BENİGN VE MALİGN NEOPLASTİK DERİ LEZYONLARINDA EPİDERMAL LANGERHANS HÜCRELERİ VE DERMAL MAST HÜCRELERİ

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ÖZET

AMAÇ: Langerhans hücreleri ve mast hücreleri kemik iliği kökenli hücreler olup dermis ve epidermisin immun sisteminde kritik rol oynarlar. Biz bu çalışmada benign ve malign skuamoid tümörlerde mast hücre ve Langerhans hücrelerinin rolünü değerlendirmeyi amaçladık.

GEREÇ ve YÖNTEM: Bu araştırmaya 12 seboreik keratoz, 9 aktinik keratoz, 15 keratoakantom ve 33 skuamöz hücreli karsinom olgusu alındı. Dermal mast hücreleri ve epidermal peri ve intratümöral Langerhans hücreleri sırasıyla mast hücre triptaz ve CD1a antikorlarıyla boyandı.

BULGULAR: Mast hücre sayısı skuamöz hücreli karsinomda seboreik keratoza göre anlamlı olarak yüksekti (p=0.001). Mast hücre sayısı diğer tümör grupları arasında farklılık göstermedi. İntratümöral Langerhans hücreleri seboreik keratozda skuamöz hücreli karsinoma göre önemli oranda artmış saptandı (p=0.026). Peritümöral Langerhans hücreleri ise skuamöz hücreli karsinom (p=0.007, p=0.001) ve keratoakantomda (p=0.001, p=0.001), aktinik keratoz ve seboreik keratoza oranla artmış olarak saptandı.

SONUÇ: Sonuç olarak biz çalışmamızda skuamöz hücreli karsinom ve keratoakantomda peritümöral Langerhans hücreleri ve mast hücrelerini sayıca artmış bulduk. İmmün sistemde görevleri bulunan epidermal Langerhans hücreleri ve dermal mast hücrelerinin benign ve malign deri tümörlerinde farklı rolleri olduğunu düşündürmektedir.

Anahtar sözcükler: Mast hücreleri, Langerhans hücreleri, deri tümörleri

Epidermal Langerhans Cells and Dermal Mast Cells in Bening and Malignant Neoplastic Skin Lesions

SUMMARY

PORPOSE: Langerhans cells and mast cells are bone marrow derived cells. They represent a critical role in the immune system in the epidermis and dermis. In this present study, we aimed to evaluate the role of mast cells and Langerhans cells in benign and malignant squamous tumors.

MATERIALS and METHODS: This study included 12 patients with seborrheic keratosis, 9 patients with actinic keratosis, 15 patients with keratoacanthoma and 33 patients with squamous cell carcinoma. Dermal mast cells and epidermal peri and intratumoral Langerhans cells were labeled with mast cell tryptase and CD1a antibodies respectively.

RESULTS: The number of mast cells were significantly increased in the patients with squamous cell carcinoma in comparison with seborrheic keratosis (p=0.001). However, this parameter did not show significant difference between patients of other study groups. The number of intratumoral Langerhans cells in seborrheic keratosis were significantly increased in comparison to squamous cell carcinoma (p=0.026). The peritumoral Langerhans cells were increased in squamous cell carcinoma (p=0.007, p=0.001) and keratoacanthoma (p=0.001, p=0.001) in comparison with actinic keratosis and seborrheic keratosis, respectively.

CONCLUSION: This immunohistochemical study demonstrates that peritumoral Langerhans cells and mast cells are increased in number in keratoacanthoma and squamous cell carcinoma. Epidermal Langerhans cells and dermal mast cells which have roles in the regulation of immune system might have different functions in benign and malignant skin tumors.

Key words: Mast cells, Langerhans cells, skin tumors

Immune cells of the epidermis and dermis participate in the tumor progression and development. Langerhans cells (LH cells) are bone marrow derived dendritic cells that are typically localized in the basal and suprabasal layers of the epidermis [1]. They are epidermal antigen presenting dendritic cells and they protect skin against toxins, infections and development of neoplasias [2-4]. These cells represent a critical role in the immune system at the interface to the external environment. The LH cells are activated by antigens, they invade and migrate through the dermis and enter lymphatic capillaries. Than they travel to the regional lymph nodes, where they present antigenic peptides to T cells and trigger an antigen specific immune response. For that reason LH cells are referred to as "professional antigen-presenting cells" [1,5,6].

Mast cells originate from the bone marrow and represent a critical role in the immune system in the dermis. They are often localized at highest density in the perivascular area, and in close proximity to peripheral nerves [7]. They produce, store and release

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cytokines including keratinocyte growth and differentiation factors, fibrogenic factors and angiogenic factors [8]. Studies have shown that mast cells contribute to the tumorigenesis of cutaneous malignancies through many mechanisms including immunosupression, enhancement of angiogenesis, degradation of the extracellular matrix and promotion of tumor cell mitosis [9].

In the present study, we aimed to evaluate mast cells and peri and intratumoral Langerhans cells in some skin lesions including seborrheic keratosis (SK), keratoacanthoma (KA), actinic keratosis (AK) and squamous cell carcinoma (SCC).

MATERIALS and METHODS

This study included 12 patients with SK, 9 patients with AK, 15 patients with KA and 33 patients with SCC. The distribution of histology, mean number of mast cells and mean number of intratumoral and peritumoral LH cells are demonstrated in Table I. Dermal mast cells and epidermal LHs were labeled with mast cell tryptase and CD1a antibodies.

Immunohistochemistry: The formalin fixed, paraffin embedded sections were cut into 4μm sections. The sections were deparaffinized with xylene and hidrated through graded alcohols into water. 3% hydrogen peroxide was applied for 30 minutes to inhibit endogenous peroxidase activity. The primer antibody for Tryptase (Dako, Hamburg, Germany) at a concentration of 1/50 was added and slides were incubated for one hour. Then, they were incubated in biotinylated goat anti-polyvalent (Labvision, Fremont. CA, USA) for ten minutes and in streptavidin peroxidase (Labvision, Fremont. CA, USA) for twenty minutes. Finally the slides were treated with AEC substrat system (Labvision, Fremont. CA, USA) for tree minutes. The sections were rinsed with water and was counterstained with Mayer's hematoxyline.

Evaluation of immunostaining:

The entire tumor was scanned under low-power magnification by light microscope. The number of tryptase positive mast cells counted in three representative, non overlapping, x200 microscopic fields of the peripheral and central regions of each tumor section. The means of mast cell numbers were then calculated. Than the LH cells were counted in three peritumoral and three intratumoral area and the mean number were calculated.

Statistical analysis

Kolmogorov-Smirnov test was used to analyse whether mast cells, intratumoral and peritumoral LH cells had normal distribution. According to this test, mast cells and LH cells had normal distribution. Descriptive statistics were demonstrated as mean±standard deviation (SD). One way analysis of variance was used in the comparison of groups. Tukey-HSD test was used for post hoc test. The difference was considered as significant if $p<0.05$. The correlation test was used to correlation between the tumor subgroups.

RESULTS

The number of the mast cells was significantly increased in the patients with SCC in comparison with SK ($p=0.001$). However, this parameter did not show significant difference between patients with SK and AK and between patients with SK and KA ($p=0.739$, $p=0.427$). The number of the mast cells obtained from patients with SCC did not show significant difference among patients with AK and KA ($p=0.090$, $p=0.083$). Additionally the number of mast cells in AK and KA did not show significant difference ($p=0.987$) (Fig 1, 2).

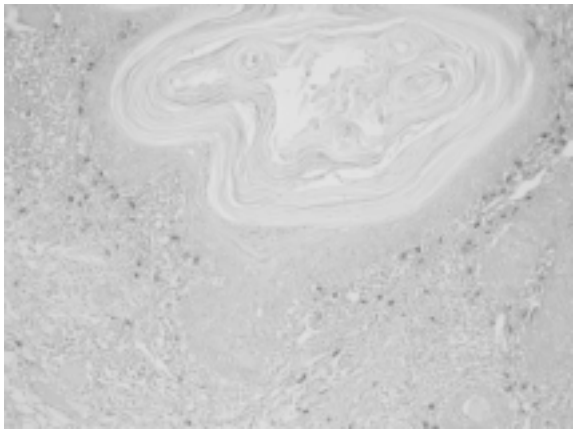

Fig 1. Mast cells in KA (x400, tryptase).

The number of the intratumoral LH cells obtained from the patients with SK was significantly increased in comparison with SCC ($p=0.026$). This parameter did not show significant difference between patients with SK and AK and between patients with

Table I. The distrubition of histology, mean number of mast cells and mean number of peri and intratumoral Langerhans cells.

Histology	Number of patients	Mean number of mast cells	Mean number of peritumoral LH cells	Mean number of intratumoral LH cells
Seboreic keratosis	12 (17%)	24.4±9.3	26.5±12	48.5±22.5
Actinic keratosis	9 (13%)	29.6±14.2	27.1±13.5	29.3±7.9
Keratoacanthoma	15(22%)	31.3±11.2	50.9±20	34.1±19.7
Squamous cell carcinoma	33(48%)	40.1±11.8	45.5±12.5	29.8±19.4

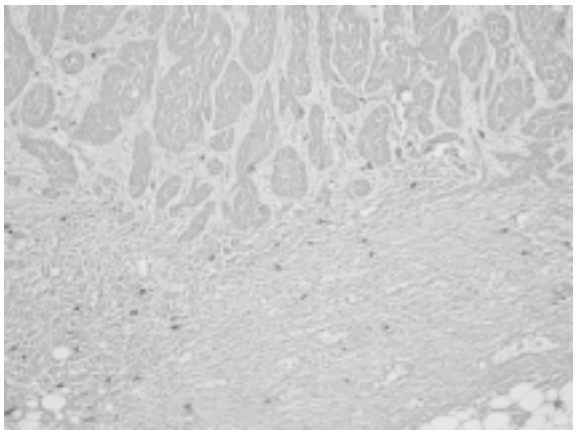

Fig 2. Peritumoral mast cells in SCC (x400, tryptase).

SK and KA (p=0.114, p=0.221). Any difference was not observed between the patients with AK and KA (p=0.933), AK and SCC (p=1.00) (Fig 3a, 3b, 3c).

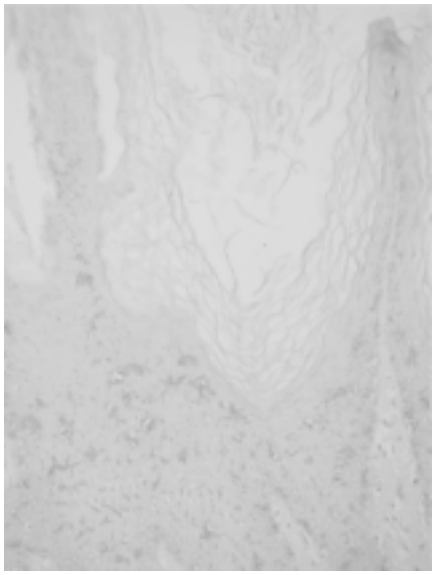

Fig 3a. Numerous intratumoral LH cells in SK (x200, Cd1a).

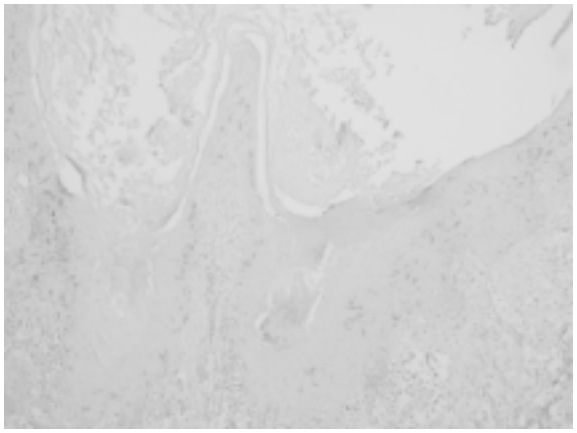

Fig 3b. In this KA the intratumoral LH cells were increased (x100, Cd1a).

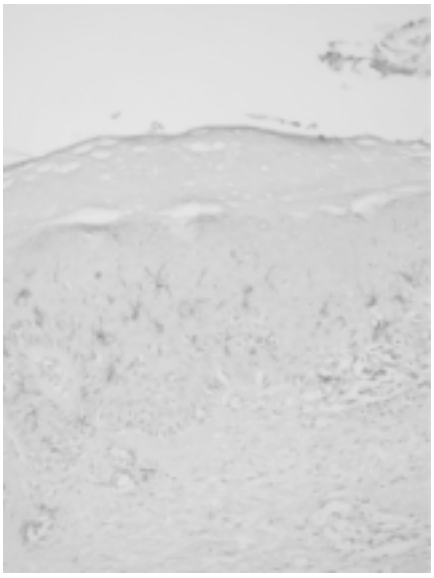

Fig 3c. Increased intratumoral LH cells in AK (x200, Cd1a).

Considering peritumoral LH cells, there was significant difference between SK and KA (p=0.001), SK and SCC (p=0.001), AK and KA (p=0.001), AK and SCC (p=0.007). The number of peritumoral LH cells were statistically insignificant between KA an SCC (p=0.631). Peritumoral LH cells were highger in KA and SCC compared with all other skin tumor groups, and lowest in SK (Fig 3d).

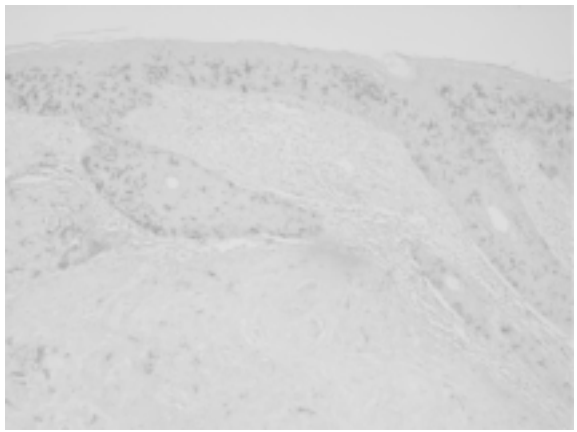

Fig 3d. Peritumoral LH cells are more in number than intratumoral LH cells in SCC (x100, Cd1a).

When SK cases evaluated separately there was not positive correlation between mast cells and peri-intratumoral LH cells (p=0.868, p=0.469). Additionally intra and peritumoral LH cells has no correlation (p=0.856). In AK mast cells and peri-intratumoral LH cells has no correlation (p=0.627, p=0.842) but between peri and intratumoral LH cells was positive correlation (p=0.006, r=0.830). In KA mast cells and peri-intratumoral LH cells has no correlation (p=0.580, p=0.694) but between peri and intratumoral LH cells was positive correlation

($p<0.001$, $r=0.924$). In SCC mast cells and peri-intratumoral LH cells has no correlation ($p=0.285$, $p=0.062$) but between peri and intratumoral LH cells was positive correlation ($p=0.002$, $r=0.525$).

DISCUSSION

The stromal microenvironment, which in benign and malign neoplasms is composed of vessels, fibroblasts and several kinds of inflammatory cells, influences tumor growth and progression. The skin immune system play a central role of the cutaneous tumor surveillance. The LH cells are an important component of skin immune system [10]. LH cells protect skin against toxins, infections and development of neoplasias [2]. The most important biological role of LH cells is to initiate an immune response. Following uptake of antigen, LH cells mature and migrate into draining lymph nodes. Down regulation of E-cadherin or IL-1β expression from keratinocytes is a key event of LH cells migration. In the lymph nodes they enter the afferent lymphatic vessels to initiate systemic immune responses. In T cell compartments of lymph nodes, they activate T cells and trigger an antigen specific immune response [1,6,10].

Recent studies have showed that LH cells are reduced or increased in some cutaneous tumors [3,11,12]. Prior studies have shown a low density of LH cells in SCC [13-15]. Melo et al. suggested that LHs are more numerous in normal skin and benign skin tumors in comparison with malign skin tumors [2]. Our immunohistochemical study has demonstrated that intratumoral LH cells are increased in number in SK. The number of the intratumoral LH cells obtained from the patients with SK was significantly increased in comparison with SCC. In the other groups were not difference. It remains unknown why LH cells are increased in benign lesions and decreased in SCCs, although it is suggested that it relates to immune evasion by the tumor.

In the present series, peritumoral LH cells were more numerous in KA. In SK and AK the number of peritumoral LH cells were lower in number in comparison with KA and SCC. The KA is a benign epithelial tumor. It is sometimes difficult to distinguish KA and SCC by histopathological examination [16]. Some believe that KA is a variant of SCC [17-19]. This is not generally accepted and many authors believe that the two entities must be distinguished, because KA have the potential to involute without treatment. Regression is immunologically mediated and activated by a variety of molecular mechanisms [20]. Are they a link between peritumoral LH cells and regression in KAs? That is an important point to understand of the tumor biology and regression in some tumors including KAs.

Moreover there was strong positive correlation between peri and intratumoral LH cells in AK, KA and SCC. The intratumoral LH cells were more numerous from peritumoral LH cells in SK. However the peritumoral LH cells were more numerous than intratumoral LH cells in SCC. Tumor infiltration by LH cells has a positive prognostic implication because of their increased ability to stimulate T cell responses against the tumor [14].

Mast cells are multifunctional effector cells of immune system. Mature mast cells produce and release a wide variety of soluble mediators, such as tryptase and chymase, histamine, heparin, leukotriene C4, prostaglandin D2 and multifunctional cytokines including TNF-α, IL-4, IL-10 that enable their participation in both adaptive and innate immune system. The effects of the mast cell mediators alter the dermal microenvironment[7,8,21,22].

It is suggested that ultraviolet-B activates mast cells. Ultraviolet-B stimulates neuropeptide secretion from dermal sensory nerves. These neuropeptides release mediators from mast cells such as Histamine and TNF-α[7,9,23]. Mast cells are the major source of TNF-α in human skin. It is suggested that TNF-α is a key mediator in ultraviolet-induced local immunosupression. TNF-α is increased in sun exposed skin and it may act by altering LH cell morphology and function[24]. Hart et al. suggest that mast cell histamine is an important mediator of systemic suppression in mice[25]. Mast cells have been observed to accumulate around the margin of many tumors and cutaneous malignancies[26-28]. Recently Grimbaldeston et. al suggested that mast cell products may contribute to the initiation and development of malignant melanoma[29]. Additionally the number of mast cells has been shown to be increased in the basal cell carcinomas[30]. It is suggested that a higher density of dermal mast cell is a predisposing factor for the development of basal cell carcinoma [7,31]. However a similar correlation has not been found for patients with SCC [8]. Our results show that mast cells were significantly increased in the patients with SCC in comparison with SK ($p:0.001$). The highest number of mast cells were in SCC (Table 1). However there was no significant difference between the other groups. Aditionally there was no correlation between mast cells and intra peritumoral LH cells.

These data suggest that mast cells can interact with other immune cells and thereby contribute to the development of cutaneous malignancies [22]. In the present study, the number of mast cells, peritumoral and intratumoral LH cells indicated that there was some variability in skin tumors. In addition, peri and intratumoral LH cells have positive correlation in KA and SCC.

In our opinion both epidermal Langerhans cells and dermal mast cells which have some contributions in the immune system, may have role in progression or development of some skin tumors.

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