The expression of a mutant epidermal growth factor receptor in prostatic tumours

E. O. OLAPADE-OLAOPA
Leicester General Hospital, Leicester, UK

Introduction

The article is a synopsis of a thesis [1] presented at the University of Leicester. Carcinoma of the prostate is now the most commonly diagnosed cancer in men and is thus a significant cause of morbidity and mortality [2]. However, the factors that govern the natural history of the disease remain poorly understood. Research efforts are therefore increasingly focused on the molecular and cellular pathways that regulate normal and abnormal growth of the gland.

Peptide growth factors and their receptors are cellular signalling molecules that are an integral part of the regulatory pathways/mechanisms in solid-organ homeostasis [3]. Of these proteins, wild-type EGFR (WT-EGFR) has been most commonly implicated in carcinogenesis, but studies on WT-EGFR expression in prostatic tumours have produced conflicting results [4,5]. Despite this, more specific investigative methods now indicate a progressive depletion of the receptor as prostatic tissues become increasingly malignant [6]. The progressive loss of WT-EGFR in prostatic tumours has remained unexplained, although several mechanisms have been proposed, including the expression of a mutated receptor. The most common mutant EGFR found in human cancers is the Type III mutant (EGFRvIII), which is a constitutively active tyrosine kinase that is able to initiate cell division independently of the ligand, and thus (potentially) independently of hormonal control [7]. This aberrant EGFR has been implicated in the pathogenesis and progression of several cancers but has not been detected in prostate cancer.

Methods

The present project assessed the hypothesis that, in addition to the normal receptor, prostatic tumours also express an abnormal EGFR, and that the contradictory findings in previous studies arise through the detection of this mutant receptor by some but not all of the different techniques used. Normal (19), BPH (19), high-grade prostatic intraepithelial neoplasia (HGPIN, 14), prostate cancer (38) and metastatic prostatic (12) tissues were scrutinized retrospectively for the presence of EGFRvIII and WT-EGFR using western blotting and immunohistochemical techniques, as previously described [8,9]. The levels of expression of both receptors within these neoplasms were scored using a modification of the H-scoring system [10]. The WT-EGFR and EGFRvIII scores were analysed statistically using paired and unpaired two-sample t-tests as appropriate.

Results

The tests confirmed the presence of the EGFRvIII protein in altered prostatic glands (BPH, HGPIN, cancer and metastatic cancer) but not in normal glands, with markedly higher levels in the malignant tissues \( (P < 0.001) \). There was a significant inverse relationship between the levels of expression of the variant EGFR and the wild-type protein in each prostatic histotype \( (P < 0.001; \text{Fig. 1}) \). Significantly, the staining patterns in the sections showed a spatial difference between the patterns of expression of the two EGFRs. WT-EGFR was expressed mainly on the cell membrane, whilst EGFRvIII was expressed mainly by the cytoplasmic or perinuclear area. In addition, the prostatic cell type which expressed EGFR the most, wild-type or mutant, depended on the histology of individual glands (basal cells in normal/atrophic and BPH glands, and luminal cells in malignant glands). The expression of WT-EGFR therefore gave the impression of an outer rim surrounding the gland (Fig. 2a) whilst EGFRvIII was expressed as an inner rim (Fig. 2b).

The clinical significance of EGFRvIII in prostate cancer was assessed by correlating the level of expression with accepted prognostic indices of the disease (age, serum PSA level, histological grade and clinical stage) [11], using Spearman’s correlation coefficient (univariate analysis) and Cox’s proportional hazards regression (multivariate evaluation). There was a significant association between EGFRvIII expression, serum PSA
level and the time to disease progression (increasing clinical stage; \( P = 0.005 \) and 0.45, respectively) only. Staining of serial sections with anti-Ki67 (a marker of cell proliferation and survival in prostate cancer [12]) also showed a direct relationship between EGFRvIII expression and the Ki-67 index (\( P = 0.006 \)). This provides further (indirect) evidence that this aberrant protein may be involved in determining the biology of prostate cancer.

**Discussion**

Despite the relatively few samples included in the study, these findings confirm the hypothesis that prostatic tumours express a mutant EGFR (EGFRvIII) that may be a useful histological marker for prostate cancer cells. Furthermore, the expression of this constitutively active receptor represents a potential mechanism for the hormone-independent proliferation in prostate cancer and could be predictive of an aggressive disease phenotype. Taken together, the evidence from this project suggest that this variant EGFR may be of clinical significance in patients with prostate cancer and could be a target for modern anticancer regimens [8]; this provides the focus of a future study.

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**Fig. 1.** Wild-type EGFR (green) and EGFRvIII (red) expression in prostatic tissues (CaP, prostate cancer; Mets, metastatic disease).

**Fig. 2.** a, WT-EGFR expression in a BPH gland and b, EGFRvIII expression in prostate cancer.
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**Author**

E.O. Olapade-Olaopa, DM, FRCS. Currently Lecturer, Section of Urology, University of Michigan, 1500 E. Medical Center Drive, Ann Arbor, MI 48109, USA. Formerly, Oncology Research Fellow, Leicester General Hospital, Gwendolen Rd, Leicester, UK.

Correspondence: Mr EO Olapade-Olaopa DM, FRCS. 45 Greystones Hall Road, Sheffield, S11 7BA, UK.
e-mail: okeoffa@yahoo.com