

Original Research Article

Hypoxia-inducible factor 1 alpha expression is an indicator of invasiveness in uterine cervical tumors

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ABSTRACT

Background: Hypoxia is a common feature of cancers. Hypoxia-inducible factor 1A (HIF1A) is a causative agent that changes the transcriptional response of tumors under hypoxia. Some alterations lead to an increase in HIF1A activity and this supports other critical pathways leading to angiogenesis, metabolic adaptation and tumor progression. This retrospective study was designed to evaluate the differences of tissue expressions of HIF1A in a spectrum of cervical neoplasms.

Methods: Tissue expression of HIF1A was studied in a total of 107 formalin-fixed, paraffin-embedded uterine cervical tumors specimens and its association with different clinicopathologic parameters was evaluated.

Results: In this series, there were 30 low and 29 high grade cervical intraepithelial neoplasms (CINs), 27 squamous cell carcinomas, 15 adenosquamous carcinomas and 6 adenocarcinomas. Strong and diffuse nuclear staining was evaluated as positive HIF1A expression. Positive HIF-1 alpha expression was detected in 7 (25.9%) of squamous cell carcinomas, 1 of adenocarcinomas (16.7%) and only 1 of HGSILs (3.4%). Statistically it was determined that the positivity rate of strong nuclear HIF1A expression was significantly higher in invasive carcinomas when compared with in non-invasive squamous cell carcinomas ($p=0.07$). Contrary, there was no statistically significant difference according to the subtypes of carcinomas due to scarce number of cases with adenocarcinoma ($p=0.188$).

Conclusions: Our findings were demonstrated to link of nuclear HIF1A expression and the invasive characters of uterine neoplasms. As a result, HIF-1 alpha expression may be important in foreseeing of the invasion and tumor progression.

Keywords: Adenosquamous carcinoma, Adenocarcinoma, HIF 1A, SCC, Uterine cervix

INTRODUCTION

HIF-1 transcription factor is one of the fundamental mediators of human tissues exposed to transcription factor. It involves in rapid gene expression in response to lower oxygen levels. The most prevalently seen etiology of tissue hypoxia is inflammation and/or circulatory failure or its combination. Lower glucose concentrations, and hypoxia have been detected on the periphery of inflamed tissues, and malignant tumors. Under hypoxic

conditions, alpha and beta subunits of HIF-1 form active heterodimers which involve in transcription process of more than 60 genes important in cell survival, adaptation, anaerobic metabolism, immune reaction, cytokine production, vascularization, and overall tissue homeostasis.¹ Cancer cells can survive in hypoxic conditions, and hypoxia can activate adaptive cellular response which may contribute to tumor progression. Biological effects related to HIF-1 alfa (HIF1A) are required for tumor progression.²

Cervical carcinoma ranks fourth among cancer-related deaths among women.³ Squamous cell carcinoma (SCC) is the most frequently seen histologic type which constitutes 75-80% of invasive cervical carcinomas.^{4,6} Adenosquamous cell carcinoma (ASC) is the 2. most frequently seen (3.6-25%) cervical carcinoma whose prevalence is higher in young women.^{6,7} Another type of cervical cancer is pure adenocarcinoma (AC).^{4,7}

In this study, we evaluated HIF1A immune expression, and its role in differential diagnosis in low-, and high grade squamous cell intraepithelial lesion (SCIL), and various histologic types of uterine cervical tumor.

METHODS

At Tepecik Education and Research Hospital, 107 patients with the diagnosis of cervical CIN, SCC, ASC and AC based on immunohistochemical analysis of pathology specimens were included in the study. The study was approved by the Local Ethics Committee of the Hospital.

For immunohistochemistry (IHC), hemotoxylin and eosin (HandEz) staining was used to select appropriate paraffin blocks and to identify the viable tumor areas. IHC was performed using streptavidine-biotin peroxidase method (Invitrogen, Camarillo, 85-9043, CA, USA). Serial 5-µm-thick sections were obtained and these slides were baked overnight at 60°C, dewaxed in xylene, hydrated with distilled water, then treated with decreasing concentrations of alcohol. All slides were subjected to heat-induced epitope retrieval procedure in the microwave (in 10mM/L citrate buffer, pH 6.0, for minutes, followed by cooling at room temperature for 20 minutes) so as to block endogenous peroxidase and biotin activities. The purified monoclonal mouse antibodies against hypoxia-inducible factor 1, alpha subunit (Anti-HIF1A Antibody (HPA001275: Atlas) were used at a dilution of 1:300. Laboratory tests were evaluated by researchers (GD, SS) blinded to the clinical features of the patients. Strong nuclear staining indicated presence of HIF1A expression. Chi-square test was performed for statistical analysis using SPSS 15.0 software statistical package program. P values less than 0.05 were considered to be statistically significant.

RESULTS

In the present study we detected cases with 30 (28%) low (CIN1), and 29 (27.1%) high grade SILs (CIN 2 and CIN 3), 27 (25.2%) SCC, 15 (14%) ASC, and 6 (5.6%) AC. Mean age of the patients was 47.9±12.8 years (range, 20-80 years). Mean age of the patients with invasive carcinoma (52.6±13.2 years: range 30-80) was higher than those with intraepithelial neoplasia (44.01±11.1 years: range, 20-66 years). Mean age was similar in all cases with invasive carcinomas.

HIF1A expressions were focal and limited in the basal cells in many SILs (Figure 1) and these expression were not accepted as positive HIF1A expressions. Strong and diffuse nuclear HIF1A expression was detected in various percentages in cases with HGSIL (3.4%), SCC (25.9%), and adenocarcinoma (16.7 %) (Figure 2). Contrary strong and diffuse HIF1A expression was not detected in LGSIL, and adenosquamous cell carcinoma (Figure 3).

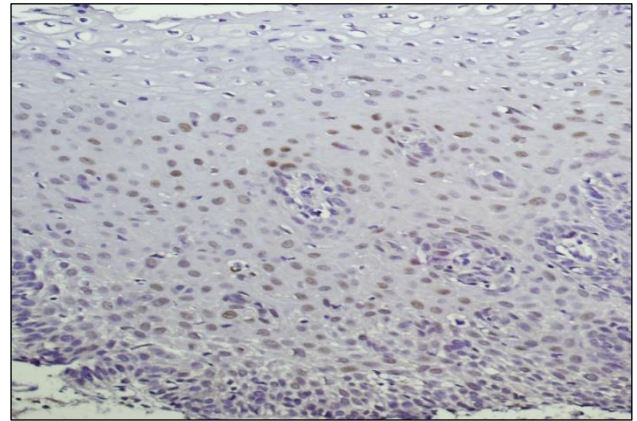


Figure 1: Strong nuclear HIF1A expression in a HSIL sample (DAB X200).

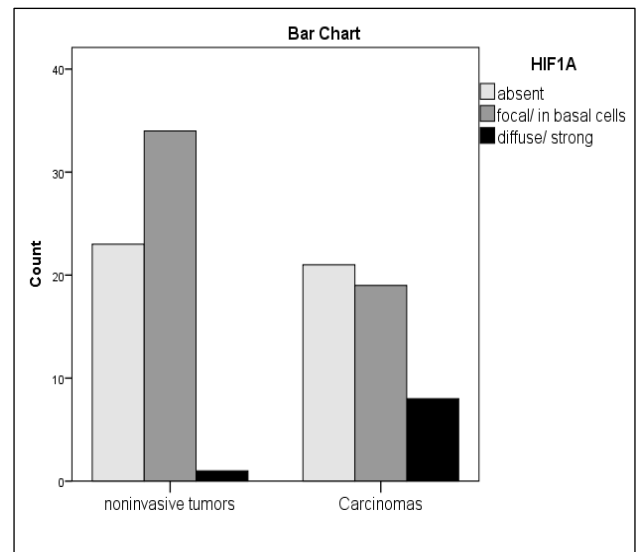


Figure 2: Strong nuclear HIF1A expression was determined in many carcinomas, while it was negative in nearly all SILs (p= 0.07).

Fisher Exact test results demonstrated that the positivity rate of strong nuclear HIF1A expression was significantly higher in invasive carcinomas when compared with in noninvasive squamous cell carcinomas (p=0.07). Statistically it was determined that there was no statistically significant difference according to the subtypes of carcinomas due to scarce number of cases with adenocarcinoma (p=0.188).

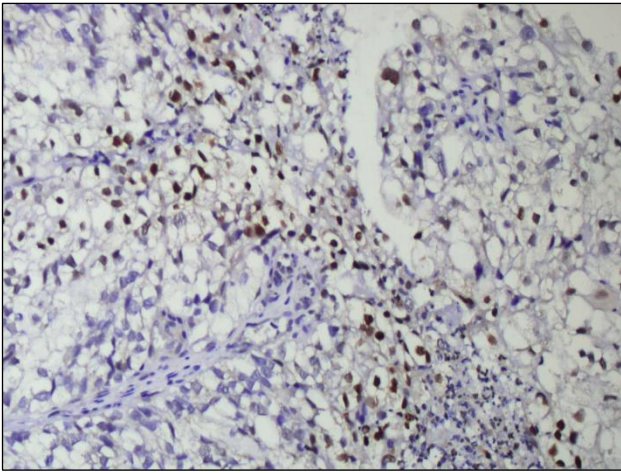


Figure 3: Strong nuclear HIF1A expression in a SCC sample (DAB X400).

DISCUSSION

Tumor hypoxia is now recognized as a key factor driving the development of malignancy, and hypoxia-inducible factor-1 (HIF-1) is the main protein component regulating the response of cells to changing oxygen levels. Hypoxia-inducible factor-1 α (HIF1A) activity appears to be a very early event in carcinogenesis and the protein is expressed before histological evidence of angiogenesis or invasion is manifest. Zhong et al first observed HIF-1 α expression in a few cases of premalignant breast, prostatic and colonic lesions.⁸ Subsequent studies with greater number of patients have showed that HIF1A expression is involved in many pathologic processes, and progressively increased HIF1A expression levels have been observed in the premalignant phases and developmental steps of breast, skin and cervical cancers.^{2,8} Multiple studies have suggested an important role for HIF-1A in malignant progression of cervical cancer. Birner et al observed HIF-1A expression in 80% of CIN-III lesions and early-stage invasive cervical cancers. In contrast, HIF-1A is not expressed in normal cervical samples. In 20% of the cases, non-dysplastic squamous cell epithelium directly adjacent to invasive cancer showed weak expression of HIF-1A in the basal and intermediate cells. These observations suggest HIF-1A may play a facilitator role in premalignant progression.⁹ Similarly, in the present study, we usually determined higher expression of HIF1A protein in invasive tumors. Therefore we speculated that the presence of relationship between the HIF1A expression and the cervical carcinogenesis.

The human papillomavirus (HPV) might be an important co-player in the development of tumorigenic properties of HIF1A in cervical cancer. The HPV E6 oncoproteins commonly inactivate p53.¹⁰ Also, the overexpression of HPV E6 and the loss of p53 promote HIF1A protein accumulation in human cervical cancer cells.¹¹ In our study, we compared HIF1A expressions in cervical carcinoma, and premalignant cervical lesions. In 8.4%

(8/48) of the cases with cervical carcinoma strong, and diffuse nuclear immune reactivity was detected. In only 1.69% (1/59) of the cases with squamous intraepithelial lesions, strong immune reactivity was seen. Therefore HIF1A may be an indicator of tumor progression in HIF1A positive cervical carcinomas.

Tissue hypoxia is a common feature of most solid tumors, often with heterogeneous O₂ levels within different regions of the individual tumors. Cellular adaptation to hypoxic stress is highly complex and depends on the up-regulation of genes supporting anaerobic metabolism and neovascularization.¹²⁻¹⁴ Under hypoxic conditions, cells secrete a variety of cytokines and growth factors that induce proliferation, migration, and blood vessel formation by endothelial cells.¹⁵ The cellular response to hypoxia is primarily regulated through the activity of the HIF1 transcriptional factor, which targets the transcription of over 70 genes.¹⁶⁻¹⁷ Other factors independent of hypoxia can also promote HIF1A protein accumulation via translational or post-translational mechanisms.¹⁸ Several studies have shown that many types of human cancers express an elevated level of HIF1A protein, which is closely associated with a more aggressive tumor phenotype and offers resistance to radiotherapy and anticancer chemotherapy.¹⁹⁻²⁴ Angiogenesis also plays a key role in tumor growth and metastasis and angiogenic factors may help to identify patients with a poor prognosis.²⁵ Aberrant new blood vessels lead to tumor hypoxia limiting normal cell growth. However, tumor cells can adapt to hypoxic conditions, by transforming to a more malignant phenotype and/ or demonstrating poor response to radio-, and chemotherapy.²⁶ VEGF is one of the main factors involved in tumor vascularization and can be upregulated in response to hypoxia, inducing the angiogenic switch.²⁷⁻²⁸ Several studies have indicated hypoxia as a predictor of adverse outcomes in cervical cancer patients receiving radiotherapy.²⁹ Differences in local control were not apparent on multivariate analysis, however. Two smaller studies yielded conflicting data regarding the impact of hypoxia on disease-free survival.³⁰⁻³³ In the current study, we only evaluated the histopathological parameters of cases. Therefore we couldn't arrive at a conclusion about the association between HIF1A expression and the prognosis of cervical cancers.

In addition, we found that there were no significant associations between HIF1A expression and FIGO stage, histological grade, tumor size and lymphatic node involvement. While multiple studies showed that patients with strong HIF1A expression had a significantly shorter overall survival time, disease-free interval and only partial response to radiotherapy.³²⁻³⁴ But other studies could not confirm these findings.³⁵ Different results could be due to the varying patient groups included and different HIF1A scoring methods applied in these studies. Overall, these results suggest HIF1A expression to be a prognostic marker in uterine cervical cancer. Further investigations on HIF1A expression patterns should be

conducted before drawing any definite conclusions. The prognostic values of other above-mentioned hypoxia markers are less convincing.

CONCLUSION

In summary, we determined that HIF1A was weakly and focally expressed in most of the noninvasive lesions, however in invasive tumors it was strongly expressed with diffuse, and higher staining intensity. Therefore this biomarker may be associated with tumor progression in different stages of the tumor. Besides, staining intensities of different histologic types of tumors also attracted our attention. Strong and diffuse nuclear staining was not observed in cases with adenosquamous carcinoma, however it was seen in 25.9%, and 16.6% of the cases with SCC, and ASC, respectively which suggested potential differences in carcinogenesis of tumors demonstrating various histologic types. In conclusion, HIF1A may aid in the differential diagnosis of especially invasive, and noninvasive squamous lesions. However studies with larger series should be conducted to elucidate this issue.

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