Immuno Expression of PAKs in Actinic Cheilitis and in Lip Squamous Cell Carcinoma

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Abstract

Lip squamous cell carcinoma (LSSC) may develop from a potentially malignant disorder known as Actinic cheilitis (AC). The p21-activated kinases (PAKs) are possible targets for cancer therapeutics. The LSSC and AC samples were analyzed by immunohistochemistry in order to detects endogenous levels PAKs group I (PAK1 and PAK2) and group II (PAK4, PAK5 and PAK6) only when they were in phosphorylated form (active). Keratinocytes of the basals stratum showed intense staining for phosphoPAKs 1/2, while the most superficial cells of the stratum spinosum and the surface layer showed moderate immunostaining for PAKs 4/5/6 phosphorylated in the epithelium adjacent to injured area. AC did not immunopress acts PAKs 1/2 and PAKs 4/5/6. LSSC did not immunoexpress phosphoPAKs 1/2, but some degenerating cells and cells in the necrotic area showed intense staining. Our results suggest that PAKs 1/2 not participate in the regulation of AC and LSSC pathogenesis, while PAKs 4/5/6 are involved in the regulation of cell death in LSSC.

Keywords: Actinic cheilitis; Oral squamous cell carcinoma; PAKs 1/2, PAKs 4/5/6

Introduction

Oral squamous cell carcinoma is ranked eighth in the overall incidence of cancer in the world with high mortality and morbidity rates. Treatment is still based on surgery, radiotherapy and chemotherapy. Lip squamous cell carcinoma (LSSC) may develop from a potentially malignant disorder known as Actinic cheilitis (AC), cheilitis exfoliativa, solar cheilosis, solar keratoses, and actinic keratosis of the lips [1,2]. AC comprises clinical and histological changes of the lower lip vermilion that occurs almost exclusively in fair-skinned due to chronic exposure to the sun [3,4].

PAKs are possible targets for cancer therapeutics. Several broad-range kinase inhibitors show potent PAKs inhibition. Six mammalian p21-activated kinases (PAKs) are serine/threonine-specific intracellular protein kinases that are involved in oncogenic signaling pathways. PAKs have a conserved carboxy terminal serine/threonine kinase domain with a single phosphorylation site and an amino terminal regulatory domain. Group I (PAK1, PAK2 and PAK3) and group II (PAK4, PAK5 and PAK6) are structurally distinct and this implies different mechanisms that regulate the activity of these proteins [5].

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Materials and Methods

This retrospective study was approved by Ethical Committee of the Federal University of Triângulo Mineiro (UFTM). Archived tissue blocks from specimens of LSSC patients (n=36; male-to-female ratio =3:1; mean age=57.83 ± 18.82 years) and AC patients (n=8; male-to-female ratio=1:1; mean age=59.75 ± 14.52 years) submitted to biopsy at Clinical Hospital of UFTM were analyzed. The diagnoses of AC and LSSC samples were performed according to World Health Organization histological criteria [6].

Immunohistochemistry

Sections were dewaxed and treated with H2O221:1 Methanol, 10% non-immune goat serum. Samples were incubated with: PhosphoPAK1 (Ser199/204)/2 (Ser192/197) antibody (Cell Signaling Technology, Inc., Danvers, MA, USA) and PhosphoPAK4 (Ser474)/5 (Ser602)/6 (Ser560) antibody (Cell Signaling Technology, Inc., Danvers, MA, USA) at 1:50 overnight, Biotin-SP-conjugated AffiniPure Goat Anti-Rabbit IgG (Jackson Immunoresearch Laboratories, West Grove, PA, USA) 1:200, and the avidin-biotin-peroxidase complex (kit ABC Elite®, Vector Laboratories, West Grove, PA, USA). 3,3ʹ-Diaminobenzidine - DAB (Sigma-Aldrich, St. Louis, MO) was used as substrate, and stained with Hematoxylin. Negative controls consisted of the absence of primary antibody. Results were analyzed using an Axiophot microscope (Zeiss®).

Results and Discussion

Histopathological changes detected in AC consist in squamous cell hyperplasia of the epithelium, with different degrees of keratinization, disordered maturation increased mitotic activity and cytologic atypia [7], inflammatory infiltration [8] and basophilic changes in the stroma.
The dysplastic feature of the disease can lead to the development of the LSSC [10]. In this study, the samples of actinic cheilitis showed atrophic epithelium with hyperkeratosis and dysplasia. In the lamina propria were observed: basophilic degeneration of collagen (solar elastosis) and inflammatory infiltrate (Figures 1 and 2). In this study, in the epithelium adjacent to injury area, keratinocytes of the basal stratum showed intense staining for PAKs 1/2 protein in active form and keratinocytes of other epithelial layers did not immunoexpress these proteins (Figure 1B), while only the most superficial cells of the stratum spinosum and the surface layer showed moderate immunostaining for PAKs 4/5/6 phosphorylated (Figure 2B). Our results suggest that PAK1 or 2 is involved in the regulation of proliferation as expected, since PAK1 is the best known member of the PAK family, was identified as a protein that interacts with the cell division cycle, activated by the Rho GTPases family members: Cdc42 and Rac1 [11,12]. In the other hand, the keratinocytes of AC did not express the PAKs 4/5/6 phosphorylated (Figure 2C).

![Figure 1: Immunoexpression PAKs 1/2 activated in actinic cheilitis and lip squamous cell carcinoma. Reaction of positivity seen in brown. (A) Negative control of the reaction, (B) stratified squamous epithelium adjacent to injury area, (C) actinic cheilitis, and lip squamous cell carcinoma: well-differentiated (D), moderately differentiated (E), and poorly differentiated (F).](image1)

Although PAK1 isoform is the most established in human cancer, the role of other members of the PAK family has been established, especially PAK4. This protein is overexpressed in cell lines derived from several types of cancer. The PAK4 activity induced by Ras is necessary for the uncontrolled cell growth [13]. In fact, it is known the relationship between PAK4 and Ras in tumor formation: PAK4 amplification plays a role in promoting Ras-mediated tumorigenesis, as well as the deletion of PAK gene seems induce decrease in tumorigenesis ans progression [14].

![Figure 2: Immunoexpression PAKs 4/5/6 activated in actinic cheilitis and lip squamous cell carcinoma. Reaction of positivity seen in brown. (A) Negative control of the reaction, (B) stratified squamous epithelium adjacent to injury area, (C) actinic cheilitis, and lip squamous cell carcinoma: well-differentiated (D), moderately differentiated (E), and poorly differentiated (F).](image2)

Both PAK4 as PAK1 usually located in genomic regions are amplified in cancer cells. The activation of the Ras could be the general mechanism of activation of PAKs in carcinomas [15].

PAK2 negatively regulates the expression of MYC, an oncogenic protein often involved in human cancers, also it can act in a positive or negative regulation of apoptosis [16]. The PAK4, a member of the group of PAKs group II, has action in oncogenesis. The PAK4 activity induced by Ras is necessary for the uncontrolled cell growth [13]. PAK5 can bind to Cdc42 and Rac, though not under the influence of these GTPases in the regulation of their activities, it has different effectors depending on their roles, working in various functions such as cell survival and in human cancers [17,18]. PAK6 is expressed in most primary and metastatic prostate cancers and contributes to the development of prostate cancer [19], as basal kinase activity regardless of binding to Rac or Cdc42 [20].

Cells from carcinoma well differentiated oral squamous, moderately differentiated and poorly differentiated showed no immunostaining for PAKs 1/2 phosphorylated. However, stromal cells were immunostained for these proteins (Figure 1D-1F). Also the majority of carcinoma cells (well differentiated, moderately differentiated and poorly differentiated) showed no immunostaining for PAKs 4/5/6 phosphorylated, the active form. Interestingly, some degenerating cells and cells in the necrotic area showed intense staining (Figures 2D-2F). Therefore, it might be understood that PAK4/5/6 overexpression is absent in cells of LSCC that survive and evade apoptosis. This in contrast with data from literature showing the role of PAK activation and amplification in promoting tumorigenesis, cytoskeletal organization, survival of tumor cells and cancer progression [21-23].

Some features of cancer cells are high rate of cell proliferation, cell survival and great ability to invade neighboring tissues. Most cancers is
controlled by oncogenesis and signaling pathways. The process of determining the signaling pathways has been the study of therapeutic mechanisms. The most successful drugs for the treatment of LSCC are directed to the inhibition of protein kinases [15].

The PAKs are important effector protein of Rac1/Cdc42, their interaction with Rac/Cdc42 generates a change in the ownership of these proteins by phosphorylation. Their effects on the cytoskeleton can be dependent or independent of their kinase activity [24]. The PAKs proteins are involved in the formation of filopodia and lamellipodia, leading to cell motility [25]. Considering that this study was conducted only with lip carcinoma, which usually has a better prognosis, future studies may elucidate the involvement of the PAKs family in carcinogenesis of head and neck cancer.

Although, literature describes the role of PAK activation and amplification in promoting tumorigenesis, cytoskeletal organization, survival of tumor cells and cancer progression, this study did not found overexpression of PAKs. But, our results suggest that PAKs 1/2, group I, not participate in the regulation of pathogenesis in actinic cheilitis and lip squamous cell carcinoma, while PAKs 4/5/6, group II, are involved in the regulation of cell death in oral squamous cell carcinoma.

Acknowledgements

We are grateful for financial support of Fundação de Amparo à Pesquisa do Estado de Minas Gerais - FAPEMIG, and Fundação de Ensino e Pesquisa de Uberaba - FUNEPU.

References