

## CDX2 Expression in Cutaneous Metastatic Carcinomas and Extramammary Paget's Disease

VIVIANA LORA and JEAN KANITAKIS

*Laboratory of Dermatopathology, Ed. Herriot Hospital Group, 69437 Lyon cedex 03, France*

**Abstract.** *Background: CDX2 is a gene involved in the regulation of intestinal cell proliferation/differentiation. It is considered specific for enterocytes and has been used for the diagnosis of primary and metastatic colon adenocarcinoma. The aim of this study was to assess the usefulness of CDX2 in the diagnosis of cutaneous metastatic tumors and extramammary Paget's disease. Materials and Methods: The immunohistochemical expression of CDX2 was studied in 68 cutaneous metastatic tumors of various origins and 14 specimens of extramammary Paget's disease. Results: CDX2 expression was found in 3/6 metastases of colon adenocarcinoma, 1/1 metastasis of urothelial carcinoma and 1/2 extramammary Paget's disease. Conclusion: CDX2 appears to be specific for cutaneous metastases from intestinal and urothelial carcinomas and is a useful diagnostic marker of these tumors. However, its sensitivity is modest, and we advocate its use in conjunction with additional immunohistochemical markers. CDX2 seems useful for the diagnosis of extramammary Paget's disease associated with an underlying colorectal tumor.*

Cutaneous metastases develop in 4.5% of all patients with visceral tumors. The histopathological diagnosis of metastasis and the definition of the primary origin are usually easy when the patient has a known history of malignancy, and when the metastasis shows histological features similar to those of the primary. However, in a non-negligible proportion of patients, cutaneous metastases are the presenting manifestation of the internal malignancy, a fact rendering histological determination of the primary tumor challenging, especially when metastatic cells are poorly differentiated (1, 2). Immunohistochemical examination represents an effective procedure in reaching the

diagnosis, namely by demonstrating tissue-specific antigens within tumor cells, such as prostate-specific antigen (PSA), uroplakin, renal cell carcinoma antigen and Hep Par-1 in tumors of the prostate, urinary tract, kidney and liver, respectively (3). Colonic adenocarcinoma (ADC) is the second most common origin of cutaneous metastases after breast and lung cancer in women and men, respectively (2). The main markers used for the diagnosis of metastatic colonic ADC are keratins since most cases are K7/K20<sup>+</sup>. However, occasional tumors may show significant K7 expression; conversely, expression of K20 may be seen in a variety of non-colorectal carcinomas, including mucinous ovarian, cutaneous neuroendocrine (Merkel-cell) and urothelial carcinomas (4, 5). More specific markers of colorectal differentiation are therefore needed. Such a marker introduced recently is CDX2, an intestinal epithelia-specific nuclear transcription factor regulating cell proliferation and differentiation, that functions as a tumor-suppressor in colorectal ADC (6, 7). CDX2 is expressed in the gastrointestinal tract, with highest expression in the small intestine and cecum, and lower expression in the distal colon (6). It can be detected on paraffin-embedded tissue sections with CDX2-88 monoclonal antibody that has been used for the diagnosis of colorectal ADC and of metastases arising therefrom (8-14). Recent studies have shown that CDX2 may also be expressed by non-colonic ADC, such as mucinous ovarian, primary intestinal-type endocervical and urinary bladder ADC (8, 9, 15). The expression of CDX2 has been studied in colorectal ADC metastatic to the lungs (10) and liver (16). Regarding the skin, CDX2 has been studied in extramammary Paget's disease (EMPD), where it can serve as a marker of colorectal-derived Paget's cells (17, 18); however, CDX2 expression has not been studied in metastatic skin tumors. The aim of this study was to assess the usefulness of CDX2 in the diagnosis of cutaneous metastatic tumors and EMPD.

### Materials and Methods

*Tissues studied.* These included 68 biopsy or excision specimens of cutaneous metastases and 14 cases of EMPD excised from a total of 34 male and 31 female patients. The specimens had been collected in our dermatopathology laboratory over the past 20 years, and were formalin-fixed and paraffin-embedded (Table I). The diagnosis had been established by examination of hematoxylin-

*Correspondence to:* Dr. Jean Kanitakis, MD, Laboratory of Dermatopathology, Ed. Herriot Hospital Group, 69437 Lyon cedex 03, France. Tel: +33 472110301, Fax: +33 472110323, e-mail: jean.kanitakis@univ-lyon1.fr

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eosin-stained sections, supplemented (when needed) with appropriate immunostains, namely for K7, K20, gross cystic disease fluid protein 15 (GCDFP15), Her-2, PSA, S100 protein, human melanoma black 45 (HMB45) and Hep Par-1. Clinical data (sex and age of patients, presence of a primary) were retrieved from the patients' medical records. Primary tumor sites at the time of metastasis diagnosis were known in 33 patients and unknown in the remaining 31 (Table I). Among the 14 cases of EMPD studied, 12 expressed a cutaneous phenotype (GCDFP15+/K20-) and the remaining two (perianal) an endodermal one (K20+/GCDFP15-). One of them was associated with rectal ADC.

**Immunohistochemistry.** Immunohistochemical labeling was performed on deparaffinized and rehydrated 5 micron-thick sections. The Ventana ES automated AEC immunohistochemistry system (Ventana Medical Systems Inc., Tucson, AZ, USA) was used to investigate the reactivity of the IgG1 kappa monoclonal antibody CDX2-88 (Biogenex, San Ramon, CA, USA) to the CDX2 antigen. Specimens of colonic ADC served as positive controls. Negative controls were obtained by omitting the primary antibody and proved to be consistently negative.

**Results**

The expression of CDX2 in primary colonic ADC was visualized as brown staining within nuclei, and this pattern was considered specific. The same specific reactivity pattern was observed in 3/6 cases of cutaneous metastases of colonic ADC, with considerable variations in the percentage of immunoreactive cells (from 40 to 100%) (Figure 1). Two metastases of colonic ADC and one of rectal ADC proved to be CDX2 negative. The primary tumor of one of these CDX2-negative cases was available for study; it corresponded to an undifferentiated colon ADC that showed CDX2 expression in 50% of the tumor cells. Specific CDX2 immunoreactivity was also found in 40% of tumor cells in one metastatic urothelial ADC (Figure 2). In 4 metastases of unknown origin, granular cytoplasmic labeling was observed, but this was not associated with nuclear staining and was therefore considered non-specific (Figure 3). The remaining cases of metastases did not express CDX2.

Regarding EMPD, CDX2 was positive in one perianal (endodermal-type) EMPD associated with an underlying dysplastic rectal adenoma (Figure 4). The second case of endodermal-type EMPD (with no known underlying tumor) and all 12 cutaneous-type EMPD specimens proved unreactive.

**Discussion**

CDX-2 is a homeobox gene related to the *Drosophila melanogaster* gene caudal, which is essential for the axial patterning and intestinal development of the fruit fly. Two CDX homeobox genes have been identified in humans; they encode a transcription factor that regulates proliferation and differentiation of fetal and adult, normal and neoplastic

Table I. Expression of CDX2 in metastatic skin tumors and extramammary Paget's disease.

Type/Origin of primary tumor	CDX2+/Total cases
Colorectal ADC	3/6
Urothelial/bladder carcinoma	1/1
Endodermal type EMPD (perianal)	1*/2
Cutaneous type EMPD**	0/12
Pancreas ADC	0/3
Breast ADC	0/7
Uterine cervical ADC	0/2
Hepatocellular carcinoma	0/2
Lung ADC	0/3
Lung epidermoid carcinoma	0/2
Kidney clear cell carcinoma	0/4
Various ***	0/5
Unknown	0/33

\*Associated with rectal ADC; \*\*from the vulva (n:10), perineum (n:1) and thorax (n:1); \*\*\*one case each of skin melanoma, laryngeal carcinoma, thymic carcinoma, neuroendocrine carcinoma, prostatic ADC.

intestinal epithelial cells (6, 7). CDX2 has been used for the diagnosis of colorectal ADC, since over 70% of cases express this marker (9, 10, 13, 19, 20). This percentage seems to be lower in poorly differentiated tumors (11, 12, 14, 19), although studies on rectal ADC were unable to confirm any correlation between CDX2 expression and differentiation (14, 19). CDX2 has also been found to be regularly expressed in metastatic colorectal ADC to the liver and lungs (9, 10, 16). However, none of these studies included cutaneous metastases.

In our study, we found that cutaneous metastases of colorectal ADC also show specific CDX2 expression, although the overall sensitivity appears modest (3/6). In keeping with previous studies (12), it seems that CDX2 is preferentially expressed in metastases of well-differentiated ADC, since the three CDX2-negative metastases of colorectal ADC of our study originated from poorly differentiated primaries. One of the CDX2-negative skin metastases originated from a primary that showed positivity in only 50% of cells, suggesting that CDX2 may be down-regulated during progression to the metastatic stage. Alternatively, it can be speculated that (cutaneous) metastases may originate from a CDX2-negative tumor cell clone. In fact, the results of previous studies (19, 21) suggested that CDX2 could act as metastasis-suppressor gene; this is consistent with a lower incidence of CDX2 expression in metastases compared with their corresponding primaries, since loss of CDX2 would promote metastasis. On the other hand, a recent study found that CDX2 loss is significantly associated with female gender (19). The rather limited number of colorectal ADC metastases we studied does not allow conclusions to be drawn as to the influence of sex on the prevalence of CDX2 positivity in our group of tumors.

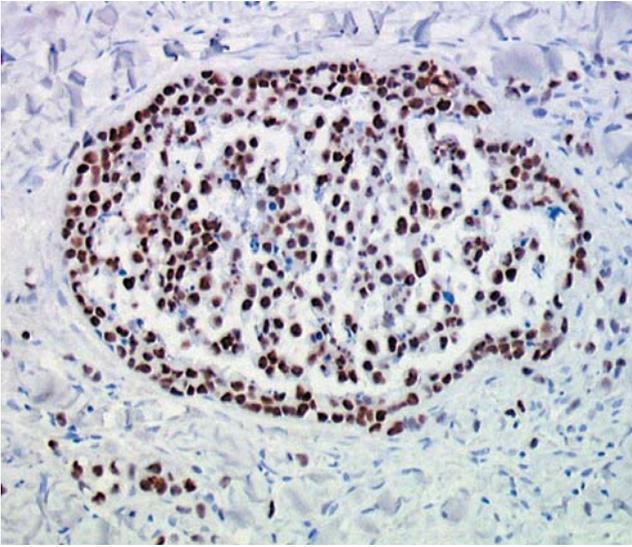


Figure 1. Nuclear expression of CDX2 by all tumor cells in a cutaneous metastasis of colorectal ADC (immunoperoxidase revealed with aminoethylcarbazole, original magnification  $\times 250$ ).

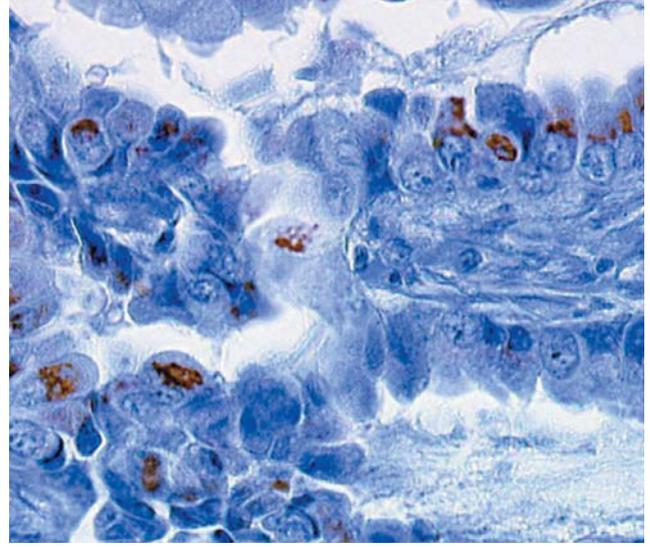


Figure 3. Nuclear expression of CDX2 by Paget's cells in a case of perianal EMPD associated with an underlying rectal ADC (immunoperoxidase revealed with aminoethylcarbazole, original magnification  $\times 400$ ).

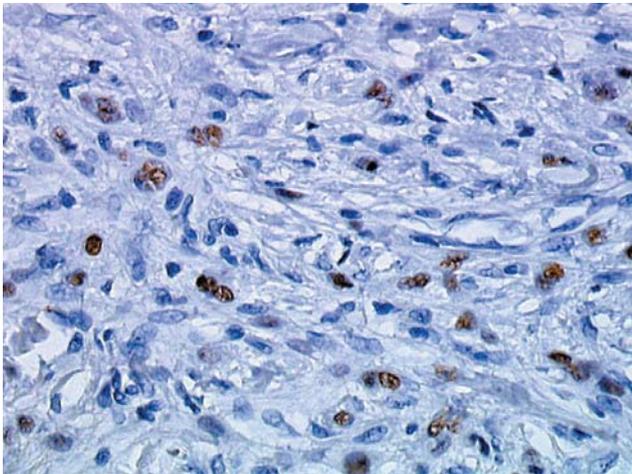


Figure 2. Nuclear expression of CDX2 by tumor cells in a cutaneous metastasis of urothelial ADC (immunoperoxidase revealed with aminoethylcarbazole, original magnification  $\times 250$ ).

Besides colorectal ADC, we found strong CDX2 expression in one cutaneous metastasis of urothelial ADC, consistent with CDX2 expression by urothelial ADC (9). The remaining metastases we studied (including ADC of the pancreas, breast, lung and uterine cervix) did not express CDX2, even though CDX2 expression has been reported in a variable percentage of the corresponding primaries, including those of the pancreas (9, 10), endocervix (15) and lungs (22). In the cases with an unknown primary, we

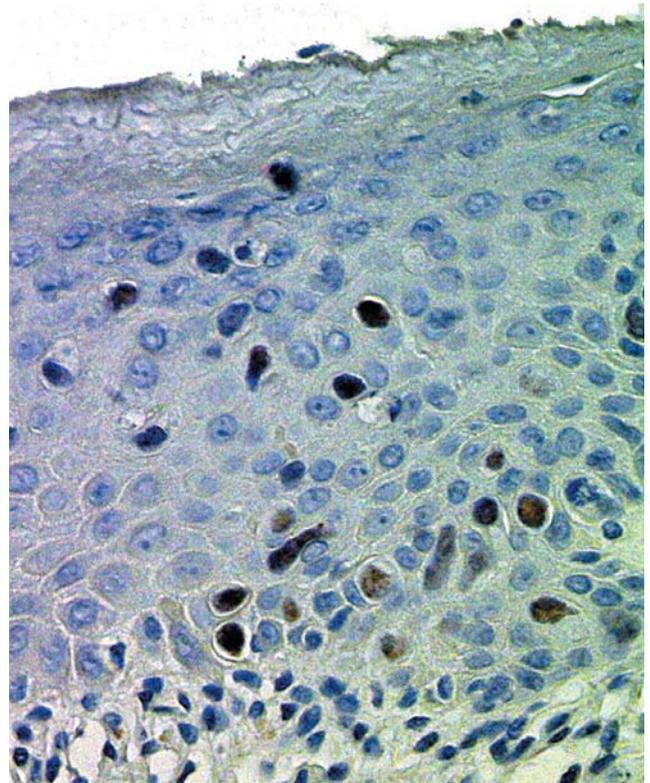


Figure 4. Non-specific cytoplasmic staining obtained with CDX2-88 antibody in a cutaneous metastasis of unknown ADC (immunoperoxidase revealed with aminoethylcarbazole, original magnification  $\times 250$ ).

observed no specific CDX2 expression. Reviewing the corresponding routinely stained sections, we found that none of them showed features of colonic ADC, consistent with the non-expression of CDX2.

EMPD deserves special mention. This unusual condition is in most cases purely cutaneous, but may more rarely be associated with a distal carcinoma; in the latter case, it has been regarded as an intraepithelial metastasis of the underlying tumor. Immunohistochemical stains for tissue-specific antigens may reveal the differentiation/origin of Paget's cells (*e.g.* uroplakins and PSA for urothelial- and prostate-derived EMPD, respectively) (23). CDX2 positivity is considered indicative of the origin of Paget's cells from an underlying intestinal carcinoma (17, 18). In our study, the perianal endodermal-type EMPD associated with an underlying rectal tumor expressed CDX2. This is in keeping with the results of the aforementioned studies, and highlights the diagnostic usefulness of CDX2 detection in EMPD. All remaining EMPD cases were CDX2 negative, suggesting that CDX2 is specific for cases associated with an underlying gastrointestinal tumor. Theoretically, urothelial-type genital EMPD could also express CDX2, because CDX2 is also expressed by urothelial ADC (9). In such cases, additional immunostains (*e.g.* for uroplakins) would be helpful in separating the two types of EMPD (colonic *vs.* urothelial).

Of note, we observed that immunostaining with CDX2-88 can occasionally produce a granular cytoplasmic staining, which seems to be non-specific. This pattern should be recognized and differentiated from the specific nuclear staining, so as not to be a source of misinterpretation.

In conclusion, we consider that CDX2 is a useful marker of cutaneous metastasis of colorectal and urothelial ADC, since it is rather specific for these tumor types. However, its sensitivity appears to be modest, and we advocate that CDX2 be used in conjunction with other, more sensitive immunohistochemical markers (such as K7 and K20). The diagnostic usefulness of CDX2 seems to be higher in the case of EMPD thanks to better sensitivity and specificity compared with cutaneous metastases, and we recommend its use in all perianal-genital endodermal-type EMPD cases.

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