Получена: 2 февраля 2017 / Принята: 20 февраля 2017 / Опубликована online: 28 февраля 2017

UDC 616.44-006+577.112+612.086

IMMUNOHISTOCHEMICAL ANALYSIS EXPRESSION TP73 IN ONCOCYTIC FOLLICULAR ADENOMA OF THE THYROID

Zhanna U. Kozykenova, **http://orcid.org/0000-0001-7420-2279**

Semey State Medical University, Semey, Kazakhstan

Abstract

Oncocytic follicular adenomas (FAs) of the thyroid are cells neoplasms of follicular origin that predominantly consist of large polygonal cells with eosinophilic and granular cytoplasm. According to some scientific studies, TP73 might play a certain role in tumor genesis (oncogenesis). Moreover, due to the absence of p53, oncogenes may attract p73 to induce apoptosis in tumor cells. TP53 is a crucial tumor suppressor in preventing the cancerous transformation of cells. There was also a particular focus on the amplification of 1p36 chromosome in oncocytic FA, which contains a tumor nidus of TP73 protein, which is a member of the p53 family, involved as a factor of the cancerous tumor development.

The aim: To evaluate the extent of the genomic instability in oncocytic follicular adenoma of the thyroid.

Materials and Methods: Twenty-four surgically resected formalin-fixed, paraffin-embedded (FFPE) thyroid tumors, including 12 oncocytic and 12 normal FAs, were available for the current study. As it was a retrospective research with minimal risk to the participants, informing patients and taking their consent was not necessary. The study was authorized by the ethics committee of the Biomedical Sciences High School of Nagasaki University (protocol No. 15062617) and permitted by the ethics committee of the State Medical University, Semey (Protocol No. 5, December 15, 2015). The study of archival data of patients with follicular adenoma of the thyroid was conducted between 2014 and 2016 in the department of pathomorphology and cytochemistry of the "Regional Oncology Center" (Semey city, Kazakhstan) and in the scientific laboratory of the Nagasaki University (Japan).

For processing of the research results were used the Mann-Whitney-U test, the exact Fisher measure, Pearson's correlation analysis. The calculation was made with a help of SAS software (version 8:2, SAS Institute, Cary, NC, USA). All tests were unilateral and $p < 0,05$ was considered to be statistically true measure.

Results: Patients with oncocytic follicular adenoma were older than with normal follicular adenoma ($p = 0.03$) and there was no statistically significant difference in tumor size. The study showed, that the frequency of unstable expression of 53BP1 was vastly higher in the oncocytic follicular adenoma than in the usual one ($p = 0.0028$). Our work demonstrated that the level of TP73 immunoreactivity was greatly higher in oncocytic than in normal FA ($p= 0,0001$). We have found a significant positive correlation ($r = 0.5983$, $p = 0.0020$) between the percentage of tumour cells expressing unstable type of 53BP1 and the percentage of cells being positive for TP73 expression in oncocytic FA.

Conclusion: It is important to note that we have found a significant positive correlation between the percentage of tumour cells expressing unstable type of 53BP1 and the percentage of cells being positive for TP73 expression in oncocytic follicular adenoma. All these data indicate that the oncocytic follicular adenoma demonstrates an elevated level of TP73 protein, which correlates with the appearance of a DNA double strand break.

Key words: Thyroid tumor, oncocytic, 53BP1 expression, genomic instability.

Резюме

ИММУНОГИСТОХИМИЧЕСКИЙ АНАЛИЗ ЭКСПРЕССИИ TP73 ПРИ ОНКОЦИТАРНОЙ ФОЛЛИКУЛЯРНОЙ АДЕНОМЕ ЩИТОВИДНОЙ ЖЕЛЕЗЫ

Жанна У. Козыкенова, **http://orcid.org/0000-0001-7420-2279**

Государственный медицинский университет города Семей, г. Семей, Казахстан

Введение. Онкоцитарная фолликулярная аденома щитовидной железы является новообразованием клеток фолликулярного происхождения, преимущественно состоящих из крупных полигональных клеток с эозинофильной зернистой цитоплазмой. Некоторые данные свидетельствуют о том, что TP73 может играть определенную роль в онкогенезе. Кроме того, при отсутствии p53, онкогены могут привлекать p73, чтобы индуцировать апоптоз в опухолевых клетках. TP53 является решающим супрессором опухоли для предотвращения злокачественной трансформации клеток. Также особое внимание было уделено на усиление хромосомы 1p36 в онкоцитарной фолликулярной аденоме, которая содержит очаги для опухоли белка 73, как часть семейства p53, вовлеченого в качестве фактора в развитии злокачественных опухолей.

Цель: Оценить степень геномной нестабильности при онкоцитарной фолликулярной аденоме щитовидной железы.

Материалы и методы: Дизайн - ретроспективное научное исследование. Для достижения поставленной цели были исследованы 24 образца ткани опухоли щитовидной железы, из которых 12 онкоцитарных и 12 обычных фолликулярных аденом, полученных путем хирургической резекции, фиксированные формалином, залитых в парафин.

На проведение исследования было получено разрешение этического комитета Высшей школы биомедицинских наук университета Нагасаки (протокол №15062617) и разрешение этического комитета Государственного медицинского университета города Семей (протокол №5 от 15.12.2015г). Исследование архивных данных пациентов с фолликулярной аденомой щитовидной железы проводилось в период с 2014 по 2016 годы в отделении патоморфологии и цитохимии КГКП «Региональный Онкологический диспансер г. Семей» (Казахстан) и в научной лаборатории Университета Нагасаки (Япония).

Для обработки результатов исследования были применены тест Манна-Уитни, точный критерий Фишера, корреляционный анализ Пирсона. Для расчетов использовали программное обеспечение SAS (версия 8.2; SAS Institute, Cary, NC, USA). Все тесты были односторонними и значение $p < 0,05$ считалось статистически значимым.

Результаты исследования: Пациенты с онкоцитарной фолликулярной аденомой были старше, пациентов с обычной фолликулярной аденомой ($p = 0,03$). Статистически не было достоверных различий в размерах опухоли. Частота нестабильной экспрессии 53BP1 была значительно выше в онкоцитарной фолликулярной аденоме, чем в обычной ($p = 0,0028$). Наша работа показала, что уровень TP73 иммунореактивности был значительно выше в онкоцитарной, чем в обычной фолликулярной аденоме ($p = 0,0001$). Мы обнаружили значимую положительную корреляцию ($r=0,5983$, $p=0,0020$) между процентом опухолевых клеток, в нестабильной экспрессии 53BP1 и процент клеток, позитивных для экспрессии TP73 в онкоцитарной фолликулярной аденоме.

Выводы: Важно отметить, что мы нашли значимую положительную корреляцию между процентом опухолевых клеток, экспрессирующих нестабильный тип 53BP1 и процент клеток, позитивных для TP73 экспрессии в онкоцитарной фолликулярной аденоме. Все эти данные указывают на то, что онкоцитарная фолликулярная аденома демонстрирует повышенный уровень TP73 белка, что коррелирует с возникновением разрыва двойной нити ДНК.

Ключевые слова: опухоли щитовидной железы, онкоциты, экспрессия 53BP1, геномная нестабильность.

Тұжырым

ҚАЛҚАНША БЕЗІНІҢ ОНКОЦИТАРЛЫҚ ФОЛИКУЛЛЯРЛЫҚ ІСІКТЕРІ КЕЗІНДЕ TP73 ЭКСПРЕССИЯСЫНЫҢ ИММУНДЫГИСТОХИМИЯЛЫҚ ТАЛДАУЫ

Жанна У. Козыкенова, **http://orcid.org/0000-0001-7420-2279**

Семей қаласының Мемлекеттік Медицина Университеті, Семей қ, Казахстан

Қалқанша безінің онкоцитарлық фолликулярлы аденомасы фолликулярлы текті жасушалар ісіктері, ірі көпбұрышты, эозинофильді түйіршікті цитоплазмалы жасушалардан тұрады. Кейбір зерттемелер деректері бойынша TP73 онкогенезде маңызды рөл атқаратыны туралы мәліметтер белгілі. Сонымен қатар, p53 болмаған жағдайда, ісік жасушаларында апоптозды тудыру үшін онкогендер p73 қамтуы мүмкін. TP53 жасушаларда қатерлі трансформацияны болдырмау үшін аса маңызды ісіктер супрессоры болып табылады. Сондай-ақ, қатерлі ісіктерінің дамуына қатысты факторы ретінде, p53 отбасына жататын 73 ісік ақуызы ошақтары болуына байланысты онкоцитарлық фолликулярлы аденомада 1p36 хромосоманың үдуіне ерекше көңіл аударылды.

Мақсаты: Қалқанша безінің онкоцитарлық фолликулярлық ісіктері кезінде геномдық тұрақсыздықтың дәрежесін бағалау.

Материалдар мен әдістері: Жұмыстың дизайні – ретроспективті ғылыми зерттеме. Зерттеменің мақсатына жету үшін хирургиялық резекциямен алынып, формалинде бекітіліп, парафин-ендірілген 24 қалқанша безінің ісіктері, соның ішінде 12 қарапайым және 12 онкоцитарлық фолликулярлық аденома осы зерттеме үшін қол жетімді болды (қалқанша безі тіндері). Зерттеме өткізуге Нагасаки университетінің биомедициналық ғылымдары жоғары мектебінің (№ 15062617 хаттама) және Семей қаласының мемлекеттік медицина университеті этикалық комитетінің келісімі алынды (№5 хаттама 15.12.2015ж). Қалқанша безі фолликулярлық аденомасымен науқастардың архивті мәліметтерімен зерттеме 2014 жылдан 2016 жылға дейін Семей қаласының (Қазақстан) онкологиялық диспансеріндегі патологиялық морфология және цитохимиялық зертханасында және Нагасаки университетінің (Жапония) ғылыми зертханасында өткізілді. Зерттеме нәтижелерін өңдеу үшін Манна-Уйтни тесті, Фишер критериі, Пирсонның корреляциялық анализі қолданылды. Есептеулерге SAS бағдарламасы (SAS институты, Cary, NC, АҚШ 8.2 нұсқасы) қолданылды. Барлық сынақтар бір жақты, және p <0,05 статистикалық мәнді болды.

Зерттеме нәтижесі: Онкоцитарлық фолликулярлы аденомасымен науқастардың жасы қарапайым аденомамен ауыратын науқастармен салыстырғанда жастары үлкен болды (p = 0,03), және де ісіктер көлемінде статистикалық нақты айырмашылығы болған жоқ. Зерттемеде 53BP1 тұрақсыз экспрессиясының жиілігі қарапайым түрімен салыстырғанда онкоцитарлық түрінде жоғары екендігі (p = 0,0028) анықталды. Біздің жұмысымыздың қорытындысы бойынша TP73 иммундыреактивтілік қарапайым фолликулярлы аденомамен салыстырғанда онкоцитарлық түрінде мәнді жоғары деңгейін көрсетті (p = 0,0001). Сонымен қатар, онкоцитарлық фолликулярлы аденомада 53BP1 тұрақсыз экспрессиясы ісік жасушалары пайызы арасында мәнді оң корреляция (r=0,5983, p=0,0020) және TP 73 экспрессиясында позитивті жасушалар пайызы анықталды.

Қорытынды: Бастысы, онкоцитарлық фолликулярлық аденомада 53BP1 тұрақсыз экспрессиясы ісік жасушалары пайызы арасында мәнді оң корреляциясы және TP 73 экспрессиясында позитивті жасушалар пайызы анықталды. Барлық осы деректер онкоцитарлық фолликулярлық аденомада ДНК қос бұрымында үзіліс пайда болуына байланысты TP73 ақуызының жоғары деңгейін көрсетеді.

Негізгі сөздер: қалқанша безі ісіктері, онкоциттер, 53BP1 экспрессиясы, геномдық тұрақсыздық.

Библиографическая ссылка:

Козыкенова Ж.У. Иммуногистохимический анализ экспрессии TP73 при онкоцитарной фолликулярной аденоме щитовидной железы / / Наука и Здравоохранение. 2017. №1. С. 64-73.

Kozykenova Zh.U. Immunohistochemical analysis expression TP73 in oncocytic follicular adenoma of the thyroid. *Nauka i Zdravookhranenie* [Science & Healthcare]. 2017, 1, pp. 64-73.

Қозыкенова Ж.У. Қалқанша безінің онкоцитарлық фоликуллярлық ісіктері кезінде TP73 экспрессиясының иммундыгистохимиялық талдауы / / Ғылым және Денсаулық сақтау. 2017. №1. Б. 64-73.

Introduction

Oncocytic follicular tumors of the thyroid, also known as oxyphilic or Hürthle cell tumors, are neoplasms of follicular cell origin predominantly, or entirely, composed of large polygonal cells with oxyphilic features related to the presence of eosinophilic and granular cytoplasm that is rich in mitochondria [5,15,21]. Encapsulated thyroid lesions with no evidence of capsular or vascular invasion and no nuclear features of papillary carcinoma are diagnosed as oncocytic follicular adenomas (FAs), and those that exhibit vascular and/or capsular invasion in the absence of diagnostic nuclear features of papillary carcinomas are diagnosed as oncocytic follicular carcinoma [6,17,22,29]. However, the clinical significance of oncocytic change in thyroid tumors remains unclear and controversial. Some studies have indicated that oncocytic carcinomas behave more aggressively in comparison with the usual variants of well differentiated thyroid cancers, and result in a higher incidence of metastases and a lower survival rate; hence they recommend aggressive surgical treatment regime for all oncocytic follicular tumors [12,16,24]. On the other hand, other reports suggest that oncocytic follicular tumors are not more aggressive than their conventional counterparts [7,11,25].

Tumor protein 73 (*TP73*) is encoded within 1p36, and belongs to the p53 protein family [31] dysregulation of the latter plays a critical role in tumorigenesis and significantly affects tumor response to therapy. Significant up-regulation of TP73 transcription, which involves transcription factors that also regulate various vital biological processes including cell differentiation, proliferation, and cell death/apoptosis, has been demonstrated using quantitative reverse transcription-PCR (qRT-PCR) in human papillary thyroid carcinomas.

However, analysis of 1p36 and TP73, especially in association with 53BP1, remains unexplored in oncocytic FA.

P73 is a tumor suppressor protein. It is a member of the p53 family that is composed of p53, p63 and p73 [14,30]. p53 is the most well-known tumor suppressor and has been suggested to be mutated in over half of the human cancers [3]. All p53 family members display similar domain structures, having an N-terminal transactivation (TA) domain, a DNA-binding domain (DBD) and an oligomerization domain (OD). p73 and p63 share a sterile alpha motif domain (SAM) and an inhibitory domain (ID) at their C-termini. In addition to the similarity in the domain structures, p73 and p63 have a higher sequence homology as compared with p53. Although p53, p63, and p73 share similar domain architecture and sequence identity, their differences in vivo are striking. While p53 is frequently mutated during tumorigenesis (in over 50% of human tumors), p63 and p73 are rarely mutated [18]. There are nine possible isoforms for p53, six for p63, and 35 for p73 that can arise through a combination of promoter usage and alternative splicing [1,2,19]. For p63 and p73, two classes of isoforms exist that either contain (TA) or lack (ΔN) the transactivation domain required for full activation of target genes (Fig. 1); The purported active isoform of p73, TAp73, is of particular interest because it is frequently expressed in human tumors and can be inhibited by either ΔNp63 or ΔNp73 (Fig. 1) [8]. In addition, tumor-specific forms of p53 have the ability to bind and inhibit p73 (Fig. 1) [9]. Thus the ability of _Np63, _Np73, or mutant p53 to inhibit TAp73 mayobviate the need for mutation of p73 during tumorigenesis.

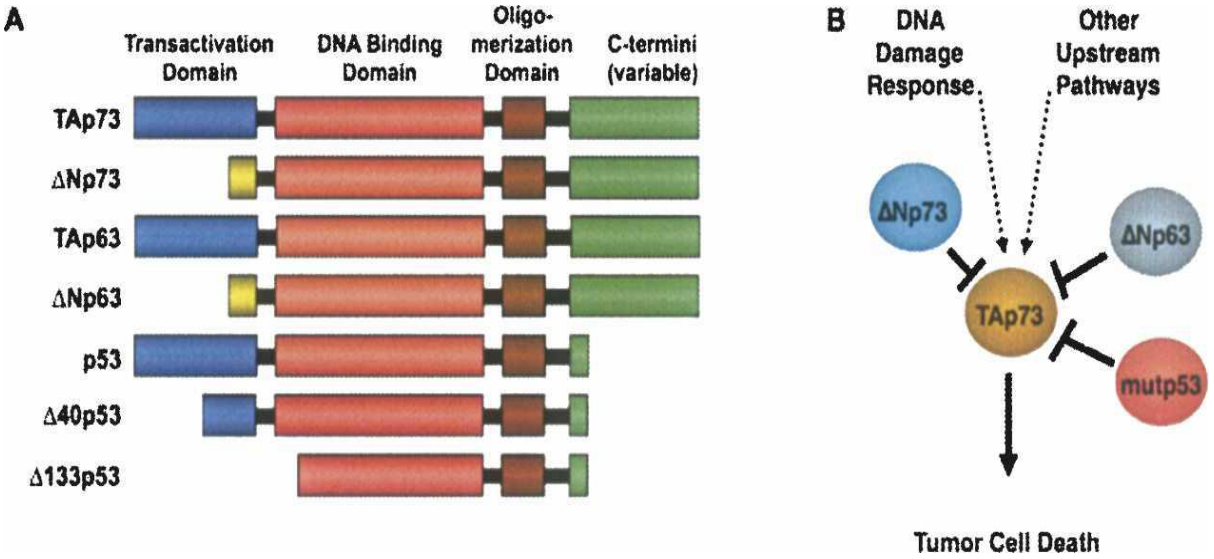

Figure 1. Isoform-based model of p53 family function. (*A*) Active isoforms of the p53 family of transcription factors (p53, p63, and p73) contain a transactivation domain, whereas inhibitory isoforms lack a transactivation domain. (*B*) Other family member isoforms may inhibit TAp73 in cells, thus preventing TAp73 from engaging in tumor-suppressive functions and reducing selective pressure for mutation of p73 during tumorigenesis.

Significant up-regulation of tumor protein 73 (TP73) transcription, which involves transcription factors that also regulate various vital biological processes including cell differentiation, proliferation, and cell death/apoptosis, has been demonstrated using quantitative reverse transcription-PCR (qRT-PCR) in human papillary thyroid carcinomas. However, analysis of TP73, especially in association with p53-binding protein1 (53BP1), remains unexplored in oncocytic FA.

In an attempt to clarify the potential pathological mechanisms underlying the aggressiveness of oncocytic FA, we analyzed the type of 53BP1 expression using immunohistochemical analysis of TP73 expression.

Thus, the **aim of this study** was to evaluate the extent of the genomic instability in oncocytic follicular adenoma of the thyroid.

Materials and Methods

Twenty-four surgically resected formalin-fixed, paraffin-embedded (FFPE) thyroid tumors including 12 oncocytic and 12 conventional FAs were available for the present study.

As this was a retrospective research study involving minimal risk to the participants, informed consent for the analysis was not obtained from each patient. In addition, following the guidelines of the Ethical Committee's official disclosure system, detailed information of the research was released to the public on the institution's homepage.

Inclusion criteria of patients into research are the following:

1. Archival histological materials of patients with confirmed histological diagnosis
2. Histological picture of oncocytic adenoma
3. Picture of thyroid neoplasia at subsequent thin-needle biopsy
4. Agreement of patient being involved into research

Exclusion criterion of patients into research is the following:

1. Rejection of patient in participation

An immunohistochemistry study was performed to determine the level of TP73 expression in the patient samples. After antigen retrieval by heating the tissue sections in a microwave for 20 minutes in citrate buffer (pH 6.0), the sections were immersed in 0.3 % H_2O_2 solution for 30 minutes to block endogenous peroxidase activity and incubated for 1 hour at room temperature with an anti-p73 rabbit monoclonal antibody (Abcam, Tokyo, Japan) at a 1:50 dilution in a humidified chamber.To detect the immunostaining, Histofine Simple Stain™ MAX PO (MULTI) (Nichirei Biosciences Inc., Tokyo, Japan) was used according to the manufacturer's instruction. For evaluation of TP73 expression, we counted the number of positively stained cells in 10 fields at ×400 magnification per

section and calculated the mean percentage of positive cells, which was defined as TP73 immunoreactivity in each case. The level of TP73 immunoreactivity was categorized into four groups according to the percentage of positive cells as follows: 1) negative: 0<5%; 2) low: 5<30%; 3) moderate: 30<60%, or 4) high: ≥60%.

Comparisons of age, gender, and tumor size between the patients with oncocytic and conventional FA were carried out using the Mann-Whitney U test. Associations between the type of 53BP1 expression (stable, intermediate, or unstable) and histologic type (oncocytic or conventional FA) were assessed by the Fisher's exact test. The Cochran-Armitage test was used to compare the level of TP73 immunoreactivity (negative, low, moderate, or high) between histologic types.

Correlations between the type of 53BP1 expression and the percentage of tumor cells exhibiting unstable 53BP1 expression and the level of TP73 immunoreactivity in FA were evaluated by Pearson's correlation analysis. The PHREG procedure in SAS software (version 8.2; SAS Institute, Cary, NC, USA) was used for calculations. All tests were one-tailed, and a *p*-value <0.05 was considered statistically significant.

Results

Patients with oncocytic FA were older than those with conventional FA (p = 0.03) and there were statistically no differences in tumor size identified by the Mann-Whitney-U test. Representative images of TP73 expression in both oncocytic and conventional FA obtained using immunohistochemistry are depicted in Fig. 2. Relative to the stable type of 53BP1 expression, the unstable pattern has been shown to increase during carcinogenesis.

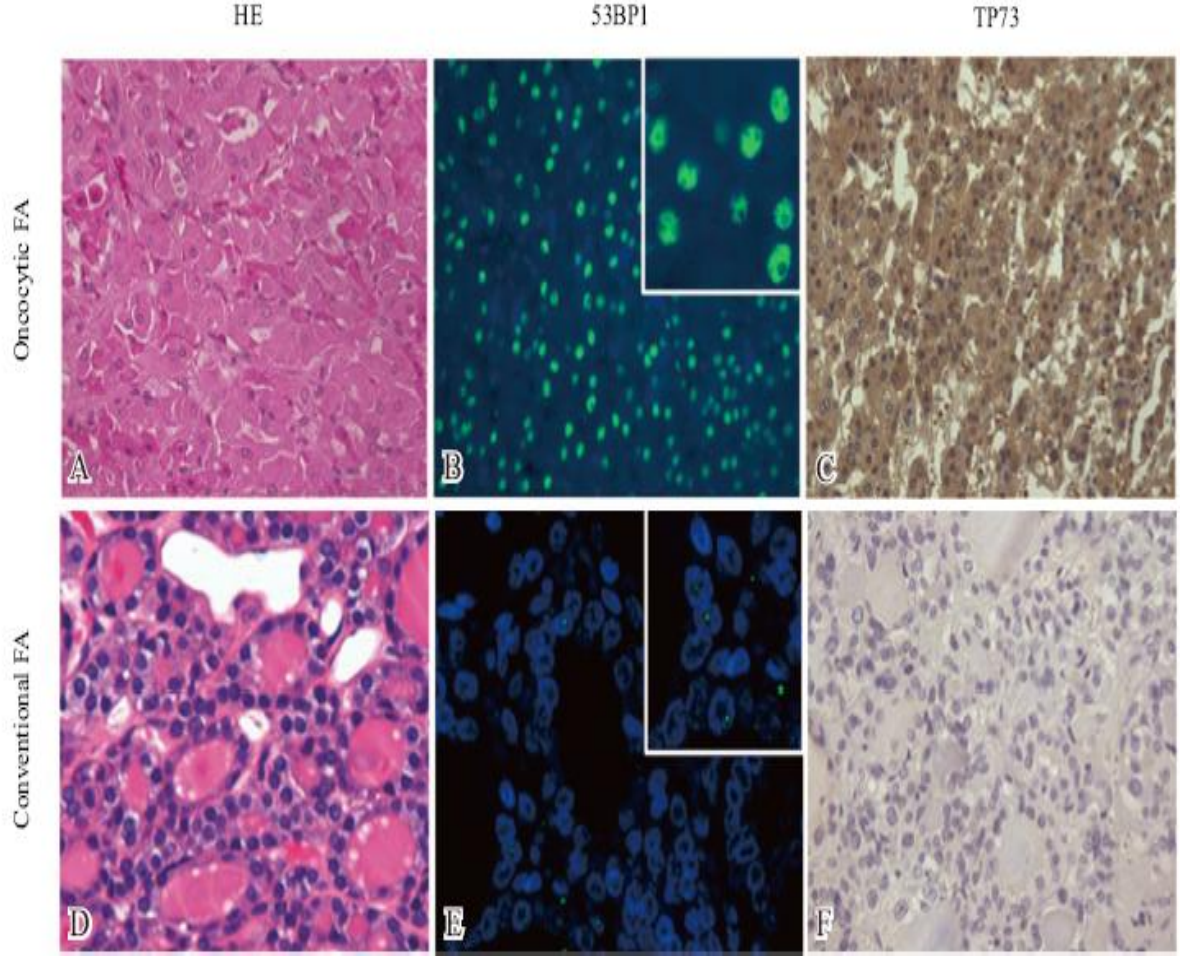

Fig. 2 Comparison of 53BP1 expression patterns and TP73 immunoreactivities between oncocytic FA (A-C) and conventional FA (D-F). H&E staining of oncocytic and conventional FA (A, D). A strong TP73 immunoreactivity (C) is observed in oncocytic FA showing unstable 53BP1 expression (B), while TP73 immunostaining (F) is faint in conventional FA with intermediate 53BP1 expression (E).

Notably, in the current study, the incidence of unstable 53BP1 expression was significantly higher in oncocytic FA than in conventional FA as assessed by the Fisher's exact test (p = 0.0028). Based on the aCGH results, we further looked into the specific chromosomal locations wherein DNA amplification occurred. Notably, analysis demonstrated amplification of chromosome 1p36 in 3 of 4 oncocytic FA cases, but not in 4 conventional FA cases. To verify this finding, we assessed the amplification of chromosome 1p36 using the LSI 1p36 probe by employing FISH analyses. These results suggested that amplification of 1p36 might be one of the defining genomic features of oncocytic FA. The LSI 1p36 probe used in the FISH analysis contains sequences that extend from near the SHGC 57243 locus through the *TP73* and *MEGF6* genes, and ends at a point telomeric to the *MEGF6* locus. These data led us to focus on the tumor protein TP73 in 1p36, which has been shown to be frequently dysregulated during carcinogenesis in various malignancies.

Our work demonstrated that the level of TP73 immunoreactivity was substantially higher in oncocytic than in conventional FA (p=0.0001).

Importantly, we found a significant positive correlation (r=0.5983, p=0.002) between the percentage of tumor cells exhibiting unstable 53BP1 expression and the percentage of cells positive for TP73 expression in oncocytic FA as evaluated using Pearson's correlation analysis (Fig. 3).

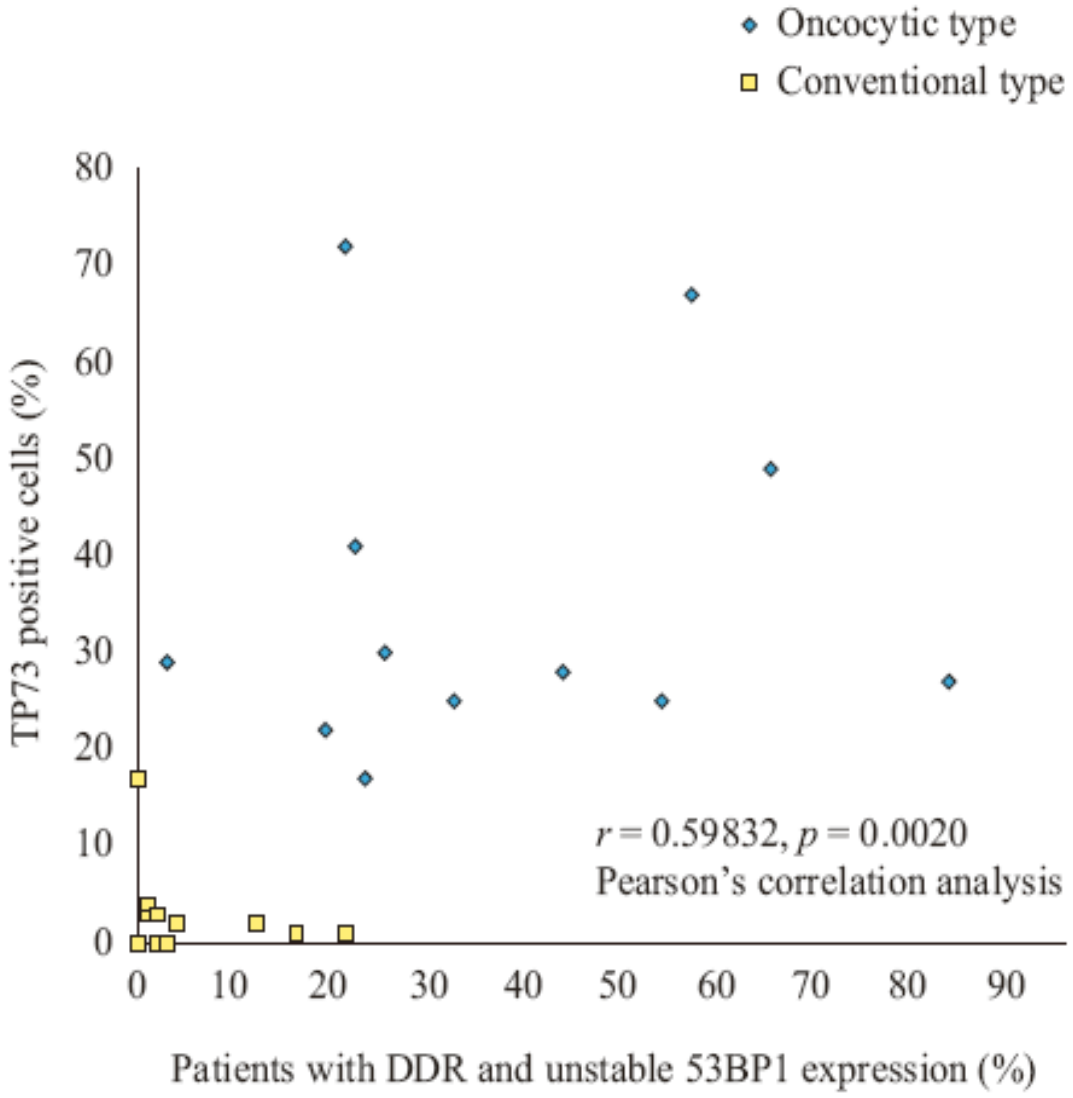

Fig. 3 Positive correlation between unstable 53BP1 expression and TP73 immunoreactivity in follicular adenoma (r = 0.59832, p = 0.0020, by Pearson's correlation analysis).

Taken together, these data indicated that oncocytic FA demonstrates an elevated TP73 protein level that correlates with the occurrence of DNA double strand breaks.

Discussion

The present study demonstrates for the first time the existence of differences in the type of 53BP1 expression between oncocytic and conventional FA. The prevalence of unstable 53BP1 immunoreactivity, which is suggestive of the induction of endogenous DDR mechanisms, was significantly higher in oncocytic than conventional FA, indicating a higher level of genomic instability in oncocytic FA. This study also revealed a higher incidence of CNA in the tumor DNA of oncocytic FA exhibiting unstable 53BP1 expression, providing further evidence for a role of genomic instability during oncocytic FA tumorigenesis and its association with the pattern of 53BP1 expression. Previous CGH analyses found that chromosomal aberrations were common in oncocytic FA/Hürthle cell adenoma [Frisk T, Kytola S, Wallin G, (1999) Low frequency of numerical chromosomal aberrations in follicular thyroid tumors detected by comparative genomic hybridization. *Genes Chromosomes Cancer* 25: 349-353.]. Although some studies have found that carcinomas have more chromosomal gains and losses than adenomas, in others the differences were not statistically significant [Tallini G, Hsueh A, Liu S, Garcia-Rostan G, et al. (1999) Frequent chromosomal DNA unbalance in thyroid oncocytic (Hurthle cell) neoplasms detected by comparative genomic hybridization. *Lab Invest* 79: 547-555.]. It is unclear why oncocytic FAs exhibit increased genome instability compared to conventional FAs. We note that patients with oncocytic FA were older than those with conventional FA, consistent with previous findings regarding the general population of patients with oncocytic FA [Rosai J (2004) Thyroid gland. In: Rosai J (ed) Rosai and Ackerman's Surgical Pathology (9th). Mosby, New York: 544-547.]. Although we cannot completely exclude the possibility that age difference contributed to the differential expression of 53BP1, our previous studies indicated that 53BP1 expression pattern is largely influenced by pathological grade and the pattern of neoplasms [Nakashima M, Suzuki K, et al. (2008) Foci formation of P53-binding protein 1 in thyroid tumors: activation of genomic instability during thyroid carcinogenesis. *Int J Cancer* 122: 1082-1088].

Our aCGH analysis demonstrated the amplification of chromosome 1p36 in oncocytic FA tumors showing unstable 53BP1 expression, but not in conventional FA tumors showing stable 53BP1 expression. Amplification of chromosome 1p36 in oncocytic FA was additionally confirmed by FISH. This is consistent with the report by Wada *et al*., who also found a gain of chromosome 1p36 in oncocytic FA by CGH analysis. In the current study, we further demonstrated a significant positive correlation between the percentage of tumor cells in FA expressing unstable 53BP1 expression and the percentage of cells positive for the expression of TP73, which is encoded by a gene located on chromosome 1p36.2-3. Previous studies have also suggested an impact of TP73 gene alteration on human thyroid tumorigenesis [Hemmer S, Wasenius VM, Knuutila S (1998) Comparison of benign and malignant follicular thyroid tumours by comparative genomic hybridization. *Br J Cancer* 78: 1012-1017]. The TP73 gene has been shown to encode a large variety of diverse transcripts that are regulated by extensive alternative splicing. These transcripts can be generally categorized into two main groups, encoding transcriptionally active and N-terminally truncated (ΔN) isoforms [Vilgelm AE, Washington MK, Wei J, et al. (2010) Interactions of the p53 protein family in cellular stress response in gastrointestinal tumors. *Mol Cancer Ther* 9: 693-705.]. ΔNp73 plays a dominant-negative role in inhibiting the transcriptional and other biological activities of the transcriptionally active isoforms, which are linked to cancer development. Accordingly, ΔNp73 is upregulated in many human cancers including liver, ovarian, breast, and melanoma [Castillo J, Goni S, Latasa MU, Perugorria MJ, et al. (2009) Amphiregulin induces the alternative splicing of p73 into its oncogenic isoform DeltaEx2p73 in human hepatocellular tumors. *Gastroenterology* 137:1805-1815.]. The correlation of 53BP1 nuclear expression pattern and TP73 does not explicitly indicate that unstable 53BP1 expression underlies the aberrant TP73 expression.

In summary, this study demonstrated the unstable pattern of 53BP1 expression in oncocytic FA and its association with a higher

incidence of copy number aberration (CNA) as assessed by array comparative genomic hybridization (aCGH). Although further studies are required to determine the pathological and clinical roles of 53BP1 nuclear foci in follicular cell-derived neoplasms, the results of the current study suggest that oncocytic FA exhibits elevated genomic instability compared to nononcocytic FAs.

References:

1. *Bourdon J.C.* 2007. p53 and its isoforms in cancer. Br. J. Cancer 97: 277–282.

2. *Bourdon J.C., Fernandes K., Murray-Zmijewski F., Liu G., Diot A., Xirodimas D.P., Saville M.K., Lane D.P*. p53 isoforms can regulate p53 transcriptional activity.Genes & Dev. 2005. 19: 2122–2137.

3. *Bullock A.N., Fersht A.R.* Rescuing the function of mutant p53. Nat. Rev. Cancer., 2001. 1, 68–76.

4. *Castillo J., Goni S., Latasa M.U., Perugorria M.J., Calvo A. et al.* Amphiregulin induces the alternative splicing of p73 into its oncogenic isoform DeltaEx2p73 in human hepatocellular tumors. Gastroenterology. 2009. 137:1805-1815.

5. *Carcangiu M.L., Bianchi S., Savino D., Voynick I.M., Rosai J.* Follicular Hurthle cell tumors of the thyroid gland. Cancer. 1991. 68: 1944-1953.

6. *Canberk S., Griffin A.C., Goyal A., Wang H., Montone K. et al.* Oncocytic follicular nodules of the thyroid with or without chronic lymphocytic thyroiditis: An institutional experience. Cytojournal. 2013. 10: 2.

7. *Chao T.C., Lin J.D., Chen M.F.* Surgical treatment of Hurthle cell tumors of the thyroid. World J Surg. 2005. 29:164-168.

8. *DeYoung M.P., Ellisen L.W.* p63 and p73 in human cancer: Defining the network. Oncogene 2007. 26: 5169–5183.

9. *Di Como C.J., Gaiddon C., Prives C.* p73 function is inhibited by tumor-derived p53 mutants in mammalian cells. Mol. Cell. Biol. 1999. 19: 1438–1449.

10. *Frisk T., Kytola S., Wallin G.* Low frequency of numerical chromosomal aberrations in follicular thyroid tumors detected by comparative genomic hybridization. Genes Chromosomes Cancer 1999. 25: 349-353.

11. *Ghossein R.A., Hiltzik D.H., Carlson D.L., Patel S., Shaha A. et al.* Prognostic factors of recurrence in encapsulated Hurthle cell carcinoma of the thyroid gland: a clinicopathologic study of 50 cases. Cancer. 2006. 106: 1669-1676.

12. *Haq M., Harmer C.* Differentiated thyroid carcinoma with distant metastases at presentation: prognostic factors and outcome. Clin Endocrinol (Oxf). 2005. 63:87-93.

13. *Hemmer S., Wasenius V.M., Knuutila S., Joensuu H., Franssila K.* Comparison of benign and malignant follicular thyroid tumours by comparative genomic hybridization. Br J Cancer 1998. 78: 1012-1017.

14. *Levrero M., De Laurenzi V., Costanzo A., Gong J., Wang J.Y., Melino G.* The p53/p63/p73 family of transcription factors:overlapping and distinct functions. J. Cell Sci., 2000. 113, 1661–1670.

15. *Maximo V., Lima J., Prazeres H., Soares P., Sobrinho-Simoes M.* The biology and the genetics of Hurthle cell tumors of the thyroid. Endocr Relat Cancer. 2012. 19: R131-147.

16. *McDonald M.P., Sanders L.E., Silverman M.L., Chan H.S., Buyske J.* Hurthle cell carcinoma of the thyroid gland: prognostic factors and results of surgical treatment. Surgery. 1996. 120: 1000-1004.

17. *Montone K.T., Baloch Z.W., LiVolsi V.A.* The thyroid Hurthle (oncocytic) cell and its associated pathologic conditions: a surgical pathology and cytopathology review. Arch Pathol Lab Med 2008. 132: 1241-1250.

18. *Moll U.M. Slade N.* p63 and p73: Roles in development and tumor formation. Mol. Cancer Res. 2004. 2: 371–386.

19. *Murray-Zmijewski F., Lane D.P., Bourdon J.C.* p53/p63/p73 isoforms: An orchestra of isoforms to harmonise cell differentiation and response to stress. Cell Death Differ. 2006. 13: 962–972.

20. *Nakashima M., Suzuki K. et al.* Foci formation of P53-binding protein 1 in thyroid tumors: activation of genomic instability during thyroid carcinogenesis. Int J Cancer. 2008. 122: 1082-1088.

21. *Petric R., Gazic B., Besic N.* Prognostic factors for disease-specific survival in 108 patients with Hurthle cell thyroid carcinoma: a single-institution experience. BMC Cancer. 2014. 14: 777.

22. *Rosai J., Kuhn E., Carcangiu M.L.* Pitfalls in thyroid tumour pathology. Histopathology. 2006. 49: 107-120.

23. *Rosai J.* Thyroid gland. In: Rosai J (ed) Rosai and Ackerman's Surgical Pathology (9th). Mosby, New York: 2004. 544-547.

24. Shaha A.R., Loree T.R., Shah J.P. Prognostic factors and risk group analysis in follicular carcinoma of the thyroid. *Surgery*. 1995. 118: 1131-1136.

25. *Sugino K., Ito K., Mimura T., Kameyama K., Iwasaki H. et al.* Hurthle cell tumor of the thyroid: analysis of 188 cases. World J Surg 2001. 25: 1160-1163.

26.*Tallini G., Hsueh A., Liu S., Garcia-Rostan G. et al.* Frequent chromosomal DNA unbalance in thyroid oncocytic (Hurthle cell) neoplasms detected by comparative genomic hybridization. Lab Invest. 1999. 79: 547-555.].

27. *Vilgelm A.E., Washington M.K., Wei J., Chen H., Prassolov V.S. et al.* Interactions of the p53 protein family in cellular stress response in gastrointestinal tumors. Mol Cancer Ther. 2010. 9: 693-705.

28. *Wada N., Duh Q.Y., Miura D., Brunaud L., Wong M.G. et al.* Chromosomal aberrations by comparative genomic hybridization in hurthle cell thyroid carcinomas are associated with tumor recurrence. J Clin Endocrinol Metab. 2002. 87: 4595-4601.

29. *Wei S., LiVolsi V.A., Montone K.T., Morrissette J.J., Baloch Z.W.* PTEN and TP53 Mutations in Oncocytic Follicular Carcinoma. Endocr Patho.l 2015. 26: 365-369.

30. *Yang A., Kaghad M., Caput D. McKeon F.* On the shoulders of giants: p63, p73 and the rise of p53. Trends Genet, 2002. 18, 90–95.

31. *Yang A., Kaghad M., Wang Y., Gillett E., Fleming M.D. et al.* p63, a p53 homolog at 3q27-29, encodes multiple products with transactivating, death-inducing, and dominant-negative activities. Mol Cell. 1998. 2: 305-316.

Контактная информация:

Козыкенова Жанна Укошовна - докторант PhD по специальности «Медицина» Государственного медицинского университета города Семей, г. Семей, Республика Казахстан

Почтовый адрес: 071400, Республика Казахстан, г.Семей, ул.Дулатова 145, кв. 55.

E-mail: alicher-02@mail.ru

Телефон: 7 777 583 56 17