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Overexpression of Insulin-like Growth Factor-1 Receptor is Associated with Penile Cancer Progression

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Abstract

Objective—To evaluate insulin-like growth factor-1 receptor (IGF1R) expression in penile cancer and its association with oncologic outcomes.

Methods—Tissue microarrays were constructed from 53 patients treated at our institution. Expression of IGF1R was evaluated using a Her2-like scoring system. Overexpression was defined as 1+ or greater membranous staining. Association of IGF1R expression with pathologic features was assessed with comparative statistics, and association with local recurrence, progression to nodal or distance metastases or death was assessed with Kaplan-Meier survival analysis and Cox proportional hazard regression models.

Results—Overall, IGF1R overexpression was seen in 33 (62%) cases. With a median follow-up of 27.8 months, IGF1R overexpression was associated with inferior progression-free survival (PFS) ($p=0.003$). In a multivariable model controlling for grade, T stage, PNI and lymphovascular invasion, IGF1R expression was independently associated with disease progression (HR 2.3, 95% CI 1.1–5.1, $p = 0.03$). Comparing patients without IGF1R over-expression to those with over-expression, 5 year PFS was 94.1% vs 45.8%.

Conclusions—IGF1R over-expression was associated with inferior PFS in penile cancer. Drugs that target IGF1R and downstream messengers may have a therapeutic benefit in patients that exhibit IGF1R over-expression.

Keywords

penile cancer; biomarkers; prognostics; risk stratification

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Introduction

Penile cancer is a relatively rare malignancy in North America, with an estimated 1,640 new diagnoses and 320 deaths in the United States in 2014.¹ Over 95% of penile cancers are squamous cell carcinoma (SCC), which are associated with several known risk factors including the presence of foreskin, phimosis, infection with human papilloma virus (HPV), lichen sclerosis et atrophicus (balanitis xerotica obliterans) and smoking.^{2–4}

Oncologic outcomes in penile cancer are driven by tumor grade and stage, with the presence of inguinal nodal metastases being the most important prognostic factor for survival.^{5, 6} While localized penile SCC without nodal involvement has a 5-year survival rate of 95%, cancers that metastasize to inguinal lymph nodes have a 5-year survival of 76%, while pelvic lymph node involvement has a 5-year survival of less than 10%.⁷ Currently, controversy exists as to which patients with clinically negative lymph nodes (cN0) should undergo inguinal lymph node dissection (ILND). The National Comprehensive Cancer Network (NCCN) recommends ILND in intermediate (T1b) and high risk (any T2 or grade 3–4) cN0 disease.⁸

Multiple molecular markers have been investigated in penile cancer, including HPV, p53, P16^{INK4a}, SCC antigen, Ki-67, E-cadherin and MMP as recently reviewed by Muneer *et al.*⁹ While both SCC antigen and Ki-67 expression are associated with increased risk of lymph node involvement, neither marker is predictive of disease progression nor routinely used in clinical practice.

Insulin-like growth factor-1 receptor (IGF1R) is a transmembrane tyrosine kinase receptor that is over-expressed in several malignancies, including urothelial¹⁰, prostate¹¹, breast¹², colorectal¹³ and lung cancer.¹⁴ IGF1R plays a critical role in cell proliferation, differentiation and malignant transformation.¹⁵ The role of IGF1R in penile SCC carcinogenesis is unknown.

Because of a paucity of prognostic molecular markers in penile cancer, we sought to evaluate the expression of IGF1R in penile SCC and to determine if IGF1R expression is associated with oncologic outcomes.

Methods

Patient cohort

Our institutional review board-approved penile cancer database (1985 to 2013) was retrospectively queried for cases of penile cancer treated at our institution with archival tissue from the primary lesion available for review. Out of 89 identified cases, 36 did not have archival tissue available, leaving 53 to form our study cohort. The cases underwent pathological re-review by two genitourinary pathologists (S.M.B and G.J.N.). Representative formalin-fixed, paraffin-embedded archival blocks were used to construct high-density tissue microarrays (TMAs).¹⁶ Tumors and paired non-neoplastic tissue were spotted 3 to 5 times each using 1.6-mm cores.

Treatment algorithm

Patients with biopsy proven penile cancer were counseled to undergo partial penectomy when technically amenable. For proximal tumors, total penectomy was recommended. Proximal lesions with concomitant urethral involvement were recommend to undergo pelvic exenteration. Bilateral inguinal lymph node dissection was recommended for patients with with stage pT1b lesions or patients with palpable lymphadenopathy. A bilateral, modified ILND as described by Catalona was performed in these cases.¹⁷ Sentinel node biopsy is not utilized at our institution.

IGF1R Expression Analysis

IGF1R expression was evaluated by immunohistochemistry (G11, Ventana Medical Systems) by two genitourinary pathologists (S.M.B., and G.J.N). Membranous staining was assessed using a scoring system analogous to HER2 staining in breast carcinoma: score 0, no staining or staining in <10% of tumor cells; 1+, incomplete staining in >10% of tumor cells; 2+, moderate and complete membranous staining in >10% of tumor cells or strong and complete membranous staining in <30% of tumor cells; and 3+, strong and complete membranous staining in >30% cells.¹⁸ The highest score at the TMA spots was assigned as the case's score and used for statistical analysis. Cases with IGF1R score 1+ were considered to show IGF1R over-expression as in previous studies.^{19, 20}

Statistical analysis

Descriptive statistics were used to summarize patient characteristics and pathologic features. Continuous variables were compared with the Wilcoxon rank-sum or Kruskal-Wallis tests and categorical variables with Fisher's exact test.

Survival analysis was performed using the Kaplan-Meier method to determine recurrence-free survival (RFS), progression-free survival (PFS) and overall survival (OS). The log rank test was used to compare survival curves. For the purposes of this analysis, recurrence was defined as development of a new tumor in the same location as the prior tumor, while progression was defined as the development of local or distant metastasis after the primary treatment. All patients were prescribed a follow-up regimen based on the National Comprehensive Cancer Network guidelines with physical exam every 3–6 months depending on nodal stage. Cancer and vital status were determined by both clinical follow-up at our institution and by a query of the Social Security Death Index.

Multivariable Cox proportional hazard models, including IGF1R expression, patient age, tumor grade, stage, lymphovascular invasion (LVI), and perineural invasion (PNI), were constructed to adjust for potential confounding. Staging was based on the American Joint Committee on Cancer, 7th edition²¹. All statistical analysis was performed with Stata 13 (StataCorp, College Station, TX). Two-sided p values < 0.05 were considered significant.

Results

Overall 33 (62%) cases demonstrated IGF1R overexpression. The distribution of IGF1R expression scores were: 0, 20 cases (38%); 1+, 22 (41%); 2+, 8 (15%); 3+, 3 cases (6%). Figure 1 depicts representative IGF1R staining patterns.

The clinicopathological characteristics of the cohort are summarized in table 1. Overexpression of IGF1R was associated with younger age ($p = 0.02$), increasing pathologic T stage ($p = 0.005$), LVI ($p = 0.01$), PNI ($p = 0.01$), and increasing nuclear grade ($p < 0.0001$).

At a median follow-up of 27.8 months, there were 4 (8%) patients that experienced local recurrence, 18 (34%) with progression to nodal or distant metastases, and 29 (55%) that died. Among patients with IGF1R over-expression, there were 16 progression events compared to 2 in patients without IGF1R over-expression ($p = 0.006$).

On Kaplan-Meier survival analysis, overexpression of IGF1R was associated with inferior PFS ($p = 0.003$) (Figure 2) and cancer-specific survival (CSS) (0.03), but not recurrence ($p = 0.3$) or overall-survival ($p = 0.1$) (not shown). CSS and PFS were further analyzed in multivariate models proportional hazard models. In unadjusted Cox proportional hazard models, the median IGF1R expression score was associated with progression (HR 3.8, 95%CI 1.9–7.6, $p < 0.0001$), but not death from disease ($p = 0.1$). After adjusting for potential confounders, IGF1R over-expression remained an independent predictor of progression (HR 2.3, 95%CI 1.1–5.1, $p = 0.03$) but not CSS (HR 1.0, 95%CI 0.4–2.8, $p = 0.9$) (Table 2). “Among the subset of patients who underwent inguinal lymph node dissection, after controlling for nodal status, IGF1R overexpression remained an independent predictor of progression (HR 2.8, 95%CI 1.1 – 7.3, $p = 0.03$).”

Discussion

Because of a lack of molecular prognostic markers in penile cancer, we evaluated the association of IGF1R expression and oncological outcomes in patients with penile cancer. We found that IGF1R expression was associated with pathologic features of disease aggressiveness and inferior PFS. Furthermore, after adjusting for pathologic features, IGF1R expression remained independently predictive of progression to nodal and metastatic disease. If confirmed in additional cohorts, our finding is of potential prognostic value and would be of value in patient risk-stratification.

The role of IGF1R in malignant transformation and cell growth has been well studied in multiple solid malignancies and cell lines.^{22, 23} After binding of IGF1R by its ligands insulin-like growth factor-1 or -2 (IGF1, IGF2), two downstream pathways are activated: the Ras-Raf-ERK/MAPK pathway and phosphoinositide 3-kinase (PI3K/AKT) pathway.^{15, 24} Activation of these pathways results in decreased apoptosis and increased mitogenic activity.^{25, 26} Dysregulation of these pathways have been associated with both penile cancer development and progression.²⁷ However, ours is the first study to analyze IGF1R expression in penile cancer.

Previous molecular markers including SCC antigen and Ki-67 have been correlated with disease burden and the presence of inguinal nodal metastases⁹; however, these markers were unable to predict the development of nodal or distant disease or survival.²⁸ In contrast, IGF1R expression was predictive of disease progression in our study.

Several studies have evaluated IGF1R as a potential drug target, utilizing monoclonal antibodies to target the extracellular domain and tyrosine kinase inhibitor to target the intracellular kinase domain.²⁹ In preclinical models, IGF1R monoclonal antibody therapy has resulted in inhibition of breast³⁰, pancreatic, renal cell and colorectal tumors in xenograft models³¹. Both monoclonal antibody and small molecular inhibitors are currently being investigated in phase 1 clinical trials. Early phase 1 results support safety and tolerability of the drugs.^{32, 33} Our finding of IGF1R overexpression in a subset of penile squamous cell carcinoma and its association with aggressive disease would suggest IGF1R merits assessment as a potential therapeutic target for this malignancy.

Limitations of this study include the relatively small sample size and retrospective nature of the study; however, given the low incidence of penile cancer, this is not unexpected for an initial study. To be of clinical benefit, these findings will ultimately need confirmation in other cohorts and ultimately in a multi-institutional, prospective study. Additionally, the use of TMAs, rather than whole tissue section, could be considered a limitation, as tissue heterogeneity may impact IGF1R expression. However, the use of multiple tissue spots to insure adequate tissue representation is well-supported.³⁴ Additionally, the referral patterns of tertiary cancer centers mean that many patients choose to follow-up with their referring physician which could limit the detection of progressive disease and death. This limitation is tempered by the thorough follow-up augmented by the institutional cancer registry. Lastly, although potential confounding factors were included in the multivariable analysis, it is possible that additional features not accounted for influence survival and recurrence in this cohort of patients.

Conclusion

Overexpression of IGF1R was seen in 62% of a North American cohort of men with penile cancer and was associated with inferior progression-free survival. If confirmed in additional cohorts, IGF1R may be a useful prognostic adjunct to traditional histopathological staging.

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Figure 1. Insulin-Like Growth Factor-1 Receptor (IGF1R) immunohistochemical membranous expression in penile squamous cell carcinomas. A, absence of expression (0); B, weak intensity (1+); C, moderate intensity (2+); D, strong intensity (3+).

Figure 2.
(Progression-Free Survival and by IGF1R over-expression. Membranous staining of 1+ or greater was considered over-expression.

Table 1

Demographic and clinicopathologic characteristics of 53 penectomy patients

	Entire Cohort (n=53)	No IGF1R Expression < 1+ (n=20)	IGF1R Expression 1+ (n=33)	P value
Age at surgery,	65 (57–75)	70 (64–78)	62 (56–73)	0.02
Race, n (%)				0.3
White	37 (69.81)	12 (60)	25 (75.8)	
Black	12 (22.64)	5 (25)	7 (21.2)	
Other	4 (7.55)	3 (15)	1 (3)	
Intervention				0.3
Partial penectomy	48 (90.6)	19 (95)	29 (87.9)	
Total penectomy	2 (3.8)	0	2 (6.1)	
Shave biopsy	1 (1.9)	1 (5)	0 (0)	
Pelvic exenteration	2 (3.8)	0	2 (6.1)	
Histologic Subtype				0.09
Usual	33 (62.26)	10 (50)	23 (70)	
Warty	5 (9.43)	2 (10)	3 (9)	
Basaloid	6 (11.32)	2 (10)	4 (12)	
Warty-basaloid	2 (3.77)	0	2 (6)	
Verrucous	3 (5.66)	3 (15)	0	
Papillary	2 (3.77)	2 (10)	0	
Other	2 (3.77)	1 (5)	1 (3)	
pT Stage				0.005
Ta	1 (1.89)	1 (5)	0	
T1a	17 (35.85)	12 (60)	7 (21)	
T1b	1 (1.89)	1 (5)	0	
T2	22 (41.51)	5 (25)	17 (52)	
T3	9 (16.98)	1 (5)	8 (24)	
T4	1 (1.89)	0	1 (3)	
+ LVI	17 (32.08)	2 (10)	15(45.5)	0.01
+ PNI	17 (32.08)	2 (10)	15(45.5)	0.01
Grade				< 0.0001
Low	11 (20.75)	10 (50)	1 (3)	
Intermediate	24 (45.28)	8 (40)	16 (48.5)	
High	18 (33.96)	2 (10)	16 (48.5)	
Lymph node dissection				0.16
None	31 (58.5)	15 (75)	16 (48.5)	
Prophylactic	10 (18.9)	2 (10)	8 (24.2)	
Post-surgical	12 (22.6)	3 (15)	9 (27.3)	
Adjuvant Chemotherapy	6 (11.3)	0	6 (18.2)	0.1

	Entire Cohort (n=53)	No IGF1R Expression < 1+ (n=20)	IGF1R Expression 1+ (n=33)	P value
Adjuvant Radiation	6 (11.3)	0	6 (18.2)	0.1

All values are expressed in medians (interquartile range) unless otherwise specified

LVI: lymphovascular invasion; PNI: perineural invasion

Table 2

Cox proportional hazard model for progression or death from disease by IGF1R over-expression.

	Progression HR (95% CI)	P value	Death from disease HR (95%CI)	P value
Unadjusted median IGF1R expression	3.8 (1.9–7.6)	< 0.0001	1.8 (0.9–3.6)	0.1
Adjusted median IGF1R expression *	2.3 (1.1–5.1)	0.03	1.0 (0.4–2.8)	0.9

* Adjusted for grade, T stage, presence of lymphovascular invasion, perineural invasion