

Distinctive Clinical and Pathologic Features of Immature Teratomas Arising from Induced Pluripotent Stem Cell Injection in a Patient with type 2 Diabetes: A Case Report

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Research Article

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Abstract

Background: As a new potential cure for diabetes, induced pluripotent stem cells (iPSCs) are characterized by self-renewal capacity and the ability to differentiate into pancreatic islet β -cells, which will hopefully secrete insulin and rebuild blood glucose balance. The safety and validity of iPSC treatment for diabetes remain controversial. One of the most serious risks is teratoma formation arising from undifferentiated stem cells, but clinical reports are rare.

Case presentation: Here, we report a distinctive case of immature teratoma after iPSC treatment for diabetes, which was accidentally treated in our soft tissue sarcoma centre. The patient received islet β -cell injection, in which the cells were differentiated from autologous iPSCs, into the deltoideus muscle. Two months later, a mass located in the injection area formed with enlarged axillary lymph nodes. Here, we present the different clinical, radiological and pathological features of this immature teratoma in detail. Distinctive from typical immature teratomas, the tumour was characterized by rapid growth and local lymph node metastasis. The tumour was not sensitive to typical chemotherapy regimens for immature teratomas. MRI scanning showed heterogeneous enhancement and a rich blood supply to the tumour. The histopathology showed immature endoderm, mesoderm and ectoderm tissues composed of osseous, cartilaginous, vascular and adenoid tissues, which have more cellular atypia than typical teratomas. Staining for both Oct-4 and Sox-2 was positive in the tumour cell nucleus by immunofluorescence assay, but insulin staining was negative. Next-generation sequencing showed many missense mutations, but abnormal gene rearrangement or copy number was not observed.

Conclusions: More attention should be given to teratoma formation after iPSC treatment for diabetes, which is more aggressive than typical teratomas. The safety and validity of iPSC treatment for diabetes should be confirmed by more standardized clinical trials.

Background

Diabetes mellitus (DM) is a type of metabolic disease characterized by hyperglycaemia, insulin resistance and defective blood glucose regulation, which can damage various organ functions. According to statistics from the World Health Organization, in 2030, the number of diabetic patients in the world will double, causing a serious socioeconomic burden [1]. Type 2 diabetes (T2DM) accounts for 85–95% of all cases and is characterized by insulin resistance in insulin-responsive tissues and impaired insulin secretion by pancreatic β -cells[2]. The majority of patients need to inject insulin for their lifetime to control blood sugar, which always results from serious complications caused by irreversible pancreatic β -cell damage [3]. In recent years, an increasing number of experts have begun to use regenerative medicine to treat irreversible pancreatic damage to β -cells in pancreatic islets using human pluripotent stem cells (iPSCs)[4]. Because autologous iPSCs are easy to obtain and expandable and can be differentiated into any desired cells, they can avoid ethical conflicts and immune rejection. iPSC technology is used in many fields and has created a new frontier, including regenerative therapy, disease modelling, drug toxicity assessment and developmental biology, and also represents a potential cure for T2DM[5, 6].

However, the tumorigenicity of iPSCs hinders the wide application of this technology in clinical research. It is difficult to clarify the validity and safety of iPSC treatment based on the limited number of clinical trials (i.e., NCT03403699)[7]. In in vitro and in vivo iPSC experimental models, wide genomic instability has been detected, including chromosomal aberrations, copy number variations, and single nucleotide variations [8, 9]. A few clinical cases have reported tumour or tumour-like lesion formation after stem cell transplantation [10, 11]. However, there are few clinical reports about tumour formation after iPSC treatment for T2DM.

Here, we report a case of immature teratoma formation after iPSC treatment for T2DM. The patient received islet β -cell injection into the deltoideus muscle, where the cells were differentiated from autologous iPSCs at another hospital. Two months later, a mass located in the injection area formed with enlarged axillary lymph nodes. The patient continued to be treated in our soft tissue sarcoma centre. Herein, we present the different clinical, radiological and pathological features of this immature teratoma in detail.

Case Presentation

Initial hospital experience

The patient was a 49-year-old male with T2DM for 17 years. The patient used 20 U insulin degludec every morning with poor blood sugar control. He received iPSC treatment in Chennuo Medical Hospital, Beijing, China. In short, somatic cells were isolated from his own blood. The patient-derived somatic cells were reprogrammed pluripotent stem cells (PSCs). PSCs were induced into islet β -cells, which were expanded and injected into the deltoid muscle of the left upper arm. However, two months later, the blood sugar regulation and insulin levels of the patient did not improve. The patient found a mass located in the injection area with enlarged axillary lymph nodes. He felt no discomfort and visited our soft tissue sarcoma centre of Yunnan Province.

Soft Tissue Sarcoma Centre of Yunnan Province

Clinical treatment

The patient complained of a growing mass in the left upper arm without any discomfort. Physical examination showed that a solid lump in the deltoid muscle was 6 cm×5 cm×5 cm with hard texture, unclear boundaries, poor mobility, and no redness or inflammation. A variety of imaging examinations in the outpatient department suggested a benign or intermediate tumour. Therefore, the patient underwent total excision biopsy of the mass in the left upper arm, and the pathological diagnosis confirmed immature teratoma. Further PET-CT examinations revealed ipsilateral axillary and cervical lymph node metastasis. After routine BEP regimen (bleomycin 30 U d1, etoposide 100 mg/m² d1-5 and cisplatin 20 mg/m² d1-5) chemotherapy for 2 cycles, the tumour progressed. Then, axillary and neck lymph node dissection was performed, which revealed 4/28 lymph node metastases with extracapsular invasion.

Adjuvant radiotherapy (intensity modulation 50 Gy, 25 times) was performed on the neck and axillary surgery area combined with the TIP regimen (albumin-bound paclitaxel 250 mg/m² d1, ifosfamide 2 g/m² d1-3 and cisplatin 30 mg/m² d1-5) chemotherapy for 4 cycles. To date, there has been no recurrence or distant metastasis.

Imaging features

Colour Doppler ultrasound in the outpatient department showed subcutaneous hypoechoes of 7.5 cm×6.7 cm×5.1 cm with an intermediate blood supply. An X-ray scan showed a soft tissue shadow with no clear bone destruction of the humerus (Fig. 1a). A plain CT scan showed a round, mixed-density mass in the deltoid muscle with an unclear boundary (Fig. 1b). An enhanced CT scan showed uneven and moderate enhancement (Fig. 1c), which indicated benign or intermediate tumours in the diagnosis. Plain MRI scan showed iso-signal on T1W imaging and high-signal on T2W imaging of the soft tissue mass in the deltoid muscle and enlarged axillary lymph nodes, both of which were uneven and obviously enhanced in enhanced MRI scan (Fig. 1d, e). After excision biopsy of the mass, a PET/CT scan showed ipsilaterally axillary, supraclavicular and neck lymph node metastasis with a maximum diameter of 2.1 cm (SUV_{max} 5.4). There were no abnormalities in the whole body, including the testis.

Pathological features

The histopathology of the soft tissue tumour of the left upper arm showed immature endoderm, mesoderm and ectoderm tissues composed of osseous, cartilaginous, vascular and adenoid tissues, which had more cellular atypia than typical teratomas (Fig. 2a). Lymph node dissection specimens showed tumour metastasis (left axillary 2/3, left apical lymph node 0/4, left thoracic intermuscular lymph node 1/1, left supraclavicular lymph node 1/11 and left cervical root lymph node 0/9 positive with extracapsular invasion) (Fig. 2b). Postoperative immunohistochemistry of the left axillary mass (X2020-16410) showed CK (-), Vim (+), Ki-67 (approximately 15%) Actin (part +) SMA (part +) Des (part +) S-100 (-) CD10 (part +) ER (-) CD34 (blood vessel +) Syn (small cell +) CgA (-). To confirm iPSC differentiation, the key markers of Oct4 and Sox2 gene amplification were detected by immunofluorescence assay, both of which were positive in the nucleus (Fig. 2c, d). However, immunohistochemical staining of insulin was negative in tumour cells.

Next-generation sequencing (NGS) of tumour tissues showed 16 gene missense or frameshift mutations, including ABCB4, CDC73, CROT, DNMT3A, HCK, KMT2D, MSH2, MSH6, NCOR2, PRDM1, PREX2, RPS6KB2, TRAF7, ZFH3, ZNF703 and ZRSR2. However, there was no gene arrangement or copy number variation. The genetic test results did not match the potentially beneficial targeted drugs. The percentage of PD-L1-positive cells was 1–2% in tumour tissue. The mismatch repair (MMR) assay by immunohistochemistry showed proficiency (pMMR).

Discussion And Conclusions

iPSC treatment has obtained amazing effects in preliminary clinical trials of immune or neurodegenerative disease [12, 13]. It also provides a new treatment model for T2DM [14]. Compared with other stem cell treatments, iPSCs have the advantages of easy acquisition, rapid expansion, low immunogenicity, and no ethical issues [15]. However, tumourigenicity is the greatest risk of iPSC treatment, which blocks its further application [16, 17].

Increasing evidence shows that reprogramming of iPSCs carries the risk of tumourigenesis, such as genome insertion of genetic recombination vectors, overexpression of oncogenic transcription factors, and overall hypomethylation similar to cancer[18]. In addition to the inherent tumourigenic risk induced by pluripotency, a long-term culture environment produces genomic abnormalities, and the final result is that the propagation of cultures has stable and heterogeneously acquired tumourigenic potential[19]. In addition, it is necessary to strike a balance between tumourigenicity and immunogenicity to obtain complete and mature pancreatic β -cells to prevent the occurrence of teratomas.

Although there are few clinical trials on tumour formation after stem cell transplantation, it is still uncertain whether a real tumour is formed. We firstly reported a clinical case of immature teratoma formed after iPSC treatment in T2DM. Unlike mature teratomas, immature teratomas have the potential for recurrence and metastasis. Therefore, they can be localized anywhere except in the limbs and lymph nodes[20]. Pashankar F et al. have shown that postoperative chemotherapy is standard in immature teratoma disease[21]. The tumour in the left upper arm developed rapid growth and local lymph node metastasis but was insensitive to chemotherapy. Okur FV et al. found that all iPSC lines expressed pluripotency-related genes, and disease-related mutations were retained in patient-specific iPSCs[22]. However, there was more cell atypia than in typical teratomas, which meant an increase in the components. The positive Oct4 and Sox2 indicators proved that the injected cells were not fully differentiated, and the negative insulin showed no insulin secretion function, which indicated that the differentiation state induced by iPSCs in vitro does not represent the state after implantation in vivo. A standardized good manufacturing practices (GMP) protocol strengthens the effective supervision and standardization of cell production processes to produce pancreatic β -cells and improves the effectiveness and safety of iPSC treatment[23].

Although iPSCs initiated the dawn of diabetes treatment, their tumourigenicity seriously hindered their clinical application and expansion. To the best of our knowledge, there is no clinical practice guidance for the appropriate treatment of teratomas caused by iPSC injection. We chose chemotherapy regimens in accordance with highly aggressive sarcoma[24]. Although radiation therapy is sometimes used in addition to surgery and chemotherapy, its benefits are unclear[25]. In this case, combined with the relevant genetic test results, we consider that the tumour is not sensitive to immunotherapy. At present, a large number of researchers are working on developing new reprogramming methods or establishing new mutation screening procedures (such as introducing non-tumourigenic genes and viruses) and adopting more standardized GMP production processes (such as 3D culture) to avoid tumour formation and immune response after transplantation[26]. In addition, vaccines based on iPSCs may provide a new

cancer immunotherapy option, but their efficacy and related immune mechanisms still need to be further studied and confirmed[27].

Based on the few clinical trials for T2DM currently available, the safety and validity of iPSC treatment need to be confirmed by more standardized clinical trials. More attention should be given to teratoma formation after iPSC treatment for T2DM, which is more aggressive than typical teratomas.

Abbreviations

iPSCs: induced pluripotent stem cells; MRI: Magnetic Resonance Imaging; OCT4: Recombinant Octamer Binding Transcription Factor 4; Sox-2: Octamer binding factor 4; GMP: Good Manufacturing Practice; DM: Diabetes mellitus; T2DM: Type 2 Diabetes Mellitus; BEP: bleomycin,etoposide and cisplatin; TIP: albumin-bound paclitaxel ifosfamide and cisplatin; PET/CT Positron Emission Tomography/Computed Tomography; SUV standard uptake value; PD-1 programmed death 1.

Declarations

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Authors' contributions

Lei Han and Hao He were major contributors to data collection and the writing of the manuscript. Yihao Yang completed immunohistochemistry assay. Qingyin Meng completed immunofluorescence assay. Fan Ye and Gong Chen contributed to Figures collection. Jing Zhang designed the main treatment plan and revised the manuscript. All authors have read and approved the final manuscript.

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Availability of data and materials

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

Competing interests

All authors declare that they have no competing interests.

Consent for publication

Consent was obtained from the patient to allow their information and images to be published.

Ethics approval and consent to participate

Treatment in tumor hospital of Yunnan province: Consent was obtained. A case report does not require approval from our institutional Research Board.

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Figures

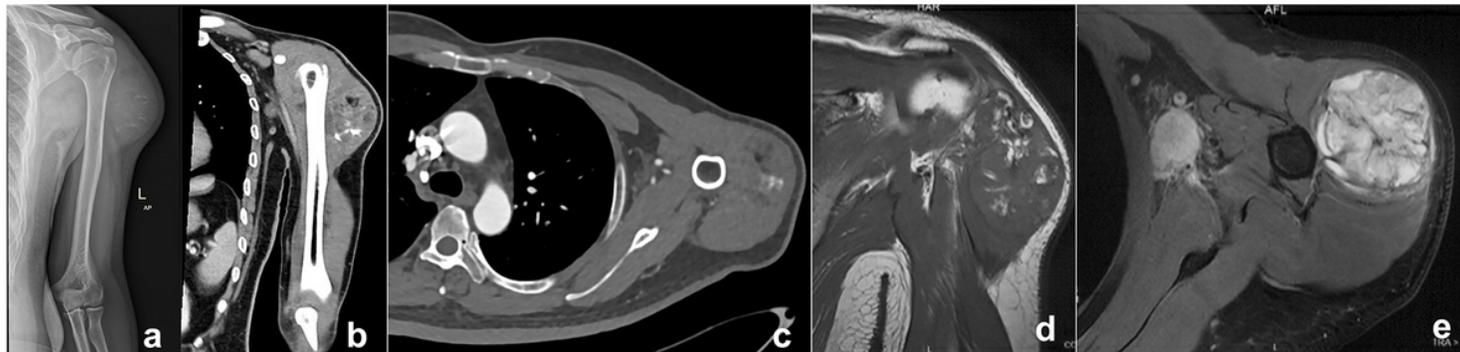


Figure 1

Imaging features before treatment. a X-ray image. In the deltoid muscle of the left upper arm, a round, mixed-density mass with an unclear boundary of 9.6 cm×5.2 cm was observed. The humeral cortex is intact. b, c Plain CT scan showed a round, mixed-density mass in the deltoid muscle with an unclear boundary. An enhanced CT scan showed uneven and moderate enhancement. d-e MRI T1W and T2W1 images. T1W was dominated by iso-signals, with patchy high- and low-signal areas. In addition, T2W1 also showed high-signal and patchy low-signal areas. The left axilla had multiple lymph node swelling, the signal was fair on plain scan, and the enhancement was roughly uniform.

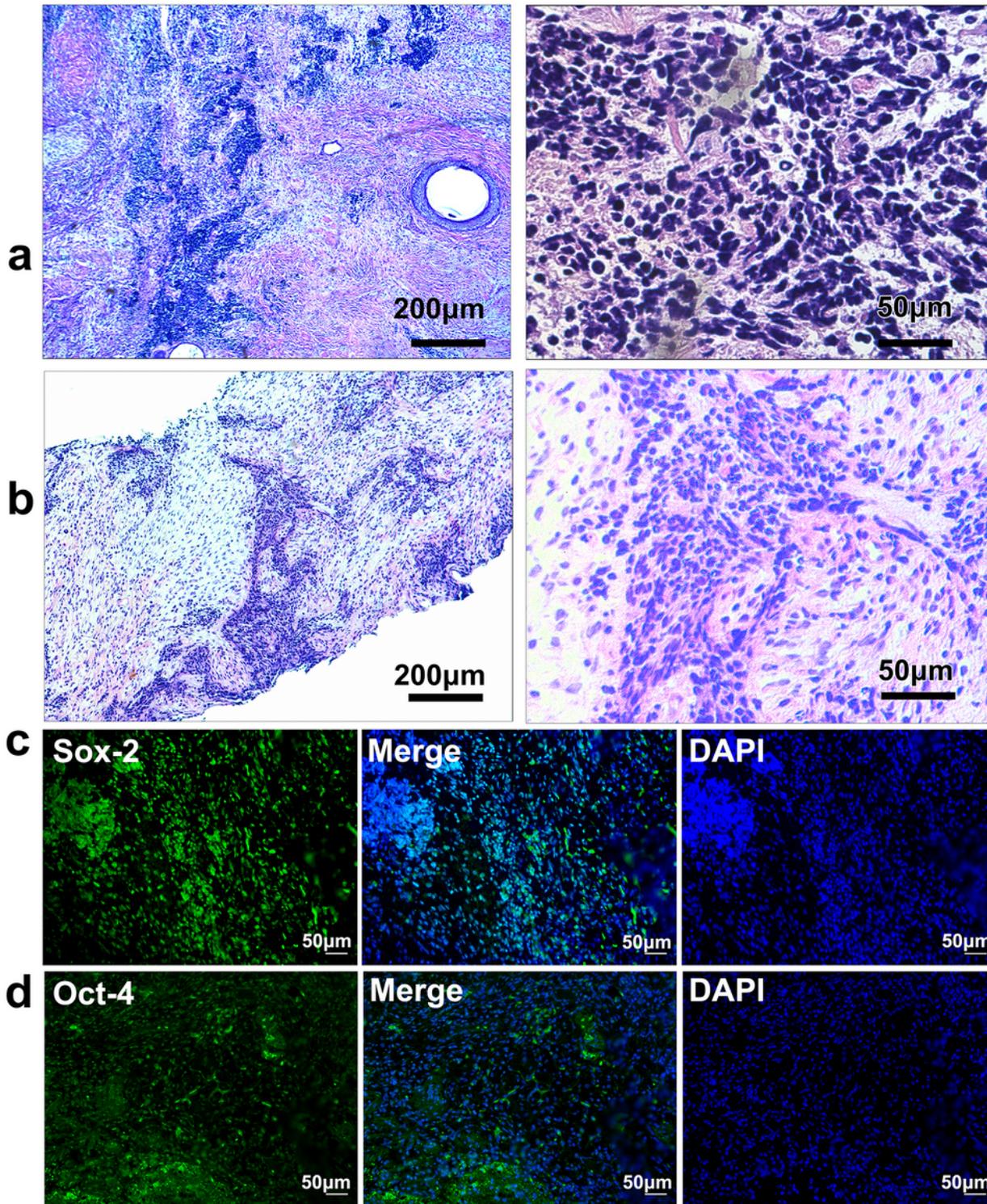


Figure 2

Pathology before and after treatment. a HE staining of the left upper arm tumour. Bone, cartilage, vasculature and adenoid tissue and other components, as well as the neuropillar structure and small round cells distributed in small pieces. The various components are arranged in a disorderly manner, and the shape of the lesion conforms to an immature teratoma, grade II. b HE staining of metastatic axillary lymph node. Different immature teratoma cells mixed in among small round lymphocytes. c-d

Immunofluorescence staining of iPSC differentiation markers. Both Oct-4 and Sox-2 staining showed nuclear positivity in paraffin sections of the left upper arm tumour.