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CYCLOOXYGENASE-2 EXPRESSION IN ORAL SQUAMOUS CELL CARCINOMA

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Cyclooxygenase (COX), the key enzyme in prostaglandin cascade, is expressed in two isoforms: the constitutive COX-1 and the inducible COX-2. Hyper-expression of COX-2 has been implicated in the pathogenesis of colon-rectal cancer in humans but it appears to play a significant role as a tumour progression factor also in other forms of human cancer, including oral cancer. The aim of this study was to analyze the expression of COX-2, at the protein level, in 45 cases of oral squamous cell carcinoma. Standard immunohistochemical streptavidin-biotin peroxidase analysis was carried out with a highly specific antibody against human COX-2 and cell specific markers, in 45 oral squamous cell carcinomas. Our study revealed a moderate to high COX-2 expression in 35 out of the 45 oral squamous cell carcinoma specimens (77.8%). COX-2 expression appeared particularly abundant in the superficial ulcerated layers of relatively well differentiated carcinomas. However, we were unable to assess any statistically significant association between COX-2 hyper-expression and tumor site, tumor grading, tumor size, presence of lymph node metastases, tumor stage and age at onset, respectively. Interestingly, COX-2 expression was detected not only in areas of epithelial dysplasia adjacent to the primary layers (86% of the cases) but also in normal-appearing epithelium at the boundaries of squamous cell carcinomas (77%), indicating a possible involvement in tumour progression by the apparently normal tissue surrounding the lesion. Moreover, intense COX-2 staining was observed in endothelial cells of intra-tumour vessels and extra-tumour vessels adjacent to the tumour nests, in a high proportion of cases (82%). COX-2 positivity was associated with CD34 and VEGF positivity, indicating that these vessels were probably neo-formed.

From this study, as well as from other works, it appears that COX-2 is over-expressed in this important human malignancy. However, further studies are necessary to understand the exact magnitude of this over-expression and, mostly, the possible role of COX-2 in the pathogenesis and progression of oral cancer.

Several lines of evidence suggest that prostanoids may be involved in the pathobiology of human oral carcinogenesis (1-3). The enzyme cyclooxygenase (COX) catalyzes the first two steps of a cascade of biochemical reactions, leading to prostaglandin formation from arachidonic acid. COX is the rate-limiting enzyme in prostaglandin biosynthesis and is expressed in a constitutive (COX-1) and in an inducible (COX-2) isoform (4). Enhanced biosynthesis of prostaglandins, as a

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consequence of up-regulation of COX-2, can increase cell proliferation, promote angiogenesis and inhibit immune surveillance against cancer. Furthermore, over-expression of COX-2 may suppress apoptosis and enhance the ability of cancer cells to invade the surrounding tissues (5-7). In particular, COX-2 up-regulation seems to be involved in the mechanisms of bone destruction by growing cancer cells and tumour pain (8). Finally, COX-2 catalyzes the oxidation of the tobacco pro-carcinogen benzopyrene-7,8-dihydrodiol to benzopyrene-diol epoxide, a highly mutagenic carcinogen (9).

The aim of this immunohistochemistry study was to evaluate COX-2 gene expression at the protein level in 45 cases of oral squamous cell carcinoma (OSCC), in particular with regard to the distribution of this enzyme not only to the tumor cells but also to the surrounding tissues, such as normal-appearing epithelium, hyperplastic epithelium, dysplastic epithelium and neoformed blood vessels, as well as to the chronic inflammatory infiltrate accompanying the tumors. Various cell type markers were selected for immunohistochemical identification of the COX-2 expressing cells and a double immunostaining technique was employed in order to verify co-localization of cell type markers and COX-2.

MATERIALS AND METHODS

Selection of cases

Samples from 45 OSCC surgical resection specimens were obtained with a protocol that was reviewed and approved by the ethical review boards of Institutions 1 and 5. Patients had not previously been treated and received surgical treatment. Cases were included in this study only when a complete clinical history, ranging from 3 to 13 years was available. The study group consisted of 31 men (mean age = 63 years) and 14 women (mean age = 65 years). The histopathological grading and the morphological characteristics of inflammatory cells were assessed on paraffin H- and E-stained sections.

Immunohistochemistry

Single immunostaining was performed on 4μm sections by linked streptavidin biotin horseradish peroxidase (LSAB-HRP) and linked streptavidin biotin alkaline phosphatase (LSAB-AP) techniques. When needed, various primary Abs were used, applied on serial sections. Immunoreactive COX-2 was detected by a rabbit polyclonal Ab highly specific for the human protein, which recognized the unique 18 aminoacid C-terminus sequence of human COX-2 (4) (Cayman Chemical Co., Ann Arbor, MI, U.S.A.). This Ab as well as the other primary Abs was used under optimal dilution and incubation time conditions (dilution was performed in 0.05 M Tris-HCl buffer, pH 7.2-7.6, containing 1% bovine serum albumin). Thus, the rabbit polyclonal anti-human COX-2 Ab was diluted 1:250 and the incubation was prolonged for 120 min at 24 °C. Mouse monoclonal cell specific Abs (Novocastra Ltd, Newcastle, UK) were used in 60 min incubation at 24 °C, with the following dilutions: anti-CD1a Ab (RTU-CD1a-235) was used neat; anti-CD3 Ab (RTU-CD3-PS1) neat; anti-CD15Ab (RTU-CD15) neat; anti-CD20 Ab (RTU-CD-20-L26) 1:200; anti-CD34 Ab (RTU-END) 1:50; anti-CD57 Ab (NCL-NK1) 1:50; anti-CD68 (RTU-CD68) neat. Finally, an anti-vascular endothelial growth factor (VEGF) Ab (Santa-Cruz Biotechnology, Santa Cruz, CA) was also employed, diluted 1:100; the incubation was carried out for 60 m at 24 °C. Sequential double immunostaining was performed to further verify co-localization of COX-2 with the cell type markers. A biotinylated goat anti-rabbit Ig secondary Ab was used to detect the polyclonal anti-COX-2 Ab, followed by visualization by streptavidin conjugated to horseradish peroxidase. A biotinylated goat anti-mouse Ig secondary Ab was employed to detect the monoclonal cell marker specific Abs; positive reactions were revealed by streptavidin conjugated to alkaline phosphatase. Sections were counterstained with Mayer's haematoxilin and mounted using an aqueous mounting medium.

Immunostained sections were analyzed by a double-headed Leitz light microscope by two investigators (G.P. and L.L.M.), using a 40 x objective. The areas studied were selected by inspection at low-power magnification with the aid of a random table. A total number of 1,000 cells were evaluated for each case and for each Ab. The COX-2-positive fraction was determined, irrespective of staining intensity. Sections were examined without previous knowledge of the clinical and history data.

Statistical analysis

Multiple observations are presented as arithmetic means with standard deviations. The inter-rater reliability between the two investigators examining the immunostained sections was assessed by the Cohen's Kappa test, yielding kappa values higher than 0.70 in almost all instances. Normal distribution of the data was analyzed by the Kolmogorov-Smirnov test. Comparison of results was attained by the one-way ANOVA as well as by Kruskal-Wallis test followed by the Dunn's post test. Values of $p > 0.05$ were considered non-significant.

RESULTS

We investigated COX-2 expression in 45 cases of OSCC, with localization to the alveolar process (14 cases), to the lip (11 cases), to the tongue (8 cases), to the buccal mucosa (4 cases), to the floor of mouth (4 cases) and to the palate (4 cases). See Tab. I. As regards tumor grading, 16 cases could be classified as G1, 20 cases as G2 and 9 cases as G3 (Tab. I). Finally, based on the International Union Against Cancer (UICC) TNM classification of malignant tumors, 21 cases were assigned stage I, 10 cases stage II, 5 cases stage III and 9 cases stage IV (Tab. I).

COX-2 expression in OSCC

Immunohistochemical analysis of the specimens, carried out with a highly specific Ab, raised against a synthetic peptide including the unique 18 residue C-terminus sequence of the human COX-2 (4), revealed a substantial expression of this protein in OSCC cells (Fig. 1, panels A-C, and 3A). A positive COX-2 immunostaining was detected in 35 out of 45 OSCC examined (77.8%; Tab. I), with a distribution pattern characterized by localization of the immunoreactive protein at the cytoplasmic and perinuclear level (Fig. 1C) and a more intense positivity in the areas of the tumors with prevalence of well differentiated cells (Fig. 1A-B). Interestingly, COX-2 was strongly expressed in superficial ulcerated layers of well differentiated carcinomas (Fig. 1C).

In particular, 11 out of the 14 tumors localized on the alveolar process were COX-2 positive (average number of COX-2-positive cells, out of 1,000 cells examined, = 420 ± 366); 7 out of the 11 tumors localized on the lip (average number of COX-2-positive cells = 210 ± 247); 6 out of the 8 tumors localized on the tongue (average number of COX-2-positive cells = 365 ± 342); 4 out of the 4 tumors localized on the buccal mucosa (average number of COX-2-positive cells = 544 ± 403); 4 out of the 4 tumors localized on the floor of mouth (average number of COX-2-positive cells = 500 ±280) and 3 out of the 4 tumors localized on the palate (average number of COX-2-positive cells 440 ± 481). See Table 1. However, although the tumors localized on the lip seemed to include less COX-2 immunoreactive cells, on average, none of the differences observed was found to be statistically significant, when analyzed by both one-way ANOVA and Kruskal-Wallis test.

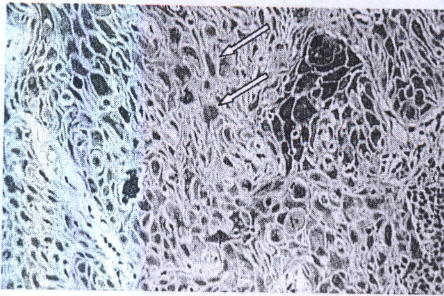
Also statistically non-significant were the slight differences between the average number of COX-2-positive cells (out of 1,000 cells evaluated) in the 16 relatively well differentiated OSCC assigned G1 (446 ± 355) and those of the 20 cases assigned G2 and the 9 cases assigned G3: 332 ± 338 and 363 ± 341 COX-2-positive cells, respectively.

As regards the association between COX-2 expression and tumor size, the average number of COX-2-positive cells in the T1 tumor subset ($n = 23$) was 368 ± 319, while the average numbers for the T2 subset ($n = 18$) and the T3 subset ($n = 4$) were 421 ± 354 and 255 ± 464 COX-2-positive cells, respectively. Also in this case the differences were statistically non-significant.

Furthermore, when the presence of lymph node metastases was considered, an average number of cells with COX-2 immunoreactivity of 343 ± 336 (out of 1,000) could be calculated for the subgroup of 33 cases with absence of metastases (assigned N0), in comparison with an average cell number of 600 ± 412, calculated for the 4 cases with homolateral positive lymph nodes (assigned N1) and 415 ± 326, calculated for the 8 cases with controlateral positive lymph nodes (assigned N2). However, also in this case the statistics suggested that differences observed were not significant. As expected, we also failed to detect statistically significant differences when the average number of COX-2-positive cells relevant to the 21 tumors assigned to stage I (340 ± 319), the 10 tumors assigned to stage II (384 ± 375), the 5 tumors assigned to stage III (422 ± 485) and the 9 tumors assigned to stage IV (441 ± 318) were compared

Tab. I. *Site, grading and TNM staging in the 45 OSCC studied. Also shown are the counts of COX-2 positive cells (out of 1,000 cells evaluated) in tumor areas and in dysplastic epithelium, normal-appearing epithelium and the inflammatory infiltrate, associated to the tumors.*

Case number and tumor site	Grading and TNM staging	Tumor	Dyspastic epithelium	Normal epithelium	Associated inflammation
1. Alveolar process	G2/T3N2M0 (IV)	<10		50	<10
2. Alveolar process	G3/T1N0M0 (I)	<10			
3. Alveolar process	G1/T1N0M0 (I)	700		450	<10
4. Alveolar process	G1/T1N0M0 (I)	950			375
5. Alveolar process	G1/T1N0M0 (I)	975	775		125
6. Alveolar process	G1/T1N0M0 (I)	575	850		50
7. Alveolar process	G2/T2N0M0 (II)	100	450	450	175
8. Alveolar process	G3/T2N2M0 (IV)	700			
9. Alveolar process	G2/T2N0Mx (II)	50	975		975
10. Alveolar process	G3/T2N2M0 (IV)	125			100
11. Alveolar process	G2/T2N2M0 (IV)	750		<10	100
12. Alveolar process	G2/T1N2M0 (IV)	575	500	350	
13. Alveolar process	G2/T2N1M0 (II)	350			125
14. Alveolar process	G3/T3N0M0 (III)	<10		150	<10
15. Lip	G2/T1N0M0 (I)	300		100	125
16. Lip	G1/T1N0M0 (I)	75	75	<10	200
17. Lip	G1/T1N0M0 (I)	300	550	275	<10
18. Lip	G1/T1N0M0 (I)	<10	175	125	<10
19. Lip	G1/T1N0M0 (I)	725		<10	<10
20. Lip	G1/T1N0M0 (I)	<10		200	<10
21. Lip	G2/T1N0M0 (I)	<10	550	150	300
22. Lip	G1/T1N0M0 (I)	<10	425	<10	<10
23. Lip	G1/T1N0M0 (I)	575	<10		50
24. Lip	G1/T1N0M0 (I)	225	75	50	<10
25. Lip	G2/T2N0M0 (II)	75	50	125	100
26. Tongue	G2/T2N0M1 (IV)	650	525		100
27. Tongue	G2/T2N1M0 (III)	950	350		750
28. Tongue	G3/T1N0M0 (I)	275	<10	<10	150
29. Tongue	G2/T1N0M0 (I)	<10			
30. Tongue	G2/T2N0M0 (II)	50	950	975	900
31. Tongue	G2/T2N2M0 (IV)	<10	100	100	250
32. Tongue	G1/T1N0M0 (I)	425	350	300	75
33. Tongue	G1/T1N0M0 (I)	550	250		
34. Buccal mucosa	G3/T1N0M0 (I)	350	400	700	
35. Buccal mucosa	G1/T2N0M0 (II)	950	550	300	300
36. Buccal mucosa	G2/T2N0M0 (II)	800	575	350	
37. Buccal mucosa	G1/T1N0M0 (I)	75			
38. Floor of mouth	G3/T2N1M0 (III)	150	950		100
39. Floor of mouth	G2/T2N0M0 (II)	750	900		425
40. Floor of mouth	G3/T2N0M0 (II)	700	350	600	225
41. Floor of mouth	G2/T2N2M0 (IV)	400			<10
42. Palate	G2/T3N0M0 (III)	50			575
43. Palate	G2/T2N0M0 (II)	<10	<10	<10	50
44. Palate	G2/T1N2M0 (IV)	750	<10	275	300
45. Palate	G3/T3N1M0 (III)	950	975		625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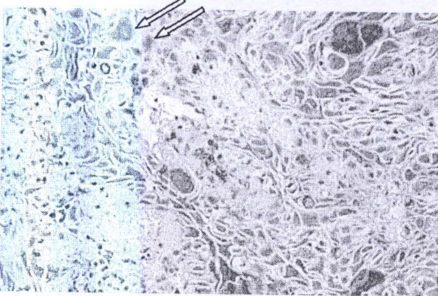
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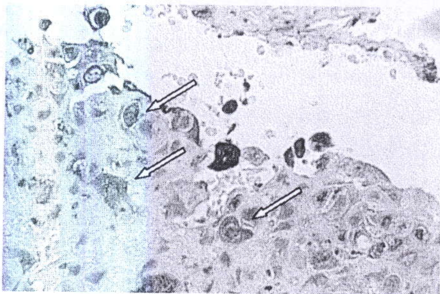
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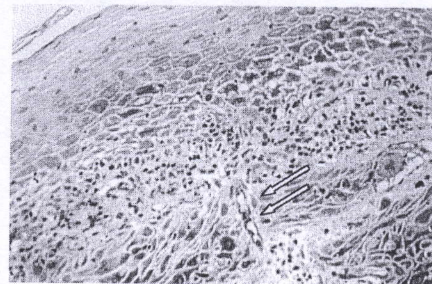
Fig. 1. *COX-2 expression in OSCC.*
Panel A. *Substantial expression of immunoreactive COX-2 in deeply infiltrating tumor nests. COX-2 appears to be expressed mostly in areas with well differentiated cells with a large cytoplasm. Sporadic aneuploid cells with highly hyperchromic nuclei are almost devoid of specific staining (arrows). Discrete COX-2 positivity is appreciable in some of the cells of the inflammatory infiltrate (right, down). LSAB-HRP. 400 x magnification.*
Panel B. *Well differentiated COX-2-positive elements, with a large cytoplasm, with sporadic anaplastic COX-2 negative cells (arrows). LSAB-HRP. 300 x magnification.*
Panel C. *Positive COX-2 immunostaining in approximately one half of the cancer cells in an ulcerated area of OSCC. Granular cytoplasmic and perinuclear positivity (arrows). LSAB-HRP. 300 x magnification.*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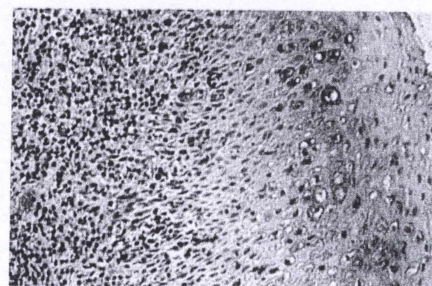
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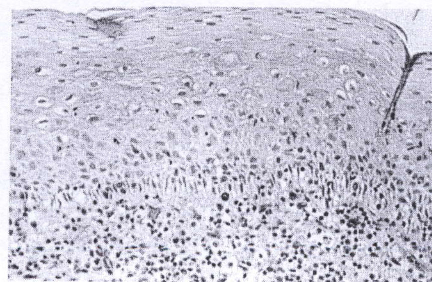
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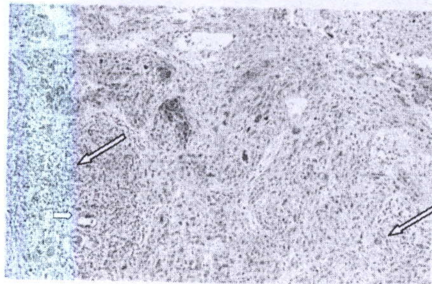
Fig. 2. *COX-2 expression in hyperplastic or dyskeratosic epithelium and in normal-appearing epithelium, associated to OSCC.*
Panel A. *High level COX-2 expression in hyperplastic, parakeratosic epithelium (above), next to the tumor infiltration (below, right). The dense inflammatory infiltrate between the hyperplastic and the neoplastic areas is prevalently COX-2-negative. Two vessels with COX-2 positive endothelial cells within the invasion area (arrows). Note that the intensity of the immunoreactive COX-2 staining of the tumor cells is comparable to that of the hyperplastic epithelium. LSAB-HRP. 300 x magnification.*
Panel B. *Strong COX-2 positivity in dyskeratosic areas (right), in contrast with the absence of staining in more superficial layers. Inflammatory infiltrate with some COX-2-positive elements (particularly macrophages), close to the lower epithelial layers, which appear largely hyperpalstic and, in certain areas, dysplastic. LSAB-HRP. 300 x magnification.*
Panel C. *Normal appearing epithelium, associated to OSCC. Of note, the faint COX-2 positivity of the majority of the well differentiated cells of the spinous layer. Inflammatory infiltrate with focal positivity (prevalently macrophages). LSAB-HRP. 300 x magnification.*



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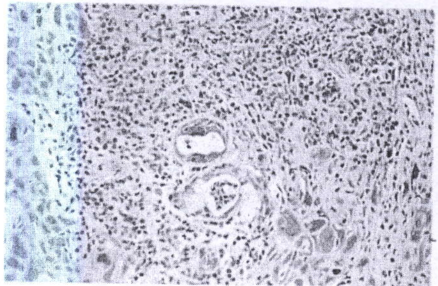

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Fig. 3. *COX-2 expression in endothelial cells of neo-formed vessels.*
Panel A. *Infiltrating tumor cells with high-level COX-2 expression. Of note, the contrast in COX-2 positivity between tumor areas and inflammatory infiltrate areas (arrows). In the central part of one of these inflammatory areas, there are vessels with COX-2-positive endothelial cells (small arrow). LSAB-HRP. 150 x magnification.*
Panel B. *At higher magnification the two vessels described in Fig. 3A, surrounded by COX-2-negative inflammatory cells with some infiltrating cancer cells. LSAB-HRP. 300 x magnification.*

with each other.

Finally, no association between the age of the patients and COX-2 score was found (data not shown). Moreover, we examined 3 lymph-node metastases. COX-2 was expressed at a high level in all of them.

COX-2 expression in OSCC surrounding tissues

COX-2 immunostaining was visualized in hyperplastic or dysplastic oral mucosa as well as in normal appearing epithelium at the boundaries of OSCC (Fig. 2A-C and Tab.I).

Twenty-nine of 45 OSCC specimens displayed different degrees of epithelial dysplasia in the areas surrounding the tumor (low, moderate, strong, as defined according to the WHO criteria). In 25 of these 29 cases (86.2%), COX-2 immunostaining was detected in the dysplastic areas surrounding the tumors, whereas only in 4 cases we failed to detect COX-2 positivity in homologous dysplastic areas. Furthermore, the intensity of the immunostaining in these dysplastic areas adjacent to the tumors was prevalently moderate to strong (Fig. 2A and B and Tab. I).

Normal-appearing epithelium surrounding the tumor was present in 26 cases out of 45. In 20 of these 26 cases, the normal-appearing epithelium expressed COX-2 (76.9%), with a variable degree of immunostaining intensity, which ranged essentially from weak to moderate (Fig. 2C and Tab.I). Positive cells were localized prevalently in the superficial epithelium layers, whereas basal cells were mostly negative (Fig. 2C).

COX-2 expression in inflammatory cells

Inflammatory infiltration was clearly appreciated in 37 out of the 45 OSCC samples. COX-2 expression in inflammatory cells was detected in 27 of these cases (73%) (Fig. 1A, 2A-C and 3A-B). However, COX-2 immunostaining was only focally distributed within the inflammatory infiltrate, as a large proportion of inflammatory cells stained negative, particularly cells of the lymphocytic and granulocytic lineage (Fig. 2B and 3A). Instead, COX-2 appeared to be expressed in monocytes, macrophages (Fig. 2B-C), plasma cells and fibroblasts, as shown by double immunostaining. However, only a fraction of the cells belonging to these cell types stained positive for COX-2.

COX-2 and tumor vascularization

In 38 cases out of 45, neoformed vessels within and around the tumor were seen. In 31 of these 38 cases (81.6%), a significant COX-2 positivity was detected in the endothelial cells of

the intratumoral and the extratumoral vessels adjacent to the tumor nests, particularly CD-34+, VEGF+ neoformed vessels, specially abundant in the areas of tumor invasion (Fig. 2A and 3 A-B).

DISCUSSION

Up-regulation of COX-2 and sustained release of prostaglandins in the cellular micro-environment have been related to oncogenesis through a variety of different mechanisms, including increase in cell proliferation, promotion of angiogenesis, inhibition of apoptosis, transformation of potential carcinogens into active mutagenic carcinogens, decrease in immune surveillance, enhancement of the ability of cancer cells to invade the surrounding tissues and participation in the process of bone destruction and in the genesis of tumour pain (5-8). Moreover, there is evidence of a causative role of COX-2 and prostanoids in several important human malignancies, including sporadic colorectal adenocarcinoma, gastric cancer and lung cancer (10-12).

Moreover, COX-2 was also found to be up-regulated in the squamous cell carcinoma of the head and neck (1, 3, 13-18), one of the leading causes of cancer-related death in both Western countries and developing countries (1, 19-20). However, the pathogenetic role and the prognostic relevance of COX-2 over-expression in this neoplastic condition have not been fully addressed.

In 1999, Chan and co-workers were the first to report an average 150-fold increase in COX-2 mRNA expression in tumor specimens obtained from 24 patients with head and neck squamous cell carcinoma, in comparison with normal oral mucosa obtained from 17 healthy volunteers, as assessed by RT-PCR. Interestingly, COX-2 appeared to be over-expressed also in the normal-appearing epithelium adjacent to the cancer lesions (50-fold increase in RT-PCR signal). They also analyzed 10 of these tumors by immuohistochemistry and found that all of them expressed immunoreactive COX-2. Notably, COX-2 immunoreactivity was also present in the epithelial cells of the normal-appearing epithelial surrounding the tumors.

We studied 45 cases of human squamous cell carcinoma of the oral cavity by immunohistochemistry performed by a highly specific COX-2 Ab and found that over-expression of COX-2 at the protein level occurred in a large proportion of the specimens examined (77.8%) but not in all, suggesting that COX-2 indeed plays a significant role in human OSCC but, perhaps, in combination with other mechanisms essential for oncogenesis and progression. Our results are in agreement with two other studies, which reported COX-2 expression in 71.1% (14) and in 88% of the specimens studied (17), respectively, but in conflict with other works. In particular, in one study all of the 37 OSCC examined expressed immunoreactive COX-2 (3), whereas, in another one, COX-2 was expressed at a low level in 30 specimens and at a high level only in 10 specimens out of the 72 OSCC specimens examined (13.9% and 41.7%, respectively) (18). In conclusion, although there is little doubt that COX-2 expression is increased in OSCC, large discrepancies exist on the magnitude and, consequently, the importance of this over-expression. These discrepancies reflect perhaps the need for more extensive and thorough investigations aimed at assessing this essential point.

The staining pattern of COX-2 immunoreactivity was essentially cytoplasmic and perinuclear in our study, in agreement with the fact that COX-2 is a loosely membrane-bound protein, associated to the endoplasmic reticulum and the outer nuclear membrane (4).

We also investigated the possible correlation between COX-2 over-expression and tumor site in OSCC but failed to detect any significant association, in agreement with two of the studies cited above (14, 18) but in contrast with another paper, which stated that buccal mucosa tumors had the highest COX-2 immunoreactivity (3) (while in a fourth study only OSCC localized to the tongue were analyzed (17)).

Likewise, we were unable to assess any associations between over-expression of this gene and tumor grading, tumor size, presence of lymph node metastases, tumor stage and age of the patients at presentation, respectively. However, two of the immunochemistry-based studies already quoted reported a significant positive association between COX-2 up-regulation and presence of lymph node metastases (14,18), while another one failed to detect this association (3). Regarding differentiation of the tumors, one of these studies claimed that COX-2 immunoreactivity was more frequently

positive in well differentiated tumors (18); another one maintained that COX-2 expression tended to be higher in poorly differentiated tumors (14); whereas a third one found no association (3). Similarly, one study suggested that COX-2 could be a marker associated with advanced stage OSCC (14), in contrast with another work, which stated that early stage tumors tended to have higher COX-2 expression. Once more, these differences solely underline the necessity of more research work aimed at assessing the real mechanistic significance of COX-2 expression in this important form of human cancer and its possible implications from the clinical point of view.

Interestingly, we observed that COX-2 was expressed in dysplastic areas adjacent to the tumors, in accordance with immunohistochemistry data from other studies (1,18) and in the normal-appearing epithelium surrounding the neoplastic lesions, as reported also by Chan and co-workers (1). This peritumoral COX-2 expression may play an important role in tumor progression, in that the consequent increase in prostaglandin biosynthesis at the boundaries of the tumor, particularly PGE_2, may lead to local immunosuppression, allowing the tumor to evade immune surveillance, as shown, for example, in a murine Lewis lung carcinoma model, in which suppression of COX-2 activity by various inhibitors led to marked lymphocytic infiltration of the tumor and reduced tumor growth(21). COX-2 inhibition was accompanied by a significant decrement in the potent immunosuppressive cytokine IL-10 and a concomitant restoration of IL-12 production by antigen presenting cells (21).

Our data regarding COX-2 up-regulation in dysplastic lesions and normal-appearing epithelium next to the tumors are also conceptually in agreement with the observation that in oral pre-malignancies COX-2 seems to be over-expressed specifically in high-risk lesion, as defined by the their aberrant DNA content (17).

We also found focal, albeit sometime intense, COX-2 expression in the inflammatory elements infiltrating the tumors, particularly macrophages. However, it is unclear whether or not COX-2 expression in inflammatory cells may reflect the normal activation of this gene within the context of the inflammatory response or, rather, play a specific role in the promotion of tumor growth.

Angiogenesis appears to be one of the principal mechanisms for invasive tumor growth and metastasis and constitutes an important point in the control of cancer progression (22). COX-2 is thought to play a role also in the promotion of tumor neoangiogenesis (23-24). Our study showed that neoformed vessels (VEGF+ and CD34+) are present in a high quantity in the areas of tumour invasion. Endothelial cells in these areas also stained positive for COX-2, in most cases, supporting the notion that this protein is implicated in neoangiogenetic processes also in OSCC, as it has been suggested by other studies (14, 25).

In conclusion, convincing evidence shows that disregulation of COX-2 expression is associated with human OSCC and it appears plausible that over-expression of this gene plays a pathogenetic role in the genesis and, particularly, in the progression of this malignancy. If so, it is foreseeable that COX inhibitor, particularly the potent and selective COX-2 inhibitors or coxibs, might be evaluated not only as chemopreventive agents in patients at high risk of developing oral cancer (20) but also as chemotherapic agents in the treatment of established tumors, by limiting their growth and metastatic potential and enhancing their sensitivity to radiotherapy and chemotherapy (19). Particularly interesting appear those experimental approaches in which the combination of COX-2 inhibition with suppression of other cellular pathways leads to enhanced antitumoral and antiangiogenetic effects (19, 26). However, before new therapeutical strategies based on COX-2 inhibition may be realistically considered for the treatment of oral cancer a number of questions need to be addressed. Among them, three seem particularly important: the prognostic relevance of COX-2 expression in this specific malignancy; the dosages and the therapeutic regimens to be employed in selective COX-2 inhibition and the potential co-operative role of COX-1, especially in the promotion of tumor-associated angiogenesis.

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