Expression of p63 in Various Hyperproliferative Skin Diseases

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The keratinocytes in human epidermis are replaced by a population of stem cells located in the basal layer of the epidermis and one candidate stem cell marker is the transcription factor p63. We studied the expression of p63, immunohistochemically, in various hyperproliferative skin diseases (10 poorly differentiated metastatis squamous cell carcinomas (SCCs), 10 non-metastatic primary cutaneous SCCs, 10 cases of Bowen's diseases, 10 actinic keratosis, and 10 melanomas) and also observed the change of p63 expression in psoriasis after cyclosporine treatment. p63 was normally expressed in basal layer cells. Poorly-differentiated metastatic SCC showed the highest expression in most of the tumor cells, while psoriasis, actinic keratosis, Bowen's disease and primary SCC showed an increased expression in the basal and suprabasal area compared to in normal epidermis. The cyclosporine treatment in psoriasis reduced the expression of p63 to a normal level. This data suggests that p63 expression may influence tumor cell differentiation and proliferation without a direct tumorigenesis effect in epithelial tissue. (Ann Dermatol (Seoul) 18(2) 64~69, 2006)

Key Words: Cutaneous neoplasm, p63, Psoriasis, Stem cell

INTRODUCTION

The keratinocytes in human epidermis are constantly turned over and replaced by a population of stem cells located in the basal layer of the epidermis. One class of candidate stem cell markers is the transcription factor p63. It has been shown that p63 is highly expressed in the basal cells of human epithelial tissue[1,2]. It is also known that p63 is essential for ectodermal differentiation during embryogenesis[3-5]. Studies have shown that p63 is a nuclear transcription factor that triggers keratinocyte differentiation and is downregulated in terminally differentiated cells *in vitro*[1,6,7].

p63 may block the apoptosis-inducing activity of p53 and could help to maintain the proliferative capacity of basal or progenitor cells[1-4]. p63 may also play a role in the regulation of proliferation and differentiation in premalignant and malignant lesions of epithelial origin. So, p63 appears to play an oncogenic role in the development of human cancer.

Herein, we studied expression of p63, immunohistochemically, in various hyperproliferative skin diseases. We also observed the change of p63 expression after cyclosporine treatment and UV irradiation.

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MATERIALS AND METHODS

Immunohistochemical staining, according to the streptavidin-biotin-peroxidase technique, using mouse monoclonal antibody 63P03(NeoMarkers, Fremont, USA) raised against all known p63 isotypes, was performed. The expression of p63 was evaluated in epidermal cells and skin appendages by semiquantitative analysis of p63 expression in the various hyperproliferative skin diseases (10 primary

non-metastatic cutaneous squamous cell carcinomas (SCCs), 10 poorly differentiated metastatic SCCs, 10 cases of Bowen's diseases, 10 actinic keratoses, and 10 basal cell carcinomas (BCCs)). We also evaulated the change of p63 expression in 10 psoriatic skin lesions, before and after cyclosporine treatment. Nuclear staining was considered as specific. The percentage of positive cells (-. 1+ : < 5%, 2+ : 5 ~ 25%, 3+ : 25 ~ 50%, 4+ : > 50%) and staining patterns (negative, basal, suprabasal, diffuse and peripheral) were assessed.

RESULTS

In normal control skin, p63 was expressed (1 + ~ 2+) in the nuclei of epidermal basal and suprabasal cells, in the cells of the germinative hair matrix and the external root sheath of hair follicles, in the myoepithelial cells and basal cells of the sweat glands, and also in the basal cells of the sebaceous glands. All terminally-differentiated cells were negative for p63.

All primary cutaneous SCCs, actinic keratoses and psoriasis before cyclosporine treatment showed 2+ ~ 3+ immunoreactivity. BCC, Bowen's disease, and poorly-differentiated metastatic SCC showed 3+ ~ 4+ positivity constantly. Terminally-differentiated squamous cell carcinoma showed less expression of p63 than an undifferentiated one. Terminally-differentiated squamous cell carcinoma also showed a peripheral staining pattern. Malignant melanoma showed only a slightly increased expression (Fig. 1, Table 1).

Psoriatic lesional skin after cyclosporine treatment (12 weeks) was similar to normal control skin. On the other hand, the psoriatic lesion before and after cyclosporine treatment (3 weeks, 6 weeks) showed 2+ ~ 3+ positivity (Fig. 2, Table 2).

DISCUSSION

The p53 gene family includes p53, p63 and p73 genes based on gene sequence homologies. p63 is comprised of at least six different protein isoforms that lead to two fundamentally different roles[8-10]. Three of the p63 isoforms (TAp63) encode proteins with roles similar to p53, including transactivation and induction of apoptosis[8]. The other three isoforms

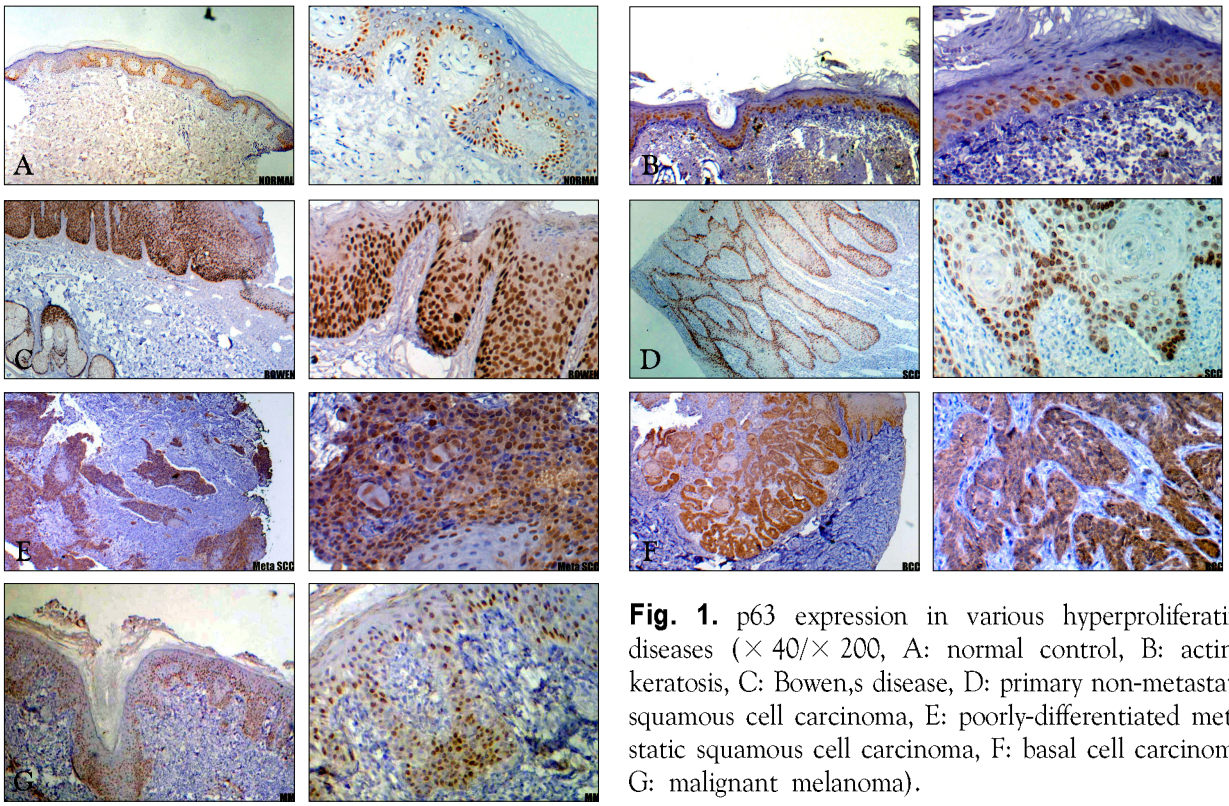

Fig. 1. p63 expression in various hyperproliferative diseases (× 40/× 200, A: normal control, B: actinic keratosis, C: Bowen,s disease, D: primary non-metastatic squamous cell carcinoma, E: poorly-differentiated metastatic squamous cell carcinoma, F: basal cell carcinoma, G: malignant melanoma).

Table 1. p63 Staining Pattern and Degree in Various Hyperproliferative Diseases

	Normal control (N=10)	Actinic keratosis (N=10)	Bowen disease (N=10)	Squamous cell carcinoma (N=10)	Pooly diff. metastatic SCC (N=10)	Basal cell carcinoma (N=10)	Malignant melanoma (N=10)
Staining Pattern	SB, B	SB, B	SB, B or D	B, SB or P	D	D	SB, B
Staining Degree	1+ ~ 2+	3+	3+ ~ 4+	2+ ~ 3+	4+	4+	2+ ~ 3+

SB: Suprabasal, B: Basal, P: Peripheral, D: Diffuse

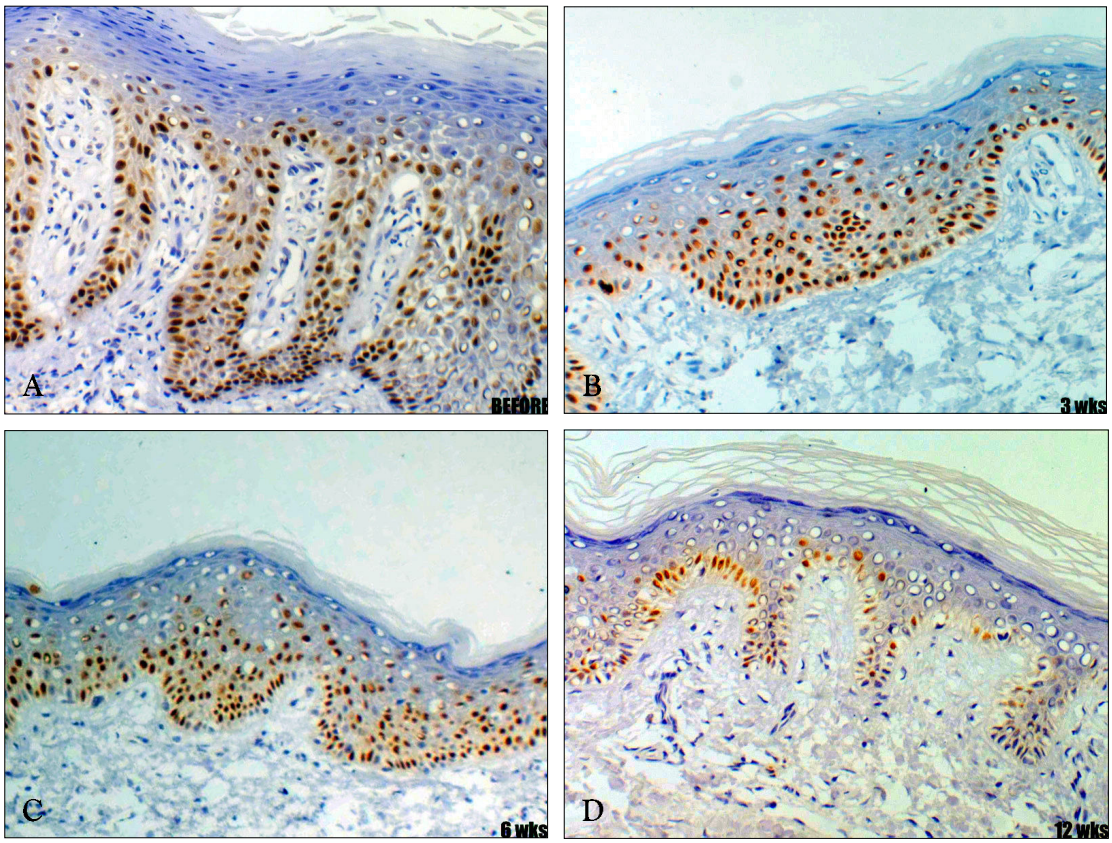

Fig. 2. p63 expression in psoriasis, before and after cyclosporine treatment (× 200, A: before treatment, B: 3 wks after treatment, C: 6 wks after treatment, D: 12 wks after treatment).

(ΔNp63) have an inhibitory effects on p53 activity. Therefore, p63 could act either as a tumor suppressor gene, or an oncogene[11].

It has been shown that p63 is highly expressed in the basal cells of human epithelial tissue[1,2]. p63 expression has also been identified in many normal tissues including urothelium, bronchial epithelium and the myoepithelial layers of breast, prostate and submucosal glands[12-15]. p63 expression has also been established in several neoplasms, including squamous, urothelial, endometrial, and papillary thyroid carcinomas and thymomas[16-23].

It has been demonstrated that p63 is essential for ectodermal differentiation during embryogenesis[3-5].

Table 2. p63 Staining Pattern in Psoriasis, before and after Cyclosporine Treatment

	Psoriasis with cyclosporin treatment			
	Before Tx (N=10)	3 wks (N=10)	6 wks (N=10)	12 wks (N=10)
Staining pattern	SB, B	SB, B	SB, B	B
Staining degree	2+ ~ 3+	2+ ~ 3+	2+	1+

SB: suprabasal, B: basal, P: peripheral, D: diffuse

The expression of p63 in normal human epidermis, cutaneous appendages and skin carcinomas has been assessed and it has been suggested that analysis of p63 expression may help in the differential diagnosis of primary cutaneous tumors compared to metastatic cutaneous tumors[24-26]. In this study, the expression of p63 in malignant melanoma did not increase, which implied the specificity of p63 with stratified epithelium.

The p63 staining of metastatic SCCs and SCCs was highly increased when compared with that of other non-malignant specimens. A diffuse p63 staining pattern was evident in metastatic SCCs. A suprabasal p63 staining pattern was found in keratinocytes within the entire range of noninvasive lesions. Poorly-differentiated squamous cell carcinoma showed a diffuse increased pattern, while well-differentiated squamous cell carcinoma showed peripheral staining. These finding imply that p63 is involved in keratinocyte differentiation. p63 was constituitively expressed in basal cells, its overexpression in squamous dysplasia and neoplasia may reflect immaturity of the tumor cell lineage, and it was observed that p63 staining was more uniform and homogeneous in less-differentiated tumor areas. Studies have shown that p63 is a nuclear transcription factor that triggers keratinocyte differentiation and is downregulated in terminally differentiated cells *in vitro*[1,6,23]. p63 expression was mainly noted in the peripheral cells of tumor nests in well-differentiated tumor areas[27,28]. p63 proteins could be used as an immunohistochemical marker for the differential diagnosis of poorly-differentiated and undifferentiated squamous cell carcinoma[27].

p63 expression was greater in metastatis SCCs when compared with that of normal skin and actinic keratosis. A diffuse high level of p63 expression in metastatic SCCs suggested that the degree of the p63-positive stems are correlated with the poor differentiation of malignant SCCs. p63 has been identified in keratinocyte stem cells.1 It is thought that stem cells are involved in the formation of malignant tumors[31]. p63 locus, chromosome 3q27-ter is frequently amplified in squamous cell carcinoma of the skin, lungs and esophagus[32-34]. Therefore, p63 appears to play an oncogenic role in the development of human cancer.

Basal cell carcinomas were shown to have high levels of p63 expression with minimal variability in their staining pattern. This correlates to the report that basal cell carcinomas show less variation in differentiation than squamous cell carcinomas[24,29].

In psoriasis, there was an increased expression of p63, but after 12 weeks of cyclosporine treatment, the expression of p63 was found to be normal. Furthermore all malignant lesions, except malignant melanoma, showed an increased expression. This data suggests that p63 expression influences tumor cell differentiation and may be associated with cancer development in epithelial tissue. But p63 positive cells did not always express Ki-67[24,29]. Thus, p63 positive cells were not undergoing active mitosis. p63 might block the apoptosis-inducing activity of p53 and could help to maintain the proliferative capacity of basal or progenitor cells[1,2,20,21]. Later stages of wound healing were associated with higher levels of ΔNp63 expression within basal keratinocytes, implying a crucial role in maintaining proliferative potential[30].

p63 was normally expressed in basal cells. Poorly-differentiated metastatic SCC showed the highest expression involving most of the tumor cells, while psoriasis, actinic keratosis, Bowen's disease and primary SCC showed slightly increased expression in the basal and suprabasal area in comparison to normal epidermis. The cyclosporine treatment in psoriasis reduced the expression of p63 to a normal level. This data suggests that p63 expression may be associated with differentiation and progression in skin epithelial tumors. High expression of p63 in

benign skin lesions such as psoriasis suggest that in comparison to p53, p63 is not involved in early carcinogenesis of epithelial skin cancer.

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