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Factor XIIIa-positive dendrocytes and proliferative activity of cutaneous cancers

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Abstract Factor XIIIa-positive dendrocytes present at the periphery and inside epithelial neoplasms are an heterogeneous group of cells. They are subsets of mesenchymal cells, cancer-associated macrophages and antigen-presenting cells. Factor XIIIa, other tissue transglutaminases, α2-macroglobulin and tumour necrosis factor-α represent a complex network of mediators influencing tumour progression in the skin. In the present study we searched for the presence of dendrocytes and α2-macroglobulin deposits inside and in the vicinity of cutaneous carcinomas (90 basal cell carcinomas and 46 squamous cell carcinomas) and malignant melanomas (69 primary and 28 metastatic tumours). We also studied the proliferation of the same neoplasms by Ki-67 immunohistochemistry. Dendrocytes were numerous, abutting on and infiltrating most basal cell carcinomas and thin malignant melanomas. In contrast, they were present in only low numbers or even absent in thick primary malignant melanomas and in their metastases. They appeared unmodified around squamous cell carcinomas compared with the surrounding skin. Extracellular deposits of α2-macroglobulin were often found in locations where dermal dendrocytes were numerous. No correlation was found between the Ki-67 indices of carcinomas and the density of peritumoral dendrocytes. In contrast, negative relationships were found between the Ki-67 indices and the number of dendrocytes present inside basal cell carcinomas and thin malignant melanomas. This study has yielded circumstantial evidence to link the density of factor XIIIa-positive dendritic cells and a low proliferative rate of neoplastic cells in basal cell carcinomas and malignant melanomas.

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Introduction

Carcinogenesis is a multi-step process in which a series of alterations occurs in sequence. There is evidence that oncogene expression or tumour suppressor gene inactivation is not always sufficient for the phenotypic expression of malignancy, and that stromal cells may play a key role in carcinogenesis [20, 32, 33]. In the steps of tumour progression, the size of the cell growth fraction appears to be related to the degree of malignancy in some carcinomas [1, 25] and in malignant melanomas (MM) [10, 21, 29, 30]. The relationship between the proliferation of neoplastic cells and the nature and function of cells in their stromal environment is poorly understood during the stepwise development of neoplasms.

The stroma of malignant neoplasms consists of a heterogeneous cell population of varied embryological origin. In the past, many of these cells were considered to be of mesenchymal origin, belonging to the connective tissue component of the organs and generally called fibroblasts [33]. However, the fibroblast-like cells of the skin represent different cell lineages showing distinct aspects of differentiation which may be explored by immunohistochemistry [27]. Dendritic cells exhibiting immunoreactivity for factor XIIIa are called dendrocytes, many of which belong to the monocyte-macrophage lineage and show phagocytic and antigen-presenting functions [2, 5, 6, 17, 18]. Other dendrocytes with similar functions may be seen in epithelial tissues [5, 23, 24, 26]. Another dendrocyte subset in the dermis does not express monocyte markers, and probably differentiates in situ from primitive mesenchyme [17, 18, 31]. One of their putative functions is to control fibroblasts in their synthetic activity for the extracellular matrix [12, 22]. This depends on the extracellular release of the factor XIIIa transglutaminase followed by its binding to membrane receptors on fibroblasts. Another major substrate

for this transglutaminase is α2-macroglobulin [7] which presence could therefore interfere with the control regulation of the stroma. In addition, it has been shown that α2-macroglobulin is present in some malignant neoplasms in amounts that relate to the risk of metastasis [16]. Furthermore, an inverse relationship has been suggested between metastatic potential and cytosolic transglutaminase activity [13].

In the present study, the nature and rate of proliferation of cutaneous carcinomas and MM were compared to both the presence of dendrocytes abutted to or infiltrating the neoplasms, and to the extent of α2-macroglobulin deposits. We also speculate as to possible interactions between intradermal and intratumoral dendrocytes and cutaneous malignancies.

Materials and methods

Ninety basal cell carcinomas (BCC), 46 squamous cell carcinomas (SCC), 69 primary MM and 28 metastatic MM to the skin were excised and fixed in neutral buffered formalin. We also treated, as a distinct group 30 carcinomas that had developed over fibrotic or sclerotic tissues, including old scars, lichen sclerosus, dermatofibromas, radiodermatitis and PUVA-induced photosclerosis (Table 1).

After embedding in paraffin, sections were cut and stained with haematoxylin and eosin. Other sections were dewaxed in xylene and hydrated through graded alcohols. They were pre-incubated with 0.05% pronase E (protease type XXV, Sigma, St Louis, Mo.) in PBS for 10 min. Endogenous peroxidase was blocked with 3% H_2O_2 in methanol. The avidin–biotin complex (ABC) technique was used with the anti-factor XIIIa polyclonal antibody (1:300, Behring-Werke) and the anti-α2-macroglobulin (1:150, Dakopatts) according to classic procedures. Ki-67 immunoreactivity was determined using the MIB-1 antibody after antigen retrieval in a microwave oven set at 750 W [1, 10]. Sections of normal skin were used as positive controls. Negative controls were performed by omitting or substituting reagents of the immunoperoxidase technique.

Image analysis was used to assess the factor XIIIa and Ki-67 immunoreactivities. Morphometric evaluations were conducted on non-necrotic, randomly selected fields of 0.1 mm^2 at the extension border of the neoplasms and in the peritumoral stroma. The number of Ki-67-positive nuclei and the number and stained area of factor XIIIa-positive dendrocytes were evaluated using spectral discrimination based on the hue and saturation of colour (WCUE 2 System Olympus). Data were averaged for each neoplasm.

Means, standard deviations and medians were calculated for each variable. Regression analysis models were applied to evaluate the relationship between neoplastic cell proliferation and factor XIIIa immunoreactivity. The best model (linear, logarithmic, exponential or power) was chosen on the basis of the highest coefficient of correlation, r. A P-value lower than 0.05 was considered significant.

Table 1 Number of carcinomas in relation to fibrotic or sclerotic tissue

Primary disease	Basal cell carcinoma	Squamous cell carcinoma
Old scar	1	5
Lichen sclerosus	–	3
Dermatofibroma	2	–
Radiodermatitis	2	4
PUVA-induced photosclerosis	–	13

Results

All omission and substitution controls were negative while the positive controls gave uniform cytoplasmic factor XIIIa staining of dendrocytes, a positivity of endothelial cells for α2-macroglobulin and nuclear Ki-67 positivity in about 5% of basal keratinocytes.

In BCC, dermal dendrocytes abutting on basaloid cells were usually numerous in the remodelled stroma (Fig. 1a). There was no correlation between the histological type of BCC and the numerical density of dermal dendrocytes. Abundant extracellular α2-macroglobulin was most often present in the peritumoral stroma (Fig. 1b). No correlation (r=0.12, NS) was found between stromal factor XIIIa immunoreactivity and the number of proliferating tumoral cells.

In 39% of BCC, intraepithelial dendrocytes were also dispersed in the neoplastic masses (Fig. 1c). In these locations, a negative logarithmic correlation (r=–0.86, P<0.001) was found between the numerical densities of factor XIIIa-positive dendrocytes and Ki-67-positive neoplastic cells (Fig. 2).

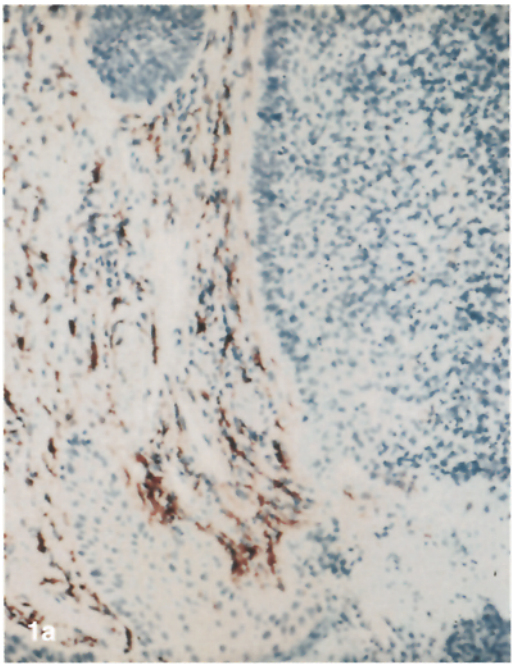
SCC only harboured rare dendrocytes. Compared with the surrounding skin, the microenvironment of SCC did not contain a strikingly abnormal number of dermal dendrocytes. Nor were abnormal deposits of α2-macroglobulin present. The number of Ki-67-positive malignant cells was highly variable and unrelated to the factor XIIIa expressivity in the peritumoral stroma (r=0.09, NS).

Numerous dendrocytes were located underneath and inside thin (<0.5 mm) primary MM with a density 2- to 20-fold that of the surrounding skin. Their dendritic aspect was sometimes poorly developed (Fig. 3). Many melanophages, when present, were factor XIIIa-positive. α2-Macroglobulin was identified in the highly vascularized stroma and inside isolated or clustered neoplastic cells in about 35% of the tumours. In thicker MM (including 2 desmoplastic lesions) the density of dermal dendrocytes was low. These cells were almost absent in metastatic MM. At the site of focal spontaneous regression of primary MM, dermal dendrocytes and extracellular α2-macroglobulin were abundant in the remodelled stroma.

In primary MM, an inverse linear relationship (r=–0.74, P<0.001) was found between the numbers of Ki-67-positive neoplastic cells and the intratumour factor XIIIa-positive dendrocytes (Fig. 4a). The ratio between these two populations of cells was clearly influenced by the tumour thickness (Fig. 4b). In addition, the relationship between the number of Ki-67-positive neoplastic

Fig. 1a–c Basal cell carcinoma. The peritumoral stroma is rich in factor XIIIa-positive dermal dendrocytes (**a**) and in α2-macroglobulin deposits (**b**). Factor XIIIa-positive dendrocytes are dispersed inside the neoplastic epithelial aggregates (**c**)

Fig. 3 Factor XIIIa-positive dendrocytes inside (**a**) and underneath (**b**) a malignant melanoma



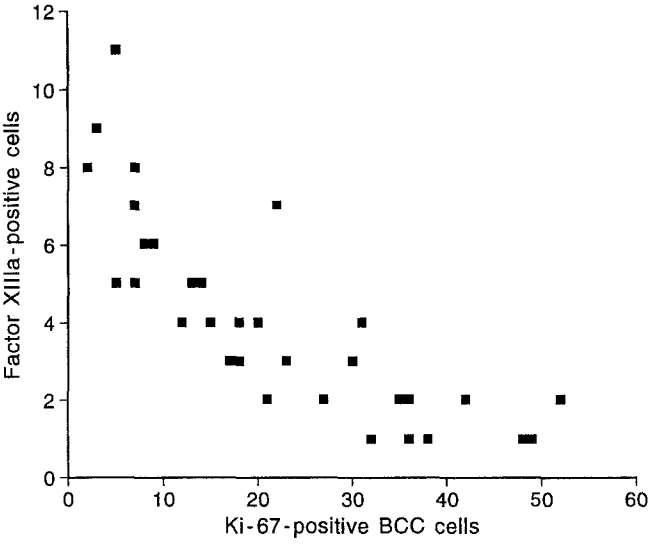
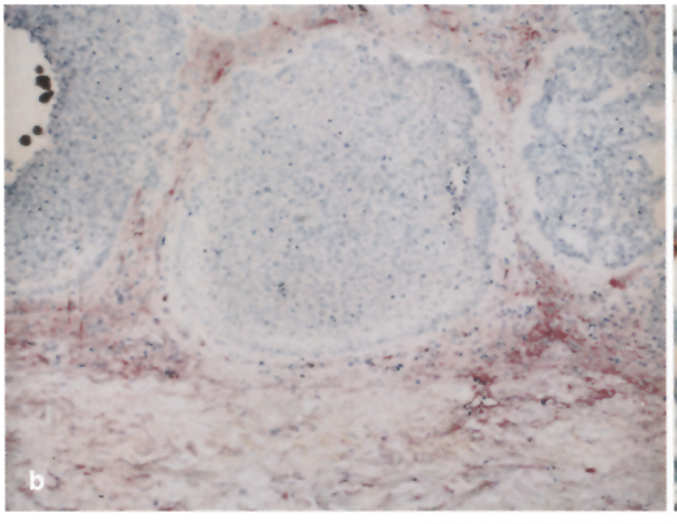
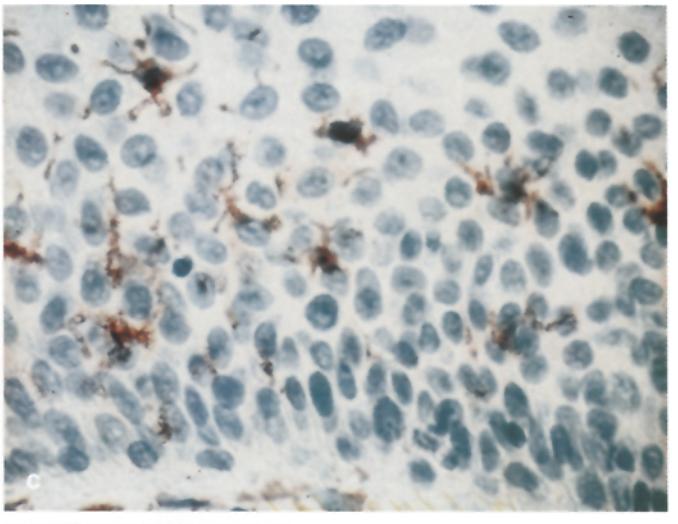
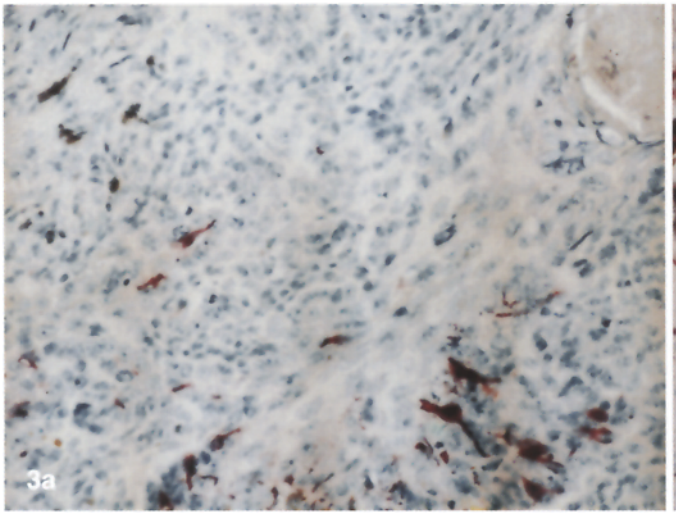
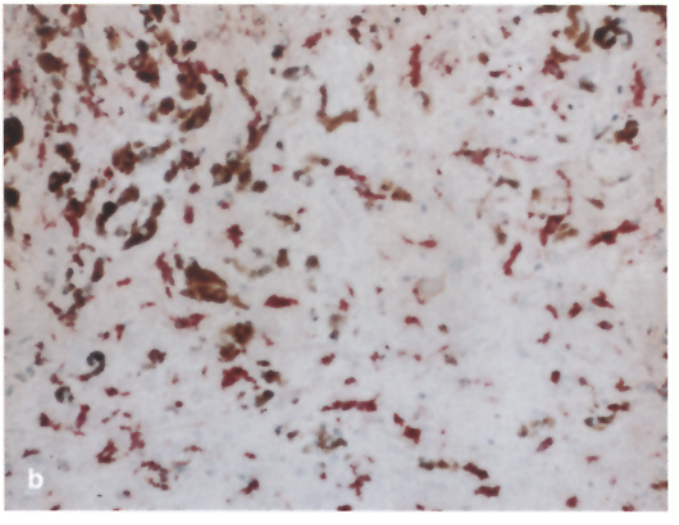


Fig. 2 Basal cell carcinoma. Negative logarithmic correlation between the number of intraepithelial factor XIIIa-positive dendrocytes and the number of cycling neoplastic cells









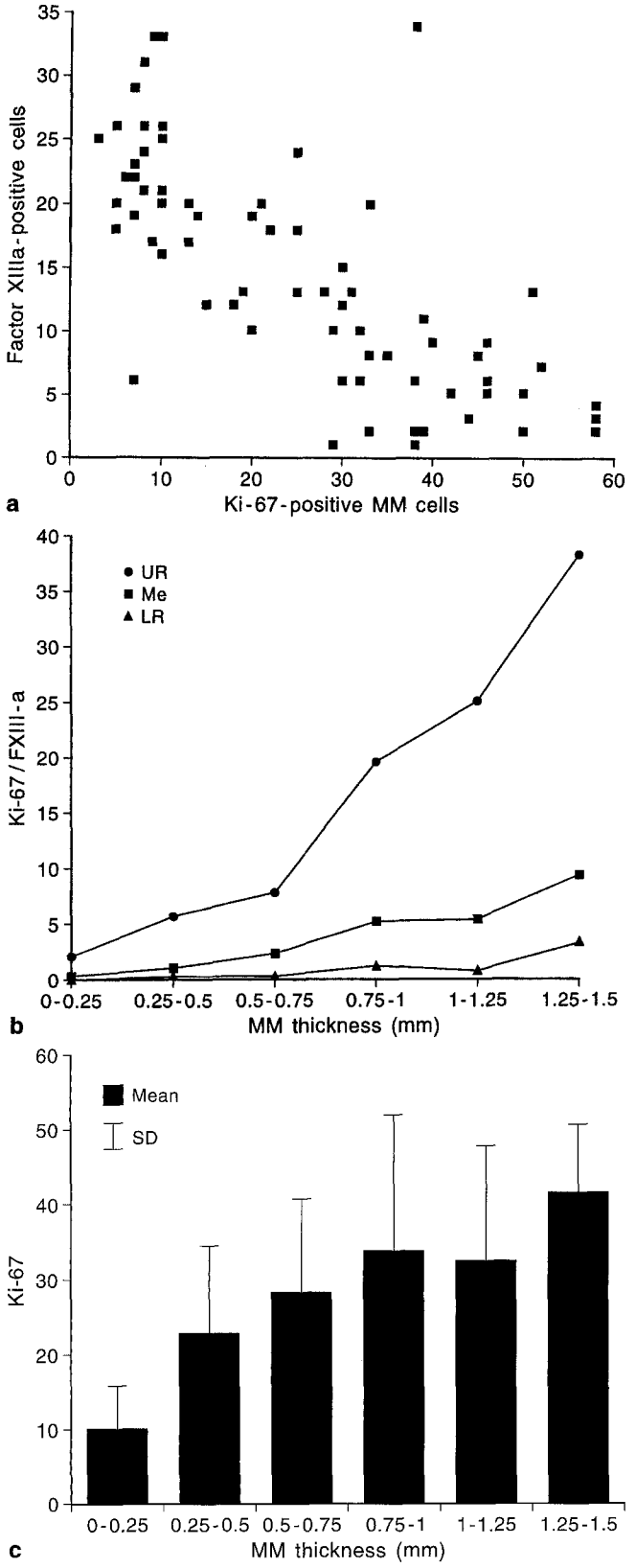


Fig. 4a–c Malignant melanoma. **a** Negative linear correlation between the number of intratumour factor XIIIa-positive dendrocytes and the number of cycling neoplastic cells. **b** The ratio between the number of cycling neoplastic cells and the number of factor XIIIa-positive dendrocytes is related to the tumour thickness (*LR* lower range, *Me* median, *UR* upper range). **c** Relationship (mean±SD) between the number of cycling neoplastic cells and tumour thickness

cells and tumour thickness (Fig. 4c) fits an exponential curve (r=0.98, P<0.001).

Dermatofibromas and some cases of chronic radiodermatitis were loaded with dermal dendrocytes. These cells were most often plump with elongated dendrites. In contrast, old scars, long-standing lichen sclerosus, PUVA-induced photosclerosis and some cases of chronic radiodermatitis were poor in dendrocytes. The nature of the primary stromal alteration influenced the nature of the carcinoma. The dermal dendrocyte population underneath BCC was always denser than that underneath SCC.

Discussion

Host-tumour interactions clearly influence the ultimate behaviour and prognosis of malignancies. Beside the crucial role of the immune response, the tumour stroma with various cytokines and enzymes is involved in the control of tumour growth, regression and metastatic progression [3, 11, 14, 15, 33]. Our study addresses two main questions, namely the nature of macrophages and fibroblast-like cells interacting with cutaneous malignancies, and the correlations between dermal and intratumour dendrocytes and some biological aspects regulating neoplastic evolution.

Several lines of evidence suggest that the stroma may display both stimulatory and restrictive effects upon tumour progression. The mode of action of stromal cell is controversial and enigmatic. The presence of dendrocytes in malignant cutaneous neoplasms has been of increasing interest during the past several years [2, 9, 17, 27]. Given the histogenetic diversity of cell types expressing factor XIIIa, ranging from bone-marrow- to mesenchyme-derived cells, several specific cellular functions may be involved in cutaneous cancers. In particular, they may produce a variety of cytokines, such as TNFα [19], which may operate through a paracrine mechanism upon other stromal cells and the malignant lineage. As far as the phagocytic function is concerned, it has been shown that cellular factor XIIIa differentiates into tissue-type transglutaminase during maturation of monocytes into macrophages [28]. This aspect of differentiation is probably associated with transglutaminase-dependent phagocytosis. The immunohistochemical method used in the present study does not identify this subset of the factor XIIIa-positive cell family.

Our study shows that the highest density in dermal dendrocytes was found in BCC, a cancer usually confined to its site of origin and with rarely reported metastasis. The neoplasm is characteristically cuffed by a remodelled stroma with a thin and delicate network of collagen [25], which may be related to the inhibiting effect of factor XIIIa on collagen synthesis [12, 22]. The restricted local invasiveness and the specificity of the very occasional metastases to particular organs may imply that local growth restrictions are operating. As suggested in the present study, intratumour dendrocytes might play a part in actively limiting tumour growth, perhaps by the release of TNFα, which has been shown to exert a

marked antiproliferative effect on transformed keratinocytes [8]. Intratumour dendrocytes, which are presumably of the monocyte-immunocompetent lineage, can be viewed as growth-restricting cells in BCC. Conversely, tumour growth and metastasis may not be fully possible if the appropriate stromal dendrocytes are absent at the lodging site. These cells might act as growth-promoting cells in remodelled dendrocyte-rich stroma such as that found in dermatofibroma [4, 27] and some cases of radiodermatitis. It is likely that such stromal tissue provides growth signals for BCC genesis.

In contrast to BCC, cutaneous SCC is a neoplasm lacking evident interactions and interdependence with stromal and intratumour dendrocytes. In addition, dendrocyte-poor remodelled stroma in scarring tissues is more probably at risk for development of SCC than of BCC.

The presence of dendrocytes in MM has been reported previously [2, 9, 27]. The present findings confirm these observations. In addition we show that the density of dermal and intratumoral dendrocytes decreases with increasing thickness and proliferative rate of the neoplasm. The extent of dendrocyte infiltration in MM might therefore be another way to establish a prognosis for this neoplasm. It remains unclear whether the dendrocyte rarefaction is a cause or a consequence of the progression of the disease.

In most cutaneous neoplasms, extracellular deposits of α2-macroglobulin were found in locations where dermal dendrocytes were numerous. This may illustrate one facet of the multiple biological interactions at the progression edge of malignancies. In addition, intracellular α2-macroglobulin was found in neoplastic cells of some MM. It was previously shown that the expression of α2-macroglobulin by neoplastic cells could be a harbinger of poor prognosis [16].

Factor XIIIa-positive dendrocytes may not be passive bystanders in BCC and MM. Their function may differ according as whether they are located in the stroma or inside the neoplasm. Intraepithelial dendrocytes are associated with a low proliferative rate of BCC and MM, and we suggest that they may exert a growth-restricting role. In contrast, stromal dendrocytes may be involved in the invasiveness and metastatic spread of the cutaneous malignancies.

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