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Original Article

Cathepsin K in the immunohistochemical diagnosis of melanocytic lesions

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Abstract: Recent studies have demonstrated that cathepsin K seems to be a powerful marker in identifying the microphthalmia associated transcription factor (MITF) family tumors such as renal perivascular epithelioid cell neoplasms (PEComas), alveolar soft part sarcoma, and translocation-associated renal cell carcinomas. However, the expression of cathepsin K in melanocytic lesions has not been well characterized. Our aim was to investigate the expression of cathepsin K in a wide histological spectrum of melanocytic lesions and to evaluate its potential diagnostic and molecular target therapy usefulness in comparison with other commonly used markers. 143 consecutive melanocytic lesions were selected for study including 56 primary malignant melanomas, 62 metastatic melanomas, and 25 benign nevi (16 intradermal melanocytic nevi and 9 compound melanocytic nevi). 107 of the 118 (91%) primary and metastatic melanomas displayed a high percentage of cells with moderately to strongly positive reactions for cathepsin K (mean 82%; range 0-95%). MITF, HMB45, Melan-A, and S100 were expressed in 85, 76, 78 and 96% of cases, respectively, with various percentages of positive cells (mean, 63, 49, 55 and 86%; range 0-90, 0-80, 0-90 and 0-95%). Among the benign nevi, cathepsin K, MITF, HMB45, Melan-A, and S100 were expressed in 88, 80, 36, 68 and 100% of cases, respectively. Cathepsin K appears to be consistently and strongly expressed in melanocytic lesions and valuable in distinguishing malignant melanomas from the majority of human cancers.

Keywords: Melanoma, melanocytic lesions, immunohistochemistry, cathepsin K, nevi, *MITF*

Introduction

The diagnosis of metastatic malignant melanoma may be difficult in surgical pathology because of its extremely variable morphology. Histologically, the tumor cells may have diverse features including epithelioid cell, round cell, spindle cell, rhabdoid cell, and so on. Therefore, it may be misdiagnosed as other tumors such as poor differentiated carcinomas, sarcomas, and large cell lymphomas. Based on these considerations, the diagnosis of such lesions should be based not only on morphology itself, but also on immunophenotype. Although some immunohistochemical markers such as S100, HMB45, Melan-A, and MITF are often used and shown to be helpful in the detection of these tumors, the limitations in the sensitivity and /or specificity of these available melanocytic markers still complicate this problem [1-7].

Recent researches have implicated that microphthalmia associated transcription factor (*MITF*) is required for the growth of melanocytes and melanoma [8-10]. Approximately 30% to 40% of melanomas harbor amplifications of *MITF*, although single nucleotide somatic mutations within its coding region have also been observed in human melanomas, making *MITF* the major melanoma oncogene [11-13]. *MITF* is one of four members of the MITF family, which includes *MITF, TFEB, TFEC* and *TFE3* [9, 10]. All family members share a homologous basic helix-loop-helix DNA binding domain and have overlapping transcriptional targets [9, 10]. Several distinct tumors are associated with the overexpression of this gene family, including melanoma, clear cell sarcoma, angiomyolipoma, perivascular epithelioid cell neoplasms (PEComas), alveolar soft part sarcoma, and translocation-associated renal cell carcinomas

Table 1. Immunohistochemical findings in primary and metastatic melanomas

	Cathepsin K	MITF	HMB45	Melan-A	S100
Strong (+++)	61	52	41	48	62
Moderate (++)	34	32	32	29	41
weak (+)	12	16	17	15	10
negative (-)	11	18	28	26	5

[9, 10, 14-22]. All these tumors have been considered as a member of the MITF family of tumors, owing to their histological, immunochemical and molecular genetic similarity [9, 10].

Cathepsin K is a cysteine protease from the papain family of proteases, which plays an important role in osteoclasts function and the overexpression of cathepsin K in osteoclasts is regulated by *MITF* [23]. In recent studies cathepsin K was identified as a transcriptional target of the MITF family, and was expressed consistently and strongly in *TFEB* translocation renal cell carcinomas, a wide spectrum of PEComas, and some cases of *TFE3* translocation renal cell carcinomas [19, 21, 23-25]. However, the expression of cathepsin K in melanocytic lesions has not been well characterized. We hypothesized that the overexpression of cathepsin K may have the same effect in melanocytic lesions. The aim of this study was to investigate the expression of cathepsin K in melanocytic lesions and to evaluate its potential diagnostic and molecular target therapy usefulness in comparison with other commonly used markers.

Materials and methods

Case selection

A total of 143 consecutive melanocytic lesions were selected in the archives of the Department of Pathology at Nanjing Jinling Hospital. The melanocytic cases include 56 primary malignant melanomas, 62 metastatic melanomas, and 25 benign nevi (16 intradermal melanocytic nevi and 9 compound melanocytic nevi). The metastases were located in the lymph nodes (n=13), deep soft tissues (n=15), lung (n=5), gastrointestinal tract (n=4), and bone (n=3).

To determine the prevalence of immunoreactivity for cathepsin K in control neoplasms, both tissue microarray (TMA) sections and conventional unstained whole tissue sections were used in the study. Organ-specific TMAs constructed using a manual tissue arrayer (Beecher Instruments Inc, Sun Prarie, WI, USA) contained 50-99 spots of tumor with a diameter of 2 mm. The control TMAs included 70 breast carcinomas, 99 non-small lung carcinomas (70 lung adenocarcinomas and 29 lung squamous cell carcinomas), 80 gastric carcinomas, and 50 colorectal adenocarcinomas. To analyze certain tumors for which TMAs were not available to us, we obtained unstained whole tissue sections including 20 prostate adenocarcinoma, 30 bladder urothelial carcinomas, 30 clear cell renal cell carcinomas, 20 gallbladder carcinomas, 30 hepatocellular carcinomas, 15 uterine leiomyomas, 10 schwannomas, and 15 anaplastic large cell lymphomas.

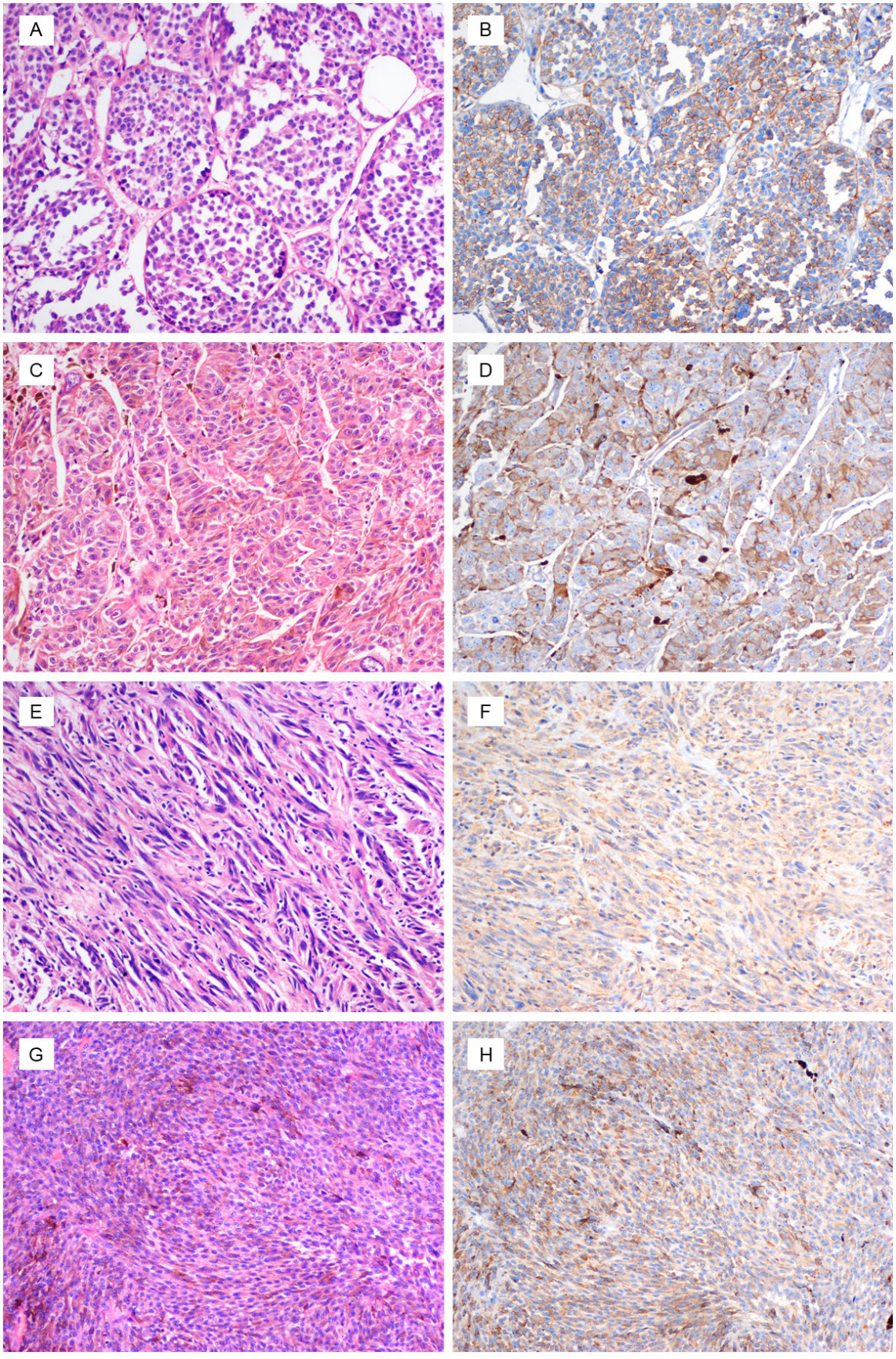
Immunohistochemistry

Tissues were fixed in 10% formalin and embedded in paraffin. Sections 3 mm thick were stained with immunohistochemistry. The following antibodies for immunohistochemistry were used: cathepsin K (3F9, Abcam, 1:300), MITF (D5, Dako, 1:100), HMB45 (HMB45, Dako, 1:500), Melan-A (A103/M2-72, Neomarkers, 1:100), and S100 (Polyclonal, Dako, 1:2000). Immunoreaction was performed using the labelled streptavidin-biotin method and overnight incubation as previously described and evaluated in a semiquantitative way assessing both staining intensity and percentage of positive cells as previously described [23, 26, 27]. For all antibodies, the resulting score was calculated by multiplying the staining intensity (0=no staining, 1=mild staining, 2=moderate staining, and 3=strong staining) by the percentage of immunoreactive tumor cells (0 to 100). The immunostaining was considered 0 or negative when the score was <25; 1+ or weak, 26 to 100; 2+ or moderate, 101 to 200; and 3+ or strong, 201 to 300.

Result

Primary and metastatic melanomas

The results of immunohistochemical findings in primary and metastatic melanomas were listed in **Table 1**. Overall, 107 of the 118 (91%) primary and metastatic melanomas with different


A
B
C
D
E
F
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H

Figure 1. Melanoma with epithelioid (A and C) and spindle cell (E and G) morphology. (B, D, F and H) The tumor strongly expresses cathepsin K. (original magnification, ×200).

types of tumors cells including rhabdoid cell, epithelioid cell, round cell and spindle cell displayed a high percentage of cells with moderately to strongly positive reactions for cathepsin K (mean 82%; range 0-95%). In contrast, the adjacent normal squamous epithelium, sweat glands, and the walls of the vessels were completely negative for cathepsin K. The MITF, HMB45, Melan-A, and S100 were expressed in melanomas as previously described, which were focally (1+) to strongly (3+) positive in 85% (100 of 118), 76% (90 of 118) and 78% (92 of 118) of melanomas, respectively, with various percentages of positive cells (MITF: mean 63%; range 0-90%; HMB45: mean 49%; range 0-80%; Melan-A: mean 55%; range 0-90%; S100: mean 86%; range 0-95%). If only moderate or strong positive staining was taken into account, cathepsin K, MITF, HMB45, Melan-A, and S100 staining were found to be moderately to strongly positive in 81%, 71%, 62%, 65%, and 87% of the melanomas (**Figure 1**).

Cathepsin K positivity in primary and metastatic melanomas that were negative for other markers

Among the MITF-negative melanomas, 7 of 21 (33%) were positive for cathepsin K. 15 of the 28 melanomas (54%) that were HMB45 negative were positive for cathepsin K. Of the Melan-A-negative melanomas, 10 of 26 (38%) were cathepsin K-positive. Similarly, 2 of 5 S100-negative melanomas (40%) were cathepsin K-positive (**Figure 2A-D**).

Benign nevi

Moderate to strong cathepsin K staining was seen in 22 of 25 (88%) cases of nevi (intradermal melanocytic nevi and compound melanocytic nevi). This trend was similar to the staining pattern of S100, which was positive in all nevi with diffuse (3+) staining pattern. 20 of the 25 (80%) nevi, 9 of the 25 (36%) nevi, and 17 of the 25 (68%) nevi showed focal (1+) to diffuse (3+) positivity with MITF, HMB45, and Melan-A respectively (**Figure 2E** and **2F**).

Control tissues

None of the breast carcinomas, non-small lung carcinomas, gastric carcinomas, colorectal adenocarcinomas, prostate adenocarcinoma, bladder urothelial carcinomas, clear cell renal cell carcinomas, gallbladder carcinomas, hepatocellular carcinomas, uterine leiomyomas, schwannomas, and anaplastic large cell lymphoma was positive for cathepsin K, except for 1 breast carcinoma and 1lung adenocarcinoma that showed moderate immunoreactivity for cathepsin K.

Discussion

In this study, we showed that cathepsin K was expressed in 107 of the 118 (91%) primary and metastatic melanomas with different types of tumors cells including rhabdoid cell, epithelioid cell, round cell and spindle cell and in 22 of 25 (88%) cases of nevi including 16 intradermal melanocytic nevi and 9 compound melanocytic nevi, most of them in a high number of tumor cells with moderate or strong positive reactions for cathepsin K. If only moderate or strong positive staining was taken into account, cathepsin K, MITF, HMB45, Melan-A, and S100 staining were found to be moderately to strongly positive in 81%, 71%, 62%, 65%, and 87% of the melanomas. The tumors displayed a higher percentage of positive reaction cells for cathepsin K than for MITF, HMB45, and Melan-A. In some melanomas for which other markers were negative, more than half of the HMB45-negative melanomas, many MITF- and Melan-A-negative melanomas, and a few S100-negative melanomas were cathepsin K-positive. In the control group, except for moderate immunoreactivity for cathepsin K in one breast carcinoma and one lung adenocarcinoma, none of the various other neoplasms used as controls was positive for cathepsin K. These findings therefore suggest that cathepsin K is a useful addition to the diagnostic panel used in possible cases of melanomas and that cathepsin K can be used as a relatively specific marker to distinguish melanomas from the majority of human cancers.

Cathepsin K is a lysosomal papain-like cystine proteinase with strong collagenolytic and elastolytic activity, which plays an important role in osteoclast function and is regulated by *MITF* by increasing mRNA and protein level of this papain-like cysteine protease [8]. As recent studies have demonstrated cathepsin K to be a transcriptional target of the microphthalmia-associ-

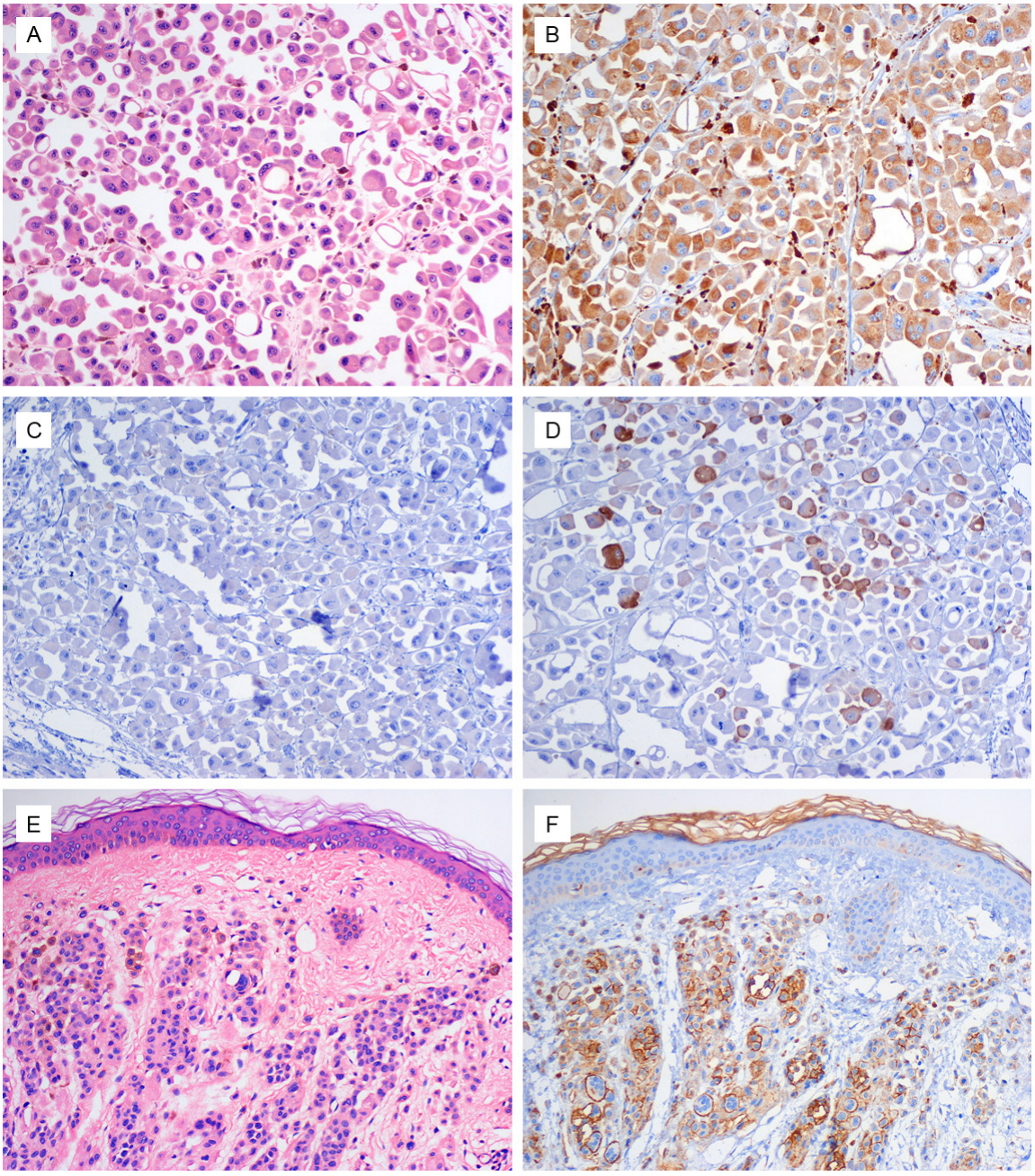

Figure 2. (A) Melanoma with rhabdoid morphology. (B) The tumour strongly expresses cathepsin K and is negative for HMB45 (C). (D) Melan-A is weakly expressed. (E) The histologic features of benign nevi. (F) Cathepsin K is diffusely expressed. (original magnification, ×200).

ated transcription factor family, immunohistochemistry antibody to cathepsin K has been utilized in the diagnosis of several microphthalmia-associated transcription factor family of tumors such as translocation-associated renal cell carcinomas, PEComas, and alveolar soft part sarcoma [19, 21, 23-25, 28]. In addition, cathepsin K is also expressed in reactive activated macrophages, but not in resident macrophages [8, 29, 30]. Consistent overexpression of cathepsin K has been demonstrated in granulomatous disorders including hypersensitivity pneumonitis, sarcoidosis, Wegener granulomatosis, berylliosis, and tuberculosis [8, 29, 30]. Our results confirmed the same effect of cathepsin K in melanocytic lesions and expanded the immunohistochemistry application of the antibody.

Recent studies have demonstrated that mammalian target of rapamycin (MTOR) is activated in the majority of malignant melanomas and may be targets for melanoma therapy [31-33]. In the study of Kneissel et al. MTOR inhibitors have efficacy in limiting the bone resorption mediated by osteoclastic cathepsin K [34]. These findings make the possibility to hypothesized that MTOR inhibitors can exert part of their activity by limiting the expression of cathepsin K [25]. In addition, cathepsin K inhibitors are being developed for cancer therapy in bone metastases, for rendering the bone a less favorable microenvironment for tumor growth by inhibiting osteoclast-mediated bone resorption [35, 36]. All these findings make novel interventional approaches with cathepsin K worth further investigation.

In conclusion, cathepsin K appears to be consistently and strongly expressed in melanocytic lesions and valuable in distinguishing malignant melanomas from the majority of human cancers. The potential target of cathepsin K for melanoma therapy remains to be clarified.

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Disclosure of conflict of interest

None.

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