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Original contribution

Overexpression of heat shock protein 27 in squamous cell carcinoma of the uterine cervix: a proteomic analysis using archival formalin-fixed, paraffin-embedded tissues

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Summary Proteomic analysis of squamous cell carcinoma of the uterine cervix was performed using total protein from archival formalin-fixed, paraffin-embedded tissues. A wide range of proteins with molecular weights of 10 to greater than 200 kd was extracted from formalin-fixed, paraffin-embedded tissues using a recently developed protocol based on the heat-induced antigen retrieval technique. The extracted proteins from normal squamous epithelium (n = 53) and squamous cell carcinoma (n = 21) were fluorescently labeled and separated using 2-dimensional gel electrophoresis. We identified 728 differentially expressed proteins, with 144 up-regulated and 584 down-regulated as compared with normal squamous epithelial tissue samples. Nine proteins showing pronounced up-regulation in squamous cell carcinoma were analyzed on liquid chromatography–tandem mass spectrometry. Among the candidate proteins identified, minichromosome maintenance 8, a disintegrin and metalloproteinase domain 18, and heat shock protein 27 were analyzed in Western blotting, resulting in significant overexpression of heat shock protein 27 in squamous cell carcinoma over normal mucosa ($P < .05$). Furthermore, immunostaining revealed heat shock protein 27 overexpression not only in squamous cell carcinoma but in various stages of cervical intraepithelial neoplasia (grades 1-3, n = 90), including dysplasia and carcinoma in situ. The expression levels of heat shock protein 27 in cervical intraepithelial neoplasia grades 1 to 3 and squamous cell carcinoma were significantly higher than that in normal mucosa ($P < .05$). In the neoplastic lesions, heat shock protein 27 expression levels in cervical intraepithelial neoplasia grade 3 and squamous cell carcinoma were significantly higher than that in cervical intraepithelial neoplasia grade 1 ($P < .05$). These results may suggest a role of heat shock protein 27 in tumor development and progression in the cervical intraepithelial neoplasia–squamous cell carcinoma sequence. Future experiments using formalin-fixed, paraffin-embedded tissue-based proteomic analysis will be a powerful tool for various pathologic studies.

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1. Introduction

Cervical carcinoma is the second most common cancer in women worldwide. Human papillomavirus (HPV) is thought to be the etiologic agent for most premalignant and malignant cervical lesions, and high-risk HPV types can be detected in almost all cases of cervical dysplasia and carcinoma [1]. HPV testing has been widely adopted for patients after an abnormal cervical cytology screening test (Papanicolaou smear or liquid-based cervical cytology). Epidemiologic and laboratory data support the conclusion that HPV is the etiologic agent for most cervical intraepithelial neoplasia (CIN) and squamous cell carcinoma (SCC), as HPV DNA could be detected in 95% to 100% of these cases. Hence, HPV testing is increasingly used for screening in conjunction with cervical cytology [2,3].

Recent molecular studies have also shown an association of HPV infection with the occurrence of CIN and SCC [2,3]. In general, persistent HPV infection with strong, constitutive expression of the viral oncogenes *E6* and *E7* is a critical step for malignant transformation [4]. There are more than a hundred known genotypes of HPV, but only some are associated with infection of the general mucosa. Low-risk types can associate with benign genital warts, whereas high-risk types can cause CIN. In most cases, HPV infections are clinically benign and cleared by the host immune system; but approximately 3% to 10% of women cannot clear their HPV infections. These women become HPV carriers and are at high risk for developing cervical cancer.

Genomic studies have been incorporated into oncology research; and now, in the postgenomic era, there is a strong drive to incorporate proteomic technologies as well [5,6]. Proteomics is a promising approach in the identification of proteins with changed levels that may be useful as diagnostic and therapeutic markers for cancer. Nevertheless, there are only 2 proteomic studies for cervical cancer [7,8]. One is an in vitro study using cultured cell lines, showing altered expression of several proteins after administration of chemotherapeutic agents [8]. The other in vivo study using frozen biopsy tissues from normal mucosa and SCC revealed several up- or down-regulated proteins in SCC [7]. Although this study provided important insights into the development of cervical SCC, its use of frozen tissues makes it difficult for other investigators to replicate these studies.

Although fresh and/or frozen tissue samples are more ideal samples for proteomic investigation, they are often difficult to obtain in large numbers and relatively expensive to store in a stable form. Formalin fixation and paraffin embedding of tissue are the standard processing methodologies practiced in pathology laboratories, resulting in a highly stable form of tissue that is easily stored because of its inherent stability at room temperature. Formalin-fixed, paraffin-embedded (FFPE) samples have been stored worldwide for over 100 years and are a huge potential resource as a tissue bank [9-12] for conducting retrospective proteomic analysis [9,12]. However, such samples are believed to be refractory to protein extraction because formalin fixation causes high level of covalent cross-linking of proteins [13]. This problem, however, is being largely overcome by a newly developed protocol using the heat-induced antigen retrieval technique, which is widely applied to immunohistochemistry for FFPE tissue sections [9,14]. A few recent studies based on this approach have shown successful extraction of high-quality proteins suitable for biochemical assays from FFPE tissues [9,12].

In the present study, we describe a proteomic analysis of normal squamous epithelia and SCC of the uterine cervix using FFPE tissue proteins in liquid chromatography–tandem mass spectrometry (LC/MS/MS). Moreover, we examined the immunohistochemical expression of the identified protein in various grades of CIN and SCC to study its involvement in the development of these neoplastic lesions.

2. Materials and methods

2.1. Tissues

SCC specimens were obtained from 21 patients (mean age, 49.3 ± 13.9 years). Normal cervical tissue samples were obtained from 53 patients who underwent hysterectomy for benign leiomyoma (mean age, 46.5 ± 6.3 years). CIN samples, which included mild to severe dysplasia and carcinoma in situ [1], were obtained from 90 patients (CIN1: n = 28; mean age, 38.8 ± 11.2 years; CIN2: n = 20; mean age, 37.4 ± 12.7 years; CIN3: n = 42; mean age, 41.3 ± 12.39 years). Tissue samples were fixed in 10% buffered formaldehyde for 24 to 48 hours and embedded in paraffin. Archival human tissue blocks of these specimens were stored from 1989 to 2005. Our current study protocol was approved by the Human Ethics Review Committee of the St Marianna University School of Medicine.

2.2. Protein extraction from FFPE tissues

To minimize contamination of stromal cells, we selected the SCC block that contained the largest amount of tumor tissue in each case. As for normal mucosa, thin squamous epithelial layers were cut from paraffin blocks using a cutter knife; and excised tissues from 3 to 5 patients were reembedded together in paraffin to generate a new "epithelial" block. Extraction of crude proteins from these FFPE tissues was carried out as described [9], with minor modifications. Briefly, 10 section pieces (10 μm each) were deparaffinized by adding 1 mL xylene with gentle agitation for 5 minutes. After removing xylene, 1 mL 100% ethanol was added and agitated for 5 minutes, and centrifuged at 15000*g* for 10 minutes; and the supernatant was removed. The resulting pellet was thoroughly dried under vacuum for 10 minutes. Two hundred microliters of 20 mmol/L Tris-HCl (pH 7.0) containing 2% sodium dodecyl sulfate (SDS) was added to the dewaxed tissue pellet, followed by heating at