Original Article
Food Science and Technology

ISSN 0101-2061 (Print)
ISSN 1678-457X (Online)

DOI: https://doi.org/10.1590/fst.125521

Expression and correlation of cycde-2 protein and nuclear factor kB in serum of patients with papillary thyroid carcinoma

Yuanjing LI[1†], Xing WEN[2†], Jie WU[3*], Debing XIANG[1], Jin QUAN[1]

Abstract

To determine the clinical prediction role of the serum level of cyclooxygenase-2 (COX-2) and nuclear factor-κB (NF-κB) in papillary thyroid carcinoma (PTC) patients. 60 cases of PTC from February 2015 to May 2017 were collected to perform retrospective analysis and 10 patients with normal thyroid function were included as control group. We detected two immune factors: COX-2 and NF-κB in serum and analyse its relation to disease-free survival, overall survival, clinicopathological of PTC. COX-2 expression was associated with tumor tissue grade ($p = 0.029$), which was more abundant in III or IV stage ($p = 0.087$) patients. Relative to control group, PTC patients exhibited low NF-κB level. Survival analysis showed that the total survival time and disease-free survival time of low NF-κB level was shorter than that of control group ($p < 0.05$). Low COX-2 expression was associated with highly differentiated tumors ($p = 0.015$). However, COX-2 expression exerted little link with tumor grade, type, recurrent, age and clinical stage of PTC ($p > 0.05$).Further, regression analysis showed COX-2 and NF-κB expression is a risk factor for PTC, affecting prognosis. PTC patients exhibited low COX-2 and NF-κB expression, proving to correlate with the PTC development.

Keywords: cyclooxygenase-2; nuclear factor-κb; papillary thyroid carcinoma; serology.

Practical Application: The practical application statement: Our study shows that PTC patients exhibited low COX-2 and NF-κB expression, proving to correlate with the PTC development. However, large cohort clinical studies are required to confirm the findings in the future.

1 Introduction

Papillary thyroid carcinoma (PTC) refers to a conventional endocrine system tumor with gradually rising prevalence recently. Its prognosis through ultrasound and puncture examination is well established, proving to be accurate and effective, and the survival time within 5 years can exceed 90% following treatment (Perisa et al., 2017). However, with the increasing prevalence and mortality, diagnostics and therapeutics should be continuously developed to improve surgical techniques to better achieve the purpose of treating diseases (Scarpino et al., 2009). In recent years, several studies have demonstrated the anti-cancer properties of Cheddar cheese (Rafiq et al., 2020) or synbiotic sheep milk ice cream (Balthazar et al., 2021), indicating that they might be a novel agent for the treatment of cancers. However, the mechanism of PTC currently remains poor understood (Xing, 2013). Therefore, we should dig into its possible pathogenesis and use modern biological techniques and serological detection techniques to improve the diagnosis and prognosis of PTC (Erdem et al., 2011). Obtaining new diagnostic methods to improve the diagnosis and treatment of PTC has also become the direction of scholars to explore (Schneider & Chen, 2013). Cyclooxygenase-2 (Cox2) exerted a positive correlation with tumor and inflammation, which can increase the specific expression and release various kinds of inflammatory factors by activating the immune response in vivo. Low nuclear factor κB (NF-κB) level is related to tumorigenesis, which is an important manifestation and effective response to tumor growth and proliferation. A large number of studies have proved that NF-κB activated the expression of related cytokine genes, exerting an immune regulatory role in tumor growth, and inhibited tumor growth and promoted its apoptosis. To further explore the biological manifestations of two factors in PTC is expected to play an important role in evaluating tumor harm. Hence, this topic aimed to analyze the expression of COX-2 and NF-κB in patients with PTC. Molecular biology and immunohistochemical techniques are proposed to reveal the relationship between COX-2, NF-κB and clinical data of PTC patients. We analyze important pathological features, tumor immune regulation and tumorigenesis to reveal its prognostic relevance to PTC. We aimed to evaluate whether it is a good predictor for early diagnosis, condition monitoring, and preoperative auxiliary diagnosis of PTC.

Received 07 Nov., 2021
Accepted 09 Jan., 2022
[1] *Department of Oncology, Chongqing Jiangjin District Central Hospital, Chongqing, China*
[2] *Dialysis Room, Chongqing Jiangjin District Central Hospital, Chongqing, China*
[3] *Department of Critical Care Medicine, The Ninth People's Hospital of Chongqing, Chongqing, China*
*Corresponding author: miqian2848260046@126.com
[†] *Contributed equally to this work*

2 Materials and methods

2.1 Clinical information

All the subjects signed informed consent, a total of 60 patients with PTC admitted to our hospital from February 2015 to May 2017. In the same period, 10 cases of normal thyroid were included as control (Figure 1). Grading: G3, 25 cases; G2, 14 cases; G1, 21 cases, Stage II:26 cases, Phase III:14 cases, Stage IV:20 cases; General information: average 50 years old; Age 29-78, 32 males, 28 women. Approved by the hospital ethics committee.

Inclusion criteria: Confirmed PTC cases (Gonzalez-Gonzalez et al., 2011); Consult to staging principles for TNM malignant tumors (Krawczyk-Rusiecka et al., 2011); No diabetes, infectious diseases, hypertension; abnormal thyroid function in liver and kidney.

2.2 Reagents and instruments required

Reagent

COX-2 and COX-2 sheep anti-mouse HRP antibody, NF-κB ELISA detection kit were from Shanghai Ruizi Biotechnology; Goat anti-rabbit IgG was from Wuhan Ph.D. biotechnology; IgG of HRP labeled goat anti-mouse and HRP labeled goat anti-mouse

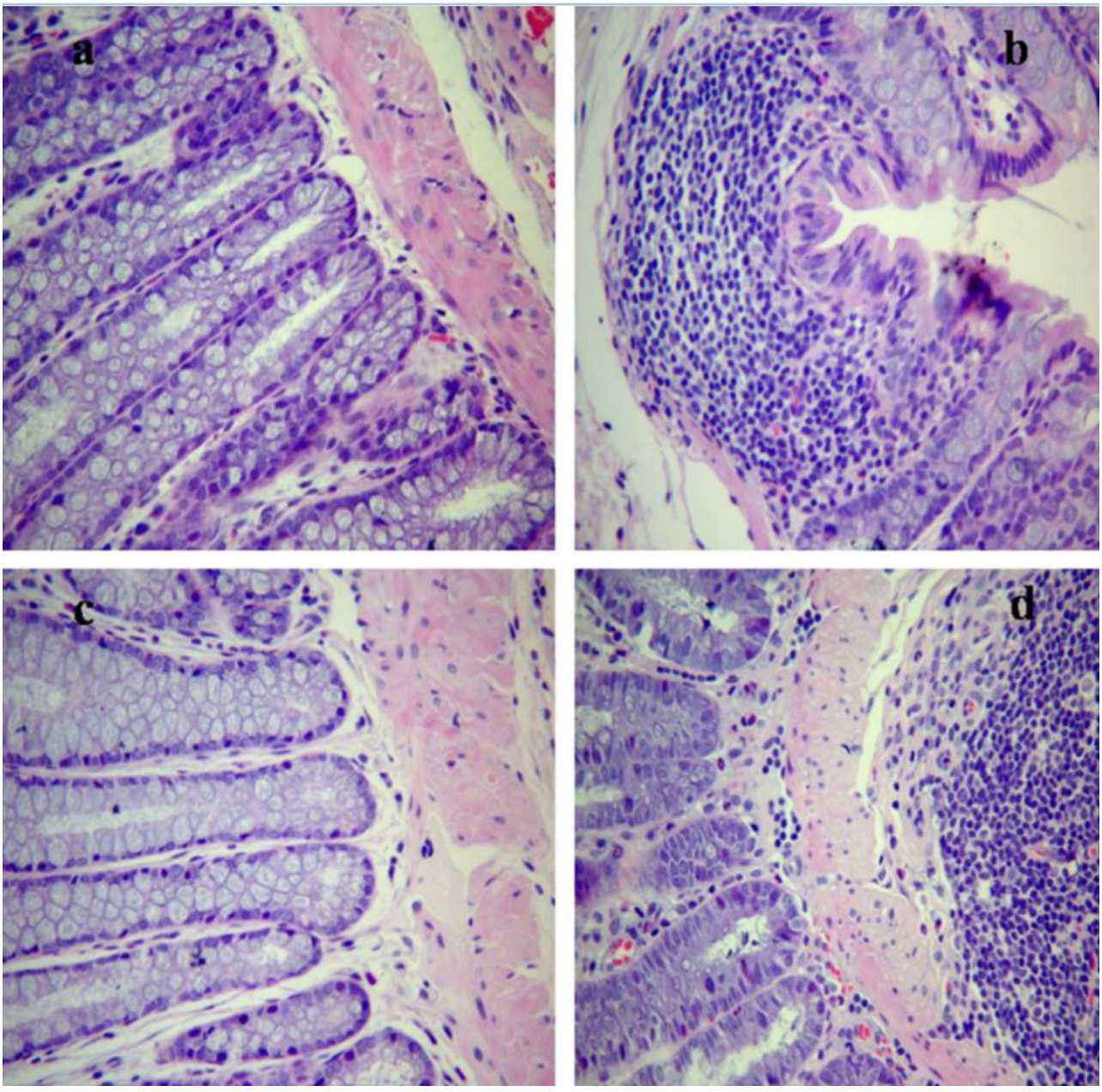

Figure 1. Flow chart of the study. COX expression in normal group (A), in PTC (B); NF-κB expression in normal group (C) and in PTC (D). A group and B group, $p < 0.05$; C group and D group, $p < 0.05$.

IgG were from Abcam (American); Immunohistochemical kit and NF-κB antibody were from Shanghai Biyuntian Biological.

Principal instruments

High speed freezing centrifuge and gel imager came from ABI (USA); -80 °C-4 °C refrigerator came from Panasonic (Japan); Table centrifuge, automatic immunoassay system came from Biofugestratos (German); Inverted phase contrast microscope was from thermo company (German); Enzyme marker was from Beijing 61 instrument factory; Veriti fast instrument was from Olympus (Japan).

2.3 Method

Detection COX-2 and NF-κB by ELISA

The serological extraction kit was used to extract serum, and the expression of serological COX-2 and NF-κB was detected by ELISA kit. Absorbed 50 μL serological samples into 96-well plate, incubated at 37 °C for 1 h; Added 50 μL enzyme labeled antibody, incubated at 37 °C for 1 h and chromogenic solution were mixed. The OD value was determined by enzyme labeling instrument.

Immunohistochemistry analysis for COX-2 and NF-κB

Tissue samples of PTC were repaired and sealed, transparent in the PBS. Added PBS to dilute COX-2 and NF-κB antibody, incubated for 2~3 h or 4 °C overnight. Add 0.05% triton x-100 of final concentration to the slide to wash. Then incubated in 1:3000 anti-sheep and mouse second antibody for 1 h. After washing, employed DAB for final coloration.

Statistical methods

All data were performed analysis statistics in spss23.0. Multivariate Cox proportional risk regression was employed for survival rate analysis. The relationship between clinicopathological parameters and the two genes in this study was measured by Pierce test. Measurement data were expressed as the mean of ± standard deviation representation. Logistic regression analysis was used to analyze the expression level of two genes and influencing factors of PTC. $p < 0.05$ indicates a significant difference.

3 Results

3.1 Expression of COX-2 and NF-κB in PTC

ELISA was employed to evaluate COX-2 and NF-κB abundance, and their link with various pathological factors stratification factors in PTC was analysed. Immunohistochemical revealed d COX-2 preferred to located in cytoplasmic site of epithelial cells. High abundance of COX-2 exhibited low correlation with III or IV stage ($P = 0.087$), however exerted better correlation with low grade of tumor tissue ($P = 0.029$). Relative to control group, PTC patients presented low abundance of NF-κB, which owned correlation with clinical status ($P < 0.05$), But showed Not little correlation with recurrent and clinical staging, tumor type and grade of patients ($P > 0.05$). Seen in Table 1.

3.2 Survival analysis

Tumor high differentiation is associated with low expression of COX-2. The disease-free survival time and total survival time in patients with low NF-κB expression were shorter than those in the control group ($p < 0.05$). Both the low COX-2 and NF-κB in PTC can be used as independent prognostic factors for PTC, as shown in Table 2 and Table 3.

3.3 ssessed risk factors and low COX 2 expression in PTC by logistic regression analysis

Seen in Table 4, patients with low COX-2 expression exhibited increased the risk of pathogenesis by 16.00%. In terms of metastasis risk, low COX-2 expression increased the risk of lymphatic metastasis by 16.00% in PTC.

Table 1. Relationship between COX-2 and NF-κB expression with clinicopathological factors in PTC.

Factors	Cases	High COX-2 expression	p value	High NF-κB expression	p value
Total cases	60	39.4%(23/60)		47.9%(28/60)	
Age/year					
≤50	24	44.7%(10/24)	0.357	42.1%(10/24)	0.442
>50	36	35.7%(13/36)		51.8%(18/36)	
Histological grade					
1/2	35	54.3%(19/35)	0.029	40.0%(14/35)	0.275
3	25	30.5%(7/25)		52.5%(13/25)	
FigureO staging					
II	26	50.8%(14/26)	0.087	34.6%(9/26)	0.166
III/ IV	34	33.8%(11/34)		52.9%(18/34)	
Lymph node status					
Positive	20	36.4%(7/20)		59.1%(12/20)	
Negative	36	55.6%(20/36)	0.179	41.7%(15/36)	0.276
Unknown	4	50%(2/4)		50%(2/4)	

3.4 Logistic regression analysis of low NF-κB expression and risk factors for the pathogenesis and metastasis of PTC

Seen in Table 5, patients with low NF-κB expression presented raised risk of pathogenesis by 28.00%. In regard with metastasis risk, low COX-2 expression enhanced the risk of lymphatic metastasis by 27% in PTC.

4 Discussion

The incidence of PTC, the common head and neck tumors, is constantly increasing, which affects the health of the patients. Therefore, the development of sensitivity indicators to improve early diagnosis has been a hot issue constantly explored by clinicians and biologists (Grogan et al., 2010). Early differential diagnosis is of positive significance for early diagnosis and treatment, and is of great help in improving the survival rate of patients (Kaur et al., 2012). At the same time, the diagnosis technology of PTC is improved, but the prognosis is still not ideal. Owing to the early detection is not timely, most of them are advanced visits, so often corresponding with the distal metastasis, the best diagnosis and treatment opportunities are lost, which brings difficulties to the treatment and prognosis of the disease. The standard treatment protocol for this disease is to reduce tumor cells in order to improve the body's immunity, thus Figurehting the development of cancer cells and reducing the possibility of distant metastasis (Xing, 2013). Early prediction of early tumor metastasis and recurrence, and the discovery of early detection accuracy and reliable indicators are important methods to improve the prediction effect (Chatterjee & Zetter, 2005; Eccles & Welch, 2007). At present, the study of gene level is still in its infancy, and the sensitivity of biomarkers, early diagnostic indicators and prognostic metastasis indicators need to be found to improve the evaluation effect of disease. For metastatic and recurrent PTC, effective gene and molecular markers are of great value in the development of targeted therapeutic drugs (Kajita et al., 2005). Finding good biomarkers is important for targeted therapy, so this study aims to find targets in combination with clinical experience to provide guidance for prognostic judgment in disease therapy.

NF-κB expression is reported to exist in PTC according to the literature (Pacifico & Leonardi, 2010). but the role in PTC has not been fully elucidated (Gao et al., 2018). NF-κB, as an important transcription factor, receives the regulation of interferon signal. As a nuclear transcription factor, it can directly modulate the upstream and downstream signaling pathways and directly enter the nucleus, thus activating the expression of multiple target genes and multidirectional nuclear translocation, thus combining different DNA structures and identifying DNA sequences. A large number of studies have confirmed that NF-κB has the biological function of participating in tumor, plays the role of apoptosis regulation, and has good functional expression. Downstream signaling pathways of NF-κB also have some effects. COX-2 is an important marker of expression in a class of immune cells and can specifically bind to cytokine expression. Studies have shown that COX-2 level is low in (Cheng et al., 2018) lung cancer, liver cancer and bowel cancer. By activating the intracellular ERK1/2 pathway and phosphorylation, it plays an important role in regulating tumor growth. NF-κB and COX2 are correlated. COX-2 can stimulate immune-related signals to stimulate the expression of NF-κB and play a positive feedback lease. At the same time, NF-κB can also promote tumor angiogenesis. The expression of COX-2 is affected. The two can affect each other

Table 2. Analysis of survival time without disease of patients by multivariable Cox proportional risk regression model.

Variables	Risk ratio (95% CI)	p value
Low COX-2 expression	0.395 (0.186–0.835)	0.015
Low NF-κB expression	0.716 (0.295–1.725)	0.456
>2 level	4.495 (1.448–13.975)	0.009
>I stage	0.726 (0.51–1.026)	0.069
>60 years	2.155 (1.098–4.274)	0.028

[CI]Confidence Interval.

Table 3. Analysis of total survival time of patients of patients by multivariable Cox proportional risk regression model.

Variables	Risk ratio (95% CI)	p value
Low COX-2 expression	0.304 (0.14–0.633)	0.002
Low NF-κB expression	0.825 (0.35–1.92)	0.647
>2 level	4.35 2(1.39–13.63)	0.013
>I stage	0.704(0.49–1.01)	0.059
>60 years	2.105 (1.06–4.16)	0.035

[CI]Confidence Interval.

Table 4. Assessed risk factors and low COX 2 expression in PTC by logistic regression.

Variables	β	SE	X2	p value	OR	95% CI
Low COX-2 expression						
Morbidity	1.098	0.465	5.289	0.023	2.957	1.176-7.548
Lymphatic metastasis	0.032	0.295	0.012	0.014	1.036	0.576-1.869

[SE]Standard Error. [OR]Odd Ratio. [CI]Confidence Interval.

Table 5. Logistic regression analysis of low NF-κB expression and risk factors for the pathogenesis and metastasis of PTC.

Variables	β	SE	X2	p value	OR	95% CI
Low NF-κB expression						
Morbidity	1.076	0.463	5.498	0.019	2.936	1.198-7.487
Lymphatic metastasis	0.615	0.247	6.256	0.015	1.852	1.148-2.973

[SE]Standard Error. [OR]Odd Ratio. [CI]Confidence Interval.

and contribute to a better understanding of the biological process of PTC, but there is a lack of relevant reports.

This study first investigated the expression of NF-κB and COX-2 in PTC. The pathological and survival time of the disease was associated with the expression of NF-κB and COX-2. The results showed that NF-κB expression was consistent, low in PTC, and positively correlated with the degree of malignancy. There was a low correlation between tissue grade and tumor type, stage, lymphatic metastasis and NF-κB expression. COX-2 functions as potential tumor suppressor (Lin et al., 2018). Other studies have confirmed, COX-2 regulated cell cycle. COX-2 overexpression promotes periodic arrest (Li et al., 2017), functioning as G2/M checkpoints. As a result, elevated expression of COX-2 and NF-κB plays an important role in tumor resistance (Zhao et al., 2015). COX-2 and NF-κB were also reported to own a correlation with microvessel density, when the expression was decreased, angiogenesis was obvious. However, the mechanism of COX-2 and NF-κB in tumor regulation needs further clarification. According to the results of this study, COX-2 and NF-κB are potential molecular markers, important for tumor prediction, whose low expression (Lv et al., 2016) in PTC were forward to in-depth monitoring and exploration for the diagnosis and treatment of high-risk patients to provide more targeted treatment (Benvenga & Koch, 2014).

5 Conclusion

To sum up, COX-2 and NF-κB expression decreased in PTC patient, reducing disease-free and overall survival time, but the relationship with PTC needs to be further explored to better guide clinical precision judgment and improve prognosis judgment.

References

Balthazar, C. F., de Moura, N. A., Romualdo, G. R., Rocha, R. S., Pimentel, T. C., Esmerino, E. A., Freitas, M. Q., Santillo, A., Silva, M. C., Barbisan, L. F., Cruz, A. G., & Albenzio, M. (2021). Synbiotic sheep milk ice cream reduces chemically induced mouse colon carcinogenesis. *Journal of Dairy Science*, 104(7), 7406-7414. http://dx.doi.org/10.3168/jds.2020-19979. PMid:33934866.

Benvenga, S., & Koch, C. A. (2014). Molecular pathways associated with aggressiveness of papillary thyroid cancer. *Current Genomics*, 15(3), 162-170. http://dx.doi.org/10.2174/1389202915999140404100958. PMid:24955023.

Chatterjee, S. K., & Zetter, B. R. (2005). Cancer biomarkers: knowing the present and predicting the future. *Future Oncology (London, England)*, 1(1), 37-50. http://dx.doi.org/10.1517/14796694.1.1.37. PMid:16555974.

Cheng, X., Yao, X., Xu, S., Pan, J., Yu, H., Bao, J., Guan, H., Lu, R., & Zhang, L. (2018). Punicalagin induces senescent growth arrest in human papillary thyroid carcinoma BCPAP cells via NF-kappaB signaling pathway. *Biomedicine and Pharmacotherapy*, 103, 490-498. http://dx.doi.org/10.1016/j.biopha.2018.04.074. PMid:29677534.

Eccles, S. A., & Welch, D. R. (2007). Metastasis: recent discoveries and novel treatment strategies. *Lancet*, 369(9574), 1742-1757. http://dx.doi.org/10.1016/S0140-6736(07)60781-8. PMid:17512859.

Erdem, H., Gundogdu, C., & Sipal, S. (2011). Correlation of E-cadherin, VEGF, COX-2 expression to prognostic parameters in papillary thyroid carcinoma. *Experimental and Molecular Pathology*, 90(3), 312-317. http://dx.doi.org/10.1016/j.yexmp.2011.01.008. PMid:21335003.

Gao, Y., Elamin, E., Zhou, R., Yan, H., Liu, S., Hu, S., Dong, J., Wei, M., Sun, L., & Zhao, Y. (2018). FKBP51 promotes migration and invasion of papillary thyroid carcinoma through NF-kappaB-dependent epithelial-to-mesenchymal transition. *Oncology Letters*, 16(6), 7020-7028. PMid:30546435.

Gonzalez-Gonzalez, R., Bologna-Molina, R., Carreon-Burciaga, R. G., Gomezpalacio-Gastelum, M., Molina-Frechero, N., & Salazar-Rodriguez, S. (2011). Papillary thyroid carcinoma: differential diagnosis and prognostic values of its different variants: review of the literature. *ISRN Oncology*, 2011, 915925. http://dx.doi.org/10.5402/2011/915925. PMid:22432054.

Grogan, R. H., Mitmaker, E. J., & Clark, O. H. (2010). The evolution of biomarkers in thyroid cancer-from mass screening to a personalized biosignature. *Cancers (Basel)*, 2(2), 885-912. http://dx.doi.org/10.3390/cancers2020885. PMid:24281099.

Kajita, S., Ruebel, K. H., Casey, M. B., Nakamura, N., & Lloyd, R. V. (2005). Role of COX-2, thromboxane A2 synthase, and prostaglandin I2 synthase in papillary thyroid carcinoma growth. *Modern Pathology*, 18(2), 221-227. http://dx.doi.org/10.1038/modpathol.3800285. PMid:15475935.

Kaur, S., Baine, M. J., Jain, M., Sasson, A. R., & Batra, S. K. (2012). Early diagnosis of pancreatic cancer: challenges and new developments. *Biomarkers in Medicine*, 6(5), 597-612. http://dx.doi.org/10.2217/bmm.12.69. PMid:23075238.

Krawczyk-Rusiecka, K., Wojciechowska-Durczynska, K., Cyniak-Magierska, A., Adamczewski, Z., Galecka, E., & Lewinski, A. (2011). COX-2 expression in papillary thyroid carcinoma (PTC) in cytological material obtained by fine needle aspiration biopsy (FNAB). *Thyroid Research*, 4(1), 3. http://dx.doi.org/10.1186/1756-6614-4-3. PMid:21214962.

Li, W., Ming, H., Sun, D., Li, W., Wang, D., Zhang, G., & Tan, J. (2017). The relationship between BRAFV600E, NF-kappaB and TgAb expression in papillary thyroid carcinoma. *Pathology, Research and Practice*, 213(3), 183-188. http://dx.doi.org/10.1016/j.prp.2016.12.022. PMid:28214213.

Lin, Y., Ma, Q., Li, L., & Wang, H. (2018). The CXCL12-CXCR4 axis promotes migration, invasiveness, and EMT in human papillary thyroid carcinoma B-CPAP cells via NF-kappaB signaling. *Biochemistry and Cell Biology*, 96(5), 619-626. http://dx.doi.org/10.1139/bcb-2017-0074. PMid:29316404.

Lv, N., Shan, Z., Gao, Y., Guan, H., Fan, C., Wang, H., & Teng, W. (2016). Twist1 regulates the epithelial-mesenchymal transition via the NF-kappaB pathway in papillary thyroid carcinoma. *Endocrine*, 51(3), 469-477. http://dx.doi.org/10.1007/s12020-015-0714-7. PMid:26289126.

Pacifico, F., & Leonardi, A. (2010). Role of NF-kappaB in thyroid cancer. *Molecular and Cellular Endocrinology*, 321(1), 29-35. http://dx.doi.org/10.1016/j.mce.2009.10.010. PMid:19879919.

Perisa, M. M., Sarcevic, B., Troselj, K. G., Grsic, K., Sitic, S., & Seiwerth, S. (2017). Expression of nm23-H1 and COX-2 in thyroid papillary carcinoma and microcarcinoma. *Oncology Letters*, 13(5), 3547-3555. http://dx.doi.org/10.3892/ol.2017.5876. PMid:28521457.

Rafiq, S., Gulzar, N., Huma, N., Hussain, I., & Murtaza, M. S. (2020). Evaluation of anti-proliferative activity of Cheddar cheeses using colon adenocarcinoma (HCT-116) cell line. *International Journal of Dairy Technology*, 73(1), 255-260. http://dx.doi.org/10.1111/1471-0307.12665.

Scarpino, S., Duranti, E., Stoppacciaro, A., Pilozzi, E., Natoli, G., Sciacchitano, S., Luciani, E., & Ruco, L. (2009). COX-2 is induced by HGF stimulation in Met-positive thyroid papillary carcinoma cells and is involved in tumour invasiveness. *The Journal of Pathology*, 218(4), 487-494. http://dx.doi.org/10.1002/path.2556. PMid:19391129.

Schneider, D. F., & Chen, H. (2013). New developments in the diagnosis and treatment of thyroid cancer. *CA: a Cancer Journal for Clinicians*, 63(6), 374-394. http://dx.doi.org/10.3322/caac.21195. PMid:23797834.

Xing, M. (2013). Molecular pathogenesis and mechanisms of thyroid cancer. *Nature Reviews. Cancer*, 13(3), 184-199. http://dx.doi.org/10.1038/nrc3431. PMid:23429735.

Zhao, S., Wang, Q., Li, Z., Ma, X., Wu, L., Ji, H., & Qin, G. (2015). LDOC1 inhibits proliferation and promotes apoptosis by repressing NF-kappaB activation in papillary thyroid carcinoma. *Journal of Experimental & Clinical Cancer Research*, 34, 146. http://dx.doi.org/10.1186/s13046-015-0265-z. PMid:26637328.