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Zhu, Li

Wada, Maiko

Usagawa, Yuko

Yasukochi, Yumi

他

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Overexpression of Cathepsin D in Malignant Melanoma

Li Zhu, Maiko Wada, Yuko Usagawa, Yumi Yasukochi, Asuka Yokoyama, Naoko Wada, Maiko Sakamoto, Tomoko Maekawa, Reiko Miyazaki, Etsuko Yonenaga, Mari Kiyomatsu, Maho Murata and Masutaka Furue

Department of Dermatology, Graduate School of Medical Sciences, Kyushu University, Fukuoka, 812-8582, Japan,

Abstract

Cathepsin D is an aspartic lysosomal endopeptidase present in most mammalian cells. Overexpression of cathepsin D is associated with the progression of several human cancers including melanoma. We examined the expression levels of cathepsin D in 20 primary malignant melanomas, 20 metastatic malignant melanomas, 20 benign nevus pigmentosus and 10 normal skin samples in Japanese. In normal skin, granular or dotted pattern of positive staining was observed along the granular layer of epidermis and hair follicle with apparent moderate to strong staining in sebaceous and eccrine glands. The percent positivity and staining intensity of cathepsin D in primary and metastatic malignant melanomas were significantly higher than that of nevus pigmentosus. Moreover, the expression levels of cathepsin D in metastatic malignant melanomas were significantly higher than those of primary malignant melanomas. Data from our and previous reports strongly supports a notion that the upregulation of cathepsin D may be critically involved in the malignant transformation and progression of melanocytic tumors.

Key words : Cathepsin D・Malignant melanoma・Nevus pigmentosus・Skin

Introduction

Cathepsin D is an aspartic lysosomal endopeptidase present in most mammalian cells. Overexpression of cathepsin D is associated with the progression of several human cancers including gastric carcinoma[1)~3)], colorectal carcinoma[4)] and ovarian cancer[5)]. Cathepsin D has also been comprehensively studied in breast cancer where overexpression of mRNA and protein has been observed and been shown to be an independent marker of poor prognosis[6)~8)]. In malignant melanoma (MM), a significantly elevated concentration of cathepsin D was measured in the tumor cells as compared to adjacent normal tissue[9)]. Podhajcer et al. reported that cathepsin D was expressed in all of the dysplastic nevi and primary and metastatic MMs but in only 18% of nevus pigmentosus (NP), whereas normal melanocytes showed no cathepsin D expression[10)]. Herein, we examined whether the expression levels of cathepsin D were elevated in MMs than in NPs in Japanese patients.

Material and Methods

Tissue samples

We examined 20 primary MMs (10 nodular melanomas, 7 acral lentiginous melanomas, 3 superficial spreading melanomas ; head : 6, trunc : 2, extremities : 12), 20 metastatic MMs (13 lymph node metastasis, 7 skin metastasis), 20 benign NP (3 junctional, 7 compound and 10 intradermal ; head : 9, trunc : 6, extremities : 5) and 10 normal skin specimens. From 4 patients, primary and metastatic lesions were consecutively obtained. Tumor thickness of primary MM was classified as

Correspondence author : Masutaka Furue, MD, Ph.D.
Department of Dermatology, Graduate School of Medical Sciences, Kyushu University, Maidashi 3-1-1, Higashiku, Fukuoka, 812-8582, Japan,
TEL : +81-92-642-5581 FAX : +81-92-642-5600
E-mail : furue@dermatol. med. kyushu-u. ac. jp

pTis ; 4, pT1 ; 2, pT2 ; 2, pT3 ; 6 and pT4 ; 6. All formalin-fixed and paraffin-embedded tissues were obtained from the archives of the Department of Dermatology of Kyushu University Hospital, Japan. Clinical and demographic data were retrieved from the patient files.

Immunohistochemical analysis

All samples were fixed with 10% buffered formalin. The archival paraffin-embedded tissue blocks were cut into 4-μm thick tissue sections. The sections were deparaffinized with xylene for 10 min and rehydrated through a graded ethanol series. Antibody-binding epitopes were retrieved by pressure cooking the tissue sections in 10 mmol L-1 sodium citrate buffer (pH 7.0) (Yatoron, Tokyo, Japan) for 10 min, and nonspecific binding was blocked using 10% normal goat serum. The sections were then incubated with monoclonal antibody against cathepsin D (4G2, 1 : 100, Novus Biologicals LLC, U.S.A.) at 4℃ overnight. Immunodetection was conducted using a standard streptavidin-biotin amplification method and 3-amino-9-ethylcarbazole as a chromogen followed by light counterstaining with hematoxylin. Washes with Tris-buffered saline or phosphate-buffered saline were performed between each step according to the manufacturer's protocol. Appropriate positive and negative controls were included in each assay.

After immunohistochemical staining, 2 dermatologists who were blinded to patient details evaluated the stained sections. In each specimen, 3 high-power fields (HPFs, × 200) were randomly selected, 100 tumor cells were counted in each field, and the average percentage of positively stained cells in each of the 3 HPFs was computed for each sample. Expression levels were graded semiquantitatively as negative (-), weak (+), moderate (2 +), or strong (3 +) when the percentage of positively stained tumor cells was 0%, 1-25%, 26-50%, or 51-100%, respectively. We also evaluated the staining intensity of the specimens using the staining intensity of sebaceous or eccrine glands as internal positive control. The staining intensity was semiquantitatively classified as negative, mild, moderate and strong.

Statistical analysis

Statistical analysis using the one-way ANOVA was performed to determine whether there was a significant difference in the expression levels of cathepsin D among the metastatic MM, primary MM and NP specimens. A p-value of < 0.05 was considered statistically significant.

Results

The expression of cathepsin D was detected in a granular or dotted pattern along the epidermis and hair follicle with moderate to strong staining in sebaceous and eccrine glands (Fig. 1A). Dermal dendritic cells were also positively stained (Fig. 1B). Normal melanocytes did not express cathepsin D. The NP cells generally expressed low amounts of cathepsin D with 36.12 ± 6.28 percent positivity (Fig. 1B). The staining intensity of primary and metastatic MMs was moderate to strong (Table 1)(Fig. 1C and 1D). The percent positivity of cathepsin D in primary and metastatic MMs were 73.25 ± 5.24 and 89.14 ± 2.13, respectively, which were significantly higher than that of NP (primary MM vs NP ; $p < 0.05$, metastatic MM vs NP ; $p < 0.01$). Moreover, the expression levels of cathepsin D in metastatic MMs were significantly higher than those of primary MMs ($p < 0.05$). The staining intensity of primary and metastatic MM was mostly moderate to strong, which was significantly stronger than that of NP ($p < 0.05$) (Table 1). There was no apparent difference in immunohistochemical staining pattern of cathepsin D according to the clinicopathologic classifications and sites in primary MMs as well as NPs. In addition, the immunohistochemical staining pattern was similar between primary and metastatic MMs even in those obtained from same patients. No statistical difference was noted in the staining intensity between thin (pTis-pT2) and thick (pT3 and pT4) primary MM.

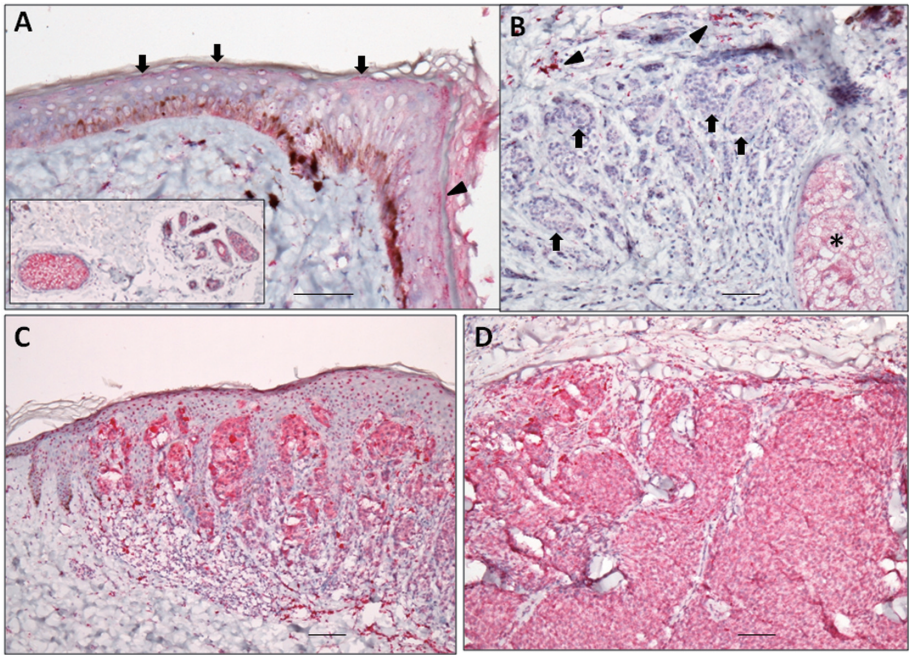

Fig. 1 Immunohistological expression of cathepsin D. A : The expression of cathepsin D is detected in a granular or dotted pattern along the epidermis (arrow) and hair follicle (arrow heads) with moderate to strong staining in sebaceous and eccrine glands (insert). B : The NP cells generally express low amounts of cathepsin D (arrow). Dermal dendritic cells (arrow head) and sebaceous gland (*) are strongly immunolabeled. C : The staining intensity of primary MMs is moderate to strong. D : The staining intensity of metastatic MMs is generally strong. Black bar = 100μm.

Table 1 Expression of cathepsin D in MM and NP

Percent expression	0%	1-25%	26-50%	>50%
NP (n=20)	3	7	4	6
Primary MM (n=20)	0	1	4	15
Metastatic MM (n=20)	0	0	0	20

Intensity of staining	Negative	Weak	Moderate	Strong
NP (n=20)	3	6	10	1
Primary MM (n=20)	0	1	6	13
Metastatic MM (n=20)	0	0	4	16

MM, malignant melanoma; BN, benign melanocytic nevus. After immunohistochemical staining, 3 high-power field images (HPFs, ×200) were randomly selected in each specimen, 100 tumor cells were counted in each field, and the mean percentage of positively stained cells from the 3 HPFs was calculated. Percent expression was graded semiquantitatively as 0%, 1–25%, 26–50% or >50% of the tumor cells were stained. Intensity of staining was graded semiquantitatively as negative , weak, moderate, or strong, respectively.

Discussion

The so-called "catheptic activity" (derived from the Greek word kathépsein, meaning to digest or to boil down) was first described in the gastric juice during the 1920s. Today, cathepsins are classified based on their structure and catalytic type into serine (cathepsins A and G), aspartic (cathepsins D and E), and cysteine cathepsins (cathepsins B, C, F, H, K, L, O, S, W, V and Z)[11]. With regards to aspartic cathepsin D and E, recent genetic and pharmacological studies have particularly suggested that cathepsin E plays an important role in host defense against cancer cells and invading microorganisms[12], while cathepsin D has been shown to be associated with tumor progression (Liaudet-Coopman).

In the normal skin, cathepsin D is the main endolysosomal enzyme which is thought to be crucially involved in the activation of transglutaminase 1 leading to final stage of desquamation[14]. Although the expression of cathepsin D was immunodetected in the entire normal epidermis in the previous study using rabbit polyclonal antibody[15], the present monoclonal antibody exhibited a granular or dotted staining mainly along the upper granular layer of the epidermis and the hair follicle. In contrast, much stronger expression was confirmed in the sebaceous and eccrine glands, which is consistent to the previous reports[16][17].

As documented in other cancers[1]~[8], intense immunolabeling of cathepsin D was observed in MMs than in benign NPs. Moreover, the expression levels of cathepsin D in metastatic MMs were significantly higher than those of primary MMs. These results in Japanese patients were almost identical with those from Slovenia and Argentina[9][10]. Podhajcer et al. have already demonstrated that all of 11 primary and 10 metastatic MMs, but in only 5 of 27 NPs, expressed cathepsin D[10]. In addition, normal melanocytes showed no cathepsin D expression as has been found in the present study. Our study also found that apparent expression of cathepsin D in 7 acral lentiginous melanomas, a preponderant melanoma type for Asian ethnic[18]. The role of cathepsin D in tumor progression still remains unknown. However, it is considered to directly degrade the extracellular matrix and to remodel the basement membrane and interstitial stroma and to indirectly activate other enzymes participated in the proteolysis process[4].

In conclusion, data from our and previous reports[9][10] strongly supports a notion that the upregulation of cathepsin D may be critically involved in the malignant transformation and progression of melanocytic tumors.

Conflict of Interest

The authors have no conflicts of interest to declare.

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（和文抄録）

悪性黒色腫におけるカテプシンDの過剰発現

九州大学大学院医学研究院皮膚科学分野

朱　　理，和田麻衣子，宇佐川祐子，安河内由美，横山翌香，和田尚子，坂本麻衣子，
前川朋子，宮崎玲子，米永悦子，清松真理，村田真帆，古江増隆

カテプシンDはリソゾーム内に存在するエンドペプチダーゼであるが，胃癌，乳癌，卵巣癌などでは周辺正常組織に比べ，過剰発現していることが知られている．悪性黒色腫においても，その過剰発現が2編の文献報告で知られているが，日本人を対象とした検討は行われていない．我々は，20例の原発性悪性黒色腫，20例の転移性悪性黒色腫，20例の良性色素細胞母斑，10例の健常皮膚を用いて，カテプシンDの発現を免疫組織学的に検討した．正常皮膚では，カテプシンDは表皮および毛嚢の上層・顆粒層に顆粒状に発現しており，脂腺やエクリン汗腺では強い発現を認めた．原発性悪性黒色腫や転移性悪性黒色腫のカテプシンDの発現は，良性色素細胞母斑のそれよりも有意に上昇していた．また転移性悪性黒色腫では，原発性悪性黒色腫よりも有意に高い発現が認められた．これらの結果から，カテプシンDの発現は色素細胞の悪性化と腫瘍進展に関与していると考えられた．