Pathol. Oncol. Res. (2010) 16:7–10
DOI 10.1007/s12253-009-9179-4

Proliferating Activity in Paget Disease of the Nipple

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Received: 17 June 2008 / Accepted: 19 May 2009 / Published online: 17 December 2009

Abstract Paget disease of the nipple is a rare disease characterized by the presence of malignant glandular cells within the squamous epithelium of the nipple. The most common hypothesis to explain the development of Paget disease is an intraepithelial epidermotropic migration of malignant epithelial cells originating from an underlying intraductal carcinoma. If the immunohistochemical properties of the Paget cells in the nipple have been extensively studied, their proliferating characteristics remain paradoxically poorly studied. In the present study we have investigated the proliferating activity of Paget cells in the nipple by using double stain immunohistochemistry with both Ki67 (a protein which is expressed in all active parts of the cell cycle) and cytokeratin 7 (a highly sensitive marker of Paget cells). Ten cases of Paget disease and in their associated intraductal carcinomas (n=10) and/or invasive carcinomas (n=4) were tested. The mean Ki67 index was in Paget disease (26%±10), in intraductal carcinomas (23%±8) and/or in invasive carcinomas (20%±8) (p>0,05). This is the first report to convincingly demonstrate by specific double stain immunohistochemistry that Paget disease and underlying intraductal carcinomas share a close proliferating activity.

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Keywords Paget disease · Nipple · Breast · Proliferation · Ki-67 · Double stain immunohistochemistry

Introduction

Paget disease of the nipple is rare disease of the breast characterized by the presence of malignant glandular cells within the squamous epithelium of the nipple [1–3]. Clinically, the classical manifestation of Paget disease range from local reddening to eczematous or psoriasiform lesion of the nipple, soon extending to the mammary areola and then to the surrounding skin [1–3]. Paget disease is almost always associated with underlying intraductal carcinoma of the lactiferous duct and more rarely with invasive carcinoma deep in the underlying breast [4–6]. Two majors hypothesis have been suggested for the development of PD. Firstly, Paget disease may represent an intraepithelial epidermotropic migration of the malignant epithelial cells from the intraductal carcinoma to the nipple. Secondly, for certain authors, failure to detect underlying carcinoma in a small number of cases, suggest that PD may result from situ neoplastic transformation of multi-potential cells present in the basal layer of the epidermis [1–3, 7–11]. If the immunohistochemical properties of Paget cells in the nipple have been extensively studied, their proliferating characteristics remain poorly understood because to assess more specifically the proliferating activity in the Paget's cells and not in the adjacent keratinocytes remains conflictual [12]. To clarify the issue, we have used double stain immunohistochemistry with both cytokeratin 7 (CK 7) and Ki-67. CK 7 is a well recognized and highly sensitive marker of Paget cells and KI-67 is a human nuclear protein, which is expressed in all active parts of the cell cycle G1, S, G2 and mitosis but is absent in resting and quiescent cells

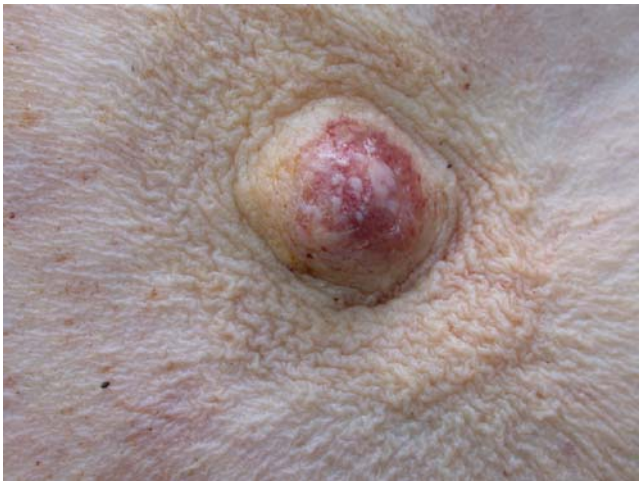

Fig. 1 Paget disease. Typical clinical features. Eczematiform reddening aspect of the nipple

(G0) [13–15]. The Ki-67 proliferating index of Paget cells has been compared with those of the underlying intraductal carcinomas and/or invasive carcinomas.

Material and Method

Ten cases of Paget disease were retrieved from the surgical pathology department of Erasme University Hospital and the material was collected according with the rules of the local ethical committee. All the patients were female. The mean age was 68 years (range 44–90). The clinical data which were available from the dermatology department consisted on erythematous rash and/or eczematous-psoriasiform lesions of the nipple with extension to the areola in four patients (Fig. 1). In all the patients, underlying ductal intraepithelial neoplasia grade 2 or 3 (DIN 2-3 according to the WHO 2003) were present. In addition, in four patients (3/8: 37%) , an invasive ductal carcinoma was also present (3 grade 2 invasive carcinomas and 1 grade 3 invasive carcinoma according to the semi-quantitative method for assessing breast carcinoma from Elston and Ellis) [16].

Table 1 Ki67 expression in Paget's disease of the nipple and corresponding intraductal carcinoma or invasive ductal carcinoma

	Paget's disease $N=10$	Intraductal carcinoma $N=10$	Invasive ductal carcinoma $N=4$	
Ki67 (%±SD)	26±10	23±8	20±8	$p>0,05$ (NS)

Immunohistochemistry for Double Stain

For the quantification of proliferating endothelial cells, a Ki-67/CK7double-labelling immunohistochemical staining was performed by using the EnVision G/2 double stain system (DAKO, Glostrup, Denmark). A monoclonal antibody directed against Ki-67 (clone MIB-1, dil 1:50, DAKO,Glostrup, Denmark) was applied to rehydrated paraffin sections and allowed to incubate for 1h at room temperature. Endogenous peroxidase was blocked. A secondary antibody linked to peroxidase and DAB was used to visualize the binding of the first antibody. The sections were then incubated for 1h with an antibody

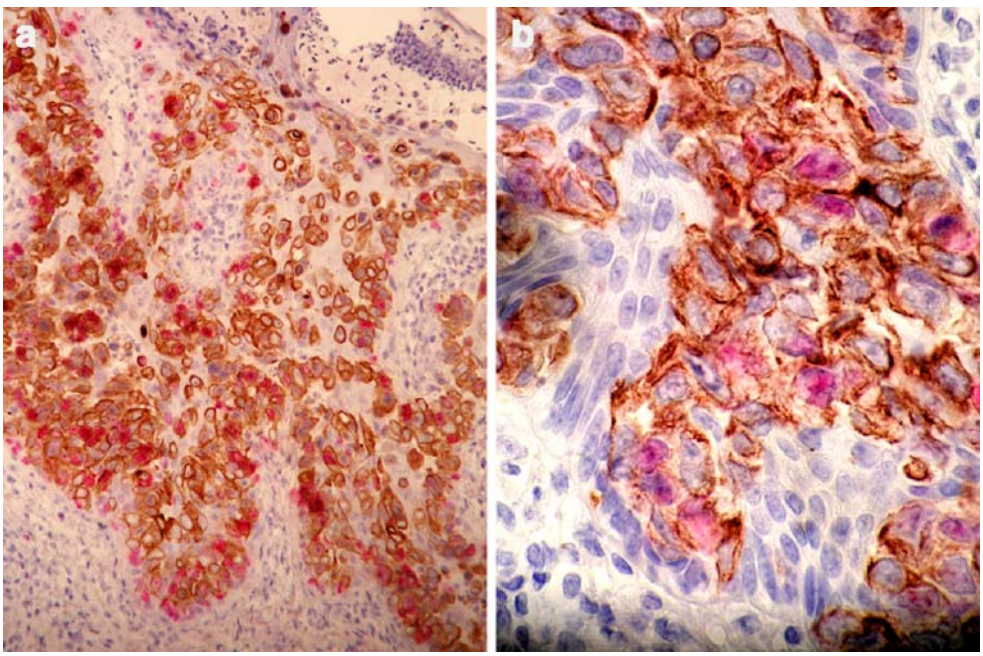

Fig. 2 Double immunostaining of Paget cells with cytokeratin 7 (membranous and cytoplasmic staining in brown) and Ki-67 (nuclear staining in *red*) at low power (**a**) and high power (**b**) view

against CK 7 (clone OV-TL 12/30, dil 1:100, BioGenex, San Ramon, USA). Alkaline phosphatase linked to a secondary antibody and fuchsin as substrate chromogen system were used to complete the second immunostain.

The slides were examined for KI-67 reactivity under x20 objective by two independent observers (JCN and IF) who were blinded to outcome. A minimum of 300 nuclei per case of Paget disease and 500 nuclei for DIN 2-3 and invasive ductal carcinoma were counted. There was agreement between observers in more than 90 of cases but disagreements in evaluation were resolved by review and discussion at a multiheaded microscope.

Statistical Analysis

Comparison of the data was performed using the Student's t-test (two-tailed). Statistical significance was defined as $p<$ 0,05.

Results

The Paget cells showed a strong immunoreactivity both for the cytokeratin 7 and Ki67 (Fig. 2a and b).

The mean Ki67 index was higher in Paget diseases (26 %±10) than in underlying intraductal carcinomas (23%±8) and/or in invasive carcinomas (20%±8) (Table 1). However, the comparison of the data was not significant with a $p>$0,05.

Discussion

Paget disease of the breast is rare and occurs in about 1% of all the patients with breast cancer [1–3]. In this disease, the epidermis of the nipple is invaded by large neoplastic cells presumed to originate from underlying in situ intraductal carcinoma. Indeed, considerable evidences demonstrate that immunohistochemically Paget cells show similar properties to the underlying intraductal carcinoma cells with a positivity for CK 7, low molecular weight cytokeratin, carcinoembryonic antigen, epithelial membrane antigen and HER2/NEU protein in a vast majority of cases [1–3, 10, 11]. The attraction of Paget cells to epidermis has been explained by the fact that normal epidermal cells produce heregulin-alpha, a motility factor which exerts a chemotactic effect on Paget cells previously proved to express heregulin receptors HER2/NEU as well as HER3 and/or HER4, both of which function as co-receptor of HER2/NEU [7, 8].

The proliferating activity of Paget's cells remains conflictual probably because by their nature they are entrapped among non neoplastic squamous cells in the epidermis rending difficult the analysis of proliferating cells with classical immunohistochemitry for individual antibody such as Ki-67 [12]. The only publication done on the subject described a Ki-67 index ranging from 16% to 19% but with a high standard deviation (±28 %) rending the interpretation of the results extremely difficult. In addition no comparison between the Ki-67 index and underlying intraductal or invasive carcinomas has been performed. The use of double stain immunohistochemistry with CK 7 and Ki-67 ensure an increase of specificity because CK 7 is an effective marker for Paget cells [1–3]. In the present study, we have showed that Paget cells are not only able to demonstrate migratory properties but also proliferating capacities and interestingly the proliferation Ki-67 index in Paget disease is relatively similar to those of underlying intraductal carcinoma. Naturally, our data should be carefully though out because if CK 7 is an effective marker for Paget cells, it is not 100% specific. Indeed, CK 7 is also a marker for Toker cells which are considered however for certain authors as precursors of Paget cell carcinoma [9].

In conclusion, in the present study, we have clearly demonstrated for the first time by double stain immunohistochemistry, that Paget cells have not only the same immunophenotype that underlying intraductal carcinoma but also share similar proliferating properties [4].

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