様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９　（共通）

科学研究費助成事業　　研究成果報告書

令和　４ 年　 ６ 月 ２７ 日現在

機関番号：１７３０１
研究種目：若手研究
研究期間：2019～2021
課題番号：１９Ｋ１６５８７

研究課題名（和文）分子異常をエビデンスとした高リスク乳頭がんの形態学的形質分析

研究課題名（英文）Analysis for histological phenotype of high-risk papillary thyroid carcinoma based on molecular abnormalities

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交付決定額（研究期間全体）：（直接経費）　　3,200,000円

研究成果の概要（和文）：甲状腺乳頭癌は予後良好な疾患であるが、一部の症例に再発、転移をきたすため、その症例の拾い上げが重要である。Ki-67発現やTERT promoter変異が予後を規定する有力な因子として報告された。本研究は342例の切除乳頭癌を対象に、これらの分子マーカーと臨床病理学的因子、特に形態学的形質との関係を明らかにした。結果として BRAF変異とTERT promoter変異との重変異の頻度はKi-67標識率の高値、高細胞成分、desmoplasiaを背景とするhobnail成分、好酸性細胞成分を伴う乳頭癌で有意に高率であることが判明した。これらは組織学的予後不良因子であることを示唆している。

研究成果の学術的意義や社会的意義
甲状腺癌の90%を占める乳頭癌の大部分は予後良好であり、甲状腺癌の場合、早期発見と早期治療は予後の改善に寄与しないことが明らかになっている。甲状腺癌の過剰診断や過剰治療（切除や放射性ヨード内照射療法）を抑制し、最適化するためには予後不良指標の同定が必要である。現在、Ki-67標識率やBRAF変異およびTERT promoter変異が予後を規定する有力な因子として報告されているが、一般的な病理検査室で遺伝子変異解析を日常的に行うことは困難である。本研究成果はこれらの分子異常をエヒデンスに、乳頭癌の組織像を特徴付け、日常の甲状腺がん病理診断学に汎用できる高悪性度の組織像を明らかにした。

研究成果の概要（英文）：The 342 surgically-resected, formalin-fixed, paraffin-embedded (FFPE) thyroid PTCs of Japanese cases were collected. All cases were evaluated for BRAF, TERT-promoter and Ki-67. Mutations in BRAF and the TERT-promoter (C228T and C250T) were analyzed with digital PCR and Ki-67 labeling index by immunohistochemistry.The clinicopathological features, including TNM stage assessed, size of tumor, and detailed histopathology features of cases were analysed.Next step is statistical analysis of prevalence and association of molecular analysis with clinicopathologic statuses.

研究分野：人体病理学

キーワード：Papillary thyroid cancer　Ki-67 expression　TERT-promoter mutations　BRAF mutation

１．研究開始当初の背景

The prevalence of thyroid cancer has increased rapidly worldwide due to improvements in ultrasonic diagnostic imaging techniques, and the increase in prevalence of papillary cancer has become a problem. From the viewpoint of histopathological diagnosis tumors include "Indolent or regressive cancer" and "Progressive cancer" that are extremely important in pathological diagnosis for avoiding overdiagnosis and overtreatment. It is well known that the prognosis of well-differentiated cancers such as papillary cancer and follicular cancer is generally good, but about 10% of cases relapse, and among them, they become resistant to radioiodine therapy and molecular targeted therapy. There are cases of death from the primary disease. At the same time, over 90% of well-differentiated cancers are papillary carcinomas that includes diffuse Sclerosing type, Tall cell type, Columnar cell type, Oncocytic type and Solid type were known as highly malignant tissue types, and recently Hobnail type was added in the 2017 WHO classification. The Hobnail type was reported to have frequent recurrence and a high distant metastasis (Asioli S, et al, Am J Surg Pathol 2010; 34: 44-52). These morphological features reflect the loss of polarity / loss of cell cohesiveness (LOP/LCC) of cancer cells and lead to the acquisition of metastatic and infiltrative ability. Moreover, epithelial-mesenchymal transition (EMT) is a trait that is often observed in advanced infiltration of papillary carcinoma (Tang W, et al, Pathol Int 2003; 53: 204-13, Kakudo K, et al. , J Clin Pathol 2004; 57: 1041-6, Bai, et al, Cancer Sci 2008; 99: 1908-15), and in some reports, the proportion of Hobnail pattern was 20% or more, which was recognized as a poor prognosis factor.

In recent years, it has become clear that Ki-67 expression and TERT promoter mutations are the leading molecular pathological factors that determine the prognosis. According to the 2017 WHO classification, Ki-67 labeling rate recognized <5% is low-risk, 5-10% is moderate-risk, 10-30% is high-risk,> 30% for invasive cancers larger than 1 cm in size. More recently, double mutants with BRAF and TERT promoter mutations have been reported to be highly malignant and have a poor prognosis, and revealing that the combination of the TERT promoter mutation and Ki-67 labeling rate could be an accurate marker (Matsuse M, et al, Sci Rep 2017; 7: 41752).

Based on the above background, the academic "question" that forms the core of the research subject is to elucidate the pathologically poor prognosis traits of papillary thyroid cancer based on molecular-histological evidence, and application to "daily thyroid cancer pathological diagnosis".

２．研究の目的

By conducting histological analysis and molecular analysis in papillary cancer, and

clarifying the relationship with clinical pathological factors, we hope to define the morphological features of high-risk papillary thyroid cancer cases that harbor molecular abnormalities.

３．研究の方法

(1) Histological analysis: evaluated histological features with a microscope.

(2) Molecular analysis: BRAF mutation and TERT promoter mutation, Ki-67 labeling rate which are prognostic risk factors were analyzed. BRAF and TERT promoter mutations (C228T and C250T) detected using droplet digital PCR. The Ki-67 labeling rate is calculated by immunostaining with a 400-fold field of view and hot spots.

(3) Clinicopathological factors: obtained information from database such as age, sex, race, family history, medical history, background thyroid disease (Basedow disease, chronic thyroiditis, thyroid hormone function, etc.), treatment, TNM factor, stage, recurrence, etc.

(4) Currently analyze of statistical significance test together with the above results are collecting.

４．研究成果

Current statistical evaluation showed:

(1) The BRAFV600E was detected in 273 (80%), TERT-promoter C228T in 49 (15.1%) and C250T in 7 (2.2%), and cases with double mutation (BRAFV600E and TERT promoter C228T+C250T mutations) in 52 (46%) patients. Among 273 patients with the BRAFV600E, 78% and 82% were <55 and ≥55 of age (p=0.42), while TERT promoter mutations, were 2.4% <55 vs 28% ≥55 of age at their first surgery for primary PTC (p<0.000) (Table). No significant differences in gender were found in patients with the BRAFV600E or TERT promoter mutations. More patients with larger tumors (>2.6 cm; p<0.000) at their first PTC surgery had TERT promoter mutation than patients with non-mutated TERT promoter and BRAF. Although double mutated shows significant association with ≥55 of age and larger size of tumor (65.3%, p<0.000; >2.8, p<0.000) (Table).

(2) Ki67 expression was distributed to 3 groups, ≤5%, 5-10% and >10%. A comparison of the Ki67 expression intensity between the wild and mutant cases of PTC showed that the mutant cases were significantly higher than that of the wild. In cases with BRAFV600E and double mutated Ki67 expression intensity in mutant groups were higher than in wild (75.7%, 88.7%, 87.5%, p<0.03 and 20.3%, 78.6%, 80%, p<0.000, correspondingly) (Table). The overall Ki67 was lower in patients with TERT promoter mutations than in those who had non-mutated TERT promoter, however, increased in proportion to increase the rate of marker in mutant groups (6.6%, 34.8%, 43.6%; p <0.0000) (Table).

(3) For histopathologic characteristics we included giant/spindle cells component, tall cell component, solid type, hobnail (LOP/LCC with desmoplastic background) and oxyphilic component. The results overall show giant/spindle cells component in 5 (1.5%), tall cell

component in 22 (6.5%), solid type in 16 (4.7%), hobnail (LOP/LCC with desmoplastic background) in 113 (33.1%) and oxyphilic component 5 (1.5%). Cases with tall cell component and hobnail (LOP/LCC with desmoplastic background) additionally were divided to 3 groups according to percentage of occupied area in the tumor (<5%, 5-10%, 30+% and <10%, 11-20%, 21+%, correspondingly) (Table).

A comparison of the histology components showed that the BRAFV600E cases were significantly higher in harboring tall cell component, hobnail (LOP/LCC with desmoplastic background) and oxyphilic component (85.7%, p=0.008; 91.1%, p<0.000; 100%, correspondingly). Same significantly high was double mutated cases (75%, p=0.03; 78.7%, p<0.000; 100%, correspondingly) (Table).

No significant differences with all histological features were detected in TERT promoter mutant cases (Table). However, hobnail (LOP/LCC with desmoplastic background) component with >30% of occupied area in the tumor was found in case with TERT promoter mutation (1 (100%)) (Table).

No significant differences in giant/spindle cells component, tall cell component, and solid type were detected between patients with wild and mutant cases (Table).

Table. Summary of results

	BRAF, n=341			**TERT promoter, n=324**				**BRAF+TERT, n=113**		
	wt, n=68 (20%)	**mu, n=273 (80%)**	**p-value**	**wt, n=268 (82.7%)**	**C228T, n=49 (15.1%)**	**C250T, n=7 (2.2%)**	**p-value**	**wt, n=61 (54%)**	**mut, n=52 (46%)**	**p-value**
Sex			1.000				0.808			1.000
F	57 (20.5%)	221 (79.5%)		220 (83%)	37 (14.1%)	6 (2.3%)		52 (55.9%)	41 (44.1%)	
M	11 (17.7%)	51 (82.3%)	0.73	48 (80%)	11 (18.3%)	1 (1.7%)	0.78	9 (47.4%)	10 (52.6%)	0.62
Mean age	49.5	54.4	0.03	49.9	69.2		0.000	47.9	68.9	0.000
Age<55	38 (21.9%)	135 (78%)		162 (97.6%)	4 (2.4%)	0		36 (90%)	4 (10%)	
Age≥55	30 (17.9%)	137 (82%)	0.42	106 (67.5%)	44 (28%)	7 (4.5%)	0.000	25 (34.7%)	47 (65.3%)	0.000
Mean size (range)	18.4 (70;2)	18.4 (83;4)	0.98	16.5 (75;3)	28.1 (70;2)	20.6 (33;10)	0.000	17.9 (70;3)	27.5 (70;5)	0.000

Ki-67 LI									
≤5%	55 (24.3%)	171 (75.7%)		198 (93.4%)	12 (5.7%)	2 (0.9%)		51 (79.7%)	13 (20.3%)	
5-10%	8 (11.3%)	63 (88.7%)		45 (65.2%)	22 (31.9%)	2 (2.9%)		6 (21.4%)	22 (78.6%)	
10+%	5 (12.5%)	35 (87.5%)	0.030	22 (56.4%)	14 (35.9%)	3 (7.7%)	0.000	4 (20%)	16 (80%)	0.000
Histology									
giant/spindle cells	3 (60%)	2 (40%)	0.66	4 (80%)	1 (20%)	0	0.609	3 (75%)	1 (25%)	0.000
tall cell	3 (14.3%)	18 (85.7%)	0.008	11 (55%)	8 (40%)	1 (5%)	0.56	3 (25%)	9 (75%)	0.03
<5%	3 (20%)	12 (80%)		8 (57.1%)	5 (35.7%)	1 (7.1%)		3 (33.3%)	6 (66.7%)	
5-30%	0	4 (100%)		2 (50%)	2 (50%)	0		0	2 (100%)	
>30%	0	2 (100%)		1 (50%)	1 (50%)	0	0.87	0	1 (100%)	0.08
solid	7 (43.8%)	9 (56.3%)	0.62	10 (62.5%)	5 (31.3%)	1 (6.3%)	0.061	7 (53.9%)	6 (46.1%)	0.59
hobnail	10 (8.93%)	102 (91.1%)	0.000	71 (65.7%)	31 (28.7%)	6 (5.6%)	0.002	10 (21.3%)	37 (78.7%)	0.000
<10%	9 (9%)	91 (91%)		65 (67.7%)	25 (26%)	6 (6.3%)		9 (22.5%)	31 (77.5%)	
11-20%	1 (9.1%)	10 (90.9%)		6 (54.5%)	5 (45.5%)	0		1 (16.7%)	5 (83.3%)	
>21	0	1 (100%)	0.002	0	1 (100%)	0	0.000	0	1 (100%)	0.000
oxyphilic	0	5 (100%)		4 (80%)	1 (20%)	0	0.000	0	1 (100%)	

５．主な発表論文等

〔雑誌論文〕　計2件（うち査読付論文　2件／うち国際共著　0件／うちオープンアクセス　2件）

１．著者名 Mussazhanova Z、Rogounovitch T、Saenko V、Krykpayeva A、Espenbetova M、Azizov B、Kondo H、Matsuda K、Kalmatayeva Z、Issayeva R、Yeleubayeva Z、Madiyeva M、Mukanova A、Sandybayev M、Bolsynbekova S、Kozykenova Z、Yamashita S、Nakashima M	４．巻 11
２．論文標題 The Contribution of Genetic Variants to the Risk of Papillary Thyroid Carcinoma in the Kazakh Population: Study of Common Single Nucleotide Polymorphisms and Their Clinicopathological Correlations	５．発行年 2021年
３．雑誌名 Frontiers in Endocrinology	６．最初と最後の頁 543500
掲載論文のDOI（デジタルオブジェクト識別子） 10.3389/fendo.2020.543500	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 －

１．著者名 Sato Ayako、Matsuda Katsuya、Motoyama Takahiro、Mussazhanova Zhanna、Otsubo Ryota、Kondo Hisayoshi、Akazawa Yuko、Higuchi Miyoko、Suzuki Ayana、Hirokawa Mitsuyoshi、Miyauchi Akira、Nagayasu Takeshi、Nakashima Masahiro	４．巻 10
２．論文標題 53BP1 expression as a biomarker to differentiate thyroid follicular tumors	５．発行年 2021年
３．雑誌名 Endocrine Connections	６．最初と最後の頁 309～315
掲載論文のDOI（デジタルオブジェクト識別子） 10.1530/EC-20-0630	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 －

〔学会発表〕　計3件（うち招待講演　0件／うち国際学会　0件）

１．発表者名 黒濵大和、松田勝也、本山高啓、ムサジャノワジャンナ、進藤久和、山下弘幸、中島正洋
２．発表標題 甲状腺Well-differentiated carcinoma, NOSの1例: 細胞診所見と分子病理学的特徴
３．学会等名 第35回長崎県臨床細胞学会総会および学術集会・がん検診従事者指導講習会
４．発表年 2021年

１．発表者名 黒濵大和、松田勝也、本山高啓、ムサジャノワジャンナ、大坪智恵子、松岡優毅、新藤久和、山下弘幸、中島正洋
２．発表標題 腫瘍内不均一のみられる甲状腺Well-differentiated carcinoma(WDC)、NOSの1例
３．学会等名 第110回日本病理学会総会
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１．発表者名 大坪智恵子、松岡優毅、黒濵大和、上木　望、松田勝也、ムサジャノワジャンナ、赤澤祐子、谷岡真司、山根裕介、中島正洋
２．発表標題 腎Clear cell sarcomaの鑑別に難渋した腎malignant rhabdoid tumorの1例
３．学会等名 第110回日本病理学会総会
４．発表年 2021年

〔図書〕　計0件

〔産業財産権〕

〔その他〕

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６．研究組織

	氏名 （ローマ字氏名） （研究者番号）	所属研究機関・部局・職 （機関番号）	備考

７．科研費を使用して開催した国際研究集会

〔国際研究集会〕　計0件

８．本研究に関連して実施した国際共同研究の実施状況

共同研究相手国	相手方研究機関