GTPases Rho distribution in intraepithelial and invasive neoplasias of the uterine cervix

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Summary

Purpose of investigation: To evaluate the distribution of GTPases RhoA, RhoB, and Cdc42 in cervical intraepithelial neoplasias (CIN) and invasive neoplasias of the uterine cervix. *Materials and Methods:* samples of neoplastic lesions of the uterine cervix of 44 patients were classified in: CIN I (n=10), CIN II (n=10), CIN III (n=09), and invasive carcinoma (n=15). Antibodies anti-RhoA, anti-RhoB, and anti-Cdc42 were used and staining was classified as: negative, mild, moderate, and intense positive. *Results:* When compared with dysplastic cells, superficial cells showed: higher expression of RhoB in CIN I ($p = 0.0018$), and lower expression of Cdc42 in CIN I ($p = 0.0225$). The authors observed higher expression of RhoA ($p = 0.0002$) and RhoB ($p = 0.0046$) in CIN dysplastic cells when compared with invasive carcinoma cells. *Conclusions:* GTPases Rho may be involved with the regulation of biological processes, important to the progression of cervical neoplasias. Probably, RhoA is important for maintenance of cell differentiation and RhoB protects cells from malignant cervical neoplasia.

Key words: GTPases Rho; Cervical neoplasias; Uterine cervix.

Introduction

Carcinomas of the uterine cervix are frequently preceded by precancerous lesions denominated cervical intraepithelial neoplasias (CIN). These lesions may remain in a non-invasive phase for a long period of time, releasing abnormal cells that are detected by cytologic examinations [1].

GTPases Rho regulate a large variety of signal transduction pathways in eukaryotic cells [2, 3]. Twenty different GTPases Rho have already been identified in mammals [4]. Among the most widely studied members of this family are: RhoA (*Ras homologous member* A), RhoB (*Ras homologous member* B), Rac1 (*Ras-related C3 botulinum toxin substrate* 1) and Cdc42 (*cell division cycle* 42) [5]. Several studies have shown the involvement of GTPases Rho in most malignant neoplasias onset and progression, including acquisition of unlimited proliferative potential, survival, tissue invasion, establishment of metastasis, and stimulation of angiogenesis [4, 6].

Through the characterization of the expression pattern of GTPases RhoA, RhoB, and Cdc42, possible diagnostic biomarkers or target molecules to new modalities of treatment of uterine cervix neoplasias may be identified.

Materials and Methods

This study was approved by the Triângulo Mineiro Federal University (UFTM) Research Ethics Committee. Samples of neoplastic lesions of the uterine cervix of 44 patients subjected to biopsy in the Clinical Hospital of UFTM were used in this study. The cases were revised by a pathologist and classified as: CIN I (n=10), CIN II (n=10), CIN III (n=09), and invasive carcinoma (n=15).

For the immunostaining of GTPases RhoA, RhoB, and Cdc42, antibodies anti-RhoA, anti-RhoB, and anti-Cdc42 1 : 50 PB 0.1M/triton 0.2% were respectively used and incubated for 16 hours at room temperature. Non-specific sites were previously blocked with non-immune goat serum 1 : 10 PB 0.1M/triton 0.2% for an hour. The secondary antibody Goat anti-rabbit biotinylated IgG was incubated in 1 : 200 PB 0.1M/triton 0.2% concentration for 90 minutes. Avidin-biotin-peroxidase complex was used and the reaction was evidenced with 3,3'-diaminobenzidine - DAB.

The immunostained cells were assessed in three random fields by two independent observers. Staining was quantified according to the intensity of impregnation of the chromogenic substance and was considered negative (-), mild positive (+), moderate positive (++), and intense positive (+++). The assessment of immunohistochemical reactions was performed with microscope and figures were created with Photoshop 7.0.1.

The results were analysed using Graphpad Prism 5 program. Variance analysis (ANOVA) and Tukey's post test were used for the expression pattern of GTPases Rho in the groups CIN I, II, and III; and t-test to compare the CIN dysplastic cells with the invasive carcinoma cells. The significance level determined was $p < 0.05$.

Results

The patients' mean age in the different groups was: CIN I (32.80 ± 13.47 years), CIN II (24.70 ± 6.21 years), CIN III (34.67 ± 8.84 years), and invasive carcinoma (47.20 ± 16.56 years).

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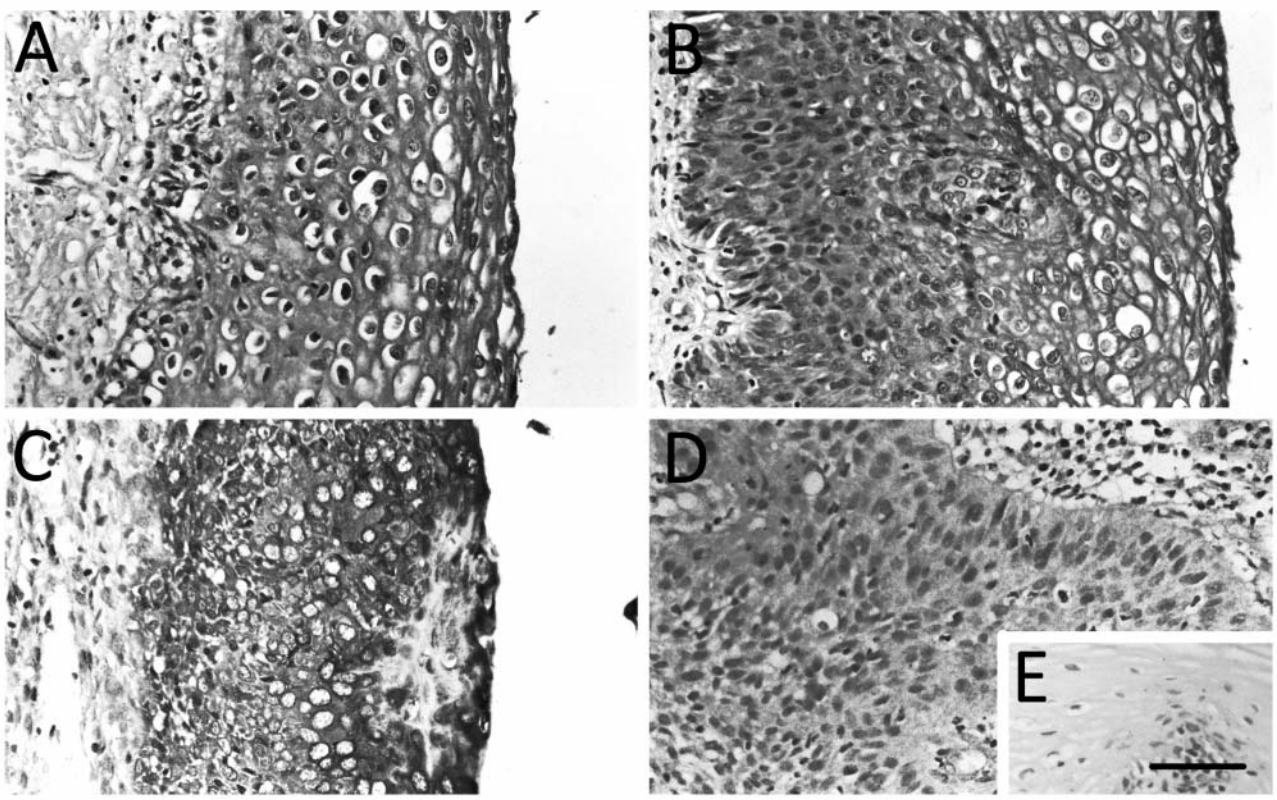

Figure 1. — Expression pattern of GTPase RhoA in intraepithelial and invasive carcinoma of the uterine cervix. Positivity of immunohistochemical reaction in brown, and nuclei stained with hematoxylin. (A) CIN I; (B) CIN II; (C) CIN III; (D) invasive carcinoma; (E) negative control. Bar = 25 μm.

The mean staining intensity for RhoA was not different between dysplastic and superficial cells in the groups CIN I ($p = 0.0541$), CIN II ($p = 0.7730$), and CIN III (moderate marked); nevertheless, the staining of dysplastic cells appeared to gradually decrease. Also no difference ($p = 0.1166$) was observed between in the groups CIN I, II, and III. Interestingly, invasive carcinoma cells expressed significantly less RhoA ($p = 0.0002$), when compared with CIN dysplastic cells, and were seen moderately stained (Figure 1).

Although the mean staining intensity for RhoB in dysplastic and superficial cells were strongly marked in CIN I, a statistical difference was observed ($p = 0.0018$), whereas no statistical difference between the staining of dysplastic and superficial cells in CIN II ($p = 0.4486$) and CIN III ($p = 1.0000$) was observed. Also no difference ($p = 0.1659$) was observed between CIN I, II, and III. Cells of CIN dysplastic were moderately or strongly stained and a statistically significant difference ($p = 0.0046$) was seen when compared to invasive carcinoma cells, observed as weakly stained (Figure 2).

Dysplastic cells showed strong staining for Cdc42 in the groups CIN I, II, and III, however only in group CIN I ($p = 0.0225$) was a statistical difference observed, when compared to superficial cells. No significant difference ($p = 0.3756$) was observed between the mean staining intensity in CIN I, II, and III. Also no difference ($p = 0.0564$) was observed between CIN dysplastic cells and invasive carcinoma cells (Figure 3).

Discussion

This study showed that cells from CIN grades I, II, and III, and from invasive cervical cancer express GTPases RhoA, RhoB, and Cdc42. Interestingly, invasive cancer cells expressed less RhoA and Rho B than dysplastic cells from CIN grades I, II and III. Some studies have previously demonstrated the involvement of these proteins in altered signaling pathways in cell lines derived from cervical neoplasias [7-10]. Previously, RhoA was found overexpressed in high-grade squamous intraepithelial when compared to cervical epithelium without squamous intraepithelial lesions, and Cdc42 as not associated with low-grade or high-grade squamous intraepithelia [11].

The immunohistochemical analysis of lesions of the uterine cervix showed RhoA protein expression in all the groups observed. However, the cells from invasive lesions presented less intense staining than CIN I, II, and

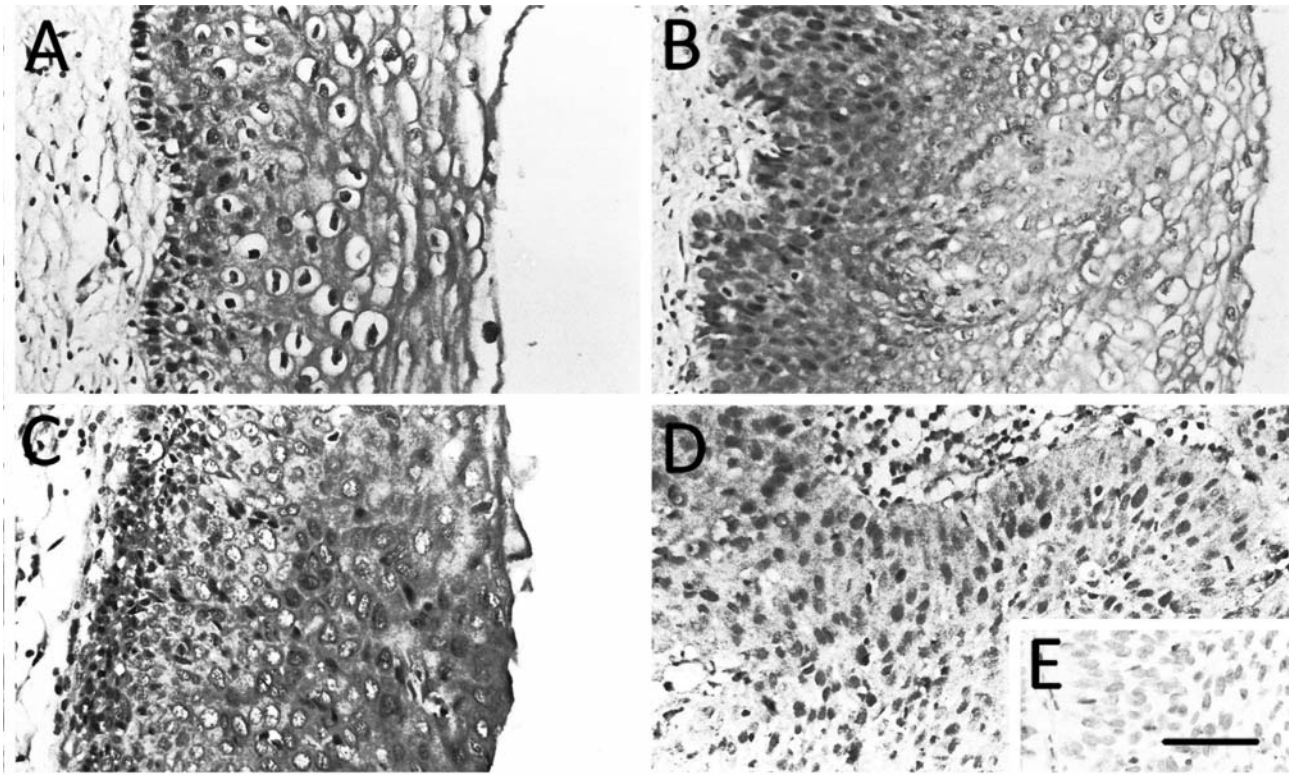

Figure 2. — Expression pattern of GTPase RhoB in intraepithelial and invasive carcinoma of the uterine cervix. Positivity of immunohistochemical reaction in brown and nuclei stained with hematoxylin. (A) CIN I; (B) CIN II; (C) CIN III; (D) invasive carcinoma; (E) negative control. Bar = 25 μm.

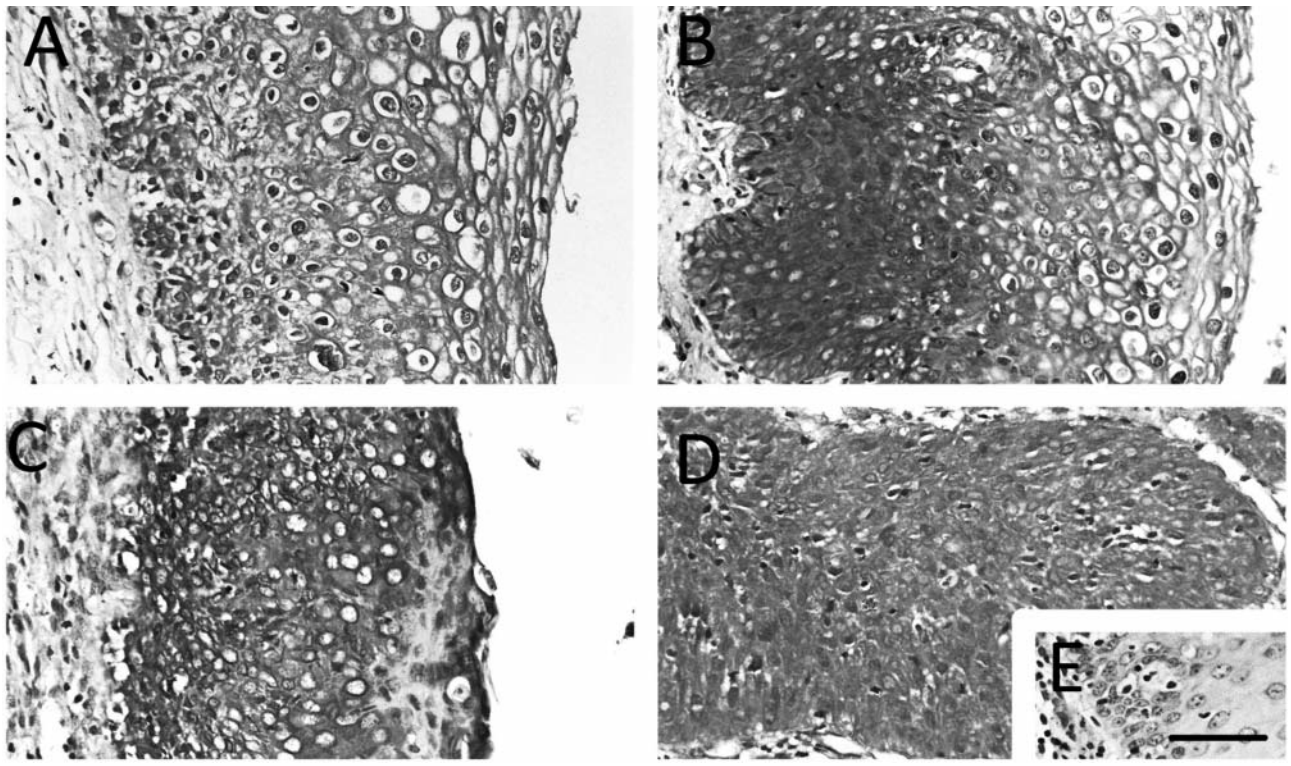

Figure 3. — Expression pattern of GTPase Cdc42 in intraepithelial and invasive carcinoma of the uterine cervix. Positivity of immunohistochemical reaction in brown and nuclei stained with hematoxylin. (A) CIN I; (B) CIN II; (C) CIN III; (D) invasive carcinoma; (E) negative control. Bar = 25 μm.

III cells. These results suggest that the expression of RhoA decreases as the lesions progress. Therefore, the action of GTPase RhoA in carcinomas of the uterine cervix and precursor lesions may be related to maintenance of cell differentiation. RhoA is involved in all stages of cancer progression, including transformation, survival, and proliferation of tumor cells [12]. The formation of stress fibers mediated by GTPases Rho in a cell line derived from cervical adenocarcinoma (HeLa) was demonstrated [7].

As for protein distribution in the different cell compartments, both cytoplasmic and nuclear immunostaining for GTPases RhoA were observed in this study. In the cytoplasm, RhoA regulates the signaling pathways involved in actin cytoskeleton remodeling. Nuclear expression may be associated with the activation of transcription factors. Localization of RhoA protein in the nucleus has already been demonstrated in cell lines derived from cervical adenocarcinoma (HeLa) [13]. A study using subcellular fractionation technique with HEK293 and HeLa cell lines showed that the necessary signals for RhoA activation originate in the nucleus [13].

The immunhistochemical analysis for RhoB protein in CIN I, II, and III groups and invasive carcinoma showed GTPase expression in all cases. The variation in staining intensity was low amongst CIN I, II, and III groups. However, in the invasive carcinoma group, the staining intensity was lower. RhoB protein has been regarded as a tumor suppressor. This GTPase is activated in response to several stress stimuli, such as damage to DNA or hypoxia and may inhibit tumor growth, cell migration and invasion, besides having proapoptotic functions [4].

The nuclear staining for RhoB was more intense in cells of undifferentiated aspect in the deeper layers of the epithelium in CIN I, II, and III cases. In the invasive carcinoma group the nuclear staining was lighter. In the present study there was staining for RhoB in the cytoplasm, nucleus, and plasmatic membrane of dysplastic cells, and predominantly cytoplasmatic in normal cells. RhoB seems to protects cells from malignant cervical neoplasia.

Depending on subcellular localization of the GTPases, different signaling pathways may be activated [14]. The GTPase RhoB is predominantly located in the plasmatic and endosomal membranes [15, 26], indicating that this GTPase plays a role in the endocytic signaling pathways, which favors the transport of signaling molecules to the nucleus, lysosomes, and cell surface [17, 18]. It was demonstrated that RhoB nuclear expression may be associated with DB1 transcription factor [19]. Despite being expressed in all eukaryotic cells, the participation of the Rho family proteins in biological processes may vary according to cellular type and extracellular matrix composition (ECM) [20]. Studies of neoplasias in other sites also demonstrated different results from the ones obtained in this paper.

In this study, the authors observed through immunohistochemical analysis that Cdc42 protein is more widely expressed than the other GTPases Rho analysed, either in precursor lesions or in invasive carcinoma of the uterine cervix. Moreover, the staining was intense in all the CIN I, II, and III groups studied, as well as in invasive carcinoma, suggesting that Cdc42 protein appears to be involved in the regulation of cell proliferation in intraepithelial and invasive cervical cancer.

One of the best known cellular functions of Cdc42 is to regulate cellular proliferation [3, 21]. It is known that Cdc42 protein may stimulate the transformation induced by Ras oncogene in vitro, probably due to its effect in the traffic and degradation of receptors [22]. In cell cycle, the authors observed that Cdc42 and its effector mDia3 are involved in the biorientation and stabilization of the attachment of spindle microtubules to kinetochores and also regulate chromosome alignment in metaphase [23]. Cdc42 expression is increased in breast tumors [24]. Therefore, Cdc42 contribution for cancer progression may be tissue-specific.

The results in the present study demonstrated that the majority of dysplastic and superficial cells presented cytoplasmic staining for Cdc42, whereas only some cells showed nuclear staining for this protein. In consequence of the stimulus of some signaling molecules, such as the platelet-derived growth factor (PDGF), Cdc42 may migrate from the perinuclear region to cell periphery [25]. In this study there was cytoplasmic and nuclear staining in dysplastic cells for RhoB and Cdc42, which possibly indicates an association between both GTPases. Studies suggest that RhoB takes part in the activation of Cdc42 and may influence its localization, since the endosomal RhoB contributes to the redistribution of Cdc42 and for actin remodeling, which is an important event in cell migration [25].

Conclusions

The present results suggest that GTPases Rho participate in signal transduction pathways that may be involved with the regulation of biological processes, important to the progression of cervical neoplasias. RhoA is most likely important for maintenance of cell differentiation and RhoB protects cells from malignant cervical neoplasia. Cdc42 protein appears to be involved in the regulation of cell proliferation in intraepithelial and invasive cervical cancer.

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