Oral Presentation VIII

Serum Levels of Activin A and Expression of its Receptors in Thyroid Tissue in Female Patients with Thyroid Papillary Carcinoma

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BACKGROUND AND AIMS

Activin, a member of the transforming growth factor beta protein family, was originally isolated from gonadal fluids and stimulates the release of pituitary follicle-stimulating hormone (FSH). Activin has numerous functions in both normal and neoplastic cells. Although it was suggested that gonadal tissue is the primary site of activin production, several extragonadal sources have subsequently been identified, including human thyrocytes.

METHODS

In our study, serum levels of activin A were measured by chemiluminescence, and the expression of receptors of activin A in thyroid tissue was detected by immunohistochemistry in female patients with thyroid papillary cancer [stage I (n = 60), stage II (n = 60), stage III (n = 60), stage IV (n = 60)] and in normal controls (n = 60).

RESULTS

The serum levels of activin A were no significantly different between patients with thyroid papillary cancer and normal controls as well as different stages. But the expressions of receptors of activin A were greater in patients with thyroid papillary cancer than in normal controls. And the positive rates of receptor expression were significantly more in stage III and IV than in stage I and II.

CONCLUSION

In conclusion, this study demonstrates that serum levels of activin A undergo no significant changes when thyroid papillary cancer occurs. The thyroid gland is not the predominant source of activin A. The expressions of receptors of activin A were significantly greater in patients with thyroid papillary cancer than in normal controls. And the positive rates of receptor expressions were associated with the severity of cancer. Because activin A may exert negative action on thyrocyte proliferation, it is conceivable that the increase in the receptor of activin A in thyroid papillary cancer might represent a counteracting mechanism.

Genomic Evidence of Reactive Oxygen Species Elevation in Papillary Thyroid Carcinoma with Hashimoto Thyroiditis

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BACKGROUND AND AIMS

Elevated levels of reactive oxygen species (ROS) have been proposed as a risk factor for the development of papillary thyroid carcinoma (PTC) in patients with Hashimoto thyroiditis (HT). However, it has yet to be proven that the total levels of ROS are sufficiently increased to contribute to carcinogenesis. We hypothesized that if the ROS levels were increased in HT, ROS-related genes would also be differently expressed in PTC with HT.

METHODS

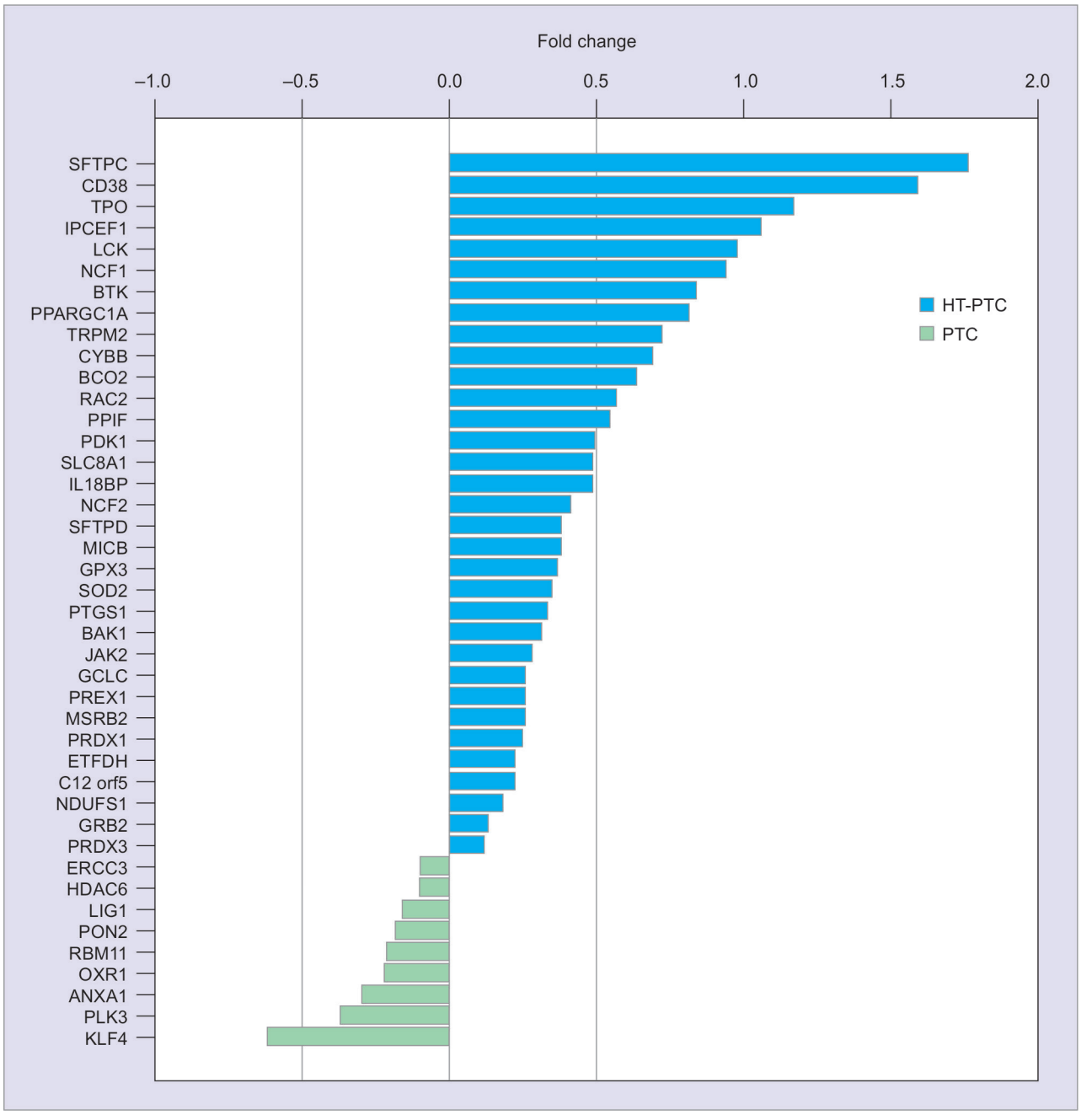
To find differentially expressed genes (DEGs) we analyzed data from the Cancer Genomic Atlas, gene expression data from RNA sequencing: 33 from normal thyroid tissue, 232 from PTC without HT, and 60 from PTC with HT. We prepared 402 ROS-related genes from three gene sets by genomic database searching. We also analyzed a public microarray data to validate our results.

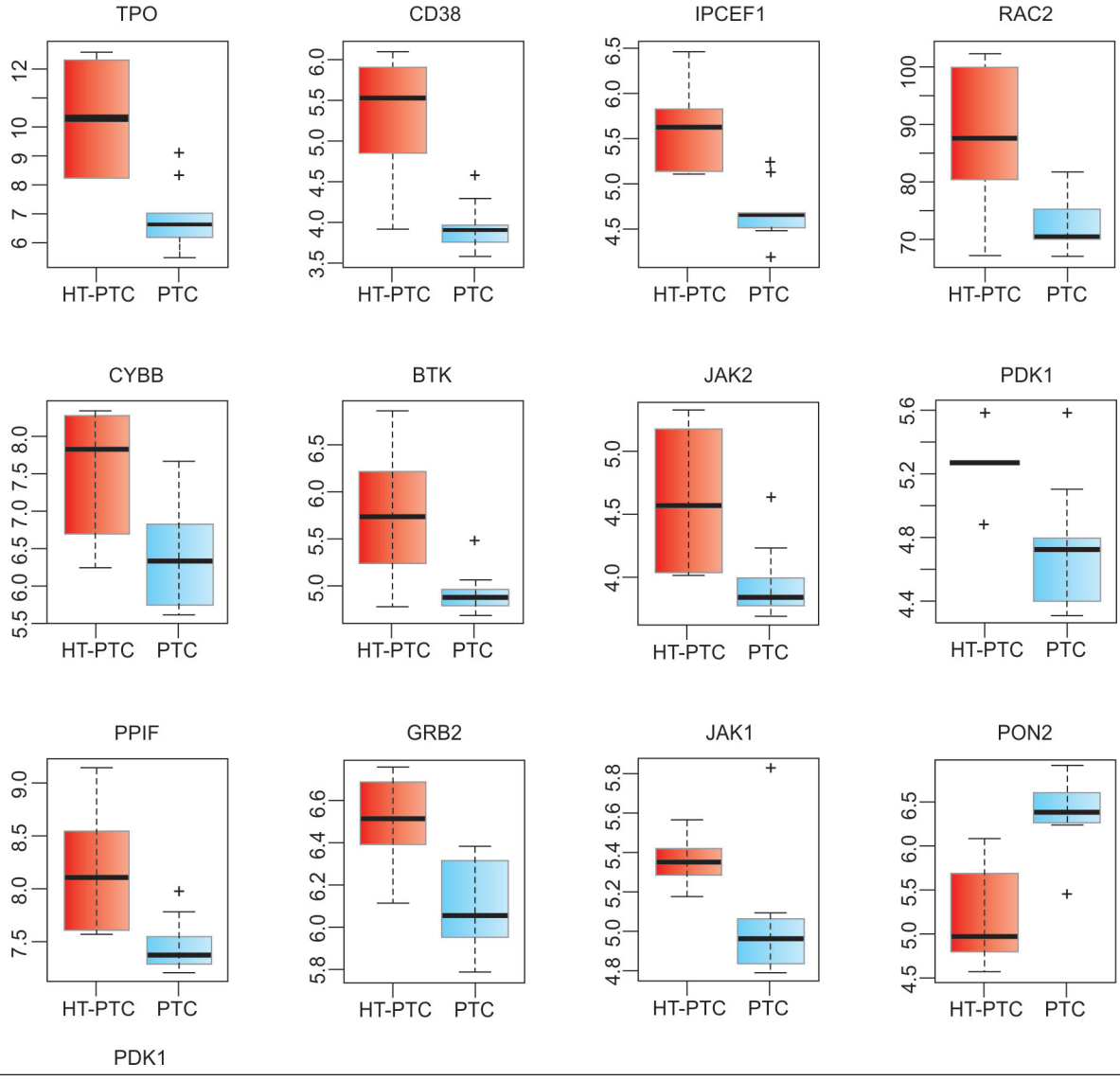
RESULTS

Thirty-three ROS-related genes were upregulated in PTC with HT, whereas there were only nine genes in PTC without HT (chi-square p value < 0.001). The mean log 2 fold changes of upregulated genes was 0.562 in the HT group and 0.252 in the PTC without HT group (t-test p value = 0.001). In microarray data analysis, 12 of the 32 ROS-related genes showed the same differential expression pattern with statistical significance. In gene ontology analysis, upregulated ROS-related genes were related with ROS metabolism and apoptosis. Immune function-related and carcinogenesis-related gene sets were enriched only in the HT group in the Gene Set Enrichment Analysis.

CONCLUSION

Our results suggested that ROS levels may be increased in PTC with HT. Increased levels of ROS may contribute to PTC development in patients with HT.





Celecoxib Potentiates the Effect of Chemotherapeutic Drug Doxorubicin on Medullary Thyroid Carcinoma *in vivo*

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BACKGROUND AND AIMS

Celecoxib enhances the effect of doxorubicin on tumor therapy *in vivo*, but the effect on human medullary thyroid carcinoma (MTC) therapy was not evaluated.

METHODS

Herein, we found that doxorubicin alone decreased and combined with celecoxib further decreased MTC weight in the xenografted nude mice. Celecoxib combined with doxorubicin also enhanced tumor weight-based inhibitory ratio.

RESULTS

Analyzing the body weight of nude mice after drug treatment, there was no obvious difference between doxorubicin, celecoxib, and celecoxib combined with doxorubicin groups, indicating that celecoxib combined with doxorubicin on MTC treatment does not increase the side effect. The MTC metastasis to the liver of nude mice can be inhibited with doxorubicin. However, celecoxib combined with doxorubicin can further inhibit the metastasis. Mechanistic analysis shows that metastasis marker MMP9 and proliferation marker Ki-67 were decreased, while tumor inhibitor P53 was increased after treatment with doxorubicin alone,

particularly in celecoxib combined with doxorubicin. MMP9 and Ki-67 were decreased, but P53 was not increased at mRNA level with treatment of doxorubicin alone or combined with celecoxib, indicating MMP9 and Ki-67 are regulated at transcriptional levels, but P53 is not.

CONCLUSION

Used together, celecoxib enhances tumor therapy of doxorubicin on human MTC by decreasing MMP9 and Ki-67 expression and increasing P53 expression.

Synergistic Anti-cancer Activity of the HDAC Inhibitor, N-hydroxy-7-(2-naphthylthio) Hepatonomide (HNHA) and Sorafenib on Anaplastic Thyroid Cancer *in vivo* and *in vivo*

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BACKGROUND AND AIMS

Anaplastic thyroid carcinoma (ATC) although rare is the most deadly form of thyroid cancer. The fatality rate for ATC is high pitched, and the survival rate at 1 year after diagnosis is <20%. Control of ATC is severely hard and widespread with unpredictability. We previously proved that histone gene reviser and epigenetic changes play significant parts in papillary and anaplastic thyroid cancer tumorigenesis. The goal of this study was to investigate the antitumor activities of a histone deacetylase (HDAC) inhibitor, N-hydroxy-7-(2-naphthylthio) hepatonomide (HNHA) alone and in combination with sorafenib in anaplastic thyroid cancer cells *in vitro* and *in vivo* and to explore its effects on apoptotic cell death pathways.

METHODS

Two ATC cell lines were exposed to sorafenib in the presence or absence of HNHA, and cell viability was determined by MTT assay. Effects of combined treatment on cell cycle and intracellular signaling pathways were assessed by flow cytometry and western blot analysis. The ATC cell lines xenograft model was used to examine the antitumor activity *in vivo*.

RESULTS

Our data showed that HNHA and sorafenib synergistically decreased cell viability in ATC cells, and also significantly increased apoptotic cell death in these cells, as proved by the cleavage of caspase-3 and DNA fragmentation. MPT0E028 altered the global modifications of histone and nonhistone proteins regardless of the presence of sorafenib. HNHA induced histone H3 acetylation and reduced anti-apoptotic factor in ATC. Thus, sorafenib is well known as a multikinase inhibitor that targeted the vascular endothelial growth factor receptor family (VEGFR-2 and -3) and platelet-derived growth factor receptor family (PDGFR-beta and Kit), which play key roles in tumor progression and angiogenesis. Combination therapy with HNHA and sorafenib significantly decreased vessel density and most significantly reduced tumor volume and increased survival in ATC xenografts.

CONCLUSION

These results propose that HNHA in combination with sorafenib has significant anti-cancer activity in preclinical models, potentially suggesting a new clinical approach for patients of advanced thyroid cancer.

Improving the Management of Post-thyroidectomy Bleeding in Hospital Interns

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BACKGROUND AND AIMS

Post-thyroidectomy bleeding (PTB) is an uncommon, but life-threatening complication. We assessed interns' baseline knowledge of identifying and managing PTB and examined the efficacy of a brief educational program in improving their knowledge.

METHODS

During one of our routine educational sessions for junior medical staff, interns were recruited for this protective study. They completed a de-identified pretest of eight questions regarding the clinical features and management of PTB. A 30-minute interactive

multimedia presentation including photos and a video on PTB was given. Three months later, the interns repeated the initial test. The pre and posttests data were matched, and the results analyzed using McNemar's test.

RESULTS

There were 31 participants. Pretest findings demonstrated that interns had a good baseline understanding of priority, diagnosis, and action for PTB. Following the presentation, posttest data showed improvement in almost all questions. Significant improvement was shown in the diagnosis, action, and location of the thyroid tray, which were the most clinically relevant components. Interns scored poorly in the question regarding causes of stridor both in the pre and posttest, which may have been due to poor understanding of the question.

Questions	*Pretest (%)*	*Posttest (%)*	*p exact (2-sided)*
How common	10 (32%)	10 (32%)	>0.050
Priority	21 (68%)	24 (77%)	0.25
Symptoms & Signs	14 (45%)	19 (61%)	0.063
Diagnosis	17 (55%)	25 (81%)	0.008
Action	20 (65%)	28 (90%)	0.008
Stridor	7 (22%)	6 (19%)	>0.050
Thyroid tray	10 (32%)	21 (68%)	0.001
Layers	17 (55%)	22 (71%)	0.063

CONCLUSION

We have demonstrated that providing a brief, interactive lecture on an uncommon but critical surgical complication of PTB improves sustained knowledge.

Single *vs* Dual Modalities in Parathyroid Localization: Impact on Surgical Approach

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BACKGROUND AND AIMS

Preoperative imaging in patients with primary hyperparathyroidism is used primarily to facilitate targeted parathyroidectomy. Ultrasound has been the mainstay method in preoperative localization. Recently since 2014 Tc-99m sestamibi with SPECT/CT (MIBI) has been readily available and dual methods have become our practice. The aim of this study is to analyze the significance of MIBI in preoperative localizations.

METHODS

A total of 355 patients with biochemical evidence of primary hyperparathyroidism underwent surgery at Hospital Putrajaya from 2002 to 2015. The first 292 patients (Group A) had only a single method of preoperative localization using surgeon-performed ultrasound (s-US). Subsequent 63 patients (Group B) had dual methods using s-US and MIBI. In group A, unilateral exploration was performed in positive ultrasound finding and terminated if the diseased gland was found. A negative intraoperative finding was converted to bilateral neck exploration (BNE). In group B, focus approach was performed if both modalities were concordant. Cure of patients who underwent the focus approach was considered successful.

RESULTS

In group A, 245 patients with positive ultrasound finding were planned for unilateral exploration; however, 60 patients were converted to BNE intraoperatively. A total of 175 (59.9%) patients were successfully explored and cured. Eighteen patients with positive ultrasound finding did not cure and failure rate was 6.5%. The overall cure rate was 91.8%. In group B, concordant ultrasound and MIBI findings were found in 34 cases (54%). Thirty patients underwent focus approach and were cured. Only one patient in group B did not cure. Successful focus approach rate in group B was 47.6% with failure rate of 1.6%. The overall cure rate in group B was 98.4%.

CONCLUSION

The combination of s-US and MIBI has lower eligible patients for focus approach, but the surgery is more successful compared to bilateral neck exploration. Therefore, focus approach is only recommended in patients with concordant s-US and MIBI findings.

Potential Relationship between Hashimoto's Thyroiditis and BRAFV600E Mutation Status in Papillary Thyroid Carcinoma Patients

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BACKGROUND AND AIMS

Concomitant papillary thyroid carcinoma (PTC) and Hashimoto's thyroiditis (HT) are frequent occurrences. Whether these two conditions are linked and whether PTC with concurrent HT has distinct clinicopathological characteristics are still debated issues. Lymphocytic infiltration is abundant in HT and might be relevant in the pathogenesis and progression of PTC. BRAFV600E mutation is associated with a more advanced PTC at diagnosis; however, its role in the clinicopathological characteristics of PTC with concurrent HT is unknown. The purpose of this study was to evaluate the potential relationship between Hashimoto's thyroiditis and BRAFV600E mutation status in patients with PTC.

METHODS

A total of 201 patients who underwent surgery for PTC between January 2013 and June 2013 were enrolled in this study. BRAFV600E mutation analysis was performed using polymerase chain reaction (PCR)–based amplification of DNA extracted from paraffin-embedded tumor specimens.

RESULTS

BRAFV600E mutation and HT were detected in 139 (69.1%) and 69 patients (34.3%) respectively. BRAFV600E mutation was not correlated with HT ($p = 0.749$). Lymph node metastasis was more frequent in BRAFV600E mutation patients (OR = 2.04, $p = 0.038$). However, age, tumor size, extrathyroidal extension, and multifocality were not significantly associated with BRAFV600E.

CONCLUSION

The results of our study suggest that BRAFV600E mutation was associated with aggressive PTC. However, there was no clinicopathological association between BRAFV600E mutation and HT.

Differential Expression of Type IV Collagen Alpha 3, 4, 5 (IV) Chains in Non-medullary Thyroid Carcinoma

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BACKGROUND AND AIMS

Thyroid cancer is the most common endocrine malignancy. With the exception of medullary carcinoma, the majority of thyroid cancers are derived from thyroid follicular epithelial cells. Type IV collagen, the major component of the basement membrane (BM), is typically fully or partially absent near epithelial elements of most invasive carcinomas. The classic network consists of alpha 1, 1, 2 (IV) chains and is expressed in all BMs. Recently, a novel tissue-specific network of type IV collagen consisting of alpha 3, 4, 5 (IV) chains or alpha 5, 5, 6 (IV) chains was characterized by molecular cloning. We reported that the expression of alpha 5, 5, 6 (IV) chain form of this second network is associated with the invasive potential of breast cancer.

METHODS

To evaluate the distribution of the second network of type IV collagen in non-medullary thyroid carcinoma, we performed immunohistochemistry with alpha (IV) chain-specific antibodies to examine the cellular regulation of these alpha (IV) chains in 69 well-differentiated thyroid carcinomas (WDTC) (58 papillary, 11 follicular), and eight poorly differentiated and undifferentiated thyroid carcinomas (PUDTC) (three poorly differentiated, five undifferentiated).

RESULTS

In normal thyroid tissues near cancers, BMs of the thyroid gland were composed of linear alpha 1, 1, 2 (IV) chains and discontinuous alpha 3, 4, 5 (IV) chains. Similar immunostaining profiles were observed in 61 cases (88%) of WDTC. In seven cases (88%) of PUDTC, although alpha 1, 1, 2 (IV) chains were present continuously throughout the cancer nests, the assembly of alpha 3, 4, 5 (IV) chains was completely inhibited. Staining for alpha 6 (IV) chain was negative in all cases.

CONCLUSION

In addition to the classic network, the thyroid gland contains a second network consisting of alpha 3, 4, 5 (IV) chains rather than alpha 5, 5, 6 (IV) chains. Expression of the second network may be associated with the differential potential of non-medullary thyroid carcinoma.

Diffuse Sclerosing Variant of Papillary Thyroid Carcinoma: Major Genetic Alterations and Prognostic Implication

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BACKGROUND AND AIMS

Diffuse sclerosing variant of papillary thyroid carcinoma (DSV-PTC) is an uncommon variant of PTC. However, their prognostic significance remains controversial. The aim of this study was to investigate the major genetic alterations of DSV-PTC and their prognostic implication.

METHODS

We retrieved 37 patients with DSV-PTC who underwent thyroid surgery and had formalin-fixed paraffin-embedded samples available. We tested for the panel of genetic alterations including BRAFV600E, NRAS codon 61, HRAS codons 12/13/61, and KRAS codons 12/13 point mutations and RET/PTC1, RET/PTC3, and PAX8/PPARγ rearrangements using reverse transcription real-time PCR. All samples that tested positive for genetic alterations were confirmed by Sanger sequencing. The associations between the identified genetic alterations and clinicopathologic characteristics were evaluated.

RESULTS

The associations between the identified genetic alterations and clinicopathologic characteristics were evaluated. Among 37 cases of DSV-PTC, 17 were positive for RET/PTC1 (46%), 6 for RET/PTC3 (16%), and 9 for BRAFV600E (24%). All mutations/rearrangements were mutually exclusive. The remaining five cases had none of the above genetic alterations. RET/PTC3 rearranged DSV-PTC was associated with advanced stage, including T4 and distant metastasis ($p < 0.05$). Patients with PET/PTC3 showed a higher frequency of persistent disease ($p < 0.01$). In contrast, DSV-PTC with RET/PTC1 was associated with a higher prevalence of disease remission ($p < 0.05$) and coexistent Hashimoto's thyroiditis ($p < 0.01$).

CONCLUSION

Taken together, RET/PTC rearrangement was the major genetic alteration, and subtyping of RET/PTC rearrangement may be of prognostic importance in patients with DSV-PTC.

The Establishment of Multicellular Tumor Spheroids of Thyroid and the Expression of E-Cadherin, YAP1, and STAT3 in Thyroid Spheroids

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BACKGROUND AND AIMS

The study of cells in a 3D context can provide insights not observed in traditional 2D monolayers. To successfully investigate the pathobiology of human cancer, it is necessary to maintain or recreate in culture the typical 3D architecture of the tissue.

METHODS

The human papillary thyroid carcinoma cell line SNU790 was provided by Korean cell line bank. Cells were cultured in the "spheroid medium" in 60 mm polystyrene petri culture dishes [BD Falcon, Becton Dickinson (BD), Franklin Lakes, NJ, USA]. This "spheroid medium" consisted of a 1:1 mixture of Dulbecco's Modified Eagle's Medium (DMEM) (high glucose content; Gibco) and F12 nutrient [1:1 (v/v), Sigma Chemical C], including glucose (25 mM), sodium bicarbonate (3 mM), L-glutamine (2 mM), penicillin 50 U/l, and streptomycin (50 µg/ml). Blots were developed with Immun-Star WesternC chemiluminescence kit (BIO-RAD) and visualized by using ChemiDoc MP Imaging System (BIO-RAD).

RESULTS

The number of spheroids was about 4.0 × 103/60 mm dish. We could observe spheroids at the 5th and 10th day and identify increase in the size and cell number over time. Shows the expression of E-cadherin, YAP1, STAT3, and beta-actin across SNU790 cell line and SNU790 spheroid cell line. Blots revealed that two cell lines expressed E-cadherin, YAP1, STAT3, and beta-actin, but

SNU790 spheroid expressed less E-cadherin and STAT3 than SNU790 cell line. It was suggested that SNU790 spheroid has more characteristics of cancer stem cell than SNU790 original cell line.

CONCLUSION

This was the preliminary study for thyroid organoid and its response to several anticancer drugs. We succeeded in thyroid spheroid culture from original thyroid cancer cell line. It was suggested that thyroid spheroids had more cancer stem cell characteristics than original cell line because they were cultured as spheroid and expressed less E-cadherin and STAT3 than the original cell line.