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LETTER TO THE EDITOR

Establishment of an orthotopic perirenal space xenograft mouse model of retroperitoneal sarcoma

Dear Editor,

Soft tissue sarcomas are a heterogeneous group of rare malignancies with mesenchymal origin that can arise at any anatomic site. They account for almost 1.5% of all malignancies in humans, with an increased incidence in young adults [1, 2]. The retroperitoneum is the primary site of 15%–20% of soft tissue sarcomas [3, 4]. There have been limited advancements in the diagnosis and treatment options available to patients with retroperitoneal sarcomas, compared to other cancers, due to a lack of basic and clinical researches, especially appropriate cell and animal models[5]. The use of patient-derived xenograft, cell-derived xenograft, and transgenic animal models is limited due to the difficulties in sample acquisition and animal raising and the complexity of genetic mutations[6]. Subcutaneous xenograft models are the most commonly used models, but they cannot reflect the occurrence and development of tumors to the same extent as orthotopic xenograft models. A simple animal model that closely mimics the clinical features of retroperitoneal sarcoma with good short-term efficiency is necessary for investigating the development and progression, molecular biology, imaging, drug screening, and sensitivity testing for retroperitoneal sarcomas [7]. The purpose of the present study was to establish an orthotopic perirenal space xenograft mouse model of retroperitoneal sarcoma for further study. The detailed materials and methods for this work can be found in the Supplementary file.

In this study, we transplanted the fibrosarcoma cell line HT1080 and liposarcoma cell line SW872 into the perirenal space of athymic nude mice (Figure 1A) to establish a mouse model of retroperitoneal sarcoma. This model presents new *in situ* tumor growth that is easily accessed, presents faster growth, and has greater resemblance to the real-life situation when compared to subcutaneous xenograft models. The perirenal space bulged slightly after transplantation of cells as trypan blue staining indicated (Figure 1B). HT1080 and SW872 cells formed solid tumors within 15 and 30 days, respectively (Figure 1C), which showed clear boundary (Figure 1D) with little adhesion to the surrounding organs (Figure 1E).

To examine the differences in tumorigenicity between perirenal space inoculation and subcutaneous inoculation, the same amount of cells were injected into the perirenal space and subcutaneous space. Both HT1080 and SW872 cells inoculated in the perirenal space had stronger tumorigenic ability (malignant proliferation) than cells inoculated subcutaneously ($P < 0.001$) (Figure 1F). Abundant Ki67- and mouse double minute 2 homolog (MDM2)-positive cells were observed in HT1080 and SW872 cell-derived tumor tissues, suggesting that the tumors in these models had basic characteristics of retroperitoneal tumors (Supplementary Figure S1A). Perirenal space and subcutaneous SW872 xenograft tumors showed no difference in the rate of positive Ki67 staining (Supplementary Figure S1B). However, there were significantly fewer TUNEL-positive cells in perirenal space xenograft tumors than in subcutaneous xenograft tumors (Supplementary Figure S1C). These results suggest that the perirenal space could be more suitable for the survival of tumors when compared to the subcutaneous space.

To further understand the changes in molecules that regulate the tumorigenic ability of cells in the perirenal and subcutaneous spaces, three solid SW872 tumors formed in the perirenal space and three in the subcutaneous space were selected to construct transcriptome sequencing libraries. A total of 18,983 genes were detected in these tumors (Supplementary Table S1). The volcanic map shows differentially expressed genes (Supplementary Figure S2A). A total of 216 genes were associated with tumorigenesis, and the range in variation of these genes reached 1.3 times (Figure 1G, Supplementary Table S2). A total of 216 genes were allocated to six main Kyoto Encyclopedia of Genes and Genomes (KEGG) metabolic pathways

Abbreviations: STS, soft tissue sarcomas; RPS, retroperitoneal sarcoma; MRPS, malignant retroperitoneal sarcoma; CDX, cell-derived xenograft; PDX, patient-derived xenograft; ps-CDX, perirenal-space-cell derived xenograft; WDLPS, well-differentiated liposarcomas; DDLPS, dedifferentiated liposarcomas

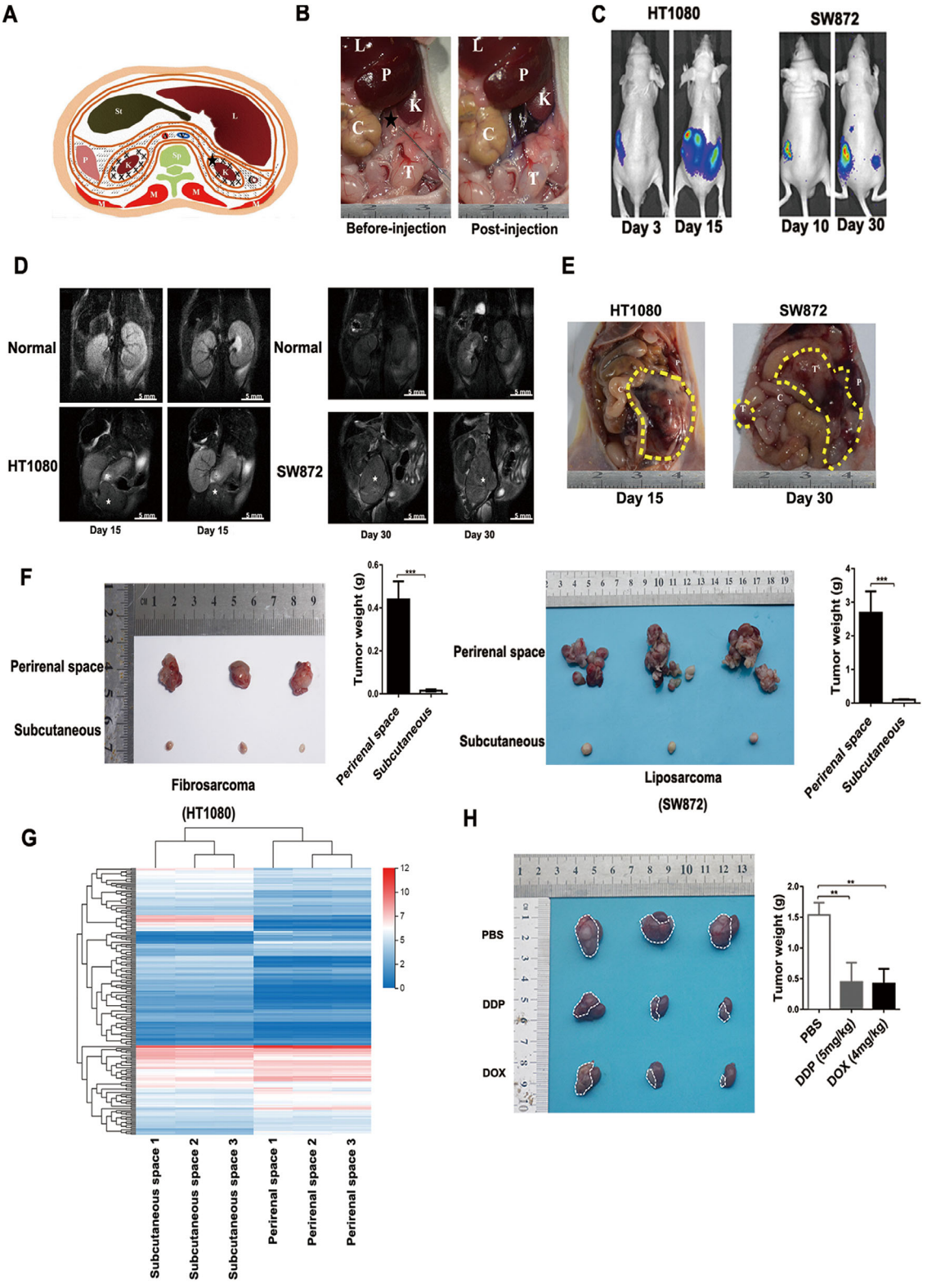

FIGURE 1 **Establishment of a perirenal space xenograft mouse model of retroperitoneal sarcoma.** (A). Pattern of the retroperitoneal space (L, liver; St, stomach; P, pancreas; K, kidney; Sp: spine; C: colon; M: muscle; A: artery; V: vein; the dotted line indicates the retroperitoneal space; crosswire indicates the perirenal space, and the pentastar indicates the grafted site). (B). Macroscopic images of the site of retroperitoneal sarcoma cell transplantation, as indicated by the yellow dotted line. Trypan blue staining on the right image highlights the post-injection appearance (L, liver; P, pancreas; K, kidney; C, colon; T, testis; the pentastar indicates the grafted site). (C). Bioluminescence imaging of tumor-bearing mice. (D). In vivo T1-weighted magnetic resonance imaging (MRI) for HT1080-grafted mice and T2-weighted MRI for SW872-grafted mice. Pentastar indicates the tumor. (E). Abdominal anatomy of HT1080-grafted mice on day 15 and SW872-grafted mice on day 30; the dotted line indicates the solid tumor (P, pancreas; C, colorectum; T, tumor). (F). Macroscopic images and weight of perirenal space

(Supplementary Figure S2B). The main altered pathways were the tumor necrosis factor (TNF) signaling pathway and the nuclear factor-kappa B (NF-κB) signaling pathway (Supplementary Figure S2C). The molecules found to have altered expression in tumors are mainly involved in the regulation of cell growth and death.

To test whether the responses of the perirenal space cell-derived xenograft models to chemotherapy drugs were consistent with clinical manifestations, doxorubicin (DOX) and cisplatin (DDP) were injected intraperitoneally, and their therapeutic effect was assessed by examining changes in bioluminescence signal intensity, tumor weight, and bodyweight of tumor-bearing mice. We injected 100 μL of phosphate-buffered saline (PBS) containing 1×10^5 HT1080 fibrosarcoma cells into the perirenal space of nude mice. The mice were divided into three groups that received an intraperitoneal injection of PBS, DDP (5 mg/kg), and DOX (4 mg/kg), respectively, since the third day after the initial cell injection. The use of DDP and DOX had reduced fluorescence signal intensity (Supplementary Figure S3A) and resulted in significant decreases in body weight (Supplementary Figure S3B) and tumor weight (Figure 1H).

In this study, we successfully established an orthotopic retroperitoneal xenograft model by inoculating liposarcoma and fibrosarcoma cells into the primary origin sites of retroperitoneal sarcoma in nude mice. We found that retroperitoneal sarcoma cells inoculated in the perirenal space had stronger tumorigenic ability than those inoculated subcutaneously. This model has several characteristics. Firstly, the cells were inoculated *in situ*, which could mimic the environment of retroperitoneal sarcoma. The model also allows the visualization of tumor cells with GFP-Luc labeling, which reflects the development of the tumor more accurately and comprehensively. Therefore, it can be used to evaluate new chemotherapeutic drugs, immunotherapies, surgical procedures, and diagnostic methods. Secondly, the observed enhancement of the MDM2/tumor protein p53 (TP53) signaling pathway was similar to that observed in the clinic. Therefore, this model can be used to study the mechanisms of tumorigenesis. Finally, the mice used in this model were ordinary nude mice, providing great advantages in terms of handing, feeding conditions, and cost, compared to severely defective nude mice such as severe combined immune deficiency (SCID) nude mice. Furthermore, unlike previous patient-derived xenograft models, the cells used in this model were commercial cell lines. Therefore, tissues do not need to be obtained from patients, which involves an extended waiting time for appropriate surgery and strict ethical review approval.

DECLARATIONS

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AUTHORS' CONTRIBUTIONS

WL, CL, CY, and FX contributed to the conception or design of the work; FX conducted data acquisition and analysis and drafted the paper; DQ, LL, XX conducted data acquisition, analysis, or interpretation; ML and XK participated in writing and revised the manuscript critically.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This research study was approved by the Ethics Committee of the Xiang'an Hospital of Xiamen University.

CONSENT FOR PUBLICATION

Not applicable.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AVAILABILITY OF DATA AND MATERIALS

All data generated or analyzed during this study are included in this article.

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and subcutaneous xenografts. (G). The heat map shows the frequency of the proteins present in the process of tumorigenesis. (H). The tumor weight in the PBS group was significantly higher than those in the cisplatin (DDP) and doxorubicin (DOX) groups. For each experiment, a minimum of three mice were used per group, and the results are expressed as average ± standard deviation. $^{**}P < 0.01$; $^{***}P < 0.001$.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.