ORIGINAL ARTICLE

Expression of EGF and its receptor and histomorphometric analysis of epithelial tissue in gingival fibromatosis

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Abstract:

Gingival fibromatosis, a relatively rare condition, develops as a slowly progressing, benign, and localized or generalized enlargement of keratinized gingiva. The purpose of this study was to investigate the expression of epidermal growth factor and the epidermal growth factor receptor and perform histomorphometric analysis of epithelial tissue in gingival fibromatosis. Immunohistochemistry with antibodies against the aforementioned antigens was performed in gingival tissues from a family with hereditary gingival fibromatosis and a family with syndromic dental anomaly-associated gingival fibromatosis ; normal gingiva was used for comparison. The height of epithelial papillae and area and perimeter of epithelial layers were measured for histomorphometric analysis. Immunoreactivity to epidermal growth factor was found in the cytoplasm of epithelial cells, and immunopositivity for epidermal growth factor receptor was detected in the cytoplasm and membrane of epithelial cells. No differences in the expression of these proteins were observed among the groups. The gingival fibromatosis groups had higher epithelial papillae and larger epithelial areas that the normal gingiva group. Our findings suggest that enlargement of epithelial layers is associated with both forms of gingival fibromatosis.

Keywords: epidermal growth factor, epidermal growth factor receptor, gingival fibromatosis, normal gingiva.

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INTRODUCTION

Gingival fibromatosis (GF) is a relatively rare condition characterized by diffuse enlargement of the maxillary and mandibular keratinized gingiva. It is slowly progressing and benign and shows expansion and accumulation of connective tissue with increased number of cells occasionally[1,2]. GF occurs as an isolated disease, in hereditary gingival fibromatosis (HGF)[3], or is associated with some rare syndromes or chromosomal disorders such as the Zimmerman-Laband syndrome[4], juvenile hyaline fibromatosis[5], hypertrichosis[6], cherubism[7], and the Rutherford syndrome[8]. Recently, we described a syndrome characterized by GF associated with dental anomalies[9].

Growth factors and their specific cell-surface receptors with related tyrosine kinase activities are naturally occurring biological mediators that play a fundamental role in the control of tissue remodeling[10]. Epidermal growth factor (EGF) is a protein that plays an important role in cell growth control. It may activate DNA synthesis and cellular proliferation and stimulate mitosis in epidermal cells. Its receptor, EGFR, is a transmembranous glycoprotein that is activated by the binding of EGF[11]. The EGFR is endogenously expressed in numerous cell types and is an important factor in the control of many fundamental cellular processes, including the cell cycle, cell migration, cell metabolism and survival, and cell proliferation and differentiation[12]. Several studies have shown that these proteins are associated with proliferation disturbances in several pathological conditions[12-14].

The purpose of this study was to investigate the expression of EGF and the EGFR and perform histomorphometric analysis of epithelial tissue in GF.

MATERIALS AND METHODS

Study design and tissue specimens

This cross-sectional study was performed by using archived tissue blocks obtained by gingivectomy or gingivoplasty from 4 patients with HGF[15], 4 patients with syndromic dental anomaly-associated GF (SGF)[9], and 4 patients with normal gingiva (NG) who underwent periodontal treatment. The control samples were derived from noninflamed or hyperplastic gingival tissues. All samples were fixed in formalin and embedded in paraffin. Five-micrometer-thick sections were cut, deparaffinized, and stained with hematoxylin and eosin for histological and histomorphometric analyses. Ethical approval for this study was obtained from the University Ethics Committee.

Histomorphometry

Histomorphometric analysis was performed with a computer-assisted image analyzer (Nikon NIS-Elements-2.35, Nikon Corporation, Melville, NY, USA). The height of epithelial papillae and area and perimeter of epithelial layers were measured in 3 fields at ×100 magnification for each sample, as previously described by Araujo et al.[14].

Immunohistochemistry

For the immunohistochemical reactions, 3-µm-thick sections were mounted on organosilane-coated slides. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide for 15 min and was followed by incubation with 10 mM citric acid (pH 6.0) in an electric pressure cooker for 5 min at 121 °C for antigen retrieval. Primary mouse monoclonal antibodies were used against EGF (Santa Cruz Biotechnology, Santa Cruz, CA, USA) and the EGFR (Dako, Glostrup, Denmark) in 1:50 dilution overnight. The antibodies were detected with an LSAB visualization kit (Dako) by using diaminobenzidine for color development. The slides were finally counterstained with Mayer's hematoxylin and mounted. Negative controls were obtained by substituting the primary antibodies with ultrapure water. A sample of breast carcinoma (previously shown to be strongly positive for the antibodies under investigation) served as the positive control. Only cells that exhibited brown cytoplasmic or nuclear staining were considered to show immunopositivity.

Analysis of immunostained samples

Immunostained samples were analyzed as previously described[16] with the image analyzer (Nikon NIS-Elements-2.35). The orientation of each sample and tissue sites were determined at ×100 and ×400 magnifications; five sites were defined and used to determine the percentage of immunopositive cells at ×1000 magnification. The sites were chosen to represent the basal and suprabasal cell layers of the epithelium, and the absence of staining was considered a negative finding. The expression of the proteins in each tissue section was then calculated by multiplying the proportion of cells at each staining intensity by the numerical value of that intensity. Each slide was scored according to the following scale: negative score, 0–0.5; low score, 0.6-1.0; and high score, 1.1-4.0.

Statistical analysis

The expression of EGF and the EGFR and histomorphometric data were assumed to have nonparametrical distributions and were compared by using the Mann-Whitney and Kruskal–Wallis tests. Spearman's correlation analysis was employed to assess the relationships between the biomarkers and the histomorphometric parameters. All analyses were performed with SPSS 17.0 (IBM Corporation, Chicago, IL, USA); statistical significance was set at $p < 0.05$.

RESULTS

Histological examination of the GF specimens revealed a well-structured epithelium with elongated and thin papillae extending into deep fibrous connective tissue. In the HGF and SGF groups, cementicles and odontogenic epithelial rests were observed in the connective tissue. In the HGF group, the height of the papillae and area and perimeter of the epithelial layers ranged from 504.35 to 778.26 μm (mean, 653.80 ± 56.73 μm); 571.48 × 10^3 to 878.42 × 10^3 $μm^2$ (mean, 729.37 ± 76.31 × 10^3 $μm^2$), and 5.14 × 10^3 to 10.00 × 10^3 μm (mean, 6.57 ± 1.14 μm), respectively. In the SGF group, the height of the papillae and area and perimeter of the epithelial layers ranged from 563.04 to 750.00 μm (mean, 663.69 ± 42.07 μm); 450.63 × 10^3 to 816.59 × 10^3 $μm^2$ (mean, 630.74 ± 90.14 × 10^3 $μm^2$), and 5.04 × 10^3 to 7.10 × 10^3 μm (mean, 5.98 ± 0.49 μm), respectively. In the NG group, the height of the papillae and area and perimeter of the epithelial layers ranged from 343.48 to 550.72 μm (mean, 402.53 ± 49.58 μm); 270.23 × 10^3 to 477.86 × 10^3 $μm^2$ (mean, 356.92 ± 43.92 × 10^3 $μm^2$), and 5.49 × 10^3 to 7.03 × 10^3 μm (mean, 6.20 ± 0.31 μm), respectively. The HGF and SGF groups showed significantly higher epithelial papillae than the NG group ($p \leq 0.05$; Table 1).

Table 1. Mean values of the papillary height and epithelial area and perimeter in the NG and GF groups.

	Height of papillae (μm)	*p* value	Area (X10^3 $μm^2$)	*p* value	Perimeter (X10^3 μm)	*p* value
NG	402.53		356.93		6.20	
HGF	653.80	0.039	729.37	0.026	6.57	0.794
GF and DA	663.78		630.75		5.98	

In bold, significant *p* value < 0.05. Analysed using the Kruskal·Wallis test.

The expression of EGF and the EGFR was frequently noted in the basal and parabasal layers of all samples. Most specimens from the different groups consistently demonstrated strong cytoplasmic and nuclear staining from the basal layer to the surface layer (Figure 1). In the HGF group, the expression of EGF ranged from 0.975 to 1.0 (mean, 0.989 ± 0.125) and the intensity ranged from 2.1 to 4.0 (mean, 3.2 ± 0.8); the expression of the EGFR ranged from 0.900 to 1.0 (mean, 0.956 ± 0.05) and the intensity ranged from 1.5 to 3.7 (mean, 2.2 ± 0.9). In the SGF group, the expression of EGF ranged from 0.779 to 1.0 (mean, 0.944 ± 0.110) and the intensity varied from 0.6 to 4.0 (mean, 2.1 ± 1.7); the expression of the EGFR ranged from 0.980 to 1.0 (mean, 0.994 ± 0.110) and its intensity varied from 0.7 to 3.1 (mean, 1.7 ± 1.1). In the NG group, the expression of EGF ranged from 0.398 to 1.0 (mean, 0.711 ± 0.303) and the intensity ranged from 0.5 to 3.9 (mean, 1.6 ± 1.5); the expression of the EGFR ranged from 0.980 to 1.0 (mean, 0.995 ± 0.01) and its intensity varied from 2.3 to 3.9 (mean, 1.9 ± 0.6). No significant associations in the expression of EGF and the EGFR were observed between the groups (Table 2). Moreover, Spearman's correlation analysis showed no significant associations between the biomarkers. Associations between the proteins and the histomorphometric parameters were also not observed.

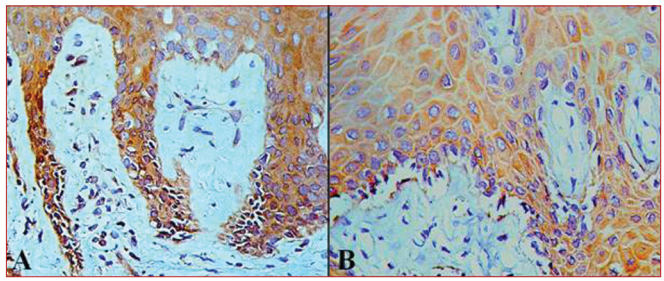

Figure 1. Immunohistochemical findings. A. Immunohistochemical staining for EGFR in an HGF sample showed cytoplasmic and mainly membranous staining of epithelial cells. B. Immunohistochemical staining for EGF in an SGF sample showed cytoplasmic staining of epithelial cells (magnification, ×400).

Table 2. Expression of EGF and the EGFR in the GF and NG groups.

Group	EGF				EGFR			
	Mean	*p*	Intensity	p	Mean	p	Intensity	*p*
NG	0.711		1.6		0.995		3.9	
SGF	0.944	0.224	2.1	0.285	0.994	0.476	1.79	0.298
HGF	0.989		3.2		0.956		2.2	

Data were analyzed by using the Kruskal–Wallis test.

DISCUSSION

GF belongs to a group of benign disorders characterized by enlargement of gingiva. It may occur as an isolated finding or in association with syndromic conditions[17]. GF is a disease of genetic origin, but the mechanism underlying the accumulation of excessive amounts of gingival tissue is unknown[17]. Therefore, this increased tissue is an important focus to understand GF better and establish new forms of treatment. In this sense, knowledge about the factors associated with the growth of gingival tissue, specifically EGF and the EGFR, may contribute to better understanding of this pathology.

Histologically, GF shows a considerable increase in connective and epithelial components[14,18-20]. In this study, the HGF and SGF groups showed similar degrees of gingival enlargement in terms of the height of the papillae and areas of epithelial tissue and connective tissue, including hyperplasia of dense connective

tissue rich in collagen fibers. However, a greater number of epithelial projections and larger area of epithelial tissue were observed in the GF groups than in the NG group ($p < 0.05$).

Previous studies of HGF showed an increase in the number and size of epithelial papillae[14,21,22]. This interesting finding has been interpreted as an inflammatory reaction in epithelial tissue, because areas of inflammatory infiltration were observed in some histological analyses[23]. However, other studies showed that even in GF specimens, the enlarged epithelial tissue was histopathologically and clinically free of inflammatory reactions[14]. Therefore, in GF, irrespective of the hereditary or syndromic form, independent mechanisms may control epithelial proliferation, which may not be mediated by inflammatory factors.

EGF and its receptor are important regulators of epithelial proliferation[24,25]. The response of target cells to growth factors depends on the expression of their specific receptors. These receptors are transmembranous antigens, which, on binding of their respective growth factors, produce a cascade of intracellular signals that stimulate chemotaxis, cell growth and differentiation, and production of the extracellular matrix[26]. Our results showed no significant associations in the expression of EGF and the EGFR between the groups. However, a trend of increasing intensity and amount of EGF was observed in the GF groups compared with the NG group.

The increased expression of these proteins is associated with several malignant and benign proliferative lesions, such as lung cancer, dysplastic lesions, adenomas, ameloblastomas, and gingival growth[27-29]. However, we found no correlation between the immunohistochemical and the histomorphometric data in the GF groups (data not shown). A study of HGF and NG samples showed that high expression of EGF and the EGFR in epithelial cells may have a stimulatory effect on tissue proliferation, resulting in deep projections of epithelial tissue in the underlying stroma[14]. This finding was also suggested by our results.

Recently, a study of the pathway of EGF and the EGFR showed their association with the regulation of collagen--degrading enzymes, which play a critical role in the turnover of gingival connective tissue by regulating the production of matrix metalloproteinases, specifically MMP1 and MMP2[30]. Therefore, these proteins may have a direct relationship with the increase in connective tissue and structural changes in GF. This finding may be a novel molecular mechanism for matrix remodeling in gingival cells, specifically in GF. Histologically, the GF samples from the affected individuals showed similar features. The gingival epithelium in GF differs from NG in that it often exhibits longer, thinner rete ridges that extend more deeply into the underlying fibrous connective tissue. Similarly, we found that the area of the epithelial layers and height of the papillae were larger in GF than in NG.

CONCLUSION

Gingival tissue from patients with HGF and SGF showed similar histological features. The gingival epithelium in GF exhibits longer, thinner rete ridges that extend more deeply into the underlying fibrous connective tissue; moreover, the epithelial area and height of the papillae in GF are larger than those in NG. The expression pattern of EGF and the EGFR indicates that more studies are required to determine the biological interactions that occur in the regulation of epithelial proliferation in GF.

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