CASE REPORT

The role of single-cell RNA sequencing in cardiac tumour - a case report

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Abstract

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. The patient was discharged on the 4th day of tumour resection, without any complications At the 18 months follow-up, she suffered from kidney and lung tumours. She refused any treatment and passed away. 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 (cGMP-PKG) signalling pathway, transcriptional misregulation in cancer, and the p53 signalling pathway may be related to the growth of this tumour. scRNA-seq is a good approach to analyse growth patterns of cardiac tumours and helpful for distinguishing the nature of the tumour.

Keywords: Sequence Analysis, RNA, Cardiac tumour.

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Introduction

Cardiac tumour is a rare disease. The estimated frequency for primary cardiac tumours is 1:2000 autopsies and for secondary tumours 1:100 autopsies. Approximately 10% of the primary cardiac tumours are malignant and 90% benign (mostly myxomas).[1] Treatment for cardiac tumours is generally surgical resection, which leads to a better prognosis in benign tumours than malignant tumours.[2] However, we often do not analyse the cell components and the cause for the growth of 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,[3] 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.

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Case Description

A 65-year-old (62 kg) female patient was admitted to our department in the First affiliated hospital of Bengbu medical college, Bengbu, China 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 the past 10 years. Two masses in the right atrium were observed on echocardiography. 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. After incision of the right atrium, the base of the larger tumour was localized to the right atrial wall, filling the right atrium (Figure 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 (Figure 1, B). The tumour sizes were $3.0 \times 7.0 \times 7.0$ cm^3 and $2.0 \times 4.0 \times 6.0$ cm^3 (Figure 1, C). 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 the 4th postoperative day. Echocardiography at postoperative month 6 and 12 showed an ejection fraction of 55%-57%, without recurrent tumours. However, the patient suffered from kidney and lung tumours 18 months after the surgery. She visited our outpatient department on 30 April 2023, and refused any treatment. She expired on 15 May 2023.

The tumour was rich in cellular myxoma with chondroid metaplasia and sarcomatoid differentiation (Figure 1, D). Immunohistochemistry showed the following: Vimentin (+), Filami-1 (FIL-1) (+), Cluster of differentiation 99 (CD99) (+), Cluster of differentiation 34 (CD34) (+), Neuron

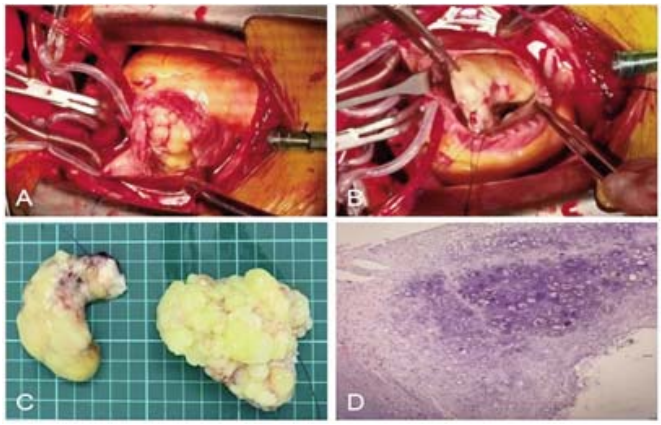

Figure-1: A The larger tumour was located in the right atrial wall and filled the right atrium. B The base of the smaller tumor was located above the tricuspid valve annulus. C Photograph shows a macroscopic view of the tumour, which were 3.0 × 7.0 × 7.0 cm3 and 2.0 × 4.0 × 6.0 cm3, respectively. D Immunohistochemistry detected tumor was rich in cellular myxoma with chondroid metaplasia and sarcomatoid differentiation.

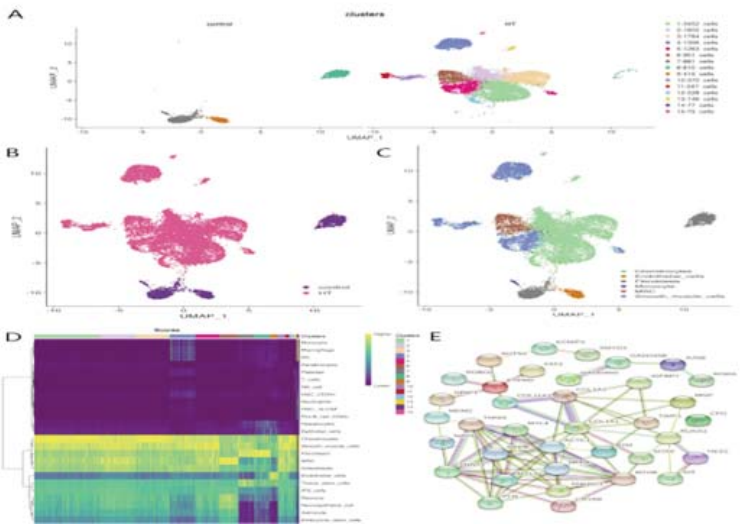

Figure-2: A Cell subgroups and cell numbers. B Dimension Reduction Clustering grouping display between heart tumour and normal heart. C Cell type diagram. D Heat map of cell type identification correlation. E Protein-protein interaction of 36 hub genes

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 after informed consent and approval from the ethics committee of Bengbu Medical College. The method of scRNA-seq is summarized below.

According to previous studies, the tumour sample was prepared.[4] Then, Chromium System (10x Genomics, Pleasanton, California) was applied following the manufacturer's instruction.[4,5] The sequencing and bioinformatics analysis were mentioned previously and performed by OE Biotech Co., Ltd. (Shanghai, China).[6]

After quality control by eliminating multicellular and apoptotic cells, the transcriptomes of 2095-11815 cells were analysed. A total of 3564 Differentially expressed genes (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, Gene Ontology (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. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment showed that the cyclic guanosine monophosphate-cyclic (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 (Figure 2).

Discussion

According to the classification of cardiac tumours from WHO in 2015, myxomas is one of the most common benign cardiac tumours. 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.[2]

During the operation, we suspected 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. [7]

Current scRNA-seq technology in cardiovascular disease

still has many challenges. Most notably, intact adult cardiomyocytes (CMs) cannot be applied to droplet-based systems, such as chromium systems and drop-seq.[8] To overcome this limitation, researchers have extracted CM nuclei to perform high-throughput scRNA-seq in this system. However, there is full of gene content in the mitochondrion of CMs. Extracting nuclei will cause the loss of mitochondrial gene information.[9] Thus, a proper isolation protocol is critical to ensure the preservation of nuclear genetic information.

Despite the limitations, scRNA-seq is a powerful tool for clinicians to devise tailored treatment for the tumour. Although the patient passed away during the follow up, we cannot disclose the relationship between cardiac tumour and new-onset tumour, this does not hinder the potential of scRNA-seq in the treatment of malignant cardiac tumours. Not surprisingly, cell heterogeneity in tumour tissue varies greatly among patients, which will influence diagnosis and treatment. Previous studies have shown that one defect in personalized medicine is that patients do not respond uniformly to treatment.[10] With the emergence and development of scRNA-seq technology, we will understand the composition of cells in tumour tissue deeply and carry out precision medicine to prevent tumour spread and recurrence, no matter benign or malignant.

Conclusion

Although our patient refused further treatment, we applied scRNA-seq to diagnosis and analyze the charactistics of atrial tumour. The cGMP-PKG signalling pathway, transcriptional misregulation in cancer, and the p53 signalling pathway may be related to the growth of this tumour. We believe that scRNA-seq is a good approach to analyse growth patterns of cardiac tumour and helpful for distinguishing the nature of the tumour.

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.

Consent for publication: The patient provided written, informed consent.

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Disclaimer: This case study has not been published or presented in any journal or conference.

Conflict of Interest: None.

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