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Original Articles

Correlation between Grade in Transitional Cell Carcinoma (TCC) and Expression of Epidermal Growth Factor Receptor (EGFR)

**MR Jallali Nadoushan[1], F Heidary[1], F Zaeri[2], H Ahmadi[2]*

[1]*Dept. of Pathology, Shahed University, Medical School, Tehran, Iran*
[2]*Dept. of Social Medicine, Shahed University, Medical School, Tehran, Iran*

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Abstract

Background: The present study was undertaken to investigate the correlation of Epidermal Growth Factor Receptor (EGFR) expression with grade of Transitional Cell Carcinoma (TCC).

Methods: Tumor samples of 75 patients from Mostafa Khomaini Hospital with Transitional Cell Carcinoma of the bladder were analyzed by immunohistochemistry for expression of EGFR. In this context, we assigned the bladder tumors a grade according WHO classification. Results analyzed for possible correlation with the expression status of the Epidermal Growth Factor Receptor (EGFR).

Results: This cross-sectional study showed that all grades of Transitional Cell Carcinoma expressed EGFR, and 14 cases were LMP (18.9%) which 10 cases among them had negative cells according EGFR point of view(71.4%) and 4 cases had reported positive (28.6%). Thirty five cases were low grade (46.7%) which 18 cases among them had reported negative cells (51.4%) and 17 cases had positive cells (48.6%). Twenty six cases were high grade (34.7%) that 9 cases among them had reported negative cells (34.6%). Seventeen cases had positive cells (65.4%). Mann-Witney test showed relation between grade and expression of EGFR ($P<0.05$).

Conclusions: This study showed that expression of EGFR is correlated with grade of tumor.

Keywords: *Epidermal growth factor receptor (EGFR), Carcinoma, Transitional Cell*

Introduction

Transitional Cell Carcinoma (TCC) of the bladder is one of the commonest cancer in Iran and according latest National Cancer Registration Report the annual incidence of new cases has been cited as 2619 in men and 545 in women (1). TCC is the fifth most common solid malignancy in the United States. Meanwhile, it is diagnosed in approximately 54,000 patients and mortality rate of this tumor is approximately 12,000 cases per year (2).

Clinical studies which evaluating the significance of EGFR expression in human TCC have shown that more than 50% of human TCCs were assay over express the EGFR. Results of these studies indicated that the level of expression directly correlated with tumor grade, stage, and survival (3).

Growth factors consist of a broad group of low-molecular-weight polypeptides that are involved in several cellular mechanisms such as proliferation, motility and transformation. All responsive cells have membrane receptors that specifically bind the appropriate ligand. An increasing number of studies showed the involvement of activated protooncogenes and abnormal growth factor production in the induction of tumor cell growth, and their association with an aggressive phenotype in many tumor groups (4).

EGFR and its ligands are implicated in the oncogenesis of certain tumors of epithelial origin (breast, uterine cervix, bladder), and that expres-

***Corresponding author:** Tel: +98 21 88964792, +98 21 88966310, E-mail: jalalinadooshan@yahoo.com

sion of these factors are marker of poor prognosis (5, 6).

This study was conducted to investigate the correlation of Epidermal Growth Factor Receptor (EGFR) expression with grade of Transitional Cell Carcinoma (TCC).

Materials and Methods

Seventy five samples, 49 male (65.3%) and female (34.7%) were obtained from archives of Mostafa Khomeini Hospital, Pathology Department, Tehran, Iran from January 2001 till August 2003. All patients with Transitional Cell Carcinoma were evaluated. Samples were obtained by transurethral resection (TUR) or biopsy. Cancer was graded according World Health Organization (WHO) classification (7) by an expert pathologist.

Specimens were fixed in 10% neutral buffered formalin and embedded in paraffin. Serial sections were obtained at 5 micrometer thickness from each specimen for hematoxylin-eosin and immunohistochemical stains. Slides were deparaffinized in xylene, rehydrated in graded alcohol, and incubated in 0.3% hydrogen peroxide in methanol to quench the endogenous peroxidase activity, which was followed by a 10 min enzymatic digestion of tissue with 0.1% trypsin/ ethylenediaminetetraacidic acid (EDTA). Nonspecific binding sites were blocked by 20- min incubation with nonimmune horse serum. Primary antibody for the detection of EGFR was a polyclonal rabbit immunoglobulin-G (IgG) (Ab-4, Oncogene Sciences, Cambridge, Mass). With a working dilution of 1: 100, tissue samples were incubated for 2 h at room temperature (RT). Staining was completed with avidinbiotin-peroxidase complex method after 30 min incubation with secondary antibody. Color development was achieved using chromogen 3, 39-diaminobenzidine (DAB). Incubation of samples for 2 h at RT with primary antibody at a working dilution of 1: 50 was followed by incubation with secondary antibody for 30 min (8).

Statistical analysis was carried out by Mann Whitney test and Chi square. P less than 0.05 considered to indicate statistical significant. Analyses were performed with SYSTAT 10 for windows (SPSS, Chicago, IL).

Results

The majority of cases were male. Minimum age was 39 yr and maximum was 80 (mean range= 54.34 yr). The lowest grade was G1 (18.7%) and the highest G3 (34.7%).

This cross-sectional study had showed that the majority of cases were in Low Grade and statistical analysis indicated relation between grade and expression of EGFR ($P<0.05$). Meanwhiles, 49.3% of cases were negative in EGFR and 50.7% were positive (Table 1).

Table 1: EGFR immuno-staining in tumour samples according to grade

Histological grade	Number EGFR Negative (%)	Number EGFR Positive (%)	Total n (%)	*P* Value
Grade 1 (LMP)	10 (71.4 %)	4 (28.6%)	14(18.7)	0.027
Low grade	18(51.4 %)	17(48.6%)	35(46.7)	0.027
High grade	9(34.6 %)	17(65.4%)	26(34.7)	0.027

Discussion

In a study, ten cancer types both express elevated levels of EGFR relative to normal tissues and have been showed in sufficient depth to allow sound judgements to be made concerning the association between EGFR and patient outlook (9). The EGFR was found to act as a strong prognostic indicator in head and neck, ovarian, cervical, bladder and oesophageal cancers. In these cancers, increased EGFR expression was

associated with reduced recurrence-free or overall survival rates (9). It has been reported that there was a connection between EGFR expressions, cell proliferation and high tumor grade, and also between EGFR/MIB-1 expression and poor prognosis of bladder cancer. However, the predictive value of EGFR expression is not independent of tumor stage and grade or proliferation (10). In the case of human bladder cancers, the analysis of EGFR content by means of immunohistochemistry (IHC) has suggested that EGFR positively is strongly associated with high-grade and deep-seated tumors and has been proposed as a prognostic marker for tumor progression (11,12).

Our study showed that expression of EGFR had correlation with grade in TCC. Meanwhile, widespread expression of EGFR in Transitional Cell Carcinoma makes this molecule a good target for antigrowth factor or gene therapy.

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