The Expression of c-erbB-1 and c-erbB-2 in the Various Skin Tumors

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Background **: c-erbB-1 (epidermal growth factor receptor) and c-erbB-2 oncoprotein have common tyrosine kinase activities, and alteration in their expression has been defined in various visceral tumors. However, relatively little is known about their expression in skin tumors.**

Objective **: Our aim was to evaluate the distribution and expression pattern of c-erbB-1 and c-erbB-2 in skin neoplasms.**

Methods **: We have undertaken an immunohistochemical survey of c-erbB-1 and c-erbB-2 in the tissue specimens of keratoacanthoma (KA), actinic keratosis (AK), squamous cell carcinoma (SCC) and basal cell carcinoma (BCC).**

Results **: Membranous c-erbB-1 expression had a tendency to be down-regulated in some specimens of SCC and dysplastic portions of AK. In invasive lesions of SCC, we observed increased cytoplasmic accumulation of c-erbB-1. Most specimens of BCC showed rather decreased expression of c-erbB-1 compared with other skin tumors. c-erbB-2 oncoprotein showed strong cytoplasmic staining in SCC, especially in the invasive tumor mass, and in some deeply dysplastic or hyperplastic portions of AK, though the difference of intensity was not striking between tumors. BCC revealed relatively weaker expression of c-erbB-2 than other skin tumors, which was similar in pattern to c-erbB-1.**

Conclusion **: The expression patterns of c-erbB-1 and c-erbB-2 are altered in various skin neoplasms, and seem to be related to the dysplastic status or differentiation level of tumor cells.**

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Key Words : c-erbB-1, c-erbB-2

There are three members of the c-erbB receptor family including c-erbB-1, c-erbB-2 and c-erbB-3. These oncoproteins possess the tyrosine kinase activity, and their functional or structural abnormalities may be responsible for deranged keratinocyte proliferation and differentiation that lead to variable tumorous conditions[1].

c-erbB-1 (epidermal growth factor receptor) is known to induce receptor clustering, internalization of receptors and activation of the receptor kinase activity when it binds ligands such as epidermal growth factor (EGF) or transforming growth factor-alpha (TGFα)[2]. c-erbB-2, sharing many properties with c-erbB-1, has been suggested to be a receptor of some yet unidentified growth factor and involved in malignant transformation of cells[1]. Previous studies showed overexpression of c-erbB-2 protein in some tumors of breast[3], ovary[4], bladder[5] or salivary gland[6], and supported the role of c-erbB-2 in tumorigenesis. However, there is no report about the expression pattern of above oncoproteins in the skin tumors. The aim of this study was to examine the distribution and expression of c-erbB-1 and c-erbB-2 immunohistochemically in skin tumors.

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MATERIALS AND METHODS

Materials

We examined sections of formalin-fixed, paraffin-embedded tissue from 5 cases of keratoacanthoma (KA), 7 cases of actinic keratosis (AK), 11 cases of squamous cell carcinoma(SCC) and 12 cases of basal cell carcinoma (BCC). 5 specimens from normal volunteers were also examined as control. Monoclonal antibody to c-erbB-1 (Oncogene Science, Uniodale, USA) and monoclonal mouse antibody to c-erbB-2 (NCL-CB11, Europath, Bude, U.K.) were used as primary antibodies.

Immunohistochemical Staining

c-erbB-1 and c-erbB-2 were detected by the streptavidin-biotin peroxidase system (Dako LSAB kit, Kyoto, Japan). After being deparaffinized in xylene and ethanol, the sections were placed in a jar filled with 10 mmol/l citrate buffer, pH6, heated twice in a microwave oven for 5 minutes and kept in citrate buffer for 20 minutes at room temperature. Then they were treated with 3% hydrogen peroxide solution to inhibit non-specific background staining by endogenous peroxidase and subsequently incubated with the primary antibodies respectively (dilution 1:100), followed by application of biotinylated link and streptavidin peroxidase. 3-amino-9-ethyl carbazole was used as chromogen. All sections were counterstained with hematoxylin, and examined under the microscope. The staining intensity was evaluated by two observers using an ordinary 0 to 3 scales, where 0 was assigned to no staining; 1 to minimal staining, 2 to moderate staining, and 3 to maximal staining. We subdivided the scales by 0.5 for the intermediate expression between each grade.

RESULTS

c-erbB-1 Expression

In normal skin, c-erbB-1 was expressed in basal cells and lower stratum malpighii, showing predominant labelling on the cell membrane with very faint cytoplasmic staining (Fig. 3a). The patterns in KA revealed slightly more exaggerated expression than the normal control, showing increased membranous or cytoplasmic staining (Fig. 3b). In AK, overall pattern was similar to KA, but some portions of the lesions showed decreased membranous labelling and increased cytoplasmic staining, especially in the relatively dysplastic foci (Fig. 3c), which was not the usual finding with KA. In BCC, 6 of 12 specimens revealed markedly decreased expression than KA or AK (Fig. 3d), but a few portions of them showed focal increase in their cytoplasmic staining. The staining pattern in SCC were somewhat heterogeneous, 4 of 11 specimens revealed minimal or markedly decreased membranous labelling, while 4 other specimens showed increased or strong cytoplasmic expression compared with other epidermal tumors (Fig. 3e). The details of c-erbB-1 labelling are shown in figure 1.

c-erbB-2 Expression

In normal skin, very little c-erbB-2 protein was expressed in the epidermis, and sebaceous gland and eccrine gland often showed faint labelling (Fig. 4a). Each specimen of KA showed different staining patterns from minimal to extensive expression (Fig. 4b). Though most specimens of AK revealed similar or mildly increased expression compared with KA, they usually showed focal areas of extensive cytoplasmic staining (Fig. 4c), which corresponded to dysplastic foci on hematoxylin and eosin staining sections. BCC showed decreased intensity of staining compared with other skin tumors (Fig. 4d). In SCC, markedly increased expression was observed, and 4 of 11 specimens showed very strong cytoplasmic staining (Fig. 2e). The stained cells in SCC revealed cytoplasmic expression predominantly with or without membranous accentuation, and their strong expression patterns were prominent in the periphery of invasive tumor mass. The details of c-erbB-2 labelling are shown in figure 2.

DISCUSSION

Groves *et al.*[2] defined two types of keratinocyte expression of c-erbB-1, that is, basal keratinocyte expression (type I) with both cytoplasmic and membrane staining, and prickle cell expression (type II) with membrane staining only. They observed that well differentiated tumors showed both expression patterns, while some poorly differentiated tumors showed loss of membrane staining and increased cytoplasmic accumulation. They also proposed the explanation about the membrane to cytoplasmic internalization of c-erbB-1. According to their theories, in the very initial stages of keratinocyte malignancy, membranous c-erbB-1 would be down-regulated, and this down-regulation would be overcome as the dysplastic process progresses,

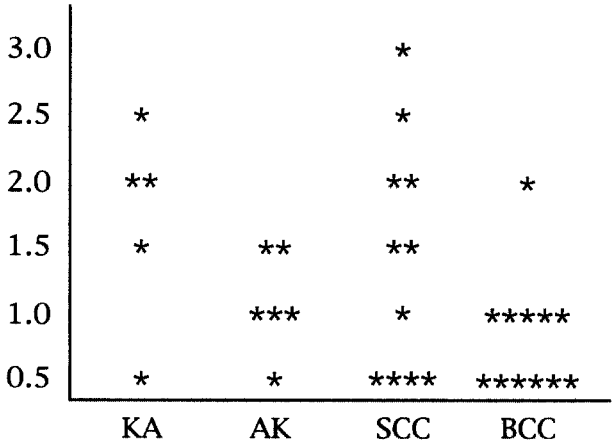

Fig. 1. The expression of c-erbB-1 in skin tumors.

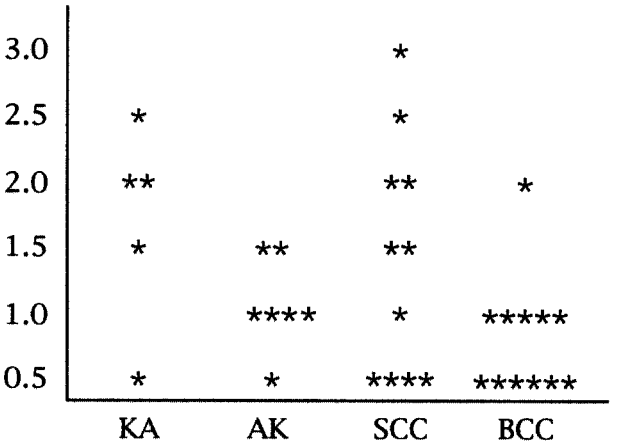

Fig. 2. The expression of c-erbB-2 in skin tumors.

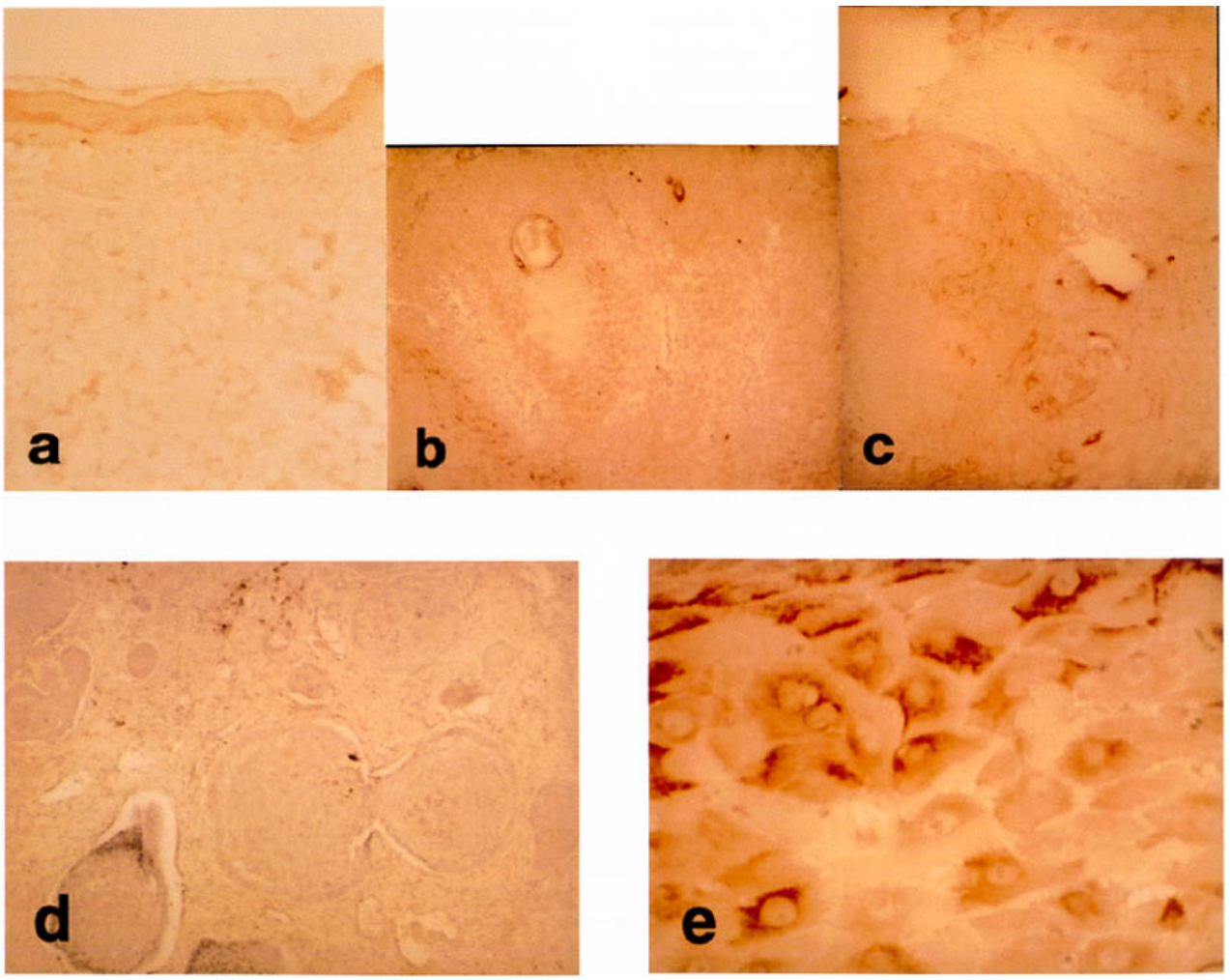

Fig. 3. Staining of c-erbB-1 showing faint expression in basal cells of normal epidermis (a, ×100), mildly increased membranous expression in KA (b, ×200), mixed membranous and focal increased cytoplasmic staining in AK (c, ×200), decreased expression in BCC (d, ×200), and markedly increased cytoplasmic expression in SCC (e, ×400).

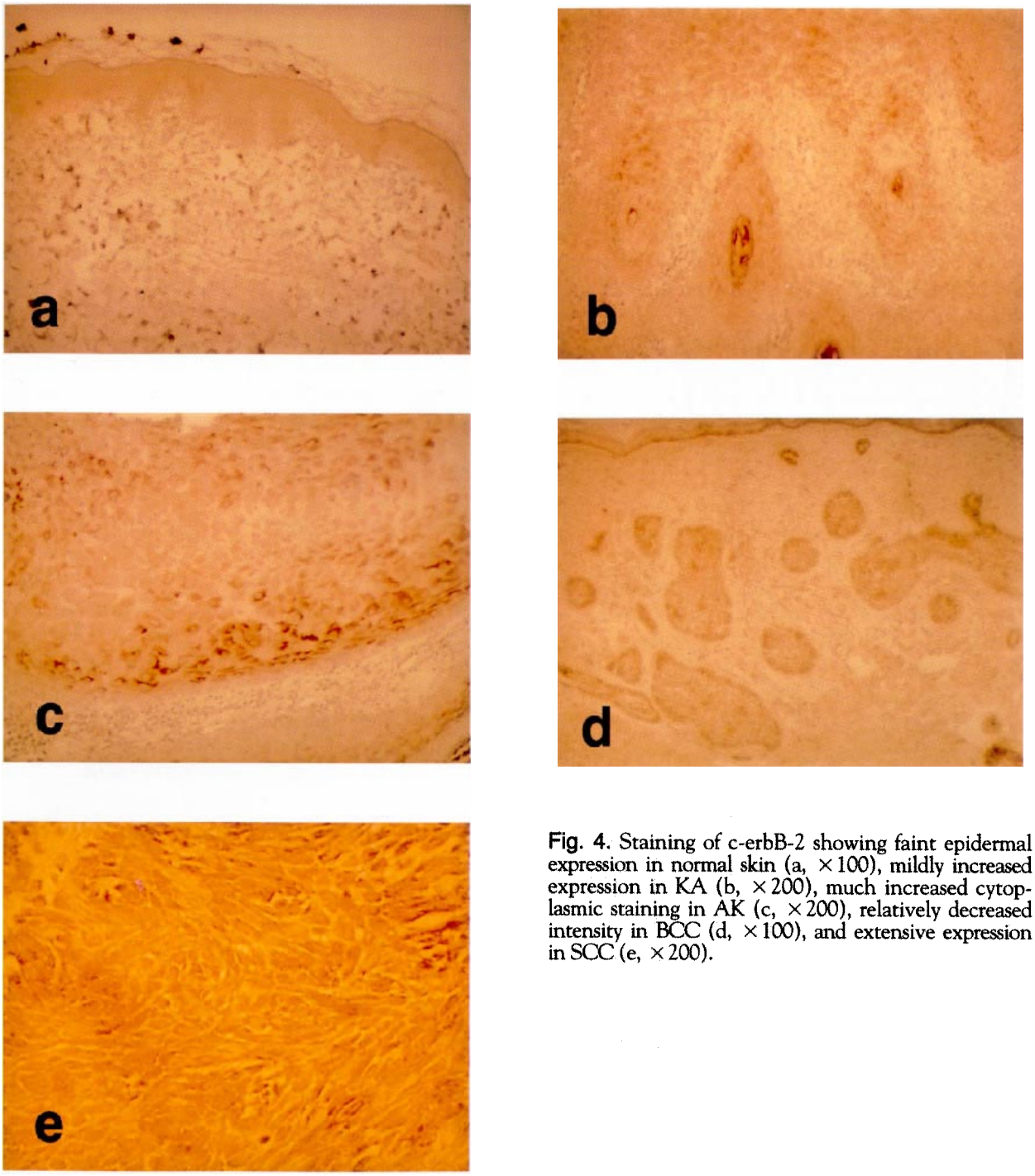

Fig. 4. Staining of c-erbB-2 showing faint epidermal expression in normal skin (a, ×100), mildly increased expression in KA (b, ×200), much increased cytoplasmic staining in AK (c, ×200), relatively decreased intensity in BCC (d, ×100), and extensive expression in SCC (e, ×200).

and finally, c-erbB-1 would accumulate in the cytoplasm when the cells become fully dysplastic[2]. The ligands such as EGF and TGF-α are thought to be one of the causative factors that bind to c-erbB-1 and initiate the internalization process of the receptors[7]. These findings suggest that the expression patterns of c-erbB-1 should be related to the cell' s dysplastic state in a given tumor[8], and the idea is also supported by

some experimental evidences that the expression of c-erbB-1 was closely linked to the differentiation capacity of keratinocytes[9] and that the cells transfected with c-erbB-1 gene showed ligand-dependant transformation[10].

In this study, some of SCC showed intense cytoplasmic accumulation of c-erbB-1 compared with KA or AK, while other specimens of SCC revealed somewhat attenuated (minimal or faint) membranous labelling. In AK, overall intensity of staining was intermediate among various tumors, but some foci were observed with decreased membranous labelling or with increased cytoplasmic staining, and they generally corresponded to a clump of dysplastic keratinocytes. Most specimens of BCC showed decreased membranous labelling compared with KA, AK or SCC. As a whole, our findings were in accordance with previous observations[2,8] that dysplastic states of the tumors were related to the c-erbB-1 expressions which varied from down-regulated membranous expression of initial dysplasia to cytoplasmic accumulations of progressive dysplasia. We could not clearly explain in this study why only some specimens of SCC, the highly dysplastic epidermal tumor, showed strong cytoplasmic accumulation. It may be that SCC specimens in our cases were rather well differentiated, or that our primary antibody detected membranous c-erbB-1 mainly instead of cytoplasmic protein.

The c-erbB-2 gene is known to have sequence homology to the growth factor receptor gene and be involved in malignant transformation of cells[11,12]. Gene amplification is reflected in the overexpression of c-erbB-2 oncoprotein, which has been observed in some tumorous conditions including prostate and uterine cervical cancer[13], mammary and extramammary Paget' s disease[14], and radiation-induced skin ulcers[15]. Ahmed *et al.*[1] showed that, in skin tumors, poorly differentiated tumor cells were strongly stained especially with cytoplasmic accentuation, whereas differentiated cells were weakly to negatively stained. They proposed that overexpression of c-erbB-2 enhance the metastatic process or promote the invasiveness of malignant tumors, and Sanjay *et al.*[16] supported the theory by showing a significant correlation between the oncoprotein c-erbB-2 and proliferative marker of Ki-67 and AgNORs(silver-stained nuclear organizer region).

The result of our study showed that c-erbB-2 expression markedly increased in SCC compared with BCC or other skin tumors. Although the overall degree of difference was not striking between SCC and other skin tumors, SCC specimens with aggressive invasion revealed much stronger cytoplasmic staining in the tumor nests. In KA or AK, some portions of severe downward proliferation or marked dyskeratotic maturation also showed increased intensity of staining. These findings support the previous idea that the c-erbB-2 expression should be directly related to differentiating level and invasiveness of tumor cells[1].

Considering both c-erbB-1 and c-erbB-2 expression together in this study, invasive SCC specimens with increased cytoplasmic accumulation of c-erbB-1 were mostly related to increased c-erbB-2 expression also. In addition, some dysplastic foci in AK or KA with loss of membranous c-erbB-1 generally corresponded to mildly increased expression of c-erbB-2. Most BCC specimens showed rather decreased expression of both c-erbB-1 and c-erbB-2 than SCC or other skin tumors. The relatively lower expression of these oncoproteins in BCC is thought to be due to the slow growing, locally malignant nature of the tumor, which was also suggested by Ahmed et al[1].

Taken together, the expression patterns of c-erbB-1 and c-erbB-2 in various skin tumors should be closely related to differentiation level, invasiveness or metastatic potency of tumor cells. For further studies, some consideration about established factors including clinical stage, histologic type and grade, and biologic marker will confer better results in investigating the role of c-erbB oncoproteins in skin tumorigenesis.

REFERENCES

1. NU Ahmed, M Ueda, M Ichihashi: Increased level of c-erbB-2/neu/HER-2 protein in cutaneous squamous cell carcinoma. Br J Dermatol 136:908-912, 1997.
2. RM Groves, MH Allen, DM Macdonald: Abnormal expression of epidermal growth factor receptor in cutaneous epithelial tumors. J Cutan Pathol 19:66-72,1992.
3. Venter DJ, Kumar S, Tuzi NL, Gullick WJ: Overexpression of the c-erbB-2 oncoprotein in breast carcinomas: immunohistochemical assessment correlates with gene amplification. Lancet ii:69-71, 1987.
4. Tyson FL, Boyer CM, Kaufman R et al.: Expression and amplification of the HER-2/neu (c-erbB-2) proto-oncogene in epithelial ovarian tumors and cell lines. Am J Obstet Gynecol 165:640-646, 1991.
5. Gardiner RA, Samaratunga MLTH, Walsh MD: An immunohistochemical demonstration of c-erbB-oncoprotein expression in primary urothelial bladder

cancer. Urol Res 20:117-120, 1992.
6. Dardick JR, Briek LE: Demonstration of c-erbB-2 oncogene overexpression in salivary gland neoplasms by in situ hybridization. J Oral Pathol Med 23:226-231, 1994.
7. KC Choi, YK Yoon, CN Oh: Immunohistochemical study of TGFα, EGF and EGF receptor on the epithelial tumors of the skin. Kor J Dermatol 32(3):438-445, 1994.
8. MN Nazmi, PJ Dykes, R Marks: Epidermal growth factor receptors in human epidermal tumors. Br J Dermatol 123:153-161, 1990.
9. Boonstra J, de Laat SW, Ponec M: Epidermal growth factor receptor expressiojn related to differentiation capacity in normal and transformed keratinocytes. Exp Cell Research 161:421, 1985.
10. Velu TJ, Beguinot L, Vasa WC et al: Epidermal growth factor-dependent transformation by a human EGF receptor proto-oncogene. Science 238:1408, 1987.
11. Bargmann CI, Hung MC, Weinberg RA: The neu oncogene encodes an epidermal growth factor receptor-related protein. Nature 319:226-230, 1986.
12. Bargmann CI, Hung MC, Weinberg RA: Multiple independent activation of the neu oncogene by a point mutation altering the transmembrane domain of p185. Cell 45:649-657, 1986.
13. Costa MJ, Walls J, Trelford JD. c-erbB-2 oncoprotein overexpression in uterine cervix carcinoma with glandular differentiation. A frequent event but not an independent prognostic marker because it occurs late in the disease. Am J Clin Pathol 104:634-642, 1995.
14. Nakamura G, Shikata N, Shoji T et al. Imm unohistochemical study of mammary and extramammary Paget' s disease. Anticancer Res 15:467-470, 1995.
15. Zhao P, Yang Z, Wang DW et al.: Overexpression of c-erbB-2 and p21 oncoproteins in human radiation-induced skin ulcers. J Environ Pathol Toxicol Oncol 14:21-23, 1995.
16. SK Sinha, UR Sinha, A Bhatia: C-erb B2 oncoprotein expression: Correlation with the Ki-67 labelling index and AgNOR count in breast carcinoma on fine needle aspiration cytology. Acta Cytologica 40(6):1217-1220, 1996.