



Case Study

Next-Generation Sequencing of Locally Advanced Squamous Cell Penile Carcinoma Reveals Novel Druggable Targets

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ABSTRACT

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Background

Penile squamous cell carcinoma (PSCC) is a relatively rare cancer that originates in the epithelium of the inner prepuce and glans. The underlying biology and molecular drivers of PSCC are poorly understood.

Case Presentation

A 58-year-old male presented with penile lesion and hematuria prompting imaging studies. A positron emission tomography (PET) scan showed hypermetabolic lymph nodes involving the left internal iliac chain and left inguinal lymph nodes. He underwent computed tomography guided lymph node biopsy and pathologic examination revealed squamous cell carcinoma. Immunohistochemical (IHC) analysis showed the tumor demonstrates strong p63, CK5 and p16 positivity and negative for CK7, CK20, and GATA-3, consistent with poorly differentiated squamous cell carcinoma. Clinical targeted-exome sequencing (Foundationone) identified multiple gene alterations in PI3K and DNA damage repair pathway.

Conclusions

While alterations in PIK3CA, and EGFR are common in squamous cell penile carcinoma, we report additional novel genomic alterations for the first time in a newly diagnosed advanced squamous cell penile carcinoma. Our study further highlights the value of comprehensive genomic analyses in identifying patient-specific novel druggable targets.

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INTRODUCTION:

Penile squamous cell carcinoma (PSCC) is a relatively rare cancer that originates in the epithelium of the inner prepuce and glans. Although rare in developed countries, it is a significant health problem in developing countries [1-3]. In comparison to squamous cell carcinomas of other sites, the underlying biology and molecular drivers of PSCC are relatively poorly understood. [4-5] Patients with local and low-stage nodal metastasis can be treated with laser therapy, surgery, or radiation. Locally advanced or metastatic disease are treated with combination of surgery, radiation and chemotherapy.[6-12] Systemic chemotherapy for metastatic PSCC yields poor outcomes, with a median overall survival (OS) of 6 to 9 months [10]. Second-line chemotherapy with taxanes is marginally active [10, 11]. Epidermal growth factor receptor (EGFR) inhibitors have exhibited modest activity [13–15]. Hence, substantial advances in systemic therapy are likely to emerge only if clinical trials are informed by improved knowledge of tumor biology.

Next-generation sequencing (NGS) technology is rapidly evolving, and has begun to unravel the unique complexities of genitourinary cancer such as PSCC [16,17]. Moreover, advances in technological capacity and bioinformatics have made it possible to use genomic information to guide therapeutic decision for individual cancer patients, based on their tumor's unique molecular alterations that can be specifically targeted with available drugs.

Here we describe a case of PSCC for which NGS of a tumor biopsy revealed several novel genomic alterations including PI3K E545K mutation and rearrangement as well as alteration in INPP4B, a tumor suppressor of the PI3K pathway. In addition, we also report mutations in gene DNA damage repair pathway such as RAD50, BRIP1 that had not previously been implicated in PSCC. This report demonstrates the clinical value of using NGS to find targeted therapies for patients with rare genitourinary cancer malignancies such as PSCC.

CASE PRESENTATION

A 58-year-old man presented to community emergency department complaining of scrotal swelling, penile lesion and hematuria. Because the lesions were suspicious for squamous cell carcinoma (Figure 1), the patient was referred to urology for further medical attention.

The patient's medical history included hypertension, a cerebral vascular accident with residual right-sided

weakness secondary to uncontrolled Diabetes mellitus (DM) in 2015, chronic kidney disease, and genital warts. He reported that he smoked one pack of cigarettes per day for more than 30 years.

Laboratory tests revealed prostate-specific antigen 0.40 ng/mL and negative urine analysis. All other laboratory values were within normal range except decreased glomerular filtration rate (GFR). A CT scan of the chest, abdomen, and pelvis revealed significant pelvic and inguinal adenopathy. A positron emission tomography (PET) scan revealed hypermetabolic lymph nodes involving the left internal iliac chain with a max SUV of 14.8. There are several enlarged left inguinal lymph nodes with a max SUV of 10.7, as well as sub cm hypermetabolic right inguinal lymph node. (Figure 2).

The patient underwent biopsy of the penile lesion which revealed invasive, poorly differentiated squamous cell carcinoma. He subsequently underwent computed tomography guided left external iliac lymph nodes biopsy, the pathology consistent with metastatic squamous cell carcinoma. Immunohistochemical (IHC) analysis showed the tumor demonstrates strong p63, CK5 and p16 positivity and negative for CK7, CK20, and GATA-3, consistent with poorly differentiated squamous cell carcinoma. (Figure 3).

The patient was advised to have a total penectomy, bilateral groin dissection, and pelvic lymphadenectomy. He declined these procedures. His case was discussed at a multidisciplinary tumor board meeting. Considering his multiple comorbidities, impaired kidney function and poor tolerance, the multidisciplinary team (MDT) recommended a combination of anti-EGFR and chemotherapy. Due to concern of side effects, patient elected to pursue anti-EGFR treatment (Panitumumab) alone.

Figure 1: Photography of penile lesion.



Figure 2: PET-CT at the time of disease presentation demonstrated enlarged cluster of lymph nodes involving the left internal iliac chain with a max SUV of 14.8 (Fig 2A). There are several enlarged left inguinal lymph nodes with a max SUV of 10.7 (Fig 2B).

Fig 2A

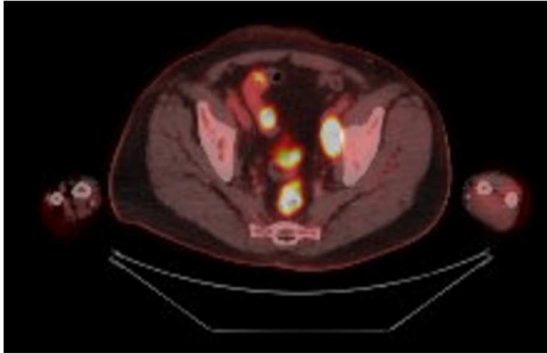


Fig 2B

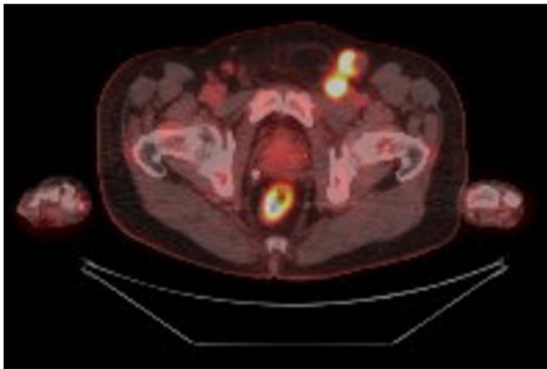


Figure 3: Histopathological examination of the left external iliac lymph node core biopsy show lymph nodes revealed poorly differentiated squamous cell carcinoma. Haematoxylin and eosin (HE) staining (Fig 3A), Immunohistochemical (IHC) analysis showed the tumor demonstrates strong p63 (Fig 3B).

Fig 3A

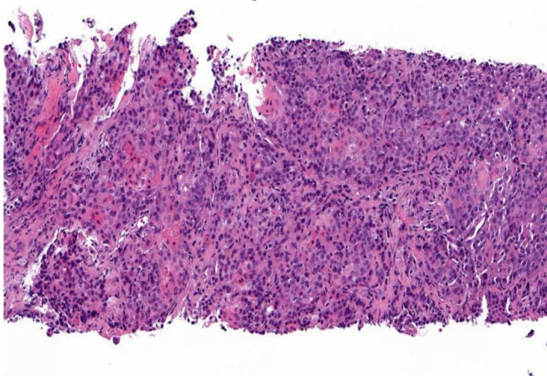
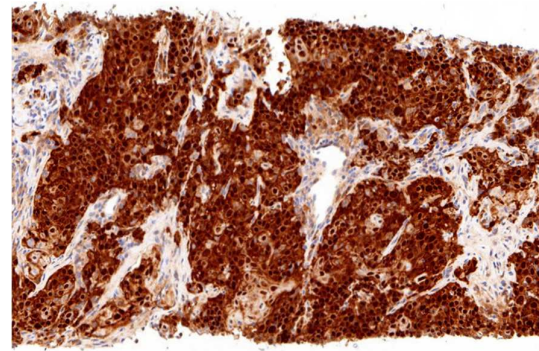


Fig 3B



Next-Generation Tumor Profiling

Tissue from a left external iliac lymph node biopsy was analyzed through a commercial next generation sequencing (NGS) based assay, which included the complete coding DNA sequences of 406 genes, as well as selected introns of 31 genes involved in rearrangements (Foundation One, Boston, MA). The mutational analysis revealed gene alteration in PIK3CA (E545K) and PIK3CA rearrangements. Additionally, one or more variants of unknown significance (VUS) in 12 genes were detected in this patient's tumor, including: TP53, RAD50, BRIP1, TSC1, KIT, NOTCH1. The Tumor Mutation Burden (TMB) was 4 Muts/Mb.

DISCUSSION

PSCC is a highly aggressive malignancy characterized by early locoregional spread with tendency of subsequent distant dissemination [1-5]. Long-term survival is rare for patients with PSCC involving the inguinal or pelvic lymph nodes. [6-10] The treatment of advanced PSCC represent an unmet need.[10]

Incorporating genomic analyses into clinical management of penile carcinoma patients may provide an avenue for identifying novel targets. Three recent investigations [18-20]with modest sample size (tumors from 11- 43 patients) reported gene alterations in EGFR, PIK3CA, NOTCH1, CDKN2A, CCND1, AR, JAK2, JAK3, ALK, PTEN and BRCA. A comprehensive genomic profiling in a cohort of 20 patients with advanced PSCC demonstrated twenty-five percent of patients with advanced PSCC harbor alterations in PIK3CA, these patients harboring PIK3CA and/or FBXW7 alterations (30%) did not also harbor EGFR amplifications (25%) because these groups of alterations occurred in a mutually exclusive fashion[18]. Our findings appear partly concordant

with previous studies suggesting the potential importance of signaling in the PI3K pathway, and deficits in DNA damage repair due to RAD50 alterations. Interestingly, we noted PIK3CA rearrangement in addition to E545K mutation, as well as alteration in INPP4B, a tumor suppressor of the PI3K pathway in this patient. The phosphatidylinositol 3-kinase (PI3K)/Akt/mammalian target of rapamycin (mTOR) signalling cascade plays a very important role in HPV-induced carcinogenesis by acting through multiple cellular and molecular events [21,22]. PIK3CA activating mutations or amplification may lead to activation of the PI3K/AKT/mTOR pathway. Therefore, inhibitors of PI3K, AKT, and/or mTOR may provide clinical benefit for patients with tumors harboring PIK3CA mutation or amplification. Given its high frequency in PSCC, the PI3K has raised interest as both a diagnostic and potentially druggable therapeutic target.

Another interesting finding in our case is the identification of molecular alteration to the DNA damage repair pathway. Mutations in were identified in RAD50, BRIP1 (BRCA1 Interacting Protein C-terminal Helicase 1), these genes had not previously been implicated in PSCC. In this context, it is known that cisplatin-based chemotherapy appears to be the most optimal first-line regimen, which accords with the presence of defects in DNA damage repair engendered by BRCA alterations [12]. Additionally Poly (ADP-ribose) polymerase (PARP) inhibitors may warrant evaluation in those patients with somatic BRCA, RAD50, BRIP1 alterations. As genome wide sequencing strategies become more widely available in the clinical setting, infrequent mutations and/or rare fusion events may collectively represent a PSCC patient population that can be managed with targeted therapies approved for use in other tumor types. This strategy may pave the way for improving outcomes in a small subset of penile carcinoma patients.

CONCLUSION

Herein we describe a case of PSCC, for which NGS of a tumor biopsy revealed several novel genomic alterations that had not previously been implicated in PSCC. This report demonstrates the clinical value of using NGS to find targeted therapies for patients with rare genitourinary cancer malignancies such as penile carcinoma. Our case study further highlights the value of comprehensive genomic analyses in

identifying patient-specific targetable mutations and rearrangements.

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