SciTeMed
Publishing Group

ORIGINAL ARTICLE

Archives of Otorhinolaryngology
Head & Neck Surgery

Presence and Quantity of COX-2 in Lymph Node Metastases of Patients with Head and Neck Cancer

Mehtap Boduc, MD[1]; Marion Roessler, MD[2]; Robert Mandic, MD[1]; Christoph Netzer, MD[1]; Christian Güldner, MD[1]; Ute Walliczek-Dworschak, MD[1]; Magis Mandapathil, MD[1,3*]

[1]Department of Otorhinolaryngology, Head and Neck Surgery, University of Marburg, Germany
[2]Department of Pathology, University of Marburg, Germany
[3]Department of Otorhinolaryngology, Head and Neck Surgery, Asklepios Clinic St. Georg, Hamburg, Germany

Abstract

Introduction: Cyclooxygenases (COX) catalyze the synthesis of prostanoids from arachidonic acid, present as two isoforms, namely COX-1 and COX-2. COX-2 has been shown to be associated with tumor progression in various cancer patients. The most prevalent type of cancer in the head and neck region is squamous cell carcinoma, with an overall poor outcome in part due to the early spread of metastatic cells.

Materials and Methods: Primary tumor specimens as well as lymph node specimens harvested during neck dissection of 289 patients with a diagnosis of HNSCC were analyzed and subjected to immunohistochemical and H-score analysis of COX-2 expression. Demographics, diagnoses, histopathology and succeeding outcome were subsequently analyzed.

Results: The primary cancer was squamous cell carcinoma in all patients (oral cavity n: 16, oropharynx n: 28, hypopharynx n: 11 and larynx n: 10 [stage III n=18; stage IVA n=45; stage IVB n=2]). H-score for COX-2 expression in the primary lesion as well as metastatic lymph nodes was significantly higher in the advanced stages compared with the early stages, with no significant differences among tumor locations. High COX-2 expression in primary lesions as well as metastatic lymph nodes was associated with poorer overall survival rates at a mean follow-up of 83.4 months (6 - 204 months).

Conclusion: COX-2 expression in HNSCC varied from the anatomical site, correlated positively with tumor stage and was associated with poor overall survival rates. Therefore, COX-2 expression in primary lesions as well as lymph node metastases appears to identify HNSCC patients at higher risk in all tumor sites. Adjuvant therapeutic approaches targeting COX-2 might be a promising tool in this patient population.

Introduction

Head and neck cancer represents the sixth most common cancer worldwide with an incidence of approximately 630,000/year [1]. More than 95% of these tumors are squamous cell carcinomas (SCC). Despite advances in the major therapeutic areas, such as surgery, radiotherapy and chemotherapy, the survival rates for patients suffering from this disease have not significantly improved within the past decades [2]. Therefore, identifying new molecular targets in head and neck squamous cell carcinomas (HNSCC) might contribute to improving cancer treatment and patients' overall prognosis.

Cyclooxygenases (COX) catalyze the synthesis of prostanoids from arachidonic acid. There are two isoforms of COX, namely COX-1 and COX-2 [3]. COX-1 is constitutively expressed in various cells, whereas COX-2 is an inducible enzyme. The induction of the COX-2 gene is stimulated by various factors, such as cytokines, oncogenes and carcinogens. It is reported to be predominantly induced and activated in numerous pathological conditions, such as inflammation and cancer [4,5]. It has been revealed from various studies that COX-2 is overexpressed in numerous human tumors, including HNSCC [6-8]. Further, COX-2 seems to play an important role in carcinogenesis and tumor progression, as it has been shown to be upregulated in transformed cells, premalignant as well as malignant lesions [9].

In oral squamous cell carcinoma, it has been shown by Pandey et al., that COX-2 expression has been significantly upregulated in OSCC compared to normal mucosa and oral dysplasia [10]. Further, COX-2 has been shown to promote tumor progression by inducing various pathways in critical stages of malignant disease [11]. Its overexpression and activity stimulate cell division, angiogenesis and metastases [12,13]. Studies suggest that COX-2 contributes to tumor progression by modulating the immune system to reduce anti-tumor immune responses [14,15]. Also, it has been suggested to act on tumor cells by promoting their mitotic activity and subsequently aid the conversion of premalignancy to invasive tumors [16]. Further, COX-2 has been described to induce angiogenic factors, such as vascular endothelial growth factor (VEGF) and fibroblastic growth factor, and therefore promotes tumor angiogenesis, tumor cell migration, and the formation of local and distant metastases [17,18]. Overexpression of COX-2 in primary tumor tissues has been shown to correlate negatively with patients' outcome and positively with tumor progression and recurrence rates in numerous tumors, including HNSCC [19,20].

***Correspondence:** Magis Mandapathil, MD, PhD
Department of Otorhinolaryngology, Head and Neck Surgery, University of Marburg; Department of Otorhinolaryngology, Head and Neck Surgery, Asklepios Clinic St. Georg, Hamburg, Germany.
Email: m.mandapathil@asklepios.com

Received: June 8, 2017; **Accepted:** July 10, 2017; **Published:** August 11, 2017

Archives of Otorhinolaryngology-Head & Neck Surgery. 2017;1(2):1
DOI: 10.24983/scitemed.aohns.2017.00024

However, its role in lymph node metastases in HNSCC has not been well established so far. The aim of the present study was to investigate COX-2 expression in primary tumors and lymph node metastases in HNSCC patients of various tumor sites and correlate these to patients' overall survival.

Materials and Methods

Patient Population

Following approval by the Institutional Review Board, the records of 289 patients, with a diagnosis of squamous cell carcinoma of the head and neck cancer between 1999 and 2012, were identified from an institutional database. Inclusion criteria were no prior treatment as well as surgical resection of the tumor, including at least a unilateral neck dissection for clinically metastatic disease. Patients, who did not undergo surgery, including a neck dissection as the primary treatment modality, were excluded from the study. Also, patients with a pathological N0 neck were excluded from the study. Altogether, 65 patients met these criteria and were included in the study.

Immunohistochemistry

Representative slides from each block were cut and stained as indicated below. Slides were reviewed by a pathologist (MR) to confirm histology. Blocks were sliced and deparaffinized sections were subjected to immunohistochemical staining using the following antibodies: anti-COX-2 (Novus Biological, Wiesbaden, Germany) and anti-mouse-IgG1 (DAKO, Santa Clara, CA, USA). Appropriate negative controls (without primary antibodies) were included for each specimen.

The staining was evaluated independently by two investigators blinded to the sample type. COX-2 expression was assessed in the primary tumor lesions as well as metastatic lymph nodes using the semi-quantitative scoring method. Whole sections were observed under the microscope [low power (LP) and high power (HP)] and positive cells were counted in four successive fields selected randomly in primary tumor lesions as well as metastatic lymph node (400 × HP). The scoring standard for staining was described as follows: negative (0, no staining in an HP field), weakly positive (1, staining only in an HP field), moderately positive (2, staining in an LP field), and strongly positive (3, positive staining in an LP field). The scores of a section in four successive HP fields were averaged (0, positive cell percentage <5%; (1) positive cell percentage = 6–25%; (2) positive cell percentage = 26–50%; (3) positive cell percentage = 51–75%; (4) positive cell percentage >75%). The scores of positive cell density and percentage were summed up to give the total score. For subgroup analysis, an H-score of ≤ 200 was considered "low" and an H-score of >200 was considered "high".

Statistical Analysis

Statistical analysis was performed using the student t test to compare variables within groups. The Kaplan-Meier estimator was employed for survival analysis and the generated curves were compared with Cox's F-test. The endpoint for the study was overall survival (OS). OS was defined as the time from sample collection to death or censoring. Censoring was defined as loss of follow-up or alive at the end of follow-up. A p-value of ≤ 0.05 was considered statistically significant.

Results

Patient Cohort

Patient demographics and tumor characteristics are shown in Table 1. 65 patients (55 males and 10 females; 59 ± 9 years) were retrospectively identified with a surgically treated tumor of the head and neck. The histology was squamous cell carcinoma in all patients, with 16 oral cavity lesions, 28 oropharyngeal, 11 hypopharyngeal and 10 laryngeal lesions.

Table 1. Clinicopathological Data of Patients Enrolled

Variable	No.
Number enrolled	65
Sex	
male	55
female	10
Tumor stage	
T-stage	
T1	20
T2	32
T3	10
T4	3
N-stage	
N1	21
N2a	5
N2b	27
N2c	10
N3	2
Tumor location	
Hypopharynx	11
Oropharynx	28
Larynx	10
Oral cavity	16
Tumor differentiation	
G1	2
G2	51
G3	12
Mean age at diagnosis (years)	59

COX-2 expression in primary tumor lesions and lymph node metastases

COX-2 expression using the H-score was determined for primary tumor and lymph node metastases. Figure 1 shows representative staining for COX-2 in the primary lesion. Laryngeal lesions appear to show the highest extent of COX-2 expression in the primary lesions and lymph node metastases, however, without significant differences compared with other sites (Figure 2). Adjacent tissues, as well as normal mucosa, did not show any significant positivity for COX-2 (data not shown). As shown in Figure 3A, B and C, COX-2 expression correlated positively with T stage, N stage and tumor stage (Figure 3A, 3B and 3C).

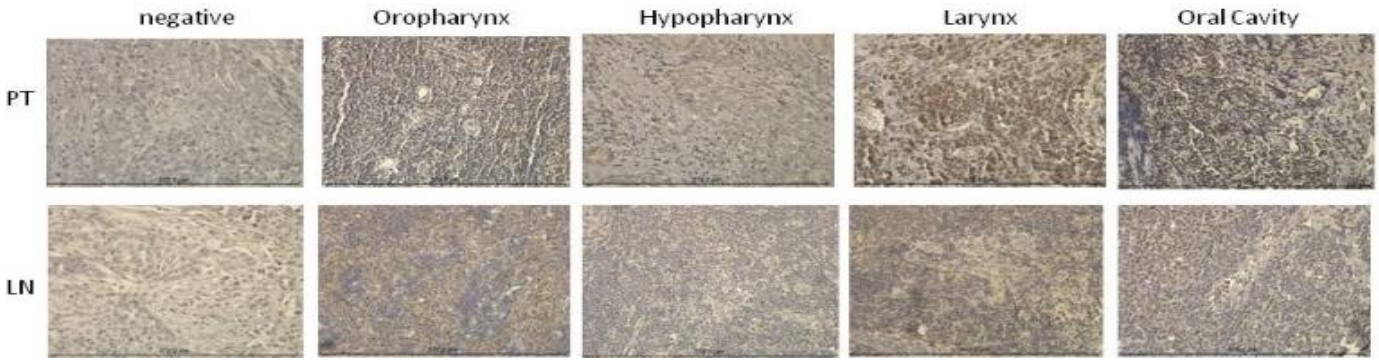

Figure 1. COX-2 staining patterns in primary tumor and lymph node specimens. Immunohistochemical analysis of COX-2 expression in primary tumor as well as lymph node specimens is shown. Representative slides of staining from the oropharynx, hypopharynx, larynx, and oral cavity with their lymph node specimens are shown respectively.

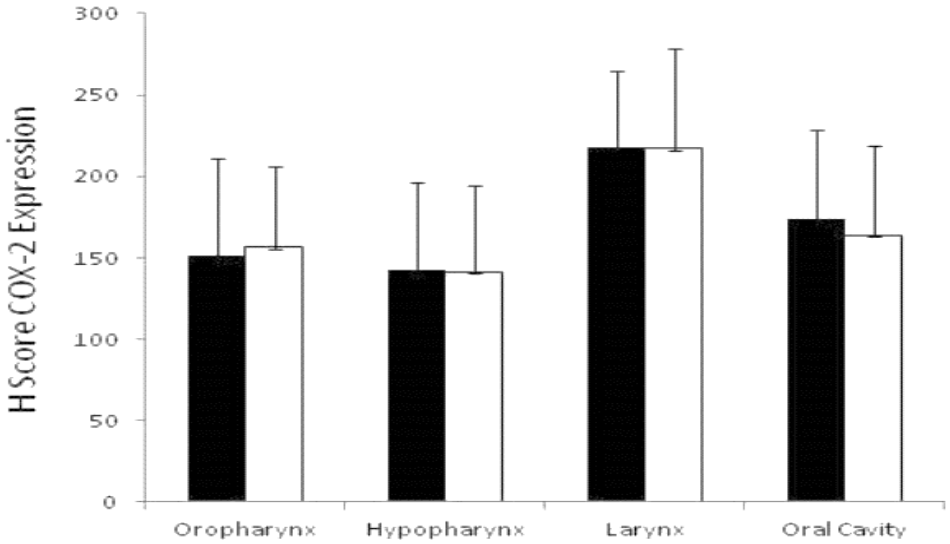

Figure 2. COX-2 expression in primary tumors and lymph node metastases in different HNSCC tumor locations. Primary tumor specimens and lymph node specimens harvested during a neck dissection were stained for COX-2 and immunohistochemically analyzed. Expression of COX-2 among different tumor locations was analyzed. The data are from 65 HNSCC patients.

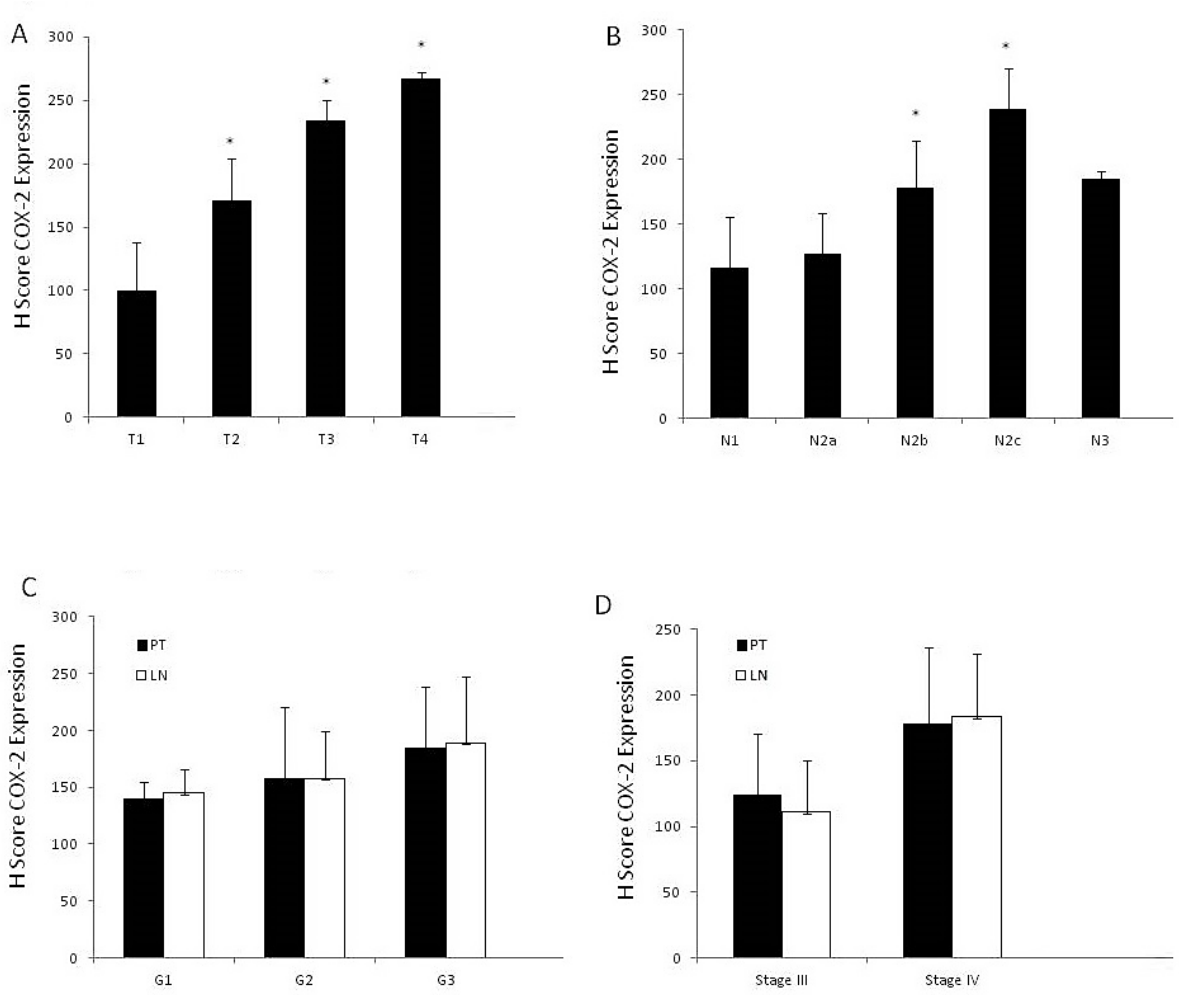

Figure 3. COX-2 expression in primary tumors and lymph node metastases. (A) Primary tumor specimens were stained for COX-2 and immunohistochemically analyzed. The data are from 65 HNSCC patients. **(B)** Lymph node specimens harvested during a neck dissection were stained for COX-2 and immunohistochemically analyzed. The data are from 65 individuals with HNSCC tumors **(C)** Immunohistochemical analysis of COX-2 expression in primary tumor specimens as well as lymph node specimens according to tumor stages. The data are from 65 HNSCC patients. Asterisks indicate a p-value of < 0.05.

Correlation of infiltrates to prognosis and outcome

Next, we wished to analyze the correlation of COX-2 expression in primary tumors and lymph nodes with the overall survival of the patients. Therefore, patients with a low H-score for COX-2 expression were compared with patients with a high H-score, as described in Materials and Methods. As shown in Figure 4A, high COX-2 expression in primary tumor specimens was associated with shorter overall survival (OS) ($p < 0.05$). Also, high COX-2 expression in metastatic lymph nodes showed significantly decreased OS rates ($p < 0.05$) (Figure 4B). High H-scores for the primary tumor as well as lymph node specimens did not correlate to disease recurrence in the analyzed patient population (Figure 4A and 4B).

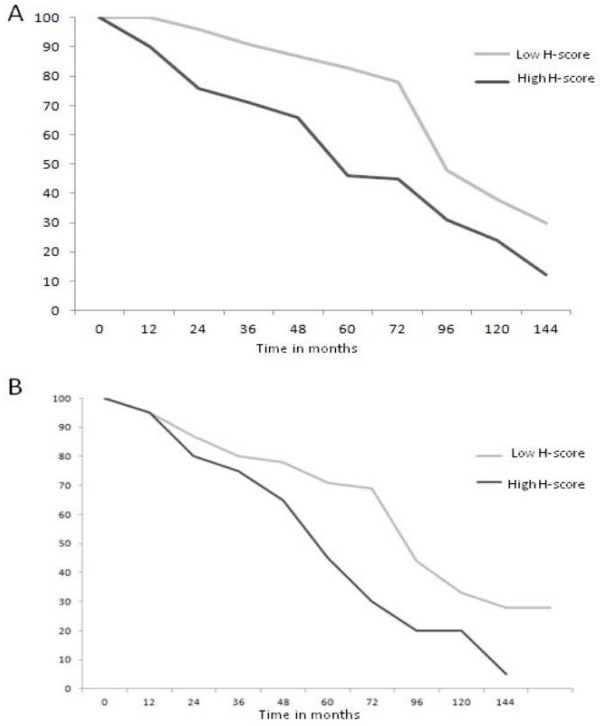

Figure 4. Survival analysis of HNSCC patients. (A) Kaplan-Meier overall survival curve, according to COX-2 expression (low versus high) in the primary tumor lesion. (B) Kaplan-Meier overall survival curve, according to COX-2 expression (low versus high) in the metastatic lymph nodes. The data are from 65 individuals with HNSCC tumors.

Discussion

The results of the present study show a higher expression for COX-2 in the advanced HNSCC compared with the early-stage HNSCC in the primary tumor as well as metastatic lymph nodes. Further, our data suggests not only a negative correlation of COX-2 expression in primary tumor to patients' overall prognosis in HNSCC, but also for COX-2 expression in metastatic lymph nodes to patients' overall prognosis. An overexpression of COX-2 has been reported previously for many various tumor types, including HNSCC [6-8]. In some of these malignancies, overexpression of COX-2 is associated with poor prognosis and low survival rate [21,22].

In the present study, overexpression of COX-2 in primary tumors as well as metastatic lymph nodes was associated with decreased OS rates compared to low COX-2 expression. The mechanisms of COX-2 overexpression during carcinogenesis are still not well understood. However, it has been shown in other tumor types that COX-2 overexpression can be a result of the inactivation of the tumor suppressor genes, like p53 [23] or activation of certain proto-oncogene, such as Ras [24]. The formation of regional and distant metastases is favored by the capacity of tumors to progress locally, which subsequently invade lymphatic and blood vessels, and promote neo-angiogenesis. Morita et al. showed that COX-2 promotes tumor lymphangiogenesis and lymph node metastasis in oral squamous cell carcinoma [25]. In their study, they showed a positive correlation of COX-2 expression to VEGF-C expression, a potent stimulator of lymphangiogenesis. Prostaglandin E2, as a major product of COX-2 overexpression and activity, has also been shown to increase tumor cell proliferation, angiogenesis, invasion, and metastasis in various tumors [26]. Prostaglandin E2 has been shown to inhibit tumor necrosis factor α (TNFα) and induce interleukin-10 (IL-10), and therefore contributing to profound immunosuppression [27,28]. Based on these findings, targeting the COX-2 pathway with selective COX-2 inhibitors seems to be a legitimate approach in anti-tumor therapy. Inhibitory effects of these agents on the growth and metastasis of tumors have been shown in various models [28,29]. Further, the positive effects of COX-2 inhibitors on the anti-cancer effects of radiation therapy as well as chemotherapy have been described in animal models [30,31]. Also, an increased expression of COX-2 in oral mucosal dysplasia and an overexpression in oral cancer reflect its role in the early stages of oral carcinogenesis as well as tumor progression [10]. In esophageal carcinoma, COX-2 overexpression was described to be associated with increased tumor invasion, more advanced tumor stages, poorer differentiation and prognoses, compared with cases with low COX-2 expression levels [32,33]. These results all indicate a crucial role of COX-2 in carcinogenesis and metastases.

In the present study, a positive correlation was found between the expression of COX-2 in advanced HNSCC of all sites, as well as a negative correlation to patients' outcome. Higher expression levels of COX-2 were present in the patients with advanced primary tumors and advanced lymph node metastasis, compared with those patients with early tumors. The increased expression of COX-2 in the advanced primary, as well as the metastatic disease, may reflect its role in the development and progression of HNSCC. Thus, according to the obtained results, COX-2 may be used for the molecular target in adjuvant therapy in HNSCC. It seems that selective COX-2 inhibitors may be beneficial in preventing the transformation of premalignant lesions to malignancies, and further tumor progression as well as the formation of metastases. However, there appears to be a wide range of pathways and factors that were associated with tumor progression comparable to COX-2, such as e. g ectonucleotidases [34]. Therefore, further studies are required to identify patient populations, who would benefit from adjuvant treatment modalities targeting the COX-2 pathway.

Conclusion

COX-2 expression in primary lesions, as well as lymph node metastases, appears to identify HNSCC patients at higher risk in all tumor sites. Adjuvant therapeutic approaches targeting COX-2 might be a promising tool in this patient population.

Article Information

Conflict of Interest Disclosures: None
Funding: None

Keywords

Head and neck cancer; lymph node metastases; cycloxygenase-2.

Reference

1. Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. CA Cancer J Clin 2005; 55: 74–108.
2. Argiris A, Karamouzis MV, Raben D, Ferris RL. Head and neck cancer. Lancet. 2008; 17; 371(9625): 1695-1709.
3. Agúndez JA, Blanca M, Cornejo-García JA, García-Martín E. Pharmacogenomics of cyclooxygenases. Pharmacogenomics. 2015; 16(5): 501-522.
4. Yu T, Lao X, Zheng H. Influencing COX-2 Activity by COX Related Pathways in Inflammation and Cancer. Mini Rev Med Chem. 2016; 16(15): 1230-1243.
5. Ricciotti E, FitzGerald GA. Prostaglandins and inflammation. Arterioscler Thromb Vasc Biol. 2011; 31(5): 986-1000.
6. Lin PC, Lin YJ, Lee CT, Liu HS, Lee JC. Cyclooxygenase-2 expression in the tumor environment is associated with poor prognosis in colorectal cancer patients. Oncol Lett. 2013; 6(3): 733-739.
7. Miglietta A, Toselli M, Ravarino N, Vencia W, Chiecchio A, et al. COX-2

expression in human breast carcinomas: correlation with clinicopathological features and prognostic molecular markers. Expert Opin Ther Targets. 2010; 14(7): 655-664.
8. Bonhin RG, de Carvalho GM, Guimarães AC, Rocha VB, Chone CT, et al. Histologic correlation of VEGF and COX-2 expression with tumor size in squamous cell carcinoma of the larynx and hypopharynx. Ear Nose Throat J. 2017; 96(4-5): 176-182.
9. Harris RE. Cyclooxygenase-2 (cox-2) and the inflammogenesis of cancer. Subcell Biochem. 2007;42: 93-126.
10. Pandey M, Prakash O, Santhi WS, Soumithran CS, Pillai RM. Over-expression of COX-2 gene in oral cancer is independent of stage of disease and degree of differentiation. Int J Oral Maxillofac Surg. 2008; 37(4): 379-383.
11. Cheng J, Fan XM. Role of cyclooxygenase-2 in gastric cancer development and progression. World J Gastroenterol. 2013; 19(42): 7361-7368.
12. Hugo HJ, Saunders C, Ramsay RG, Thompson EW. New Insights on COX-2 in Chronic Inflammation Driving Breast Cancer Growth and Metastasis. J Mammary Gland Biol Neoplasia. 2015; 20(3-4): 109-119.
13. Wang MT, Honn KV, Nie D. Cyclooxygenases, prostanoids, and tumor progression. Cancer Metastasis Rev. 2007; 26(3-4): 525-534.
14. Fulton AM, Ma X, Kundu N. Targeting prostaglandin E EP receptors to inhibit metastasis. Cancer Res. 2006; 66(20): 9794-9797.
15. Chen EP, Smyth EM. COX-2 and PGE2-dependent immunomodulation in breast cancer. Prostaglandins Other Lipid Mediat. 2011; 96(1-4): 14-20.
16. Mandapathil M, Whiteside TL. Targeting human inducible regulatory T cells (Tr1) in patients with cancer: blocking of adenosine-prostaglandin E_2 cooperation. Expert Opin Biol Ther. 2011; 11(9): 1203-1214.
17. Watson AJ. Chemopreventive effects of NSAIDs against colorectal cancer: regulation of apoptosis and mitosis by COX-1 and COX-2. Histol Histopathol. 1998; 13(2): 591-597.
18. Zhi YH, Liu RS, Song MM, Tain Y, Long J, et al. Cyclooxygenase-2 promotes angiogenesis by increasing vascular endothelial growth factor and predicts prognosis in gallbladder carcinoma. World J Gastroenterol. 2005; 11(24): 3724-3728.
19. Hu H, Han T, Zhuo M. et al. Elevated COX-2 Expression Promotes Angiogenesis Through EGFR/p38-MAPK/Sp1-Dependent Signalling in Pancreatic Cancer. Sci Rep. 2017; 28;7(1): 470.
20. Güler SA, Uğurlu MÜ, Kaya H, Şen S, Nazlı Y, Güllüoğlu BM. Impact of cyclooxygenase-2 over-expression on the prognosis of breast cancer patients. Ulus Cerrahi Derg. 2015 Jun 1;32(2):81-88.
21. Kuźbicki Ł, Lange D, Stanek-Widera A, Chwirot BW. Intratumoral expression of cyclooxygenase-2 (COX-2) is a negative prognostic marker for patients with cutaneous melanoma. Melanoma Res. 2016; 26(5): 448-456.
22. Song J, Su H, Zhou YY, Guo LL. Cyclooxygenase-2 expression is associated with poor overall survival of patients with gastric cancer: a meta-analysis. Dig Dis Sci. 2014; 59(2): 436-445.
23. Lin PC, Lin YJ, Lee CT, Liu HS, Lee JC. Cyclooxygenase-2 expression in the tumor environment is associated with poor prognosis in colorectal cancer patients. Oncol Lett. 2013; 6(3): 733-739.
24. Choi EM, Kim SR, Lee EJ, Han JA. Cyclooxygenase-2 functionally inactivates p53 through a physical interaction with p53. Biochim Biophys Acta. 2009; 1793(8): 1354-1365.
25. Moazeni-Roodi A, Allameh A, Harirchi I, Motiee-Langroudi M, Garajei A. Studies on the Contribution of Cox-2 Expression in the Progression of Oral Squamous Cell Carcinoma and H-Ras Activation. Pathol Oncol Res. 2017; 23(2): 355-360.
26. Morita Y, Hata K, Nakanishi M, Nishisho T, Yura Y, et al. Cyclooxygenase-2 promotes tumor lymphangiogenesis and lymph node metastasis in oral squamous cell carcinoma. Int J Oncol. 2012; 41(3):885-892.
27. O'Callaghan G, Houston A. Prostaglandin E2 and the EP receptors in malignancy: possible therapeutic targets?. Br J Pharmacol. 2015; 172(22): 5239-5250.
28. Kim R, Emi M, Tanabe K. Cancer immunosuppression and autoimmune disease: beyond immunosuppressive networks for tumour immunity. Immunology. 2006; 119(2): 254-264.
29. Cathcart MC, O'Byrne KJ, Reynolds JV, O'Sullivan J, Pidgeon GP. COX-derived prostanoid pathways in gastrointestinal cancer development and progression: novel targets for prevention and intervention. Biochim Biophys Acta. 2012; 1825(1): 49-63.
30. de Souza Pereira R. Selective cyclooxygenase-2 (COX-2) inhibitors used for preventing or regressing cancer. Recent Pat Anticancer Drug Discov. 2009; 4(2): 157-163.
31. Fujimura T, Ohta T, Oyama K, Miyashita T, Miwa K. Cyclooxygenase-2 (COX-2) in carcinogenesis and selective COX-2 inhibitors for chemoprevention in gastrointestinal cancers. J Gastrointest Cancer. 2007; 38(2-4): 78-82.
32. Choy H, Milas L. Enhancing radiotherapy with cyclooxygenase-2 enzyme inhibitors: a rational advance?. J Natl Cancer Inst. 2003; 95(19): 1440-1452.
33. Konturek PC, Kania J, Burnat G, Hahn EG, Konturek SJ. Prostaglandins as mediators of COX-2 derived carcinogenesis in gastrointestinal tract. J Physiol Pharmacol. 2005; 56 Suppl 5: 57-73.
34. Mandapathil M. Adenosine-mediated immunosuppression in patients with squamous cell carcinoma of the head and neck. HNO. 2016; 64(5): 303-309.