Basement Membrane Components in Malignant Melanoma

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ABSTRACT

To investigate changes in the basement membrane (BM), malignant melanoma (MM) were studied immunohistochemistry by immunofluorescent staining with antisera to laminin, collagen type 4 and fibronectin. In MM in situ, these BM components were preserved as a continuous band on the dermo-epidermal junction, whereas fibronectin was remarkably decreased in the papillary dermis. In invasive MM, the BM components were actively synthesized, and two distribution patterns were observed: they were irregularly distributed on the surface of the tumor cells, or surrounded the cells or nest.

Key word: malignant melanoma, basement membrane, extracellular matrix

INTRODUCTION

MM is a malignant neoplasm of tremendous interest to pathologists as a model for the study of tumor evaluation. Recent investigation suggest that melanoma cells in the vertical growth phase (VGP) have more markedly increased proliferative activity and capacity to metastasize than those in the radial growth phase (RGP)1). The Basement Membrane Components (BMCs) are related to the invasion and metastasis of tumor

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Kaneko Y: BM components in malignant melanoma cells\(^2\). Therefore, changes in the BMC may be observed in tumor progression.

In the present study, changes in the BMCs, such as collagen type 4 (C-4), laminin (LN) and fibronectin (FN) were investigated with special reference to tumor progression.

MATERIALS AND METHODS

**Antibodies** Anti-LN, C-4 and FN rabbit antisera were obtained as previously described\(^3\). Rhodamine-conjugated goat anti-rabbit IgG was purchased from Tago Inc. (Burlingame CA).

**Samples** The specimens obtained from biopsied or resected tumor, 5 MM in situ, 2 superficial spreading melanoma, 6 acral lentiginous melanoma, 5 nodular melanoma and 6 skin metastasis, were embedded and quickly frozen in liquid nitrogen. The sections were sliced at 6 μm thick in a cryostat and dried at room temperature for 30 min.

**Immunohistochemistry** For staining of the BMCs, the sections were incubated with anti-C-4, anti-FN, anti-LN, or normal rabbit serum, and followed by the rhodamine-conjugated second antibody for 30 min at room temperature. The sections were rinsed in phosphate-buffered saline (PBS) three times for 5 min between these incubations.

RESULTS AND DISCUSSIONS

In the normal skin, C-4 and LN were observed as a linear and continuous band on the BMs of the dermo-epidermal junction (DEJ), blood vessels and appendages (Fig 1a) as reported previously\(^4\). FN was observed in a fine fibrillar pattern in the papillary dermis (Fig 1b), as previously reported\(^5\).

In MM in situ, C-4 and LN were localized continuously on the DEJ, although their thickness varied and their intensity was slightly decreased (Fig 2a). In addition, the amount of FN was apparently decreased in the papillary dermis (Fig 2b) compared with that of normal
Fig. 1 Indirect immunofluorescent micrograph of normal skin stained with anti-laminin antisera (a) and anti-fibronectin antisera (b). Laminin is observed as linear fluorescence on the dermo-epidermal junction (J), vessels (V) and appendages (A) (a x40). Fibronectin is observed in a fine fibrillar pattern in the papillary dermis (b x100).

Fig. 2 Indirect immunofluorescent micrograph of malignant melanoma in situ lesion stained with anti-collagen type 4 antisera (a) and anti-fibronectin antisera (b). C-4 is localized continuously on the DEJ, although the thickness varied and intensity is slightly decreased in part (a x250). The amount of fibronectin is apparently decreased in the papillary dermis (b x250).
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Fig. 3 Individual tumor cells in RGP stained with anti-laminin antisera. The BMC clearly surrounds each of them (x400).

Fig. 4 Two distribution patterns of BMCs around tumor cells in VGP and skin metastasis. In the first pattern, laminin are irregularly distributed on the surface of almost every tumor cell, in a granular pattern in some places and in a linear pattern in other places (a x250). In the second pattern, the several tumor cells are surrounded by relatively intact BMCs; linear and continuous immunofluorescence of laminin is observed (b x250).
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In the cases of invasive melanoma, there is a difference between tumor nests of the RGP and VGP. In the RGP, the BMCS surrounded small nests or single tumor cells as continuous lines (Fig 3). In the VGP and metastatic lesions, two patterns in the distribution of the BMCS were demonstrated: the BMCS were distributed irregularly on melanoma cells (Fig 4a) or surrounded the tumor cells as large nests (Fig 4b). Although both patterns were observed, either pattern predominated. The loss of the BMCS was more extensive in the poorly differentiated squamous cell carcinomas than in the well differentiated one2). Recent investigations indicate that tumor cells in the VGP are biologically more malignant than those in the RGP1); the former cells grow rapidly and metastasize frequently, while the latter cells have a low growth-rate and no tendency to metastasize. However, these data indicate that even the more invasive tumor cells actively synthesize the BMCS. The metastatic lesions as well as tumor nests of the VGP in the metastatic cases reveal both the distribution patterns of tumor nests, suggesting that the pattern of the BMCS is not related to the metastatic potential of tumor cells.

REFERENCES
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