Expression of cytokeratins 8 and 17 as a diagnostic marker of cervical intraepithelial neoplasia

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SUMMARY

Cytokeratins belong to the most fundamental markers of epithelial differentiation. Their composition reflects both a cell type and the differentiation status. The aim of this study was to investigate the expression of keratins 8 and 17 in normal cervical epithelium, mature and immature metaplastic epithelium as well as in various grades of intraepithelial neoplasia and squamous cell carcinomas. Fifty-eight smears representing 20 normal, 23 LGSILs, 12 HGSILs and 3 cervical carcinomas were stained with anticytokeratin 8 (clone 35bH11) and anticytokeratin 17 (clone Ks17E3). Expression of both keratins was examined and the percentages of immunoreactive normal, metaplastic, intraepithelial neoplastic and malignant cells were determined. Evaluation of tissue sections was also performed. Keratin 17 was identified in all SILs and carcinomas. It was also present in 3/20 (15%) of normal cervical smears that contained immature metaplastic cells. Keratin 8 was found in the majority of LGSIL cases 20/23 (86.9%), in all HGSIL and malignant lesions as well as in endocervical columnar epithelial cells and in 5/20 (25%) normal smears with immature metaplastic cells. Both keratins showed a more extensive and intense expression in severe lesions. Evaluation of tissue sections revealed expression of CK8 and CK17 of various intensity in most of the premalignant and malignant cases. Premalignant and malignant cells showed similarities in cytokeratins 8 and 17 expression. Both CKs were not expressed in normal ectocervical epithelium. The study of the expression of CK8 and CK17 may contribute in detection of cervical intraepithelial neoplasia.

Key words: *Uterine Cervical Neoplasms; Cervical Intraepithelial Neoplasia; Keratin-17; Keratin-8; Tumor Markers, Biological; Cell Differentiation*

INTRODUCTION

Cytokeratins belong to the most fundamental markers of epithelial differentiation. They build up the cytoskeleton of almost all eukaryotic cells. Keratins play an important role in mechanical stability of epithelial cells, whereas some of them are involved in intracellular signaling pathways such as apoptosis, wound healing, and protection from stress. There are fifty-four functional keratin genes (1). They are highly complex with various molecular weights. Cytokeratins from CK1 to CK8 belong to the neutral (type II) cytokeratins, whereas cytokeratins from CK9 to CK20 belong to the acidic (type I) cytokeratins. Cytokeratin polypeptides are usually found in pairs comprising a type I CK and a type II CK. Keratins typical of ectocervical squamous epithelium are CK4, CK5, CK13, and CK14. Mature squamous metaplastic epithelium shows a similar keratin distribution pattern. The basal layer of the stratified squamous epithelia mainly expresses CK5 and CK14.

Endocervical columnar cells are found to contain significant amounts of keratin 16. The subcolumnar reserve cells express considerable amounts of CK15, CK16, and frequently CK6.

The aim of this study was to investigate the expression of keratins 8 and 17 in normal cervical epithelium, mature and immature metaplastic epithelium as well as in various grades of intraepithelial neoplasia and squamous cell carcinomas.

MATERIALS AND METHODS

Fifty-eight cases from the archives of the Departments of Cytology and Pathology, General Hospital of Volos, Greece, representing 20 normal, 23 LGSILs, 12 HGSILs and 3 cervical carcinomas were analyzed immunocytochemically using monoclonal antibodies: anticytokeratin 8, clone 35bH11 (Diagnostic BioSystems, Pleasanton, CA 94566) and anticytokeratin 17, clone Ks17E3 (Biocare Medical, LLC, USA) according to the protocol recommendations of their manufacturer.

Expression of both keratins was examined and the percentages of immunoreactive cells were determined in normal cases as well as in various grades of intraepithelial lesions and in malignant cases. In addition to expression, the distribution of both cytokeratin polypeptides in different types of epithelial cells was observed.

Evaluation of formalin-fixed paraffin embedded cervical tissue specimens obtained by punch biopsies (minimum 3 samples from each patient) from the same patients was also performed.

RESULTS

Keratin 17 was identified in all HGSILs and carcinomas (Table 1).

Table 1. Expression of cytokeratins

CASES	TOTAL	CYTOLOGY CK8	CYTOLOGY CK17	HISTOLOGY CK8	HISTOLOGY CK17
NORMAL	20	5	3	2	1
LGSIL	23	20	23	19	22
HGSIL	12	12	12	11	12
Ca	3	3	3	3	3

It was also present in 3/20 (15%) of normal cervical smears that contained immature metaplastic cells. Cytokeratin 17 was observed throughout the full thickness of immature squamous metaplastic epithelium, in the basal layer of ectocervical epithelium of 3 normal smears.

In premalignant lesions, cytokeratin 17 was found in 83% of the SIL cases (Figure 1) and in 3 carcinomas (100%). The intensity of immunostaining was variable and related to the severity of the lesion (Figure 2).

Keratin 8 was found in the majority of LGSIL cases 20/23 (86.9%), in all HGSIL and malignant lesions (Figure 3) as well as in endocervical columnar epithelial cells (Figure 4) and in 5/20 (25%) of normal smears with immature metaplastic cells. Both keratins showed more extensive and intense expression in severe lesions.

Evaluation of tissue sections revealed positivity for CK8 correlated with increasing lesion grade: 19/23 (82.6%) LGSIL, 11/12 (91.6%) HGSIL and 3/3 (100%) Ca.

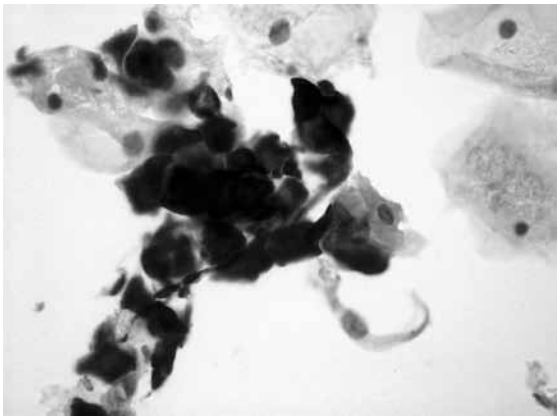

Figure 1. LGSIL case showing positive reaction in cytokeratin 17 (x 400)

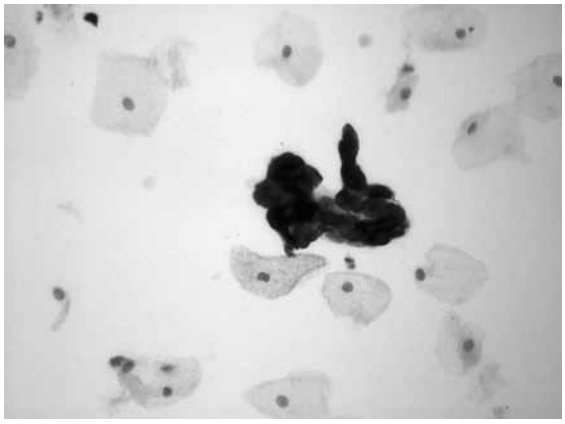

Figure 2. Epithelial cells from a HGSIL positive for cytokeratin 17 (x 400)

Figure 3. Dysplastic cervical cells positive for cytokeratin 8 (x 400)

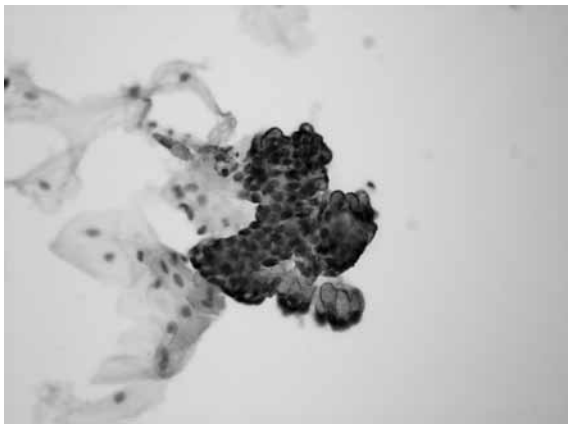

Figure 4. Endocervical columnar epithelial cells positive for cytokeratin 8 (x 400)

DISCUSSION

Expression of cytokeratins is frequently tissue specific and depends mainly on the terminal differentiation and the stage of development. The cytokeratin profile by immunocytochemistry techniques is a valuable tool widely accepted and used for tumor investigation (2-6). The expression of high molecular weight cytokeratins is predominantly localized in the basal layer of squamous epithelium, where the progenitor cells are found (7,8). These cells become the target for cancer causing mutations. The changes that happen in the intermediate filament protein expression reflect changes in the substantial cellular properties (9-10).

The results of the present study show that:

1. Premalignant and malignant cells showed similarities in cytokeratins 8 and 17 expression.
2. More severe lesions presented a more extensive and intense expression of keratin 8 (of simple epithelia).
3. Cytokeratin 8 gave a positive reaction in endocervical cells and CK17 in reserve cells.
4. Both CKs were not expressed in normal ectocervical epithelium.
5. The study of the expression of CK8 and CK17 may contribute in the detection of cervical intraepithelial neoplasia.

Data from the literature showed that the study of the expression of cytokeratins 8 and 17 harbors a significant prognostic potential (2,3,9,11,12). There are also different views. According to Regauer and Reich (13), who disagree with Smedts et al. (4), CK17 expression in pseudostratified epithelia merely reflects a metaplastic phenotype process. Kastsuhide Ikeda et al. (14) found that immunostaining was significantly correlated with increasing lesion grade of cervical intraepithelial neoplasia and squamous cell carcinoma.

Recently Fillies et al. (12) noted that the expression of CK8/18 in squamous cell carcinomas of the oral cavity is an independent prognostic marker and indicates a decreased overall and progression free survival.

Future research would be helpful in further evaluating the utility of clinical application of cytokeratins in distinguishing SILs and carcinomas from benign lesions of squamous epithelium.

Conflict of interest

We declare no conflicts of interest.

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