Human Pathology (2017) **61**, 97–104

ELSEVIER

Human PATHOLOGY

www.elsevier.com/locate/humpath

Original contribution

Epidermal growth factor receptor as an adverse survival predictor in squamous cell carcinoma of the penis☆,☆☆

Alice Muglia Thomaz da Silva Amancio PhD[a], Isabela Werneck da Cunha MD, PhD[a], José Ivanildo Neves MSc[a], Josiane da Silva Quetz PhD[b], Dirce Maria Carraro PhD[a], Rafael Malagoli Rocha PhD[a], Stenio Cássio Zequi MD, PhD[c], Antonio Leopoldo Cubilla MD[d], Francisco Paulo da Fonseca MD, PhD[c], Ademar Lopes MD, PhD[c], Maria do Perpétuo Socorro Saldanha da Cunha MD[b], Marcos Venício Alves Lima MD, PhD[e], José Vassallo MD, PhD[a,f], Gustavo Cardoso Guimarães MD, PhD[c], Fernando Augusto Soares MD, PhD[a,g,*]

[a]*Department of Anatomic Pathology, A.C. Camargo Cancer Center, 01508-010, São Paulo, SP, Brazil*
[b]*Department of Pathology, Cancer Institute of Ceara, 60351-010, Fortaleza, CE, Brazil*
[c]*Urology Division, Department of Pelvic Surgical Oncology, A.C. Camargo Cancer Center, 01508-010, São Paulo, SP, Brazil*
[d]*Instituto de Patologia e Investigacion, Universidad Nacional de Asuncion, 1617, Asuncion, Paraguay*
[e]*Department of Urology, Cancer Institute of Ceara, 60351-010, Fortaleza, CE, Brazil*
[f]*Laboratory of Molecular and Investigative Pathology, Faculty of Medical Sciences, State University of Campinas Medical School, 13083-970, Campinas, SP, Brazil*
[g]*General Pathology, Faculty of Dentistry, University of São Paulo, 05508-000, São Paulo, SP, Brazil*

Received 2 March 2016; revised 7 July 2016; accepted 15 July 2016

Keywords:
EGFR;
Penile cancer;
Immunohistochemistry;
FISH;
Prognosis

Summary Penile carcinoma (PC) is more frequent in underdeveloped countries, generally is diagnosed at an advanced stage when therapeutic options are restricted, and thus is associated with high morbidity/mortality rates. Recent studies have demonstrated clinical benefits with epidermal growth factor receptor (EGFR)–targeted therapy in patients with PC, although there is no test that provides accurate patient selection. The aim of the present study was to evaluate the prognostic value of EGFR gene and protein status in tumor samples from patients with primary penile squamous cell carcinoma. We assessed the expression of wild-type and 2 mutant EGFR isoforms (delA746-E750 and mL858R) by immunohistochemistry in 139 samples, of which 49 were also evaluated for *EGFR* copy number by fluorescence in situ hybridization (FISH). Positive immunohistochemical staining of wild-type and mutant EGFR was evidenced by complete and strong membranous staining. For FISH analysis, cases were considered unaltered, polysomic, or amplified, as determined by signals of the *EGFR* gene and chromosome 7. An independent cohort of 107 PC samples was evaluated for mutations in *EGFR*, *KRAS*, and *BRAF*. Protein overexpression was noted in nearly

☆ Competing interests: The authors declare no conflicts of interest.
☆☆ Funding/Support: The work described in this manuscript was supported by grants from FAPESP #2009/52088-3 and #2012/09845-0.
* Corresponding author at: Department of Anatomic Pathology, A.C. Camargo Cancer Center, #109 Antonio Prudente St, Hilda Jacob Building, First Floor, São Paulo, SP 01509-010, Brazil.
E-mail address: fasoares@cipe.accamargo.org.br (F. A. Soares).

http://dx.doi.org/10.1016/j.humpath.2016.07.041

half of the cases and was associated with cancer recurrence (P = .004) and perineural invasion (P = .005). Expression of the 2 mutated EGFR isoforms was not observed. The FISH status was not associated with protein expression. Altered FISH (polysomy and gene amplification) was an independent risk factor for dying of cancer. Only 1 patient of 107 presented *KRAS* mutations, and no mutations of *EGFR* or *BRAF* were observed.

1. Introduction

Penile carcinoma (PC) is relatively frequent in underdeveloped countries such as Brazil, where its diagnosis usually is made in advanced stages of the disease when therapeutic options are limited [1-3]. Distant metastases are rare; the presence of lymph node metastases (LNMs) is the most significant prognostic variable [1,4], as 5-year survival rate declines to less than 40% in patients with tumorous lymph nodes [5].

Several histologic variants of PC have been recognized [6-8], some of which present higher rates of regional metastasis and adverse outcome [1,6,8-10], whereas others rarely invade the lymph nodes or cause death [1,10]. Usually, squamous cell carcinoma (SCC) is the most frequent histologic type, in which the mortality rate and the risk of LNMs are heterogeneous [10]. Other morphologic aspects such as high histologic grade, presence of vascular/perineural invasion, and a dissociative invasion front are associated with a poor prognosis [2,10-14]. Although several biomarkers have been reported as useful predictors of LNMs, few are in wide use.

The molecular pathways involved in penile carcinogenesis are poorly understood. The 2 main molecular subtypes are characterized by the presence or absence of human papillomavirus. As for SCC from other primary sites, there is a chromosomal instability background that results in different patterns of gene alterations. Epidermal growth factor receptor (EGFR) has been implicated as an important target based on reports of the clinical benefit of EGFR-targeted therapy in patients with advanced PC [15-19].

Therapies targeting EGFR may be based on monoclonal antibodies, with similar application of treatment of metastatic colorectal cancer (in patients who harbor wild-type [wt] *RAS* and *BRAF*) and head and neck carcinoma (in which protein overexpression and gene gains are associated with a poor prognosis). Another therapeutic strategy is represented by small tyrosine kinase inhibitors for lung cancer (in patients with activating mutations in exons 19 to 21 of *EGFR*, which encode the tyrosine kinase domain). Patients with increased *EGFR* gene copies may benefit from both kinds of therapy [20-22].

Most PC lesions express EGFR [23-26]. Its phosphorylated form has been associated with activation of the AKT pathway, but not with gene amplification, prognosis, or survival [26]. In a small series of patients with PC, no mutations in the exons that encode the tyrosine kinase domain have been reported, either immunohistochemically [27] or by sequencing [28].

Because EGFR-targeted therapies are promising in the treatment for patients with advanced PC, it is desirable to evaluate this gene and protein further. Hence, it was our purpose to evaluate the status of *EGFR* and the encoded protein in tumor samples from patients with primary penile SCC and their association with patient prognosis. The immunohistochemical expression of wt *EGFR* and of 2 isoforms with activating mutations (delA746-E750 in exon 19 and mL858R in exon 21) was analyzed in a large cohort of patients with PC. A subset of these cases also was tested to determine *EGFR* copy number. In an independent set of patients, mutations in exons 18 to 21, the sequence that encodes the tyrosine kinase domain, were investigated using the cobas EGFR Mutation Test (Roche Molecular Systems, Pleasanton, CA).

2. Materials and methods

2.1. Tissue samples

Paraffin-embedded tissue samples from 139 cases of primary penile SCC with at least 5 years of follow-up were obtained from the files of the Department of Pathology, A. C. Camargo Cancer Center, São Paulo, Brazil. The mean age of the patients was 53.8 years (range, 21-87 years). Most patients were untreated initially and underwent surgery as the primary treatment per our PC protocol [1]. This cohort is part of a larger group of patients already studied by our group [10]. Seven cancers from this cohort had been tested for *EGFR* mutations, as reported elsewhere [28]. Most patients had not undergone circumcision, nor did the tumors present lymphatic or perineural invasion. Tumors were mainly in the T2 and T3 stages according to the American Joint Committee on Cancer TNM system [29]. Other clinical and histologic details are shown in the Supplementary Table.

EGFR mutation was assessed in a different cohort of 107 patients from Instituto do Cancer do Ceara (a state in the northeast part of Brazil that has a higher incidence of PC) to validate our previously published data on *EGFR* direct sequencing [28]. This cohort also was investigated for mutations in downstream effectors of the EGFR pathway, *KRAS* and *BRAF*.

The institutional ethics committee approved this study (Protocol # 1096/08).