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Up-regulation of VEGF, c-fms and COX-2 expression correlates with severity of cervical cancer precursor (CIN) lesions and invasive disease

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Abstract

Objectives. To describe the expression of vascular endothelial growth factor (VEGF), proto-oncogene macrophage colony-stimulating factor receptor (c-fms) and cyclooxygenase-2 (COX-2) in cervical carcinogenesis and to analyze the correlation of VEGF with c-fms and COX-2 expression.

Methods. In this study, 26 cases of benign cervix, 28 low-grade cervical intraepithelial neoplasia (CIN; CIN 1), 30 high-grade CIN (CIN 2/3) and 28 squamous cervical carcinomas (SCC) were examined by immunohistochemistry (IHC) and analysis was performed separately for epithelium and stroma.

Results. Positive epithelial expressions in normal cervix, low-grade CIN, high-grade CIN and SCC were, respectively: VEGF — 11.5%, 39.3%, 53.3% and 75% ($P<0.001$); c-fms — 0%, 10.7%, 40% and 67.9% ($P<0.001$); COX-2 — 7.7%, 39.3%, 80% and 100% ($P<0.001$). Stromal VEGF expression was higher than epithelial expression in all CIN grades and was also associated with the lesion grade, while c-fms and COX-2 stromal expression was weak. VEGF expression was statistically correlated to c-fms and COX-2 expression in high-grade CIN ($P=0.020$ and $P=0.027$, respectively) and SCC ($P=0.015$ and $P=0.005$, respectively).

Conclusions. On the basis of our findings, these factors may participate in the development and progression of CIN lesions, with possible interaction of c-fms and COX-2 on VEGF expression, and may be potential molecular targets for studies of cervical cancer prevention and treatment.
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Keywords: Vascular endothelial growth factor; Macrophage colony-stimulating factor receptor; Cyclooxygenase-2; Cervical intraepithelial neoplasia; Cervical cancer; Angiogenesis

Introduction

For decades, neovascularization (angiogenesis) associated with progression of cervical intraepithelial neoplasia (CIN) lesions to invasive carcinoma has been described by colposcopy [1]. More recently, microscopic analysis of cervical biopsies has confirmed such an increased angiogenesis during the disease progression from the normal cervix through different grades of CIN to cancer, not only in the lesions but also in their surrounding tissues [2,3].

It is agreed that tumor growth is preceded by an increase in vascular supply to feed neoplastic tissue, when a variety of pro-angiogenic growth factors are released into the micro-environment by malignant, inflammatory and stromal cells in response to various stimuli [4,5]. Among others, vascular endothelial growth factor (VEGF) seems to be a potent angiogenic factor in gynecological tumors and many studies have indicated that VEGF expression closely correlates with the malignant transformation of cervical precancerous lesions [3,6–9].

Several factors regulate VEGF expression, including local alterations such as hypoxia, oxidative and mechanical stress,

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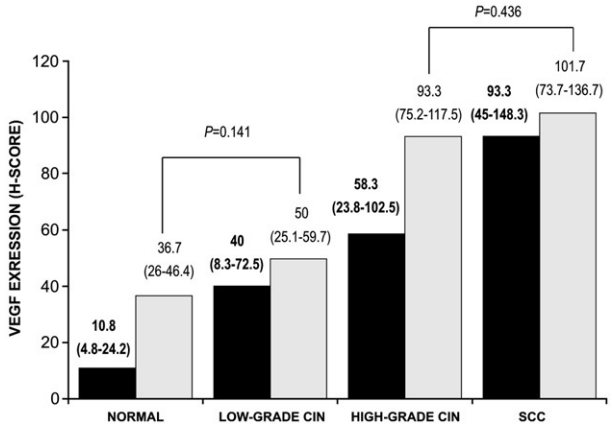

Fig. 1. VEGF expression by immunohistochemistry in epithelium (black columns) and stroma (gray columns) of normal cervix, low-grade CIN, high-grade CIN and SCC cases. VEGF epithelial expression was different between all categories (Kruskal–Wallis test, $P<0.001$). However, in the VEGF stromal expression, there were no differences between normal and low-grade CIN (Mann–Whitney U test, $P=0.141$) and between high-grade CIN and SCC lesions (Mann–Whitney U test, $P=0.436$). All other comparisons in the stroma were statistically different (Kruskal–Wallis test, $P<0.001$). The values are presented as median and 25th–75th percentile (percentiles in brackets).

glucose deprivation, acidosis, cytokines and oncogenes [5]. In tumor tissues, mutation of oncogenes, mutation of tumor suppressor genes and abnormal production of certain cytokines are frequently related to high VEGF expression and angiogenesis [5].

Of the factors that influence VEGF expression, macrophage colony-stimulating factor (CSF-1) via its receptor (proto-oncogene c-fms) and the enzyme cyclooxygenase-2 (COX-2) have been studied in various human tumors, but their expression in cervical cancer and its precursors is incompletely understood [10–13].

C-fms was first described as a receptor for differentiation, proliferation and activation of mono-nuclear phagocytes. However, abnormal expression of c-fms, with or without its ligand, macrophage colony-stimulating factor (CSF-1), has been documented in a wide variety of epithelial tumors, including carcinomas of breast, ovary, endometrium, lung, kidney, pancreas, and prostate [14,15]. C-fms is the cellular homologue of the retroviral oncogene v-fms, and its activation triggers multiple signal transduction pathways controlling cell proliferation and differentiation [16,17]. There is some tentative evidence suggesting that c-fms can induce angiogenesis by stimulating VEGF in both benign and malignant conditions [10,11,18,19]. Scanty data on the involvement of CSF-1/c-fms in CIN and cervical cancer are available, and no studies have analyzed its expression correlated to VEGF [20–23].

COX-2, an enzyme responsible for the conversion of arachidonic acid to prostaglandin, is undetectable in most normal tissues, however, COX-2 is specifically induced in inflammation and neoplasia. In neoplasia, it stimulates cell proliferation, resistance to apoptosis, inhibition to immune surveillance and can promote angiogenesis through many pathways that include an increase of VEGF production [24,25]. The close relationship between COX-2 and VEGF has been documented in many tumors, such as ovarian, breast and endometrial cancer [12,13,26,27]. There is some recent evidence suggesting that COX-2 is also involved in cervical carcinogenesis and is associated with advanced disease, metastasis and poor prognosis. However, the role of COX-2 in CIN lesions has been addressed by few studies only [7,28–33]. Similarly, reports on the relationship between COX-2 and VEGF in cervical cancer are limited [7,34–36].

The objectives of the present study are to analyze the expression of VEGF, c-fms and COX-2 in different stages of cervical carcinogenesis, from normal cervix to invasive carcinoma, and to investigate possible correlations of VEGF with c-fms and COX-2 expression.

Materials and methods

Design and subjects

Routine cervical biopsies from women who participated in cervical cancer screening between 2000 and 2003 at Hospital de Clínicas de Porto Alegre, Brazil, were selected for analysis. Lesions were categorized as normal cervix, low-grade CIN (CIN 1), high-grade CIN (CIN 2 and CIN 3) and squamous cervical carcinoma (SCC).

Normal cervix and CIN samples were selected from a cohort of patients screened for cervical cancer that is part of the Latin American Screening (LAMS) Study [37]. As in this study any colposcopic finding was submitted to biopsy, it was possible to obtain normal cervical samples from false-positive colposcopic findings. To ensure that the tissue represented healthy cervix, we selected only cases that had both normal histology and negative testing for high-risk human papillomavirus (HPV) by Hybrid Capture II (Digene Corporation; Gaithersburg, MD).

Squamous cervical carcinoma samples were selected from archival paraffin blocks, derived from women who were treated during the same period in this same hospital, but not included in the LAMS cohort. All biopsies were fixed in formaldehyde and embedded in paraffin according to local laboratory routine procedures. This study was approved by the local ethics committee.

Selection of samples and regions for analysis

Initially, paraffin blocks were cut at 4 μm and stained with H&E to review the histological diagnosis by two pathologists and were included in this study if the diagnosis was confirmed independently by both.

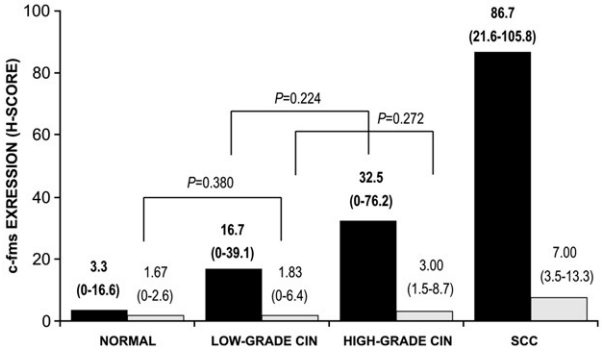

Fig. 2. C-fms expression by immunohistochemistry in epithelium (black columns) and stroma (gray columns) of normal cervix, low-grade CIN, high-grade CIN and SCC cases. C-fms epithelial expression was not different between low-grade and high-grade CIN (Mann–Whitney U test, $P=0.224$), but all other comparisons were statistically different (Kruskal–Wallis test, $P<0.001$). C-fms stromal expression was not different in the comparisons normal/low-grade CIN and low-grade/high-grade CIN (Mann–Whitney U test, $P=0.380$ and 0.272, respectively), but all other comparisons were statistically different (Kruskal–Wallis test, $P<0.001$). The values are presented as median and 25th–75th percentile (percentiles in brackets).

The reviewed sample was then submitted to consecutive cuts at 4 μm, with the first cut stained again with H&E to confirm the presence of the lesion/normal tissue and the immediately following sections stained for VEGF, c-fms and COX-2 by immunohistochemistry (IHC) at the University of Texas Health Science Center at San Antonio (UTHSCSA). From each H&E slide, three representative microscopic fields of the lesion were selected at ×200 magnification (blind to IHC results) and marked for subsequent analysis on the IHC slides. This procedure assured that exactly the same region was read for all different IHC markers.

Immunohistochemistry

Slides were dried at 60 °C for 20 min, de-paraffinized in xylene, re-hydrated through a graded ethanol series and then washed with distilled water. Antigen retrieval for COX-2 was achieved first by boiling the tissues in citrate buffer, pH 6.0, for 20 min and then by cooling the slides for 20 min. The same procedure was performed to VEGF in 1 mM EDTA buffer, pH 8.0. C-fms slides were not submitted to antigen retrieval. Slides were then placed in a semi-automated immunostainer (Sequenza, Thermo Electron Corporation; Waltham, MA).

IHC was carried out with the avidin–biotin complex (ABC), using LV's Ultravision Kit (Lab Vision Corporation; Fremont, CA), at room temperature (RT), according to the manufacturer's instructions. Briefly, Hydrogen Peroxide Block was applied for 12 min to quench endogenous peroxidase activity followed by Ultra V Block for 5 min to block avidin/biotin. Slides were incubated with the primary antibody as follows: rabbit monoclonal anti-human VEGF diluted at 1:150 for 40 min at RT (reacts with the VEGF-A121, A165 and A189 amino acid splice variants; cat #RB-9031; Lab Vision Corporation); rabbit polyclonal anti-human c-fms at 1:50 overnight at 4 °C (cat #CBL776; Chemicon International; Temecula, CA); rabbit monoclonal anti-human COX-2 diluted at 1:150 for 30 min at RT (cat #RB-9072; Lab Vision Corporation). Subsequently, biotinylatedbiotinylated secondary antibody, goat anti-rabbit, was applied for 30 min followed by streptavidin-peroxidase for 10 min. Immunoreactive complexes were detected using diaminobenzidine chromogen exposure for 5 min. Finally, slides were counterstained with methyl green for 5 min, washed in distilled water, dehydrated in graded ethanol, cleared with xylene and mounted.

Negative controls were concomitantly processed by omitting the primary antibody. External positive controls for VEGF, c-fms and COX-2 were human angiosarcoma, tonsil and colon carcinoma, respectively. Because it was technically not possible to manage all slides at the same time, each IHC batch contained exactly the same proportion of normal, low-grade CIN, high-grade CIN and SCC lesion slides.

Interpretation

IHC images were captured from the areas previously selected at H&E slides using a Nikon DXM 1200F digital camera microscope with ×200 objective lens (Nikon; Melville, NY). For each microscopic field, the percentages of cytoplasmatic staining in each category (negative, weak, moderate or strong brown staining) were counted for the totality of cells by one blinded investigator (blinded to the IHC marker and lesion grade) and the *H*-score was obtained using the following formula [38]:

$$H-\text{score} = (\%\,\text{cells unstained} \times 0) + (\%\,\text{cells stained weak} \times 1) + (\%\,\text{cells stained moderate} \times 2) + (\%\,\text{cells stained strong} \times 3).$$

The *H*-scores ranged from 0 (100% negative cells) to 300 (100% strong staining). Epithelial lesions and adjacent stroma were included equally in the microscopic field and the *H*-scores were obtained separately for each one. An *H*-score value equal to or greater than 50 was considered positive. We have recently developed a computer-assisted technique to interpret IHC staining but, at the time the current study was performed, the technique had not yet been published and, for that reason, the *H*-score was chosen.

Statistical analysis

IHC measurements were performed in two different ways: (a) to demonstrate the expression of each marker in normal, CIN and carcinoma lesions, each case was considered as a mean of all regions evaluated; and (b) to show the association between c-fms and VEGF or COX-2 and VEGF, each microscopic field was considered as an individual value in the analysis.

Kruskal–Wallis test was used for comparisons of mean IHC scores in normal, CIN and SCC lesions, and Mann–Whitney *U* test was used for single comparisons. Proportions of positive cases were compared by Pearson Chi-Square. Linear regression models using stepwise backward approach were used to test the correlation of c-fms and COX-2 to VEGF expression. In all tests, $P<0.05$ was considered statistically significant. Because *H*-score values had a non-normal distribution, they are shown in median, 25th percentile and 75th percentile.

The power analysis done before the study indicated that 28 patients were needed in each category to give a statistical power of 80%, with 95% CI, regarding epithelial expression. To confirm that the results were not by chance, we repeated the power analysis using our data and obtained 99.80% power for VEGF and 100% for c-fms and COX-2.

Results

In order to investigate the stromal and epithelial expression of VEGF, c-fms and COX-2 along the process of cervical carcinogenesis, we performed IHC for sequential cuts of normal cervix, premalignant and invasive cervical lesions. As these factors were analyzed in parallel for the same region of each lesion, it was possible to draw comparisons of their expression and therefore present possible correlations.

This study comprised 112 patients, including 26 normal, 28 low-grade CIN, 30 high-grade CIN and 28 SCC cases. A mean of 2.89 ± 0.45 fields per case was analyzed because not all cases had 3 microscopic fields available for analysis due to the small size of some lesions.

Regarding VEGF expression, we found a direct relationship between the increasing grade of the lesions and VEGF expression in both epithelium and in stroma. Normal epithelium, low-grade CIN, high-grade CIN and SCC lesions expressed VEGF in 11.5%, 39.3%, 53.3% and 75% of the cases, respectively ($P<0.001$), while adjacent stroma was positive in 15.4%, 53.6%, 96.7% and 89.3% of the cases, respectively ($P<0.001$), as shown in Fig. 1 (representative IHC stainings are shown later in Fig. 4).

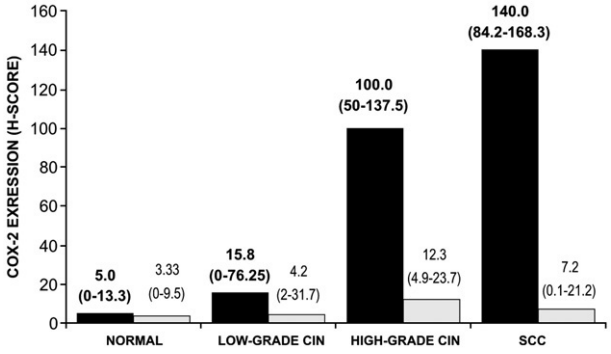

Fig. 3. COX-2 expression by immunohistochemistry in epithelium (black columns) and stroma (gray columns) of normal cervix, low-grade CIN, high-grade CIN and SCC. COX-2 epithelial expression was different between all categories (Kruskal–Wallis test, $P<0.001$) but in the stroma this difference was not identified in all stages (Kruskal–Wallis test, $P<0.054$). In the stroma, the only difference recognized was between normal cervix and high-grade CIN lesions (Mann–Whitney *U* test, $P=0.003$). The values are presented as median and 25th–75th percentile (percentiles in brackets).

Interestingly, while VEGF expression was markedly higher in the stroma as compared to the epithelium in normal tissue, development of preneoplastic lesions resulted in a drastic increase in epithelial VEGF, suggesting an early role for stromal and epithelial VEGF-induced angiogenesis, which may be a prerequisite for microinvasion during CIN progression (Fig. 1).

We have previously shown that the expression of proto-oncogene c-fms and its ligand CSF-1 is elevated in cervical carcinomas compared to normal cervical tissue, suggesting that this pathway is involved in cervical carcinogenesis [39]. In this study, we have examined the expression of c-fms along cervical malignant transformation using IHC. Our data in Fig. 2 show that epithelial c-fms expression was closely associated with the lesion grade: normal cervix, low-grade CIN, high-grade CIN and SCC showed epithelial c-fms expression in 0%, 10.7%, 40% and 67.9% of the cases, respectively ($P<0.001$). The stromal expression also correlated with the CIN severity and invasive disease, but was consistently low when compared to the epithelial expression, except in benign cases ($P=0.218$) (Figs. 2 and 4). The data suggests that the induction of the CSF-1/c-fms pathway may occur at the preneoplastic stage of cervical tumor development.

Given the inflammatory nature of cervical cancer we have also examined the expression of COX-2. The epithelial expression of this enzyme also increased in parallel to the grade of CIN analyzed, with high expression in carcinomas. Normal cervix, low-grade CIN, high-grade CIN and SCC lesions showed positive epithelial COX-2 expression in 7.7%, 39.3%, 80% and 100% of the cases, respectively ($P<0.001$).

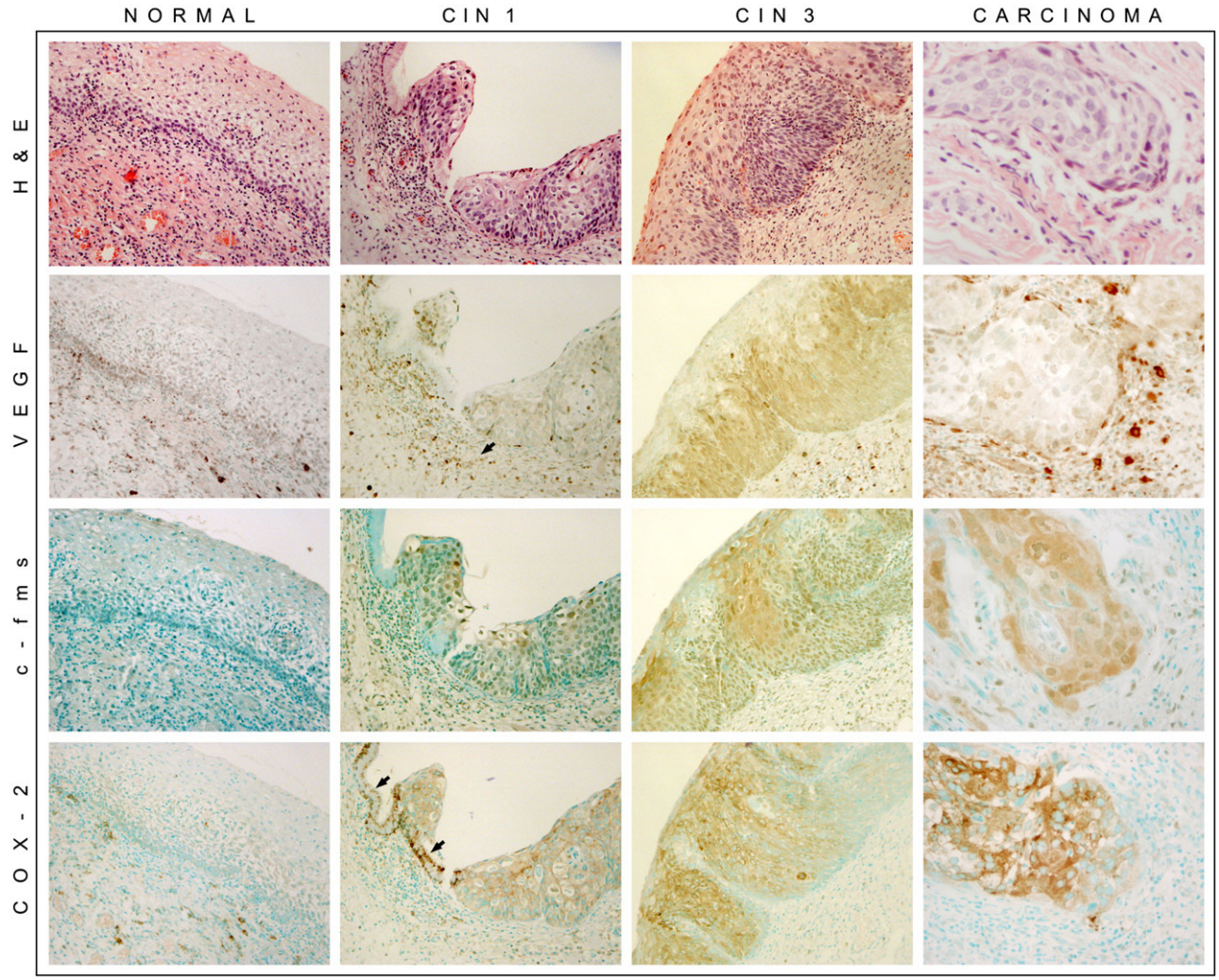

Fig. 4. H&E and respective immunohistochemistry staining of VEGF, c-fms and COX-2 in different stages of cervix malignant transformation. First line, H&E: pre-selected H&E representative areas of normal cervix, CIN 1, CIN 3 (magnifications ×200) and carcinoma (magnification ×400). Second line, VEGF: normal cervix with weak VEGF cytoplasmatic expression in basal squamous epithelial cells and moderate expression in the stroma; CIN 1 with weak positivity for VEGF in the squamous epithelium and intense expression in inflammatory cells (arrow); CIN 3 with intense staining for VEGF in 75% of squamous epithelium; carcinoma with intense stromal expression (in this example, higher than the epithelium that is weak). Third line, c-fms: expression of c-fms is negative in normal cervix; CIN 1 showing some squamous epithelial cells with cytoplasmatic staining and rare nuclear staining; CIN 3 with intense irregular positivity for c-fms at epithelium; carcinoma highly positive in the epithelium and almost undetectable in the stroma. Fourth line, COX-2: normal cervix negative for COX-2 in the epithelium but positive in few stromal cells (inflammatory cells); CIN 1 showing moderate COX-2 cytoplasmatic expression in the dysplastic epithelium and also in the normal glandular epithelium (arrows); CIN 3 noticeably positive for COX-2 with regular staining; carcinoma highly positive for COX-2 in the epithelium and negative in the stroma.

Table 1
Correlation of VEGF with c-fms and COX-2 epithelial expression in normal cervix, low-grade CIN, high-grade CIN and SCC lesions

Lesion grade	Association			
	c-fms/VEGF		COX-2/VEGF	
	Beta standardized (CI 95%)	*P*	Beta standardized (CI 95%)	*P*
Normal	0.00 (−0.66–0.66)	0.999	0.08 (−0.25–0.52)	0.480
Low-grade CIN	−0.01 (−0.39–0.35)	0.932	0.03 (−0.20–0.26)	0.809
High-grade CIN	0.25 (0.06–0.64)	0.020	0.24 (0.02–0.34)	0.027
SCC	0.26 (0.07–0.61)	0.015	0.30 (0.09–0.50)	0.005

However, such a trend was not identified in the stromal expression which was markedly lower than the epithelial expression (Figs. 3 and 4). Interestingly, we found a constant expression of COX-2 by the normal cervical glandular cells that were presented in some of our samples as represented in Fig. 4 (data analysis not shown).

Considering published evidence, suggesting possible regulation of VEGF by c-fms and COX-2, we performed an individual analysis of this hypothetical correlation. Epithelial VEGF expression was significantly correlated with both c-fms and COX-2 in high-grade CIN and SCC lesions, while no correlation was observed in low-grade CIN, as indicated in Table 1. This analysis was run separately for each stratum of cervical lesions because VEGF expression was clearly influenced by lesion grade. To illustrate this correlation, among SCC cases, VEGF expression in COX-2 positive regions had a median *H*-score of 100 (25–75th percentile; 50–100), while the same value for VEGF at COX-2 negative regions was 40 (0–100) ($P=0.024$). Similar values were obtained when we compared VEGF expression in c-fms positive regions (median *H*-score of 100 [70–150]) to c-fms negative regions (median *H*-score of 10 [0–95.5]) ($P=0.002$). Correlations of VEGF, c-fms and COX-2 were not performed for stroma because stromal expression was minimal for c-fms and COX-2, not allowing statistical comparisons.

Discussion

Expression of c-fms and COX-2 in cervical carcinogenesis

We demonstrated that expression of c-fms and COX-2 correlates with the stages of cervical carcinogenesis, increasing from normal cervix to invasive carcinoma. Our results also showed that the expression is mostly due to the involvement of the epithelial compartment that expressed levels of c-fms and COX-2 that were much higher than the stroma.

To our knowledge, the observation that c-fms expression increases in parallel with CIN grades is described for the first time here. About 70% of the cancers that we tested were positive to the proto-oncogene c-fms, while no positive cases were identified among the normal samples. Although the stromal expression also showed statistically significant difference between the lesion grades, this variation was much less than the increase detected in the epithelium.

We have shown in a previous article that c-fms expression is significantly higher in cervical carcinomas than normal cervix [39]. Only one previous study has analyzed c-fms expression in CIN lesions (but not in invasive carcinomas), in which a trend of c-fms expression increase along CIN 1, 2 and 3 was reported, but not statistically significant [40]. The difference in significance between this and our study may be attributed to antibody specificity and IHC scores (the previous study used a categorized variable while we used a continuous variable). Similar to our current study, other articles with different histological tumors types, such as bladder, prostate and breast carcinomas, have confirmed the up-regulation of c-fms in parallel with the increasing lesion grade [41–44].

Although we have not examined the expression of the c-fms ligand CSF-1, there is abundant evidence showing that this cytokine is produced by cells within cervical tissue, including infiltrating leukocytes, acting through autocrine and paracrine mechanisms, and that the deregulation of c-fms expression and ligand independent actions induced by chemical mutagenesis may lead to induction of this pathway during transformation [10,11,39,45]. Therefore, based on these observations, it is feasible to suggest that c-fms identified in our samples is potentially activated and that induction of c-fms levels in cervical lesions may lead to activation of the CSF-1 signaling pathway.

COX-2 has been implicated in a variety of cancers and associated with carcinogenesis [12,13,27]. In our study, we have observed a drastic induction of COX-2 expression in high-grade and cervical carcinomas compared to normal tissue. 100% of cervical cancers showed COX-2 expression in contrast to only 7.7% of normal samples, which suggests an important role of COX-2 in cervical carcinogenesis. A few studies have shown the positive correlation of COX-2 expression according to the severity of CIN and invasive disease, but they have not included all categories of lesions (healthy cervix, low-grade CIN, high-grade CIN and carcinoma) under the same analysis [7,28–30,32,33]. The only report that included the full spectrum of cervical lesions, differently from the previous studies, concluded that expression of COX-2 was not significantly different across histological strata. However, that study presents important limitations because unequal distribution of cases (normal cervix, 9 cases; low-grade CIN, 33 cases; high-grade CIN, 174 cases; carcinoma, 7 cases) and the use of a dichotomic variable for IHC analysis (negative/weak vs. moderate/strong) may have interfered with the detection of small variations of the enzyme expression, as indicated by the author [31].

Correlation of VEGF with c-fms and COX-2 expression

Our results show that VEGF expression increases in parallel with lesion severity, from normal epithelium to squamous cell carcinoma. A similar pattern was observed in the stromal

compartment. VEGF expression was correlated to the expression of c-fms and COX-2 in high-grade CIN and carcinoma but not in low-grade CIN, suggesting that the CSF-1/c-fms and the COX-2 pathways may induce VEGF expression during cervix transformation.

This is the first study that examines the co-expression of c-fms and VEGF in cervical carcinogenesis. Further studies are required to determine whether the CSF-1/c-fms pathway induces VEGF expression and whether this induction plays a role in progression into malignancy. Evidence in the literature do suggest a role for CSF-1/c-fms in regulating VEGF: in non-tumor studies, CSF-1 through c-fms can induce monocytes to produce and release VEGF in a dose-dependent manner as well as augment the differentiation of bone marrow cells into endothelial cells of blood vessels via VEGF [18,19]. Also, blockage of c-fms in mice with breast cancer xenografts suppressed tumor growth by 40–50% and was associated with decreased VEGF expression and reduced angiogenic activity [10].

The correlation of VEGF with COX-2 expression in high-grade CIN and SCC was also identified in our study. It has been suggested that COX-2 stimulates VEGF production mainly through the prostaglandin E2 (PGE2) pathway, one of the most abundant prostanoids detected in the majority of epithelial malignancies [28]. Moreover, the effects of COX-2-induced prostanoids on angiogenesis can probably be amplified via a feedback loop where VEGF activates both phospholipase A2-mediated release of arachidonic acid and COX-2 expression, thereby enhancing PGE2 production [46,47]. Although extensively investigated in other tumors, the positive correlation between COX-2, VEGF and cervical lesions severity was described in one study only that did not include cancer cases [7].

Although our cases were tested for high-risk HPV infection by HCII, we used these results only to select the healthy cervix samples, as described in Materials and methods. Therefore, our analysis has the limitation of being based only on histological findings and does not take into account the potential influence of type-specific HPV infection in the expression of VEGF, c-fms and COX-2. The vast majority of the studies about these markers also do not take into consideration the HPV infection [3,7,8,28–30]. Some authors who included the HPV infection in the analysis did not find a correlation with COX-2 and VEGF expression [31,35], but one author indicated a potential association of VEGF-C and high-risk HPV [48]. Thus, future studies about correlation of type-specific HPV and these markers are strongly recommended, as previously mentioned by Kulkarni et al. [28].

In our study, increase of VEGF expression was an early event, with an important contribution by the epithelial compartment and underlying stroma in CIN lesions and its expression was correlated to c-fms and COX-2 in both pre-invasive and invasive stages. Because IHC analysis cannot establish an evident cause–effect relationship, our observations that the expression of VEGF is correlated to c-fms and COX-2 in high-grade lesions and SCC require further evaluation to study the regulation of VEGF by c-fms and COX-2. *In vitro* experiments and animal models to evaluate the consequences of the selective blockage of c-fms and COX-2 in VEGF expression and angiogenesis are probably the next steps. Such studies may provide a scientific rationale for future cervical cancer research by examining those factors as potential targets for the purpose of CIN and cervical cancer management and as a possible anti-angiogenic therapeutic strategy.

Conflict of interest statement

The authors declare that they have no conflicts of interest to disclose.

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