Significance of Mitotic Cells or Clumping Cells in p53 Immunopositivity of Bowen's Disease

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Key Words

p53
Bowen's disease
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The p53 nuclear phosphoprotein is a tumor suppressor gene product that potently prevents a cell from entering into the S phase of the cell cycle [1,2]. Deletion or mutation of the normal p53 gene produces an abnormal mutant p53 protein and accelerates the growth of certain cell lines. The mutant p53 molecule has a prolonged half-life that allows us to detect it by immunohistological methods [1, 2]. In fact, the presence of immunoreactive p53 has been documented in various premalignant and malignant skin tumors, including Bowen's disease [3-6]. However, little is known of the significance of mitotic cells or clumping cells in the p53 immunoreactivity of Bowen's disease. We have examined 6 cases of p53-immunopositive Bowen's disease and 6 cases of immunonegative Bowen's disease, and have compared the number of mitotic cells and clumping cells. The mean number of mitotic cells of the immunopositive cases is $1.05 \pm 1.24/\text{HPF}$, that is not significantly different from that of immunonegative cases ($0.45 \pm 0.31/\text{HPF}$, Student's t test, $p = 0.3038$, table 1). The mean number of clumping cells of the immunopositive cases is $1.7 \pm 1.05/\text{HPF}$, and it is again not significantly different from that of immunonegative cases ($1.2 \pm 0.87/\text{HPF}$, $p = 0.3888$). We have also examined the percentage immunopositivity of p53 in mitotic cells or in clumping cells. It should be noted that the mitotic cells are exclusively unlabeled with p53, whereas the majority of clumping cells (69.2-100%) are positively stained for p53 (table 1).

Although the p53 mutation has been reported to correlate with the invasiveness of squamous cell carcinoma and basal cell carcinoma [7, 8], Szekeres and de Giacomoni [9] describe that the p53 expression is not related to the immunopositivity of the Ki-67 proliferating cell marker in Bowen's disease. In accordance with their finding, a significant difference is not evidenced in the number of mitotic cells between the immunopositive and immunonegative cases. It is also

intriguing that cells in mitosis are consistently negative for p53, a phenomenon that has previously been observed by Sim et al. [10]. Since the phosphorylation status and the spatial distribution of p53 molecules are regulated in a cell cycle-dependent manner [1,2], the qualitative and/or quantitative alteration of the p53 molecule may abrogate the immunoreactivity of the molecule. On the contrary, the present study shows that the nuclear compartment of clumping cells is highly susceptible to the retention of immunoreactive p53 molecules. The fact that the aberrant expression of p53 is not related to the formation of mitotic cells and clumping cells in Bowen's disease may further support the multistep nature of oncogenesis.

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Table 1. Mitotic cells and clumping cells in p53-positive or p53-negative Bowen's disease"

Case

p53 staining	Number of mitotic cells mean $\pm$ SD/HPF	p53 positivity in mitotic cells, %
Number of clumping cells mean $\pm$ SD/HPF		
p53 positivity in clumping cells, %		

1

2 3 4 5 6b

0.1 0.6 0.8 0.3 3.5 1.0

± 0.32

± 0.52

± 1.03

± 0.48

± 3.1

± 1.05

0.8 ± 0.63 1.0 ± 0.67 1.3 ± 0.82 3.6 ± 1.43

± 1.69

± 1.25

87.5 70.0 69.2

100 86.4

100

1.05 ± 1.24

1.7 ± 1.05

85.5 ± 13.6

9 10 11

12

0.3

0

0.3

0.8

0.5

0.8

± 0.48

± 0.48 ± 0.92 ± 0.71 ± 1.62

0.6 ± 0.7

± 0.7 0.3 ± 0.48 2.5 ± 1.18

± 1.83 0.5 ± 0.85

0.45 ± 0.31

1.2 ± 0.87

HPF = High power field. 11 The sections were immunostained with anti-p53 antibody (clone; BP53-12, Japan Tanner Co., Kobe, Japan) and Vectastain ABC kit (Vector Lab., Burlingame, Calif., USA). b Slight microinvasion was observed into the underlying dermis in case 6.

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Failure of Combination Therapy with Acitretin and Cyclosporin A in 3 Patients with Erythrodermic Psoriasis

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Key Words

Retinoids · Cyclosporin A · Erythroderma ■ Psoriasis

Psoriatic erythroderma is a serious condition which requires a fast response to therapy, preferably during an in-patient treatment. Therapeutic options include various topical treatments

and photo(chemo)-therapy, but in most cases systemic treatment is necessary [1]. Combination of cyclosporin A and methotrexate (MTX) resulted in serious adverse events after a short treatment period, and therefore is not recommended [2]. Combination of etretinate and MTX may lead to increased hepatotoxic risks, by increasing the MTX plasma levels [3,4]. Because of the different mechanisms of action and a different side effect profile combined treatment of cyclosporin A and retinoids might be successful.

Three patients with psoriatic erythroderma, who did not respond to monotherapy with acitretin in adequate dosages, were treated with combined therapy consisting of acitretin and cyclosporin A. They did not use co-medication known to interact with cyclosporin A metabolism or with nephrotoxic potentials. Serum creatinine level, liver enzymes, serum electrolyte levels, haematological parameters and blood pressure were measured before initiating combination treatment and once weekly during therapy. Clinical scores were evaluated using the Psoriasis Severity Score modified according to Perkins et al. [5].

The individual dosages, clinical efficacy and side effects of each patient are summarised in table 1.

Case 1

A 58-year-old man was admitted because of a biopsy-proven psoriatic erythroderma. He had been treated with acitretin in a dosage of 0.5 mg/kg/day during 12 weeks. Because of lack of clinical improvement, combination therapy was started with cyclosporin A in a dosage of 3 mg/kg/day. After 1 week of combined treatment, acitretin was decreased to 0.3 mg/kg/day because of a retinoid-induced dermatitis. After 6 weeks the serum creatinine level was elevated > 40%, and the serum cholesterol level and the blood pressure were raised. No clinical improvement was achieved during 6 weeks treatment. Both therapies were stopped and MTX was started at a dosage of 7.5 mg/week. After 2 weeks the blood pressure and the cholesterol level had normalised, the serum creatinine level normalised after 4 weeks. The erythroderma cleared in 5 months.

Case 2

An 83-year-old man with a history of psoriasis during 5 years was admitted with an erythrodermic psoriasis, which was treated subsequently with MTX during 2 weeks, prednisone during 2 weeks and acitretin 0.4 mg/kg/day during 3 weeks. These therapies were not successful. Cyclosporin A 3 mg/kg/day was combined with acitretin 0.4 mg/kg/day. After 4 weeks of combined treatment, only minimal clinical improvement was observed. The patient complained of dry lips and dry eyes. All serological parameters and the blood pressure were stable during therapy. Acitretin treatment was stopped and the cyclosporin A dose was raised to 5 mg/kg/day and combined with topical calcipotriol. This resulted in a good clinical improvement after 6 weeks.

Case 3

A 67-year-old man suffering from psoriasis for more than 40 years was admitted with an erythrodermic psoriasis. The psoriasis did not respond to local steroids or coal tar both in combination with acitretin 0.5 mg/kg/day during 8 months. Cyclosporin A (3 mg/kg/day) was started combined with acitretin 0.4 mg/kg/day. After 11 weeks of combination therapy the serum creatinine level was raised with 90% and the blood pressure had increased. The clinical response was unsatisfactory. Subsequently, both therapies were stopped and treatment with MTX 7.5 mg/week was initiated. However, 5 weeks later the patient had a cerebrovascular accident, complicated by fatal pulmonary embolism.

Considering the different modes of action and different side effect profiles, combination therapy of retinoids and cyclosporin A is a promising approach. However, because both retinoids and cyclosporin A are metabolised in the liver via a cytochrome P450-dependent system, a risk for interaction resulting in increased cyclosporin A levels is present. Recently, the effect of etretinate on the cyclosporin A metabolism was studied in an in vitro assay, using microsomes from normal human liver [6, 7]. With cyclosporin A levels of 5 μ M and etretinate levels of 100 μ M no metabolic interaction could be shown [6]. However, in a similar study with 100 μ M etretinate and cyclosporin A levels up to 30 μ M cyclosporin A metabolism was inhibited by 33-45% [7]. If this finding is of relevance in cyclosporin A treat-

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