Clinical Trial/Experimental Study

Medicine

OPEN

Production of parathyroid-like cells from thyroid stem cells in co-culture environment

Özge Karabıyık Acar, PhD[a,b], Gülçin Delal Nozhatzadeh, MSc[a,b], Alperen Tuncer, MSc[a], Gamze Torun Köse, PhD[a], Ezgi Hacıhasanoğlu, MD[c], Fikrettin Sahin, PhD[a], Erhan Aysan, MD[d,*]

Abstract

Background: Parathyroid-like cells were aimed to be developed using cells isolated from thyroid since their embryological origins are the same.

Method: Activin A and sonic hedgehog (Shh) are the proteins used in differentiation (dif) medium. Parathyroid and thyroid cells were cultured in a 3-dimensional environment and divided into five groups: thyroid standard (st) medium, thyroid dif medium, parathyroid st medium, thyroid-parathyroid co-culture st medium, and thyroid-parathyroid co-culture dif medium. Throughout 28 days of incubation, groups were investigated by carrying out the live dead assay, confocal microscopy, real-time PCR, immunohistochemistry and biochemical assays.

Results: Thyroid-parathyroid co-culture cells grown in dif medium exhibited upregulated expressions of parathormone (PTH) (5.1-fold), PTH1R (3.6-fold), calcium sensing receptor (CaSR) (8.8-fold), and loss of thyroid-specific thyroid transcription factor 1 (TTF1) expression when compared to the thyroid st medium group. PTH secretion decreased by 35% in the parathyroid st medium group and 99.9% in the thyroid-parathyroid co-culture st medium group but decreased only 3.5% in the thyroid-parathyroid co-culture dif medium group on day 28.

Conclusion: Using Activin A and Shh proteins, thyroid stem/progenitor cells were differentiated to parathyroid-like cells successfully in a co-culture environment. A potentially effective novel method for cell differenatiation is co-culture of cells having the same embryological origin.

Abbreviations: BF = bright field, CaSR = calcium sensing receptor, dif = differentiation, FBS = fetal bovine serum, PBS = phosphate-buffered saline, PTH = parathormone, PTH1R = parathyroid hormone receptor 1, RT = room temperature, Shh = sonic hedgehog, st = standard, TPO = thyroid peroxidase, TSHR = thyroid stimulating hormone receptor, TTF1 = thyroid transcription factor 1.

Keywords: co-culture, differentiation, parathormone (PTH), parathyroid, stem cell, thyroid

1. Introduction

The smallest organ of the human body is the parathyroids. Healthy parathyroid tissues are oval-shaped organs with a diameter of only 4 to 5 mm and are located posterior to the thyroid tissue, attached to the thyroid capsule.[1] Parathyroid and thyroid are endocrine organs that have a common embryological origin. Both organs differentiate from the third pharyngeal pouch during the embryological period and travel together down to the anterior neck region.[2]

In vitro studies with parathyroid cells have begun quite a long time ago[3,4] and cells were first isolated from bovine parathyroid tissues.[5,6] The first in vitro study that performed using human viable parathyroid was harvested from the parathyroid adenoma tissue.[7] The term parathyroid organoid was first introduced to the literature by the study of Ridgeway et al[8] and they formed multicellular aggregates in the monolayer culture system using bovine parathyroid cells. Afterwards, there were limited studies in the literature for the development of parathyroid organoids using 3-dimensional culture and differentiation (dif) of stem cells to parathyroid-like cells. Due to the small size and having only one functional unit (chief cells), parathyroids can be pioneers in organoid studies.

In this study, we aimed to develop parathyroid-like cells from the cells isolated from the thyroid due to their common origin in the embryological period and close anatomical relationships in the adult human body. In the literature it was shown that a large amount of stem/progenitor cells exist within the thyroid tissue and have both intrinsic abilities to generate thyroidal cells and a potential to produce non-thyroidal cells.[9] After

The authors have no funding and conflicts of interest to disclose.

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

[a] Department of Genetics and Bioengineering, Faculty of Engineering, Yeditepe University, Istanbul, Turkey, [b] Department of Genetics and Bioengineering, Faculty of Engineering and Natural Sciences, Istanbul Okan University, Istanbul, Turkey, [c] Department of Pathology, Faculty of Medicine, Yeditepe University, Istanbul, Turkey, [d] Department of General Surgery, Faculty of Medicine, Yeditepe University, Istanbul, Turkey.

** Correspondence: Erhan Aysan, Department of General Surgery, Faculty of Medicine, Yeditepe University, ATA-2 Sitesi, Akasya Cad. No:25, Cengelkoy, Uskudar, Istanbul 34900, Turkey (e-mail: erhanaysan@hotmail.com).*

How to cite this article: Karabıyık Acar Ö, Nozhatzadeh GD, Tuncer A, Torun Köse G, Hacıhasanoğlu E, Sahin F, Aysan E. Production of parathyroid-like cells from thyroid stem cells in co-culture environment. Medicine 2022;101:48(e32009).

Received: 7 May 2022 / Received in final form: 3 November 2022 / Accepted: 3 November 2022

http://dx.doi.org/10.1097/MD.0000000000032009

induction, we also attributed the parathyroid-like behavior of the organoids to the stem/pregenitor cells that were coming from thyroid tissue. We evaluated the results with mainly three different techniques by measuring parathormone (PTH) secretion, determining gene expression profile using real-time PCR and performing immunohistochemistry.

2. Methods

2.1. Ethics approval

Before the operations, informed consent from the patients and the approval of the local human ethics committee were obtained. This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Clinical Research Ethics Committee of Republic of Turkey for human trials, SBÜ Istanbul Training and Research Hospital, 11.03.2022, #94.

2.2. Tissue removal

In this study, human parathyroid tissues were acquired from two patients with indications for subtotal parathyroidectomy and indication for total thyroidectomy. The diagnosis of the parathyroid donor patient was secondary hyperparathyroidism due to chronic renal failure. Nearly 6 cm^3 parathyroid tissue was excised from the patient, 2 cm^3 volume was separated and placed in a sterile falcon tube that contained harvest medium (Dulbecco's Modified Eagle Medium including 1000 unit/mL penicillin/streptomycin) and transferred to Yeditepe University Thyroid, Parathyroid and Organoid Laboratory. The diagnosis of the thyroid donor patient was a nodular goiter. After thyroid tissue was excised, a total of 2 cm^3 volume taken from the part of the healthy parenchyma section that did not contain nodules was transferred to the laboratory with the same protocol. Cell isolations from parathyroid and thyroid tissues were applied with the protocols described below.

2.3. Parathyroid cell isolation

Parathyroid tissue was cut into small pieces using a scalpel in 1X Phosphate-buffered saline (PBS) solution. After washing, the minced tissue was taken into a solution containing collagenase type II, DNase-I, and BSA at a concentration of 1 mg/mL, 0.05 mg/mL, and 5 mg/mL, respectively, and was shaken at a constant speed of 80 rpm in a 37°C water bath for 40 minutes. After shaking, the tissue was precipitated. The supernatant was washed with 1X PBS solution and centrifuged at 300 × g for 5 minutes. After centrifugation, the supernatant was removed and the pellet was dissolved in an isolation medium that contains RPMI 1640 (Gibco®) supplemented with 10% Fetal bovine serum (FBS) (Gibco®) and 100 U/mL of penicillin/streptomycin (Pan Biotech®, Germany) and passed through a sterile strainer with 40 µm pore sizes.

2.4. Thyroid cell isolation

Thyroid tissue was cut into small pieces using a scalpel in 1X PBS solution. After washing, the minced tissue was taken into a solution containing collagenase type IV and DNase I at a concentration of 1 mg/mL and 0.1 mg/mL, respectively, and was shaken at a constant speed of 80 rpm in a 37°C water bath for 30 minutes. The tissue was precipitated after shaking. The supernatant was washed with 1X PBS solution and centrifuged at 300 × g for 5 minutes. After centrifugation, the supernatant was removed and the pellet was dissolved in an isolation medium and passed through a sterile 100 µm strainer. The filtered cell suspension was precipitated and the pellet was incubated with erythrocyte lysis buffer which was removed by 1X PBS washing.

2.5. Cell characterization

For characterization of the isolated cells, cellular morphology was evaluated by bright field (BF) microscopy. Specific marker proteins were detected by immunofluorescence. Cells (1000 cells/well) were seeded in a 3-well chamber slide (Ibidi) and allowed to attach and proliferate for 96 hours. For BF microscopy imaging, cells were captured to assess cellular phenotype without fixation. For immunofluorescence imaging, to visualize cell-specific marker proteins, the following primary and secondary antibodies were used: rabbit polyclonal anti-calcium sensing receptor (CaSR) antibody (Abcam®, ab137408; 1:500), rabbit polyclonal anti-parathyroid hormone receptor 1 (PTH1R) antibody (Abcam®, ab75150; 1:500), rabbit polyclonal anti-thyroid stimulating hormone receptor (TSHR) antibody (Abcam®, ab202960; 1:500), rabbit monoclonal Anti-thyroid transcription factor 1 (TTF1) antibody [SP141] (Abcam®, ab227652; 1:25), mouse monoclonal anti-parathyroid hormone antibody [rPTH/911] (Abcam®, ab234415; 1 µg/mL), goat anti-mouse IgM cross-adsorbed secondary antibody (DyLight® 594) (Invitrogen®, SA5-10152; 1:200) and goat anti-rabbit IgG H&L (Alexa Fluor® 488) (Abcam®, ab150077; 1:1000). For cytoskeleton staining Alexa Fluor™ 647 Phalloidin (Invitrogen®; 1:1000), and for nuclei staining DAPI (Sigma-Aldrich®; 1 µg/mL) were used.

Cells were fixed with 3.7% formaldehyde and permeabilization was only applied for the internal protein detection. For reducing the nonspecific staining, blocking was performed for 10 minutes. Cells were individually incubated with the primary antibodies for 1 hour at room temperature (RT) and appropriate secondary antibodies were added for 1 hour at RT. Cells were washed using 1X PBS and to identify nuclei DAPI was used. After staining, an antifade mountant (Invitrogen®, Thermo Scientific) was added to the cells to minimize photobleaching. Images were taken using confocal microscopy with the fitting filters.

2.6. Organoid preparation and groups

The isolated cells were divided into five groups as follows:

Group-1: Thyroid cells grown in standard (st) medium

Group-2: Thyroid cells grown in dif medium

Group-3: Parathyroid cells grown in st medium

Group-4: Thyroid-Parathyroid co-culture cells grown in st medium

Group-5: Thyroid-Parathyroid co-culture cells grown in dif medium

For the generation of 3-dimensional organoids, each cell groups were seeded in ultra-low attachment multiwell plates (96-well format, U shape; Corning®) in a st medium containing Dulbecco's Modified Eagle Medium/Ham's F-12 (Gibco®) supplemented with ITS Premix (Corning®) and 100 U/mL of penicillin/streptomycin (Pan Biotech®, Germany). Cell seeding densities were applied to a total of 20,000 cells. On day 2, specified medium changes were applied. For thyroid cells, thyroid st medium containing Coon's modification of Ham's F-12 medium (Sigma-Aldrich®) supplemented with 5% FBS (Gibco®), a five-hormone mixture consisting of insulin, human recombinant (10 µg/mL, Sigma-Aldrich®), hydrocortisone (10 nmol/L, Sigma Aldrich®), transferrin, human (5 µg/mL, Sigma-Aldrich), Gly-His-Lys acetate salt (10 ng/mL, Sigma-Aldrich), somatostatin (10 ng/mL) and 100 U/mL of penicillin/streptomycin (Pan Biotech®, Germany) were used. RPMI 1640 (Gibco, Invitrogen®) supplemented with 5 % (v/v) FBS (Gibco®, Invitrogen), 100 ng/mL Activin A (R&D Systems®), 100 ng/mL sonic hedgehog (Shh) (R&D Systems®) and 100 U/mL of penicillin/streptomycin (Pan Biotech®, Germany) was used as a dif medium. For all groups, culture mediums were changed every three days and this process continued for 28 days.

2.7. Live/dead viability assay

The viability assay was performed by the Live/Dead® Viability/Cytotoxicity Kit (Invitrogen®) according to the manufacturers' instructions. The assay solution was prepared by combining 2 µM Calcein-AM and 4 µM ethidium homodimer-1 in PBS. Cultures were rinsed with PBS and organoids were incubated with the assay solution in dark for 35 minutes. Upon incubation, the assay solution was diluted with PBS, and images were captured using a fluorescence microscope after 14 days of incubation.

2.8. Gene expression profile by real-time PCR

Total RNA isolation was performed from the organoid cultures using a NucleoSpin RNA XS isolation kit (Macharey-Nagel®, Germany) after 14 and 30 days of incubation. The manufacturer's instructions were followed. Purity was monitored using a NanoDrop spectrophotometer (Thermo Scientific®). A reverse transcription reaction was performed using the iScript cDNA Synthesis Kit (Bio-Rad®) using a thermal cycler according to the manufacturer's description. Gene expression profiles of the selected parathyroid-specific markers PTH, CaSR, and PTH1R, thyroid-specific marker Thyroid peroxidase (TPO) were investigated. Real-time PCR was carried out using iTaq Universal SYBR Green Supermix (2X) (Bio-Rad®). The reaction setup was prepared based on the manufacturer's instructions. β-actin gene was employed for normalization and internal control purposes. The reaction was performed with Bio-Rad CFX96 C1000 Touch thermal cycler (Bio-Rad®). Data analysis was carried out by the comparative Ct method and data were expressed as fold change. Primer sequences and amplicon lengths are shown in Table 1.

2.9. Histology and immunohistochemistry

For histological examination, organoids were fixed in 10% formalin for at least 24 hours and embedded in 4% low melting temperature agarose gel (Sigma-Aldrich®). Thereafter, agarose embedded organoids were processed using st protocols and embedded in paraffin wax. Immunohistochemistry were performed using primary antibodies against calcium-sensing receptor (CaSR, Abcam®; 1:100 dilution), parathyroid hormone [rPTH/911] (PTH, Abcam®; 1:100 dilution), TSH Receptor antibody (TSHR, Abcam®; 1:100 dilution), and TTF1 (Abcam®; 1:100 dilution). Immunohistochemical reactions were performed on tissue sections using an automated immunohistochemical stainer (Leica BOND-MAX), according to the manufacturer's protocol. Detection was enabled by the BOND Polymer Refine Detection Kit (Leica DS9800).

2.10. PTH measurement

At specified incubation time points, the medium was collected from each organoid group and stored at –80°C until use. Before PTH measurement, thawed media were centrifuged and supernatants were used for the detection of secreted PTH concentration (pg/mL). Blank datum of each medium was subtracted from the measurements individually. PTH measurement was performed using human PTH enzyme-linked immunoassay kit (Abcam, ab230931).

3. Results

Before the dif protocol, BF and confocal microscopy images were investigated (Figs. 1–3). The cellular morphology of the isolated cells that were seeded to the tissue culture plastic were evaluated under a BF microscope. Parathyroid cells were

Table 1

Forward (F) and reverse (R) primer sequences employed in real-time PCR and expected amplicon lengths.

Primers		Sequence (5'→3')	Product length (bp)
PTH	F	GACATTGTATGTGAAGATGATACC	191
	R	GCTTCTTACGCAGCCATTCTA	
TPO	F	CGGGTCATCTGTGACAACAC	448
	R	CGGAGTCTACGCAGGTTCTC	
CaSR	F	GGACAGCGGGAACAGGATTTGAGAG	211
	R	CCCAGTTAGTCCCGGTTCCTTCACC	
PTH1R	F	AGGTGGTTCCAGGGCACAA	320
	R	CAACTCTTCCTCCGTGAGGC	
β-actin	F	TGATCCACATCTGCTGGAAGGT	142
	R	GACAGGATGCAGAAGGAGATTACT	

CaSR = calcium sensing receptor, PTH = parathormone, PTH1R= parathyroid hormone receptor 1, TPO = thyroid peroxidase.

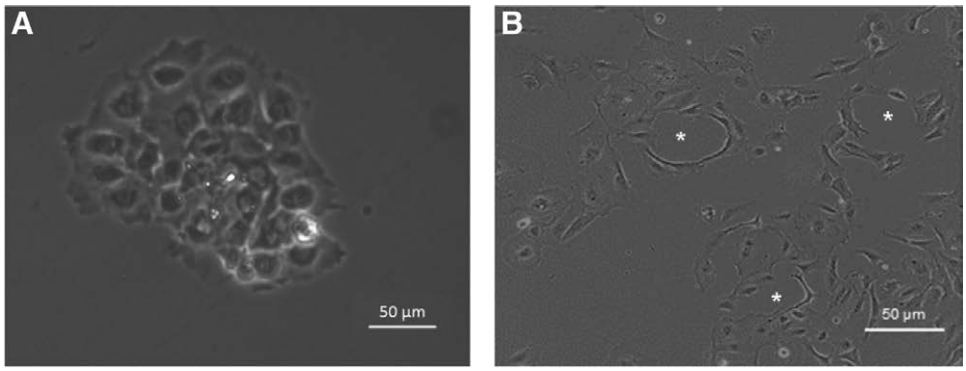

Figure 1. Before differentiation protocol, BF microscopy of cells in monolayer culture. (A) Parathyroid cells in polygonal and (B) Thyroid cells in follicular structures. Follicules are shown by asterisk (*). Scale bars represent 50 µm. BF = bright field.

observed as natural polygonal structures (Fig. 1A), while thyroid cells had a follicular structure (Fig. 1B). In immunofluorescence staining CaSR (Fig. 2A), PTH (Fig. 2B), and PTH1R (Fig. 2C) proteins were revealed positive in parathyroid cells, and TSHR (Fig. 3A), TTF1 (Fig. 3B) proteins were revealed positive in thyroid cells.

After characterization, cells were successfully integrated into spheroid cultures using ultra low attachment 96 well plates. Live/Dead assay was applied for the evaluation of cell viability in organoids that were stained with calcein AM (live) in green and ethidium homodimer-1 (dead) in red. On days 14 and 28, over 90% viability was observed in all groups.

For the organoids prepared using thyroid cells (both grown in st and dif medium), there was neither PTH gene expression (Table 2) nor PTH secretion detected (Table 3). However, after 28 days of induction, TPO gene expression was downregulated 18.1-fold, while PTH1R gene expression was upregulated 2.3-fold in thyroid cells (dif medium) when compared with cells grown in st medium.

When thyroid-parathyroid co-culture groups after 28 days of incubation in dif and st medium compared, TPO gene expression was decreased 49.5-fold in st medium, however, there was no TPO expression observed in the co-culture dif medium group. In addition to that, when dif medium was used for the thyroid-parathyroid co-culture cultivation, PTH gene expression increased from 2.5 to 5.1-fold, PTH1R gene expression increased from 1.2 to 3.6-fold and CaSR expression increased from 1.0 to 8.8-fold (Table 2).

As seen from Table 3, PTH secretion was only observed in parathyroid, thyroid-parathyroid co-culture st medium, and dif medium groups. As it was expected, PTH release in the parathyroid cells was higher than the ones in the other groups. However, this secretion decreased by 11% on day 14 and by 35% on day 28. In the thyroid-parathyroid co-culture st medium group, PTH secretion decreased by 95.5% on day 14 and 99.9% on day 28. In the thyroid-parathyroid co-culture dif medium group, although PTH secretion decreased by 96.5% on day 14, PTH secretion approached the initial level and showed a decrease of only 3.5% on day 28.

For the organoids prepared using thyroid cells (st and dif medium), TTF1 protein was positive on day 3, however, no apparent staining was observed on day 28 when dif medium was used. In co-culture cells grown in dif medium, PTH staining remained positive (Fig. 4A) and TTF1 became negative (Fig. 4B) after 28 days of incubation, whereas TTF1 protein was detected as still positive (Fig. 4C) with the co-culture cells grown in st medium (Table 4).

4. Discussion

In the literature, different medium formulations were applied for parathyroid-like cell dif. Those formulations were derived

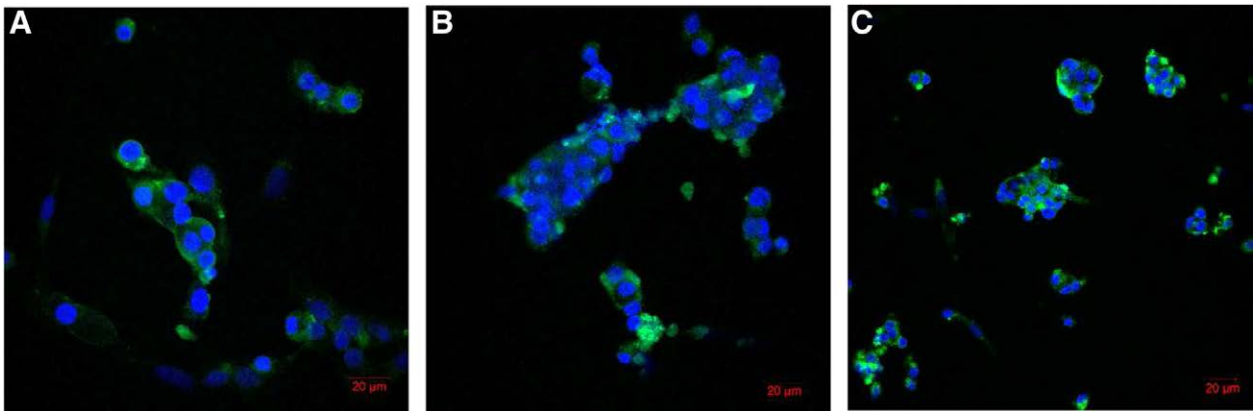

Figure 2. Immunofluorescence staining of proteins in parathyroid cells using confocal microscopy before differentiation protocol. (A) CaSR (membrane), (B) PTH (internal) and C) PTH1R (membrane), parathyroid cells stained in green for all proteins and counterstained in blue for nuclei. Objective 40 × original magnification, scale bars represent 20 µm. CaSR = calcium sensing receptor, PTH = parathormone, PTH1R= parathyroid hormone receptor 1.

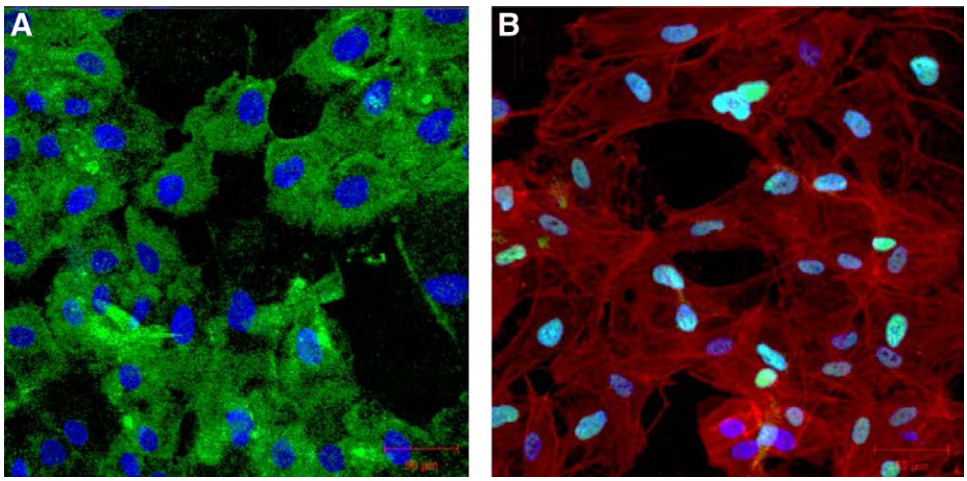

Figure 3. Immunofluorescence staining of proteins in thyroid cells using confocal microscopy before differentiation protocol. (A) TSHR (membrane) and (B) TTF1 (nuclear), Thyroid cells stained in green for all proteins and counterstained in blue for nuclei. The cytoskeleton was stained with phalloidin red to observe cell morphology. Objective 40 × original magnification, scale bars represent 50 µm. TSHR = thyroid stimulating hormone receptor, TTF1 = thyroid transcription factor 1.

Table 2

Real time PCR results of the organoid groups after 28 days of incubation.

Fold change $2^{-\Delta\Delta Ct}$	PTH gene expression	TPO gene expression	PTH1R gene expression	CaSR gene expression
Thyroid organoids in st medium	0.0	14.9	0.0	144.0
Thyroid organoids in dif medium	0.0	–18.1	2.3	–1.5
Parathyroid organoids in st medium	1112.8	0.0	6.5	7.5
Co-culture organoids in st medium	2.5	–49.5	1.2	1.0
Co-culture organoids in dif medium	5.1	0.0	3.6	8.8

CaSR = calcium sensing receptor, dif = differentiation, PTH = parathormone, PTH1R= parathyroid hormone receptor 1, st = standard, TPO = thyroid peroxidase.

Table 3

PTH (pg/mL) measurement results of the organoid groups (n = 2).

PTH (pg/mL)	Day 3	Day 14	Day 28
Thyroid organoids in st medium	0	0	0
Thyroid organoids in dif medium	0	0	0
Parathyroid organoids in st medium	1752	1561	1134
Co-culture organoids in st medium	852	38	1.3
Co-culture organoids in dif medium	531	18	512

dif = differentiation, PTH = parathormone, st = standard.

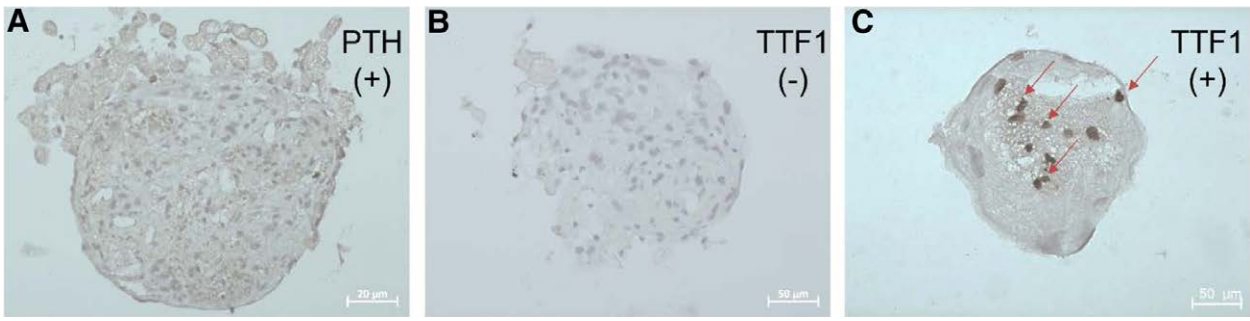

Figure 4. Immunohistochemical staining of co-culture groups after 28 days of incubation. (A) Positive PTH staining was observed as brown cytoplasmic color in thyroid-parathyroid co-culture organoids in dif medium. (B) TTF1 staining was observed negative in thyroid-parathyroid co-culture organoids in dif medium. (C) TTF1 staining was observed positive with brown nuclear color (red arrows) in thyroid-parathyroid co-culture organoids in st medium. Scale bars represent (A) 20 µm and (B–C) 50 µm. dif = differentiation, PTH = parathormone, st = standard, TTF1 = thyroid transcription factor 1.

from the D'Amour protocol and the Bingham protocol, which include either only Activin A or Activin A with Shh in varying amounts.[10,11] Activin A is a widely used transforming growth factor-beta superfamily protein that enables cell dif through multiple pathways.[12,13] Shh is a definitive endoderm induction protein that has a pivotal role in cell dif during development.[14] Parathyroid-like cells differentiated from human embryonic stem cells and thymic epithelial cells before.[11,15] Bingham et al (2009) included 100 ng/mL Activin A for 12 days in the medium and succeeded in vivo dif of human embryonic stem cells to a parathyroid-like phenotype.[11] Ignatoski et al (2010) differentiated human embryonic stem cells to parathyroid cells using Activin A and Shh.[16]

In this study, we aimed to differentiate stem/progenitor cells isolated from thyroid into parathyroid-like cells by using Activin A and Shh. We evaluated this dif by detecting the presence of CaSR, PTH1R, TPO, and PTH gene expressions using real-time PCR, the presence of TTF1 and PTH by immunohistochemical stainings, and PTH measurement from the culture medium by enzyme-linked immunoassay.

CaSR is highly expressed in the parathyroid and renal tubule.[17] However, the gene is widely expressed at lower levels in other tissues, for example in the liver, bone, lung, breast, placenta, and gut.[18] Also, PTH1R is expressed in most tissues, such as the parathyroid, bone, kidney, ovary, intestine, thymus, and liver.[19] TPO gene is specifically expressed only in thyroid tissue. It is the key physiological function in the biosynthesis of thyroid hormones.[20] PTH is a hormone consisting of 84 amino acids that is exclusively secreted from parathyroid chief cells.[21]

When we evaluated the changes in the thyroid organoids in dif medium; we found that TPO decreased 18.1 times. This result revealed that thyroid cells lost their specific properties and underwent an apparent change. In addition, while PTH1R was not expressed in the thyroid organoids in st medium, its positivity at the level of 2.3-fold with dif medium showed that this transformation was in the direction of the parathyroid lineage. However, the absence of PTH gene expression and PTH secretion showed that this transformation was not at the desired level. From this point of view, we decided to form thyroid-parathyroid co-cultures. It is known that the co-culture environment ensures cellular interaction (cross-talk) between the cultured cells and this interaction may be more effective for the same embryological originated cells.[22]

Two specific markers for thyroid and parathyroid cells were used in the immunohistochemical analysis. TTF1 is the protein that regulates transcription of thyroid cell-associated genes, and stains cell nuclei in brown color.[23,24] TTF1 is very useful in separating the thyroid from the parathyroid.[25] PTH staining is specific for parathyroid chief cells and produces brown cytoplasmic staining.[25]

While TTF1 was initially positive on day 3 in the organoid groups formed using co-culture cells (st and dif medium) it

Table 4

Immunohistochemical (IHC) staining results of the organoid groups throughout 28 days of incubation.

	Day 3		Day 28	
IHC Staining	**PTH staining**	**TTF1 staining**	**PTH staining**	**TTF1 staining**
Thyroid organoid in st medium	–	+	–	+
Thyroid organoid in dif medium	–	+	–	Unclear
Parathyroid organoid in st medium	+	–	+	–
Co-culture organoid in st medium	+	+	+	+
Co-culture organoid in dif medium	+	+	+	–

dif = differentiation, PTH = parathormone, st = standard, TTF1 = thyroid transcription factor 1.

became negative on day 28 with the induction of dif medium due to dif of thyroid stem/progenitor cells into parathyroid-like cells.

5. Conclusion

In conclusion, in the first part of this research, we revealed that cells isolated from thyroid had the potential to differentiate into parathyroid-like cells by using Activin A and Shh, but this dif was not at the functional level. In the second part, parathyroid cells were co-cultured with thyroid cells, and it was revealed that dif was more pronounced and functional. We hypothesized that this dif might be related to microenvironmental factors due to the same embryological origin of the stem/progenitor cells.

In summary, our findings showed that using Activin A and Shh proteins, thyroid stem/progenitor cells differentiated to parathyroid-like cells successfully in a co-culture environment. Co-culture of cells with the same embryological origin may be a promising new technique for cell dif.

Author contributions

Conceptualization: Erhan Aysan, Gamze Torun Köse.
Data curation: Erhan Aysan.
Formal analysis: Erhan Aysan.
Funding acquisition: Fikrettin Sahin, Gamze Torun Köse.
Investigation: Alperen Tuncer, Gülçin Delal Nozhatzadeh, Gamze Torun Köse, Özge Karabıyık Acar.
Methodology: Alperen Tuncer, Erhan Aysan, Ezgi Hacıhasanoğlu, Gülçin Delal Nozhatzadeh, Özge Karabıyık Acar.
Project administration: Fikrettin Sahin, Gamze Torun Köse, Özge Karabıyık Acar.
Resources: Fikrettin Sahin.
Supervision: Gamze Torun Köse.
Visualization: Ezgi Hacıhasanoğlu, Özge Karabıyık Acar.
Writing – original draft: Gülçin Delal Nozhatzadeh, Özge Karabıyık Acar.
Writing – review & editing: Gamze Torun Köse.

References

[1] Guyton A, Hall J. Thyroid Metabolic Hormones. In: Gruliow R, (ed). Guyton and Hall Textbook of Medical Physiology. 11th ed. Philadelphia Pennsylvania: Elsevier; 2006:931–43.
[2] Tisell LE. Surgery of the thyroid and parathyroid glands. G. W. Randolph (ed.). 220 × 285 mm. Pp. 620. Illustrated. Philadelphia: Saunders. 2004. £125·00. British Journal of Surgery. 2005;92(2):255.
[3] Hamilton JW, Cohn D. Studies on the biosynthesis in vitro of parathyroid hormone: I. Synthesis of parathyroid hormone by bovine parathyroid gland slices and its control by calcium. J Biol Chem. 1969;244:5421–9.
[4] Raisz LG. Regulation by calcium of parathyroid growth and secretion in vitro. Nature. 1963;197:1115–6.
[5] Brown EM, Hurwitz S, Aurbach GD. Preparation of viable isolated bovine parathyroid cells. Endocrinology. 1976;99:1582–8.
[6] Capen CC. Fine structural alterations of parathyroid glands in response to experimental and spontaneous changes of calcium in extracellular fluids. Am J Med. 1971;50:598–611.
[7] Dietel M, Dorn-Quint G. By-pass secretion of human parathyroid adenomas. A particular intracellular form of rapid adaptation to external stimuli. Lab Invest. 1980;43:116–25.
[8] Ridgeway RD, Hamilton JW, MacGregor RR. Characteristics of bovine parathyroid cell organoids in culture. In Vitro Cell Dev Biol. 1986;22:91–9.
[9] Fierabracci A, Puglisi MA, Giuliani L, et al. Identification of an adult stem/progenitor cell-like population in the human thyroid. J Endocrinol. 2008;198:471–87.
[10] D'Amour KA, Agulnick AD, Eliazer S, et al. Efficient differentiation of human embryonic stem cells to definitive endoderm. Nat Biotechnol. 2005;23:1534–41.
[11] Bingham EL, Cheng SP, Woods Ignatoski KM, et al. Differentiation of human embryonic stem cells to a parathyroid-like phenotype. Stem Cells Dev. 2009;18:1071–80.
[12] Sulzbacher S, Schroeder IS, Truong TT, et al. Activin A-induced differentiation of embryonic stem cells into endoderm and pancreatic progenitors–the influence of differentiation factors and culture conditions. Stem Cell Rev Rep. 2009;5:159–73.
[13] Rodríguez-Martínez G, Molina-Hernández A, Velasco I. Activin A promotes neuronal differentiation of cerebrocortical neural progenitor cells. PLoS One. 2012;7:e43797.
[14] Wu SM, Choo ABH, Yap MGS, et al. Role of Sonic hedgehog signaling and the expression of its components in human embryonic stem cells. Stem Cell Res. 2010;4:38–49.
[15] Ignatoski KMW, Bingham EL, Frome LK, et al. Directed trans-differentiation of thymus cells into parathyroid-like cells without genetic manipulation. Tissue Eng Part C Methods. 2011;17:1051–9.
[16] Ignatoski KMW, Bingham EL, Frome LK, et al. Differentiation of precursors into parathyroid-like cells for treatment of hypoparathyroidism. Surgery. 2010;148:1186–90.
[17] Brown EM, Gamba G, Riccardi D, et al. Cloning and characterization of an extracellular Ca2+-sensing receptor from bovine parathyroid. Nature. 1993;366:575–80.
[18] Brennan SC, Thiem U, Roth S, et al. Calcium sensing receptor signalling in physiology and cancer. Biochim Biophys Acta. 2013;1833:1732–44.
[19] Bisello A, Chorev M, Friedman PA, et al. Parathyroid hormone receptors (version 2019.4) in the IUPHAR/BPS guide to pharmacology database. IUPHAR/BPS Guide to Pharmacol CITE. 2019;2019(4 SE-Summaries). Available from: http://journals.ed.ac.uk/gtopdb-cite/issue/view/275.
[20] Benvenga S, Tuccari G, Ieni A, et al. Thyroid gland: anatomy and physiology. Encyclopedia Endocr Dis. 2018;4:382–90.
[21] Brown EM. Extracellular Ca2+ sensing, regulation of parathyroid cell function, and role of Ca2+ and other ions as extracellular (first) messengers. Physiol Rev. 1991;71:371–411.
[22] Pastuła A, Marcinkiewicz J. Cellular interactions in the intestinal stem cell niche. Arch Immunol Ther Exp (Warsz). 2019;67:19–26.
[23] Dobrescu A, Tarta C, Cornianu M, et al. Thyroid transcription factor–1 (TTF-1) immunoexpression in thyroid carcinoma with follicular origin. Revista de Chimie (Rev Chim). 2020;71:185–91.
[24] Gomez-Fernandez C, Jorda M, Delgado PI, et al. Thyroid transcription factor 1: a marker for lung adenocarcinoma in body cavity fluids. Cancer Cytopathol. 2002;96:289–93.
[25] Erickson LA, Mete O. Immunohistochemistry in diagnostic parathyroid pathology. Endocr Pathol. 2018;29:113–29.