

Immunohistochemical Detection of the Alternate *INK4a*-Encoded Tumor Suppressor Protein p14^{ARF} in Archival Human Cancers and Cell Lines Using Commercial Antibodies: Correlation with p16^{INK4a} Expression

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The *INK4a* locus encodes two structurally unrelated tumor suppressor proteins, p16^{INK4a} and p14^{ARF}. Although the former is one of the most common targets for inactivation in human neoplasia, the frequency of p14^{ARF} abrogation is not established. We have developed an immunohistochemical assay that allows the evaluation of p14^{ARF} expression in formalin-fixed, paraffin-embedded tissues, using commercially available antibodies. p14^{ARF} positive cells showed nuclear/nucleolar staining, which was absent in all cell lines and tumors with homozygous deletions of the *INK4a* gene. The assay was applied to 34 paraffin-embedded cell buttons, 30 non-small cell lung cancers and 28 pancreatic carcinomas, and the staining results were correlated with p16^{INK4a} expression. Loss of p14^{ARF} expression was common but less frequent than down-regulation of p16^{INK4a} (53% versus 76% of all specimens). The p14^{ARF} and p16^{INK4a} expression pattern was concordant in 65 of 92 cases (71%). Significantly, 24 cases were p16^{INK4a}-/p14^{ARF}+, while the opposite staining pattern was observed in three cases, consistent with the notion that the two proteins have nonredundant functions. The immunohistochemical assay described here may facilitate studies on the prevalence and significance of aberrant p14^{ARF} expression in human tumors.

KEY WORDS: Antibodies, Immunohistochemistry, *INK4a*, p14^{ARF}, p16^{INK4a}.

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The *INK4a* gene on chromosome 9p21 is one of the most common targets for inactivation in human neoplasia. The gene is unusual in that it encodes two structurally unrelated proteins, p16^{INK4a} and p14^{ARF}, the human homologue of murine p19^{ARF}. Two different first exons are spliced in different reading frames to common exon 2 (1). p16^{INK4a} acts as a retinoblastoma protein (pRB) agonist by inhibiting the phosphorylation of pRB by activated cyclin-dependent kinases 4 and 6 (2). The principal methods of p16^{INK4a} inactivation are homozygous deletion of the gene, promoter methylation of exon 1 α , and intragenic mutation (3). The frequency of p16^{INK4a} inactivation in human neoplasia rivals that of p53. We previously demonstrated that immunohistochemistry (IHC) is a sensitive and specific method of detecting the absence of functional p16^{INK4a} in formalin-fixed, paraffin-embedded tumors, whatever the underlying mechanism (4, 5). In contrast, p14^{ARF} primarily acts as a p53 agonist by inhibiting the MDM2-mediated degradation of the latter (6, 7). p14^{ARF} can also be inactivated by homozygous deletion, promoter hypermethylation, and, presumably, intragenic mutation, although no mutations selectively targeting exon 1 β have been described (1). Promoter methylation of exons 1 α and 1 β appear to be independent events (8), and comparatively few data exist on the frequency of p14^{ARF} inactivation in human neoplasia. An important reason for this relative lack of data may be the unavailability of an assay that would allow the evaluation of p14^{ARF} expression in archival tissues. Here we describe such an assay, which utilizes commercially available reagents and which should be ap-

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plicable in any immunohistochemical laboratory. We demonstrate the validity of our method and its application to paraffin-embedded cell lines and tumors, and we provide evidence that, at least in some human cancers, p14^{ARF} abrogation, although important, may be less common than, and independent of, p16^{INK4a} inactivation.

MATERIALS AND METHODS

Cell Lines and Tissues

All lung cancer and mesothelioma cell lines (designated by the prefix "H") were originally established at the National Cancer Institute—Navy Medical Oncology Branch (9). Breast cancer cell lines MCF-10A, SKBR3, BT474, T47D, MDA-MB-231, MDA-MB-361, and MDA-MB-468 were provided by the Imperial Cancer Research Fund Clare Hall Laboratories (London, UK). Colorectal carcinoma cell lines SW480, SW620, SW837, SW1463, RKO and DLD-1, and cell lines PC-3 and U2OS, as well as a nude mouse xenografts of cell lines H417 and H2009, had been used in previous immunohistochemical studies (4, 10, 11). The non-small cell lung cancers (NSCLC) were part of a cohort of well-characterized tumors from Australia (12). The pancreatic carcinomas were from the pathology files of the Johns Hopkins Medical Institutions (5, 13). Normal breast, skin, colon, appendix, and tonsil and the phyllodes tumor were from the Department of Cellular Pathology at the John Radcliffe Hospital (Oxford, UK). The cell lines, xenograft, human tumors and normal tissues had been fixed in 10% buffered formalin, processed, and embedded in paraffin using routine procedures.

Materials

Mouse monoclonal anti-p16^{INK4a} antibody Ab-7 and monoclonal anti-p14^{ARF} antibodies 14PO2 and 14P03, as well as polyclonal Ab-1 were obtained from LabVision/NeoMarkers (Fremont, CA). Rabbit polyclonal anti-p14^{ARF} antibodies Ab-1/PC409 and ZF14 were obtained from Oncogene Research Products (via CN BioSciences, Nottingham, UK) and Zymed Laboratories (South San Francisco, CA), respectively. The Elite ABC detection kit was purchased from Vector Laboratories (Burlingame, CA).

Immunohistochemistry

Five μm thick paraffin sections were cut onto coated slides and stored at 4°C until used. The experiments were carried out in a Shandon Sequenza immunostainer. The immunohistochemical assay for detecting p16^{INK4a} in fixed and paraffin-embedded tissues has been described in detail elsewhere (4, 5, 14). Briefly, after antigen

retrieval in 0.1 M EDTA pH8.0 (20 minutes at 95 to 100°C), the sections were reacted with the anti-p16 monoclonal antibody at 1 $\mu\text{g}/\text{mL}$ at 4°C overnight. Some of the p16^{INK4a} staining data had been included in two earlier studies (5, 12). For p14^{ARF} IHC, we initially tested all five antibodies (see Results). We chose to optimize reaction conditions for one of them, *i.e.*, monoclonal antibody 14PO2. After dewaxing and rehydration, the endogenous peroxidase activity was quenched for 20 minutes with 0.3% H₂O₂ in methanol. The sections then underwent antigen retrieval in 0.01 M citrate buffer pH6.0 at 95 to 100°C for 20 minutes (cell blocks) or 40 minutes (tissues). After blocking with 1% horse serum for 20 minutes, the sections were reacted with primary antibody at 1 $\mu\text{g}/\text{mL}$ (cell blocks) or 4 $\mu\text{g}/\text{mL}$ (tissues) at 4°C overnight. The detection reactions for both p16^{INK4a} and p14^{ARF} followed the Vectastain Elite ABC protocol as suggested by the manufacturer. Diaminobenzidine (from Vector) with hematoxylin counterstain was used for color development. Negative antibody controls were stained under identical conditions. External positive controls for p14^{ARF} included normal breast, colon, appendix, and tonsil, a phyllodes tumor, and nude mouse xenografts of lung cancer cell lines H417 and H2009. Several cell lines and tumors with known homozygous *INK4a* deletions served as external negative controls. A specimen was considered positive for p16^{INK4a} or p14^{ARF} if there was nuclear staining above any cytoplasmic background; cytoplasmic staining itself was disregarded (5). If the cells of interest failed to show distinct nuclear reactivity, the specimen was considered negative for the respective protein. In tissue sections, admixed stromal, inflammatory, and normal epithelial cells served as positive internal controls.

RESULTS

Development of an Immunohistochemical Assay for p14^{ARF}

In preliminary experiments, we tested five anti-p14^{ARF} antibodies (three polyclonals and two monoclonals) obtained from three companies. After antigen retrieval in 0.1 M EDTA and primary incubation overnight at 1:400 (polyclonals) or 2 $\mu\text{g}/\text{mL}$ (monoclonals), all five antibodies produced the expected nuclear staining pattern in positive controls. Because the monoclonal antibodies appeared to be more sensitive, we optimized the reaction conditions for one of them, 14PO2 from NeoMarkers. To validate the IHC assay, we used the positive and negative control specimens detailed in the Methods. Variables

tested included primary antibody concentration, different antigen retrieval techniques, and different detection reactions, among others. We found it necessary to employ a longer antigen retrieval time and higher primary antibody concentration for archival tissues, compared with formalin-fixed, paraffin-embedded cell buttons. In cells expressing p14^{ARF}, the staining pattern was predominantly nuclear, sometimes with nucleolar accentuation, usually associated with some cytoplasmic staining (Fig. 1, A and C). In some tumors and cell lines, there was strong nucleolar staining. In many cell types, the staining intensity was less than for p16^{INK4a}, but there seemed to be tissue specific variability. p14^{ARF} levels appeared to be relatively high in the breast (Fig. 1A), but lower in other tissues. The protein was expressed by non-neoplastic epithelium of the breast, skin, tonsil, colon and appendix. Nuclear staining could also be detected in a subset of lymphocytes, fibroblasts and endothelial cells serving as convenient internal positive controls in tumor sections. No such staining was observed on negative antibody

control stains. Cells devoid of p14^{ARF}, e.g., those with a homozygous *INK4a* deletion, typically showed nonspecific cytoplasmic reactivity, but no nuclear staining above the background (Fig. 1B).

Immunohistochemical Evaluation of p14^{ARF} Expression in Formalin-Fixed, Paraffin-Embedded Cell Lines and Tumors and Correlation with p16^{INK4a} Expression

To demonstrate the general applicability of our p14^{ARF} assay to archival specimens, we stained 34 cell buttons and 58 carcinomas. To study the relationship between p14^{ARF} and p16^{INK4a} expression in neoplastic cells, the same 92 specimens were stained for p16^{INK4a} as well. As detailed in Tables 1 and 2, six cell lines were positive, and 18 were negative for both proteins. Of the six p14^{ARF}+ cell lines, all but one (H719) had previously been shown to have a normal *INK4a* status. In addition, nude mouse xenografts of two lung cancer cell lines without detectable *INK4a* abnormalities reacted posi-

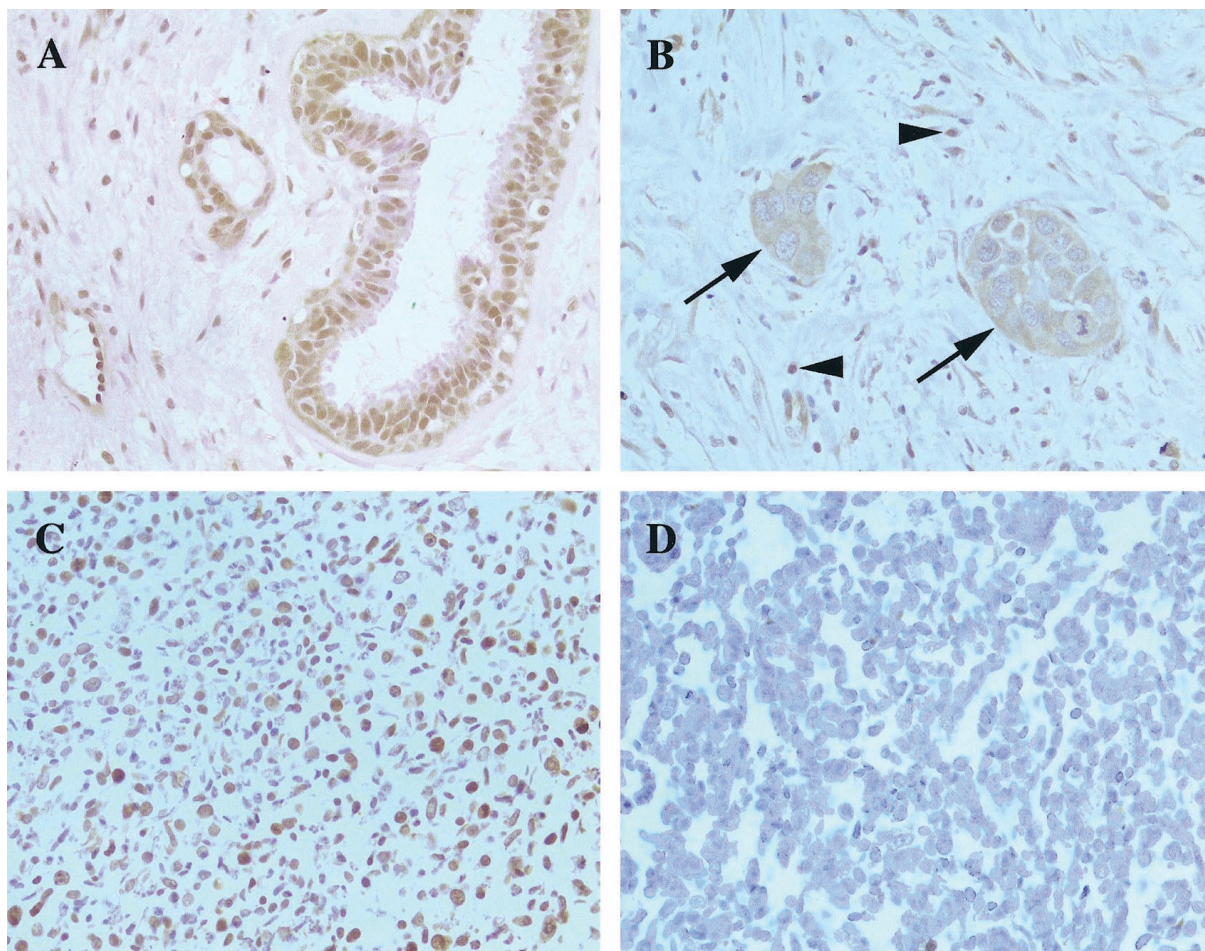


FIGURE 1. p14^{ARF} staining patterns in archival tissues and cell lines. **A**, Strong nuclear and weak cytoplasmic reactivity in most epithelial and stromal cells of a phyllodes tumor stained for p14^{ARF}. **B**, The tumor cells in a p14^{ARF} negative NSCLC show moderate cytoplasmic but no nuclear reactivity (*arrows*); adjacent stromal and inflammatory cells are positive (*arrowheads*). **C**, **D**, Paraffin-embedded cell line U2OS shows positive nuclear staining for p14^{ARF} (**C**) but not for p16^{INK4A} (**D**). Original magnifications, 400 \times .

TABLE 1. p16^{INK4a} and p14^{ARF} Immunocytochemical Staining Patterns in Formalin-Fixed, Paraffin-Embedded Cell Lines

Cell Line	p16 ^{INK4a} IHC	p14 ^{ARF} IHC
H719	+	+
H1926	+	+
H2171	+	+
H2172	+	+
SKBR3	+	+
MDA-MB-468	+	+
MCF-10A	-	+
T47D	-	+
BT474	-	+
MDA-MB-361	-	+
SW480	-	+
SW620	-	+
SW1463	-	+
PC3	-	+
U2OS	-	+
H125	-	-
H441	-	-
H513	-	-
H841	-	-
H865	-	-
H1339	-	-
H1915	-	-
H2107	-	-
H2373	-	-
MDA-MB-435	-	-

IHC, immunohistochemistry; -, No nuclear staining; +, positive nuclear staining.

TABLE 2. Molecular Status of the *INK4a* (p16/*ARF*) Locus and Expression of p16^{INK4a} and p14^{ARF} Determined by IHC in Nine Human Cancer Cell Lines

Cell Line	p16		ARF	
	DNA	Protein	DNA	Protein
H792	HD	-	HD	-
H1264	HD	-	HD	-
H2122	HD	-	HD	-
MDA-MB-231	HD	-	HD	-
RKO	MSNK	-	Methyl	-
DLD-1	Methyl	-	Methyl	-
SW837	MSNK	-	Unmethyl	+
H157	Mutation	-	Mutation	-
H661	Mutation	-	Mutation	-

IHC, immunohistochemistry; -, no nuclear staining by IHC; +, positive staining by IHC; HD, homozygous deletion (9); MSNK, methylation status not known; methyl, promoter methylated (8); unmethyl, promoter unmethylated (8).

tively. Some cell lines showed prominent nucleolar reactivity on the p14^{ARF} stains. Interestingly, 10 cell lines were negative for p16^{INK4a} but expressed p14^{ARF} (Fig. 1, C and D); the opposite staining pattern was not observed. We then studied 30 NSCLC and 28 pancreatic adenocarcinomas. Among the latter, all

were p16^{INK4a} negative, but nine expressed p14^{ARF} (Table 3). Among the lung cancers, 16 cases expressed p16^{INK4a}, and 18 were positive for p14^{ARF}. However, the staining pattern for these two proteins did not coincide in eight of the 30 tumors (Table 3). Considering all test cell lines and tumors, the concordance rate for p16^{INK4a} and p14^{ARF} expression was 71% (19/92 positive and 46/92 negative for both). Although the expression patterns of these two proteins were significantly correlated ($P < .0001$, Fisher's exact test), it is noteworthy that 24 cases were p16^{INK4a} negative/p14^{ARF} positive, while three cases were p16^{INK4a} positive/p14^{ARF} negative. In p14^{ARF} and/or p16^{INK4a} positive cell lines and tumors, a variable number of cells were devoid of nuclear reactivity.

Correlation between ARF Status and p14^{ARF} Expression

For nine cell lines and 20 pancreatic carcinomas, information about *ARF* abnormalities at the DNA level was available. No nuclear staining was observed in cell lines with homozygous deletions of the *INK4a* locus ($n = 4$), promoter methylation of exon 1 β ($n = 2$) or an intragenic mutation ($n = 2$) (Table 2). Colorectal cancer cell line SW837 has no known *ARF* abnormality and has an unmethylated exon 1 β promoter (8), and this cell line expressed p14^{ARF}. Similarly, all 12 pancreatic adenocarcinomas with *INK4a* deletions were p14^{ARF} negative (Table 4). No data were available on the exon 1 β methylation status in these cases. One silent and two frameshift mutations were associated with absence of nuclear staining. In contrast, three missense mutants produced positive nuclear immunoreactivity; two of these cases had identical mutations (Table 4). Two pancreatic carcinomas had *INK4a* mutations affecting only the p16^{INK4a} open reading frame but not *ARF*, and both of these showed a positive immunohistochemical reaction pattern for p14^{ARF}.

DISCUSSION

p16^{INK4a}, one of the two proteins encoded by the *INK4a* gene, is one of the most frequent targets of inactivation in human cancers. The other *INK4a*-encoded protein, p14^{ARF}, appears to have physiological functions that only partially overlap with

TABLE 3. p16^{INK4a} and p14^{ARF} Immunohistochemical Staining Patterns in Archival Lung and Pancreatic Carcinomas

Tumor Type	p16+/p14+	p16+/p14-	p16-/p14+	p16-/p14-	Total
NSCLC	13	3	5	9	30
Pancreatic ca	0	0	9	19	28
Total	13	3	14	28	58

NSCLC, non-small cell lung cancer; p16, p16^{INK4a}; p14, p14^{ARF}; +, positive for protein expression; -, negative for protein expression.

TABLE 4. Molecular Status of the *ARF* Gene and p14^{ARF} Expression Determined By IHC in 20 Pancreatic Adenocarcinomas

Pancreatic Carcinoma	ARF Abnormality	p14 ^{ARF} IHC
PX92/PX101	None ^a	+
PX20	Mutation (Silent)	-
PX16/PX65	Mutation (Missense) ^b	+
PX67	Mutation (Missense)	+
PX24	Mutation (Frameshift)	-
PX122	Mutation (Frameshift)	-
PX23	HD	-
PX28	HD	-
PX56	HD	-
PX64	HD	-
PX76	HD	-
PX88	HD	-
PX90	HD	-
PX91	HD	-
PX107	HD	-
PX117	HD	-
PX120	HD	-
PX121	HD	-

^a These two carcinomas contain *INK4a* mutations that affect the p16 ORF only.

^b These two carcinomas have identical *INK4a* mutations.

IHC, immunohistochemistry; HD, homozygous deletion; +, positive nuclear staining; -, no nuclear staining.

those of p16^{INK4a} (1, 6, 15). p14^{ARF} is an integral component of the p53/MDM2/p14^{ARF} pathway and has bona fide tumor suppressor activity (16). However, it is not clear to what extent p14^{ARF} is deregulated in human neoplasia.

We previously showed that IHC is an effective method to demonstrate abrogation of p16^{INK4a} function in pathologic specimens (4, 5), and we set out to develop a similar assay for p14^{ARF}. IHC allows evaluation of protein expression in specific cells and is applicable to most archival tissues. We are aware of only two previous immunohistochemical studies on p14^{ARF} expression in human cancers (17, 18). Both of these used rabbit polyclonal antibodies, which are not commercially available, and only one of them was performed on paraffin sections (17).

Here we demonstrate that p14^{ARF} expression can be evaluated in formalin-fixed, paraffin-embedded tissues with commercially available reagents. Although we optimized the assay for only one of the five antibodies tested, monoclonal 14PO2 from NeoMarkers, which has been used before in immunofluorescence assays (19), it is quite possible that comparable results may be obtained with other anti-p14^{ARF} antibodies. In agreement with earlier studies, the presence of immunoreactive p14^{ARF} was indicated by granular or diffuse nuclear staining, with or without nucleolar accentuation (17, 18); cytoplasmic staining was observed even in cells with homozygous deletions of the *INK4a* gene and may thus be nonspecific. In some normal cells, cell lines and tumors, the staining pattern was predominantly nucleolar, consistent with the recently described nucleolar sequestration of MDM2 by p14^{ARF} (7, 19, 20). The significance of the

somewhat variable subcellular localization is unclear, but it does not seem to be related to fixation. The assay seems to have adequate sensitivity. A subset of non-neoplastic cells in various tissues, as well as five cell lines and two nude mouse xenografts with an intact *INK4a* gene, reacted positively. Not all cells with an intact *ARF* gene expressed p14^{ARF} at a detectable level. It was previously shown that the intracellular level of p16^{INK4a} is partly cell cycle dependant (21). It is conceivable that intracellular p14^{ARF} levels are subject to similar variability, although we are not aware of detailed published studies on the regulation of p14^{ARF} expression in normal or neoplastic cells.

The IHC reaction pattern in cell lines and tumors correlated well with the molecularly defined status of the *INK4a* gene that was available for some cell lines and for many of the pancreatic cancers, and with previous studies on p14^{ARF} expression in some of the cell lines. All cell lines and tumors with homozygous *INK4a* deletions were devoid of nuclear reactivity above any cytoplasmic background. Colorectal carcinoma cell lines RKO and DLD-1 reportedly have methylated exon 1 β promoters (8), and both of these failed to express the protein, whereas the unmethylated cancer cell line SW837 was p14^{ARF} positive. Although some *ARF* mutations led to negative immunoreactivity, missense mutations produced positive nuclear staining (Tables 2 and 4). Thus, the presence of nuclear staining does not necessarily indicate the presence of normal p14^{ARF}. Our data are consistent with earlier findings of p14^{ARF} mRNA and protein expression in MDA-MB-468 breast cancer cells (20) and with absence of exon 1 β promoter methylation in many colorectal cancer cell lines (8). T47D breast cancer cells were previously found to be p14^{ARF} negative by immunofluorescence but positive by RT-PCR, and U20S osteosarcoma cells were reported to be negative but inducible for p14^{ARF} (19). Both cell lines showed nuclear staining in our IHC assay, indicating good sensitivity.

However, the limits of the assay's sensitivity have yet to be determined. It is possible that very low but physiologically significant levels of p14^{ARF} may not be detectable by paraffin section IHC. Other potential problems with the immunohistochemical approach include variability in stain interpretation (our simple dichotomous scoring system should be rather robust) and a relatively poor signal-to-noise ratio due to seemingly low levels of antigen, especially in non-neoplastic tissues (15), and significant nonspecific background staining. However, as has been the case with p16^{INK4a}, the latter problem is likely to be alleviated by the advent of second generation anti-p14^{ARF} antibodies with improved sensitivity and specificity.

We found loss of p14^{ARF} expression in 12 of 30 NSCLC, which is comparable with the rate of p14^{ARF} down-regulation reported in three previous

studies on this tumor type (17, 18, 22). All four studies agree that p14^{ARF} loss is less common than down-regulation of p16^{INK4a}. Five of our lung cancers were negative for p16^{INK4a} but positive for p14^{ARF}, and this phenotype was observed in two earlier studies (17, 18). Like Gazzeri *et al.* (18), but unlike Vonlanthen *et al.* (17), we also identified a smaller number of p16^{INK4a}/p14^{ARF}– NSCLC. It was previously shown that almost all pancreatic carcinomas lack functional p16^{INK4a} (13). Interestingly, nine of 30 pancreatic carcinomas were positive for p14^{ARF}, suggesting that inactivation of this protein in this tumor type may not be as critical as abrogation of p16^{INK4a}. Our data on cell lines and tumors support other studies that suggested that p16^{INK4a} and p14^{ARF} expression is differentially regulated, possibly due to methylation of only one of the two promoters in a given tumor or cell line (8). We believe our study to be the first to examine the relationship between these two markers at the protein level. In our series the concordance rate between p16^{INK4a} and p14^{ARF} immunoreactivity was 71% (24/34 cell lines, 22/30 NSCLC, 19/28 pancreatic cancers), which was statistically highly significant. However, 10 of 30 cell lines and 14 of 60 carcinomas had a p16^{INK4a}–/p14^{ARF}+ phenotype, while the opposite staining pattern was observed in three tumors. These findings support the notion that the two proteins encoded by the *INK4a* locus have nonredundant functions.

In conclusion, the present study demonstrates that it is possible to evaluate p14^{ARF} expression in formalin-fixed, paraffin-embedded tissues entirely with commercial reagents. The assay we describe should be applicable in any immunohistochemical laboratory. It may facilitate studies on the prevalence and pathobiologic and clinical significance of aberrant p14^{ARF} expression in human neoplasia. We provide preliminary evidence that down-regulation of p14^{ARF} is frequent in cancer cell lines and at least some tumors, but probably less common than p16^{INK4a} inactivation. Finally, we have shown that expression of the two *INK4a*-encoded proteins may be discordant in a significant proportion of cell lines and cancers.

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Book Review

Ackerman AB, Reddy VB, Soyer HP: *Neoplasms with Follicular Differentiation, Second Edition, 1109 pp, New York, Ardor Scribendi Publishers, 2000 (\$225.00).*

The second edition of *Neoplasms with Follicular Differentiation* is everything one would expect from Dr. Bernard Ackerman. Beautifully written and illustrated, intellectually challenging, controversial, and encyclopedic in both breadth of coverage and size, the second edition has more than 200 new photographs with increased emphasis on trichoblastoma and “trichoblastic carcinoma.” Dr. Ackerman contends the nature and accurate diagnosis of follicular neoplasms has long been obscured by incomprehensible terminology and illogical classification. I heartily agree, and his attempt to shed light on this perplexing topic is most welcome.

Dr. Ackerman proposes a new classification of follicular proliferations based on his method of pattern analysis and carefully defined categories of cyst, malformation, hamartomas, hyperplasias, benign neoplasms, and malignant neoplasms. Proliferations are deemed follicular in nature if they show microscopic evidence of differentiation toward elements of the follicle. This contrasts with classifications based on evidence of *origin* from follicular elements. This method certainly holds great appeal for those of us outside academe’s ivory towers with only our trusty microscopes to guide us, but will no doubt rankle those who feel it would be more scientifically honest to define follicular proliferations based on origin from primordial “follicle” cells.

Many of the proliferations discussed are well-accepted entities such as nevus comedonicus (malformation), fibrous papule (hamartoma), and tricholemmoma (hyperplasia). In addition to reviewing historical, clinical, and histopathologic aspects, Dr. Ackerman manages to embellish even these less controversial entities with wisdom gleaned from his own extensive

experience. For example, he notes that, in his opinion, perifollicular fibroma is simply a form of fibrous papule; similarly, most tricholemmomas and inverting follicular keratoses are described as forms of verruca vulgares.

More than half of the book is devoted to his concept of trichoblastoma and trichoblastic carcinoma. Dr. Ackerman encompasses trichoepithelioma, desmoplastic trichoepithelioma, adamantoid trichoblastoma, trichoblastic fibroma, and prototypical trichoblastoma as variants of trichoblastoma. Trichoblastic carcinoma represents its malignant counterpart, based on classic Ackerman criteria of malignancy: asymmetry, poor circumscription, etc. In addition, Ackerman considers basal cell carcinoma to be trichoblastic carcinoma.

Neoplasms with Follicular Differentiation will not appeal to everyone. Dr. Ackerman stakes out positions that are diametrically different from many other experts in dermatopathology. Controversial opinions are stated with the certainty of fact, for example: “solar keratoses are squamous cell carcinomas.” Nevertheless, I greatly enjoyed this book and would unabashedly recommend it to readers with a particular interest in dermatopathology. Dr. Ackerman presents a novel view of follicular proliferations in a wonderfully lucid, logical fashion, backing up his claims with extensive black and white and color photographs that are uniformly excellent. The text is in his own imitable style with his usual clarity and flourish. However, this is a book perhaps most appropriate for those with a fairly solid background in dermatopathology who will best appreciate and evaluate the differences between Dr. Ackerman’s classification and those of others in the field.

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