# Prostate Adenocarcinoma: are EGFR-targeted drugs of therapeutic relevance for castrate-resistant patients?

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***Abstract***- The progression of normal prostatic epithelium to androgen-dependent cancer and, eventually, castrate-resistant prostate cancer is a complex process involving many different growth regulatory signals. Activation of epidermal growth factor receptor (EGFR) has been implicated in prostate cancer cell growth.

Patients and Methods

The study was conducted in CHUM Research Centre/Montreal Cancer Institute, Montreal, Quebec, Canada. Tumor tissue sample collection started in January, 2011 and was completed in July, 2012. Specimens were obtained from 58 Moroccan castrate-resistant patients with prostate cancer, and all patients had given informed consent. Patient characteristics were retrieved from medical records, and membrane-specific EGFR expression was evaluated immunohistochemically. A statistical analysis was performed using Epi-Info software.

Results

EGFR overexpression, defined as complete membrane staining in more than 10% of tumor cells, was observed in in 36 of 58 tumors (62,06%). There was a significant association between EGFR overexpression and Gleason score and TNM stage, but no correlation with PSA level was found.

Conclusion

EGFR is predominantly expressed in castrate-resistant patients, suggesting that EGFR-targeted drugs could be of therapeutic relevance in this otherwise difficult to treat subset of prostate cancer. However, since these markers are only expressed in a fraction of advanced tumors, patients selection needs to be realized based on the EGFR profile of their tumors.

***Index Terms*** EGFR expression, Prostate cancer, Immunohistochemistry

## I. INTRODUCTION

Prostate cancer is the most commonly diagnosed malignancy and the second leading cause of men cancer mortality. Androgen withdrawal is the only effective therapy for patients with advanced disease. Approximately 80% of patients achieve symptomatic and/or objective response after androgen ablation. However, progression to androgen independence ultimately occurs in almost all patients. Although numerous non-hormonal agents have been evaluated in patients with castrate-resistant prostate cancer, these agents have limited antitumor activity with an objective response rate <20% and no demonstrated survival benefit [5]. Therefore, the identification and selected inhibition of molecular targets that mediate the progression of prostate cancer will have gear impact on future treatment concepts. Possible targeted therapies include molecules that block specific proteins ans pathways or gene therapy for insertion of wild-type genes to restore the function of defective tumor-supressor genes [6].

Both clinical and experimental data have established the importance of the epidermal growth factor receptor (EGFR) in carcinogenesis and progression of various types of solid tumors including prostate cancer [1]. Many studies have suggested that the progression to castrate-resistant disease may be associated with epidermal growth factor receptor (EGFR), epidermal growth factor (EGF), amphiregulin, and/or transforming growth factor-a (TGF-a) [2]. TGF-a and EGF bind to EGFR, which initiates tyrosine kinase activity, which then leads to the activation of the intracellular signaling pathway. This activation can lead to gene expression, cell proliferation, and cell survival [3]. Previous investigations have provided support for the contention that EGFR may be responsible for prostate cell growth. It has been suggested that monoclonal antibodies and tyrosine kinase inhibitors that target EGFR may prove to be effective in reducing the progression of some solid tumors [4].

Evidence that over-expression of EGFR in castrate-resistant prostate cancer may provide a rationale for new therapeutic strategies. The major aims of this study were: (a) to determine whether EGFR protein is expressed in human prostate cancer specially in castrate-resistant patients; (b) to assess whether EGFR expression increases with cancer progression toward androgen independence; (c) to evaluate the association between patterns of EGFR expression and standard clinicopathological parameters; and (d) to define the potential prognostic effect of EGFR.

## PATIENT AND METHODS

### Specimen collection and patients

The study was conducted in CHUM Research Centre/Montreal Cancer Institute, Montreal, Quebec, Canada. Tumor tissue sample collection started in January, 2011 and was completed in July, 2012. Specimens were obtained from 58 consenting Moroccan castrate-resistant patients, 46 with a localized prostate cancer and 12 with distant metastasis.

H&E-stained slides from each biopsy/radical prostatectomy case were reviewed, and a Gleason grade and pathological stage were assigned. Tumors were classified as high grade when the combined Gleason score was ≥7 and as low grade when the combined score was ≤6. Serum prostate-specific antigen (PSA) levels were obtained from the patient's medical records in every case.

Other patient characteristics that were also retrieved were age, lymph node status and presence of distant metastasis.

Tissue microarrays

Tissue microarrays were constructed following standard methodology. After selection of donor areas by microscopic examination, 0.6 mm punches were placed in a receptor block measuring 25x 35 mm. One microarray block containing all punches and duplicates of each tumor was produced. The block was sectioned at 3µm and the sections were floated out on a water bath at 45°C and picked up onto sequentially numbered slides. The slides were dried at room temperature overnight prior to staining.

Immunohistochemistry

EGFR Antibody specificity was confirmed by western blotting prior to staining. TMAs were incubated with the EGFR mouse monoclonal antibody (Santa Cruz Biotechnology, CA, United States of America (USA)) diluted 1:100 in antibody diluent (Ventana Medical Systems Inc., USA). Signal was developed using the UltraView DAB detection kit (Ventana Merdical Systems Ins., USA) and counterstaining was performed with hematoxylin and bluing reagent.

Staining quantification

At least two uropathologists, blinded to clinical data, scored each sample. A third investigator reviewed discordant cases. The samples analyzed were from tissue microarrays with 0.6 mm tissue cores, and the entire tumor area was used for quantification. Immunoreactivity for EGFR was interpreted without previous knowledge of any of the clinicopathological parameters. EGFR cell membrane immunoreactivities for the EGFR protein were categorized as undetectable (zero) to 3. A score of zero was defined as undetectable staining or membrane staining in less than 10% of the tumor cells. A score of 1+ was defined as faint, incomplete membrane staining in more than 10% of the tumor cells. A score of 2+ was defined as weak to moderate, complete membrane staining in more than 10% of the tumor cells. Finally, a score of 3+ was defined as strong, complete membrane staining in more than 10% of the tumor cells. EGFR protein expression was then classified as negative (scores 0 and 1+) or positive (scores 2+ and 3+).

Statistical analysis was performed using the Chi2-test. A p-value less than 0.05 was considered statistically significant. No adjustments were made.

RESULTS

We are the first to report EGFR expression in Moroccan patients with prostate cancer. The study included 58 hormone-recfractory patients. All cases were evaluated by IHC analysis. The patients' clinicopathological characteristics are listed in Table 1. The mean age of patients is 61 years (range, 50–90 years), and the mean preoperative PSA level is 12.4 ng/ml (range, 1.6–87.8 ng/ml). Of the 58 PACs, there were 10 (17.24 %) low-grade (Gleason score ≤6) and 48 (83.07%) high-grade (Gleason score ≥7) tumors. At prostatectomy, there were 37 (63. 79%) organ-confined tumors (stages I and II) and 21 (36.21%) in advanced stage (III and IV) tumors.

As for EGFR expression (Figure 1), it was detectable in 36 of 58 tumors (62,06%). Table 2 shows that EGFR expression exhibited higher levels of protein expression in PSA level over than 10 ng/mL without any statistical significance. As for Gleason score, EGFR expression was significantly higher in high-grade cancer tissues (66, 66%) compared with low-grade.

Our results also show a significant high EGFR expression in advanced TNM stages (T3a, T3b) with a 95, 23% rate.

As for lymph node and distant metastases, a strong expression localization of EGFR (score 2+/3+) was seen in 80% and 80, 33% of patients with positive-metastases, respectively.

DISCUSSION

With the availability of monoclonal antibodies and small-molecule tyrosine kinase inhibitors as potential therapeutic agents in the management of many solid cancers, the investigation of EGFR, as a possible target, has been conducted in many solid tumors including carcinomas of the colon, lung, breast, bladder, head and neck. The results of these investigations have suggested that agents that target EGFR may prove to be effective in reducing the progression of some solid tumors [7; 8; 9]. Due to its grave prognosis, additional treatment options for androgen-independent prostate cancer are sorely needed. Evidence that overexpression of EGFR occurs in castrate-resistant prostate cancer may provide a rationale for new therapeutic strategies.

In our present study of EGFR expression in tumors from hormono-refractory patients, we found that 36 of 58 cases (62,06%) stained positively for EGFR protein. These findings support the notion that EGFR gene over-expression may play a significant role in the pathogenesis of such malignancies, and suggest that therapy directed against this aspect of the malignancy may prove beneficial. Trials of anti-EGFR therapy have begun in patients with castrate-resistant prostate cancer. One group of investigators treated a group of castrate-resistant prostate cancer patients with a combination of therapeutic agents including gefitinib, estramustine, and docetaxel [10]. They demonstrated that gefitinib combined with estramustine and docetaxel had acceptable and predictable tolerability. Another group of investigators found that gefitinib had only minimal single agent activity in forty castrate-resistant patients [11].

Previous studies have established links between EGFR over-expression and castrate-resistant behavior in prostate cancer. Di Lorenzo et al. found that EGFR expression increases in prostate cancer as it becomes progressively more aggressive [12]. They compared the immunohistochemical expression of EGFR in prostate cancers from patients unexposed to hormone therapy to EGFR expression in cancer samples from patients treated with androgen ablation (by LHRH and anti-androgen), and from patients with clinically castrate-resistant prostate cancer. EGFR was expressed in 41% of cancers that had not been exposed to hormone therapy, in 76% in those exposed to LHRH and anti-androgen therapy, and in 100% of cancers from patients with metastatic hormonal-refractory disease [12].

We also concluded in our a significant high EGFR expression in advanced TNM stages (T3a, T3b) with a 95, 23% rate, which is in accordance with the results of Di Lorenzo's and Shuch's studies. Our results also show that EGFR expression exhibited higher levels of protein expression in PSA level over than 10 ng/mL without any statistical significance. As for Gleason score, EGFR expression was significantly higher in high-grade cancer tissues (66, 66%) compared with low-grade. However, in the study by Shuch and co-workers, a significant relationship was found between the EGFR gene expression and PSA but not with

grade [13]. Other authors reported a significant relationship between PSA, grade, and stage of the disease and postoperative positive margins with EGFR gene expression [14].

It remains to be demonstrated if EGFR therapeutic targeting may optimize patient outcome [15], the association between EGFR expression and high Gleason scores and advanced TNM stages suggests that EGFR may be a valuable prognostic factor. However, EGFR prognostification needs to be more fully assessed in the framework of prospective studies and future studies are needed to evaluate the overall significance of the expression of EGFR and its potential use in anti-EGFR therapies.

CONCLUSION

Summing up, our study shows that EGFR is predominantly expressed in castrate-resistant patients, suggesting that EGFR-targeted drugs could be of therapeutic relevance in this otherwise difficult to treat subset of prostate cancer. However, since these markers are only expressed in a fraction of advanced tumors, it will be necessary to assess the efficacity of EGFR targeted therapy in relation to the marker expression in the individual patients rather than looking at a raw overall efficacy. Patients selection needs to be realized based on the EGFR profile of their tumors.

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**Tables**

**TABLE1. Clinicopathological characteristics of patients with prostate adenocarcinomas (n = 58).**

| | **Clinico-pathologicalparameters** | N | **%** |
|---|---|---|---|
| **PSA level** | ≤10 ng/ml | 6 | 10,34 |
| | >10 ng/ml | 52 | 89,66 |
| **Gleason score** | <3+3 | 10 | 17,24 |
| | 3+4 | 20 | 34,48 |
| | 4+4 | 8 | 13,79 |

| | | | |
|---|---|---|---|
| | 4+5 | 20 | 34,48 |
| **TNM Stage** | Organ confined (T2a, T2b) | 37 | 63,79 |
| | T2a | 24 | 41,38 |
| | T2b | 13 | 22,41 |
| | Advanced (T3a, T3b) | 21 | 36,21 |
| | T3a | 13 | 22,41 |
| | T3b | 8 | 13,79 |
| **pN** | N0 | 48 | 82,76 |
| | N+ | 10 | 17,24 |
| **pM** | M0 | 46 | 79,31 |
| | M+ | 12 | 20,69 |

**TABLE 2 . Epidermal Growth Factor (EGFR) Immunostaining in patients with positive-prostate cancer in comparison with clinico-pathological parameters**

| | | **EGFR immunostaining** | | | | |
|---|---|---|---|---|---|---|
| | **Clinico-pathological parameters** | **EGFR -** | **%** | **EGFR+** | **%** | **p-value** |
| **PSA level** | ≤10ng/ml | 4 | 66,67 | 2 | 33,33 | 0,33 |
| | >10ng/ml | 18 | 34,61 | 34 | 65,38 | |
| **Gleason score** | Low score ≤3+3 | 6 | 60 | 4 | 40 | < 0,05 |
| | High score≥ 3+4 | 16 | 33,33 | 32 | 66,66 | |
| **TNM Stage** | Organ confined (T2a, T2b) | 21 | 56,57 | 16 | 43,24 | < 0,05 |
| | Advanced (T3a, T3b) | 1 | 4,76 | 20 | 95,23 | |
| **pN** | N0 | 20 | 41,66 | 28 | 58,33 | < 0,05 |
| | N+ | 2 | 20 | 8 | 80 | |
| **pM** | M0 | 20 | 43,47 | 26 | 56,52 | 0,46 |
| | M+ | 2 | 16,66 | 10 | 83,33 | |

**Figures**

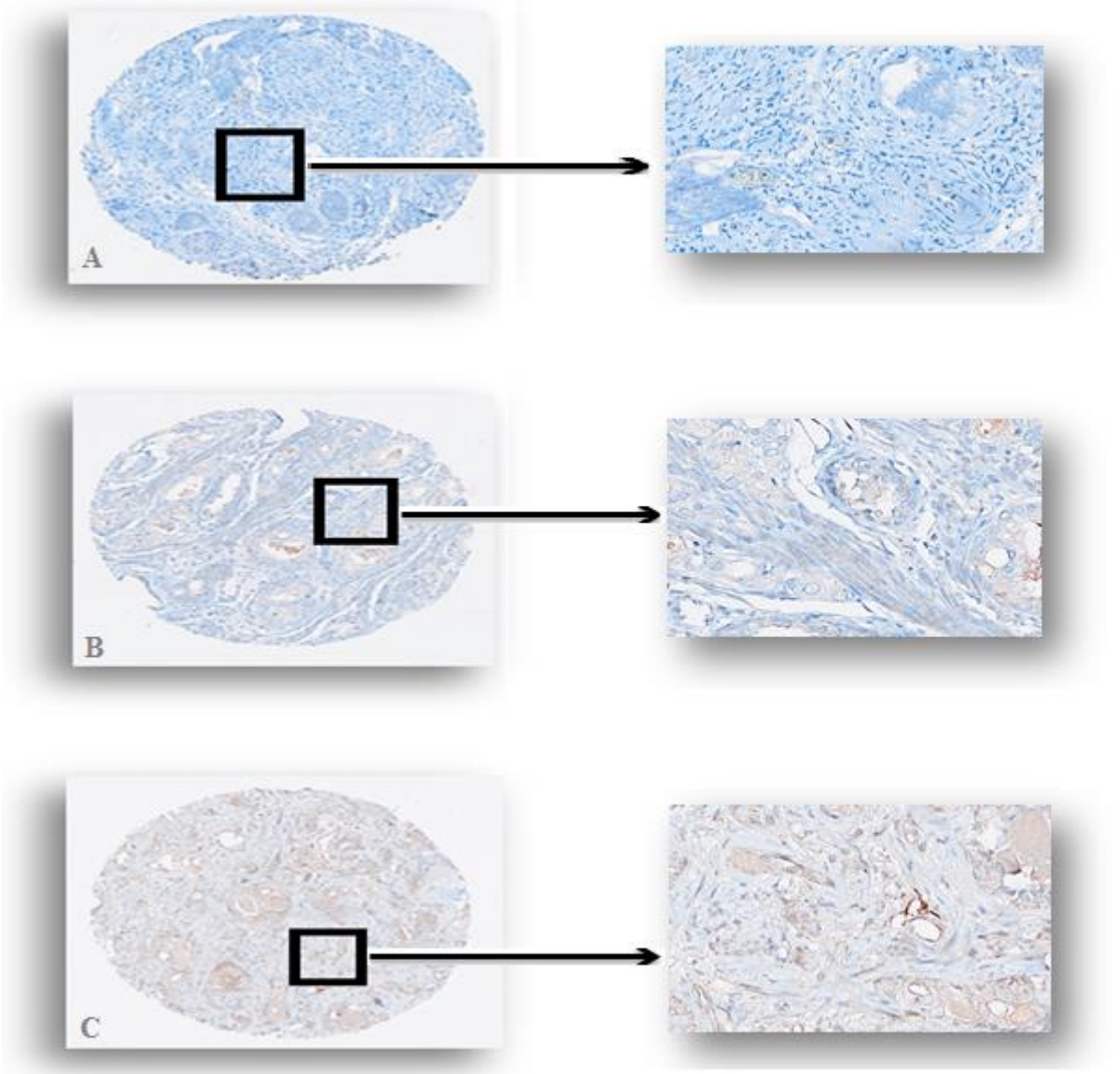


**Figure 1:** Expression of EGFR in prostate cancer tissues: **A)** Faint/barely perceptible membranous reactivity in tumor cells (original magnification, ×400) .**B)** Weak to moderate complete, basolateral, or lateral membranous reactivity in tumor cells (original magnification, ×400) **C)** Complete, basolateral, or lateral membranous reactivity of strong intensity in tumor cells (original magnification, ×400).