Review

Safety of using cultured cells with trisomy 7 in cell therapy for treating osteoarthritis

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ABSTRACT

Cell therapy is a promising alternative treatment approach currently under study for osteoarthritis (OA), the most common chronic musculoskeletal disease. However, the mesenchymal stem cells (MSCs) used in cell therapy to treat OA are usually expanded *in vitro* to obtain sufficient numbers for transplantation, and their safety has not been fully assessed from multiple perspectives. Analysis of karyotypic abnormalities, in particular, is important to ensure the safety of cells; however, chromosomal mutations may also occur during the cell-expansion process. In addition, there have been many reports showing chromosome abnormalities, mainly trisomy 7, in the cartilage and synovium of patients with OA as well as in normal tissues. The suitability of cells with these karyotypic abnormalities as cells for cell therapy has not been evaluated. Recently, we assessed the safety of using cells with trisomy 7 from the osteoarthritic joint of a patient for transplantation, and we followed up with the patient for 5 years. This study showed analysis for copy number variant and whole-genome sequencing, compared with blood DNA from the same patient. We did not find any abnormalities in the genes regardless of trisomy 7. No side effects were observed for at least 5 years in the human clinical study. This suggests that the transplantation of cultured cells with trisomy 7 isolated from an osteoarthritic joint and transplanted into the osteoarthritic joints of the same person is not expected to cause serious adverse events. However, it is unclear what problems may arise in the case of allogeneic transplantation. Different types of risks will also exist depending on other transplantation routes, such as localization to the knee-joint only or circulation inflow and lung entrapment. In addition, since the cause of trisomy 7 occurrence remains unclear, it is necessary to clarify the mechanism of trisomy 7 in OA to perform cell therapy for OA patients in a safe manner.

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Contents

1. Introduction

The safety of cells for cell therapy needs to be assessed from multiple perspectives [1–3]. Analysis of karyotypic abnormalities, in particular, is important to ensure the safety of cells. Mesenchymal stem cells (MSCs) are widely used in clinical practice as a cell source for cell therapy [4–8]. MSCs are usually expanded *in vitro* to obtain sufficient numbers of cells for transplantation; however, chromosomal mutations may occur during the expansion process [9–11]. Moreover, there have been many reports showing the presence of chromosome abnormalities, mainly trisomy 7, in cartilage and synovial cells in patients with osteoarthritis (OA; Table 1). The developmental mechanism of trisomy 7 in OA patients is not clear; however, trisomy 7 is also found in patients with rheumatoid arthritis (RA) and in normal tissues. Therefore, the patient's own cells may possess trisomy 7. The suitability of cells with these karyotypic abnormalities as cells for cell therapy has not been evaluated. Recently, we reported on safety issues related to transplantation [12]. In this review article, we focused on research studies reporting on the transplantation of cells with trisomy 7 and discussed the current situation for cell therapy in OA. We also review reports from the 1990s regarding cells with trisomy 7 in OA patients and on the use of cultured cells with chromosome abnormalities for cell therapy.

2. Osteoarthritis

OA is the most common chronic musculoskeletal disease, occurring in the knee, spine, hip, hand, foot, and shoulder, or among other joints. Arthritis is considered to be the fourth leading cause of disability worldwide [13–15]. Knee OA, the most common form of OA, is a complex disease involving knee joint components such as bone, cartilage, meniscus, and synovium. Recently, research studies on the pathology and the cellular and molecular biological processes underlying OA have increased; however, the complexity of this condition is still unclear.

When the failure of joint tissue occurs, early intervention through surgical procedures is the most effective therapeutic solution before it progresses to OA [16]. Unfortunately, when OA of a joint has fully developed and does not respond to conventional conservative therapy, joint replacement with an artificial joint is currently the accepted treatment. In the United States, an estimated 600,000 knee replacements are performed annually [17]. According to the National Database of Health Insurance Claims and Specific Health Checkups of Japan, ~150,000 cases of arthroplasty are reported, and 80,000 knee replacements are performed annually [18]. However, knee replacement surgery is not without potential serious complications [19,20]. As many as 20% of patients continue to have knee pain and other problems after joint replacement surgery [21]. The frequency of revision total arthroplasty within 10 years is 6.2–8.9% [22,23]. These problems have led to the development of alternative treatment methods.

Cell therapy is expected to be one of the alternative treatment methods for OA, and various technological developments are being attempted. Culturing of the cells used in this treatment requires considerable effort and cost because the cells need to be cultured in a clean-room environment [24]. Despite this, many clinical trials using cell therapy are currently being conducted. A search on clinicaltrials.gov using the keywords "cell therapy" and "knee osteoarthritis" in January, 2022, revealed 169 registered trials. The cells used in these studies include adipose-derived stem cells, bone marrow−derived MSCs, umbilical cord−derived MSCs, and synovium-derived MSCs [25−27].

3. Cell culture in cell therapy and chromosome abnormalities

Cell preparation for transplantation requires the isolation and culturing of cells from tissues to obtain sufficient cell numbers *in vitro*. Mass culturing to obtain a quantity of cells that can be transplanted may introduce risks. Studies using bone marrow−derived MSCs have shown chromosomal alterations after four passages [9]. Other studies revealed that karyotypic abnormalities were detected in 6 of 144 MSC samples (4%) [10]. In peripheral blood−derived MSC passaging experiments, genomic instabilities, such as single-nucleotide variations, were detected from passage 1 to passage 9 [11]. In neural stem cells, chromosome abnormalities were detected in 9 of 97 samples (9%), suggesting that instability varies by cell type [10]. Therefore, when cells are cultured and used in large quantities, evaluation of the instability prior to using the cells is important.

4. Chromosome abnormalities in osteoarthritis

Chromosome abnormalities are known to be acquired *in vivo* as well as in cell culture. Chromosome abnormalities in OA have been reported in several instances since the 1990s (Table 1). F. Mertens et al. reported that in cultured cells of osteophytes, synovium, and cartilage from patients with knee OA, chromosome abnormalities were present in about 90% of the cases [28]. Chromosome abnormalities were observed in the osteophytes of 27 of 29 patients (93%), the synovia of 16 of 17 patients (94%), and the cartilage of 8 of 9 patients (89%). These chromosome abnormalities were not related to the grade of knee OA based on radiographic evaluation. Most of these abnormalities were trisomy 7, trisomy 5, and deletion of the Y chromosome, although inversions of chromosomes 1 and 22 and translocations between chromosomes 13 and 22 were also observed. In samples of 100−200 cells, trisomy 7 was found to be particularly frequent, observed in 6 ± 5% of osteophytes, 8 ± 6% of synovial cells, and 6 ± 6% of chondrocytes. In cells isolated from patients with anterior cruciate ligament tear or meniscus tear, trisomy 7 was found in 3 ± 2% (6 of 17) patients. These results suggested that these somatic abnormalities occur early in the development of knee OA and may be involved in the pathogenesis of the disease [28].

RW Kinne et al. analyzed enzyme-digested synovial tissue cells and cultured synovial cells isolated from patients with knee OA and RA using fluorescence *in situ* hybridization (FISH), and peripheral blood and skin cells as controls [29]. In cultured synovial cells isolated from OA patients, chromosome abnormalities were observed in all 16 patients. In cultured synovial cells isolated from

Table 1
Previous reports on the detection ratio of trisomy 7 and other chromosomal abnormalities, mainly in arthritis.

Primary disease	Analysis object	No. Of cases	Chromosome abnormality ratios in cases	Major abnormalities	Chromosome abnormality ratios in cells	References
PVNS	Synovial cells	4	100%	Trisomy 7 Trisomy 5	2–42.5%	[54]
RA	Synovial cells	7	86%	Trisomy 7 Trisomy 5	0–32%	[55]
RA	Synovial cells	5	100%	Trisomy 7	0–80%	[56]
OA	Osteophytes	29	93%	Trisomy 7	0–29%	[28]
	Synovial cells	17	94%	Trisomy 5	0–50%	
	Chondrocytes	9	89%		0–18%	
OA	Synovial cells Osteophytes	10	100%	Trisomy 7 Trisomy 5	0–24%	[57]
OA	Synovial cells	9	N.A.	Trisomy 7	5–20%	[58]
RA	Synovial cells	8			5–20%	
NA	Synovial cells	5			0.50%	
OA	Synovial tissue enzyme-digested cells	13	100%	Trisomy 7	5%	[29]
	Synovial cells	16	100%	Monosomy X	5–10%	
RA	Synovial tissue enzyme-digested cells	5	100%		15%	
	Synovial cells	10	90%		15%	
NA	Synovial tissue enzyme-digested cells	1–3	0%	N.D.	N.D.	
	Synovial cells	1–3	0%	N.D.	N.D.	
OA	Synovial cells	8	100%	Trisomy 7	4–30%	[59]
PVNS	Synovial cells	2	100%	Trisomy 7	0%	
HS + CS	Synovial cells	2	100%	Trisomy 7	0%	
NA	Synovial cells	2	100%	Trisomy 7	0%	
OA	Paraffin-embedded synovia	8	100%	Trisomy 7	2–12%	
PVNS	Paraffin-embedded synovia	2	100%	Trisomy 7	3–15%	
HS + CS	Paraffin-embedded synovia	2	100%	Trisomy 7	4–5%	
NA	Paraffin-embedded synovia	2	100%	Trisomy 7	0–2%	
JCA	Synovial cells	9	11.1%	Trisomy 7	0–0.05%	[34]
OA	Synovial cells	10	50%	Trisomy 7	0–39.5%	
OA	Synovial tissue enzyme-digested cells	9	100%	Trisomy 7	1–25%	[33]
	Synovial cells	12	100%		1–42%	
	Synovial macrophages	13	92%		0–24%	
RA	Synovial tissue enzyme-digested cells	5	100%		4–12%	
	Synovial cells	6	100%		5–46%	
	Synovial macrophages	8	100%		2–9%	
JT	Synovial tissue enzyme-digested cells	N.A.	N.A.		N.A.	
	Synovial cells	3	66%		0–5%	
	Synovial macrophages	3	66%		6–7%	
OA	Synovial tissue enzyme-digested cells	44	18%	Trisomy 7 Monosomy X	4–12%	[30]

Abbreviations: PVNS, pigmented villonodular synovitis; OA, osteoarthritis; RA, rheumatoid arthritis; NA, non-arthritis; HS, hemorrhagic synovitis; CS, chronic synovitis; JCA, juvenile chronic arthritis; JT, joint trauma; N.A., not assigned; N.D., not detected.

RA patients, chromosome abnormalities were observed in all 10 patients. The most frequent chromosome abnormality, trisomy 7, was observed in $20 \pm 14\%$ of synovial cells isolated from OA patients and in $13 \pm 11\%$ of synovial cells isolated from RA patients, out of 10–50 cells examined. Chromosome abnormalities were also observed in enzyme-digested synovial-tissue cells before culture, including in all 13 patients with OA and in all 5 patients with RA, whereas peripheral-blood control samples showed no chromosome abnormalities. In RA patients, abnormalities found in chromosomes 6 and 8 of cultured synovial cells correlated with disease duration, and blood C-reactive protein (CRP) levels correlated with trisomy 7. These results indicate that chromosome abnormalities may reflect the pathogenesis of chronic inflammation [29].

Castellanos MV et al. analyzed chromosome abnormalities in cultured chondrocytes from OA patients by FISH, focusing on chromosome 7, X, and Y. In this study, chromosome abnormalities were found in 33% (7 of 21) of the patients. Trisomy 7 was detected in $2 \pm 1\%$ of the 200 cells analyzed; deletions of chromosome X were found in 57% (12 of 21) of the patients and in $4 \pm 2\%$ of the 200 cells. They also reported the presence of chromosome abnormalities in OA of the lumbar spine at a frequency similar to that of the knee [30].

The variations in the ratios of trisomy 7 in these previous reports appear to be mainly due to the limitations of the analytical techniques, including the variability in the tissues and cells, and because the number of cells that can be analyzed by the G-band method and FISH is limited to about 200 cells [31]. While several studies have reported chromosome abnormalities occurring in osteoarthritic tissue, the frequency may increase or decrease depending on the detection sensitivity.

The array comparative genomic hybridization (CGH) method can detect chromosome aberrations based on comprehensive analysis, but it cannot detect structural aberrations such as inversions and translocations. Using array-CGH, Stumm et al. found a deletion of the Y chromosome in 50% (4 of 8) of the OA patients analyzed [32]. In this analysis, the authors investigated the functional effects of chromosome abnormalities focusing on SRY-box transcription factor 9 (*SOX9*), a Y-chromosome gene involved in early chondrogenic differentiation. The incidence of deletion of chromosome Y in chondrocytes increased with age; however, the expression of *COL2A1* and *ACAN*, which are related to the extracellular cartilage matrix, did not change. Therefore, the authors considered that the presence of chromosome abnormalities did not affect function in the cultured cells [32].

4.1. Trisomy 7 in osteoarthritis and other tissues

Trisomy 7, in particular, is frequently detected in cells derived from osteoarthritic joints (Table 1). It has also been found in other

tissues, including in macrophages derived from bronchoalveolar lavage fluid in chronic obstructive pulmonary disease; therefore detection of trisomy 7 is considered to reflect an inflammatory response [33]. Trisomy 7 is also common in other tissues, such as the colon, kidneys, and skin, and its rate may increase with age [34–37]. There are other reports of trisomy 7 in cell cultures of normal kidney and brain tissue [38,39]. In colorectal cancer, trisomy 7 with aberrant expression of *EGFR* is frequently observed [40]. The correlation between chromosome 7 and OA was reported in a genome-wide association study of 1341 Dutch human cases of OA; 7q22 was detected as a novel common variant affecting the prevalence and progression of OA [41]. However, it is not clear whether chromosome 7 trisomy is a cause or a consequence of OA. Thus, the suitability of cells with trisomy 7 as cells for cell therapy needs to be further evaluated.

4.2. Trisomy 7 in cultured cells for cell therapy

Cells isolated from the synovium of OA patients also possess trisomy 7. Synovial MSCs are cells with colony-forming ability and differentiation capability isolated from the synovium and are widely used in clinical practice as a cell source for cell therapy [42–45]. However, the presence of trisomy 7 has been reported in cultured synovial MSCs (Fig. 1); these MSCs were transplanted into knee joints and followed up for 5 years [12]. Various investigations related to this study are ongoing using primary synovial MSCs and their surplus passaged cells. Cell culture growth curves were analyzed for up to 15 cell passages in the presence and absence of trisomy 7. As a safety test, synovial MSCs with trisomy 7 were subjected to the soft agar colony formation test, a transplantation test into immunodeficient mice, whole genome analysis, and DNA methylation analysis. For functional evaluation, gene expression profiles, cell surface antigens, and chondrogenic potential were analyzed. In 3 of the 10 patients, the transplanted synovial MSCs

had trisomy 7 in 5–10% of the cells. In the growth-curve analysis, no difference was observed between synovial MSCs with and without trisomy 7. The effect of synovial MSC from passage 0 to passage 15 on the proportions of trisomy 7 was variable and showed three patterns: invariant, increasing, and decreasing. Synovial MSCs are a heterogenous cell population and form diverse colonies, which may explain the various patterns of trisomy 7 [46,47]. There were no differences in the expression of tumor-related genes, such as *CDKN1A*, *CDKN2A*, *MYC*, and *KIT*; or genes on chromosome 7, such as *EGFR*, *HGF*, *IL6*, and *PPIA*, depending on the presence or absence of trisomy 7. In a transplantation study into immunodeficient mice, HT-1080 cells formed tumors, whereas synovial MSCs with trisomy 7 did not form tumors [12]. Whole genome sequence analysis and DNA methylation analysis showed no difference in the results between synovial MSCs with and without trisomy 7. No gene abnormalities were observed, regardless of trisomy 7. There were also no differences in cell-surface antigens or chondrogenic potential between synovial MSCs with and without trisomy 7. None of the 10 patients, with or without trisomy 7, exhibited any tumor formation by 5 years after transplantation. These results support the conclusion that the presence of trisomy 7 in autologous synovial MSCs does not affect subsequent transplantation safety and functional analysis, and trisomy 7 does not cause tumor formation for 5 years after transplantation.

The presence of trisomy 7 in chondrocytes derived from OA patients was described in a June 5, 2012, report reviewing J-TEC autologous cultured cartilage (JACC), a cell therapy and gene therapy product approved by the Pharmaceuticals and Medical Devices Agency (PMDA) in Japan [48]. JACC is a three-dimensionalized culture of chondrocytes in collagen gel. In an evaluation using chondrocytes isolated from the knee-joint cartilage of three patients with OA, chromosome abnormalities were observed in all pre-cultured cells, and trisomy 7 was detected in 2 out of 20 cells in all cases. In one case, trisomy 7 was detected at the same rate before
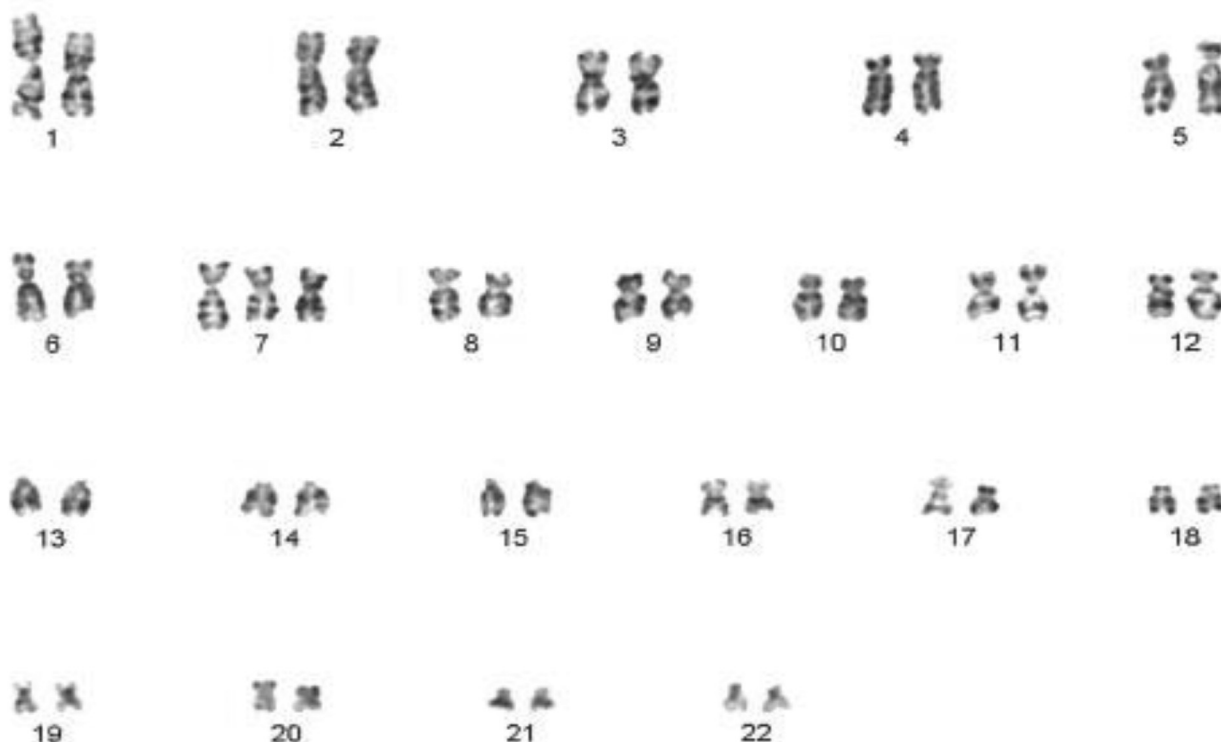

Fig. 1. Trisomy 7 observed in synovial mesenchymal stem cells in a clinical trial. Chromosome X or Y are not shown to prevent patient identification.

and after culture. Normal chromosomes were observed in the other two cases after culture. Since there were no new chromosome aberrations after culture, the report (June 5th, 2012) described that chondrocyte cytogenetic stability was not affected by the culture process using the stated manufacturing method for this product. Five years after manufacturing and marketing approval of JACC by the PMDA, no adverse events suspected to be related to karyotypic abnormalities were reported in post-marketing surveillance [49].

Yokoyama et al. evaluated chondrocytes with trisomy 7 derived from patients with knee OA and the effect of passaging as well as tumorigenicity following transplantation. The karyotype of chondrocytes cultured for a longer time period than the normal process showed no abnormal alterations based on array-CGH and G-band results. The authors transplanted cultured chondrocyte sheets into immunodeficient mice, and subsequent testing revealed no tumorigenicity associated with the chondrocyte sheets with trisomy 7. The authors confirmed by bioluminescence imaging that the transplanted chondrocyte sheets remained in the knee joint and did not transfer elsewhere for one month [50].

5. Allogeneic transplantation and route of administration

Based on these previous reports, the transplantation of cultured cells isolated from an osteoarthritic joint into one's own osteoarthritic joints is not expected to cause serious adverse events. However, it is unclear what problems may arise in the case of allogeneic transplantation. Different types of risks will exist depending on the route of transplantation, even for the same cultured cells. Therefore, each treatment target requires a unique risk-management strategy. For example, synovial MSCs implanted in the joint will remain in the joint and have no effect on the whole body [51,52]. However, intravenously administered MSCs can adhere to various tissues—such as the lungs—and be maintained for long periods of time, which may pose as-yet unknown risks in the future [53]. Even though no side effects have been observed in transplantations using cells with chromosome abnormalities in previous reports, this does not guarantee that allogenic cells with chromosome abnormalities can be safely transplanted into humans without considering the type of cultured cells or the method of administration.

6. Conclusions

The safety of cells with karyotypic abnormalities generated during culture has not yet been evaluated. Therefore, the occurrences of karyotypic abnormalities *in vivo*, by disease, and *in vitro*, by culture, need to be considered separately. Clarifying the mechanism of trisomy 7 in patients with OA will also help in providing safe cell therapy for these patients.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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