



Analysis of STAT3 Activation and Cytokine Production in Skin Cancer Patients

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Abstract

Skin cancer is a disease marked by the presence of abnormal cells within the skin tissues. These cells grow in a disorganized manner and reproduce uncontrollably at a rapid pace. Various factors contribute to the onset and progression of skin cancer; these can be genetic and environmental, psychological, microbiological, and immunological, such as inflammation and a weakened immune system. Signal transducers and activators of transcription (STATs) are a family of latent transcription factors activated in response to various cytokines and growth factors. This study aims to identify the association of STAT3 activation with pro-inflammatory cytokines such as IL-1 β , IL-6, and TNF- α . The phospho-STAT3/STAT3 ratio indicates significant activation levels of this transcription factor in skin cancer patients versus the control group. Also, we encountered higher serum concentrations of IL-1 β , IL-6, and TNF- α compared to the control group. Although our analysis did not show a correlation between STAT3 activation and cytokines, elevated levels of activated STAT3 offer new insights into the practical application of STAT3, which could help develop more effective treatments for this malignancy. In addition, the fact that patients show a higher serum concentration of cytokines highlights their key role in the pathophysiology of skin cancer.

Introduction

Skin cancer is a disease marked by the presence of abnormal cells within the skin tissues. These cells grow in a disorganized manner and reproduce uncontrollably at a rapid pace. Skin cancer is categorized into two major groups: melanoma and non-melanoma skin cancer (NMSC). Non-melanoma skin cancer is further divided into basal cell carcinoma (BCC) and squamous cell carcinoma (SCC)¹. Globally, there are 2 to 3 million cases of non-melanoma skin cancer and 132,000 cases of melanoma reported annually. The occurrence of skin cancer has risen in recent decades². Several factors aid in the onset and progression of skin cancer, including genetic (white race and history of skin cancer), psychological factors (personality and depression), environmental (ultraviolet radiation, smoking, and exposure to certain chemicals), microbiological and immunological, such as inflammation and a weakened immune system³⁻⁵.

Signal transducers and activators of transcription (STATs) are a family of latent transcription factors activated in response to various cytokines and growth factors. Inhibition of STAT3 activity in tumor-derived cell lines has been linked to growth arrest and apoptosis. Consequently, targeting STAT3 signaling pathways has

become a therapeutic strategy in pre-clinical skin cancer models ⁶. However, the number of studies examining their involvement in patients with the disease remains limited.

When activated, STAT3 promotes the expression of pro-inflammatory cytokines such as Interleukin-1 β (IL-1 β), Interleukin-6 (IL-6), and Tumor Necrosis Factor Alpha (TNF- α), creating a feedback loop that can sustain and amplify inflammation. This mechanism is fundamental in the context of chronic inflammatory diseases ⁷. Pro-inflammatory cytokines exert a critical role in the growth and progression of skin cancer, generating a microenvironment that supports the proliferation of cancer cells, inhibits apoptosis, and contributes to angiogenesis, which supplies the tumor with nutrients and oxygen ⁸. Additionally, pro-inflammatory cytokines can suppress the immune response against tumor cells, facilitate tissue remodeling, and promote metastasis, enabling cancer to disseminate more easily to other body parts ⁹.

This study aims to determine the relationship between STAT3 activation and pro-inflammatory cytokines, including IL-1 β , IL-6, and TNF- α .

Subjects and Methods

Study design and patient selection

Fifty-one adult patients diagnosed clinically and histopathologically with skin cancer were included, with the support of the Dermatological Institute of Jalisco Dr. José Barba Rubio, certified by the Mexican Council of Dermatology and the International Committee of Dermatopathology. 74.5% of the patients presented BCC, followed by SCC (19.6%); melanoma and mixed carcinoma were less frequent, with 3.9% and 2%, respectively. The control group was composed of fifty patients, most of whom presented keratosis or eczema. Exclusion criteria were pregnancy, history of chronic inflammatory or autoimmune diseases, active infectious diseases, kidney disease, diabetes, or use of immunosuppressive drugs. The study was developed according to the Helsinki Declaration World Medical Association guidelines. Each subject provided written informed consent with the approval of the Clinic department and institutional bioethics committee at Guadalajara University (Record No. 6/2017-2018) ¹⁰.

STAT3 activation

Extracts were prepared from biopsy homogenates by homogenizing 30 mg of frozen tissue, which was first minced and thoroughly rinsed in PBS to remove blood. The tissue was then resuspended in 500 μ L to 1 mL of chilled 1X Cell Extraction Buffer PTR. The samples were incubated on ice for 20 minutes, followed by centrifugation at 18,000 \times g for 20 minutes at 4°C. The supernatants were then transferred into clean tubes, discarding the pellets, and stored at -80°C. The protein concentration in the extracts was quantified

using a BCA protein assay from Bioscience (Catalog #: P011), measuring a standard curve and protein extracts at 562 nm with a microplate reader (Multiskan GO, Thermo Scientific).

Total STAT3 protein and STAT3 phosphorylation (p-Y705) were measured using the ab176655 and ab176654 ELISAs (Abcam). These assays were conducted according to the manufacturer's instructions, utilizing lysates containing 200 μ g/ml of total protein. A standard curve was created for each assay, using serial dilutions of the control lysates. The absorbance was then measured at 450 nm with a microplate reader (Multiskan GO, Thermo Scientific).

Cytokine concentrations

Blood samples (6 mL) were collected from all study participants using vacuum tubes (Vacutainer-Becton Dickinson) and allowed to clot for 1 hour at 37°C. Following centrifugation at 1,000 \times g for 10 minutes at 4°C, the serum was stored at -80°C until use. Serum concentrations of IL-1 β (Catalog #: DY201), IL-6 (Catalog #: DY206), and TNF- α (Catalog #: DY210) were determined using DuoSet ELISA kits (R&D Systems) according to the manufacturer's instructions. Absorbance for the standard curve and serum samples was measured at 450 nm with a microplate reader (Multiskan GO, Thermo Scientific).

Statistical analysis

Descriptive statistics were estimated for sociodemographic data, and distribution analysis was performed using the chi-square test. Differences in STAT3 activation and cytokine concentrations between the control and skin cancer groups were analyzed using the Student's t-test. Correlations were determined using Pearson's r coefficients. Data analysis was achieved using SPSS Statistics software version 22, with a statistically significant of p-value < 0.05 difference.

Results

General characteristics of the study population

The skin cancer group consisted of fifty-one patients of both genders (mean age of 67.30 years, SD \pm 13.20; 51.20% female). Most were married (70.50%) and not employed outside the home (51%). Concerning the Fitzpatrick skin phototype scale (FST), skin cancer patients mainly exhibited phototypes II and III (92.70%). Additionally, the control group consisted of fifty volunteers of both genders without skin cancer. No differences in age, gender, marital status, occupation, or phototype were found, allowing us to rule out these characteristics as influencing factors in the subsequent analyses (Table 1).

Tissue levels of phospho-STAT3/STAT3

The phosphorylation level of STAT3 was assessed and normalized with the total STAT3 protein level. The phospho-STAT3/STAT3 ratio indicates significant levels of activation

Table 1. General characteristics of the study population

Characteristics	Control group n (%)	Skin cancer patients n (%)	p-value
Age (mean ± SD)	59.90 ± 13.40	67.30 ± 13.20	n.s. ^a
Gender			
Female	27 (54.00)	29 (51.20)	n.s. ^b
Male	23 (46.00)	22 (48.80)	
Marital status			
Single	11 (22.00)	8 (15.70)	n.s. ^b
Married	39 (78.00)	36 (70.50)	
Divorced	0 (0.00)	1 (2.00)	
Widowed	0 (0.00)	6 (11.80)	
Occupation			
Farm workers	14 (28.00)	14 (27.50)	n.s. ^b
Home maker	23 (46.00)	26 (51.00)	
Retired	10 (20.00)	8 (15.70)	
Other	3 (6.00)	3 (5.80)	
Fitzpatrick Phototypes			
I	10 (20.00)	3 (5.80)	n.s. ^b
II	25 (50.00)	24 (47.10)	
III	15 (30.00)	23 (45.10)	
IV	0 (0.00)	1 (2.00)	

^aStudent's t-test; ^bchi-square test; n.s.= not statistically significant.

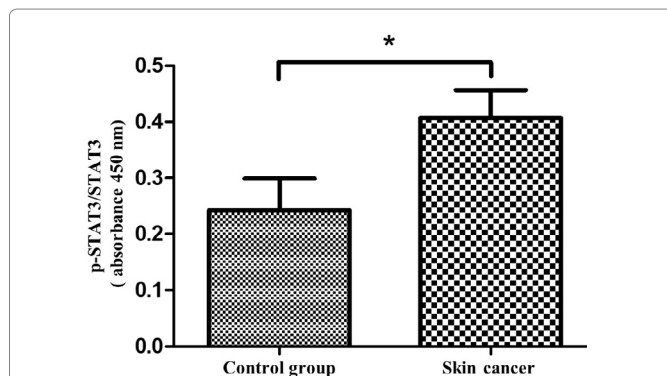


Figure 1. THE PHOSPHORYLATION LEVEL OF STAT3 NORMALIZED BY TOTAL STAT3. Data represent 450 nm Absorbance mean ± SD; Student's t-test is significant at *p-value < 0.05.

of this transcription factor in skin cancer patients (mean 450 nm absorbance ± standard error = 0.41 ± .050) versus the control group (mean 450 nm absorbance ± standard error = 0.24 ± 0.06) (p-value = 0.04) (Figure 1).

Serum concentrations of IL-1 β, IL-6, and TNF-α

Skin cancer patients tend to have higher concentrations of the pro-inflammatory cytokines compared to the control group, the means and standard errors were as follows: IL-1β (28.78 ± 8.98 versus 4.80 ± 0.62 pg/mL; p-value = 0.01), IL-6 (160.72 ± 27.66 versus 97.17 ± 35.85 pg/mL; p-value = 0.18), and TNF-α (67.15 ± 20.33 versus 16.56 ± 4.68 pg/mL; p-value = 0.02) (Figure 2).

Correlations between STAT3 activation and Cytokine concentrations

No correlations between *phospho-STAT3/STAT3* and

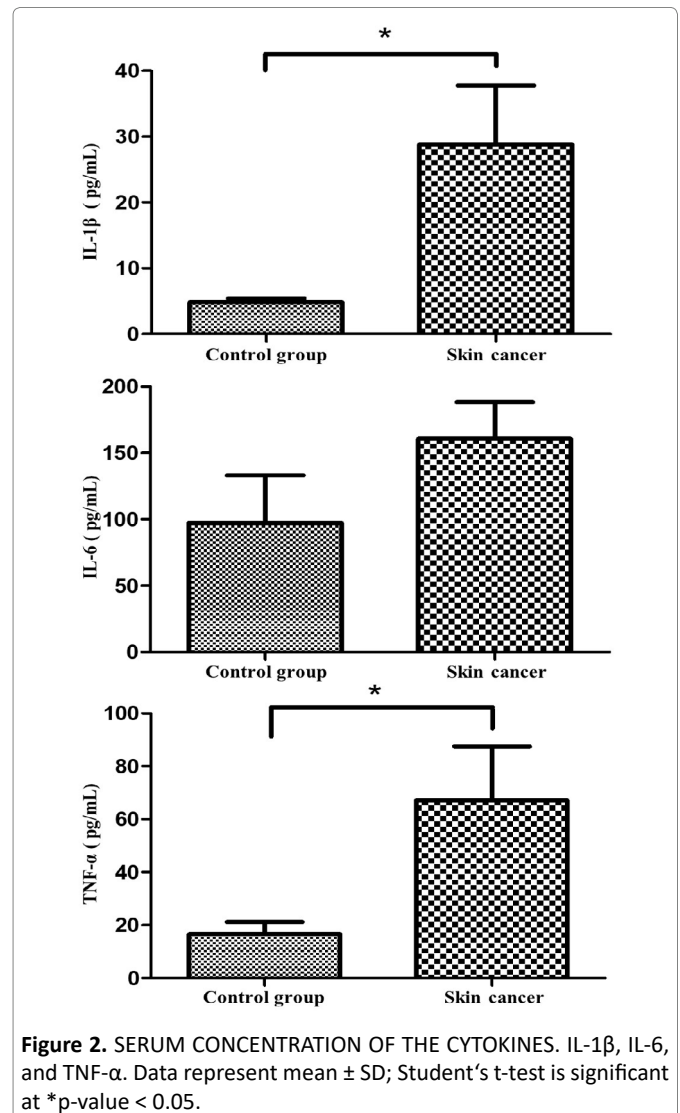


Figure 2. SERUM CONCENTRATION OF THE CYTOKINES. IL-1β, IL-6, and TNF-α. Data represent mean ± SD; Student's t-test is significant at *p-value < 0.05.

Table 2. Correlations for measures of STAT3 activation and cytokine concentrations

Measure	p-STAT3/STAT3	IL-1 β	IL-6	TNF-α
Control group				
<i>phospho-STAT3/STAT3</i>		-0.45 ^a	-0.24 ^a	-0.19 ^a
IL-1 β	1 ^a	1 ^a	0.77**^a	0.15 ^a
IL-6			1 ^a	-0.05 ^a
TNF-α				1 ^a
Skin cancer patients				
<i>phospho-STAT3/STAT3</i>		0.10 ^a	0.04 ^a	0.21 ^a
IL-1 β	1 ^a	1 ^a	0.74***^a	0.75***^a
IL-6			1 ^a	0.40***^a
TNF-α				1 ^a

^aPearson's r correlation coefficients. Correlation is significant at *p-value < 0.05, **p-value < 0.01, and ***p-value < 0.001.

cytokine concentrations were observed. How it was expected, these pro-inflammatory cytokines were correlated mainly in the skin cancer group: IL-1β versus IL-6 (r = 0.74, p-value < 0.001), IL-1β versus TNF-α (r = 0.75, p-value < 0.001) and IL-6 versus TNF-α (r = 0.40, p-value < 0.001) (Table 2).

Discussion

The frequency of cases of non-melanoma skin cancer was higher than that of melanoma, with a major prevalence of BCC than CEC, which corresponds to the epidemiology of the disease¹¹. The absence of differences in age, gender, marital status, occupation, or phototype between patients and the control group suggests that other factors, such as the degree of solar exposure, may be involved. Solar exposure is one of the most impactful elements on human skin and is particularly crucial regarding sensitivity to UV-induced DNA damage¹²⁻¹⁴.

One of the most significant observations reported in a previous study indicates that mice deficient in STAT3 were resistant to skin tumor development¹⁵. Several studies have confirmed increased expression of STAT3 in NMSC across all histopathological subtypes of BCC, comparing the expression within the adjacent epidermis to that within the epidermis of a healthy control group^{16,17}. Similarly, our results demonstrate higher levels of STAT3 activation in patients compared to the non-skin cancer group.

The role of STAT3 in inflammation and immunity, along with its involvement in various diseases such as genitourinary, gastrointestinal, lung, ovarian, and brain tumors, makes it an ideal candidate for therapeutic strategies¹⁸⁻²⁰. When we compared the cytokine levels of patients to the control group, skin cancer patients tended to have higher concentrations of the pro-inflammatory cytokines IL-1 β , IL-6, and TNF- α . Elevated serum levels of TNF- α are positively correlated in ovarian, colorectal, and lung cancer patients and enhance the risk of tumor progression and recurrence²¹. IL-6 and TNF- α have also been involved in tumor promotion in skin cancer. The soluble receptor activator of NF- κ B and IL-1 is also proposed to be useful in predicting the overall outcome for patients with advanced BCCs^{22,23}. Moreover, high levels of IL-6 have been associated with severity and worse prognosis in cancer patients; this relationship seems to be conditioned to the stage in which the patient is^{24,25}. The downstream IL-6/STAT3 axis is also associated with skin cancer, where tumor-derived IL-1 β specifically induces STAT3 activation that creates a tumor-autoinflammatory loop that amplifies IL-6 signaling in human melanoma cells²⁶. In contrast, we did not observe a connection between local activation levels in tissue and serum cytokine concentrations in skin cancer patients. Among the factors to be considered that may be influencing this connection are the type of skin cancer, disease stage, patient treatment history, or individual variability. Also, this does not rule out the possibility that the mechanism operates primarily within the tumor microenvironment.

On the other hand, the correlation between the cytokines IL-1 β and IL-6 is because the presence of IL-1 β

induces the expression of IL-6, which, in turn, is associated with tumor progression^{27,28}. It has been described in several investigations since TNF- α can trigger a signaling pathway for gene transcription of cytokines such as IL-1 β or IL-6 and vice versa²⁹. The relationship between these cytokines was also identified in regulating metabolic function, which could explain why a positive correlation was observed in the control group³⁰.

Conclusion

Although our analysis did not show a correlation between STAT3 activation and cytokines, elevated levels of activated STAT3 offer new insights into the practical application of STAT3, which could help develop more effective treatments for this malignancy. Furthermore, patients' higher serum concentrations of cytokines highlight their fundamental role in the pathophysiology of skin cancer. Limitations of this study include its cross-sectional design and the relatively small sample size. Future research with a more robust cohort could offer additional insights by ensuring that the findings are more generalizable across different populations. It could also facilitate subgroup analyses, such as examining variations based on demographic factors like age, gender, or underlying health conditions.

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Conflict of Interest

The authors declare that they have no competing interests.

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