Down-Regulation of Keratin 14 Gene Expression after v-Ha-ras Transfection of Human Papillomavirus-immortalized Human Cervical Epithelial Cells

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ABSTRACT

Keratin expression in human cervical squamous cell carcinoma (SCC) lines differed significantly from both normal and human papillomavirus (HPV) immortalized exocervical cells. Keratin 14 (K14) expression, determined by protein synthesis and mRNA levels, was dramatically down-regulated in the cervical SCC lines while keratin 5 (K5) expression remained unchanged. K14 expression was similarly down-regulated in an HPV-16 immortalized cervical cell line after tumorigenic transformation with recombinant v-Ha-ras DNA. Cultures derived from nude mouse tumor explants also exhibited an altered keratin profile and the levels of K14 protein synthesis, as well as K14 mRNA, were not detectable. In both cases K5 protein synthesis was not significantly down-regulated. In addition, neoplastic cervical SCC lines exhibited up-regulation of keratin 16 and K14 expression remained unchanged. Thus, neoplastic transformation of human exocervical cells, both in vivo (spontaneous cervical SCC) and in vitro (HPV-16- and v-Ha-ras-induced cervical SCC), is accompanied by characteristic changes in keratin expression. The specific down-regulation of K14 in these tumorigenic cervical cells, in the absence of significant changes in the expression of K5, implies that the normal coordinate regulation of K5 and K14 gene expression has been uncoupled.

INTRODUCTION

The keratin multigene family consists of over 20 epithelial-specific structural proteins which are epithelial-specific intermediate filament proteins (1-3). Keratins are obligate heteropolymers (4) and at least one type II (basic-neutral) and one type I (acidic) keratin are required to assemble the 10-nm intermediate filaments. However, while each type II keratin has a structurally preferred type I partner, permissive associations between various type II and type I keratins can occur (6, 7). For this reason, keratin genes are usually expressed in a tightly coordinated manner. In normal epidermis K14 and K10 are coordinately expressed, and in both epidermis and cervical cells K5 and K14 are expressed as a "keratin pair" (3, 8-10). Although keratins are major differentiation products in all epithelial cells, their expression differs according to cell lineage, and between two and ten keratins are expressed in any given cell type (1, 3, 11, 12). Thus all epithelial cells have a characteristic keratin profile which can be used as a molecular marker for a given cell type.

The human uterine cervix (HCX) comprises two major epithelial cell types, the outer exocervix and the inner endocervix. The keratin profiles expressed by these two cell types differ in several respects (8, 10, 13). The region between these two defined cellular entities has a mixture of both cell types and is termed the transformation zone, an area highly susceptible to tumor development (14). Two types of cervical carcinomas have been described and derived as cell lines: HeLa cells representing adenocarcinomas (15, 16) and both C4-1 and CaSkii representing squamous cell carcinomas (17, 18). It is generally accepted that adenocarcinomas develop from endocervical cells and squamous carcinomas develop from exocervical cells.

Cervical carcinoma is one of the most frequent cancers among women and a major cause of deaths attributable to cancer (19, 20). The mechanisms contributing to the development of cervical carcinoma are not completely understood (21-23) but evidence suggests a role for certain subtypes of HPV. HPV DNA has been identified in proneoplastic and neoplastic lesions of the uterine cervix in over 90% of patients examined (24, 25) and the consistent association of HPV-16 and HPV-18 with most advanced cervical intraepithelial neoplasia, as well as many cervical carcinomas, suggests an important role for these types of HPV in the carcinogenesis process (26, 27).

Keratin analysis of HCX and cervical tumor cell lines has recently substantiated the pathological data and provided a molecular marker for cervical carcinomas (8, 28-30). Keratins are well established as markers for tumor identification and can be used to monitor tumor progression in culture systems (31, 32). In recent studies of the interaction between HPV and HCX (10), the introduction of HPV-16 DNA into nontransformed exocervical cells had very little effect on the keratin profile but did lead to immortalization. Comparison with cervical carcinomas showed that the keratin profile was significantly different from normal and HPV immortalized cervical cells, indicating that keratins serve as a useful marker for transformation. In the present study, introduction of v-Ha-ras into HPV-immortalized human cervical cells produced a tumorigenic cell line with an altered keratin profile. Furthermore, keratin expression in this cell line, which was developed in vitro, is very similar to that of cervical SCC lines derived from carcinomas in vivo. The major change in keratin expression is the down-regulation of K14 which, in the absence of a major effect on K5, implies that the ras oncogene can interfere with the normal coordinate regulation of these two keratin genes.

MATERIALS AND METHODS

Epithelial Cells and Cultures. Epithelial cells were derived from the transitional zone of the HCX and from HKc as previously described (33). These cells were negative for HPV DNA and were grown in a low-calcium, serum-free medium, MCD153-LB (34). Whereas non-treated epithelial cells (HCX or HKc) senesced, transfection with pMHPV16δ, a head-to-tail dimer of HPV-16 DNA containing a neomycin resistance gene (35), resulted in immortalized cell lines such as HCX16-2 (from exocervical cells) and HPV16 (from foreskin keratinocytes and formerly designated HKc/HPV16d-1) after selection with...
the antibiotic G418 (10, 36). Subsequent to immortalization, cell lines were adapted to F-12/Dulbecco's modified Eagle's medium with 5% serum while normal cells, HKc and HCX, were maintained in serum-free medium (MCDB153-LB). The HCX16-2 line was subsequently cotransfected with a v-Ha-ras clone (pH1) and a plasmid containing a MDR gene which confers resistance to colchicine and enables a second selection. The resulting exocervical cell line (HCX16-2-pH1, originally designated HCX16-2HR) produced cystic squamous cell carcinomas after s.c. injection into nude mice (athyritic/nu-nu; Frederick Cancer Research Facility, Frederick, MD). Cell lines (e.g., HCX16-2-pH1-T1) were subsequently developed from explanted nude mouse tumors for further study (37).

Cotransfection of HPV-16 DNA and pH1 (v-Ha-ras) into secondary cultures of human foreskin keratinocytes also produced an immortal cell line with a doubling time of less than 24 h. However, this line has thus far failed to produce tumors in nude mice. Transfection of the HPV-immortalized keratinocyte cell line (HPVg) with a recombinant herpes simplex virus 2 construct (HSV-2 BgII N) also resulted in a tumorigenic line (HSV), which produced well differentiated invasive squamous cell carcinomas in nude mice (38). In addition, four known spontaneous squamous cervical carcinoma cell lines (CaSki, SiHa, C4-1 (17), QG-U, and QG-H (39)), a uterine carcinoma line, SiHa (40), and an adenocarcinoma cell line, HeLa (15, 16), were grown in F-12/Dulbecco's modified Eagle's medium supplemented with 5% fetal bovine serum.

**Protein Labeling.** Total cellular proteins were pulse-labeled for 2 h at 37°C in methionine-free MCDB153-LB containing 10 μCi/ml of [35S]methionine (1450 Ci/mmole). Cultures were then rinsed in sterile phosphate-buffered saline containing 5 μg EDTA and 5 μg ethyleneglycol bis-(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid and stored at −30°C until extracted (41, 42).

**Keratin Extraction.** Cellular cytoskeletons were prepared by treatment with Triton X-100 (1%) and high salt (1.4 M NaCl) buffers (42, 43). Total cellular proteins and cytoskeletal pellets (keratin intermediates) were extracted with 2% sodium dodecyl sulfate and 2% mercaptoethanol in 0.5 M Tris-HCl (pH 6.80) by heating at 80°C for 15–20 min, homogenizing while hot, and centrifuging at room temperature for 20 min. The supernatants were stored at −30°C until analyzed.

**Electrophoresis.** Labeled keratins and total cellular proteins extracted from all the cell lines studied were analyzed by both one- and two-dimensional electrophoresis as previously described (42, 44). Proteins were separated in the first dimension by nonequilibrium pH gradient (pH 4–9) electrophoresis and in the second dimension by sodium dodecyl sulfate-polyacrylamide gradient (7.5–17.5%) slab gel electrophoresis. The gels were treated with Enhance (New England Nuclear) and exposed to Kodak XAR-2 film at −70°C.

**Northern Blot Analysis.** RNA was isolated from subconfluent HCX lines by lysis in guanidine isothiocyanate (45) followed by centrifugation through cesium trifluoroacetate (Pharmacia Fine Chemicals, Piscataway, NJ). Total cellular or polyadenylated RNA was separated by electrophoresis in 1.5% agarose gels containing formaldehyde and the RNA was transferred to Nytran by Northern blotting. The blots were hybridized under stringent conditions to [32P]-labeled K14-specific probes; both a near full length cDNA for human keratin 14 and a highly specific 3′ noncoding probe generated from the HK14-cDNA (see Fig. 4) by the polymerase chain reaction.

**RESULTS**

**Keratin Expression.** Secondary cultures of human exocervical epithelium have a stable keratin profile similar to that of cultured normal HKc. Both cell types express four major keratins (K5, K6, K14, K16) and minor but variable amounts of keratins characteristic of simple epithelia (K7, K8, K18, K19). However, keratin expression in two cervical squamous cell carcinoma lines (C4-1 and QG-U) differed from normal exocervical cell cultures (10). In particular, K14 expression was significantly reduced while K5 expression was normal. The keratin profile of HPV-16-immortalized exocervical cells (lines HCX16-1 to HCX16-5) resembled that of normal exocervical cell cultures, and only slight changes in profile were observed (10). An increase in K7 and K19 expression was found, and this was reproducible in cell lines derived from different individuals. However, no significant effect was observed on the level of K5 or K14 expression in any of the immortalized cell lines (10, 36).

Keratin expression was examined in two other cervical squamous carcinoma lines (CaSki and QG-H) and a uterine carcinoma cell line (SiHa). The keratin profiles of CaSki and QG-H were similar and resembled those of C4-1 and QG-U (Fig. 1a). However, the uterine carcinoma line (SiHa) differed in keratin profile and the major keratins expressed by these cells were K7, K8, K16, and K18. The tumorigenic lines derived from *in vivo* carcinomas differed in profile from the cells immortalized with recombinant HPV-16 DNA and were characterized by a higher level of K7, K8, and K18 expression. Tumor lines also had a variable but consistently low level of K14 expression but K5, K6, K16, and K19 expression were not dramatically affected (Fig. 1a). A tumorigenic cervical cell line (HCX16-2-pH1), obtained after *in vitro* cotransfection of HPV-16-immortalized cervical cells (the HCX16-2 line) with a multidrug resistance gene and the v-Ha-ras oncogene (37), was also examined. Keratin expression in the HCX16-2-pH1 line differed from that observed in either the parental line (HCX16-2) or the control line (HCX16-2-MDR) made by transfecting HXC16-2 cells with the MDR selectable marker alone. In general, much lower levels of keratin synthesis were found and the relative quantities of the different keratins were altered (Fig. 1a). In particular, K14 synthesis was absent while K5, K6, and K16 synthesis was slightly reduced. In contrast, the synthesis of K7, K8, and K19 was increased. The HCX16-2-pH1 cells were tumorigenic in nude mice and a similar keratin profile was observed in cultures derived from nude mouse tumor explants (HCX16-2-pH1-T1). The expression of simple keratins (K7, K8, K19) was even more pronounced in the tumor explant cultures and K14 expression was not detected (Fig. 1a). However, as only one (HCX16-2) of the five original lines (HCX16-1 to HCX16-5) has been transfected with v-Ha-ras to date, we cannot state whether these data are representative of all five HCX16 lines.

Cotransfection of HKc with both HPV-16 DNA and the v-Ha-ras oncogene also induced a dramatic alteration in keratin profile (Fig. 1b). While the cell line that resulted (HH) also lacked K14 expression, very little K5, K6, and K16 were also expressed.

**Two-Dimensional Electrophoresis.** Keratin expression in epithelial cells is complex and two-dimensional electrophoresis is necessary to adequately resolve the individual keratins. Low levels of vimentin were found in both exocervical cultures (Fig. 2a) and in HPV-16-immortalized cell lines (Fig. 2b). No K14 synthesis was detected in the ras-transfected cervical cell cultures (Fig. 2, c and d). In the cervical squamous cell carcinoma lines examined, the level of K14 expression was always low but variable. The two examples shown (Fig. 2, e and f) represent the extremes observed with different cell lines: K14 expression was low in the C4-1 cell line and absent in the QG-H line. It is significant that in all instances where K14 expression was low or absent, the levels of K5 expression were normal or only slightly reduced. In addition to confirming the up-regulation of simple epithelial keratins (K7, K8, K18, K19), the two-dimen-
b. Keratinocytes

KERATIN EXPRESSION IN TRANSFECTED EPITHELIAL CELLS

Fig. 1. Sodium dodecyl sulfate gradient gel electrophoresis of [35S]methionine-labeled cytoskeletal extracts of human cervical cells (a) and human keratinocytes (b). Keratin intermediate filament proteins migrate between Mr 60,000 (K5) and Mr 40,000 (K19) and are numbered (type II, 5—8; type I, 14—19) as described (1). Vimentin (Vim) expression was observed in some cell lines. a, Lane 1, HPV-16 immortalized HCX (line HCX16-2); Lane 2, HCX 16-2 cells transfected with MDR control vector (MDR); Lanes 3 and 4, HCX 16-2 cells transfected with v-Ha-ras plasmid (pH1) and in a cell line derived from a nude mouse pH1-induced tumor explant (TI); Lanes 5—9, cervical squamous cell carcinoma lines (CaSki, C4-1, QG-U, QG-H, SiHa). b, Lane 1, normal human keratinocyte cultures (HKc); Lane 2, human keratinocytes cotransfected with HPV-16 DNA and v-Ha-ras DNA (HH).

As observed previously with human exocervical cells, minimal alterations occurred in keratin expression after immortalization of HKc with HPV-16 DNA alone (Fig. 3, a and b). K14 expression was not altered when HPVG cells were transfected with the BglIII N fragment of herpes simplex 2 viral DNA (Fig. 3c; tumorigenic cell line HSV). However, a dramatic reduction in K14 expression and a significant increase in the levels of K13 expression were observed in the cell line (HH) produced by cotransfection of secondary HKc cultures with HPV-16 DNA and the v-Ha-ras oncogene (Fig. 3d). In both lines, the synthesis of K5, K6, and K16 was down-regulated, while that of K7, K8, K18, and K19 were up-regulated.

Northern Blot Analysis. To determine whether the dramatic alterations in K14 expression were regulated at the RNA level, Northern blot analysis was performed. Total RNA was extracted from normal and transfected exocervical cells and cervical squamous cell carcinoma lines as well as from normal HKc and the immortalized HKc lines. Variable levels of the 1.6-kilobase human K14 mRNA were observed after hybridization of the Northern blot filters to a specific human K14 cDNA polymerase chain reaction probe (see Fig. 4). High levels of K14 mRNA were observed in exocervical cells (Fig. 4a) and HKc (Fig. 4c). Levels of K14 mRNA were decreased after immortalization with HPV-16 DNA in both HKc and HCX cells, and subsequent transfection with the MDR gene reduced K14 mRNA levels even more (HCX only). However, no K14 mRNA was detected in v-Ha-ras-transfected HPV-immortalized cervical cells (line HCX16-2-pH1; Fig. 4a) and epidermal keratinocytes (line HH; Fig. 4c). Similar results were obtained with cells derived from the nude mouse tumor explant (line HCX16-2-pH1-T1), and two cervical squamous cell carcinoma lines examined (QG-U and QG-H; Fig. 4b), while levels of K14 mRNA were barely detectable in another line (C4-1; Fig. 4b).

DISCUSSION

Examination of keratin expression in several human cervical SCC lines demonstrates that a variable but dramatically reduced level of K14 expression is a consistent finding. However, while K14 is significantly down-regulated, K5 (the in vivo partner of K14) shows little or no reduction in expression at the protein level. These two keratins are coexpressed at the same level in normal epithelial cells (1, 2, 5, 7), but this study suggests that the coexpression of these two keratin genes is uncoupled in cervical SCC lines. The regulation of keratin gene expression can occur at both levels, transcription and translation (46, 47). In general, keratin genes are under multifunctional transcriptional control which involves several promoter, enhancer, and silencer elements. Evidence also suggests that type II keratin genes (e.g., K5) can induce expression of the type I partner (K14) and that such mechanisms have a posttranscriptional component (46). However, while the precise details of keratin gene regulation are still not fully understood, the present data do infer that the normal mechanism for maintaining the coordinate regulation of K5 and K14 is aberrant in human cervical SCC lines.

In addition to the down-regulation of K14 expression, other significant alterations in keratin expression were consistently found in the cervical SCC lines. While a general increase in the expression of simple epithelial keratins (K7, K8, K18, K19) was observed, K13 was consistently up-regulated in the absence of its normal partner, K4. This represents another change in the coordinate regulation of keratin gene expression by these tumorigenic cervical SCC cells. Also, it is tempting to speculate that
because the normal coexpression of two keratin pairs (K4–K13 and K5–K14) has been disrupted, these cells may be able to assemble filaments from K5 and K13 (6, 7).

The changes in K14 expression are limited to cell lines derived from cervical tumors of squamous or exocervical origin because normal endocervical cells and adenocarcinomas do not express K14 (10). The uterine tumor line studied (SiHa) had a keratin profile resembling that of HeLa cells (adenocarcinoma). Although both cell lines expressed high levels of K7, K8, K16 (or K17), and K18, combined with no detectable levels of K14, other major differences are apparent. In particular, no expression of K5, K6, K13, or K19 is observed while all of these keratins are expressed in cervical SCC lines. Furthermore, as K16 and K17 are not separated in the gel system used, the K16 detected in SiHa cells may in fact be K17 as is the case for HeLa cells.

Variable and low expression of K14 has been reported in SCC from human skin, with the lowest levels found in poorly differentiated tumors (48). In mouse skin tumors, reports indicate that the levels of K14 expression can remain high (49), while in human keratinocytes transfected with SV40 no significant effects on K13 or K14 were reported in early passages, but later passage cells did lack K14 expression (50).

The current data indicate that cervical cells and foreskin epidermal keratinocytes respond differently to the same stimulus. HPV immortalization of both cell types has very little effect on the keratin profile and causes no significant changes in K13 or K14 expression (10, 36). Subsequent transfection of HPV-immortalized human exocervical cells with an activated oncogene (v-Ha-ras construct) produces a keratin profile similar to cell lines from in vivo cervical SCC. Analysis of one of the resulting tumorigenic cervical lines (HCX16-2-pH1) and cells from the subsequent production of tumors in the nude mouse shows that the alterations in keratin expression are maintained. In common with the in vivo cervical SCC lines, the in vitro generated line (HCX16-2-pH1) showed a dramatic decrease in K14 expression at both the protein and mRNA levels. Some decrease in K5, K6, and K16 expression and an increase in K7, K8, K18, and K19 expression were also observed. A slight increase in K13 expression was also found in these cells but not to the same extent as in the in vivo lines. Thus, this tumorigenic exocervical cell line (HCX16-2-pH1) mimics the keratin expression of cervical SCC lines derived from in vivo carcinomas.

Foreskin-derived human epidermal keratinocytes transfected with HPV-16 DNA and the v-Ha-ras construct (HH line) also had reduced K14 expression and increased K13 expression.
Keratin expression in transfected epithelial cells

Fig. 3. Two-dimensional electrophoresis of [35S]methionine-labeled cytoskeletal extracts of various epithelial keratinocytes. Proteins were separated by nonequilibrium pH gradient electrophoresis (NEpHGE; pH 4-9; left, basic) followed by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis. a, secondary culture of normal human epidermal keratinocytes (HKc); b, HPV-16-immortalized epidermal keratinocytes (line HPVG); c, herpes simplex 2-transfected keratinocytes (line HSV); d, HPV-16- and v-Ha-ras-cotransfected keratinocytes (line HH).

These cells demonstrated high levels of K7, K8, K18, and K19 expression and low levels of K5, K6, and K16 expression. Thus, although some characteristics are similar to the cervical line (HCX16-2-pH1), the overall profile resembles that of SiHa or HeLa. When this line (HH) was tested for tumorigenicity in the nude mouse assay, no tumors were found. Thus, cotransfection of human foreskin epidermal keratinocytes with HPV-16 DNA and a v-Ha-ras construct does not have the same effect on epidermal keratinocytes in terms of keratin expression and tumorigenic potential. Keratin expression has also been studied in another ras-transfected human keratinocyte line (HaCaT) which is tumorigenic but does not show a decrease in K14 expression (51). The HaCaT line has a complex keratin profile which changes according to the differentiation state, expressing K5, K6, K7, K8, K14, K16, K18, and K19 in an undifferentiated state while five additional keratins (K1, K4, K10, K13, K15) are

Fig. 4. Northern blot analysis of K14 expression in human cervical cells and derived lines (a), human cervical cell tumor lines (b), and human epidermal keratinocytes and derived lines (c). A 1.6-kilobase human K14 mRNA was detected by hybridization to a 32P-labeled polymerase chain reaction (PCR)-derived probe covering unique sequences in the COOH-terminal and 3'-noncoding regions of K14 cDNA. a, Lane 1, secondary culture of human exocervical cells (Exo); Lane 2, HPV-16-immortalized exocervical cells (line HCX16-2); Lane 3, HCX16-2 cells transfected with MDR control vector (line MDR); Lanes 4 and 5, HCX16-2 cells transfected with v-Ha-ras plasmid (line pH1) and a cultured cell line derived from a nude mouse pH1-induced tumor explant (line pH1-T1); Lane 6, HeLa cervical adenocarcinoma cell line. b, Lane 1, HPV-16-immortalized exocervical cells (line HCX16-2); Lanes 2-4, cervical tumor cell lines (QG-U, C4-1, QG-H). Kb, kilobases. c, Lane 1, normal human epidermal keratinocyte cultures (HKc); Lane 2, HPV-16-immortalized keratinocytes (HPVG); Lane 3, HPV-16 and v-Ha-ras transfected keratinocytes (HH). While HPV-16 transfection reduces K14 mRNA levels in cultured human exocervical cells and epidermal keratinocytes and a further reduction occurs in control secondary transfections, levels of K14 mRNA in HPV-immortalized cells transfected with v-Ha-ras, in common with cervical carcinoma lines, appeared to be virtually zero.
expressed as the cultures differentiate and stratify. A human foreskin epidermal keratinocyte line derived by transfection of HPV-immortalized cells with HSV-2 DNA (BgIII N fragment) is tumorigenic (38) but demonstrated neither a reduction in K14 expression nor an increase in K13 expression.

It is also interesting to speculate as to the mechanism of the down-regulation of K14 expression in the ras-transfected HPV-immortalized cervical cell line. In the cervical SCC lines studied, K14 expression was not always totally absent but was variably down-regulated. However, in the in vitro line (HCX16-2-ph1) and cells derived from the nude mouse tumors, K14 expression is virtually absent. Prior to v-Ha-ras transfection, although the cells had normal levels of K14 expression in terms of protein synthesis, Northern blotting showed that the mRNA levels were significantly reduced after HPV-16 transfection. Thus, HPV integration is sufficient to down-regulate K14 gene expression at the transcriptional level, but levels of stable K14 mRNA remain high enough to allow normal levels of K14 protein synthesis. The MDR controls also showed that subsequent transfection of the HPV-immortalized exocervical cells caused a further decrease in K14 mRNA but there were still sufficient quantities present to maintain normal levels of K14 protein synthesis. However, after v-Ha-ras transfection of the HPV-immortalized cells, K14 mRNA levels descend to virtually zero and K14 synthesis cannot be maintained. The levels of K14 mRNA are higher in exocervical cells and epidermal keratinocytes compared to their HPV-immortalized counterparts, while the level of K14 protein synthesis is similar in both, inferring that K14 mRNA is under some form of translational control in normal cells.

The ability of activated ras oncogenes to affect developmental regulation of both intermediate filament and actin gene expression has recently been described (52). An activated ras gene was shown to prevent normal up-regulation of muscle-specific gene products during myogenesis and interfere with the normal down-regulation of both vimentin and non-muscle actins. Thus, in the v-Ha-ras-transfected HPV-immortalized human exocervical cells, the presence of an activated oncogene could severely disrupt gene expression and influence the potential of these cells to execute their normal program of cellular differentiation. Whether this mechanism plays an important role in the formation of cervical tumors in vivo depends on the presence of HPV-16 DNA and an activated oncogene. The loss of one allele of c-Ha-ras has been described in heterozygous cervical tumors and mutation at codon 12 (or a mutation and a deletion) has been found in cervical tumors at an advanced stage of carcinoma. The observed down-regulation of K14 gene expression has only been observed in cultured cells and has not been demonstrated for tumors in vivo. In fact, one recent study (55) has shown that K14 is expressed in the basal and some suprabasal cells of squamous cervical tumors in situ using a monoclonal K14 peptide antibody but no direct keratin protein analysis, Northern blotting, or in situ hybridization data was presented. Also, because the presence of HPV DNA and the up-regulation of c-Ha-ras expression was not confirmed, these could represent early tumors prior to K14 down-regulation.

Thus, although changes in keratin expression suggest alterations in the differentiation potential of various normal, immortalized and tumorigenic epithelial cells, no simple rules apply. In human cervical SCC lines from in vivo tumors and the tumorigenic cervical line made in vitro by transfection with HPV-16 and v-Ha-ras DNA, the alterations in keratin expression are consistent. However, while K14 down-regulation may be a good molecular marker for cervical SCC lines, care must be exercised in extrapolating that changes in K14 expression may be useful for estimating tumorigenic potential in the broad sense. Nevertheless, these cervical cell lines represent an interesting approach for studying the effects of cellular oncogene and HPV gene expression on the coordinate regulation of keratins at the molecular level.

REFERENCES

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