The Role of Single-cell RNA Sequencing in Cardiac Tumour - A Case Report

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Case Report

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Abstract

Background: Benign cardiac tumour is a common disease and is often completely removed by surgery. However, the characteristics of these tumours have not been studied deeply. In this study, we used single-cell RNA sequencing (scRNA-seq) to analyse cell composition and growth patterns in a giant cardiac tumour.

Case presentation: A 65-year-old woman presented to our hospital with 5 days of chest tightness, dyspnoea, and lower abdominal distension. Echocardiography revealed a mass in the right atrium. An emergency operation was carried out to prevent tumour shedding. At 4 days after successful tumour resection, the patient was discharged without any complications. At the ten-month follow-up, she had recovered well, with good quality of life. scRNA-seq was applied to analyse the nature of the tumour. The cellular components of benign tumours include chondrocytes, smooth muscle cells, fibroblasts, mesenchymal stromal cells, and osteoblasts. Additionally, the cyclic guanosine monophosphate-cyclic guanosine monophosphate (cGMP-PKG) signalling pathway, transcriptional misregulation in cancer, and the p53 signalling pathway may be related to the growth of this tumour.

Conclusion: scRNA-seq is a good approach to analyse growth patterns and search for therapeutic targets. scRNA-seq results are helpful for reducing tumour recurrence and also benefit patient quality of life.

Background

Cardiac tumour is a common disease. Treatment for cardiac tumours is generally surgical resection, which leads to a better prognosis in benign tumours than malignant tumours [1]. However, we often do not analyse the cell components and the cause of growth for tumours. Therefore, for a malignant tumour, surgical resection is not sufficient to achieve satisfactory survival due to the lack of precision-targeted drugs. Recently, the scRNA-seq technique has been widely used [2], and it is helpful for understanding the potential mechanism of the growth of the tumour. Herein, we report the application of single-cell sequencing to a giant cardiac tumour.

Case Presentation

A 65-year-old (62 kg) female patient was admitted to our department on 19 September 2021, with the chief complaint of chest tightness, dyspnoea, and lower abdominal distension for 5 days. Her previous history was unremarkable, but she had hypertension for 10 years. Two masses in the right atrium were observed on echocardiography; of concern, one of the masses was lodged in the tricuspid valve orifice. To prevent pulmonary embolism caused by tumour shedding, an emergency operation was carried out.

Complete surgical resection of the tumour was performed through median sternotomy under standard cardiopulmonary bypass, superior vena cava, inferior vena cava, and aortic cannulation with aortic cross-clamping. After incision of the right atrium, the base of the larger tumour was localized to the right atrial wall, filling the right atrium (Fig. 1, A). The base of the smaller tumour was located above the tricuspid

valve annulus, and the tumour crossed the tricuspid valve and penetrated deep into the right ventricle (Fig. 1, B). The tumour sizes were 3.0 × 7.0 × 7.0 cm^3 and 2.0 × 4.0 × 6.0 cm^3 (Fig. 2, A). The tumours were removed carefully. The tricuspid valve was seriously evaluated, and intact valve function was noted. The cardiopulmonary bypass time was 66 min, and the patient was easily weaned from cardiopulmonary bypass. Postoperative echocardiogram showed good right ventricular function without tricuspid regurgitation. The patient recovered uneventfully and was discharged on postoperative day 4. Echocardiography at postoperative month 6 showed an ejection farction of 55%, without recurrent tumours.

The tumour was rich in cellular myxoma with chondroid metaplasia and sarcomatoid differentiation (Fig. 2, B). Immunohistochemistry showed the following: Vimentin (+), Filami-1 (FIL-1) (+), Cluster of differentiation 99 (CD99) (+), Cluster of differentiation 34 (CD34) (+), Neuron specific enolase (NSE) (+), Casein kinase (CK) (-), S100 (-), Epithelial membrane antigen (EMA) (-), Cluster of differentiation 117 (CD117) (-), Specific AT sequence binding protein-2 (SATB-2) (-), and KI-67 proliferation index 40%.

To identify the features of the tumour, we determined its cell composition and growth pattern via scRNA-seq. The tumour samples were used for scRNA-seq after informed consent from patient and approval from the ethics committee of Bengbu medical college (No.2021170). All methods were performed in accordance with the Declaration of Helsinki. The method of scRNA-seq is summarized below.

The tumour sample was minced on ice to less than 1 mm cubic pieces, followed by enzymatic digestion using an enzymatic cocktail [collagenase I/dispase II (1 μg/ml) tissue] with manual shaking every 5 min. The sample was then centrifuged at 300 rcf for 30 s at room temperature, and the supernatant was removed without disturbing the cell pellet. Next, 1×PBS (calcium and magnesium free) containing 0.04% weight/volume BSA (400 μg/ml) was added and then centrifuged at 300 rcf for 5 min. The cell pellet was resuspended in 1 ml lysis buffer and incubated for 15 min at 4°C. Then, the sample was resuspended in 1 ml PBS containing 0.04% BSA. Next, the sample was filtered over Scienceware Flowmi 40-μm cell strainers. Nuclei were gently collected and suspended in ice-cold PBS containing 0.4% BSA.

Chromium System (10x Genomics, Pleasanton, California) was used and processed following the manufacturer's instructions. Library construction was performed using Chromium Single Cell 3' Library & Single Cell 3 v3 Gel Beads. Libraries were pooled based on their molar concentrations. To demultiplex samples, process barcodes, align and filter reads, and generate feature barcode matrices, we used the 10x Genomics Cell Ranger (v3.1.0) pipeline according to the manufacturer's instructions in raw data quality assessment. We processed the unique molecular identifier (UMI) count matrix using the R package Seurat (version 3.0). To remove low-quality cells and likely multiplet captures, we applied criteria to filter out cells with unique molecular identifiers (UMIs)/gene numbers out of the limit of the mean value +/- 2-fold the standard deviation. We further discarded low-quality cells when a certain percentage of counts belonged to mitochondrial genes. Library size normalization was performed in Seurat on the filtered matrix to obtain the normalized count. Principal component analysis (PCA) was performed to reduce the dimensionality of the log-transformed gene-barcode matrices of the top variable genes. Cells were

clustered based on a graph-based clustering approach and visualized in 2 dimensions using tSNE. A likelihood ratio test was used to identify significantly differentially expressed genes between clusters. Differentially expressed genes (DEGs) were identified using the R package. A P value < 0.05 and |log2foldchange| > 1 were set as the thresholds for significant differential expression. Gene Ontology (GO) enrichment and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses of DEGs were performed using the hypergeometric distribution. The sequencing and bioinformatics analysis were performed by OE Biotech Co., Ltd. (Shanghai, China).

After quality control by eliminating multicellular and apoptotic cells, the transcriptomes of 2095–11815 cells were analysed. A total of 3564 DEGs were detected. Compared with the public data in published studies [2], we found that the major cell components of the tumour to be chondrocytes, mesenchymal stromal cells, and smooth muscle cells. After filtering, GO analysis of 36 hub genes showed that they are involved in multicellular organismal processes, anatomical structure development, cellular response to stimulus, and cell differentiation. KEGG pathway enrichment showed that the cyclic guanosine monophosphate-cyclic guanosine monophosphate (cGMP-PKG) signalling pathway, transcriptional misregulation in cancer, and the p53 signalling pathway are related to the growth of these tumours. Protein–protein interactions of these genes were also analysed (Fig. 3).

Discussion And Conclusions

According to the classification of cardiac tumours from WHO in 2015 [3], myxomas and papillary fibroelastomas are the most common benign cardiac tumours and are typically endocavitary. In our case, the tumour was classified as myxoma, though chondroid and sarcomatoid components were detected via immunohistochemistry. Surgery is the first choice of therapy for myxoma, with a satisfactory long-term survival rate [1].

During the operation, we suspect the tumour to be malignant based on its appearance. We chose two methods to detect its characteristics: pathological examination and scRNA-seq. However, pathology revealed the tumour as benign. We obtained much information about this tumour using scRNA-seq. First, the components mainly include chondrocytes, smooth muscle cells, fibroblasts, mesenchymal stromal cells, and osteoblasts, in agreement with the pathological examination. The tumour was benign by double confirmation. Second, through KEGG enrichment and protein–protein interaction of hub genes, we speculate that the causes of tumour growth are related to three signalling pathways, the roles of which in tumours have been widely studied [4–6].

Current scRNA-seq technology in cardiovascular disease still has many challenges. Most notably, scRNA-seq relies on reverse transcription of RNA to cDNA and is unable to detect low-copy transcripts in one cell to gain a full understanding of many regulatory processes [7]. Furthermore, intact adult cardiomyocytes (CMs) are too large to apply to droplet-based systems, such as chromium systems and drop-seq [8]. To overcome this limitation, some researchers have extracted CM nuclei for use with this system to perform high-throughput scRNA-seq. However, CMs have an exceptionally high mitochondrial gene content

compared with all other cell types. Extracting nuclei can also remove mitochondria, which are abundant in CMs [9]. Thus, tailoring the isolation protocol to the sample of interest is critical to ensure the preservation of nuclear integrity and sensitivity.

Despite the limitations, the advantage of scRNA-seq technology allows for unbiased assessment of cellular heterogeneity, identification of rare subpopulations of cells, and elucidation of dynamic cellular trajectory analysis during development and differentiation [10]. Based on the above advantages, scRNA-seq is a powerful tool for clinicians to devise tailored treatment. Although the tumour in our study was benign, this does not hinder the potential of scRNA-seq in the treatment of malignant cardiac tumours. Not surprisingly, the composition of cells in a given tumour tissue varies greatly among patients, with important implications for diagnosis and therapeutic response. Previous studies have shown that one of the major challenges in personalized medicine is that patients do not respond uniformly to treatment [11]. With a broad range of genetic and environmental factors, patient-specific cell-to-cell heterogeneity is increasingly understood to be a fundamental contributor and driver of differential drug responses among individuals, highlighting the growing importance of scRNA-seq in advancing precision medicine [12].

Although our case involved a benign tumour, a benign or malignant tumour is a postoperative diagnosis that must be kept in mind to select a proper treatment strategy. We believe that scRNA-seq is a good way to analyse growth patterns and search for therapeutic targets. scRNA-seq results can help in identifying a precision-targeted drug to reduce tumour recurrence and benefit the quality of life of patients.

Abbreviations

FIL
Filamin
CD
Cluster of differentiation
NSE
Neuron specific enolase
CK
Casein kinase
EMA
Epithelial membrane antigen
SATB
Specific AT sequence binding protein
cGMP-PKG
cyclic Guanosine monophosphate-cyclic - guanosine monophosphate

Declarations

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Authors' contributions

XJQ and TW -Writing analyzed and interpreted the scRNA-seq data. IAA, XZ and SQZ performed the histological examination of the tumour. JJM drafted the figures. YYJ and CS were contributors in writing the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

Not applicable.

Ethics approval and consent to participate

The tumour samples were used for scRNA-seq after informed consent from patient and approval from the ethics committee of Bengbu medical college (No.2021170). All methods were performed in accordance with the Declaration of Helsinki.

Consent for publication

The patient provided written, informed consent.

Competing interests

The authors declare that they have no competing interests

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Figures

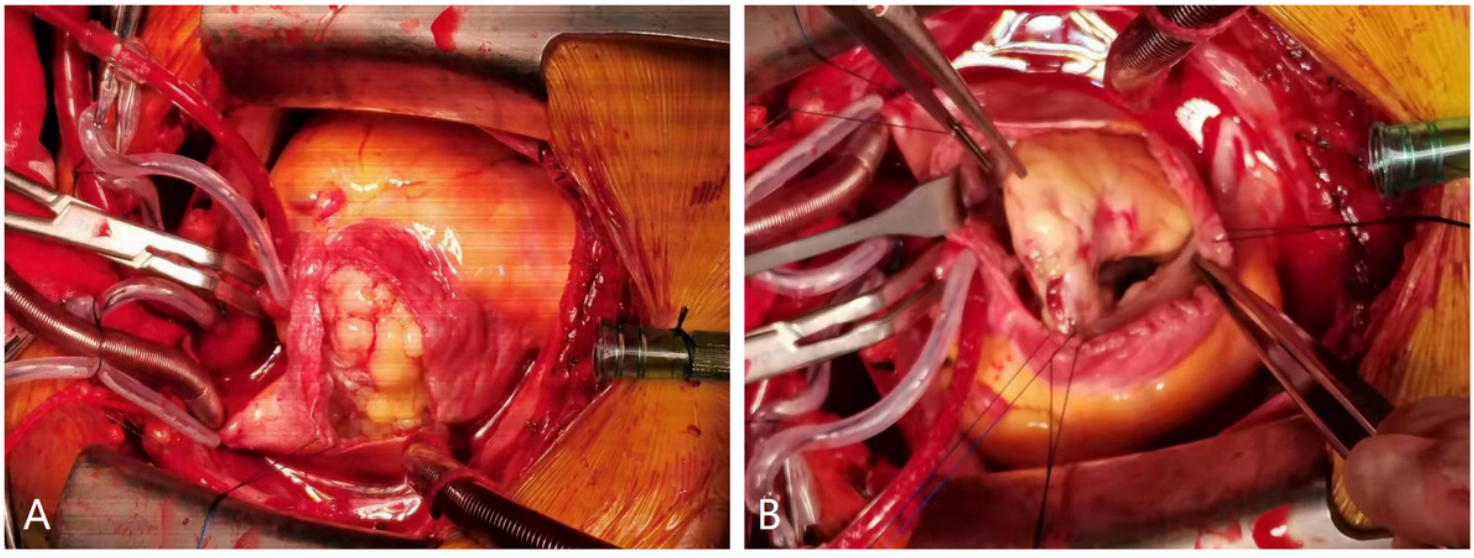

Figure 1

A The larger tumor was located in the right atrial wall and filled the right atrium.

B The base of the smaller tumor was located above the tricuspid valve annulus.

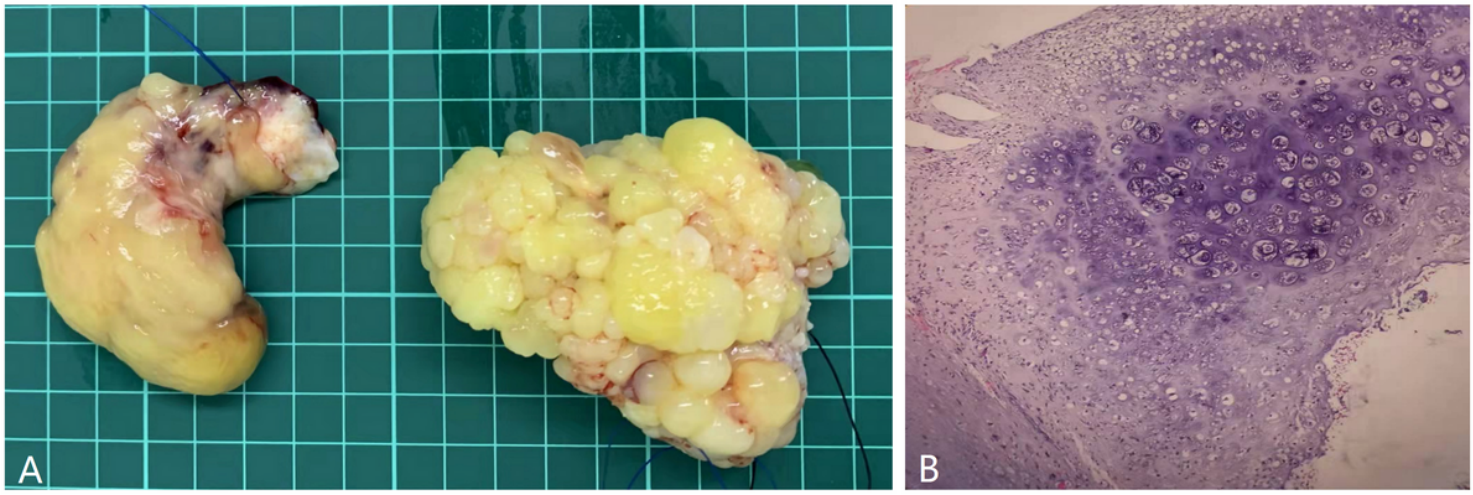

Figure 2

A Photograph shows a macroscopic view of the tumor, which were 3.0 × 7.0 × 7.0 cm^3 and 2.0 × 4.0 × 6.0 cm^3, respectively.

B Immunohistochemistry detected tumor was rich in cellular myxoma with chondroid metaplasia and sarcomatoid differentiation.

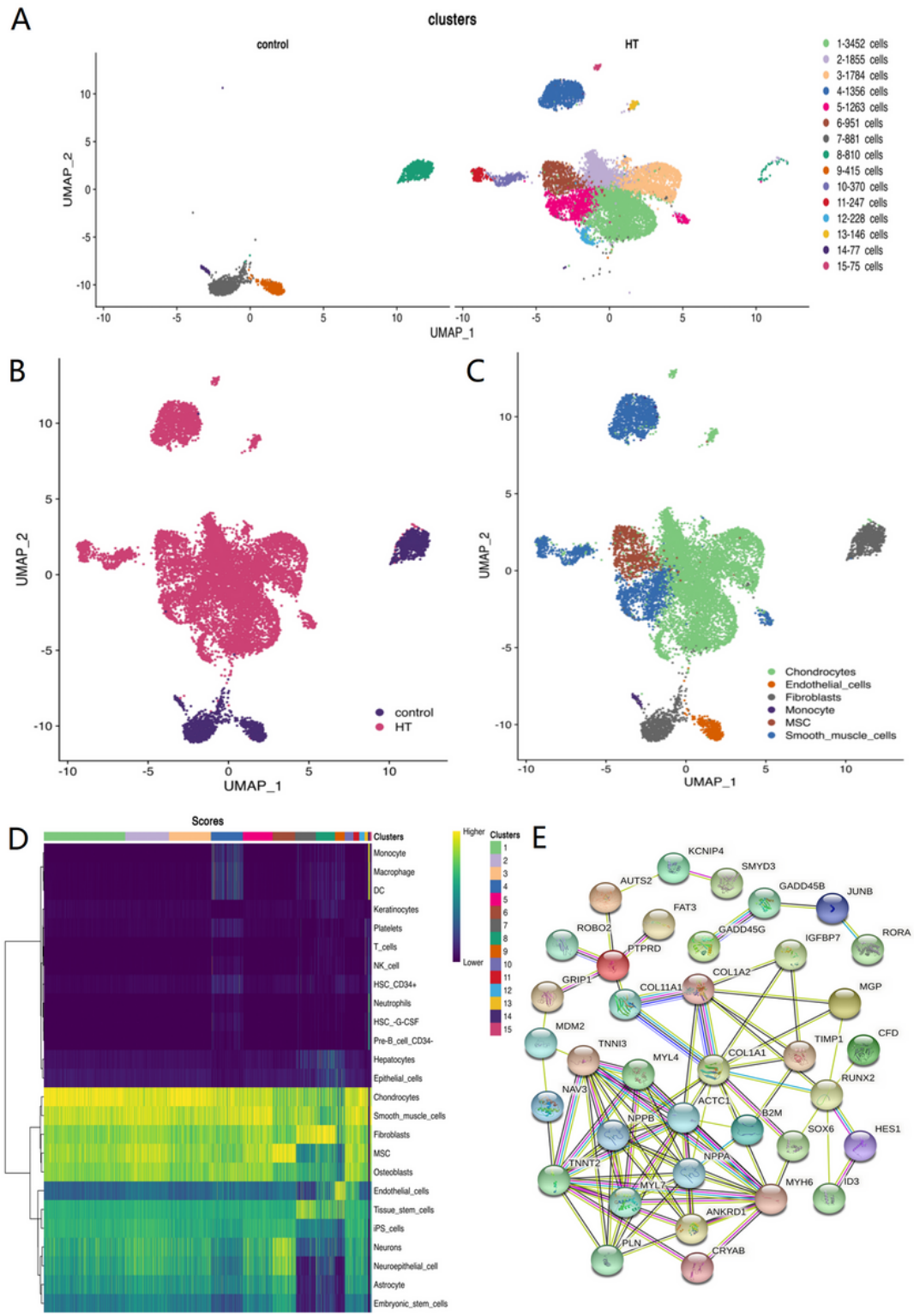

Figure 3

A Cell subgroups and cell numbers.

B Dimension Reduction Clustering grouping display between heart tumor and normal heart.

C Cell type diagram.

D Heat map of cell type identification correlation.

E Protein-protein interaction of 36 hub genes.