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Expanding the mutational spectrum of the *ABCB4* gene in inherited adult cholestatic liver disorders with four novel pathogenic variants: case reports

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Ethics statement: This work is not clinical research and is considered as routine clinical care.

ABSTRACT

Low phospholipid-associated cholelithiasis and intrahepatic cholestasis of pregnancy are two MDR3-related inherited liver disorders caused by biallelic or monoallelic *ABCB4* loss-of-function variants. Low phospholipid-associated cholelithiasis is clinically characterized by the early onset of symptomatic cholelithiasis in young adults while intrahepatic cholestasis of pregnancy is a distinct clinical entity associated with adverse fetal outcomes. Of note, patients carrying *ABCB4* sequence variations commonly exhibit phenotypic expression over a wide continuum due to

environmental and hormonal contributing factors and genetic modifiers. Patients with an early diagnosis of MDR3-related diseases could benefit from ursodeoxycholic acid treatment in order to prevent acute and chronic complications as well as adverse pregnancy outcomes. We herein report five patients with an overlapping phenotype from low phospholipid-associated cholelithiasis to intrahepatic cholestasis of pregnancy, harboring five *ABCB4* missense variants, four of which were novel. Our study highlights the phenotypic and genetic heterogeneity of inherited cholestatic liver diseases and also expands the mutation spectrum of *ABCB4* sequence variations in adult cholestatic liver diseases.

Key words: Low phospholipid-associated cholelithiasis syndrome. Intrahepatic cholestasis of pregnancy. Novel *ABCB4* loss-of-function variants. Targeted next-generation sequencing.

INTRODUCTION

ABCB4 (MIM *171060) encodes a phospholipid floppase that translocates phosphatidylcholine from the inner to the outer leaflet of the apical (canalicular) membrane of the hepatocyte. Low phospholipid-associated cholelithiasis syndrome (LPAC syndrome) (MIM #600803) is a recent entity of liver disorders characterized by a) the onset of biliary symptoms before the age of 40, b) microlithiasis or intrahepatic sludge on imaging and c) the recurrence of symptoms after cholecystectomy. The syndrome is caused by biallelic or monoallelic *ABCB4* "loss-of-function" variants. Severe biliary complications including acute pancreatitis, recurrent cholangitis, segmental spindle-shape dilatation of the biliary tree filled with gallstones, secondary sclerosing cholangitis and adverse fetal complications in intrahepatic cholestasis of pregnancy (ICP), which are commonly observed in patients with LPAC syndrome (1,2). ICP is characterized by jaundice and pruritus associated with abnormal values of hepatobiliary-injury biomarkers, including raised serum bile acid concentrations. Adverse pregnancy outcomes include spontaneous preterm labor, fetal distress, fetal asphyxia events and third trimester intrauterine death. Furthermore, the genetic heterogeneity of ICP could hamper the etiological diagnosis and clinical management. ICP (MIM #614972) is caused by heterozygous sequence variations of at least five different genes including *ABCB4*, *ABCB11*, *ATP8B1*, *NRIH4* and *TJP2* (3). We report

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CLINICAL REPORT

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WAGR syndrome and congenital hypothyroidism in a child with a Mosaic 11p13 deletion

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Wilm's tumor, aniridia, genitourinary anomalies, and mental retardation (WAGR) syndrome, a rare genetic disorder, is caused by the loss of 11p13 region including *PAX6* and *WT1*. We report novel findings in a 28-month-old boy with aniridia, Wilm's tumor, congenital hypothyroidism, and sublingual thyroid ectopia. He was found to have a mosaic 5.28 Mb interstitial deletion of chromosome 11p13 deleting *PAX6* and *WT1*. In order to clarify the mechanism underlying his thyroid dysgenesis, sequence analysis of candidate thyroid developmental genes was performed. We identified a *FOXE1*: c.532_537delGCCGCC p.(Ala178_Ala179del) variant that predisposes to thyroid ectopia. Taken together, this is the first report of mosaic 11p13 deletion in association with thyroid dysgenesis. We also propose a model of complex interactions of different genetic variants for this particular phenotype in the present patient.

KEYWORDS
congenital hypothyroidism, mosaic 11p13 deletion, thyroid ectopia, WAGR syndrome

1 | INTRODUCTION

Wilm's tumor, aniridia, genitourinary anomalies, and mental retardation (WAGR), a rare contiguous gene deletion syndrome (MIM #194072), is caused by interstitial deletions of distal 11p13 (Francke, Holmes, Atkins, & Riccardi, 1979; Riccardi, Sujansky, Smith, & Francke, 1978). In most patients, the deletion is de novo. The incidence is unknown and only a few hundred patients have been reported worldwide (Clericuzio, Hingorani, Crolla, van Heyningen, & Verloes, 2011). Haploinsufficiency of *PAX6* (OMIM *607108) and *WT1* (OMIM *607102) in this region are candidates for the clinical features observed in patients with WAGR syndrome. It is speculated that variable expressivity and reduced penetrance in genetic conditions are due in part to mosaicism with the percentage of different cell types carrying the mutation (Zlotogora, 2003). Only a single patient with mosaic deletion of 11p13 has been reported (Erez et al., 2010). Thyroid development requires coordinated action of many transcription factors. The FOXE1 (MIM *602617) transcription factor is highly expressed in thyroid tissues. FOXE1 plays an important role in the control of the survival and the migration of the thyroid precursor cells to their final destination in the neck. Murine *Foxe1*$^{-/-}$ knockdowns have a sublingual thyroid, modeling human thyroid ectopia. Furthermore, variants in *FOXE1* can predispose to a wide range of thyroid diseases including congenital hypothyroidism, thyroid ectopia, thyroid cancer, and Bamforth-Lazarus syndrome (Baris et al., 2006; Fagman & Nilsson, 2011). The *FOXE1* polyalanine tract-encoding length polymorphism is also a risk factor for congenital hypothyroidism, thyroid ectopia, and thyroid cancer (Bullock et al., 2012; Carré et al., 2007). We describe here a 28-month-old boy with congenital hypothyroidism, aniridia, and Wilm's tumor. Clinical and molecular investigations have been performed to unravel the genetic underlying mechanism and to correlate genotype–phenotype relationship.

2 | CLINICAL REPORT

A 28-month-old child was referred to clinical genetics service for evaluation of a history of mild WAGR syndrome with left sided

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RECONSTRUCTION OF BONE DEFECT WITH THE CORAL SCAFFOLD AND OSTEOBLASTS: AN EXPERIMENTAL STUDY IN RABBITS

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ABSTRACT
Demand for bone grafts to treat bone defects in cases of pathologically and injuries is increasing, but the source of bone graft derived from humans is not enough to supply the needs of patients. Therefore, research and create the equivalent of bone graft is a necessary work but also a long-term solution in the future. The present study was carried out with the goal of creating the equivalent bone graft using sea coral (*Porites lutea* species) as the substrate to contain osteoblasts derived from bone marrow to treat defects bones in the body of the rabbit femur bones. This is a controlled experimental study. Rabbits were divided into two groups, Group 1 (experimental rabbits), including the rabbit pieces were grafted by the coral contained osteoblasts. Group 2 (control group) consisting of rabbits were grafted by coral alone. Research results on the rabbits were evaluated at the time points of 1, 3 and 6 months by radiology and histology (H & E staining). From this study, we found that the group of rabbits that were grafted by coral containing autologous osteoblasts, the bone healing results occur faster and better quality of bone healing when compared with the control group at time study 1 month and 3 months. The study results will open up the prospects of clinical applications in the future for bone defects cases, this will be a long term solution and effective for patients when there is not enough bone graft source, in while autologous bone grafting is limited in terms of quantity.

KEYWORDS: sea coral, osteoblast, bone defect, grafting, reconstruction.

1. INTRODUCTION

Bone tissue is one kind of tissue in the human body that is capable of self-healing in the small bone defects. However, in the case of injury or illness caused the large bone defects, the bone tissue is not capable of self-repair without the use of interventions such as bone graft substitute.[1,2,11] However, bone grafts problems existing obstacles need to be overcome. If the use of autologous bone tissue, patients need bone tissue obtained from a different location on the body, which leads to prolonged surgery leads to the risk of bleeding, inflammation and slow wound recovery. Bone allografts are the next choice, but the type of bone tissue often fails to meet demand by depending on the number of donors and the risk of infectious diseases. Source grafts from animals is very rich but the risk of transmission of animal diseases are difficult to control.

Therefore, the application of various biomaterials for bone grafting is an essential demand. Besides, we also need to improve the quality of the bone graft substitution by adding cells to increase the efficiency of bone formation and help the bone healing occurs faster and more efficient.[1,2,4]

We conducted the study to evaluate the ability to regenerate bone tissue for bone defects by grafts created from the combination of sea coral (*Porites lutea* species) and autologous osteoblasts derived from bone marrow on the rabbit femur.[1] Since then, we have evaluated to be effective in clinical applications.

2. MATERIALS AND METHODS

2.1. Materials

Brown rabbits were collected from farms in Vietnam. Rabbit was chosen as the male rabbits, healthy, body weight from 2.0 to 2.5 kg, about 6 months old. Rabbits were housed and cared for in the same conditions.

Porites lutea corals were collected from Institute of Oceanography, they are manufactured in the bone graft substitution at The laboratory of Biomaterials, Pham Ngoc Thach University of Medicine.

2.2. Isolation of mesenchymal stem cells from the bone marrow of brown rabbit species

Rabbits were anesthetized by Zoletil 50 (VIRBAC Laboratories, France). Then, we used Betadine (Zuellig Pharma) to disinfect the area obtained in the knee bone

Case Report

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A Unique Case of Crossover Second Toe Syndrome Treated by Autologous Peripheral Blood Stem Cells

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Abstract

Plantar plate tears, hammertoe, metatarsalgia, predislocation syndrome and metatarsophalangeal joint (MPJ) instability, all refer to what is commonly known as crossover second toe syndrome (CST). The CST, despite being widely present in the population, is a highly problematic condition that involves the entire structure of the foot and ankle including bones, muscles, tendons and joints. The CST is a form of degenerative condition associated with a progressive metabolic degenerative arthritis or arthrosis that leads to multiform forefoot deformities. Although the real underlying cause of the deformity is multifactorial, only some of the leading factors have been scientifically clarified, which make the prognosis quite problematic as the procedure needs to be proceeded on different levels and plans just to achieve an acceptable outcome, due to bone deformities, tendinopathies and neuropathies. Anatomically and pathologically, this condition is the result of the aging process, gradual deprivation of bone strength and a chronic inflammatory state induced by prolonged divergent and asymmetric mechanical forces of muscles and tendons. A strong pull of the flexor tendon medially and/or the attenuation of the lateral collateral ligaments of the second MPJ complex with valgus tendency related to a dorsal pull of the long and short extensor tendons without opposition from the plantar plate will result in a hammertoe deformity with dorsal contracture or dislocation of the toe at the MPJ. It has also been found that a long metatarsal together with prolonged periods of high peak pressures during walking are associated with the deformity and tears at the site of the plantar soft tissue. An adjunctive concern is caused by the hypermobility of the first ray linked with elevated pressures from the plantar to the second metatarsal head region. The direct pressure together with adducts of the hallux may cause dorsal displacement of the second toe. A case of a 56-year-old woman was presented to us with multiple joint osteo-arthritis and painful swelling on shoulders, bilateral knees, lower back, and ankles with a visible bilateral crossover second toe condition. An MRI and X-ray were performed, objectified a multiple osteoarthritis and grade 4 dual crossover second toe syndrome with deformity associated with hallux valgus, hallux rigidus, and neuroma of the third intermetatarsal space with metatarsus sub-luxation. The patient received a treatment of autologous PB-SCs infusion during a period of 3 weeks; the results showed a complete recovery of crossover second toe condition on both left and right foot.

Keywords: Crossover second toe syndrome; Peripheral blood stem cells; Telomere measurement; Rt-PCR

Introduction

The term “second crossover toe” (SCT) was firstly introduced by Coughlin in 1987 to describe a multi-plane deformity at the MPJ [1,2]. Anatomically and mechanically, the SCT deformity is a result of the instability of the whole foot-ankle structure. Here the defection of MPJ involves feebleness resulting from hallux abducto-valgus deformity and the gastrocnemius equinus. The MPJ disability may often be associated with a subluxation and/or dislocation of the dorsal and medial direction that may lead to a definitive plantar plate rupture and collateral ligament damage [2,3].

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Research Article Open Access

Human Peripheral Blood Stem Cells can be a Solution to Diabetes Mellitus Type 2 a Preliminary Study on 14 Patients

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Abstract

Background: The use of autologous peripheral stem cell (PB-SCs) in Diabetes Mellitus type 1 (type 1 DM) was described in 2007 with promising conclusion. However a similar treatment with a positive outcome on type 2 diabetes mellitus patients (type 2 DM) has not been yet reported. The goal of this study was to determine the effect of autologous PB-SCs transplantation in treatment of DM2 patient.

Methods: Current study involved 14 patients with type 2 DM (aged 48 to 84 years) during a period of 180 days in our facility. Clinical variables (duration of DM, oral hypoglycemic drugs, time free from oral drugs) and laboratory variables (HbA1c, blood pressure, weight, cholesterol), mononuclear cells infused were assessed. Purified PB SCs were infused into major systemic vein (upper limbs or lower limbs) and subcutaneously in the abdomen. Follow-up is performed weekly after infusion during a period of 6 months.

Results: Mean HbA1c values showed a significant reduction during follow-up in all patients after autologous PB-SCs. After the treatment with stem cells the HbA1c level dropped by at least one unit relative to the HbA1c level in the medication phase, with stem cells patients have mean value of HbA1c lower than 6.5% (Mean value at time of diagnose 8.9%, at time of medication 7.9% and at time of post stem cell therapy 6.2%). Also, it was confirmed that the stem cell treatment is more efficient for the patients with higher HbA1c level in the medication phase and the level originally diagnosed. In 180 days after the combined therapy, HbA1c, cholesterol, and liver profile stay stable compare to the baseline among those patients who did not continue the LGI diet. All patients were insulin and/or oral hypoglycemic drugs completely free.

Conclusions: Therapy based on autologous PB-SCs can improve glucose control and reduce the dose of insulin and/or oral hypoglycemic drugs in type 2 DM patients, but it only improves pancreatic β-cell function transiently if the patients would not adjust their diet and life style. Further randomized controlled clinical trials involving more patients will be required to confirm these findings and the mechanism needs to be learned deeper.

Keywords: HPB-SCs; LGI diet; Type 2DM; HbA1c; MSCs; NSCs; ESCs; HSCs

Introduction

Diabetes is a disorder that strictly belongs to the Metabolic Syndrome (MS) a cluster of metabolic conditions that includes a variety of diseases such as hypercholesterolemia, liver enzyme disorders, atherosclerosis, hypertension, cardio-vascular disease (CVD) that altogether rise the risk of morbidity and life impairment among world population. Diabetes is one of the most silent and threatens disease of the modern time and it is constantly increasing in both industrialized and developing countries [1]. Better economic condition, a more sedentary life style, a higher consumption of polished rice which contains high glycemic index values, a diet richer in starchy processed food and western style-hyper energy food have contributed to a rapid evolvement of DM2 among Vietnamese population, especially in more industrialized cities such as Ho Chi Minh City and Ha Noi [1]. Data from a recent revise in Vietnam confirmed that Diabetes type 2 (DM2) increased 211% in a little more than a decade from 2002 to 2013 [1]. Today, with more than 3.3 million diabetics Vietnam is among the top ten countries in the world in terms of diabetes prevalence [1]. During the 90's few studies showed that Vietnamese diabetic patients had low or normal body mass index (BMI) and, though nowadays, this index together with obesity and abnormal fat distribution have greatly increased still remain within the normal parameters dictated by WHO [1]. Indeed, scientists have confirmed that the abnormal fat distribution and, not the BMI, have to be considered the main cause of insulin resistance (IR) among Vietnamese diabetic population [1]. Present time cell therapy is basically focused on the use of adult MSCs, stem cells of stromal origin characterized by their multipotency with the ability to self-renew [2,3], differentiate to various cell phenotypes such as osteoblasts, chondrocytes, adipocytes, fibroblasts, tenocytes [4,5], or hepatocytes, neural cells and epithelial cells [6]. Bone marrow MSCs have been the most widely studied, however MSCs can be also isolated, with similar but not identical features, from different tissues including cord blood, umbilical cord, placenta, adipose tissue, trabecular bone, dental pulp and peripheral blood [7-12]. Overall,

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Research Article Open Access

Autologous Peripheral Blood Stem Cells Increase the Telomere Length in Patient: A Case Report of 13 Patients

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Abstract

Telomere dysfunction, which leads to genomic instability, is assumed to play a major role in the development of degenerative diseases as Cancer, Multiple Sclerosis (MS), Amyotrophic Lateral Sclerosis (ALC), Alzheimer's Disease (AD), dementia and diabetes. However, the few epidemiologic studies that assessed the relationship between telomere length in blood cells and autologous peripheral blood stem cells (PB-SCs) have been quite erratic. Cell senescence process, still remains an exciting biological process that is characterized by important structural and morphological changes that involves deviations and alterations in nuclear structure, DNA activity in protein processing and metabolism, and apoptosis resistance.

The associations between telomere length from peripheral blood leucocytes before and after infusion with autologous PB-SCs were examined in 13 case studies that were conducted at our facility. Using value range from 1.5 Kb to >20 Kb as a cut point, patients showed a significant increase of telomere length compared before the infusion of autologous PB-SCs. Meanwhile, there was a substantial improvement in terms of general health such as sleeping, attention, vitality, memory and sexual activity with a significant difference in the quality of life. Overall telomere length from blood was significantly associated with the injection of autologous PB-SCs and as far as 6 months after the injection.

Keywords: Amyotrophic lateral sclerosis; Multiple sclerosis; Alzheimer's disease; Stem cells

Introduction

During the past few years, important results have been achieved in trying to corroborate the essential role of senescence process in human body [1]. Senescence may exhibit a negative impact on organ, tissue and cell regeneration through a release of host bioactive molecules, including Reactive Oxygen Species (ROS) and a wide variety of pro-inflammatory cytokines, chemokines and growth factors known as the Senescence-Associated Secretory Phenotype (SASP) [1]. Senescence process have been associated with few metabolic degenerative diseases, such as cardiovascular disease, diabetes, atherosclerosis and cancer. Currently, the major challenge in the field is to determine the association between senescent cells and age-related tissue dysfunction, define if this is just a question of correlation or there is cause-effect condition or both [1-3].

Telomeres are specialized DNA-protein arrangements that close the final parts of linear chromosomes. Functional telomeres need appropriate extension of telomeric DNA repeats to keep chromosomal stability. Any dysfunction on this part of DNA lead to chromosome end-to-end fusion, chromosomal changes and instabilities that may eventually lead to degenerative disease such as cancer [2]. Vertebrate telomeres are composed of variable numbers of a tandem repeat sequence of TTAGGG, bound to three shelterin subunits, TRF1, TRF2, and POT1 [4]. During each cell division telomeres reduce, a process supposed to be enhanced by oxidative stress and inflammation, telomerase preserve and maintains telomere length and stability. Very short or dysfunctional telomeres trigger replicative senescence, a process that may be activated by a single critically short telomere in a cell [5,6]. According to some scientist, the telomere length can be used as early predictive indicator in serious life threatening diseases, for instance very short telomeres are seen in pre-malignant lesions, atypical hyperplasia and several form of carcinoma in humans [7-11]. However, whether overall telomere length from blood cells is associated with cancer risk remains unclear; recently, two of our recent examined patient showed an abnormal extent telomere which confirmed in both cases a specific tissue lesion a Barrett's syndrome and a liver cholestasis (data not shown). As similarly described by Finley et al. in their study, an increase in telomerase expression would be compatible with an increase in histologic grade which allows the growth of telomere length [12]. In this paper we conducted a 13 independent case-control study to investigate further whether the overall telomere length from blood sample may respond to a treatment of autologous PB-SCs.

Based on our previous edited study human PB revealed the presence of different sub-groups of pluripotent and multipotent stem cells. The use of reverse transcription polymerase chain reaction (RT PCR) and fowcytometry analysis confirmed the expression of multipotent markers of both adherent and non-adherent mononucleated cells such as Oct4, Sox2, OCN, Nestin, Nanog, DMP and CD44, CD73, CD90, CD133, CD 34, CD45, CD14, Nestin, SSEA3 and Tra1. The study also quantified

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GELATIN-ALGINATE SPONGE: A POTENTIAL SCAFFOLD FOR ADIPOSE TISSUE ENGINEERING

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ABSTRACT
Gelatin-alginate sponge (GA sponge) is one of the ideal materials to be studied and applied in soft tissue regeneration, especially in adipose tissue engineering. This sponge is made from the combination of gelatin and alginate with crosslinking activity of 1-Ethyl-(3-3-dimethylaminopropyl) carbodiimide. Gelatin and alginate were mixed at ratio 8:2 with 0.3% EDC and freeze-dryed to form the GA sponge. After that, GA sponge was evaluated the structure by Hematoxylin and Eosin staining method and scanning electron microscopy imaging, the components by Fourier Transform Infra Red, the water absorption, *in vitro* biodegradation by using collagenase; *in vitro* cytotoxicity towards human fibroblasts. Finally, adipogenic differentiation potential of human adipose-derived stem cells inside the GA sponge was studied. The results show that the GA sponge with spongy and stable properties has high water absorption capacity, *in vitro* biodegradability, non-cytotoxicity. Simultaneously, human adipose-derived stem cells can adhere and differentiate into fat cells within the GA sponge. With these results, the GA sponge has properties suitable for applications in adipose tissue engineering.

KEYWORDS: Gelatin, Alginate, sponge, scaffold, tissue engineering, adipose derived stem cells.

INTRODUCTION
In recent years, adipose tissue engineering has been investigated in many researches with the goal is replacing the traditional method of tissue regeneration.[5,17] This is a modern method, a potential development in aesthetic medicine as well as to heal defects in natural tissue regeneration. Three important factors in tissue engineering are cells, scaffolds and growth factors. In adipose tissue engineering, scaffolds can be made from many different materials and they need to have the properties required to ensure the stem cells can survive, adhere, proliferate and differentiate into fat cells and then regenerate tissue. Gelatin and alginate occupy a large part in the field of regenerative medicine.[7, 23] Gelatin is derived from collagen so it is biocompatibility and biodegradability. In addition, gelatin also contains arginine-glycine-aspartic molecule segment (RGD) which forms the ligands for binding to receptors on the cell membrane in order to promote adhesion, migration, proliferation and differentiation of cells.[10] Alginate polysaccharide does not cause an immune response and have biodegradability.[15, 20] Although GA sponge has been extensively studied in different ratio of mixing, the role of this sponge as scaffold for tissue engineering has not been studied.[5] We made GA sponge and evaluated the properties of GA sponge to use it as a scaffold for adipose tissue engineering.

MATERIALS AND METHODS
Samples
The GA sponge was made from the mixing of gelatin (Sigma) and alginate (Sigma) with crosslinking of 1-Ethyl-(3-3-dimethylaminopropyl) carbodiimide (EDC) (Sigma). Human adipose derived stem cells (hADSCs) were supplied from the Laboratory of Department of Physiology and Animal Biotechnology, Faculty of Biology and Biotechnology, University of Science, Vietnam National University at Ho Chi Minh City.

Making of the GA sponge
Gelatin and alginate is completely dissolved in distilled water at 50^{o}C to create 1% gelatin and 1% alginate solutions. These solutions were then mixed with the ratio of 8 gelatin : 2 alginate in volume and incubated at -80^{o}C in 24 hours. Next, these frozen blocks were incubated in 0.3% EDC for 24 hours at 4°C in dark condition and freeze-dried. Finally, GA sponges were sterilized by irradiation in 25kGy and cut into small samples of size 3x3x3mm^3 for all experiments.

Adipose tissue can be generated in vitro by using adipocytes from human fat tissue mesenchymal stem cells seeded and cultured on fibrin gel sheet

Cong Toai Tran, Duy Thao Huynh, Ciro Gargiulo, Le Bao Ha Tran, Minh Hang Huynh, Khanh Hoa Nguyen, Luis Filgueira & D. Micheal Strong

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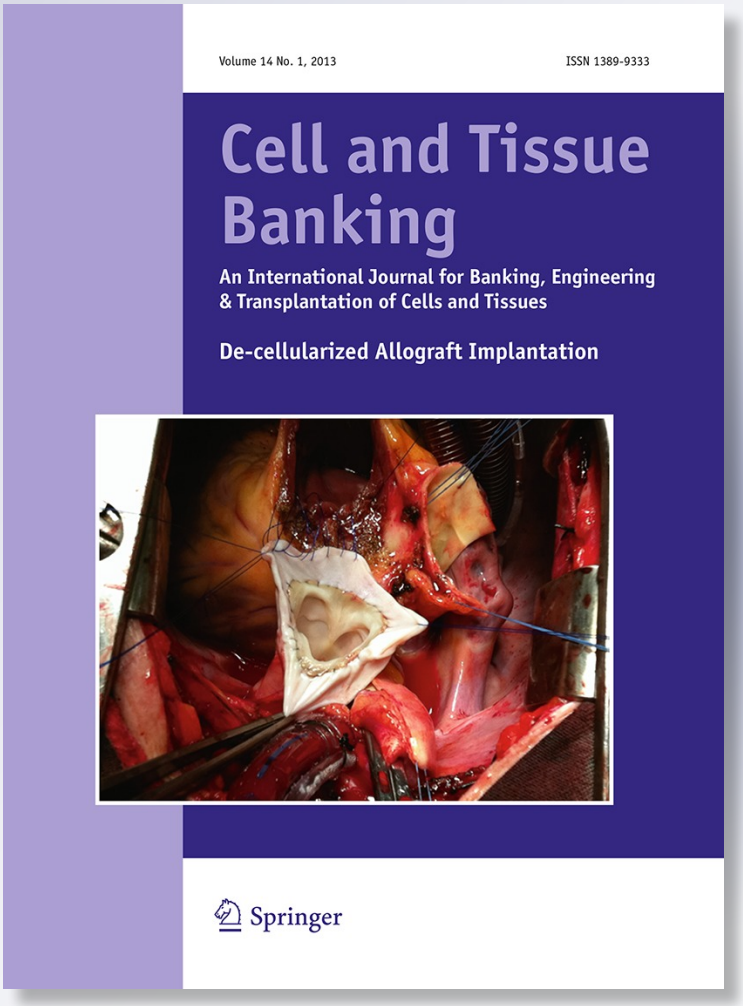

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ORIGINAL PAPER

Adipose tissue can be generated in vitro by using adipocytes from human fat tissue mesenchymal stem cells seeded and cultured on fibrin gel sheet

Cong Toai Tran · Duy Thao Huynh · Ciro Gargiulo · Le Bao Ha Tran · Minh Hang Huynh · Khanh Hoa Nguyen · Luis Filgueira · D. Micheal Strong

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Abstract The current study has developed an innovative procedure to generate ex novo fat tissue by culturing adipocytes from human fat tissue mesenchymal stem cells (hFTMSCs) on fibrin gel sheet towards applications in medicine and cosmetology. Fibrin gel has been obtained by combining two components fibrinogen and thrombin collected by human peripheral blood. By this procedure it was possible to generate blocks of fibrin gel containing adipocytes within the gel that show similar features and consistency to human fat tissue mass. Results were assessed by histological staining methods, fluorescent immune-histochemistry staining as well photos by scanning electron microscopy (SEM) to demonstrate the adhesion and growth of cells in the fibrin gel. This result opens a real possibility for future clinical applications in the treatment of reconstructive and regenerative medicine where the use of stem cell may eventually be a unique solution or in the field of aesthetic medicine where autograft fat stem cells may grant for a safer and better outcome with long lasting results.

Keywords Mesenchymal stem cells · Adipocytes · Fibrin gel · Fat tissue mass

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Introduction

Nowadays, scientists have identified different variety of sources from which it may be obtained multipotent mesenchymal stem cells (MSCs), such as bone marrow (BM), umbilical cord blood (UCB), peripheral blood, placenta and adipose tissue. Among those, due to its qualities and great availability adipose tissue is probably the higher and more attractive source of MSCs (Unguryte et al. 2010). Fat tissue is very common and abundant in the body, particularly rich in MSCs and very easy to collect with a drastically low invasive procedure. hFTMSCs have the same characteristics and features of those from BM and UCB, (Locke et al. 2009; Zuk et al. 2002; Gimble and Guilak 2003), however results either from our study or published researches have confirmed that fat tissue compared to BM and UCB contains more MSCs, for

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Culture and differentiation of osteoblasts on coral scaffold from human bone marrow mesenchymal stem cells

Cong Toai Tran · Ciro Gargiulo · Huynh Duy Thao · Huynh Minh Tuan · Luis Filgueira · D. Michael Strong

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Abstract In this paper we describe an approach that aims to provide fundamental information towards a scientific, biomechanical basis for the use of natural coral scaffolds to initiate mesenchymal stem cells into osteogenic differentiation for transplant purposes. Biomaterial, such as corals, is an osteoconductive material that can be used to home human derived stem cells for clinical regenerative purposes. In bone transplantation, the use of biomaterials may be a solution to bypass two main critical obstacles, the shortage of donor sites for autografts and the risk of rejection with allograft procedures. Bone regeneration is often needed for multiple clinical purposes for instance, in aesthetic reconstruction and regenerative procedures. Coral graft *Porites lutea* has been used by our team for a decade in clinical applications on over a thousand patients with different bone pathologies including spinal stenosis and mandibular reconstruction. It is well accepted that human bone marrow (hBM) is an exceptional source of mesenchymal stem cells (MSCs), which may differentiate into different cell phenotypes such as osteoblasts, chondrocytes, adipocytes, myocytes, cardiomyocytes and neurons. Isolated MSCs from human bone marrow were induced into osteoblasts using an osteogenic medium enriched with two specific growth factors, FGF9 and vitamin D2. Part of the cultured MSCs were directly transferred and seeded onto coral scaffolds (*Porites Lutea*) and induced to differentiate into osteoblasts and part were cultured in flasks for osteocell culture. The data support the concept that hBM is a reliable source of MSCs which may be easily differentiated into osteoblasts and seeded into coral as an optimal device for clinical application. Within this project we have also discussed the biological nature of MSCs, their potential application for clinical transplantation and the prospect of their use in gene therapy.

Keywords Osteoblast · Mesenchymal stem cell · Human bone marrow · Coral · Transplantation

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Introduction

Bone graft replacements are essential for multiple clinical purposes and worldwide the need of bone

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In vitro culture of Keratinocytes from human umbilical cord blood mesenchymal stem cells: the Saigonese culture

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Abstract There have been many attempts to acquire and culture human keratinocytes for clinical purposes including from keratotome slices in media with fetal calf serum (FCS) or pituitary extract (PE), from skin specimens in media with feeder layers, from suction blister epidermal roofs' in serum-free culture and from human umbilical cord blood (hUCB) mesenchymal stem cells (MSCs) in media with skin feeder layers. Conversely this study was designed to investigate whether keratinocytes could be obtained directly from hUCB MSCs in vitro. It is widely established that mesenchymal stem cells from human umbilical cord blood have multipotent capacity and the ability to differentiate into disparate cell lineages hUCB MSCs were directly induced to differentiate into keratinocytes by using a specific medium composed of primary culture medium (PCM) and serum free medium (SFM) in a ratio 1:9 for a period of 7 days and tested by immunostain p63 and K1-K10. Cells thus cultured were positive in both tests, confirming the possibility to directly obtain keratinocytes from MSCs hUCB in vitro.

Keywords Mesenchymal stem cell · UCB · Keratinocyte culture · Cell culture

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Introduction

MSCs from human-UCB

Human UCB is a significant source of hematopoietic stem cells and has been considered as a valid alternative for hematopoietic stem cell transplantation (Toai et al. 2009; Lee et al. 2004; Park et al. 2006; Van de Ven et al. 2007; Maurice et al. 2007; Musina et al. 2007; Sasaki et al. 2008). MSCs from hUCB have been used in a wide range of diseases such as liver disorders, myocardial infarction, central nervous system condition or in degenerative pathologies such as diabetes, Crohn's disease, osteogenesis imperfect (OI), rheumatoid arthritis (RA) and osteoarthritis (OA) (Toai et al. 2009; Lee et al. 2004;

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In vitro culture and differentiation of osteoblasts from human umbilical cord blood

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Abstract It is well accepted that human umbilical cord blood (UCB) is a source of mesenchymal stem cells (MSCs) which are able to differentiate into different cell phenotypes such as osteoblasts, chondrocytes, adipocytes, myocytes, cardiomyocytes and neurons. The aim of this study was to isolate MSCs from human UCB to determine their osteogenic potential by using different kinds of osteogenic medium. Eventually, only those MSCs cultured in osteogenic media enriched with vitamin D_2 and FGF9, were positive for osteocalcin by RT-PCR. All these cells were positive for alizarin red, alkaline phosphatase and Von Kossa. The results obtained from RT-PCR have confirmed that osteogenesis is complete by expression of the osteocalcin marker. In conclusion, vitamin D_2, at least in vitro, may replace vitamin D_3 as an osteogenic stimulator factor for MSC differentiation.

Keywords Mesenchymal stem cells (MSCs) · Human umbilical cord (UCB) · Bone marrow (BM) · Major histocompatibility complex (MHC)

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Introduction

Multipotent mesenchymal stem cells

Mesenchymal stem cells (MSCs) are a particular type of cell of embryonic mesodermal origin with a strong adherence ability and capable of differentiating into cells of different lineage tissues such as bone, cartilage, adipose tissues (Lee et al. 2004; Park et al. 2006; Bieback et al. 2004; Boissy et al. 2000; Chamberlain et al. 2007), and neural cells including astrocytes, neurons, hepatocystic and dermal tissue (Tse and Laughlin 2005; Chao et al. 2004; Koc and Lazarus 2001; Xu et al. 2004; Minguell et al. 2001; Song and Tuan 2004; Goodwin et al. 2001; Rosada et al. 2003; Kogler and Wernet 2006; Riordan et al. 2007; Kim et al. 2004; Jang et al. 2006; Kang et al. 2006; Van de Ven et al. 2007). Human MSCs have been isolated from various sources, such as adipose tissues, bone marrow (BM), umbilical cord blood (UCB), amniotic fluid, amniotic placenta, scalp tissue, amniotic membrane,