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The morphological analysis of anaplastic thyroid cancer cell line

Anaplastik tiroit kanseri hücre hattının morfolojik analizi

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ABSTRACT

Aim: Thyroid follicular cell derived cancers are classified into three groups such as papillary thyroid cancer (85%), follicular thyroid cancer (12%) and anaplastic (undifferentiated) thyroid cancer (ATC) (3%). ATCs have very rapid course, poor treatment outcomes and they are very aggressive. The aim of current study was to assess the analysis of the morphological differences of ATC cell line with the normal thyroid cell line (NTC).

Materials and Methods: NTH and ATC cells were examined with haematoxylin and eosin, the nucleus: cytoplasm (N:C) ratios were detected, and cell cycles were investigated. These cell lines were compared according to their N:C ratio and their abundance in cell cycle phases.

Results: The N:C ratio was higher in ATC than NTC. Both cell groups were mostly found in G0/G1 phase (68.4; 82.8) and have statistical difference in both G0/G1 and S phases.

Conclusion: The rapid course and the rarity of ATC are significant barriers for clinical trials. Cultured cell lines are very important to explore the behaviour in the biology of ATC cells (such as the cell cycle), to understand the course of the disease, and to find an effective target for treatment.

Keywords: Anaplastic thyroid cancer, cell cycle, apoptosis.

ÖZ

Amaç: *Tiroid foliküler hücre kaynaklı kanserler papiller tiroid kanseri (%85), foliküler tiroid kanseri (%12) ve anaplastik tiroid kanseri (ATK) (%3) olmak üzere üç kategoriye ayrılır. ATK'lerin çok hızlı seyri, kötü tedavi sonuçları vardır ve çok agresiftirler. Bu çalışmanın amacı, ATK hücre hattının normal tiroid hücre hattı (NTH) ile olan morfolojik farklılıklarının analizini değerlendirmektir.*

Gereç ve Yöntem: *NTH ve ATK hücrelerinde hematoksilen-eosin boyama ile inceleme yapıldı, nükleus sitoplazma (N:S) oranlarına bakıldı ve hücre döngüleri incelendi. Bu hücre hatları N:S oranlarına ve hücre döngüsü fazlarındaki bulunma oranlarına göre karşılaştırıldı.*

Bulgular: *ATK hızlı seyri ve nadir görülmesi nedeniyle tedavisi zor bir kanser türüdür. Kültürlenmiş hücre hatlarında çalışmak, ATK hücrelerinin biyolojisindeki davranışları keşfetmek (hücre siklusu gibi), hastalığın seyrini anlamak ve tedavi için etkili bir hedef bulmak açısından önem taşımaktadır.*

Sonuç: *N:S oranı ATK'de NTH'den daha yüksekti. Her iki hücre grubu da çoğunlukla G0/G1 fazında (68.4; 82.8) bulundu ve hem G0/G1 hem de S fazlarında istatistiksel farklılıklara sahipti.*

Anahtar Sözcükler: *Anaplastik tiroid kanseri, hücre döngüsü, apoptoz.*

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INTRODUCTION

Thyroid cancers arising from thyroid follicular cells are the most common endocrine cancers (1). Thyroid cancers consist of various histotypes with distinctive molecular profiles (2). The kinds of thyroid cancer derived from follicular cells make up approximately 95% of cases. Thyroid cancers come out mostly in women than in men (3). Thyroid follicular cell originated cancers are classified into three categories such as papillary thyroid cancer (85%), follicular thyroid cancer (12%) and anaplastic (undifferentiated) thyroid cancer (ATC) (3%) (4). On the other hand, parafollicular cell- derived forms of thyroid cancer cause medullary thyroid carcinoma (5). Surgery is the best choice of treatment for patients with differentiated thyroid cancer. Well-differentiated thyroid cancers display expanding growth, follicular architecture, various cytologic atypia and invasiveness. Low-risk cancers have good prognosis after surgery. Widely-invasive tumours necessitate total thyroidectomy and radio ablation (6).

ATCs have very rapid course, poor treatment outcomes and they are very aggressive. Disease-specific mortality in ATCs is nearly 100 percent (7). Traditional treatment modalities such as surgical resection, radiation and chemotherapy are ineffective in ATC (8). The diagnosis of ATC is usually detected by fine needle aspiration biopsy and/or of tissue taken by surgical or large-needle biopsy (9). Morphological analyses of ATC display spindle cell, pleomorphic giant cell on cytopathology. Besides, various mitotic figures and atypical mitoses exist on cytopathology (10). 47% of patients have previous history of well-differentiated thyroid carcinoma (5).

The number of studies examining cell morphology, N/C ratio and cell cycle behaviour together is limited in the literature. The aim of the current study is to link morphological features of ATC cell line and NTC with cell cycle. The nucleus to cytoplasm ratio (N:C) is one of the key value to evaluate aggressiveness of the cancer could be coupled to cell cycle process.

MATERIALS and METHODS

Cell Culture

Human normal primary thyroid follicular epithelial cell line (NTC) Nthy-ori-3-1 (ECACC 90011609) and human anaplastic thyroid carcinoma cell line (ATC) 8505c (ECACC no: 94090184) were growth in RPMI 1640 (Capricorn; RPMI-XA) medium supplemented with 1% L-glutamine and 10% FBS 1% penicillin streptomycin. Cells were incubated under 5% CO2 at 37^{o}C. Cells were monitored daily and according to their confluency; culture medium changed or passaged every 2-3 days.

Haematoxylin&Eosin staining (H&E)

8505c and Nthy-ori-3-1 cells were passaged and diluted at 2x10^{5} cells/ml and incubated for 24 hours under 5% CO_2 at 37^{o}C. The cells were passaged and washed with 1X PBS. They were fixed with 4% PFA for 30 minutes and washed with 1X PBS. Samples were rinsed with distilled water and dipped in haematoxylin (Harris, Merck) for approximately 5 minutes. After acid alcohol and water rinse, samples were stained with eosin (Carloerba, 446634) for approximately 1.5 minutes and passed through once 95% and twice 100% alcohols. Finally, the samples rinsed with xylene and mounted.

- Cell Cycle Analysis

8505c and Nthy-ori-3-1 were passaged and diluted at 2x10^{5} cells/ml and incubated for 24 hours under 5% CO_2 at 37^{o}C. The cells were passaged and washed with 1X PBS. They were fixed in ice-cold 70% ethanol. Afterwards, cells were washed with 1X PBS and incubated with Muse cell cycle reagent kit for 30 minutes. Cell cycle phases were displayed with Muse ® Cell Analyser.

Statistical Analysis

Nucleus: Cytoplasm (N:C) ratio were assessed from the H&E staining. The images were deconvoluted by ImageJ (Image analysis software, National Institutes of Health, Bethesda, MD) using color deconvolution. The image processing and analysis were performed completely blinded. The nuclear area and cell area were drawn with freehand selection tool. The measurements were converted pixel to micrometer scale and area values were measured. The nuclear area values were subtracted from the whole cellular area for cytoplasmic area values. Afterwards, nuclear area values were divided to this cytoplasmic area values. Cell cycle analysis were performed trice. Data were analyzed by IBM SPSS Statistics 25.0 and graphs were prepared by GraphPad Prism 8.4.3 program. Statistical differences were indicated with asterisk (*** $p<0.001$).

RESULTS

Morphological characterization to normal and cancer cells are important for diagnosis and arise a new therapeutic notion. At first, NTC and ATC were inspected by phase-contrast microscope (Figure-1A). NTCs displayed more spindled morphology than ATCs. Nucleus: Cytoplasm (N:C) ratio reflects the cell characteristics, especially in pathological process, these values could be clue of aggressiveness of the cells. H&E staining showed that nuclear region to cytoplasm ratio was higher in ATCs (Figure-1B) and the N:C ratios were 0.26 and 0.52 for Nthy-ori-3-1 and 8505c cells, respectively (Figure-1C). In addition, nucleus and cell area of Nthy-ori-3-1 and 8505c cells were displayed in Figure-2.

Cell cycle process is one of the most pivotal process to regulate cellular activity in both healthy and disease state. Cell cycle check points regulates the abundance of the cell percentage of the cells at different stages of this process. Here, we compared cell cycle phases in NTCs and ATCs and analysis showed that G0/G1 (68.4; 82.8), S (23,1; 11.3), G2/M (5.9; 4.2) percentages of the cells in NTC and ATC, respectively (Figure-3).

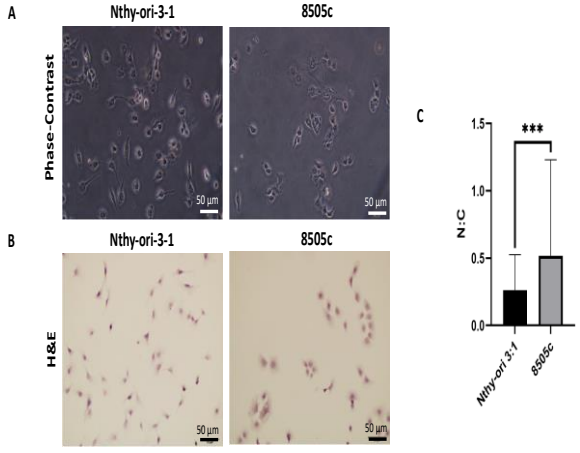

Figure-1. Representative images of Nthy-ori-3-1 and 8505c cells. A) Phase-contrast images. B) H&E images. C) N: C ratios of Nthy-ori-3-1 and 8505c cells. These two different representations of cells showed that while normal thyroid cell line Nthy-ori-3-1 cell have spindle morphology, anaplastic (8505c) cell line showed smaller and more rounded morphology. The ratio of N:C was higher in anaplastic thyroid cancer cells (Nthy-ori-3-1 0.26; and 8505c 0.52). (n:56) *** p<0.001

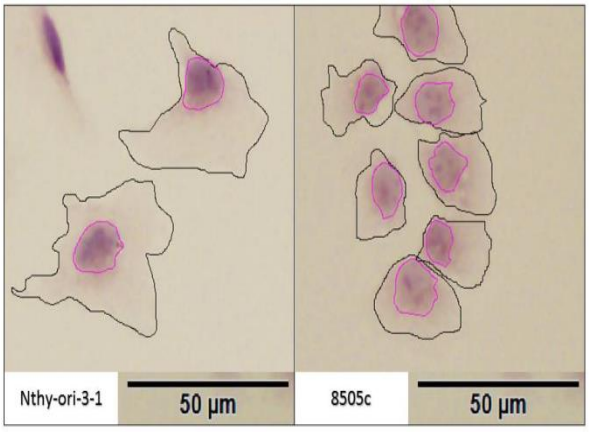

Figure-2. Representative demonstrations of nucleus and cell area of Nthy-ori-3-1 and 8505c cells. The magenta line displayed the nuclear region of the cells and the black line demonstrated the cell area.

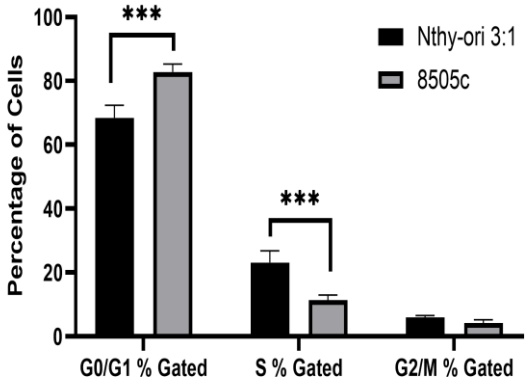

Figure-3. Cell cycle analysis of Nthy-ori-3-1 and 8505c cells. The percentage of cells in cell cycle phases (G0/G1, S and G2/M) were compared. Cells were mostly accumulated in G0/G1 phase in both cell lines. Nthy-ori-3-1 and 8505c cells were found in the cell cycle phases of G0/G1 (68.4; 82.8) S (23,1; 11.3), G2/M (5.9; 4.2), respectively. *** p<0.001

DISCUSSION

In our study, we evaluate the features of cell cycle and cell biology in ATC cell line and compare these properties with NTC line. The aggressive and rapid course of ATC causes this disorder to cure these patients impossible (11). Therefore, the use of cultured cell lines essential to determine the physiopathology of this rare disease and discover new drugs for the treatment (12).

ATC cells are known to have low apoptosis and high proliferation (13, 14). Wei et al. revealed that propranolol blockage caused decreased levels of Bcl-2 and the phosphorylated Akt in 8505c cells (15). Treatment with beta antagonist propranolol led to inhibit tumor cell proliferation, invasion, suppressed apoptosis and migration (16). In addition, Yang et al. reported that heme oxygenase-1 inhibitors induced cell cycle arrest and promoting tumor suppression in 8505c cancer cells (17). Moreover, the combination of proteasome inhibitors and TNF-related apoptosis-induced ligand (TRAIL) potentiated to induce damage of ATC cells (13). Furthermore, Flavopiridol decreased the levels of cell cycle proteins such as CDK9 and MCL-1, induced cell cycle arrest and inhibited colony formation, migration and growth in ATC cell lines (18).

Disarrangement of nuclear membrane is a pathognomonic feature of malignant cells. It was thought that cell shape abnormalities in malignancies may affect cellular functions such as cell cycle and apoptosis (19). The shape abnormalities of cells in cancers are still unknown and need to be elucidated (20). Moreover, enlarged nucleus was detected in cancer cells and resulted in the generation of the nucleus-to-cytoplasmic (N:C) ratio, determined as the ratio of the cross-sectional area of the nucleus divided by cytoplasm (21). Histology is the best way to asses for the identification of the N:C ratio however, it is not useful in practice due to analyze large populations of cells (22). The association between cell structure and function, the effect of the distribution of organelles (for example N/C ratio) in cells on cell biology, especially on the cell cycle, was evaluated in our study.

The cell cycle points out to sequence of phases through which dividing cells must give to deliver genetic material and cytoplasmic proteins and organelles to daughter cells. The cell cycle is consisted of following phases: G0 (not actively dividing), G1 (first gap, cell growth), S (DNA synthesis), G2 (further growth, second gap, reorganization of cellular contents), M (mitosis, which drives for 1 to 3 hours and lasts by formation of daughter cells. The stages of mitosis are prophase (P, chromosome condensation), metaphase (M, chromosome alignment), anaphase (A, chromosome segregation), and telophase (T, formation of daughter nuclei) (23). Similarly several cancers, studies to explore characteristic changes of the cell cycle may explain the biology of ATC. For example, mutations in p53 have been the most reported mutation in ATC (24, 25). Overexpression of cell cycle proteins such as epidermal growth factor receptor, cyclins D1 and E were detected in ATC (25-27). In addition, overexpression of some genes related with chromosomal instability and cell proliferation is a characteristic feature in ATC. This condition was not found in well-differentiated thyroid cancer and it is related with highly mutagenic ATC phenotype (28). On the other hand, Evans et al (29) revealed that decreased expression of p16 and p21 pointed out that there were several instabilities in the course of the normal cell cycle in ATC. Weinberger et al (30) reported that several cell cycle M-phase genes were extremely upregulated in ATC. In addition, they displayed dysregulation of the M-phase of the cell cycle in ATC for the first time. They suggested that treatment modalities targeting cell cycle mitosis might lead precious investigations in the future. It is still obscure and needs to be investigated concerning irregularity in cell cycle mediators of ATC whether related to rapid progression of this disease.

CONCLUSION

The rapid course and the rarity of ATC are significant barriers for clinical trials. Cultured cell lines are very important to discover the nature of this cancer and understand the course of the disease and find an effective target for treatment (12). Nowadays, it is urgent to find new treatment modalities for ATC patients.

Conflict of interest: The authors report no relevant conflict of interest or disclo-sures relevant to this viewpoint.

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