensitive disease).

Differences in antibody immunoreactivity of tissues were another possible cause for variation in immunostaining results. We have however previously reported that immunoreactivity to this anti-EGFRvIII antibody was similar in prostatic sections obtained from different centers that used similar preservation protocols[22]. All test tissues were fixed in 10% buffered formalin and embedded in paraffin at the source centers using identical methods. The UCH tissues were then transported to the LGH within 1 week of preparation and immediately processed employing the same protocols as for LGH tissues.

With the above criteria, these prostatic samples from the UK and Nigeria were considered suitable for comparison of immunoreactivity to this anti-EGFRvIII antibody.

Immunohistochemistry: Anti-EGFRvIII staining was detected by streptavidin-biotin amplified immunoperoxidase reactivity as previously described[17,20]. The sections from prostatic tissues from black and white men were stained within 3 weeks of preparation to exclude antigen degradation[22]. Briefly, after preparation and microwave exposure of the antigenic epitopes, the sections were blocked with 20% normal goat serum and then incubated with a pre-diluted solution of anti-EGFRvIII primary and secondary antibodies using the DAKO detection kit. The avidin-biotin complex was then applied to the sections followed by a freshly prepared chromogen mix of buffered 3',3' diaminobenzidine tetrahydrochloride. The sections were counterstained with Mayer's haematoxylin, dehydrated, enhanced with heavy metal to give a black positive reaction, cleared and mounted with XAM neutral mounting medium (BDH Poole, Dorset, UK). Positive and negative controls were included in all staining runs. The positive controls were sections of EGFRvIII-expressing HC2 20d/2c mouse tumour [15], whilst negative controls were slides in which the anti-EGFRvIII primary

antibody was replaced with normal rabbit serum.

Evaluation of Immunochemistry:

Immuno-reactivity of homogeneous histological areas within the sections was assessed independently, and without prior knowledge of histological grading by EOO-O and scored using a modification of the H-scoring system[17,23]. Briefly, the intensity of the reaction (0-3+) in homogeneous histological areas of the sections was weighted by the percentage of cells staining at each intensity. Thus the intensity of antibody staining had a range from 0 (no staining) to 300 (100% 3+ staining). The final scores were expressed as a percentage of 300 and classified as; 0 = negative, 1–33% = low expression, 34 – 66% = moderate expression, >66% = high expression.

Statistical Methods:

EGFRvIII immunoreactivity of the sections were reported as means±SD, and as proportions with 95% Confidence Intervals (CI). Levels of immuno-positivity were compared using paired and unpaired Student's t-tests, as appropriate. All the tests were two-sided, and were done at the 0.05 level of significance.

RESULTS

Comparative Pathology of Cancer of the Prostate Glands

Four of the thirty-six CaP glands (11%) from black men were low-grade (G1, Gleason scores 2-5) tumors, whilst the remaining 32/36 (89%) specimens were from high-grade (G2 and G3, Gleason scores 5-7 and 8-10 respectively) tumors. On the other hand, in white men 31% (11/36) were G1 tumors whilst 69% (22/36) were G2/3 cancers. Similar to our earlier report, HGPIN glands were seen in a greater portion of CaP glands (14/36 [39%]) from white men, as compared to specimens in black men (4/36 [11%]).

EGFRvIII Immunoreactivity In Prostatic Tissues:

As we have earlier reported (23), EGFRvIII pattern of staining was dependent on the histology of the individual glands within sections from both subpopulations as similar immuno reactions were seen in normal/atrophic

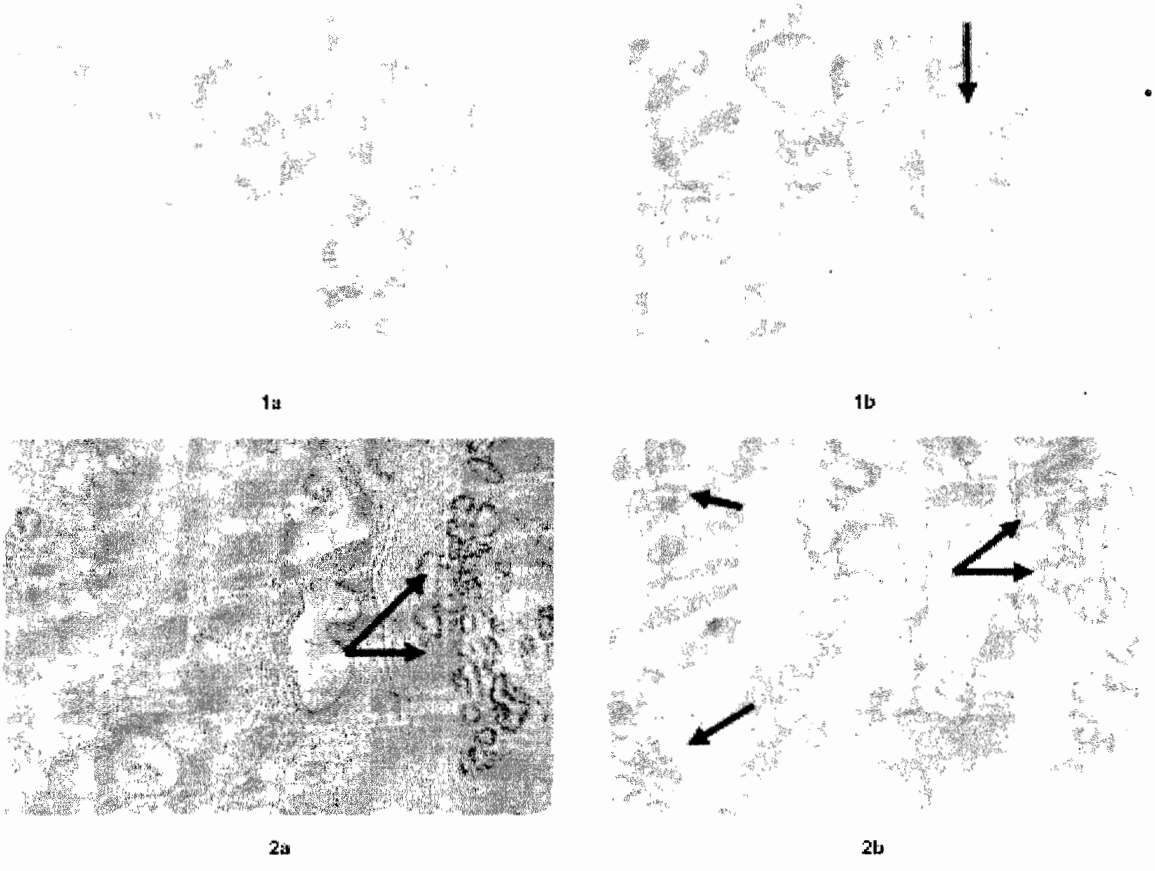

Figure: EGFRvIII Expression in Black and White Man. Upper panels: Sections of normal and Atrophic Prostatic Glands(1a) and Caucasian men (1b) showing no EGFRvIII immunostaining in the epithelium and the surrounding stroma. Note the mild EGFRvIII immunopositivity in the adjacent BPH glands in both sections. Lower panels: showing sections of BPH and CaP glands from blackmen (2a) and Caucasian men (2b). They show low expression for the receptor in BPH and high expression in the adjacent CaP epithelium (arrowed). However, similar to normal/atrophic glands there is no EGFRvIII immunoreactivity in peri-epithelial stroma. (Magnification x 100).

Table 1: EGFRvIII Expression in Normal, Benign Hyperplastic and Malignant Prostatic Epithelium in Black Men

Histology (N)	Mean Score (SD)	Percent and CI of Intensity of immunoreaction			
		Strong	Moderate	Weak	Absent
Normal/Atrophic (19)	0 (0)	0 (0, 0-18)	0 (0, 0-18)	0(0, 0-18)	100 (0, 82-100)
BPH (19)	19 (14)	0 (0, 0-17)	3 (16, 3-40)	16 (84, 60-97)	0 (0, 0-17)
CaP Cum (36)	75 (21)	28 (78, 63-92)	8 (22, 8-37)	0 (0, 0-10)	0 (0, 0-10)
G1 (4)	53 (13)	1 (25, 1-81)	3 (75, 19-99)	0 (0, 0-60)	0 (0, 0-60)
G2 (16)	78 (21)	14 (88, 62-99)	2 (12, 2-38)	0 (0, 0-21)	0 (0, 0-21)
G3 (15)	77 (19)	13 (87, 59-96)	3 (13, 4-41)	0 (0, 0-19)	0 (0, 0-19)

Table 2: EGFRvIII Expression in Normal, Benign Hyperplastic and Malignant Prostatic Epithelium in White Men

Histology (N)	Mean Score (SD)	Percent and CI of Intensity of immunoreaction			
		Strong)	Moderate	Weak	Absent
Normal/Atrophic (19)	0 (0)	0 (0, 0-18)	0 (0, 0-18)	0 (0, 0-18)	100 (0, 82-100)
BPH (19)	14 (18)	0 (0, 0-17)	2 (11, 2-33)	17 (89, 67-99)	0 (0, 0-17)
CaP Cum (36)	70 (27)	23 (64, 46-79)	11 (31, 16-48)	2 (6, 0-19)	0 (0, 0-10)
G1 (11)	57 (23)	4 (36, 11-69)	5 (46, 17-77)	2 (18, 2-52)	0 (0, 0-29)
G2 (10)	70 (20)	6 (60, 26-88)	4 (40, 12-74)	0 (0, 0-31)	0 (0, 0-31)
G3 (15)	73 (17)	13 (87, 60-98)	2 (13, 2-41)	0 (0, 0-22)	0 (0, 0-22)

CI, Confidence Interval; BPH, Benign prostatic hyperplasia; CaP, Primary prostatic cancer; Cum, Cummulative.
Tables showing mean EGFRvIII expression in different prostatic tissue histotypes.EGFRvIII expression increased with increasing malignancy in both study populations (CaP > BPH > Normal p, <0.0001). However, EGFRvIII immunoreactivity was similar in both BPH and CaP tissues in black and white men (p, 0.86 and 0.31 respectively).

and BPH glands in both BPH and CaP sections. Furthermore, the variant antigen was expressed mainly in the cytoplasm, appearing mostly as a distinct peri-nuclear deposit on the luminal surface of the cells (figures 1b and 2b). Also in keeping with the previous studies, EGFRvIII was expressed by abnormal prostatic epithelial cells only and not by normal glands or the peri-epithelial stroma in all sections. In addition, the mean expression of the receptor increased as the tumours became more malignant with the highest immunoreactivity being seen in poorly differentiated tumours (See Tables 1 and 2 and Figures 1&2).

Statistical comparison of the scores revealed significant differences between EGFRvIII expression in BPH and CaP glands [black, p = 0.006; white, p = 0.0006], and between well-differentiated (G1) and higher grade tumours (G2/3) and tumours [black, p = 0.02; white, p = 0.009].

A detailed account of EGFRvIII expression in the various histotypes in both study groups is summarized in Tables 1 and 2.

Comparison of EGFRvIII Expression in Prostatic Tissues from Black and Caucasian Men.

Figure1 and Tables 2 and 3 summarise the comparison of the EGFRvIII expression in prostatic tissue in black and Caucasian men studied.

Comparison of EGFRvIII expression in the tissues from the two subpopulations was between the main (prostatic) tissue histotypes i.e. normal, benign hyperplastic, and malignant cell types. EGFRvIII-positivity was also compared in the advanced cancers (G2/G3) in both groups as a separate analysis. However the number of CaP sections with HGPIN glands was too small for comparison between the two groups. HGPIN glands were therefore excluded from further analysis in the study.

Normal Glands. As stated above, EGFRvIII was not expressed by normal epithelium in sections from black and caucasian men.

Benign prostatic hyperplasia.. The mean EGFRvIII expression in BPH epithelium was similarly weak in both subpopulations though higher in specimens from black men as compared to those from white men (black, 19% [±14] vs caucasian, 14% [±18] {p = 0.74}).

Cancer of the Prostate. The mean EGFRvIII expression was similarly strong in CaP epithelium from both black and white men (black, 75% [±21] vs white, 70% [±27] respectively {p = 0.82}). The levels of expression of the variant protein was also similar in the higher grade cancers (G2/G3) in both subpopulations (black, 78% [±21] vs caucasian, 76% [±20] {p = 0.72}). Furthermore, EGFRvIII immunopositivity was significantly higher in CaP than in benign hyperplastic tissues in both groups of men [p < 0.0000].

DISCUSSION

We have previously reported the detection of the EGFRvIII and the correlation of its level of expression with both clinical indices of disease progression[17] and molecular markers of aggressive phenotype[24] in prostatic tumours from (UK) white men.

Considering the aggressive biology of malignant prostatic disease in black men, the postulation that this variant protein could be contributory to the CaP phenotype in this subpopulation merited investigation. In this regard, our detection of the expression of the EGFRvIII by prostatic tumours from black men is a significant finding as it provides additional information to support the hypotheses that the molecular changes that attend malignant transformation of the prostate gland are similar in black and white men.

We and others have shown that black men have a higher androgen receptor expression in neoplastic prostatic epithelium and a lower expression of the receptor in the surrounding stroma[8, 25]. We have also demonstrated that there was a direct relationship between the level of EGFRvIII expression in prostatic epithelium and the depletion of stromal AR expression in CaP tissues [20]. These studies suggest that prostatic tumours from black men would express higher levels of the EGFRvIII. As such, the similarity of the level of expression of the mutant protein in prostatic tissue from the two sub-populations in all the histotypes (normal, BPH and CaP) was surprising. This finding also differs from sub-population studies on the native EGFR that have detected higher levels of expression of the wild-type protein in malignant prostatic tissues from black men[9], and major interethnic differences in the alterations in the EGFR gene[26]. A possible explanation for the similar levels of expression of the EGFRvIII is the small sample size of our study population, and that the majority of the CaP tissues screened in both sub-populations (especially the blacks) were high-grade tumours. As the level of the EGFRvIII was similarly high in the advanced CaP tumours from both groups of men a larger sample size with larger number of low-grade tumours may be necessary to demonstrate subtle racial differences in expression of this abnormal protein.

The expression of the EGFR family of receptors in prostatic diseases has been the focus of extensive translational science research and these proteins are targets of several anti-cancer treatments[18]. Strategies directed at the EGFRvIII in particular include immunotoxins[27], antibody therapy[28], vaccines[29] and gene therapy[30]. Our findings suggest that anti-EGFRvIII therapies would be equally applicable and effective in black and white men with CaP.

The anti-EGFRvIII strategies are now undergoing clinical trials[31] and it would be necessary to conduct similar studies in black African men to confirm the clinical benefits of these laboratory findings.

The expression of EGFRvIII is similar in prostatic tissues from black and white men in all histotypes. As such anti-prostate cancer strategies directed at this variant receptor would be similarly effective in both subpopulations.

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