Use of p63 expression in distinguishing primary and metastatic cutaneous adnexal neoplasms from metastatic adenocarcinoma to skin

Abstract: p63, a recently identified homologue of the p53 gene, is mainly expressed by basal and myoepithelial cells in skin. Others and we have shown the value of p63 in distinguishing primary adnexal tumors from visceral adenocarcinomas metastatic to skin. We now investigate the pattern of p63 expression in metastases from skin adnexal carcinomas and their cognate primaries and evaluate p63 expression in a larger case series of malignant cutaneous adnexal neoplasms. Immunohistochemical analysis for p63 was performed on 13 metastases of adnexal carcinomas and their corresponding primary tumors. Twenty visceral metastatic adenocarcinomas to the skin and 7 primary mucinous carcinomas with cutaneous or visceral origin were compared. The majority (90.9%) of primary adnexal tumors strongly expressed p63 and their metastases labeled similar to their cognate primary tumors. With one exception, primary or metastatic mucinous carcinomas did not express p63. Metastases from two apocrine carcinomas lacked p63 expression. All other cutaneous metastases from internal adenocarcinomas were negative for p63. Analysis of p63 expression may assist in the differential diagnosis of primary adnexal carcinomas versus metastatic visceral adenocarcinomas to the skin. Metastases from adnexal carcinomas generally retain p63 expression similar to their associated primary tumors.


The p63 gene, a recently identified member of the p53 gene family, is located on the chromosome 3q27-29.1 In comparison with p53 gene, which essentially acts as a tumor suppressor regulating the expression of genes involved in cell cycle arrest or apoptosis in response to genotoxic damage, the p63 gene has a more complex function.2,3 This is mainly a result to the fact that p63 gene expresses at least six major transcripts that lead to two separate classes of proteins.1,2,4,5 Three of the p63 isoforms (TAp63) encode proteins with roles similar to that of p53, whereas the other three isoforms (ΔNp63) lack the acidic N-terminal transactivation domain and exert inhibitory effects on p53 activity. The analysis of RNA from cultured human keratinocytes indicated that they predominantly express truncated, dominant-negative p63 isoforms (ΔNp63).1,6 Thus, in these cells, the expression of ΔNp63 may block the apoptosis-inducing activity of p53 and thus could help maintain their proliferative capacity.6–10 Recent studies have shown that ΔNp63 is the dominant isotype in squamous cell carcinoma of head and neck region and its high expression is associated with aggressive behavior.11,12
Studies indicate that p63 plays an essential role in epithelial morphogenesis, as shown by the defective development or agenesis of squamous epithelia in the p63-deficient mice. It has been recently shown that p63 is highly expressed in the basal cells of human epithelial tissues, including skin. In addition, p63 is also expressed in other normal epithelia including prostate basal cells, uterine cervix or urogenital tract. Moreover, several studies showed that p63 is a selective nuclear marker of myoepithelial cells in breast and has significant diagnostic value in differentiating between in situ and invasive carcinomas. Interestingly, it has been shown that primary or metastatic carcinomas derived from glandular epithelia, including adenocarcinoma of the breast or prostate, consistently fail to express p63.

The expression of p63 in normal human epidermis, cutaneous appendages and skin carcinomas has been recently assessed. In these studies, p63 was detected in the epidermal and adnexal basal/myoepithelial cells and it has been suggested that p63 might be used as a diagnostic marker for epidermal or adnexal tumors in the skin. Recently, others and we have shown the value of p63 in distinguishing primary adnexal tumors from visceral adenocarcinomas metastatic to skin. Expression of p63 in cutaneous adnexal tumors metastatic to other sites has not yet been examined.

In practice, the diagnosis of a cutaneous metastasis with visceral origin is of paramount significance. On rare occasions, it may represent the first manifestation of an internal malignancy but more often are an indication of a widely disseminated disease and associated with a poor prognosis. On histological grounds alone, the distinction between such metastases and primary cutaneous neoplasms, especially adnexal tumors, may be a challenging task. Although the primary cutaneous adnexal tumors rarely metastasize, these occurrences also have significant clinicotherapeutical implications.

The purpose of this study was to investigate the pattern of p63 expression in rare cases of metastases from skin adnexal carcinomas and their cognate primaries. We have also included additional cases of metastatic visceral tumors to skin in order to further expand our knowledge regarding the utility of p63 expression in the differential diagnosis of primary adnexal carcinomas and metastatic visceral tumors to the skin.

### Materials and methods

**Patients**

The pathology databases at the University of Texas M.D. Anderson Cancer Center; St. John’s Institute of Dermatology, London; and University of Massachusetts Medical School, Worcester, were retrospectively (January 1997 to October 2005) reviewed to identify cases of primary cutaneous adenocarcinomas that subsequently metastasized, with material from both primary and metastasis available for review. The study was performed with the appropriate institutional review board approval. There were retrieved 13 metastases of adnexal carcinomas and their corresponding primary tumors: 5 eccrine carcinomas, 2 apocrine carcinomas, 2 hidradenocarcinomas and 1 each of mucinous, pilomatrixial, sebaceous carcinomas and a malignant mixed tumor. Six of the metastatic tumors were identified in the regional lymph nodes, and in the remaining seven cases, the adnexal carcinoma metastasized to another cutaneous site. Twenty metastatic adenocarcinomas to the skin (14 of them previously characterized and reported) and 7 primary mucinous carcinomas with cutaneous or visceral origin were compared.

Among the 20 cases of metastatic adenosarcomas to skin were 13 of breast origin, 5 from the gastrointestinal tract and 2 from lung. Clinical information was obtained from the medical records. The pathology reports and the hematoxylin and eosin-stained glass slides were reviewed in all cases.

**Immunohistochemical staining for p63**

Immunohistochemical analysis for p63 was performed on formalin-fixed, paraffin-embedded archival tissue using the streptavidin-biotin-peroxidase technique, as previously described. For all cases, a 4-μm histological section was deparaffinized and rehydrated in graded alcohols and distilled water. After blocking the endogenous peroxidase (3% hydrogen peroxide for 5 min), heat-induced antigen retrieval was performed in a water bath, using citrate buffer at pH 6.0 for 20 min. A mouse anti-human monoclonal antibody that reacts with all p63 isoforms (clone 4A4, dilution 1 : 200; Santa Cruz, Biotechnology Inc., Santa Cruz, CA, USA) was used. Biotin-conjugated secondary antibody was applied for 20 min, and streptavidin-biotin-peroxidase complex (Strept-AB complex, dilution 1 : 200; DAKO Corp., Carpinteria, CA, USA) was added for 20 min. Positive and negative controls were included in each staining procedure.

The distribution of the immunoreactivity in the neoplastic cells was analyzed by quantifying nuclear staining, following previous methodology. Only nuclear labeling was considered. A consensus was obtained to score the p63 nuclear staining as follows: zero (0) for less than 5% positive nuclei; 1 for 5–25% positive nuclei, 2 for 26–75% positive nuclei and 3 for over 75% positive nuclei. Positivity
Expression of p63 in primary and metastatic adnexal carcinomas

p63 expression in the primary cutaneous adnexal neoplasms and their cognate metastases is summarized in Table 1.

Eleven of 13 primary tumors were available for p63 immunostaining; p63 was strongly expressed (nuclear scores 3+ or 2+) in the eccrine carcinomas (Fig 1A, B). The pattern of p63 labeling in all five eccrine carcinomas was similar to that previously described: the outer cell layer of the proliferating basaloid cords was strongly positive, while the luminal cells in the tumor nests did not express p63. The p63 labeling was generally retained in the metastatic eccrine tumors (Fig 1C, D), with one exception in which only approximately 20% of the cells labeled for p63 falling short of the established 25% cutoff for p63 positivity.

The infiltrating lobules of basaloid cells in both cases of hidradenocarcinoma (Fig 2A, B) expressed p63 in the great majority of cells (score 3+) and their cognate metastases also strongly labeled for p63 (Fig 2C, D). The basaloid cell proliferations of the pilomatrixical and sebaceous carcinomas were also positive for p63 (nuclear scores 3+), and this expression was similar in their metastases. The primary cutaneous malignant mixed tumor expressed p63 in approximately 40% of cells and this expression was reduced in its metastasis (10% of cells, score 1+).

Of interest was the fact that the primary cutaneous apocrine carcinoma case available for immunostaining expressed p63 in the less than 25% of cells (score 1+). The metastatic apocrine adenocarcinomas were also essentially negative for p63. Similarly, the metastatic mucinous adenocarcinoma did not express p63.

No cytoplasmic labeling with p63 was observed in any of the primary or metastatic adnexal carcinomas included in the study.

### Table 1. Expression of p63 in the primary cutaneous adnexal neoplasms and their cognate metastases

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Primary</th>
<th>Metastatic</th>
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<tbody>
<tr>
<td>Eccrine carcinoma (5)</td>
<td>3+</td>
<td>3+</td>
</tr>
<tr>
<td></td>
<td>3+</td>
<td>3+</td>
</tr>
<tr>
<td></td>
<td>3+</td>
<td>3+</td>
</tr>
<tr>
<td></td>
<td>2+</td>
<td>3+</td>
</tr>
<tr>
<td></td>
<td>2+</td>
<td>1+ (20%)**</td>
</tr>
<tr>
<td>Apocrine carcinoma (2)</td>
<td>NA</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>1+</td>
<td>1+</td>
</tr>
<tr>
<td>Hidradenocarcinoma (2)</td>
<td>3+</td>
<td>3+</td>
</tr>
<tr>
<td></td>
<td>3+</td>
<td>2+ (60%)**</td>
</tr>
<tr>
<td>Mucinous carcinoma</td>
<td>NA</td>
<td>0</td>
</tr>
<tr>
<td>Pliomatrixical carcinoma</td>
<td>3+</td>
<td>3+</td>
</tr>
<tr>
<td>Sebaceous carcinoma</td>
<td>3+</td>
<td>3+</td>
</tr>
<tr>
<td>Malignant mixed tumor</td>
<td>2+</td>
<td>1+ (10%)**</td>
</tr>
</tbody>
</table>

*NA, not available; two of the primary tumors (one apocrine and one mucinous) were not available for p63 immunostaining.

**Numbers in parenthesis represent the percentages of cells positive for p63.
Expression of p63 in primary and metastatic mucinous carcinomas with cutaneous or visceral origin

Based on the initial findings of our study, we have pursued the investigation of the p63 labeling pattern in a larger case series of primary and metastatic mucinous carcinomas.

p63 expression in an additional seven cases of primary mucinous carcinomas of various origins (two cutaneous, three from gastrointestinal tract and two from breast) and in five metastatic mucinous carcinomas to skin is summarized in Table 2.

With the exception of one of the primary cutaneous mucinous carcinomas, all primary or metastatic mucinous tumors lacked p63 expression (Fig. 3A, B).

Expression of p63 in metastatic adenocarcinomas to the skin

Twenty metastatic adenocarcinomas to the skin (13 from breast, 5 from the gastrointestinal tract and 2 from lung) were included in our study. Fourteen of these were previously characterized. None of the metastatic adenocarcinomas was positive for p63. The great majority of the metastatic breast carcinomas did not express p63 in any of the cells. In three cases, focal cytoplasmic p63 positivity was identified. In the metastatic carcinomas from the gastrointestinal tract (three with colonic and two with appendiceal origin), p63 was expressed in less than 5% of the neoplastic cells. The apical cells showed mild cytoplasmic staining. The metastatic lung tumors to the skin did not label for p63.

As previously shown, differences in p63 immunolabeling between the primary cutaneous adnexal tumors and metastatic adenocarcinomas to the skin were highly statistically significant ($p < 0.001$).

Discussion

Analysis of p63 expression in normal tissue and neoplastic conditions has been recently the subject of several basic research studies and also gained special attention in the applicative pathology literature. The analysis has been recently incorporated by some
in the routine breast pathology diagnosis following demonstration of p63 as a reliable myoepithelial cell marker.\textsuperscript{18,19} Similarly, the analysis is sometimes used to identify prostatic basal cells in challenging cases.\textsuperscript{16}

It has been recently noted that the vast majority of the various visceral adenocarcinomas, including their metastases, do not express p63 or have focal and variable p63 positivity.\textsuperscript{15,29} However, there is only limited knowledge regarding the pattern of p63 expression in cutaneous adnexal neoplasms.

The expression of p63 in normal human epidermis, cutaneous appendages and skin carcinomas has been recently assessed.\textsuperscript{20–23} Recently, others and we have shown the value of p63 in distinguishing primary adnexal tumors from visceral adenocarcinomas metastatic to skin.\textsuperscript{21–23} This present study is the first to examine expression of p63 in cutaneous adnexal tumors metastatic to other sites. While this occurrence is rare, it is often raised in differential diagnosis. While patient history is often highly informative in this regard, sometimes it is not available or completely reliable. The purpose of our study was to investigate the pattern of p63 expression in rare cases of metastases from skin adnexal carcinomas and their cognate primaries and to further expand our knowledge regarding the use of p63 expression in the differential diagnosis of these tumors.

Our study confirmed and expanded the results of previously published studies, namely constant p63 expression in both epidermal and adnexal basal cells, and also eccrine myoepithelial cells. In 10 of 11 (90.9\%) primary adnexal tumors, available for immunostaining, p63 was expressed by the majority of cells (nuclear scores 2+ or 3+). Notably, there was a different pattern of staining for different tumors, as mentioned in the Results section. Excluding the apocrine and mucinous carcinomas, the metastatic adnexal tumors generally retained their p63 expression. Thus, eight (72.7\%) metastatic adnexal carcinomas labeled 2+ or 3+, similar to their associated primaries. Because p63 is present in both primary and metastatic cutaneous adnexal carcinomas (especially eccrine), p63 is not helpful in distinguishing them. However, when p63 reactivity is retained, it is helpful in their differential diagnosis.
with cutaneous metastases of visceral adenocarcinomas.

Metastases from two apocrine carcinomas showed a p63 score of 1+ or 0. While only two cases were examined, our data suggest that cutaneous apocrine carcinomas lack p63 expression and this marker is apparently not useful in distinguishing them from apocrine carcinoma metastases from breast or other organs.

In order to enhance the statistical analysis of our study, we have also included additional primary and metastatic mucinous tumors. We were intrigued by the relative lack of p63 reactivity in cutaneous mucinous adenocarcinomas and we examined p63 expression in other mucinous adenocarcinomas not of cutaneous origin. With only one exception (one primary cutaneous mucinous tumor), the primary or metastatic mucinous carcinomas did not express p63.

The reason why mucinous and apocrine neoplasms do not express p63 is not entirely clear. We hypothesize that this fact might be because of the lack of myoepithelial or basal cell phenotype in these tumors.

In concordance with our previous study, all visceral metastatic adenocarcinomas were negative for p63 (score 0). While the metastatic tumors from breast or lung included in our study did not label for p63 in any cells, some of the tumors from the gastrointestinal tract had focal p63 expression in less than 5% of cells, a level inferior to the 25% proposed as significant p63 expression. Therefore, the difference in p63 immunolabeling distinguishes primary cutaneous adnexal tumors and metastatic adenocarcinomas to the skin, being highly statistically significant (p < 0.001).

In summary, the analysis of p63 expression may assist in the differential diagnosis of primary adnexal carcinomas vs. metastatic visceral adenocarcinomas to the skin. The mucinous and apocrine neoplasms constitute the exception; p63 expression analysis is not effective in differentiating their origin. Otherwise, metastases from adnexal carcinomas generally retain p63 expression similar to their associated primary tumors.

Table 2. Expression of p63 in primary and metastatic mucinous carcinomas with cutaneous or visceral origin

<table>
<thead>
<tr>
<th>Mucinous carcinoma</th>
<th>Number of cases</th>
<th>p63 score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cutaneous</td>
<td>2</td>
<td>2+0</td>
</tr>
<tr>
<td>Colon</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Breast</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Metastatic to skin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cutaneous origin</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Gastrointestinal tract origin</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Breast</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

Fig 3. Metastatic cutaneous mucinous adenocarcinoma (A) lacking of p63 staining (B).

References