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Original contribution

Basal cell carcinoma of the prostate is an aggressive tumor with frequent loss of PTEN expression and overexpression of EGFR☆

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Summary Basal cell carcinoma (also referred to as adenoid cystic carcinoma) is a rare tumor of the prostate. Although largely characterized as indolent, poor outcomes have been reported in a considerable fraction of cases. As yet, optimum treatment strategies for this cancer have not been developed. This study investigates protein expression of common or potential molecular therapeutic targets and reports on the clinicopathological features of 9 new cases. We evaluated the expression of ERBB2, KIT, androgen receptor, PTEN, EGFR, ERG, and p53 via immunohistochemistry. We also examined *EGFR* amplification and *TMPRSS2-ERG* gene rearrangement by fluorescence in situ hybridization. The mean clinical follow-up was 44 months. We found that basal cell carcinoma behaved aggressively with almost one-half of the cases displaying high-risk pathologic features or local recurrence (44%). One patient died as a result of metastatic disease. The most consistent abnormalities included a loss of PTEN expression (56% of cases) and EGFR overexpression (67% of cases). EGFR overexpression occurred in the absence of gene amplification. The *TMPRSS2-ERG* rearrangement was not detected in any of the tumors studied, nor was ERG protein positivity identified by immunostaining. In addition, ERBB2, KIT, p53, and androgen receptor expressions were either absent or showed only weak, limited reactivity. Our results suggest that there is a high morbidity associated with this tumor, and more intense follow-up and additional treatment may be indicated. Furthermore, targeted therapies directed against the EGFR and PTEN proteins or their constitutive pathways may be promising for future clinical management.

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1. Introduction

Although it has been 40 years since Frankel and Craig's initial report, current understanding of basal cell carcinoma of the prostate remains limited [1]. Partly, this knowledge gap is due to the rarity of the tumor; less than 100 cases have been reported to date [1-14]. However, the varied histologic manifestations of basal cell carcinoma have also clouded the issue. The first pattern resembles basal cell carcinoma of the skin, composed of nests of cells with pale, microvesicular nuclei, little cytoplasm, and peripheral palisading, similar to the cytologic features of basal cell hyperplasia. The adenoid cystic pattern closely resembles its salivary gland namesake, composed of cystically dilated acini and cribriform glands with lumina containing eosinophilic hyalinized material and basophilic mucinous secretions. The 2 patterns are currently recognized as parts of the same spectrum of disease and have been merged into a single category in the current World Health Organization classification of tumors [15].

Despite being largely regarded as pursuing an indolent clinical course, death from disease has been reported in almost 10% of published cases, and other aggressive behaviors, such as recurrence or high tumor stage, have been present in an even higher proportion of patients [3,4,7,8]. Standardized nomenclature and diagnostic criteria have eluded pathologists until lately. It is no wonder then, given the lack of general experience with the tumor and ambiguity in the literature that not only is there limited understanding of the natural behavior of this entity but also no certainty as to the best course for treatment. With the growing armamentarium of molecular therapies available, we decided to evaluate some of the most common targets that have pharmaceutical treatments (*ERBB2, EGFR, KIT*, and androgen receptor) as well as other genes frequently mutated in carcinomas (*TP53, PTEN*, and *ERG*) to determine if there is a role for these or future medications in the management of basal cell carcinoma.

2. Methods

2.1. Cases

Nine cases of basal cell carcinoma of the prostate were gathered from the surgical pathology files of the participating institutions. None of the cases has been previously reported in the literature. Demographic and clinical information was obtained from medical records or by contacting the consulting pathologists. Hematoxylin and eosin–stained slides for all cases were reviewed to confirm the diagnosis. This study was approved by the Indiana University Institutional Review Board.

2.2. Immunohistochemistry

Immunohistochemical staining was performed on all cases on 5-μm-thick sections of formalin-fixed, paraffin-embedded tissue using the peroxidase-labeled streptavidin-biotin method. ERBB2 and EGFR antigen retrieval were performed according to kit instructions. The antibodies used and expected staining patterns are listed in Table 1. Positive and negative controls gave appropriate results for each procedure. Staining intensity was evaluated on the following scale: negative, absent staining or staining in less than 1% of tumor cells regardless of intensity; 1+, weak staining (visible at intermediate magnification [×100] but only well delineated at higher magnification [×200]); 2+, moderate staining (obvious at intermediate magnification [×100]); and 3+, strong staining (clearly defined at low magnification [×40]). The percentage of cells staining for each antibody was also recorded. All immunohistochemical stains were evaluated independently by 2 reviewers (N. S. and L. C.). Immunohistochemical staining was performed on either transurethral resection specimens (cases 1, 3-7, and 9) or the radical prostatectomy specimens (cases 2 and 8) to maximize the amount of tumor available for evaluation.

2.3. Fluorescence in situ hybridization

Fluorescence in situ hybridization (FISH) analysis to investigate *EGFR* amplification and *TMPRSS2-ERG* gene rearrangement was performed, as previously described [16,17]. Briefly, multiple 4-μm sections were obtained from formalin-fixed, paraffin-embedded tissue blocks containing neoplastic tissue. A hematoxylin and eosin–stained slide from each block was examined to identify areas of neoplastic tissue for FISH analysis. The slides were deparaffinized with two 15-minute washes in xylene and subsequently washed twice with 100% ethanol for 10 minutes each and air dried. The sections were heated at 95°C in 0.1 mM citric acid (pH 6) solution (Invitrogen, Carlsbad, CA) for 10 minutes, rinsed with distilled water for 3 minutes, and washed with 2× saline-sodium citrate (SSC) for 5 minutes. Tissue digestion was performed by applying 0.4 mL of pepsin (Sigma, St Louis, MO) solution (4 mg/mL in 0.9% NaCl in 0.01 N HCl) to each slide and incubating the slides in a humidified box for 40 minutes at 37°C. The slides were rinsed with distilled water for 5 minutes, washed with 2× SSC for 5 minutes, and then air dried. A probe cocktail for *EGFR* FISH was performed with centromeric α-satellite DNA probes for chromosome 7 (Centromeric Enumeration Probe, CEP 7, Spectrum Green; Vysis, Downers Grove, IL) and with probes for the *EGFR* gene at chromosome 7p12 (*EGFR*, Spectrum Orange; Vysis). The probe cocktail for *TMPRSS2-ERG* included BAC clones RP11-476D17-gold (3′ *ERG*), RP11-95I21-green (5′ *ERG*), and RP11-35C4-aqua (*TMPRSS2*) (Empire Genomics, Buffalo, NY). The *EGFR* and *ERG* probes were diluted with tDenHyb2 (Insitus, Albuquerque, NM) in a ratio of 1:50 and 1:25, respectively. Five microliters of diluted probe was applied to each slide; coverslips were placed over the slides and sealed with rubber cement. The slides were denatured at 80°C for 10 minutes