Effect of Laser Resurfacing on p53 Expression in Photoaged Facial Skin

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BACKGROUND p53 overexpression has been reported in photoaged skin. Meanwhile, p53 gene mutations have been implicated as an important factor in the pathogenesis of ultraviolet (UV) light–induced skin cancer.

OBJECTIVE The objective was to evaluate the effect of laser resurfacing on the epidermal thickness and expression of p53 in photoaged skin.

METHODS Specimens were obtained from the facial skin of 10 patients before and after 3 months and 1 year of treatment using CO_2 (five cases) and erbium (Er):YAG (five cases) lasers. Specimens were also obtained from six age-matched controls. These biopsies were used for routine histopathology, histometry, and p53 immunoperoxidase staining.

RESULTS Both CO_2 and Er:YAG lasers were found to induce a significant decrease in p53 expression in biopsies obtained after 3 months ($p = .0004$ and .002, respectively) followed by gradual increase ($p = .01$ in both groups). A significant increase ($p < .01$) in epidermal thickness was also observed after 1 year of resurfacing. This increase, however, is inversely correlated with the level of p53 expression in such patients.

CONCLUSION The decrease in epidermal p53 expression after CO_2 and Er:YAG lasers may account for some of the benefits of resurfacing on the epidermis, as well as prevention of actinic neoplasia by adjusting any disturbance in the proliferation/apoptosis balance observed in photoaged facial skin.

The authors have indicated no significant interest with commercial supporters.

p53 is a nuclear phosphoprotein that serves as a tumor suppressor. In its natural form (wild-type) p53 can bind to DNA and prevent cells from entering the S (synthetic) phase of the cell cycle so as to allow time for DNA repair. Alternatively, p53-dependent events can eliminate the cells by sending them down an irreversible apoptotic pathway.[1] Thus, p53 allows the DNA to be either repaired or ultimately destroyed before replication renders the damage permanent.[2]

Ultraviolet (UV) radiation produces damage in DNA molecules,[3] and skin responds to such UV-induced DNA damage with a p53-dependent response.[2] Although the cells contain robust systems to repair DNA, cell replacement may be a preferred alternative to DNA repair, particularly when damage is extensive. Such replacement requires that the damaged cells first die by apoptosis and are then replaced by division of nearby functional cells ultimately derived from the stem cell pool.[4] Thus programmed cell death or apoptosis is an important cellular process that may play a critical role in cutaneous aging as well as in maintaining proliferative homeostasis within the skin.[5–8]

Laser resurfacing is now accepted as a valuable rejuvenating tool for facial photoaging.[9] A recent study, however, implying dermabrasion (mechanical

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 • Published by Blackwell Publishing •
ISSN: 1076-0512 • Dermatol Surg 2007;33:668–675 • DOI: 10.1111/j.1524-4725.2007.33141.x

resurfacing) for rejuvenation of facial skin showed decreased level of epidermal p53 expression that persisted for several months after treatment. It was suggested that these changes in p53 expression may mediate the rejuvenating effects of dermabrasion on the epidermis.[10]

This study is a pilot study to evaluate changes that occur in the expression of p53 in vivo, as a regulator of the process of apoptosis, after laser resurfacing used for management of photoaged facial skin.

Materials and Methods

This study has been conducted on 10 patients, Fitzpatrick skin types IV and V, attending the dermatology outpatient clinic of Al-Minya University Hospital, Al-Minya, Egypt, for treatment from signs of photoaging. Of these patients, 4 (40%) were men and 6 (60%) were women. The age of the patients ranged from 40 to 65 years with a mean age and standard deviation (SD) of 52.3 ± 7.79. Punch biopsies were taken from the facial skin before treatment and after 3 months and 1 year of laser therapy. Skin samples were also obtained from the facial skin of 6 age-matched control volunteers (3 men and 3 women) undergoing cosmetic and dermatosurgical procedures for other causes. The mean age of the control subjects was 53.4 ± 6.2 years. Skin biopsies were fixed in formalin (10%), embedded in paraffin, and sectioned into 5-μm sections. These sections have been used for routine histopathology (H&E), histometry, and for p53 immunohistochemical staining. An informed consent had been obtained from all patients and controls. The study was approved by the Committee for Postgraduate Studies and Research of Al-Minya University, and the study protocol conformed to the guidelines of the 1975 Declaration of Helsinki.

Laser Resurfacing

Prophylactic oral antibiotics and oral acyclovir were prescribed for 2 days before and 7 days after laser resurfacing. Oral acyclovir, however, was given for 2 weeks in patients with positive history of herpes simplex. The face was covered with a thin layer of local anesthesia (EMLA cream) for 1 hour before the procedure.

For CO_2 laser resurfacing, a short-pulsed CO_2 laser device (Deka, Smartoffice, Florence, Italy) was employed. The fluence ranged from 5 to 8 J/cm^2/pass. Two passes were used for mild to moderate wrinkles whereas three passes were needed for deeper wrinkles. For erbium (Er):YAG laser resurfacing, an Er:YAG laser device (Fotona, Ljabljana, Slovania) was employed. The fluence ranged from 3 to 5 J/cm^2/pass. Up to eight passes were sometimes performed until the rhytids have been effaced. After laser resurfacing, closed dressings with topical antibiotic have been used for all patients that were changed daily until complete healing. Sunscreens were recommended for all patients with instructions to avoid sun exposure.

Measuring Epidermal Thickness (Histometry)

The histologic measurements were carried out on standard H&E-stained sections. The mean thickness between the outermost surface of the epidermis, excluding the stratum corneum, and the dermoepidermal junction through the entire length of three examined sections were determined using computer image analysis (LEICA Q550, QWin, Cambridge, England).

Immunohistochemical Staining

The following protocol[10] has been used for immunohistochemical staining of p53: After overnight incubation at 37°C, tissue sections were deparaffinized in xylene and rehydrated in ascending grades of alcohol. Endogenous peroxidase activity was exhausted by incubation of tissue sections in 0.3% H_2O_2 for 30 minutes at room temperature. Tissue sections were then treated with retrieval solution (Cat. No. S1700, DAKO, Glostrup, Denmark). Twenty percent rabbit serum in Tris-buffered saline was used for blocking. The p53 monoclonal antibody (LSAB, Cat. No. S0809,

DAKO) in 1:200 dilution in 2% rabbit serum was used to stain p53. It was incubated with the samples overnight. The ready-to-use ultravision horseradish peroxidase/diaminobenzidine chromogen detection system (Cat. No. TP-015-HD, Lab Vision Corp., Fremont, CA) was used to demonstrate p53 expression. All tissue sections were stained under similar conditions to ensure equal staining quality. Squamous cell carcinoma was used as a positive control. In the negative control samples, the primary antibody was not added.

Scoring of p53 Immunoreactivity

The level of p53 expression is evaluated according to the scoring method that was set forth by Liang and colleagues.[2] Each p53 score represents the mean value of different fields from three sections of each specimen. p53 staining of the outer root sheath of hair follicles was evaluated by the same scoring system as the surrounding epidermis. This system evaluates the degree of positivity and intensity of staining in only those specimens demonstrating a dispersed pattern, that is, the "wild-type" of p53 expression. This system results in a score ranging from 0 to 3 for both the degree of positivity (percentage of positively stained nuclei of epidermal cells: 0, $<1\%$; 1, 1%–10%; 2, 10%–50%; 3, $>50\%$) and the degree of intensity of staining (the relative intensity of color of the positively stained nuclei from faint brown for score 1 to deep brown for score 3). The sum of the two scores is used as a representative of the level of p53 expression.

Statistical Analysis

Summary data are expressed as mean $\pm$ SD. Correlation coefficient test was used to test the correlation between p53 expression and epidermal thickness. The significance of the differences was also determined by Student's two-tailed t test. All p values were two-tailed, and differences were considered significant when the p value was less than or equal to .05.

Results

Histometric Evaluation of Epidermal Thickness

CO_2 Laser Resurfacing Evaluation of epidermal thickness of facial skin before and after treatment with CO_2 laser revealed increased epidermal thickness after treatment. The epidermal thickness significantly increased ($p = .02$) from a mean of $61 \pm 10.83\ \mu$m before treatment to a mean of $80 \pm 9.35\ \mu$m after 3 months and then showed a further minor increase ($p = .01$) to a mean of $82 \pm 10.37\ \mu$m in biopsies obtained 1 year after treatment (Table 1; Figure 1).

Er:YAG Laser Resurfacing The epidermal cell layers increased in number, which was reflected as a mod-

TABLE 1. Score of p53 and Epidermal Thickness in Facial Skin Samples Obtained before Treatment and 3 Months and 1 Year after CO_2 Laser Therapy*

			p53 score			*Epidermal thickness (μm)*		
Case	*Sex*	*Age (years)*	*Before treatment*	*After 3 months*	*After 1 year*	*Before treatment*	*After 3 months*	*After 1 year*
1	Male	50	5	0	0	65	70	80
2	Male	60	5	2	3	50	75	75
3	Female	50	5	2	3	50	75	70
4	Male	65	5	3	4	75	90	95
5	Female	40	4	2	3	65	90	90
Mean		53	4.8	1.8	2.6	61	80	82
SD		9.75	0.45	1.10	1.52	10.83	9.35	10.37

*p53, $p = .0004$ after 3 months and .01 after 1 year; epidermal thickness, $p = .02$ after 3 months and .01 after 1 year. Correlation between p53 expression and epidermal thickness, $r = 0.36$ after 3 months and 0.08 after 1 year.

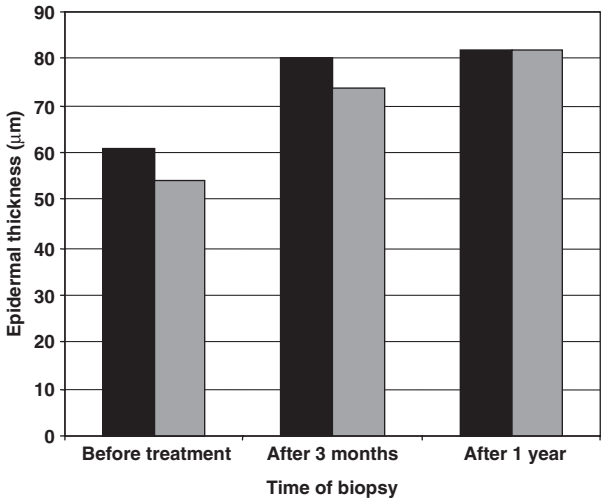

Figure 1. Epidermal thickness (mean value) before and after CO_2 (■) and Er:YAG (▩) laser resurfacing.

est, although insignificant ($p = .06$) increase in the mean viable epidermal thickness from 54 ± 9.61 μm before treatment to 74 ± 12.44 μm within 3 months after treatment. Further significant ($p = .01$) increase in epidermal thickness to 82 ± 10.37 μm, however, was observed in biopsies obtained 1 year after treatment (Table 2; Figure 1). The mean epidermal thickness observed after Er:YAG laser resurfacing was less than that observed after CO_2 laser resurfacing in biopsies obtained 3 months after treatment, but the mean epidermal thickness was equal in the two groups in biopsies obtained 1 year after treatment (Figure 1). This difference, however, was statistically insignificant ($p = .4$ and 1 after 3 months and 1 year, respectively).

Evaluation of p53 Expression

CO_2 Laser Resurfacing Immunohistochemical staining for p53 in the epidermis of facial skin revealed a dispersed pattern of staining in all patients and controls. The score of p53 expression initially showed a significant decrease from a mean of 4.8 ± 0.45 before treatment to a mean of 1.8 ± 1.10 in biopsies obtained after 3 months ($p = .0004$). This is followed by insignificant increase in the score of expression after 1 year to a mean of 2.6 ± 1.52 ($p = .4$). This score, however, was still significantly lower than the pretreatment level ($p = .01$; Table 1; Figures 2 and 3). The level of p53 expression after CO_2 laser resurfacing is inversely correlated with epidermal thickness ($r = 0.36$ and 0.08 after 3 months and 1 year, respectively).

Er:YAG Laser Resurfacing The pattern of p53 expression in facial skin before and after treatment with Er:YAG laser was similar to that after CO_2 laser resurfacing. Evaluation of p53 expression in facial skin before (4.6 ± 0.55) and after treatment with Er:YAG laser revealed a significant ($p = .002$) decrease in the level of expression in biopsies obtained after 3 months (2.4 ± 0.89), and the expression remained significantly ($p = .01$) lower after 1 year (3.2 ± 0.84) of treatment, although of its modest increase than specimens obtained after

TABLE 2. Score of p53 and Epidermal Thickness in Facial Skin Samples Obtained before Treatment and 3 Months and 1 Year after Er:YAG Laser Therapy*

			p53 score			*Epidermal thickness (μm)*		
Case	*Sex*	*Age (years)*	*Before*	*After 3 months*	*After 1 year*	*Before*	*After 3 months*	*After 1 year*
1	Female	43	4	2	2	55	75	80
2	Female	50	5	2	4	70	75	80
3	Male	50	5	2	3	50	55	75
4	Female	55	4	2	3	45	75	75
5	Female	60	5	4	4	50	90	100
Mean		51.6	4.6	2.4	3.2	54	74	82
SD		6.34	0.55	0.89	0.84	9.61	12.44	10.37

*p53, $p = .001$ after 3 months and .01 after 1 year; epidermal thickness, $p = .06$ after 3 months and .01 after 1 year. Correlation between p53 expression and epidermal thickness, $r = 0.82$ after 3 months and 0.65 after 1 year.

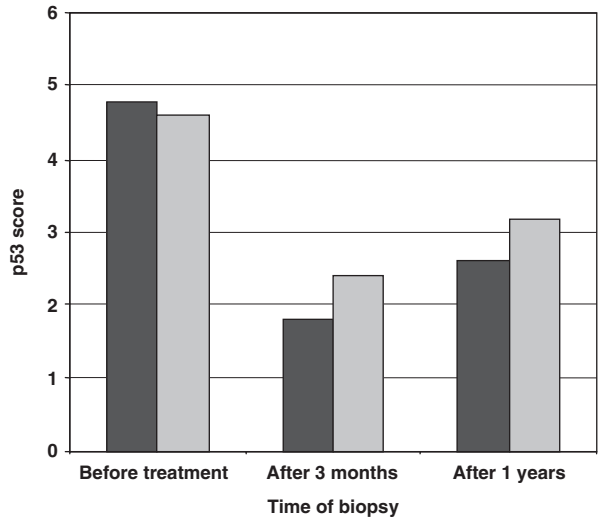

Figure 2. p53 expression (mean value) before and after CO_2 (■) and Er:YAG (▩) laser resurfacing.

3 months (Table 2; Figures 2 and 4). The decreased p53 expression observed after Er:YAG laser resurfacing was less than that observed after CO2 laser resurfacing. This difference, however, was statistically insignificant ($p = .2$ and $.5$ after 3 months and 1 year, respectively). The level of p53 expression after Er:YAG laser resurfacing is inversely correlated with epidermal thickness ($r = 0.82$ and 0.65 after 3 months and 1 year, respectively).

Control Cases

Histometric Evaluation of Epidermal Thickness No significant difference ($p > .05$) was encountered regarding epidermal thickness in facial skin of patients before treatment ($58.5 \pm 11.56\ \mu$m) when compared to their age-matched controls ($58.2 \pm 3.2\ \mu$m).

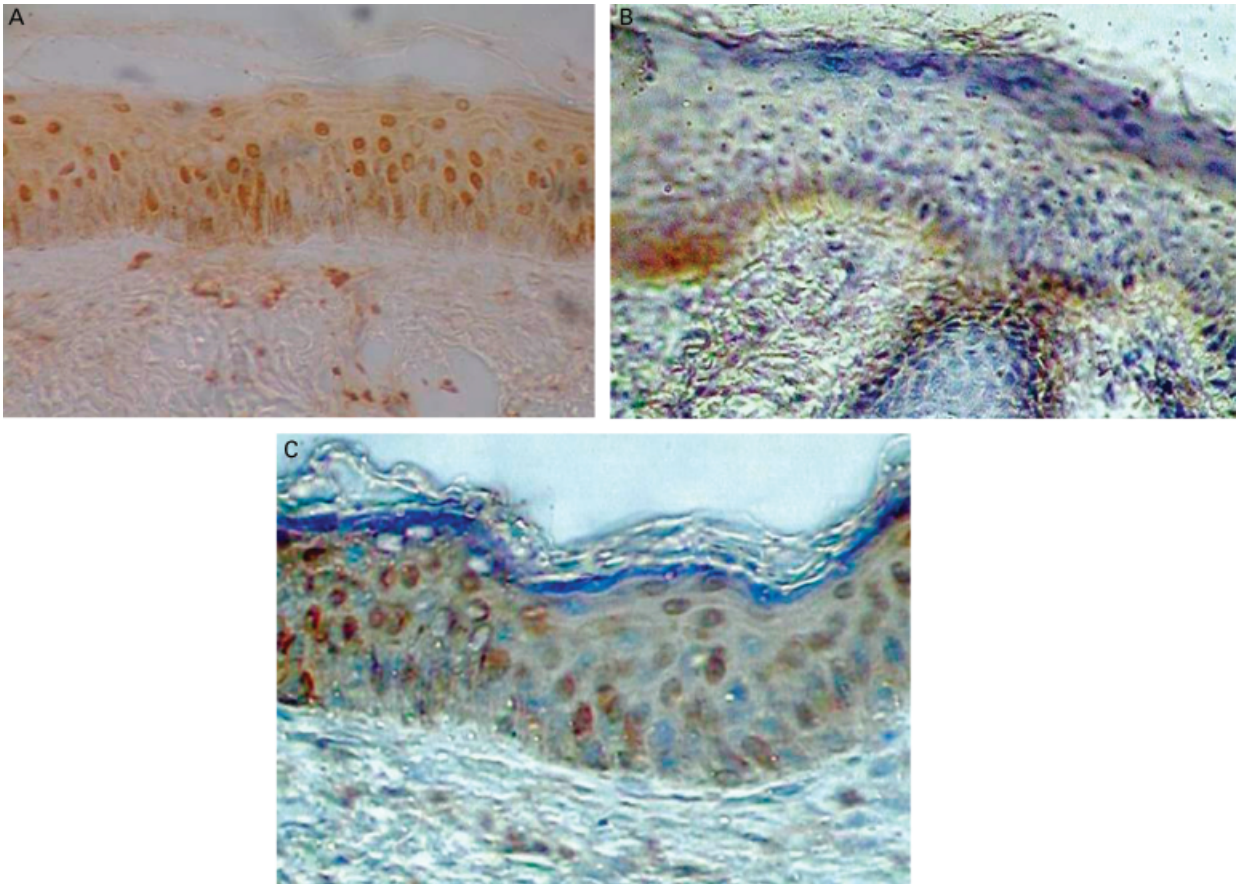

Figure 3. Dispersed nuclear expression of p53 in facial skin before (A), 3 months after (B), and 1 year after (C) CO_2 laser resurfacing (Case 2; Table 1). Immunoperoxidase; original magnification $\times 200$.

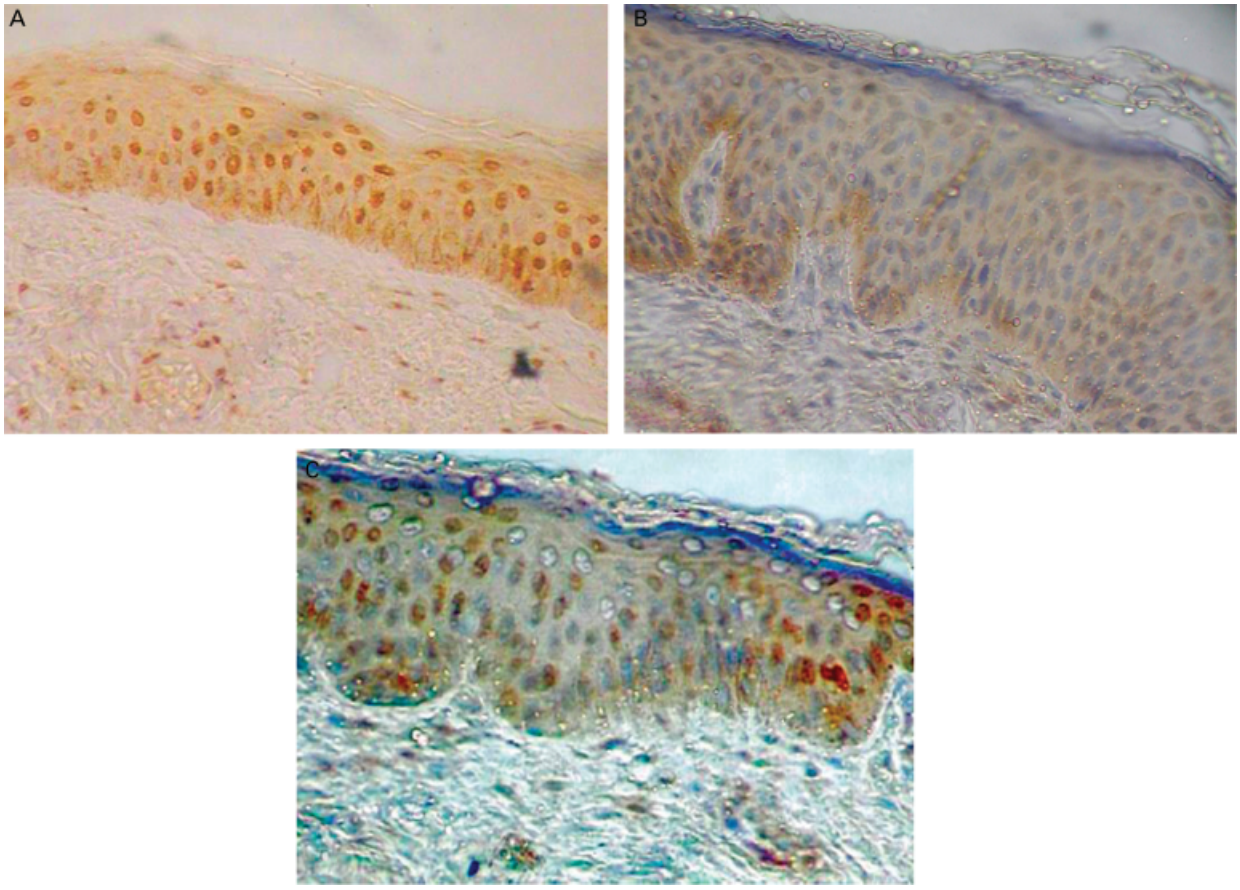

Figure 4. Dispersed nuclear expression of p53 in facial skin before (A), 3 months after (B), and 1 year after (C) Er:YAG laser resurfacing (Case 3; Table 2). Immunoperoxidase; original magnification, $\times 200$.

Evaluation of p53 Expression No significant difference ($p > .05$) was encountered regarding p53 expression in facial skin of patients before treatment (4.7 ± 0.48) when compared to controls (4.3 ± 0.3).

Discussion

Maintenance of skin homeostasis requires a delicate balance among proliferation, differentiation, and apoptosis.[6,7] The p53 gene, located on the short arm of chromosome 17, acts as a tumor suppressor gene.[11] The p53 protein product is a 393-amino-acid phosphoprotein which localizes to the nucleus. There is increasing evidence that mutations of the p53 gene are among the most common genetic alterations in human malignancies,[11] and they have been implicated as an important factor in the pathogenesis of UV light–induced skin cancer.[12] Because cellular death by apoptosis plays an important role in the process of cutaneous aging,[5–8] disturbance of p53 function as a regulator of apoptosis may also play an important role in this process.[10]

In this study we evaluate the effect of CO_2 and Er:YAG laser resurfacing on the expression of p53 in vivo to clarify one of the possible mechanisms through which these modalities could act. To the best of our knowledge no previous publications have reported the effect of these therapeutic modalities on the expression of p53 in the skin.

Evaluation of p53 expression in facial skin in both groups revealed a significant decrease in the level of expression in early biopsies obtained 3 months after

treatment. Meanwhile, late biopsies obtained after 1 year of treatment revealed a modest but insignificant increase in the level of p53 expression. The score of p53 expression, however, was still significantly lower than the pretreatment level. This suggests that both CO_2 and Er:YAG lasers result in epidermal changes that may persist for several months after treatment.

p53 accumulation in a dispersed pattern has been reported in chronically sun-exposed skin[13,14] and was found to increase in an age-dependent pattern that may reflect a cumulative insult to DNA of epidermal cells.[14] It has been also noted that such accumulation of p53 in photoaged skin is partially related to altered keratinocyte differentiation.[11,14] Thus, changes in the level of p53 expression may have a potential role in prevention of actinic neoplasia by adjusting alteration in proliferation/apoptosis balance normally observed in photoaged facial skin.[10,14]

A previous study employing dermabrasion for rejuvenating the photodamaged facial skin showed that dermabrasion causes a significant decrease in the level of expression of p53 in biopsies obtained after 3 weeks followed by a significant increase in biopsies obtained after 3 months of treatment, although it was still significantly lower than the pretreatment level.[10] In this study, the expression of p53 in the epidermis remained significantly lower than the pretreatment level for up to 12 months after treatment in both CO_2 and Er:YAG-treated groups. This suggests that the rejuvenating effect of both CO_2 and Er:YAG lasers on the epidermis may persist for longer time than dermabrasion.

In this study, both CO_2 and Er:YAG lasers were found to induce significant increase in epidermal thickness with a striking development of the rete ridges and normalization of the overall morphology of the epidermis, suggesting marked increase in the activity of keratinocytes and revitalization of the epidermis. Such epidermal changes induced by both CO_2 and Er:YAG lasers persisted for up to 12 months after treatment. The increased epidermal thickness after both CO_2 and Er:YAG laser resurfacing is inversely correlated with the level of p53 expression. The decreased level of p53 expression with its apoptotic effect may explain such an increase in epidermal thickness after CO_2 and Er:YAG laser resurfacing. The epidermal thickness observed after 3 months of Er:YAG laser resurfacing was less ($p > .05$) than that observed after CO_2 laser resurfacing; however, they became equal after 1 year.

A previous study employing dermabrasion for treatment of photodamaged facial skin showed that dermabrasion causes a significant increase in epidermal thickness measurements in biopsies obtained after complete epithelialization. Biopsies obtained 3 months after dermabrasion, however, revealed tendency of the epidermal changes to return back to the pretreatment state.[15] This may suggest that both CO_2 and Er:YAG lasers have a more persistent rejuvenating effect on the epidermis than dermabrasion.

In conclusion, modalities that are currently used in the treatment of aged facial skin such as CO_2 and Er:YAG lasers affect the level of expression of p53. This may play a role in mediating the effects of such modalities on the epidermis as well as prevention of actinic neoplasia by adjusting any disturbance in the proliferation/apoptosis balance observed in photoaged skin. Future studies are mandatory to confirm these findings and should address other apoptosis regulatory proteins that might be affected by laser resurfacing.

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