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2386 East Heritage Way, Suite B, Salt Lake City, Utah 84109 USA
Phone +1-877-628-7300 • Email—Info@PC-Project.org
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KERATIN EXPRESSION IN MOUSE EPIDERMAL TUMORS

Dennis R. Roop, Thomas Mehrel, Thomas M. Krieg, Hisayoshi Nakazawa, Christina K. Cheng, and Stuart H. Yuspa

Laboratory of Cellular Carcinogenesis and Tumor Promotion
Division of Cancer Etiology
National Cancer Institute
Bethesda, MD 20892

The process of terminal differentiation is highly regulated in mouse epidermis and occurs in stages. Terminal differentiation begins with the migration of cells from the basal layer, continues with the progression of cells through the spinous and granular layers and ends with the deposition of the mature epidermal cells (squames) in the stratum corneum. The commitment to enter the differentiation program appears to occur postmitotically since DNA synthesis and cell division are restricted to the basal layer. Changes in keratin gene expression occur during epidermal differentiation. Keratins K5 and K14 are major products of basal epidermal cells (37). K5 is a member of the Type II intermediate filament (IF) subclass and K14 is a member of the Type I IF subclass (27). The presence of both K5 and K14 is required for keratin filament formation and these filaments together with microtubles (tubulin) and microfilaments (actin) form the cytoskeleton of epidermal cells. One of the earliest changes associated with the commitment to differentiation and migration into the spinous layer is induction of another differentiation-specific pair of keratins, K1 (Type II) and K10 (Type I) (22,26,37). IF containing K1 and K10 replace those containing K5

and K14 as the major products of cells in the spinous and granular layers.

Several laboratories have reported changes in keratin expression in mouse tumors produced in the skin carcinogenesis model (17,31,35). In addition to keratins K5, K14, K1 and K10 expressed in normal mouse epidermis, papillomas express keratins K6 and K16 (11,15), which are expressed in skin and other tissues under conditions of hyperproliferation (14,33). There is a dramatic decrease in expression of K1 and K10 in malignant skin tumors as revealed by studies at the mRNA (31) and protein (31,35) level. Recent results obtained from the use of epidermal cell cultures in combination with biochemical and molecular techniques have provided a potential explanation for the lack of expression of K1 and K10, the differentiation-specific keratins, in malignant tumors. Exposure of normal mouse skin and normal keratinocytes to chemical carcinogens causes a defect in the program of epidermal differentiation which allows cells to escape some signals for terminal differentiation (39). Tumor promoters appear to selectively expand the clones of these altered cells (40). A subsequent change in these differentiation-altered cells complements the underlying biochemical events in initiation to produce the malignant tumor (7-8). At the gene level it appears that activation of the Harvey ras gene is sufficient to produce the initiated phenotype yielding cells that have both a defect in differentiation and produce a benign tumor (3,19,24). Subsequent genetic changes which complement ras activation to affect malignant conversion have not been identified (5).

Sensitive methods to monitor keratin gene expression would be very useful in skin carcinogenesis studies. Such methods might allow the detection of early changes, which proceed the appearance of benign tumors, and the recognition of malignant conversion at a preclinical stage. Therefore, we initiated a project to isolate genes encoding keratins expressed at different differentiation states. Initially, we constructed cDNA libraries to mRNA isolated from primary cultures of mouse epidermal cells grown in low calcium medium, which selects for proliferating basal cells (6), and mRNA isolated from newborn mouse epidermis, which is hyperplastic and consists primarily of differentiated cells (22). These libraries were screened to identify clones corresponding to abundant mRNA species. This approach allowed us to isolate and sequence all of the major keratins expressed in mouse epidermis which includes keratins K5 and K14, expressed predominantly in proliferating basal cells (21,28) and keratins K1 and K10, expressed predominantly in differentiated epidermal cells (27,29). In addition, we isolated a cDNA clone for keratin K6 which is expressed under hyperproliferative conditions such as cell culture, but not in normal epidermia (15).

Sequence analysis of these cloned cDNAs has revealed unique amino acids at the carboxyl terminal of individual keratin proteins (12,28-30). Synthetic peptides corresponding to these sequences have been used to elicit monospecific antibodies (20-21). These antibodies have been useful reagents to study the specific expression of individual keratin proteins within particular cell types (23,25). In addition, the sequence information has enabled us to produce specific nucleic acid probes for the analysis of transcriptional activity by in situ hybridization (24-25). In this chapter we review recent studies using these specific tools to follow keratin gene expression in normal mouse epidermis and in benign and malignant epidermal tumors.

Keratin Expression During Normal Differentiation

Prior to the development of in situ hybridization conditions, which allow the localization of specific gene transcripts within cells in different layers of the epidermis (24), it was assumed that the distribution of keratin gene transcripts would correlate with that of the proteins that they encode. It is now evident that this assumption was somewhat misleading. During gene induction, there is a good correlation between the appearance of keratin mRNA, as detected by in situ hybridization, and the detection of keratin proteins by immunofluorescence, i.e., transcription and translation appear to be tightly coupled. However, as cells proceed to the next differentiation state, there is concomitant activation of new genes and repression of keratin genes expressed in the previous differentiation state.

The exertion of this strict constraint on keratin gene transcription is best illustrated for the genes encoding keratins K5 and K14. Transcripts for K5 and K14 are essentially restricted to the proliferating basal layer of newborn mouse epidermis [only the in situ pattern for K5 is shown in Fig. 1(b)]. The detection of mRNAs for these keratins in the basal layer and not in the differentiated layers of the epidermis suggests the transcription of these genes must be repressed soon after cells commit to terminal differentiation and migrate away from the basement membrane. In addition, the abrupt disappearance of transcripts for these genes in the suprabasal layers indicate a change in the stability of these mRNAs as cells enter the suprabasal layers. It is also evident that K5 and K14 are transcribed in hair follicles, apparently in the outer root sheath. To follow the distribution of the proteins encoded by K5 and K14 mRNAs, we have exploited the existence of unique amino acids at the carboxyl termini of these proteins to produce antibodies that are highly specific for K5 and K14. In fact, we have used this approach to produce monospecific antibodies for all of the major keratins synthesized in mouse

epidermis (20,21). Indirect immunofluorescence of newborn mouse skin with a K5 antibody confirms that K5 mRNA is translated in the basal layer as well as in hair follicles [Fig. 1 (A)]. Furthermore, this analysis demonstrates that, even though K5 transcripts disappear in the suprabasal layers, the translated K5 protein is quite stable and persists throughout the living layers of the epidermis. Thus, depending on the method of analysis, very different patterns of expression are perceived.

The decision to enter the differentiation program is marked by at least two changes at the transcriptional level (1) the repression of transcription of the K5 and K14 genes and (2) the induction of transcription of keratin genes 1 and 10. This is shown in Fig. 1 (D) (only the in situ pattern for K1 is shown). Most of the K1 transcripts are detected in the first suprabasal layer. They are very abundant throughout the spinous layer and diminish as cells migrate into the granular layer. Thus, as shown for K5 an dK14, transcription of the K1 and K10 genes is repressed as epidermal cells progress to the next differentiation state. Furthermore, this transition is also most likely associated with a change in the stability of K1 and K10 mRNAs. Although most of the transcripts for the K1 and K10 genes are located suprabasally, a few basal cells contain an appreciable number of silver grains suggesting that the induction of transcription of these genes may occur in some, if not all cells, prior to migration away from the basement membrane.

It was of interest to determine if the K1 and K10 transcripts detected in some basal cells were actually translated in these cells, or only translated after cells migrate away from the basement membrane. Therefore, indirect immunofluorescence was carried out using antibodies that are specific for keratins 1 and 10 (20). Occasional basal cells (approximately 5-10%) can be stained with these antibodies [only the staining pattern for K1 is shown in Fig. 1 (C)]. These results indicate that transcription and translation are in fact tightly coupled in differentiating epidermal cells. In additional experiments (not shown) we have carried out immunofluorescent staining with a guinea pig antibody against K1 and a rabbit antibody against K10. These double label experiments permit the simultaneous localization of both proteins in the same cell and allow us to determine if expression of the K1 and K10 genes occurs at exactly the same time. All suprabasal cells reacted with both antisera. In most instances, basal cells staining with anti-K1 also stained with anti-K10. However, a few rare basal cells only stained with anti-K1. Basal cells only reacting with anti-K10 were not detected. These results suggest that, at least in these rare cells, expression of the Type II IF gene K1, occurs prior to that of the Type I IF gene, K10, (Nakazawa et al., manuscript in preparation).

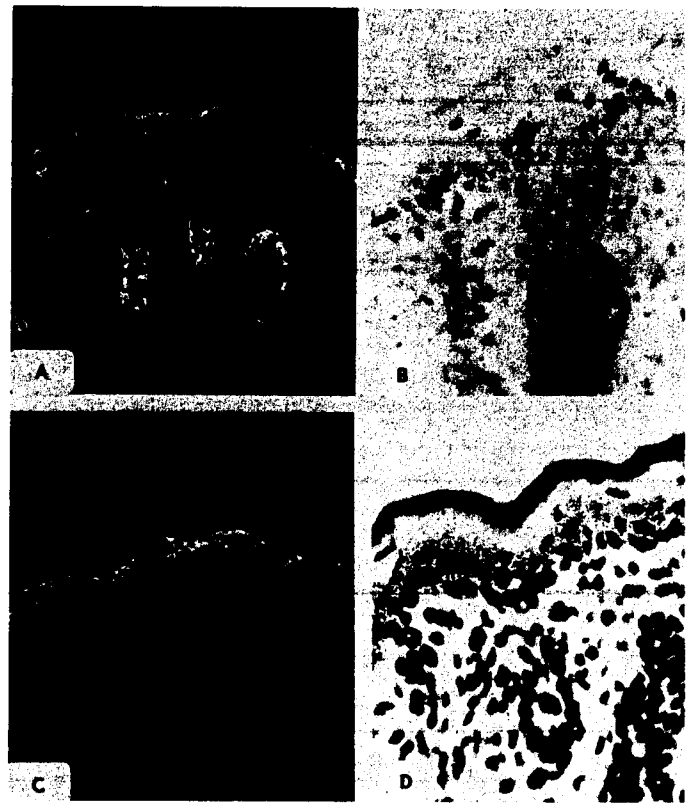

Figure 1: Analysis of keratin expression in newborn mouse skin by indirect immunofluorescence and in situ hybridization. Indirect immunofluorescence was performed with antisera against K5 (A) and K1 (C). Frozen sections of newborn mouse skin hybridized with ^{35}S-labeled RNA probes corresponding to K14 (B) and K1 (D).

Keratin Expression Under Hyperproliferative Conditions

In addition to the keratin genes associated with proliferation and differentiation, we have recently isolated and sequenced a cDNA clone which is not expressed in normal mouse epidermis but is expressed under hyperproliferative conditions such as in cell culture, benign and malignant epidermal tumors and hyperplasia induced by the tumor promoter, TPA, or wounding (15). This is most convincingly shown by indirect immunofluorescence with a monospecific antibody produced against this keratin. Normal adult mouse epidermis is not stained with this antiserum, however, hair follicles are stained. It is unclear at the present time whether this keratin is located in the inner or outer root sheath of the follicle. The expression data and amino acid sequence information, indicating that this cDNA clone encodes a Type II keratin, are consistent with this keratin corresponding to K6, which Weiss et al., (33) have previously shown to be expressed under hyperproliferative conditions.

The lack of expression of keratin K6 in normal epidermis appears to be regulated at the transcriptional level since in situ hybridization experiments and RNA blot analysis fail to detect transcripts of this gene (15). These results contrast with a report by Tyner et al., (32) suggesting that the human K6 gene is constitutively expressed in normal epidermis but is not translated. Translation presumably occurs in response to hyperproliferative stimuli such as wounding. In related experiments, mRNA for the mouse Type I keratin K16 gene, usually co-expressed with the Type II keratin K6, is not detected in normal mouse epidermis by in situ hybridization, but is detected after exposure to hyperproliferative stimuli such as treatment with TPA or wounding (11). It is unclear at this time whether the mechanism(s) regulating the expression of keratins K6 and K16 are different between the two species or whether there are technical reasons to explain the different observations.

Keratin Expression in Epidermal Tumors Analysis with Specific Antisera

As discussed in the introduction, several laboratories have documented changes in keratin expression during skin carcinogenesis at the protein (16,35) and mRNA (31) level. We have recently used the specific antisera, described in the previous section to confirm these observations by immunoblot analysis (25). An example of results obtained with selected papillomas and carcinomas is shown in Figure 2. K14 is detected in all papillomas and carcinomas. This result is in agreement with other reports suggesting that K14 and K5 are the only keratins constantly

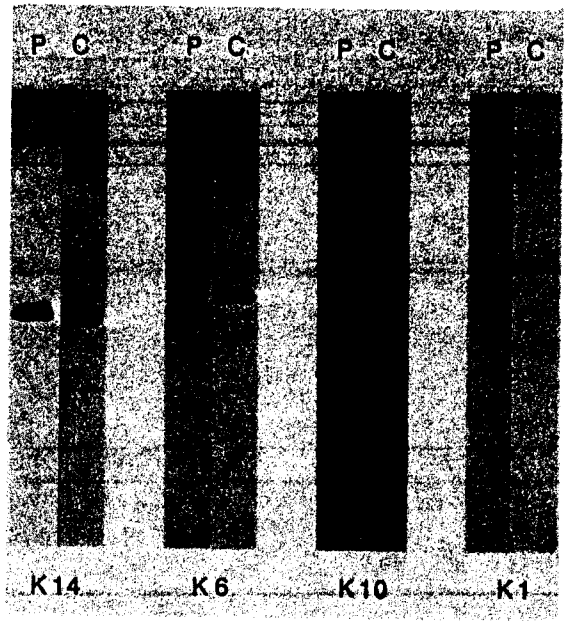

Figure 2: Immunoblot analysis of keratins from mouse skin tumors. Cytoskeletal extracts from papillomas (P) and carcinomas (C) were subjected to electrophoresis, transferred to nitrocellulose paper, and reacted with antisera specific for K14, K6, K10 and K1.

detected in all normal stratified epithelia and in all skin tumors (14,16). K6 is also detected in all papillomas and carcinomas as expected due to the hyperproliferative nature of these tumors. However, it should be emphasized that K6 is not expressed in normal epidermis.

Immunoblots employing antisera elicited against the differentiation-specific keratins K1 and K10 reveal the presence of these proteins in papillomas but not in carcinomas. Two bands were detected in papilloma extracts with the K10 antiserum. The additional band probably corresponds to K11. Huszar et al. (9) have recently produced a monoclonal antibody K_K 8.60 which also detects K10 and K11 in extracts from human epidermis. It is not known at the present time whether K10 and K11 are separate but closely related gene products or result from post-translational modification. The concentration of K10 and K11 is approximately the same in the papilloma extracts shown in Figure 3; however, extracts from newborn mouse epidermis contain predominately K10 (20). Some papillomas also show reduced levels of K1 and K10 (25) and this will be discussed below.

The availability of antisera, which were produced in two different species (guinea pig and rabbit), have allowed us to use double-label immunofluorescence to follow the expression pattern of different keratins at the same time in epidermal tumors (25). The distribution of K5 and K14 in papillomas is similar to that observed in normal epidermis in that both are prominent in the basal cell layer and persist throughout most of the suprabasal layers. K1 and K10 staining of papillomas is confined to the suprabasal cell layers and not detected in basal cells as occasionally observed in normal epidermis. In some areas of papillomas, the first and second cell layers are negative for K1 and K10. These observations are in fact consistent with previous data demonstrating that proliferative cells persist in suprabasal regions of papillomas (38). There is reduced staining of K1 in the most superficial layers, and this is most likely due to processing which also occurs in the stratum corneum of newborn mouse skin (20). K6 expression is detected in the epithelial portions of papillomas, however, in some areas, staining is restricted to the suprabasal layers.

All squamous cell carcinomas examined to date are strongly positive for K5, K14, K6. An example of the staining pattern obtained for K14 is shown in Fig. 3. Double-label immunofluorescence was performed on this section to also detect K1 expression. Note the lack of staining. Performing double-label immunofluorescence on the same section is extremely useful since the staining pattern of K14 establishes the epithelial nature of the tumor areas that are negative for K1. All carcinomas produced in the skin carcinogenesis model are essentially negative for expression of K1 and K10 (25). Individual cells have been

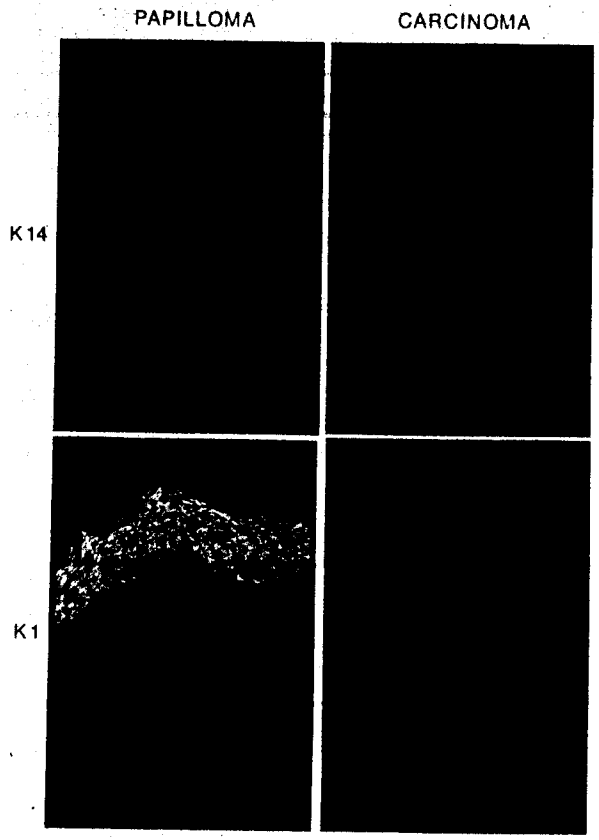

Figure 3: Analysis of keratin synthesis in papillomas and carcinomas. Double-label immunofluorescence was performed on the same section to simultaneously detect K14 and K1. The K14 antiserum was produced in guinea pigs and the K1 antiserum was produced in rabbits.

detected which are positive for the differentiation-specific keratins, but these are relatively rare and most often only stain positive for K10.

The lack of expression of K1 and K10 in carcinomas prompted us to examine papillomas at various stages of promotion to determine if the disappearance of these keratins could be detected early in conversion (Roop et al., 1988). Several papillomas exhibited the staining pattern shown in Fig. 3. The periphery of the tumor stained positive for K1 and K10 but the central area was negative. The epithelial origin of both areas was demonstrated by K14 staining. A careful examination of the histology of this tumor revealed well organized papilloma cells at the periphery, however, the central area was dysplastic. These results suggest that the failure to express the differentiation-specific keratins occurs early during conversion.

Analysis by In Situ Hybridization

It was of interest to determine if the inhibition of expression of the differentiation-specific keratins during conversion was regulated at the transcriptional or translational level. A previous report suggested that translatable K1 mRNA was present in a masked form in a transplantable squamous cell carcinoma (34), however, we failed to detect transcripts of K1 and K10 genes in RNA extracted from carcinomas resulting from initiation-promotion protocols (31). In situ hybridization analysis with probes specific for K1 and K10 failed to detect appreciable transcripts in carcinomas (25). An example of the results obtained with the K1 probe is shown in Fig. 4. In addition, the in situ pattern detected for K14 is shown to indicate the integrity of the tissue and demonstrate the abundance of transcripts for this gene in carcinomas. The results with the K1 and K10 probes indicate that expression of these genes is blocked at the transcriptional level and are consistent with a defect in the program of epidermal differentiation. The high level of expression observed for the K14 gene is indicative of the proliferative status of the tumor.

The analysis of papillomas by in situ hybridization revealed several differences in the distribution of keratin gene transcripts as compared to normal epidermis (25). First, transcripts for K14 are not restricted to the basal layer, as observed in newborn epidermis, but are distributed well into the suprabasal layers of highly stratified papillomas (Fig. 4). The persistence of expression of K14 in the upper regions of papillomas is consistent with the presence of proliferative cells in these regions. Similar results have also recently been obtained for pupoid fetus (pf/pf) mutant mice, which are genetically altered in their response to signals for epidermal diffentiation or in the generation of these signals (4 and

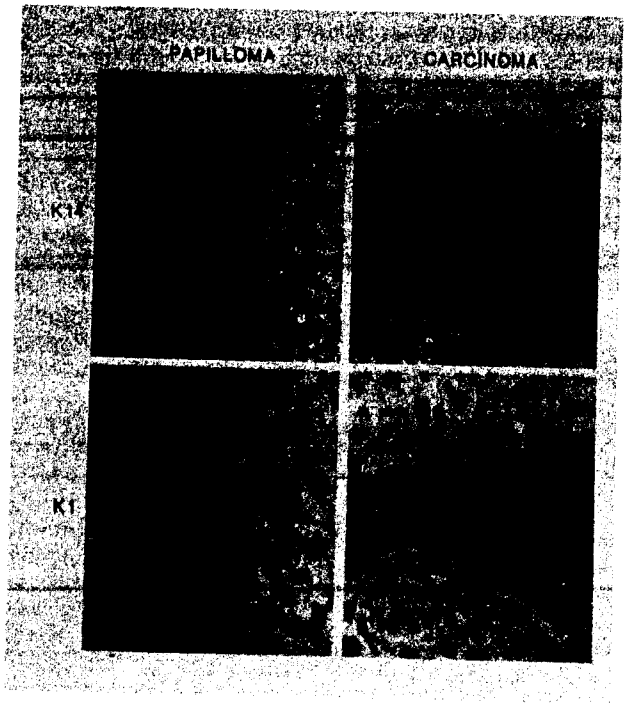

Figure 4: Detection of keratin gene transcripts in mouse skin tumors by in situ hybridization. Frozen sections of papillomas and carcinomas were hybridized with ^{35}S-labeled RNA probes corresponding to K14 an dK1.

C. Fisher and D. Roop, unpublished observations). Therefore, the expression pattern of K14 in papillomas is compatible with benign tumor cells having a constitutive defect in their response to differentiation signals (38). Second, the distribution of transcripts for the differentiation-specific keratins are in general similar in papillomas and newborn epidermis, however, the concentration of transcripts in the first suprabasal layer seems to be less in papillomas (Fig. 4). This observation is consistent with a delay in the induction of the differentiation-specific keratin genes as would be predicted due to their altered differentiation response.

Keratin Expression During Stages of Skin Carcinogenesis

Early Events Preceding the Appearance of Benign Lesions

The gene encoding keratin K6 is normally never expressed in the epidermis (15). However, its expression is quickly triggered when changes in the proliferative status of epidermal cells occur. These changes can be induced experimentally by topical application of TPA or tape-stripping. All benign and malignant epidermal tumors examined to date express K6. We suspect that expression of the K6 gene occurs very early in skin carcinogenesis. The ability to detect K6 may allow the identification of altered foci before they become apparent as a benign lesion. Experimentally, this would facilitate the identification of genetic changes which occur very early during initiation-promotion protocols.

Early Events Associated With Malignant Conversion

Three independent laboratories have documented the absence of the differentiation-specific keratins (K1 and K10) in malignant mouse skin tumors resulting from initiation-promotion protocols (17,31,35). We have recently used antibodies specific for these keratins to demonstrate that these changes occur early during conversion (25). Our observations have been confirmed independently by Aldaz et al., (2). These investigators have also correlated these changes in keratin expression with the expression of - glutamyltransferase (GGT), which has previously been shown to occur in malignant and some benign tumors (10), and changes in chromosomal status, which appear to occur progressively during skin carcinogenesis (1). Therefore, the loss of keratins K1 and K10 appears to be an early negative marker for conversion from bening to malignant status.

In addition to these keratins serving as negative markers for conversion,keratin K13 has recently been shown to be a potential positive marker for conversion (18). These observations resulted from a collaboration between our laboratory and that of

Dr. Jurgen Schweitzer. K13 is normally expressed in the suprabasal layers of internally stratifying epithelia. It is not expressed in normal epidermis nor is it expressed under hyperproliferative conditions induced in vivo or in vitro. However, a combination of immunological analysis, employing a specific antiserum produced against a unique synthetic peptide, and in situ hybridization analysis, using a specific nucleic acid probe, revealed that K13 was expressed in carcinomas resulting from initiation-promotion protocols. In addition, the number of papillomas expressing K13 was found to increase with the time of promotion. Additional experiments will be required to determine if there is a correlation between the loss of keratin K1 and K10 and the appearance of K13. Nevertheless, the appearance of K13 may provide a positive marker for malignant conversion.

The availability of markers to detect malignant conversion at stages prior to gross or histological examination would be of particular importance in experimental studies. Experiments designed to determine the potency of genotoxic agents undoubtedly underestimate their potency since animals usually die as a result of the first or second malignant tumor. The ability to screen for early signs of conversion would permit the assessment of the conversion state of all tumors at the time of death and provide a more accurate estimate of the potency of genotoxic agents. In addition to this application, the detection of conversion at an early stage may assist in the identification of primary genetic events required for conversion.

Application the the Diagnosis of Human Skin Cancer

Insufficient data are currently available to determine how useful these observations will be in the study and diagnosis of human skin cancers. The presence of K6 has been reported in human skin tumors (14,33). However, there are no reports correlating the expression of K6 with early stages of carcinogenesis. Human squamous cell carcinomas in general have reduced levels of the differentiation-specific keratins (9,13-14,16,34). However, a large percentage have minor, but significant, amounts of these proteins (9,14). K13 expression has not been observed in human skin cancers. Thus, at the present time, these changes in keratin expression are only suitable for monitoring skin carcinogenesis in the mouse model. The development of antisera and nucleic acid probes to specifically detect human keratins is required to determine if these observations will eventually be useful in the diagnosis of human skin cancer.

REFERENCES

1. Aldaz, C.M., Conti, C.J., Klein-Szanto, A.J.P., and Slaga, T.J. *Proc. Natl. Acad. Sci. USA*, 84:2029-2032, 1987.
2. Aldaz, C.M., Conti, C.J., Larcher, F., Trono, D., Roop, .R., Chesner, J., Whitehead, T., and Slaga, T.J. *Cancer Res.*, in press, 1988.
3. Bizub, D., Wood, A.W., and Skalka, A.M. *Proc. Natl. Acad. Sci. USA*, 83:6048-6052, 1986.
4. Fisher, C., Jones, A., and Roop, D.R. *J. Cell Biology*, 105:1807-1820, 1987.
5. Harper, J.R., Roop, D.R., and Yuspa, S.H. *Mol. Cell. Biol.*, 6:3144-3149, 1986.
6. Hennings, H., Michael, D., Cheng, C., Steinert, P., Holbrook, K., and Yuspa, S.H. *Cell*, 19:245-254, 1980.
7. Hennings, H., Shores, R., Wenk, M., Spangler, E.F., Tarone, R., and Yuspa, S.H. *Nature*, 304:67-69, 1983.
8. Hennings, H., Spangler, E.F., Shores, R, Mitchell, P., Devor, D., Shamsuddin, A.K.M., Elgjo, K.M., and Yuspa, S.H. *Env. Health Perspect.*, 68:59-67, 1986.
9. Huszar, M., Gigi-Letiner, O., Moll, R., Franke, W.W., and Geiger, B. *Differentiation*, 31:141-153, 1986.
10. Klein-Szanto, A.J.P., Nelson, K.G., Shah, Y., and Slaga, T.J. *JNCI*, 70:161-168, 1983.
11. Knapp, B., Rentrop, M., Schweitzer, J., and Winter, H. J. *Biol. Chem.*, 262:938-945, 1987.
12. Krieg, T.M., Schafer, M.P., Cheng, C.K., Filpula, D., Flaherty, P., Steinert, P.M., and Roop, D.R. *J. Biol. Chem.*, 260:5867-5870, 1985.
13. Moll, R., Franke, W.W., Schiller, D.L., Geiger, B., and Krepler, R. *Cell*, 31:11-24, 1982.
14. Moll, R., Moll, I., and Franke, W.W. *Arch. Dermatol. Res.*, 276:349-363, 1984.
15. Nakazawa, H., Mehrel, T., Cheng, C.K., Yuspa, S.H., and Roop, D.R. *J. Cell. Biol.*, 103-561a, 1986.
16. Nelson, W.G., Battifora, H,. Santant, H., and Sun, T.T. *Cancer Res.*, 44:1600-1603, 1984.
17. Nelson, K.G. and Slaga, T.J. *Cancer Res.*, 42:4176-4181, 1982.
18. Nischt, R., Roop, D.R. Mehrel, T., Yuspa, S.H, Reutrop, M., Winter, H., Schweitzer, J. *Molecular Carcinogenesis*, submitted, 1988.
19. Quintanilla, M., Brown, K., Ramsden, M., and Balmain, A. *Nature*, 322:78-80, 1986.
20. Roop, D.R., Cheng, C.K., Titterington, L., Meyers, C.A., Stanley, J.R., Steinert, P.M., and Yuspa, S.H. *J. Biol. Chem.*, 259:8037-8040, 1984.

21. Roop, D.R., Cheng, C.K., Toftgard, R., Stanley, J.R., Steinert, P.M., and Yuspa, S.H. *Ann. N.Y. Acad. Sci.*, 455:426-435, 1985.
22. Roop, D.R., Hawley-Nelson, P., Cheng, C.K., and Yuspa, S.H. *Proc. Natl. Acad. Sci. USA*, 80:716-720, 1983.
23. Roop, D.R., Heutfeldt, H, Kilkenny, A., and Yuspa, S.H. *Differentiation*, 35:143-150, 1987.
24. Roop, D.R., Lowy, D.R., Tambourin, P.E., Strickland, J., Harper, J.R., Balaschak, M,. Spangler, E.F., and Yuspa, S.H. *Nature*, 323:822-824, 1986.
25. Roop, D.R., Krieg, T.M., Mehrel, T., Cheng, C.K., and Yuspa, S.H. *Cancer Res.*, in press, 1988.
26. Schweitzer, J., Kinjo, M, Furstenberger, G., and Winter, H. *Cell*, 37:159-170, 1984.
27. Steinert, P.M., Parry, D.A.D., Idler, W.W., Johnson, L.D., Steven, A.C., and Roop, D.R. *J. Biol. Chem.*, 260:7142-7149, 1985.
28. Steinert, P.M., Parry, D.A.D., Racoosin, E.L., Idler, W.W., Steven, A.C., Trus, B.L., and Roop, D.R. *Proc. Natl. Acad. Sci. USA*, 81:5709-5713, 1984.
29. Steinert, P.M., Rice, R.H., Roop, D.R., Trus, B.L., and Steven, A.C. *Nature*, 302:794-800, 1983.
30. Steinert, P.M., Steven, A.C., and Roop, D.R. *Cell*, 42:411-419, 1985.
31. Toftgard, R., Yuspa, S.H., and Roop, D.R. *Cancer Res.*, 45:5845-5850, 1985.
32. Tyner, A.L, Fuchs, E. *J. Cell. Biol.*, 103:1945-1955, 1986.
33. Weiss, R.A., Eichner, R.A., and Sun, T-T. *J. Cell. Biol.*, 98:1397-1406, 1984.
34. Winter, H. and Schweitzer, J. *Proc. Natl. Acad. Sci. USA*, 80:6480-6484, 1983.
35. Winter, H., Schweitzer, J., and Goerttler, K. *Carcinogenesis*, 1:391-398, 1980.
36. Winter, H., Schweitzer, J., and Goerttler, K. *Arch. Dermatol. Res.*, 275:27-24, 1983.
37. Woodcock-Mitchell, J., Eichner, R., Nelson, W.G., and Sun, T-T. *J. Cell. Biol.*, 95:580-588, 1982.
38. Yuspa, S.H. In: *Role of Chemicals and Radiation in the Etiology of Cancer*, E. Huberman, ed., Raven Press, New York, pp. 201-208, 1985.
39. Yuspa, S.H. *J. Amer. Acad. Dermatol.*, 15:1031-1044, 1986.
40. Yuspa, S.H. In: *Accomplishments in Cancer Research 1986*, J.G. Fortner and J.E.Rhoads, eds., Lippincott, Co., Philadelphia, PA., pp. 169-182, 1986.