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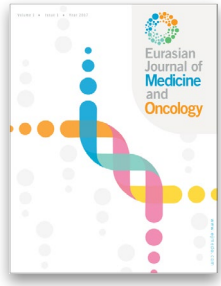

Case Report

Immunotherapy Combined with Microwave Ablation and Radiotherapy Reversed the Biological Behavior of Penile Squamous Cell Carcinoma: A Case Report and Literature Review

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Abstract

Penile squamous cell carcinoma (pSCC) is a rare malignancy of urinary system. Patients with advanced and metastasized pSCC are associated with poor prognosis due to the limited treatments options of pSCC. In our case report, we describe a patient diagnosed with early stage (stage IIA) and well differentiated pSCC. However, the well differentiated penile squamous cell carcinoma had showed an unexpected aggressiveness. The patient had two times rapid relapse with local advanced and metastasis after two radical operation. As received multimodal treatments including chemotherapy, immunotherapy and local ablation therapy and radiotherapy, the patient had got progression free survival (PFS) exceeding 26 months and overall survival (OS) exceeding 44 months. Immunotherapy combined with ablation therapy and radiotherapy may reverse the biological behavior of penile squamous cell carcinoma.

Keywords: Immunotherapy, microwave ablation (MWA), multimodal treatments, penile cancer, penile squamous cell carcinoma (pSCC), radiotherapy

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Penile cancer (PeCa) is a rare disease representing 0.4–0.6% of all male malignant cancers, and more than 95% of patients were histologically classified as penile squamous cell carcinoma (pSCC).[1] PSCC primarily presents as a painless ulcer or lump on the glans penis, recognized by invasive growth and early lymphatic metastasis.[2,3] As for

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locally advanced or metastatic pSCC, a multidisciplinary approach is usually required, including surgical resection, radio-therapy, ablation, and systemic therapy.[4,5] As so far, platinum-based regimens were most commonly adopted in penile cancer treatment,[6] including cisplatin, carboplatin, irinotecan, fluorouracil, paclitaxel and ifosfamide.[7-19] However, randomized data might be needed in current chemotherapeutic regimens to support an overall survival benefit.

Recent case reports have shown that epidermal growth factor receptor (EGFR) might be potential therapeutic targets in pSCC.[20,21] However, studies have also shown that anti-EGFR tyrosine kinase inhibitor including panitumumab and cetuximab has limited response in pSCC.[21,22] In addition, immune check point inhibitors have occupied an important place in many solid tumors over past decade. Cemiplimab and nivolumab have already been approved for treating squamous-cell cancer of skin and head and neck.[23,24] Cocks M et al. and Udager AM et al. reported frequent PD-L1 expression at respectively 40% of 53 and 62% of 37 in penile cancer.[25,26] Therefore, immunotherapies targeting the PD-1 and PD-L1 pathways, are promising treatment for penile cancer. Some case reports present benefits from treating with pembrolizumab, toripalimab, nivolumab and atezolizumab, such as better overall survival (OS), progression free survival (PFS). However, randomized data and guideline are still absent.

Here we report a recurrent metastatic pSCC patient who obtained partial response (PR) from multimodal treatments, including surgery, chemotherapy, immunotherapy, radiotherapy and microwave ablation (MWA) therapy, with a PFS exceeding 26 months and OS exceeding 44 months.

Case Report

A 38-year-old male was phimosis inborn and diagnosed with well to moderately differentiated pSCC by biopsy after finding penis neoplasm 4 years ago. On April 2018 he had undergone partial penectomy. The surgical margin was negative with no distant metastasis or metastasis of inguinal lymph nodes observed. The clinical grade was cT2N0M0 IIA according to Adapted from American Joint Committee on Cancer (AJCC) Staging Manual, 8th edition. On June 2018 he further received penectomy and inguinal lymph node dissection as he presents with a local relapse. Pathological results demonstrated well differentiated pSCC with negative of incision, lymphovascular or inguinal lymph nodes involvement (0/21 on left groin and 0/10 on the right groin). 1 month after surgery, he complained of radiating pain from the left hip region and left leg. After 6 months, computed tomography (CT) (Fig. 1) and magnetic resonance imaging (MRI) (Fig. 2) scan revealed massive bone destruction of left ischium and pubis accompanied with soft tissue swelling. At the same time a positron emission tomography computed tomography (PET/CT) showed the same tumor invasive sign in the left ischium and pubis. No other metastases were observed on the imaging examinations. Then he received extensive left-side pelvic tumor resection and hip arthroplasty. After baseline measured by magnetic resonance imaging (MRI) (Fig. 2), he was administered combination therapy including taxane-based chemotherapy, toripalimab, microwave ablation (MWA) and radiotherapy. Total 6 courses chemotherapy regimens consisted of 135mg/m^2 paclitaxel liposome on day 1 and day 8; 0.6g/m2 carboplatin on day 1 and 5mg/m^2 bevacizumab on day 1. Toripalimab and zoledronic acid using by 3mg/m^2 and 4 mg respectively, has been added to the therapeutic regimen since second course of chemotherapy. After the multimodal treatment, the pain ameliorated considerably while the MRI showed that the size of tumor-like soft tissue expanded at 2 months after the first course of chemotherapy. It was con-

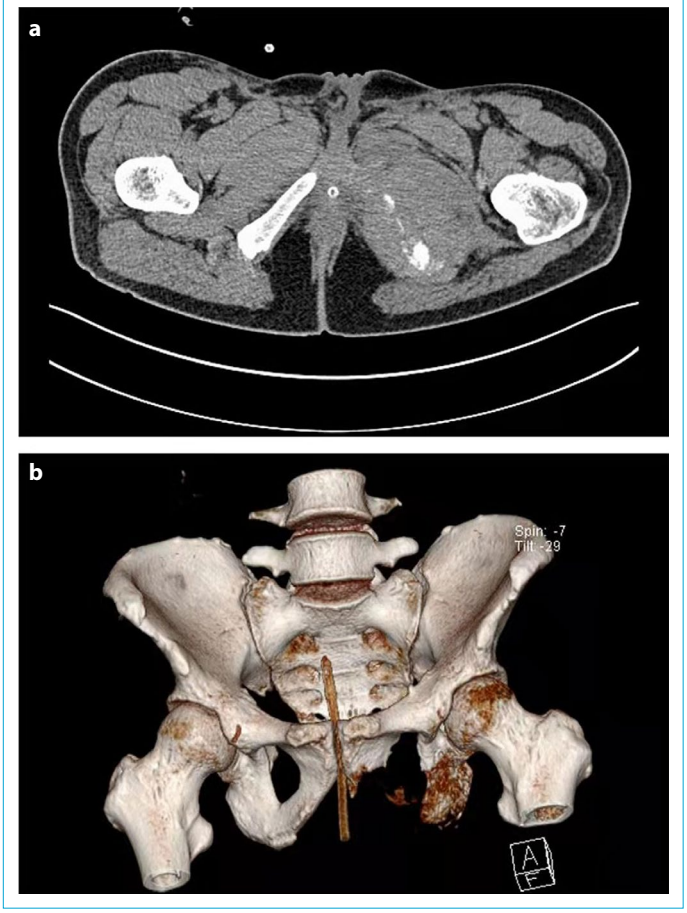

Figure 1. CT. Tumor lesion in the left ischium and pubis for the second relapse. **(a)** Cross sectional place. **(b)** Three-dimensional reconstruction.

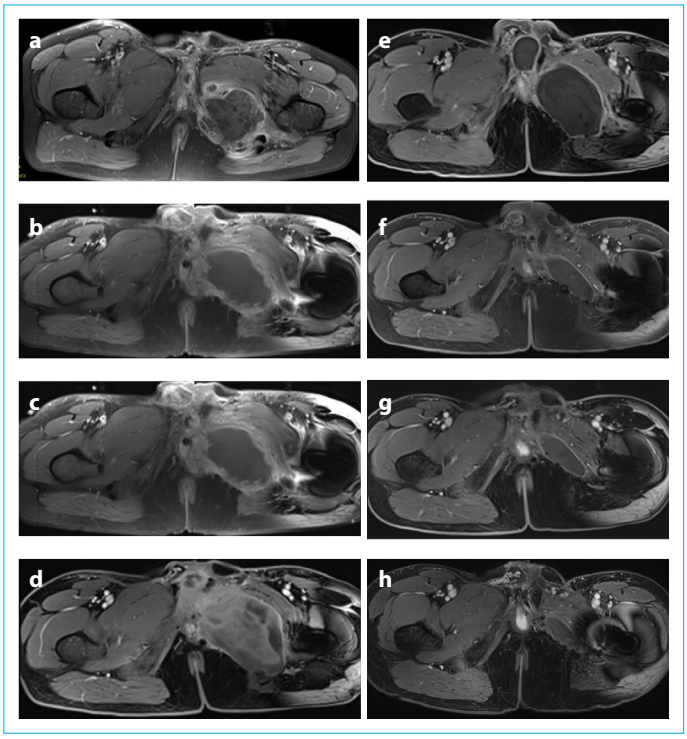

Figure 2. MRI. Tumor lesion in the left ischium and pubis before **(a)** and after surgery **(b)**, or after **(c)** 2 months, **(d)** 5 months, **(e)** 11 months, **(f)** 18 months, **(g)** 21 months, **(g)** 33 months of the surgery. Lesion continued lessening.

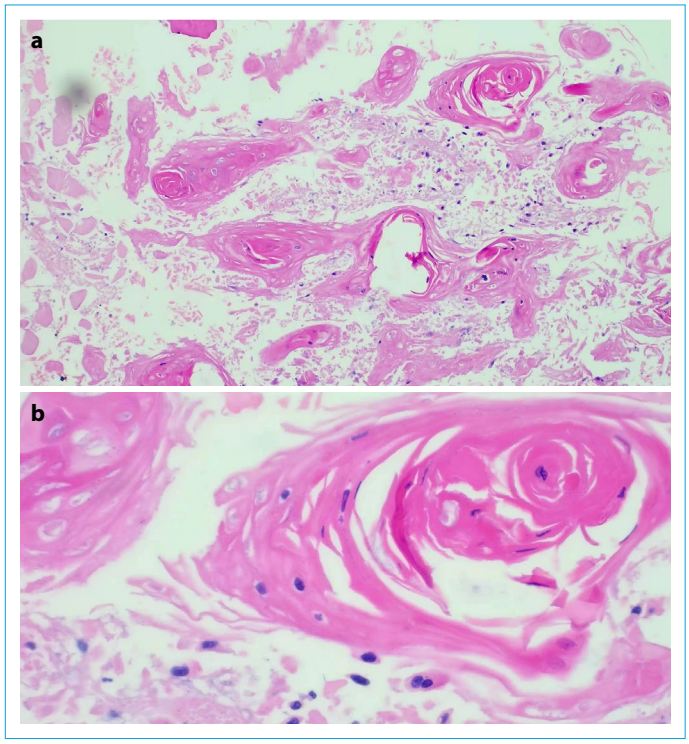

Figure 3. Typical cornified pearl under the microscope.

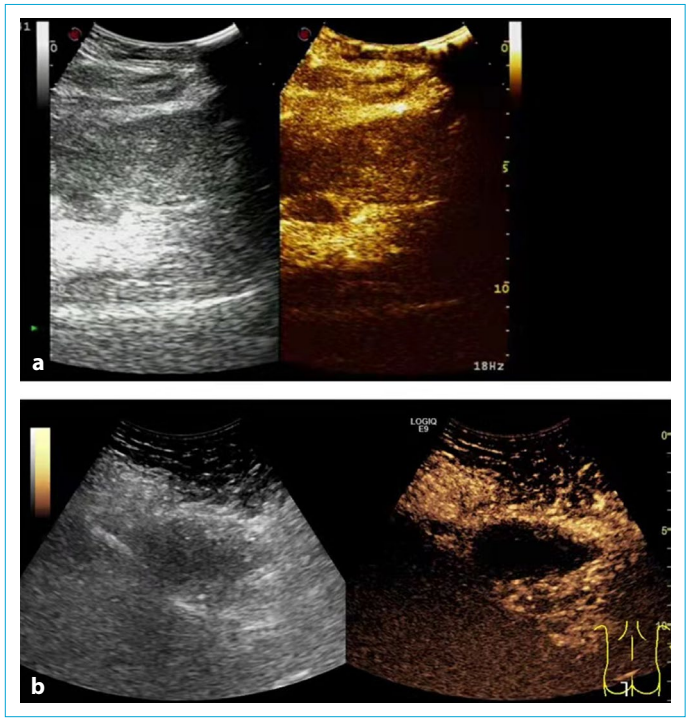

Figure 4. Contrast-enhanced ultrasound (CEUS). **(a)** 2 months after drug therapy. **(b)** 26 months after drug therapy. No abnormal perfusion foci were found.

sidered probably as PD-L1 associated pseudoprogression but differentiating pseudoprogression from true progression is difficult. The pathological biopsy from left thigh showed necrotic cells and keratin pearl, which revealed suspicious squamous cell carcinoma (Fig. 3). During chemotherapy, he accepted 2 cycles of MWA and subsequent contrast-enhanced ultrasound (CEUS) showed no abnormal perfusion foci (Fig. 4). After chemotherapy, a PET/CT scan revealed residual tumors possibly. Therefore, he received radiotherapy with a dose of 54 Gy in 27 fractions. Subsequently, the patient has continued the treatment of toripalimab last a year and zoledronate each three or four weeks with no significant sign of recurrence according to MRI, CEUS or PET/CT. Treatment course was shown in Figure 5. Next-generation sequencing (NGS) genomic study in the cancer tissue showed negative mutation of EGFR, positive expression of programmed death ligand-1 (PD-L1) (percentage marked cells score ≥1.6%) and tumor mutational burden (TMB) 7.8Mut/Mb. The patient had no previous medical history or history of smoking and drinking. He had no family history of malignant tumor. During the multimodal therapy, the patient had adverse reaction with grade 2 nausea and grade 3 granulocytopenia, which disappeared as symptomatic treatments. From the first time being diagnosed as pSCC to the last time follow-up, the overall survival and progression-free survival of the patient has been more than 44 months and 26 months respectively.

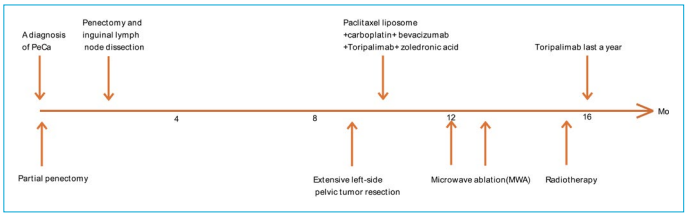

Figure 5. Treatment course.

Discussion

As so far, no standard therapy management is available in pSCC as the lack of large-scale prospective trials. Systemic chemotherapy is thought to be the preferred option when pSCC advanced or metastasized. Regimens including cisplatin plus irinotecan (IP), cisplatin plus fluorouracil (TPF), paclitaxel plus carboplatin (PC) and Paclitaxel plus ifosfamide plus cisplatin (ITP)[7-19,27] are most used as adjuvant as well as neoadjuvant chemotherapy. Each regimen showed different benefit in objective response rate (ORR), overall survival (OS), partial response (PR), pathological complete response (pCR), relapse-free survival (RFS) and time to progression (TTP). Several studies containing methotrexate, bleomycin and vinflunine showed unacceptable drug toxicity but only modest results were reported.[10-13] Current regimens in common use have not apparent difference of survival in pSCC. Therefore, we preferred to consider the toxicity in the same level treatment effect of chemotherapy. The paclitaxel liposome plus carboplatin (PC) regimen has been used as adjuvant and neoadjuvant chemotherapy reported in the studies of Carlos Bermejo 2006[10] and Vanita Noronha 2011[28], which has been revealed to improve prognosis of advanced and metastasized pSCC. Meanwhile, based on histological similarities of pSCC and head and neck SCC, we made the chemotherapeutic regimen including 6 cycles of PC.

Owing to the poor prognosis with a median OS of less than 6 months after primary chemotherapy of pSCC[18], it is necessary to find other appropriate treatments. Antiangiogenic therapy is promising as its extensive use in solid tumors. In a small cases study[29], advanced pSCC were treated with sorafenib or sunitinib, the vascular endothelial growth factor tyrosine kinase inhibitors (TKI) after second-line chemotherapy. Among all the 6 patients, one patient showed partial response and four showed stable disease. Bevacizumab, one of the angiogenesis inhibitors acting on the VEGF, has been approved in the therapy of recurrent cervical carcinoma. Recent study also showed that using chemotherapy with bevacizumab has increased response rate and led to longer PFS in the primary treatment of recurrent and metastatic head and neck squamous cell carcinoma (HNSCC).[30] In addition, the VEGF receptor activated by VEGF-A ligan is overexpressed in approximately 50% of pSCC. Given the above it, bevacizumab may be one of the effective and potential therapeutic drugs in systemic penile cancer treatment.

Radiotherapy has a clear role to play in the curative management of SCC of the penis, both in management of the primary tumor and in control of the high-risk groin or pelvis after surgical nodal staging. Previous studies showed the increased responsiveness and prognosis with chemoradiotherapy and in SCC from other sites of cervix, vulva, oropharynx and anal canal.[30-34] The shared histology suggests the rationale for pSCC with a similar approach. Adjuvant radiation is associate with better OS and locoregional control in a study with 23 patients who have received inguinal lymphadenectomy from 2002 to 2008.[35] Meanwhile, Tang Dominic et al.[36] found better survival and less recurrence received adjuvant radiotherapy in 92 penile cancer patients after lymph node dissection. It was found from American National Cancer Database that adjuvant radiotherapy has survival benefit in stage III pSCC patients after lymph node dissection especially in the patients with higher nodal stage.[37] The last study includes 93 patients with pT1-4N3M0 pSCC of penis. The study revealed that patients without extranodal extension may benefit from inguinopelvic radiotherapy in regional control.[38] However, published studies are limited by the retrospective of selection and inherent associated referral bias. Therefore, radiotherapy may continue to be considered in the multimodal treatments. It needs to be further evaluated of radiotherapeutic effect in clinical trials and the International Penis Advanced Cancer Trial (InPACT) is highly anticipated.

In this case, we present the metastatic pSCC patient with maintenance therapy of toripalimab after multimodal treatments. Immune check point inhibitors (ICB) has shown extraordinary therapeutic effect in various solid tumors as well as tumors with squamous cell histology over last decade. PDL-1 is a predictive biomarker for therapeutic response and prognostic biomarker of PD-1 and PDL-1 therapy.[39] It was reported that in pSCC patient, PD-L1 was positive in 53.4% in China.[40] Another biomarker, Tumor mutational burden (TMB) could also predict the response of therapy with an immune checkpoint inhibitor. Higher mutations increase the probability of recognition by the immune system as it generates neo-tumor-antigens.[41,42] Nowadays, pembrolizumab has approved to be used in solid tumors with a tumor mutational burden (TMB)>10 Mut/Mb and microsatellite instability (MSI-high).[43] The gene detection in our case showed the expression of PD-L1 and TMB with ≥1.6% and 7.8Mut/Mb respectively. Although the TMB was assessed lower than 10 Mut/Mb, it was much higher than the median TMB (4.5 Muts/Mb) in PSCC.[44] In our case, toripalimab has durable clinical benefit with

better overall survival and disease-free survival. It suggests that immunotherapy may be a promising option of recurrent and metastatic pSCC. However, large clinical trials are needed.

Interestingly, in the first time follow up of multimodal treatment combining chemotherapy, targeted therapy and immunotherapy, the therapeutic effect was not satisfied. The MRI scan even showed the possibility of progress. Yet after 2 cycles of microwave ablation therapy and 27 fractions of local radiotherapy, patient treated with monotherapy of toripalimab had a stable disease for over 18 months. There is no doubt that local therapy of microwave ablation and radiation played an important role in reducing tumor load. Current studies have noted the interactions between local therapy and immune therapy. Several animal experiments and clinical trials revealed that multiple tumor antigen released by plenty of tumor debris after ablation.[45,46] Furthermore, a study revealed that high-intensity focused ultrasound (HIFU) increases cytotoxicity of cytotoxic T lymphocytes (CTLs) in the H22 HCC tumor trail.[47] In theory, adaptive antitumor immune response is initiated by tumor antigen recognized, activated and presented. However, enhanced immune infiltration alone by the ablation is insufficient to generate persistent antitumor immune response. Shi L et al.[48] found in a retrospective study that T cell infiltration and PD-L1 expression increase after microwave ablation (MWA) treatment in primary colon tumors. The T cell immune response in the distant or situ can be quickly inhibited by upregulation of PD-L1 expression in a tumor-bearing mouse model. Meanwhile, therapy combining MWA with anti-PD-1/PD-L1 monoclonal antibodies leaded stronger T cell response and extended the survival of tumor-bearing mouse.[50]

Radiotherapy also influences the immunotherapy efficacy by remodeling tumor microenvironment. Radiation can upregulate the expression of type 1 interferon and proinflammatory cytokines by destroying the DNA of tumor cells. And radiotherapy also has the same augmenting antigens and antigens delivery function like ablation. Those biological effect promotes anti-tumor immune infiltration and reduces the immunosuppressive function of myeloid-derived suppressor cells.[49,50] Latest study has found a functional intersection called Ter cell–ARTN axis (tumor-promoting erythroid progenitor cells and artemin axis) of immunotherapy and radiotherapy. In this study, PD-L1 blockade and radiotherapy reduced tumor- induced Ter cells and ARTN, which in turn promoted the therapeutic effects of both anti–PD-L1 treatments and radiotherapy.[51] Together, studies revealed the regulatory effects between radiotherapy and immunotherapy which proves better therapeutic efficacy of their combining use.

In our case report, the patient with pSCC had successively received partial penectomy, penectomy and inguinal lymph node dissection. The clinical diagnosis was T2N0M0 IIA with well to moderate differentiation in histology. However, tumor relapsed after 1 month of first partial penectomy. The second relapse happened in 1 month later of the penectomy and inguinal lymph node dissection by ache and limited walk as the initial symptoms. Prior study has noted that recurrence factors include clinical N3 stage, ≥3 pathologically involved lymph nodes, and extranodal extension (ENE).[52] Niels M. Graafland et al.[3] has revealed the histological grade and lymphovascular invasion (LVI) are independent predictors for micrometastasis. Only 23% of the high-risk PeCa (≥pT2, G3, or LVI) developed with micrometastasis according to the European Association of Urology (EAU) guidelines. As the patient in our case report, he had been diagnosed in T2 stage without any other recurrence relative factors. On the contrary, tumor relapsed quickly after twice perfect surgery. If there is an unknown biological behavior of progression in the pSCC? Will the anti–PD-L1 treatments benefit from it? We need more clinical trials and foundation biology experiment to figure out it.

Conclusions

In summary, a patient with advanced pSCC had successful local controlled by treating with multimodal therapy. Local therapy combination with anti-PD-L1/PD-1 agents may be beneficial.

Disclosures

Informed consent: Written informed consent was obtained from the patient for the publication of the case report and the accompanying images.

Peer-review: Externally peer-reviewed.

Conflict of Interest: None declared.

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Availability of Data and Materials: The data and materials are collected from patient's medical record and are available from the corresponding author on reasonable request.

Authorship Contributions: Concept – L.X., X.Z., L.L., C.J., P.Z., Z.Z.; Design – L.X., X.Z., L.L., C.J., P.Z., Z.Z.; Supervision – L.X., X.Z., L.L., C.J., P.Z., Z.Z.; Materials – L.X., X.Z., L.L., C.J., P.Z., Z.Z.; Data collection &/or processing – L.X., X.Z., L.L., C.J., P.Z., Z.Z.; Analysis and/or interpretation – L.X., X.Z., L.L., C.J., P.Z., Z.Z.; Literature search – L.X., X.Z., L.L., C.J., P.Z., Z.Z.; Writing – L.X., X.Z., L.L., C.J., P.Z., Z.Z.; Critical review – L.X., X.Z., L.L., C.J., P.Z., Z.Z.

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