ORIGINAL ARTICLE

Isolation, In Vitro Expansion, And Cryopreservation of Primary Cells Derived from Human Thyroid Carcinoma

Fatimah Eliana[1]*, Sabrina Azmi[1], Anggi Puspa Nur Hidayati[1], Juhan Khalila[1]
1 Faculty of Medicine, YARSI University

*Corresponding author:
Fatimah Eliana, MD.
Faculty of Medicine, YARSI University, Jakarta, Indonesia.
Email: fatelianasppd13@gmail.com

ABSTRACT
Thyroid carcinoma is a malignant neoplasm arising from thyroid parenchymal cells and currently ranks as the fourth most diagnosed cancer in Indonesia. This study aimed to isolate thyroid carcinoma cells for in vitro expansion and long-term preservation as a reliable cell culture stock, including cryopreservation for future research applications. In addition, we sought to identify and characterize cells derived from papillary thyroid carcinoma (PTC) tissue to evaluate the presence of mutations with potential prognostic significance. Primary cell isolation was performed via enzymatic digestion using collagenase, enabling effective separation of tumor cells from adjacent non-malignant thyroid tissue. Cell proliferation was supported using Dulbecco's Modified Eagle Medium (DMEM) supplemented with 20% fetal bovine serum (FBS), selected for its high concentration of growth-promoting factors that enhance proliferation rates. For biobanking purposes, cryopreservation of the thyroid carcinoma-derived cells was conducted using a standard slow-freezing protocol. Molecular characterization was carried out through PCR amplification, gel electrophoresis, and Sanger sequencing of key oncogenic drivers, specifically the BRAF gene and five RAS gene targets: HRAS exon 2, NRAS exons 2 and 3, and KRAS exons 2 and 3. No pathogenic mutations were identified in the analyzed BRAF or RAS gene regions.

Keywords: Papillary thyroid carcinoma, primary cell culture, dan BRAF and RAS mutations

INTRODUCTION

Thyroid carcinoma is a malignant tumor arising from follicular thyroid cells.[1] Its incidence has risen markedly over the past four decades in the United States.[2] Globally, it ranks as the 13th most common cancer and the sixth most prevalent among women aged 20-45.[3] In Indonesia, according to 2015 data from the Ministry of Health, thyroid carcinoma is the fourth most frequently diagnosed cancer and the tenth leading cause of cancer-related death at Dharmais Cancer Hospital.

As the most common endocrine malignancy, thyroid cancer is categorized by differentiation, histology, and genetic mutations. Major histological types include differentiated thyroid carcinoma (papillary, follicular, and Hürthle cell subtypes), medullary thyroid carcinoma, poorly differentiated carcinoma, and anaplastic thyroid carcinoma.[4] Papillary thyroid carcinoma (PTC) accounts for about 85% of cases, while anaplastic thyroid carcinoma, though rare (1%), causes 15-40% of thyroid cancer deaths.[5,6]

The rising incidence of thyroid cancer has prompted the development of in vitro models using tumor-derived cells. These models are widely used to study tumor behavior, compare malignant and normal thyroid cells, and conduct drug testing.[7] Isolating primary cells from thyroid tumors is essential for accurate identification and further analysis. In vitro models have helped explore genetic and epigenetic drivers of thyroid tumorigenesis, assess drug response, and identify cancer stem cells.[8]

This study investigates the in vitro behavior of papillary thyroid carcinoma cells compared to normal thyroid cells, including isolation, culture, cryopreservation, identification, and mutation analysis with potential prognostic significance.

MATERIALS AND METHODS

Primary Cell Isolation from Human Thyroid Carcinoma Tissue

This in vitro study utilized cell samples obtained from human thyroid carcinoma tissues during thyroidectomy procedures. Primary cell isolation was performed on both malignant and adjacent non-malignant thyroid tissues. The culture medium consisted of Dulbecco's Modified Eagle Medium (DMEM) supplemented with 5% fetal bovine serum (FBS), 2 mM glutamine, 2.6 g/L sodium bicarbonate ($NaHCO_3$), 5 µg/mL gentamicin, 1% non-essential amino acids (NEAA), and six growth-supporting supplements: 10 mIU/mL thyrotropin (TSH), 10 mIU/mL insulin, 1 nM hydrocortisone, 2 ng/mL glycyl-histidyl-L-lysine acetate, 5 µg/mL transferrin, and 10 ng/mL somatostatin. The excised thyroid tissue (13.66 g) was decontaminated and finely minced into small fragments under sterile conditions using a surgical blade. These fragments were rinsed with Dulbecco's Phosphate-Buffered Saline (DPBS), then immersed in a 0.25% trypsin solution and stored overnight at 4°C.

On the following day, tissue fragments from both carcinoma and normal thyroid regions were transferred into 50 mL conical tubes and incubated with type I collagenase in 0.25% trypsin at 37°C for approximately 3 hours, with gentle agitation every 30 minutes. The resulting cell suspensions were filtered through a cell strainer and centrifuged at 1500 rpm for 7 minutes at 4°C to collect the cell pellets. After discarding the supernatant, the pellets were resuspended and homogenized. Due to remaining turbidity, a portion of the suspension was transferred into 15 mL tubes and re-centrifuged at 2000 rpm for 7 minutes at 4°C. Meanwhile, cells in the 50 mL tubes were resuspended in complete DMEM and plated into separate T-75 flasks designated for healthy and carcinoma-derived cells. Following re-centrifugation, visible cell pellets in the 15 mL tubes were recovered, resuspended in complete medium, and added to their respective primary cultures in the T-75 flasks.[7]

Primary Cell Culture

Primary thyroid cells were cultured and expanded under standard conditions, with medium replacement every two days. Once the cells reached confluency in T-75 flasks, subculturing was carried out. When the total cell count exceeded 5×10^6, harvesting was performed. Cells were centrifuged at 500 × g for

2 minutes, the supernatant was discarded, and the resulting pellet was retained. The cell pellet was resuspended in 100-200 µL of phosphate-buffered saline (PBS) for subsequent analysis or experimental use.[8,9]

Cryopreservation of Thyroid Carcinoma Cells
Cryopreservation was carried out using a cryoprotectant solution composed of 700 µL thyroid carcinoma cell suspension, 200 µL fetal bovine serum (FBS), and 100 µL dimethyl sulfoxide (DMSO). The cell mixture was initially frozen at -80°C overnight in a deep freezer, then transferred to a liquid nitrogen cryogenic storage system for long-term preservation.[10]

Identification and Characterization of Cells Derived from Thyroid Carcinoma Tissue The primary cells utilized in this study were isolated from histopathologically confirmed papillary thyroid carcinoma tissue.

DNA Extraction and PCR Amplification of BRAF-RAS Mutation Regions
Genomic DNA was extracted from over one million cultured cell isolates derived from patient-derived papillary thyroid carcinoma (PTC) tissue, in addition to normal human dermal fibroblasts (HDF) and the HSC-3 human tongue squamous cell carcinoma line. DNA isolation was performed using the Quick-DNA™ Miniprep Plus Kit (Zymo Research), in accordance with the manufacturer's protocol. The concentration of the extracted nucleic acids was measured using a Tecan fluorometric system. Approximately 100-200 ng of DNA was utilized for polymerase chain reaction (PCR) amplification targeting BRAF and RAS gene mutation regions. DNA samples were either used immediately for PCR analysis or stored at -20°C for future application.[11]

PCR Amplification of Six BRAF-RAS Gene Mutation Regions
Subsequent PCR amplification targeted six known mutation regions within the BRAF-RAS gene family, enabling molecular analysis relevant to thyroid carcinogenesis. The amplification of six mutation regions within the BRAF-RAS gene family was conducted using the MyTaq HS Red Mix PCR Kit (Bioline), following the manufacturer's protocol. The six targeted regions included BRAF, HRAS exon 2, NRAS exon 2, NRAS exon 3, KRAS exon 2 and KRAS exon 3.[12]

Detection of BRAF-RAS Mutations by Sanger Sequencing
DNA sequencing was conducted using an automated 96-capillary ABI 3730xl DNA Sequencer (Applied Biosystems) at Apical Scientific Sdn. Bhd., Malaysia, through PT. Genetika Science Indonesia. The resulting sequences were analyzed using the BLASTN platform to confirm alignment with the respective BRAF-RAS gene regions. Mutation screening was subsequently performed by aligning the sequence data with the corresponding reference sequences from the GenBank database. Multiple sequence alignment was carried out using ClustalW to detect nucleotide variations indicative of potential mutations.[13]

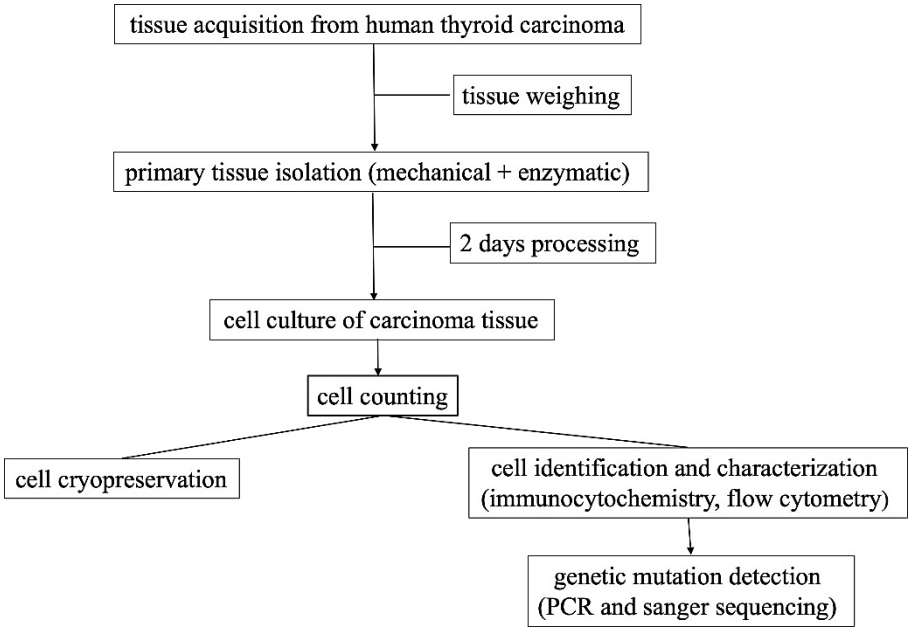

Figure 1. Research Flow

RESULTS

Primary Cell Isolation from Normal and Carcinoma Thyroid Tissue

Primary cells isolated from both normal and carcinoma thyroid tissues, previously incubated in 0.25% trypsin solution, were transferred into 50 mL conical tubes and treated with collagenase type I to promote further tissue dissociation.

Figure 2. Tissue separation process using a cell strainer

Following centrifugation, each cell suspension was resuspended in culture medium and transferred into T-75 flasks, yielding two separate cultures: one containing normal thyroid cells and the other containing carcinoma-derived thyroid cells.

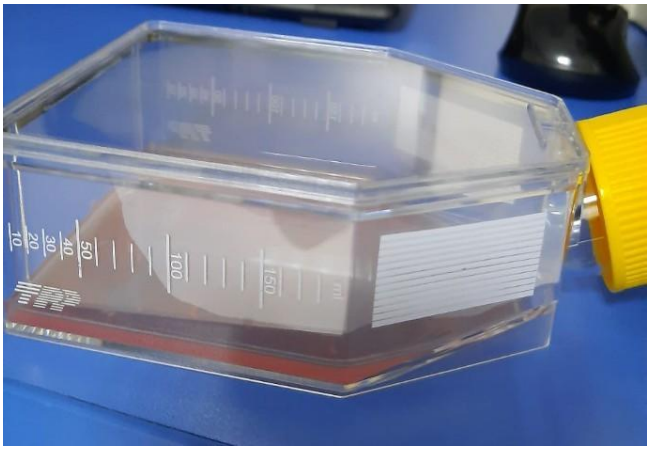

Figure 3. T-75 flask containing cells in complete DMEM medium

Observations were conducted on both thyroid-derived cell types. On day 5 of cultivation, cell growth was observed in both T75 flasks.

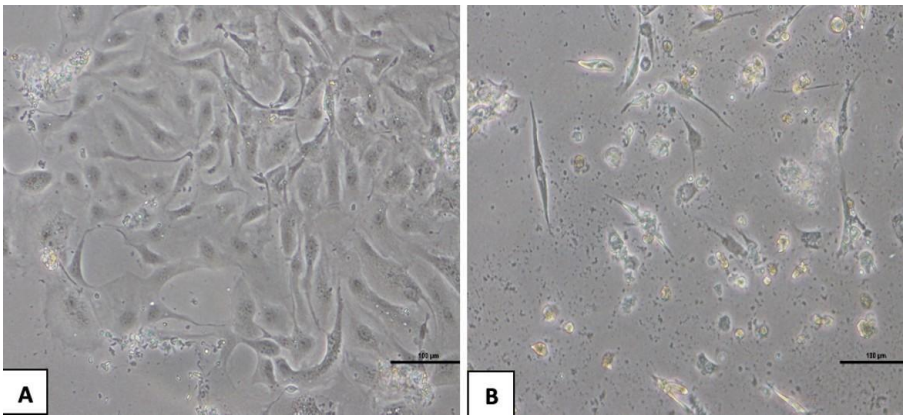

Figure 4. Cell growth derived from thyroid tissue. (A) Normal (B) Carcinoma

The above images illustrate cells derived from both normal thyroid tissue (A) and thyroid carcinoma tissue (B), exhibiting a flattened spindle or fusiform shape, with fine granular cytoplasm and elongated nuclei. In image (A), cell proliferation appears more extensive compared to image (B).

Cell Culture and Expansion of Normal and Papillary Thyroid Carcinoma Tissues

Cell observation and proliferation were carried out until day 12, by which time the cells had reached 80% confluence in the T75 flask, with cellular morphology as shown below.

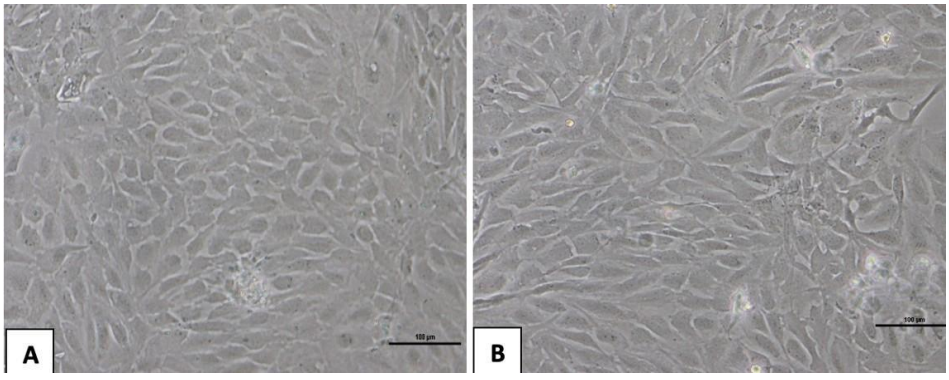

Figure 5. Proliferation of thyroid-derived cells on day 12: (A) Normal, (B) Cancerous

Figure 5 shows cells with a spindle-shaped or fusiform morphology, consistent with fibroblast-like appearance, observed in both normal and cancerous thyroid-derived cells. Once the cells reached confluence in the T75 flask, they were harvested using 0.25% trypsin, and counted with a hemocytometer. The total cell counts obtained were 1,562,500 cells from normal thyroid tissue and 3,525,000 cells from thyroid carcinoma tissue. These results indicate a higher and faster proliferation rate in cancer-derived thyroid cells.

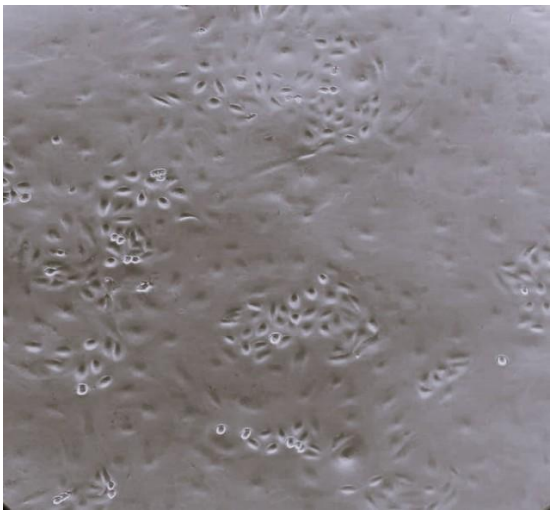

Figure 6. Morphology of thyroid carcinoma-derived cells after administration of 20% FBS

The subsequent process involved subculturing using complete DMEM supplemented with 20% FBS until the cells were ready for experimentation. The proliferation phase lasted for 10 days and resulted in cells with predominantly round morphology, some slightly flattened, forming clusters surrounded by spindle-shaped cells. This morphological appearance is suggestive of epithelial-like cells (Fig 6).

Cell Cryopreservation

Cryopreservation of thyroid tissue-derived cells requires an appropriate method to maintain cell viability and functionality after thawing. The primary methods used include (1) Slow freezing, which is the most commonly employed technique for thyroid tissue cryopreservation, as it gradually reduces ice crystal formation; (2) Vitrification, which involves ultra-rapid freezing using high concentrations of cryoprotectants, preventing ice crystal formation by converting the solution into a glass-like state; (3) Controlled-rate freezing (CRF) using a programmable freezer, similar to slow freezing but offering more precise temperature control due to specialized equipment.

Cryopreservation relies on cryoprotective agents (CPAs) to protect cells from damage caused by ice crystal formation during freezing. CPAs are generally divided into two categories (1) Penetrating (permeable) cryoprotectants, which enter the cell and protect intracellular structures by minimizing ice formation, such as dimethyl sulfoxide (DMSO), glycerol, ethylene glycol, and propylene glycol; (2) Non-penetrating (non-permeable) cryoprotectants, such as sucrose, trehalose, polyvinylpyrrolidone (PVP), and albumin. These agents are often used in combination to enhance cryopreservation efficiency and maintain cell viability post-thaw.

In this study, the cryopreservation process employed the slow freezing method and DMSO as the cryoprotectant, commonly used for stem cells and hematopoietic cells. The protocol consisted of mixing 700 µL of thyroid carcinoma cells, 200 µL of FBS, and 100 µL of DMSO. The mixture was initially stored in a deep freezer at -80°C and transferred the

following day to a cryo-freezer containing liquid nitrogen.

Identification and Characterization of Thyroid Carcinoma-Derived Cells

PCR was performed with slight modifications to accommodate the concentration of extracted DNA and the melting temperatures (Tm) of each primer used. The table below presents the primer sequences for the BRAF-RAS genes (Table 1).

Table 1. Primer Sequences of the BRAF-RAS Genes

No	Primer	Gene	Exon	Sequence 5'--> 3'	Size (bp)
1	BRAF_F BRAF_R	BRAF	15	TCATAATGCTTGCTCTGATAGGA GGCCAAAAATTTAATCAGTGGA	224
2	HRAS_ex2_F HRAS_ex2_R	HRAS	2	GACGGAATATAAGCTGGTGGTG CCTATCCTGGCTGTGTCCT	178
3	NRAS_ex2_F NRAS_ex2_R	NRAS	2	CAATTAACCCTGATTACTGG GGTGGGATCATATTCATCTACA	152
4	NRAS_ex3_F NRAS_ex3_R		3	TCCCTGCCCCCTTACCCT TTGATGGCAAATACACAGA	173
5	KRAS_ex2_F KRAS_ex2_R	KRAS	2	GTATTTGATAGTGTATTAAC CTCTATTGTTGGATCATATTCG	195
6	KRAS_ex3_F KRAS_ex3_R		3	CAGACTGTGTTTCTCCCTTCTC ATGATTTAGTATTATTTATGG	181

PCR process was carried out in the following sequence: initial denaturation at 95°C for 1 minute, followed by 35 amplification cycles at 95°C for 15 seconds. This was followed by annealing and polymerization steps at specific temperatures and durations depending on the primer used. A final extension was performed at 72°C for 3 minutes. The table below shows the modified PCR conditions for each BRAF-RAS gene.

Table 2. Modified PCR Conditions for Each BRAF-RAS Gene

No.	Mutation Region	Pre-denaturation	Denaturation/Annealing/ Polymerization (°C)	Post-polymerization	Cycles
1	BRAF	95°C 1 min	95 (15sec)/55 (15sec)/72 (10sec)	72°C 3 min	35 cycle
2	HRAS_ex2		95 (15sec)/64 (15sec)/72 (10sec)		
3	NRAS_ex2		95 (15sec)/55 (15sec)/72 (10sec)		
4	NRAS_ex3		95 (15sec)/60 (10sec)/72 (20sec)		
5	KRAS_ex2		95 (15sec)/54 (15sec)/72 (10sec)		
6	KRAS_ex3		95 (15sec)/50 (10sec)/72 (10sec)		

PCR-Electrophoresis Results of BRAF-RAS Genes

PCR results were visualized on 2% agarose gel using a 100 bp ladder marker. This PCR method successfully detected six mutation regions of the BRAF-RAS genes, as indicated by the presence of distinct white DNA bands. The DNA band lengths were as follows: BRAF gene - 224 bp, HRAS exon 2 - 178 bp, NRAS exon 2 - 152 bp, NRAS exon 3 - 173 bp, KRAS exon 2 - 195 bp, and KRAS exon 3 - 181 bp

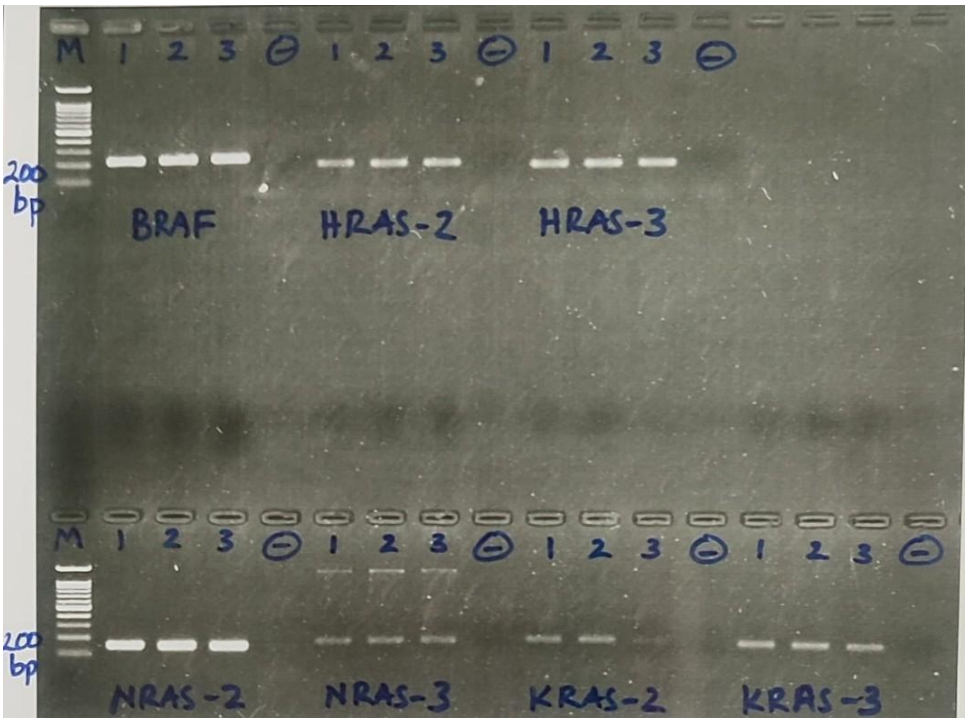

Figure 7. PCR-Electrophoresis Results of the BRAF-RAS Genes

Legend:

1 = Normal Human Dermal Fibroblast cells (HDF cells)
2 = Papillary Thyroid Cancer cells (PTC cells)
3 = Human Tongue Squamous Cell Carcinoma cells (HSC-3 cells) (-) = Negative control for the PCR reaction

Results of BRAF-RAS Gene Sequence Alignment

Figure 8. Sequence Alignment of the BRAF-RAS Genes in Papillary Thyroid Cancer Cell

Based on the sequencing results obtained from PTC cancer cells of the patient, no mutations were identified in the six analyzed genes, namely BRAF, HRAS exon 2, NRAS exon 2, NRAS exon 3, KRAS exon 2, and KRAS exon 3. This was indicated by the absence of amino acid changes at known mutation sites when compared to the corresponding amino acids in HDF cells, which represent normal or healthy individual cells.

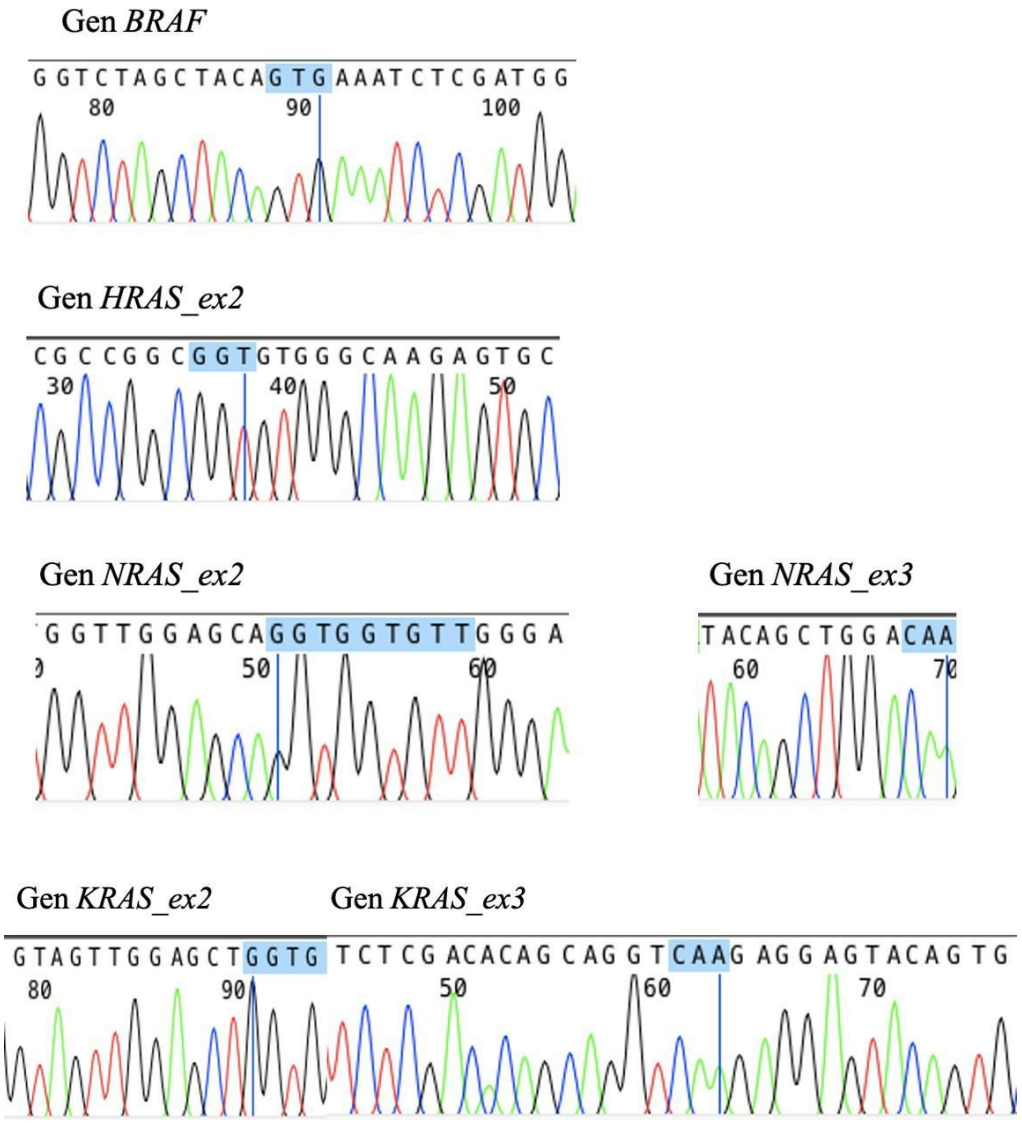

Figure 9. Sequencing Results of the BRAF-RAS Genes in Papillary Thyroid Cancer Cell

DISCUSSION

Primary Cell Isolation from Normal and Carcinoma Thyroid Tissue

The method used in this study was the enzymatic method. Enzymatic cell isolation is a technique used to dissociate cells from tissues or cultures with the aid of enzymes that digest extracellular components, such as the extracellular matrix or cell-cell adhesions. The basic principle of the enzymatic method involves using specific enzymes to break the bonds between cells in tissue, allowing individual cells to be released without compromising their structural integrity. In this study, the enzymatic method wwas used to culture primary cells derived from thyroid carcinoma tissue. The choice of enzyme depends on the type of tissue to be isolated. Common enzymes used in enzymatic cell isolation include collagenase—as used in this study—which digests collagen in the extracellular matrix, particularly in connective and muscle tissues. Other frequently used enzymes include trypsin, which cleaves proteins between cells in culture; hyaluronidase, which breaks down hyaluronic acid in connective tissue to release cells; dispase, which helps release cells from epithelial tissue or culture surfaces; and DNase, which prevents cell aggregation by digesting DNA released from lysed cells.[6,7]

Cell Culture from Normal and Carcinoma Thyroid Tissue

Cell culture using DMEM (Dulbecco's Modified Eagle Medium) and 10% FBS (Fetal Bovine Serum) is a commonly applied technique in cell biology laboratories to maintain and propagate cells in vitro. DMEM is characterized by its content of glucose, amino acids, vitamins, and mineral salts essential for cell growth. FBS, derived from fetal bovine blood, is frequently used as a supplement in culture media due to its rich content. The concentration of FBS can be adjusted depending on the cell type. In this study, a 20% FBS concentration was used due to its higher growth factor content, which accelerates the proliferation rate, albeit at a higher cost.[8,9]

The figure below compares the results of thyroid tissue-derived cell cultures using complete DMEM supplemented with 10% and 20% FBS on day 3 of cultivation. The cells exhibit a slightly flattened, fusiform shape with fine granular cytoplasm and oval nuclei. Cultures using complete DMEM with 10% FBS showed slower cell growth and a less epithelial-like morphology compared to cultures with 20% FBS.

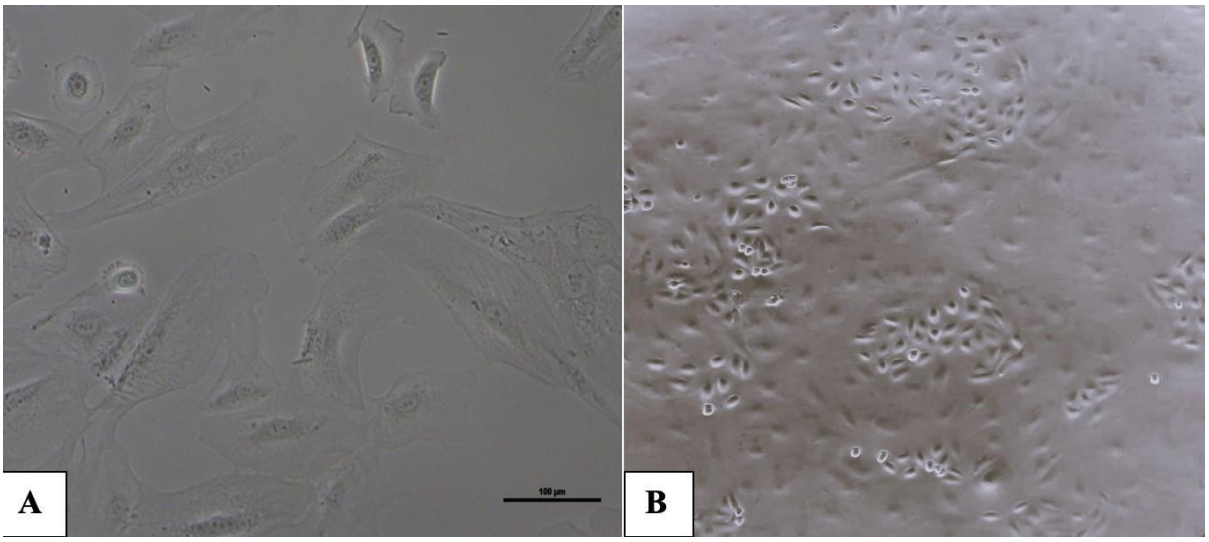

Figure 10. Growth of thyroid carcinoma-derived cells in complete DMEM on day 3 of culture with the addition of (A) 10% FBS and (B) 20% FBS.

Cryopreservation of Thyroid Carcinoma-Derived Cells

In this study, the storage of cells for subsequent experiments was conducted using the cryopreservation method. Cryopreservation aims to preserve cell viability without causing damage and is commonly used for storing embryos, sperm, oocytes, stem cells, and biological tissues for medical or research purposes. It is also applied in in vitro fertilization (IVF) programs, regenerative therapies, tissue transplantation, and the conservation of endangered species. Cryopreservation is a technique used to store cells, tissues, or organs at ultra-low temperatures to maintain their viability over extended periods. The temperature typically reaches as low as -196°C, utilizing liquid nitrogen as the freezing medium. The method of cryopreservation in this study was performed using the slow freezing method. The preservation medium consisted of 700 µL of thyroid carcinoma cell suspension, 200 µL of FBS, and 100 µL of DMSO. The mixture was first stored in a deep freezer at -80°C. On the following day, the samples were transferred to a cryofreezer containing liquid nitrogen.[14]

In this study, cryopreservation was performed using the slow freezing method. The preservation medium consisted of 700 µL

of thyroid carcinoma cell suspension, 200 µL of FBS, and 100 µL of DMSO. The samples were stored in a deep freezer at -80°C, and on the following day, transferred to a cryofreezer containing liquid nitrogen.

An alternative cryopreservation method is the controlled-rate freezer (CRF) technique, which uses specialized equipment to gradually control the cooling rate of samples. This method is standardized and widely used to preserve the viability of cells, tissues, or embryos during freezing. CRF operates by reducing the temperature in a controlled and documented manner using computer programming to ensure gradual cooling. It prevents the formation of large ice crystals that can damage cells and allows for higher post-thaw survival rates compared to rapid freezing methods. In the CRF method, the temperature typically decreases at a rate of 1°C per minute, so reaching -80°C requires only about 2 hours, after which the sample can be directly transferred to a cryofreezer. This method is highly suitable and commonly applied in biomedical fields, in vitro fertilization (IVF), and stem cell storage.[15] Identification and Characterization of Thyroid Carcinoma-Derived Cell

Thyroid carcinoma is a malignant neoplasm originating in the thyroid gland, located in the anterior region of the neck.[16] Histopathologically, thyroid cancer is classified into four main subtypes. The most common is papillary thyroid carcinoma (PTC), accounting for approximately 75-85% of all cases. PTC typically presents in women and is characterized by slow progression. Microscopically, it demonstrates papillary structures alongside enlarged nuclei, intranuclear cytoplasmic inclusions, and psammoma bodies. The second most common subtype is follicular thyroid carcinoma (FTC), comprising around 15% of cases. FTC generally affects older individuals than PTC and displays a follicular growth pattern with microfollicular or trabecular architecture. Diagnosis of FTC relies on identifying capsular and/or vascular invasion. Notably, it lacks the hallmark nuclear features of PTC.

Medullary thyroid carcinoma (MTC) arises from parafollicular or C cells responsible for calcitonin production and represents approximately 5% of thyroid cancers. Histologically, MTC consists of polygonal to spindle-shaped cells, typically arranged in solid, trabecular, or insular formations, with the presence of amyloid stroma. The least common but most aggressive subtype is anaplastic thyroid carcinoma (ATC). ATC is characterized by pleomorphic giant and spindle cells, high mitotic rates, undifferentiated growth, and extensive necrosis. Due to its rapid local invasion and metastatic potential, ATC is associated with a poor prognosis.[16,17]

In this study, histopathological analysis confirmed the presence of multifocal papillary thyroid carcinoma, including oncocytic, classic, follicular, and tall cell variants. Molecular characterization of the carcinoma was performed using PCR electrophoresis targeting the BRAF and RAS gene families. These genetic markers play an important diagnostic and prognostic role in thyroid cancer. Detection of driver mutations guides personalized therapeutic approaches, including the use of BRAF inhibitors (e.g., vemurafenib, dabrafenib) or radioactive iodine (RAI) therapy.[18]

The BRAF (B-Rapidly Accelerated Fibrosarcoma) gene encodes a serine/threonine protein kinase involved in the MAPK/ERK signaling cascade, which controls cell growth, proliferation, and differentiation. Activating mutations in BRAF, particularly the BRAF V600E mutation—caused by a substitution of valine with glutamic acid at codon 600—result in constitutive activation of the signaling pathway, promoting tumorigenesis. Found in approximately 40-80% of PTC cases, the BRAF V600E mutation is associated with aggressive tumor behavior, increased risk of metastasis, reduced sensitivity to RAI therapy, and potential resistance to BRAF-targeted treatments.[18] In the current study,

no BRAF V600E mutation was detected, suggesting a more favorable biological behavior and treatment response.

The RAS gene family—comprising HRAS, KRAS, and NRAS—encodes small GTPases involved in key cell signaling pathways, particularly RAS-RAF-MEK-ERK and PI3K-AKT, which regulate proliferation, survival, differentiation, and apoptosis. RAS proteins function as molecular switches, transitioning between an active GTP-bound state and an inactive GDP-bound state. Mutations in RAS genes lead to constitutive activation of downstream signaling, promoting unregulated cell proliferation, angiogenesis, apoptosis resistance, and metastatic spread. In thyroid cancer, specifically FTC and ATC, mutations in NRAS and KRAS are most frequently observed.[19,20]

Detection of RAS mutations can be achieved through PCR-electrophoresis, real-time PCR (qPCR), Sanger sequencing, next-generation sequencing (NGS), or immunohistochemistry (IHC) targeting mutant RAS protein expression. In this study, PCR-electrophoresis was employed for RAS mutation screening in primary thyroid carcinoma cells. The presence of RAS mutations often correlates with more aggressive clinical features and resistance to standard therapies, including BRAF inhibitors and radioactive iodine.

To further define the molecular profile, sequencing analysis was conducted to evaluate one BRAF gene region and five RAS gene exons: HRAS exon 2, NRAS exons 2 and 3, and KRAS exons 2 and 3. No pathogenic mutations were identified in either the BRAF or RAS loci analyzed, indicating a mutation-negative molecular profile. This finding supports a favorable clinical prognosis. In such cases, conventional treatments—including thyroidectomy, radioactive iodine therapy, and levothyroxine suppression—remain standard and effective.

Although targeted therapies specifically for RAS mutations are currently under clinical investigation, MEK inhibitors, such as trametinib and selumetinib, have shown promise in the treatment of RAS-mutant cancers and may offer benefit in select cases of RAS-positive thyroid carcinoma in the future.

CONCLUSION

Primary isolation of thyroid carcinoma-derived cells in this study was achieved through enzymatic digestion using collagenase, effectively separating malignant cells from adjacent normal thyroid tissue. Subsequent cell proliferation was supported using Dulbecco's Modified Eagle Medium (DMEM) supplemented with 20% fetal bovine serum (FBS), chosen for its elevated growth factor content, which facilitated enhanced cellular expansion. For long-term storage, cryopreservation was performed employing the standard slow-freezing protocol. Molecular characterization of the isolated cells was conducted via PCR-based electrophoresis followed by Sanger sequencing of key oncogenic targets: the BRAF gene and five RAS gene exons, including HRAS exon 2, NRAS exons 2 and 3, and KRAS exons 2 and 3. No pathogenic mutations were detected in the BRAF or RAS gene families (HRAS, NRAS, KRAS) in the samples analyzed.

REFERENCES

1. Kitahara, C.M., Schneider, A.B. 2022. Epidemiology of Thyroid Cancer. Cancer Epidemiology, Biomarkers & Prevention. 2022, 31: 1284-97.
2. Puxeddu, E., Tallini, G., Vanni, R. What is new in thyroid cancer: The special issue of the journal cancers. Cancers (Basel). 2020.
3. Poppe, K., Bisschop, P., Fugazzola, L., Minziori, G., Unuane, D., Weghofer, A. 2021 European Thyroid Association Guideline on Thyroid Disorders prior to and during Assisted Reproduction. Eur Thyroid J.

2021,9: 281-95.
4. Fagin, J.A., Wells, S.A. Biologic and Clinical Perspectives on Thyroid Cancer. New England Journal of Medicine. 2016, 375:1054-67.
5. Ozisik, H., Ozdil, B., Sipahi, M., Erdogan, M., Cetinkalp, S., Ozgen, G., Saygili, F., Oktay, G., Aktug, H. Stemness potency and structural characteristics of thyroid cancer cell lines. Pathol Res Pract. 2023,241:154262.
6. Fallahi, P., Ferrari, S.M., Elia, G., Ragusa, F., Patrizio, A., Paparo, S.R., Marone, G., Galdiero, M.R., Guglielmi, G., Foddis, R., Cristaudo, A., Antonelli, A. Primary cell cultures for the personalized therapy in aggressive thyroid cancer of follicular origin. Semin Cancer Biol. 2022,79:203-16.
7. Wang, Y., Li, W., Phay, J.E., Shen, R., Pellegata, N.S., Saji, M., Ringel, M.D., de la Chapelle, A., He, H. Primary Cell Culture Systems for Human Thyroid Studies. Thyroid. 2016, 26:1131-40.
8. Chew, D., Green, V., Riley, A., England, R.J., Greenman, J. The Changing Face of in vitro Culture Models for Thyroid Cancer Research: A Systematic Literature Review. Front Surg. 2020, 7. Weiskirchen, S., Schröder, S.K., Buhl, E.M., Weiskirchen, R. A Beginner's Guide to Cell Culture: Practical Advice for Preventing Needless Problems. Cells. 2023.
9. Whaley, D., Damyar, K., Witek, R.P., Mendoza, A., Alexander, M., Lakey, J.R. Cryopreservation: An Overview of Principles and Cell-Specific Considerations. Cell Transplant. 2021, 30, 096368972199961.
10. Xu, X., Quiros, R.M., Gattuso, P., Ain, K.B., Prinz, R.A. High prevalence of BRAF gene mutation in papillary thyroid carcinomas and thyroid tumor cell lines. Cancer Res. 2003, 63: 4561-7
11. Harahap, A.S., Subekti, I., Panigoro, S.S., Asmarinah, Lisnawati, Werdhani, R.A., *et al.* Profile of BRAFV600E, BRAFK601E, NRAS, HRAS, and KRAS Mutational Status, and Clinicopathological Characteristics of Papillary Thyroid Carcinoma in Indonesian National Referral Hospital, The Application of Clinical Genetics. 2023, 16: 99-110
12. Ferrari, S.M., Fallahi, P., Ruffilli, I., Elia, G., Ragusa, F., Paparo, S.R., Ulisse, S., Baldini, E., Giannini, R., Miccoli, P., Antonelli, A., Basolo, F. Molecular testing in the diagnosis of differentiated thyroid carcinomas.Gland Surg. 2018.
13. Fox, J.M., Wiggins, R.C., Moore, J.W.J., Brewer, C., Andrew, A.C., Martin, F. Methodology for reliable and reproducible cryopreservation of human cervical tissue. Cryobiology. 2017, 77, 14-8.
14. Yang, J., Gao, L., Liu, M., Sui, X., Zhu, Y., Wen, C., Zhang, L. Advanced Biotechnology for Cell Cryopreservation. Transactions of Tianjin University. 2020.
15. Crnčić, T.B., Tomaš, M.I., Girotto, N., Ivanković, S.G. Risk factors for thyroid cancer: What do we know so far? Acta Clin Croat. 2020, 59:66-72.
16. Gavryutina, I., Fordjour, L., Chin, V.L. Genetics of Thyroid Disorders. Endocrines. 2022, 3:198-213.
17. Puxeddu, E., Tallini, G., Vanni, R. What is new in thyroid cancer: The special issue of the journal cancers. Cancers (Basel). 2020.
18. Fagin, J.A., Wells, S.A. Biologic and Clinical Perspectives on Thyroid Cancer. New England Journal of Medicine. 2016, 375, 1054-67.
19. Motoi N, Sakamoto A, Yamochi T, Horiuchi H, Motoi T, Machinami R. Role of ras mutation in the progression of thyroid carcinoma of follicular epithelial origin. Pathol Res Pract. 2000;196 (1):1-7.doi: 10.1016/S0344-0338(00)80015-1.