[Short communication]

Therapy of autologous human adipose tissue-derived mesenchymal stem cells for the cerebral palsy: a case report

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Short Running Title : Therapy of autologous Had-MSCs for the cerebral palsy: a case report

ABSTRACT

This case report describes the efficacy of intravenous administration of autologous human adipose tissue-derived mesenchymal stem cells (hAdMSCs) in a female patient aged 3 years and 7 months with cerebral palsy. Our group previously demonstrated the *in vitro* differentiation capacity of hAdMSCs into adipocytes, osteoblasts and neuronal cells. This case was conducted as compassionated stem cell therapy.

Subcutaneous fat was collected from the patient's abdomen via liposuction. Isolation and cultivation of the hAdMSCs was performed as previously described. The immunophenotype and karyotype of the hAdMSCs was evaluated, followed by confirmation of their differentiation potential *in vitro*. Clinical examinations were performed immediately before and 1 year after the first stem cell intravenously infusion at intervals of 3 months, and included the following tests: 1) the Gross Motor Function Measure; 2) the Oral Motor Function Assessment; 3) the Urimal Test of Articulation and Phonology; 4) the Korean Developmental Test of Visual Perception-2; and 5) the Kaufman Assessment Battery for Children.

The patient did not have any adverse reactions during intravenous infusion of autologous hAdMSCs or at post-treatment follow-up. Gross motor function and the motor function of the tongue, jaw, and lip, all showed noticeable improvements. In particular, the motor function of the tongue was markedly increased by hAdMSC administration, leading to enhanced articulation skills post-infusion. The patient also showed improvement in the visual motor integration and general visual perception categories of the K-DTVP-2. In addition, her acquired learning skills, as assessed by the Kaufman Assessment Battery for Children, were significantly increased.

Keywords: Autologous adipose tissue-derived mesenchymal stem cell, cell therapy, cerebral palsy, systemic infusion

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INTRODUCTION

Neonatal encephalopathy due to prenatal hypoxia-ischemia occurs in one to three per 1,000 live births. Neonatal encephalopathy is associated with high mortality and morbidity, as well as life-long chronic disabilities, including cerebral palsy. Cerebral palsy in turn describes a group of movement and posture disorders attributed to non-progressive disturbances in the developing fetal or infant brain. Cerebral palsy is characterized by permanent neurologic damage and activity limitations and has no known cure.

Recently, accumulating evidence indicates that mesenchymal stem cells (MSCs) can differentiate into neural cells *in vitro* [1] and protect the brain in animal models of central nervous system (CNS) injury [unpublished article, Kim YB et al.]. Furthermore, a new clinical trial is underway involving transplantation of autologous bone marrow-derived MSCs in children with cerebral palsy, with the hope that this novel therapeutic modality will improve patient quality of life and reduce the effects of the disorder [2].

This case report now describes the efficacy of systemic intravenous infusion of autologous human adipose tissue-derived MSCs (hAdMSCs) for the treatment of cerebral palsy in children.

METHODS

A comparison is provided herein between pre-treatment and follow-up medical report data concerning the use of intravenously infused autologous MSCs in a young female patient (aged 3 years and 7 months) with cerebral palsy. This compassionate use of cell therapy was based on the confirmed *in vivo* safety profile of MSCs and their demonstrated beneficial

properties.

Prior to stem cell therapy, an informed consent form was signed by the parental guardians of the patient. By signing the informed consent form, the guardians agreed to provide the medical records for the publication of this case report.

General patient information

The patient was born by caesarean section in January, 2008, and presented with intermittent cyanosis and spasms in the eye immediately after birth. The patient was subjected to evaluation via magnetic resonance imaging (MRI) and angiography, leading to the diagnosis of a cerebral infarction that involved a portion of the left frontal lobe, the entire temporal lobe and a portion of the occipital lobe (Figure 1). Her overall development was normal for her age, but her movements and dexterity were restricted due to paralysis of the right upper limb. The patient also had an unnatural gait characterized by imbalance and coordination problems that affected the upper and lower extremities, resulting from paralysis of the right lower limb. She demonstrated facial asymmetry and loss of facial expression due to facial paralysis, and paralysis of the tongue resulted in the incorrect pronunciation of words along with inaccurate articulation. The patient has been receiving rehabilitation and physical therapy from the age of 5 months.

Source, culture, quality standards and multiple lineage cell differentiation of hAdMSCs

The patient was subjected to hematology and serological tests for liver and renal function prior to the collection of subcutaneous fat from the abdomen via liposuction. The results of the tests were normal. The patient was not infected with syphilis, human immunodeficiency virus (HIV), hepatitis B or hepatitis C, and there was no history of familial or hereditary

disease.

Isolation and cultivation of hAdMSCs was performed, as previously described, under good manufacturing practice (GMP) conditions at the Stem Cell Research Center of RNL Bio Co., Ltd. (Seoul, South Korea) [1]. Multiple hAdMSC aliquots were prepared following passage 2 and stored in liquid nitrogen vapor. Cryopreserved cells were thawed and recultured in growth medium according to the infusion schedule (see below). Cells were harvested at passage 4 and tested for cell count and viability. Cells were also screened for endotoxin and mycoplasma contaminants before each intravenous infusion. No evidence of bacterial, fungal, or mycoplasma contamination was observed in the hAdMSCs tested before infusion (data not shown).

The cells isolated from the patient's adipose tissue showed typical hAdMSC morphology. The differentiation capacity of the hAdMSCs into osteoblast, adipocyte, hepatocyte (Figure 2) and neuronal cells (Figure 3A) was confirmed *in vitro*.

The immunophenotype of the hAdMSCs was analyzed by using FACS (fluorescence-activated cell sorter), a FACS Calibur flow cytometer and CELL Quest software (BD Biosciences, San Jose, CA, USA). The distinguishing phenotype of CD29-, CD44-, CD73-, CD90-, CD105-, and HLA-ABC-positive was detected in more than 95% of the cells, and CD31, CD34, CD45, and HLA-DR antigens were expressed in less than 4% of the cells (Figure 3B). Finally, a karyotype analysis was performed at Samkwang Medical Laboratories (Seoul, Korea), by the Cytogenomic Services Facility and then was normal (Figure 3C).

Administration schedule of stem cell infusions

The autologous hAdMSCs (1×10^8 cells) were fully resuspended and mixed with normal saline (100 ml). The cells were intravenously infused, and the interval between each infusion was 3 months. The first injection was performed when the patient was 3 years and 7 months

old (2011.08.13). A total of 4×10^8 cells were infused on four separate occasions, and the last infusion (2012.07.18) was performed immediately prior to the time of this report.

Clinical examinations

Clinical examinations were performed in the clinic by a specialist who was not associated with the stem cell infusion procedure. Examinations were performed immediately before and 1 year after the first stem cell infusion. The examinations included: 1) the Gross Motor Function Measure (Korean version) [3]; 2) the Oral Motor Function Assessment (described in the Appendix); 3) the Urimal Test of Articulation and Phonology for Children [4]; 4) the Korean Developmental Test of Visual Perception-2 (KDTVP-2) [5]; and 5) the Kaufman Assessment Battery for Children (Korean version) [6].

RESULTS

Gross Motor Function Measure

The Gross Motor Function Measure [3] is an instrument comprising five dimensions (1: lying and rolling; 2: sitting; 3: crawling and kneeling; 4: standing; and 5: walking, running and jumping) to measure the gross motor function of children with cerebral palsy. A score of 100% refers to performance at full capacity. As shown in Table 1, gross motor function was maintained at 100% in the dimensions of lying, rolling, sitting, crawling, and kneeling, after autologous hAdMSC infusion, the same as prior to infusion. Function was improved from 92.3% (pre-treatment) to 100% (post-treatment) in the dimension of standing, and from 97.06% (pre-treatment) to 100% (post-treatment) in the combined dimension of walking, running and jumping.

Oral Motor Function Assessment

Oral motor function was examined at 1 year after the first stem cell treatment (2012.07.18) and showed improvements in jaw, lip, and tongue movements compared with oral motor function prior to stem cell infusion (Figure 4).

Urimal Test of Articulation and Phonology for Children

The Urimal Test of Articulation and Phonology for Children [4] was performed before and 1 year after stem cell infusion. Prior to stem cell treatment (2011.08.05), the patient demonstrated 100% accuracy in consonant and vowel pronunciation at the word level, but overall intelligibility was low at the sentence level. The reduced intelligibility was due to impaired oral motor function, including disturbances in the point of articulation and a slow rate of change in articulation placement. The patient was recommended for oral articulation therapy.

At 1 year after the first stem cell treatment (2012.07.17), verbal expression was improved and indeed, was high compared with the patient's chronological age. The child demonstrated age-appropriate phonological capabilities and did not present with any overall defects in language skills.

The Korean Developmental Test of Visual Perception-2

The Korean Developmental Test of Visual Perception-2 (KDTVP-2) [5] was scored following the assessment of three composite categories: general visual perception (GVP), reduced motor perception (RMP), and visual motor integration (VMI). The standard for each of the composite categories was as follows: 130 and over = very superior; 121 to 130 = superior; 111 to 120 = above average; 90 to 100 = average; 80 to 89 = below average; 70 to 79 = inferior; and less than 70 = severely inferior.

One year after the initial hAdMSC infusion, the results of the KDTVP-2 showed

improvements in the GVP category, from the average level (before treatment) to the superior level (after treatment) (Table 2). Furthermore, improvements were observed in the RMP category, from the above average level (before treatment) to the very superior level (after treatment). The post-treatment RMP score was above the 99th percentile and was equivalent to the average score of a child aged 9 years and 2 months (Table 2, Figure 5). Finally, the patient improved from the inferior level in the VMI category to the average level. The VMI score was at the 5th percentile before treatment vs. the 58th percentile after treatment. The post-treatment VMI score was equivalent to the average score of a child aged 4 years and 1 month (Table 2, Figure 5).

Kaufman Assessment Battery for Children

The Kaufman Assessment Battery for Korean Children [6] measures intelligence by concentrating on the child's ability to solve unfamiliar problems both simultaneously and sequentially. Simultaneous and sequential processing scores are combined to comprise the composite cognitive (mental processing) score, whereas the achievement score quantitates achievement and focuses on applied skills and facts that are learned through the school or home environment.

As shown in Table 3, the patient showed an improvement from the 63rd (pre-treatment) to the 99th percentile (post-treatment) on the overall achievement scale, whereas no clear differences were observed pre- and post-treatment on the cognitive scale.

DISSCUSSION

Accumulating evidence indicates that hAdMSCs show potential for neural differentiation and an ability to protect neural cells from damage in animal models of CNS injury. Thus, these cells represent a new approach to cell-based therapy for the management

of cerebral palsy. Especially, intravenous infusion of hAdMSCs was recently shown to improve both physical activity and cognitive defects in an animal model of HILR (hypoxia-ischemia-lipopolysaccharide reperfusion)-induced experimental cerebral palsy. These efficacy may have resulted from the secretion of growth factors and/or neurotrophic factors by the hAdMSCs, which then protected the myelin sheaths of oligodendrocytes from injury-associated damage. Furthermore, the infused hAdMSCs differentiated into Olig2-positive oligodendrocyte lineage cells and neurofilament-positive neuronal cells, but not into glial fibrillary acidic protein positive-astrocytes [unpublished article by Kim YB et al]. Thus, the regenerative capacity of hAdMSCs probably also stemmed from their ability to replace damaged oligodendrocytes and neurons without forming glial scars.

To the best of our knowledge, this is the first report regarding the safety and efficacy of stem cell transplantation in children with cerebral palsy. The subject of the current report was a young patient aged 3 years and 7 months with numerous communication impediments [7-8] associated with articulation shortcomings, such as restricted tongue movement and a limited range of motion of the tongue [9]. To this point, difficulties in fine-tuning the tip of the tongue have been shown to be associated with articulation errors [10], and the patient's oral motor function (tongue, jaw, and lip movements) was markedly improved following stem cell infusion. The movement of the tongue in particular was affected, resulting in increased articulation skills. The child also showed improvement in the VMI and GVP categories of the KDTVP-2, as well as in acquired learning skills.

CONCLUSIONS

The current results suggest that infusion of autologous hAdMSCs can be effective therapy for cerebral palsy. We anticipate that repeated administration of an adequate number of hAdMSCs will prevent further neurological damage following the onset of cerebral palsy.

ABBREVIATIONS

CNS, central nervous system; FACS, fluorescence activated cell sorter, GMP, good manufacturing practice; GVP, General Visual Perception; hAdMSC, human adipose tissue-derived mesenchymal stem cell; HILR, hypoxia-ischemia-lipopolysaccharide reperfusion; HIV, human immunodeficiency virus; HLA, human leukocyte antigen; KDTVP-2**,** Korean Developmental Test of Visual Perception-2; MSC, mesenchymal stem cell; MRI, magnetic resonance imaging; RMP, Reduced Motor Perception; VMI, Visual Motor Integration

CONFICT OF INTERESTS

Ken Nakama, Soo Won Choi, Pil Soon Yang and Kyeong Chin Song have no competing financial or personal interests in this work. Myung Soon Ko and Jung Youn Jo are employees of RNL BIO and declares no competing financial interests. Jeong Chan Ra is employee and shareholder of RNL BIO Limited.

AUTHORS' CONTRIBUTIONS

K. Nakama cared and treated patient. S.W. Choi and P.S. Yang assisted in data analysis and manuscript preparation. K.C. Song cared patient and collected the data. M.S. Ko participated in study coordination and data collection, and wrote the final version of the manuscript. J.Y. Jo characterized and performed cell differentiation of human AdMSCs. J.C. Ra involed in the preparation of AdMSCs and drafted and revised the manuscript. All authors read and approved the final manuscript.

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272 **TABLES**

273 Table 1. Gross Motor Function Measurement scores before and after hAdMSC treatment.

Category	Pre-treatment (2011.08.06)	Five months after first treatment (2012.01.20)	One year after first treatment (2012.07.15)
Lying and rolling	100%	100%	100%
Sitting	100%	100%	100%
Crawling and kneeling	100%	97.6%	100%
Standing	92.3%	97.2%	100%
Walking, running and jumping	97.06%	98.5%	100%

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275 Table 2. K-DTVP2 scores before and after hAdMSC treatment.

Category	Two months after first treatment (at 3 years and 9 months old, 2011.10.25)			One year after first treatment (at 4 years and 6 months old, 2012.07.25)		
	Score	Percentile	Level	Score	Percentile	Level
General visual perception (GVP)	96	**39**	Average	124	**95**	Superior
Reduced motor perception (RMP)	117	87	Above average	142	>99	Very superior
Visual motor integration (VMI)	75	**5**	Inferior	103	**58**	Average

276 * K-DTVP2 is the Korean Developmental Test of Visual Perception-2.

277 Table 3. Kaufman Assessment Battery for Korean Children results before and after
278 hAdMSC treatment.

Category	Percentile		
	Pre-treatment (2011.03.07)	Two months after first treatment (2011.10.26)	One year after first treatment (2012.07.16)
Sequential processing score	96.0	98.0	98.0
Hand movement	84.0	95.0	84.0
Number recall	95.0	91.0	99.6
Simultaneous processing score	99.7	99.9	99.9
Magic window	99.9	99.0	91.0
Face recognition	98.0	95.0	99.0
Gestalt closure	95.0	99.9	99.9
Cognitive score	99.7	99.9	99.8
Achievement score	**63.0**	**98.0**	**99.0**
Expressive vocabulary	96.0	99.0	99.0
Faces and places	**18.0**	**58.0**	**88.0**
Arithmetic	**86.0**	**99.0**	**96.0**
Riddles	**30.0**	**94.0**	**95.0**

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FIGURE LEGENDS

Figure 1. Brain MRI showing cerebral infarction affecting a portion of the left frontal lobe, the entire temporal lobe and a portion of the occipital lobe.

Figure 2. Human adipose derived mesenchymal stem cell have the multiple lineage differentiation potency into A) osteoblasts, B) adipocyte and C) hepatocyte. Scale bar is 100μm. PAS staining, Periodic acid schiff staining, OPG, Osteoprotegerin, ALP, Alkaline phosphatase, RUNX2, Runt-related transcription factor 2, Col Ⅰ, Type Ⅰ collagen, OPN, Osteopontin, PPARG, Peroxisome proliferator-activated receptor gamma, LPL, Lipoprotein lipase, FABP4, Fatty acid binding protein 4, AFP, Alpha fetoprotein, CK-18, Cytokeratin-18, HNF-4A, Hepatocyte nuclear factor 4 alpha, GAPDH, Glyceraldehyde-3-phosphate dehydrogenase.

Figure 3. Stem cell characteristics showing the A) neuronal cells differentiation B) immunophenotype and C) karyotype analysis of hAdMSCs. Scale bar is 100μm. TUJ1, Neuronal class Ⅲ beta-tubulin, SOX2, Sex determining region Y-box 2, NSE, Neuron specific enolase, GAPDH, Glyceraldehyde-3-phosphate dehydrogenase.

Figure 4. Oral motor function scores before and after treatment with autologous hAdMSCs. A) Total score, B) jaw subitem score, C) lip subitem score and D) tongue subitem score.

Figure 5. Changes in the A) RMP and B) VMI score before and after treatment with autologous hAdMSCs. RMP, Reduced motor perception, VMI, Visual motor integration.

Appendix. Oral Motor Function Assessment

Category	Assessment subitem
Jaw	J-1. Jaw opening
	J-2. Ability to make a sound with the upper and lower teeth
	J-3. Jaw movement from side-to-side
	J-4. Ability to cover the lower lip with the upper lip
	J-5. Ability to cover the upper lip with the lower lip
	J-6. Jaw rotation in a circular motion
Lip	L-1. Lip protrusion (kissing position followed by relaxation)
	L-2. Lip retraction (smiling followed by relaxation)
	L-3. Cheek puffing
	L-4. Opening and closing of the lips while keeping the teeth closed
	L-5. Ability to bite the lower lip with the upper teeth
	L-6. Ability to say the syllable “Pa”
Tongue	T-1. Tongue extension and retraction
	T-2. Ability to tip up the upper lip with the tongue
	T-3. Ability to tip down the lower lip with the tongue
	T-4. Ability to alternately tip up the upper lip and tip down the lower lip with the tongue
	T-5. Ability to push the right corner of the mouth with the tongue
	T-6. Ability to push the left corner of the mouth with the tongue
	T-7. Ability to alternately push the right and left corner of the mouth with the tongue

Assessment base (or score)

0 points: Impossible even with the physical assistance of a therapist.

1 point: Possible to perform action 5 times with the physical assistance of a therapist.

2 points: Possible to perform action 10 times with the physical assistance of a therapist.

3 points: Possible to perform action 5 times by oneself.

4 points: Possible to perform action 10 times by oneself.

5 points: Possible to perform action 10 times by oneself, with stability and correct position within 20 seconds.

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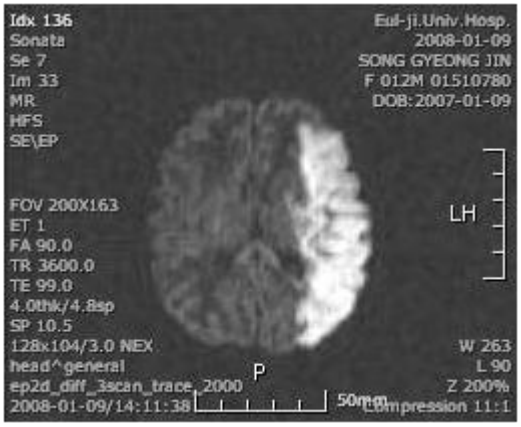

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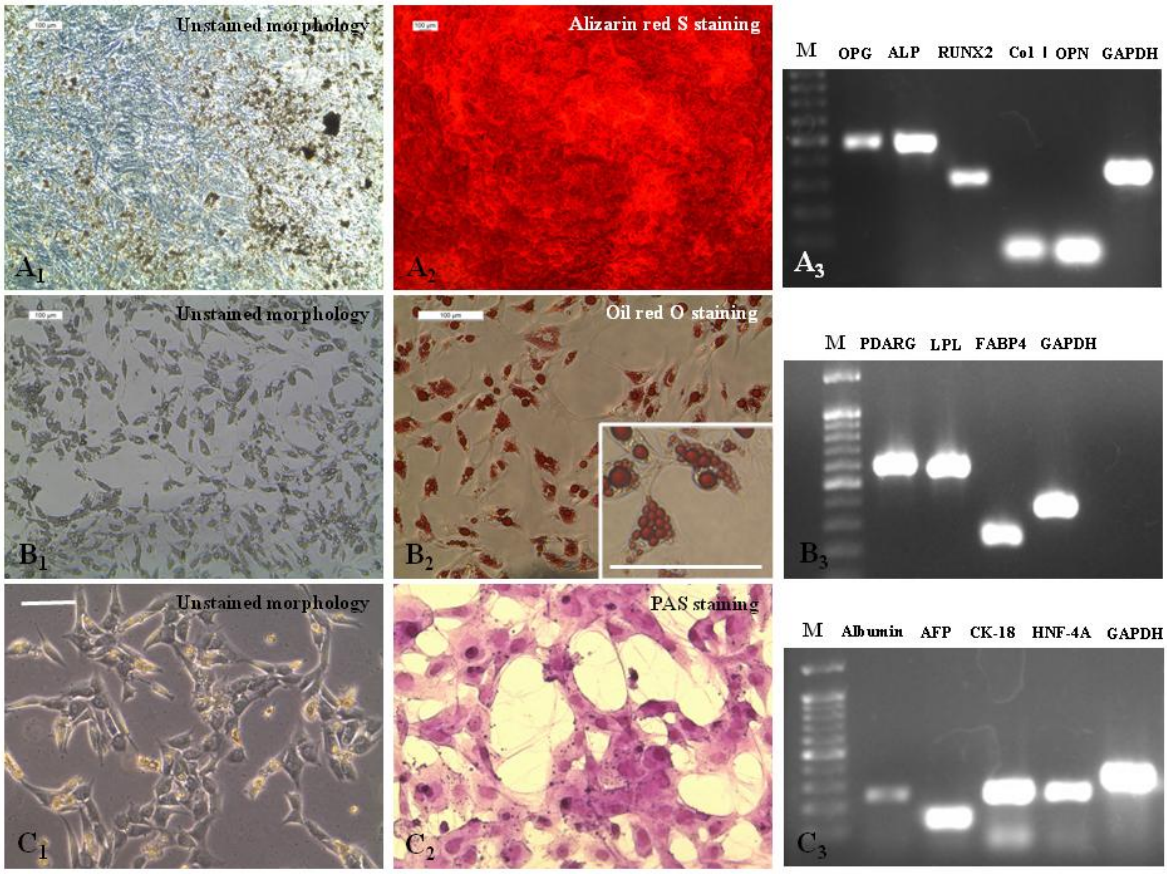

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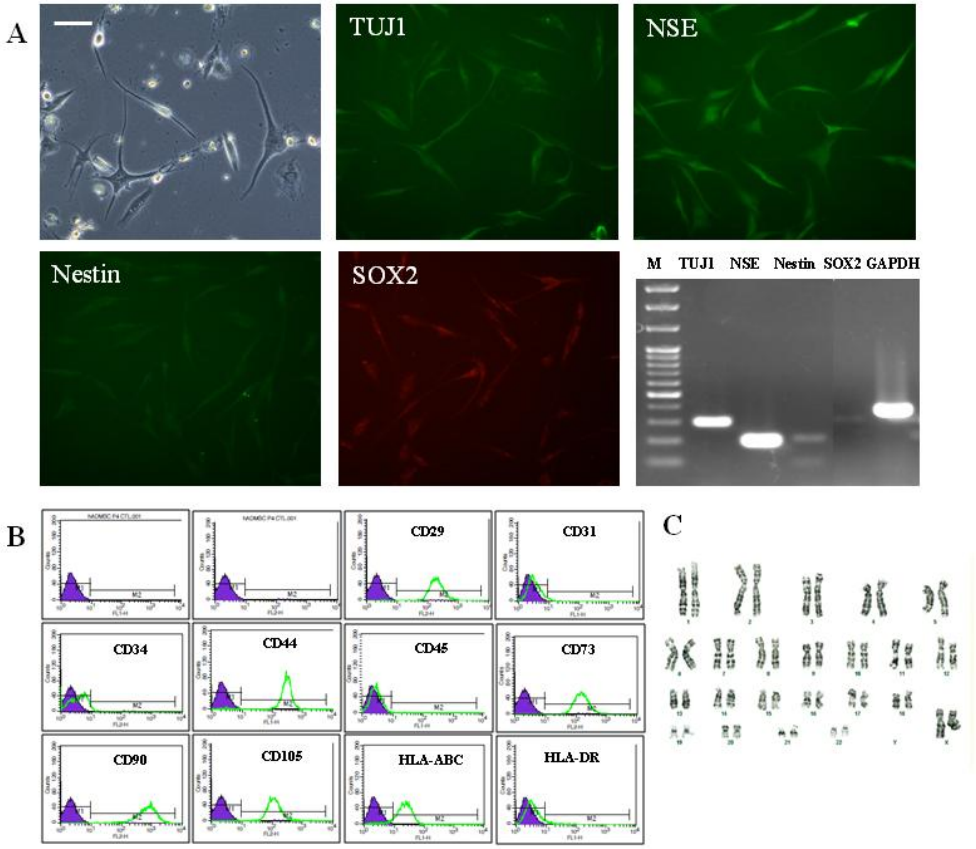
318 (fig 2)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TUJ1
NSE
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SOX2
M TUJ1 NSE Nestin SOX2 GAPDH
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HLA-ABC
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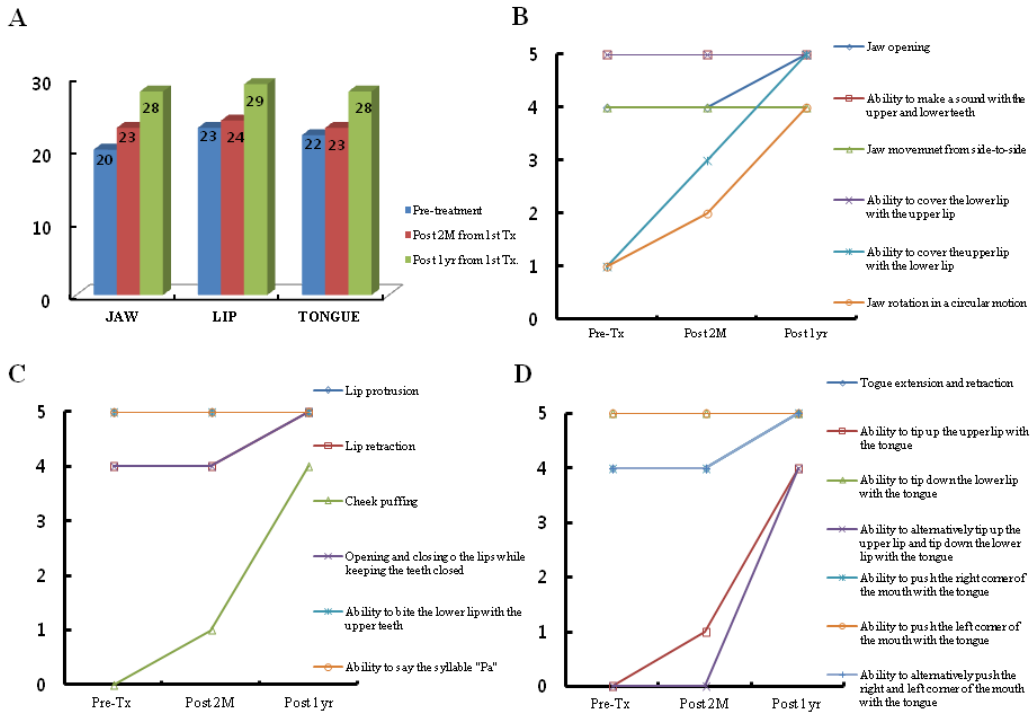
C

(fig 3)



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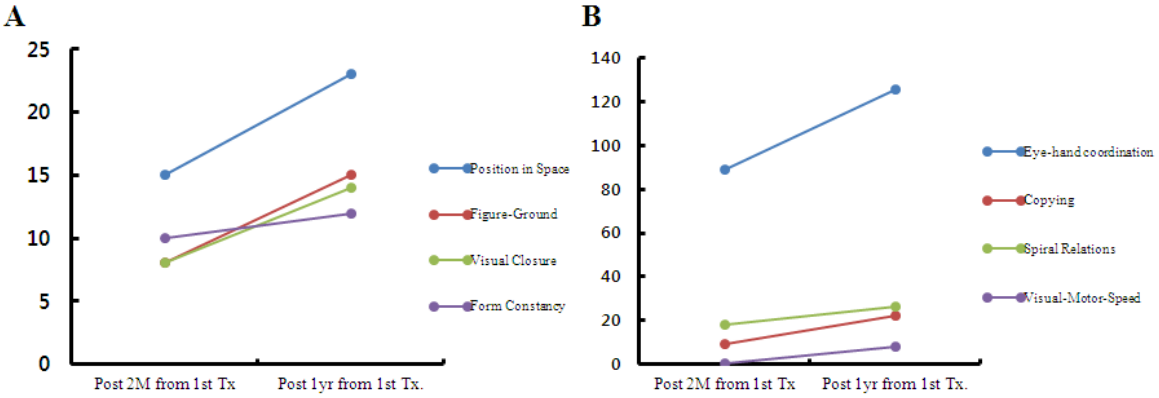

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324 (fig 5)