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Expression of telomerase reverse transcriptase in psoriatic lesional skin

Moushira A. Mahmoud[a,*], Roshdy W. Mohamed[b], Fadia M. Attia[c], Mona A. Atwa[b] and Samir M. Abdel-moneim[a]
[a]*Departments of Biochemistry, Suez Canal University, Ismailia, Egypt*
[b]*Dermatology and Andrology, Suez Canal University, Ismailia, Egypt*
[c]*Clinical Pathology, Suez Canal University, Ismailia, Egypt*

Abstract. *Background:* Little information is known about telomerase expression in the chronic benign hyperproliferative skin disease, psoriasis. Further studies are still required to investigate its usefulness as a biomarker of this skin disorder. *Aim:* To investigate the expression of human telomerase reverse transcriptase (hTERT) in psoriatic lesional skin and its relation to disease severity. *Methods:* The levels of hTERT-mRNA were quantified using real time RT-PCR in lesional versus nonlesional skin specimens from 24 psoriatic patients. *Results:* The expression of hTERT was detected in 16 psoriatic lesional specimens (66.7%), but in none of the normal skin. There was no relation between hTERT expression level and age of the patient or the duration of the disease. Among hTERT-positive patients, a significant positive correlation was observed between hTERT-mRNA levels and both the Psoriasis Area-and-Severity Index (PASI) and scaling scores ($p = 0.012$ & $p = 0.006$, respectively). *Conclusion:* Telomerase mRNA is detectable in lesional skin of most psoriatic patients and correlates with the severity of the disease and the rate of epidermal proliferation.

Keywords: Human telomerase reverse transcriptase, psoriasis, PASI score, scaling score

1. Introduction

Telomerase is a ribonucleoprotein complex located within the nucleus. It functions as a cellular reverse transcriptase to synthesize the repetitive nucleotide sequence (TTAGGG in humans) forming the telomeres at the end of chromosomes [10]. Telomeres are important for protecting the encoding DNA sequences from damage. They protect chromosomal ends from nucleolytic degradation, end to end fusions, rearrangement and ensure the proper segregation of chromosomes [12]. Without telomerase activity, each round of cellular division results in the shortening of telomeres and reaching a critical length leads to replicative senescence and a finite lifespan. Thus, telomerase appears to stabilize telomeres, leading to cellular immortality [3,6,16]. Human telomerase enzyme consists of two essential components; a protein subunit named Human Telomerase Reverse Transcriptase (hTERT) that performs the catalytic activity and a nucleotide sequence termed Human Telomerase RNA (hTR) that serves as an intrinsic template for telomeric repeat synthesis [22]. The enzyme is expressed by most malignant cells and is normally inactive in most somatic cells, with the exception of proliferative stem cells, germline cells, and activated lymphocytes. The activation of telomerase in malignant cells seems to be an important step in tumorigenesis to gain the ability of indefinite proliferation and to become immortal [9,19]. Accordingly, telomerase activity was employed to assess the proliferative capacity

*Corresponding author: Moushira Abdel-Wahab Mahmoud, Department of Biochemistry, Faculty of Medicine, Suez Canal University, Ismailia, Egypt. Tel.: +20 10 3179695; Fax: +20 64 3216496; E-mail: moushiraa@yahoo.com.

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of malignant disorders in many tissues [30] including skin [8] and was considered to be a potential marker of disease activity. Further studies revealed that this marker was also correlated with cell division and proliferation in a group of potentially malignant skin conditions like; venereal warts [27], oral lichen planus [20], and actinic-damaged skin [26]. The expression of hTR is not a predictor of telomerase activity, because hTR is expressed in all cells [2,15]. Human TERTs are large proteins (103–134 kDa) and its mRNA is expressed almost exclusively in malignant tumors as well as in stem and germ cells and correlates closely with the detection of telomerase activity [1,23,25].

Psoriasis is a common benign chronic skin disease that is characterized by hyperproliferation and abnormal differentiation of keratinocytes, as well as by infiltration of activated T cells in the epidermis and papillary dermis. In 2001, Jang et al. [14] reported the detection of telomerase activity in psoriatic lesional skin, and up to our knowledge no further work was published to confirm or further investigate their finding. In this study, we used the real-time RT-PCR technique to measure quantitatively hTERT-mRNA in psoriatic lesional skin and correlate it with clinical disease severity.

2. Methods

2.1. Patients and sample collection

Twenty-four patients of psoriasis vulgaris were included in the study that was conducted in Suez Canal University hospital. Full clinical history and examination were done for each patient and disease severity was assessed by PASI score. In addition, the average value for the scaling domain of PASI score, as determined for different body sites, was calculated for each patient and separately considered to represent the degree of epidermal proliferation in lesional skin. None of the patients received systemic therapy before the collection of skin samples. Informed consents were obtained from all patients after explanation of the study objectives and methods used.

A lesional skin specimen, located on sun-protected area, was taken from each patient by a 4-mm punch biopsy. Another specimen was taken from normal sun-protected skin that was at least 10 cm apart from any psoriatic lesion. Specimens were immediately snap frozen in liquid nitrogen, and stored at $-80\,^{\circ}$C until further processing.

2.2. Quantitative real-time RT-PCR for expression of hTERT

For the total RNA isolation from skin specimens, a High Pure RNA Tissue Kit (Roche Diagnostics, Mannheim, Germany) was used, following the manufacturer's recommendations.

Quantitative detection of hTERT mRNA was performed with the commercially available LightCycler Telo TAGGG hTERT Quantification Kit® (Roche Diagnostics, Mannheim, Germany) using the LightCycler® instrument (Roche Molecular Systems, Alameda, CA) for real-time PCR. All steps were performed according to the manufacturer's instructions. Briefly, hTERT encoding mRNA was reverse-transcribed (10 min at 60°C), followed by denaturation of the RNA/ DNA complex (30 sec at 95°C) and amplification of a 198 bp fragment of the generated cDNA in 40 PCR cycles (0.5 sec at 95°C; 10 sec at 60°C; 10 sec at 72°C) with specific primers in a one-step RT-PCR reaction. The amplicon was detected by fluorescence using a specific pair of hybridization probes that hybridize to an internal sequence of the amplified fragment during the annealing phase of the PCR cycles. One probe is labeled at the 5'-end with LightCycler-Red 640, and, to avoid extension, modified at the 3' end by phosphorylation. During fluorescence resonance energy transfer (FRET), fluorescein is excited by the light source of the LightCycler instrument, and part of the excitation energy is transferred to the LightCycler-Red 640. The emitted fluorescence of the LightCycler-Red 640 is then measured by the LightCycler instrument. The same RT-PCR for mRNA encoding the housekeeping gene for porphobilinogen deaminase (PBGD) was performed in separate tubes. The reaction product served as a control for RT-PCR and as a reference for relative quantification. To provide a positive control and establish an external standard curve, all measurements included the determination of 5 standards with *in vitro* transcribed hTERT mRNA containing 10^6, 10^5, 10^4, 10^3 and 10^2 copies/2 μl as well as total RNA purified from a hTERT mRNA-expressing cell line supplied by the detection kit. The cycle numbers of the logarithmic linear phase were plotted against the logarithm of concentration of hTERT mRNA. By comparing the crossing line intercept of an unknown sample with the standard curve, a quantitative estimate of the starting copy number of hTERT mRNA (as well as PBGD mRNA) was calculated. Total RNA was used as positive control for detection of PBGD mRNA. Probes without template that otherwise fulfilled the same re-

Table 1
Patient characteristics[1]

	Age (yr)	Disease duration (yr)	PASI score	Scaling score
Mean ± SD	45.4 ± 20.9	7.8 ± 7.3	12.3 ± 8.4	2.3 ± 0.7
(Range)	(5–75)	(0.5–25)	(0.6–29.4)	(1–3)

[1] All patients are males.

Table 2
Comparison between hTERT mRNA-positive and hTERT mRNA-negative psoriatic patients

	hTERT- positive	hTERT-negative	P-value
Patients' age (yr)	46.1 ± 23.6 (5–75)	44.0 ± 15.5 (19–70)	0.82
Disease duration (yr)	7.3 ± 7.4 (0.5–25)	8.6–7.5 (2–20)	0.69
PASI score	13.2 ± 8.5 (0.6–29.4)	10.5 ± 8.4 (3.2–24)	0.47
Scaling score	2.3 ± 0.6 (1–3)	2.2 ± 0.75 (1–3)	0.60

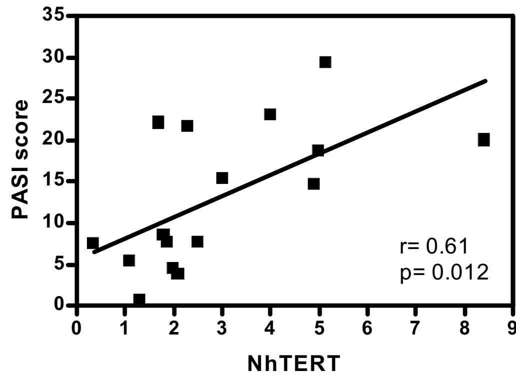

Fig. 1. Correlation between hTERT mRNA expression and PASI score in psoriatic skin lesions.

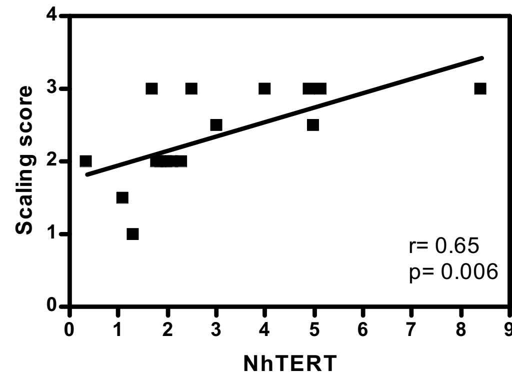

Fig. 2. Correlation between hTERT mRNA expression and scaling score in psoriatic skin lesions.

quirements were examined as negative controls. For each sample, the amount of hTERT transcript is divided by the amount of PBGD transcript to obtain a normalized hTERT (NhTERT) value, according to the formula NhTERT = hTERT mRNA copies per sample/(PBGD mRNA copies per sample/100).

2.3. Statistical analysis

Prism statistical software version 4.0 (GraphPad, San Diego, CA, USA) was used for statistical analysis. All data are expressed as mean ± SD, unless otherwise indicated. Data comparisons were performed using Student's t-test. Correlations were calculated by Pearson's correlation and simple regression tests. A P value of < 0.05 was considered significant.

3. Results

The presence of hTERT mRNA was analyzed in 24 psoriatic lesional skin specimens and 24 matched control normal skin from the same patients. Table 1 provides the characteristics of the 24 patients included in the study.

The hTERT transcripts were detected in 16 of 24 (66.7%) psoriatic lesional skin, while no expression was detected in all normal skin specimens. There was no significant difference between hTERT-positive and hTERT-negative patients regarding age, duration of the disease, PASI score or scaling score (Table 2). In hTERT-positive patients, the mean NhTERT was 2.97 ± 2.05 (0.35–8.42). There is a significant positive correlation between the level of hTERT-mRNA expression and both PASI and scaling scores (Figs 1 and 2). However, no statistically relevant correlation was found between the level of hTERT mRNA and age of the patients ($P = 0.15$), or duration of the disease ($P = 0.92$).

4. Discussion

Telomerase enzyme has shown high activity in malignant tissues, where it was suggested to have an addi-

tive diagnostic and prognostic value in most human cancers [5,18,31,34]. In addition, lower levels of telomerase activity were also detected in cycling somatic cells like; the endometrial tissue during the proliferative phase of the menstrual cycle [35] and the highly proliferative normal oral mucosa [33]. These observations raised the suggestion that telomerase activity is a biomarker of cell proliferation, rather than malignant transformation [7,21]. In support of this suggestion are some more recent studies which showed that the ectopic expression of hTERT, encoding the catalytic subunit of human telomerase, reconstitute telomerase activity and extend the life span of normal human cells without causing cellular transformation or genomic instability [11,13,36].

In the present study, we detected low expression levels of the telomerase catalytic subunit in lesional skin from 66.7% psoriatic patients, while it was completely absent in all normal skin samples from same patients. This explains the findings of Jang and his colleagues [14], who detected low levels of telomerase activity in psoriatic lesional specimens from 7 out of 10 patients (70%) and none of the nonlesional skin. Earlier work, by another group [32], has described much lower telomerase activity in other nonmalignant skin conditions than that of malignant skin tumors. Differences in the levels of telomerase expression between malignant and non-malignant skin conditions could be explained on the basis that, in malignant tissues most of the cells express telomerase, while only a peculiar subset of cells expresses the enzyme in non-malignant pathological conditions [4]. In psoriatic skin, there are six- to seven-fold more stem cells than in normal epidermis, which emerge from its normal quiescent G_0 state into active cell cycle state to become transient amplifying cells [37]. It has been suggested that telomerase is not an epidermal stem cell marker and it is much more expressed in the more actively proliferating transient amplifying cells [17]. Moreover, in situ hybridization detection of increased hTR expression in suprabasal epidermal cells of psoriatic lesions was explained by the possible migration of expanded proliferating keratinocytes from the basal layer into the suprabasal compartment [24], a proposal that was suggested many years ago [29].

The level of telomerase expression, among hTERT-positive patients, was significantly correlated with the severity of psoriasis, as indicated by PASI score and its scaling domain. PASI score includes three domains namely, erythema, induration and scaling, of which the latter is the most representative of epidermal proliferation. However, no statistically significant differences were detected between hTERT-positive and hTERT-negative patients with regards to PASI and scaling scores. It is known that telomerase activity is high in both S and M phases of cell cycle [28]. Therefore, in telomerase-negative psoriasis lesions, there is a possibility that epidermal cells bearing proliferative potential might be mainly in resting G0/G1 phase at the time of examination, and thus might exhibits little telomerase expression.

It has been found that retinoic acid suppress telomerase activity in parallel with slowing the proliferation of HaCaT cells, a commonly used model for highly proliferative epidermis [14]. The differential expression of hTERT in psoriatic skin lesions, reported here, suggest a possible diagnostic potential in certain situations where the clinical diagnosis of psoriasis may be difficult. Moreover, it proposes a potential target for therapy of this chronic disease. Further studies seem to be required to understand the cellular factors involved in regulating telomerase expression in psoriasis, which may have future diagnostic or therapeutic ramifications.

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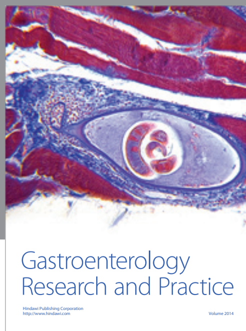

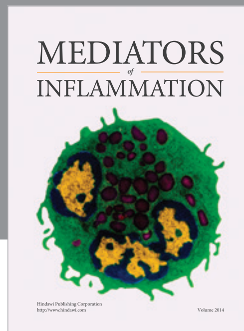

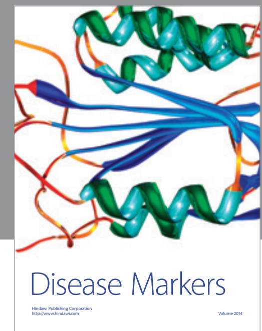

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