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ORIGINAL ARTICLE

Expression of PAX8 and NKX 2-1 Genes for Differentiation Thyroid Cell

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ABSTRACT
Stem cells are defined as a type of pluripotent or multipotent cells, which have two typical features: self-renewal and have the potential to differentiate into several different cell lineages. The mesenchymal stem cells (MSCs) are considered a promising cell source for regenerative medicine, because they have the potential to differentiate into a variety of cell type. In this study we purposed the effect of extracts from mice thyroid on differentiation of omental derived thyroid cells. As a result study demonstrated that thyroid tissue extract ofNMRI mice have potential to induce thyroid cells lineage, on the omental cells. Omentum tissue removed immediately via surgery and spited in to smaller pieces. Tissue fragments washed, centrifuged and transferred to culture flask with adequate medium (DMEM with 10% serum) and let to grow up and filled the b flask bottom. After 3 passages different the omental stem cell into thyroid cell of mice thyroid extracts. Presence of OMSCs was analyzed with both RT-PCR and specific markers (including CD90, CD44 and CD45 markers). Moreover, differentiation of OSCs to thyroid cells lineage were analyzed with both RT-PCR . In this study as a result we showed that extracts from mice thyroid could induce differentiation of miceomental stem cells and drive them to thyroid cell lineages. This study showed that omental are active cells for local proliferation, maturation , and differentiation of different cell lines .As a result ,study demonstrated that mice thyroid extract have potential to induce differentiation of omental derive mesenchymal stem cells (ODMSCs) to thyroid lineage.
Keywords: Omentum, stem cells, NMRI mice thyroid extract, thyroidcell differentiation

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INTRODUCTION

Stem cells have remarkable potential to produce into a variety of cell types within the body during early life and growth. Additionally, in several tissues they serve as a kind of internal repair system, dividing essentially without limit to replenish other cells provided that the individual or animal remains alive. Whenever a stem cell divides, each new cell has got the potential either to keep a stem cell or become another kind of cell with a far more specialized function, like a muscle cell, a red blood cell, or even a brain cell [1,2].

Stem cells are distinguished from other cell types by two important characteristics. First, they're unspecialized cells effective at renewing themselves through cell division, sometimes after long periods of inactivity. Second, under certain physiologic or experimental conditions, they could be induced to become tissue- or organ-specific cells with special functions. In certain organs, including the gut and bone marrow, stem cells regularly divide to fix and replace exhausted or damaged tissues[3-5].

The omentum is a sheet-like tissue attached to the greater lesser and curvature of the stomach and contains secondary lymphoid organs. The omentum has been used for its healing potential for years by rearranging the omental pedicle to damaged organs, but the mechanism by which omentum helps the healing process of damaged tissues is not well understood. Omental transposition has been used to treat different purposes such as infection or different wound-healing process. In addition, it has been used in brain surgeries too. The production of a variety of growth factors by the omentum provides a possibility to sustain transplanted pancreatic islets, into adult organs [6,7].

The omentum exerts has an vital role in the peritoneal defense mechanism against pathogens by providing not only the primary site for neutrophil exudation but also the local site for peritoneal leukocyte proliferation and macrophage differentiation [8,9].
The extensive range of omental applications and functions is due mainly to its unique angio- genic properties, by the way cellular mechanism of omental angiogenesis has not yet been identified in detail. Inflammatory response in an animal model, typically is associated with a rapid influx of inflammatory cells toward the peritoneal cavity, followed by angiogenesis in various peritoneal tissues, including omentum [10,11]. The omental milky spots are active sites for leukocyte migration and peritoneal leukocyte supply during inflammation [12, 13].
The angiogenesis in omentum was accompanied by the accumulation of omental mast cells, located in close contact with vessels, and their number correlated strongly to the number of omental vessels [14, 15].The mammalian thyroid contains of two endocrine cell types, the thyroid follicular cells (TFCs) that produce the thyroid hormones T3 and T4 and the C-cells that produce calcitonin. The primary function of the thyroid gland is to metabolize iodide by synthesizing thyroid hormones that are important regulators of growth, development and metabolism in almost all tissues. A follicular organization of TFCs is considered to be the requirement for efficient thyroid hormone biosynthesis. It has been shown that NKX2-1 and Pax8 function are vital for TFC survival, differentiation and function during thyroid organogenesis and in mature thyroid tissue. We studied whether over expression of the transcription factors Nkx2-1and Pax8 could stimulate differentiation of murine omental (OSCs)into TFCs and subsequent self-formation of thyroid follicles [16-18].
Many research groups are using omentum as a model for differentiation of various cell lineages [19].In this study we work out on the effect of mice thyroidto induce differentiation of omental stem cells to thyroid cell lineages.

MATERIAL AND METHODS

Omentum tissue was remote from Omentum of NMRI mice (10-14 days, weighing 20-25gr) during removing skin were opened at the belly by using micro dissecting scissors. After folding back the skin flaps with the scissors, omentum cut and the tissue lightly moved to a 60 mm petridish containing cold phosphate-buffered saline (PBS, Gibco). Tissue fragments washed with PBS, then splited into smaller pieces. The solution (DMEM and omentum) was poured in a centrifuge tube, centrifuged at 1500 rpm for 5 minutes. The supernatant was discarded, and the tissue fractions were resuspended in 10 ml DMEM supplemented with 10% FBS, 1% penicillin, and streptomycin, then cultured at a density of 105cells/cm2 seeded into 25- cm2 plastic flasks (Biofil), and incubated in 97% humidity and 5% $C0_2$ at 37 °C for 72 hours. Non adherent tissue fragments were removed and adherent fragments were thoroughly washed twice with PBS, medium and replaced every 3-4 days for 1 week, when the primary culture reached 90% confluence, they were rinsed three times with PBS and were suspended in 0.25% trypsin EDTA (Gibco) for 1–2 minutes at room temperature, followed by additional culture at a ratio of 1:3 per passage. After 3 passages (confluence 90%) different concentrations of micethyroid extracts (25, 50 ,75, 100, 200 ml) added to medium and allowed to differentiate of cells for 17 days. Present of OMSCs were analyzed with both RT-PCR (for OCT4 and WT1 genes) and flow cytometry (for CD90, CD44 and CD45 markers) methods. Moreover, differentiation of OSCs to thyroid cells lineage were analyzed with both RT-PCR (for Pax8 and NKX2-1 genes) methods (Tables 1 and 2).

Table 1: Primers specifications used for RT-PCR:

PAX8	F: 5'-GAATATTCTGGCAATGCCTACAG-3' R: 5'-AGATTCCTTTGTGTGACTCTCTG-3'
Nkx2.1	F: 5'-GTGAGCTTGCTTGTAAATACCAG-3' R: 5'-GGTGCTGCAAATACCAAACTG-3'

Table: 2- PCR program used for RT-PCR:

Cycle	**stage**	temperature	**Time**
1cycle	**Hot start**	95 °C	**5min**
35cycles	**Denaturation**	95 °C	**30 sec**
	Annealing	56 °C	**30 sec**
	Extension	72 °C	**1 min****
1cycle	**Final extension**	72 °C	**10 min**

RESULTS

Isolation of omental derived mesenchymal stem cells (ODMSCs) was confirmed with detection of CD90+ , CD44+ but not CD45- cells and also over expression of Oct4 and Wt1 genes (RT-PCR) in separated cells. (Figure1) After 3 passages (confluency 90%) different concentrations of mice thyroid extracts (25, 50 75, 100, 200 ml) added to medium and allowed to differentiate the cells for 17 days. (Figure2) Moreover, RT-PCRforPax8andNkx2-1 showed high expression of this molecule in thyroid cells. Furthermore, differentiated cells expressed both Pax8andNkx2-1. (Figure3)

As a result in this study we showed that extracts from mice thyroid could induce differentiation of mice omental stem cells drive to thyroid cell lineages.

(Figure1):

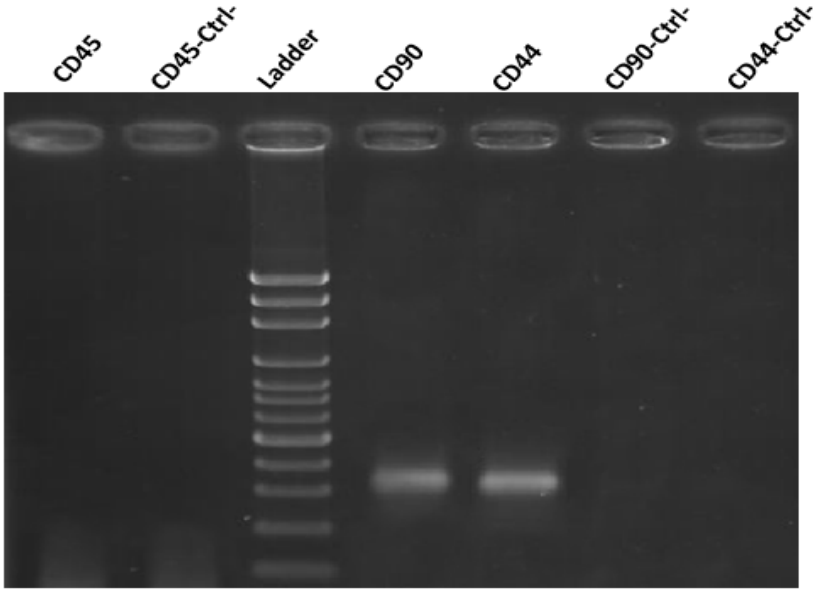

Figure1: Isolation ofomental derived mesenchymal stem cells (ODMSCs) has been confirmed with detection of CD90+ , CD44+ but has not been confirmed with $CD45^{+}$ detection.

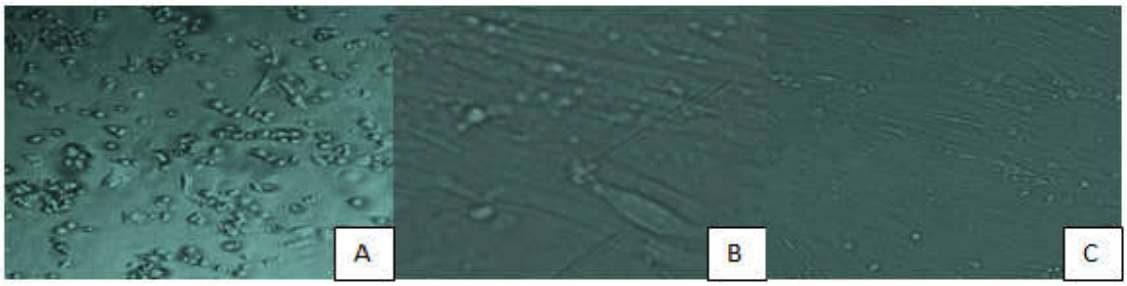

Figure2: For differentiation of omentum cells(Pen / strep, FBS, DMEM) added to medium and allowed to differentiate to omentum cells.(A) Primary cultured omentum cells(B) secondary cultured omentum cells (C) Third cultured omentum cells

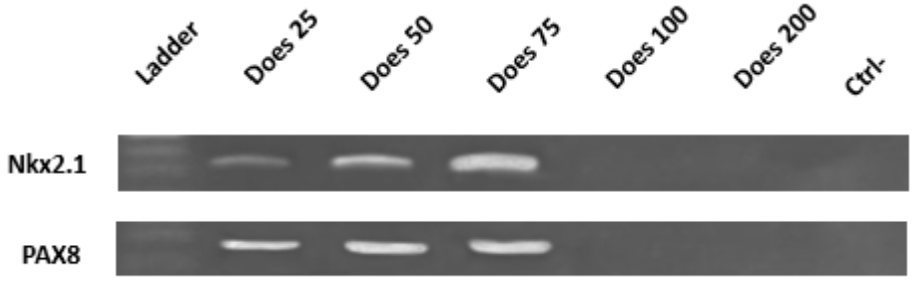

Figure3:RT-PCRforPax8andNkx2-1 showed high expression of these molecule in thyroid cells.

DISCUSSION

Omentum stromal cells have the capability to differentiate to adipogenic, neurogenic phenotypes in culture (containing some tissue extracts like thyroid gland extract.). Omentum is important organ and haveg the ability to fix injured tissues, could have retained remnants of embryonic or adult multipotent cells. Omental cells are easy to obtain in large quantities and could be harvested from the patient's own omentum, and no requirement for immunosuppressive therapy [20] .These cells are passage in culture without lack of pluripotent markers and they could be frozen in vast quantities for long-term. Omentum stem cells can express the specific positive and negative surface markers (CD markers), the CD44, CD90 and expressed adult stem cell markers including SDF-1a, CXCR4, WT-1, in addition to pluripotent embryonic stem cell markers [21].We used thyroid extract solution to induce omentum tissue to thyroid cells in vitro. Presently thyroid extract treatments have already been debated because of their rapid induction process. We previously indicated that omental milky spots are active sites for local proliferation, maturation, and differentiation of different cell lines. Some researchers have reported the inductive effect of certain material on bone marrow stem cells to differentiate into different tissue [22]. In this study a variety of factors thyroid growth factor may effect to omentum stem cell to differentiation into thyroid cell.

Some, groups of researches could differentiate adipose mesenchymal cells to thyroid withretinoic acid and growth factors [23], but in this research we used omental stem cells with thyroid tissue extract to differentiate thyroid cells. Unlike other researchers that have use chemical reagent, we used thyroid extraction method to induce differentiation *in vitro*. As a result, our study demonstrated that mice thyroid extract have potential to induce differentiation omental derived mesenchymal stem cells (ODMSCs)to thyroid lineage. Over expression of Pax88 and Nkx2-1genes (RT-PCR) in separated cells |revealed that omentum tissue has pluripotent or multipotent cells that differentiate to thyroid lineage in numerous concentration of thyroid tissue extract of NMRI mice.

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