

Tumor necrosis factor-receptor 2 and TROY gene expression patterns in cutaneous squamous cell carcinoma in a Tunisian population

Ines T. Zidi, PhD,

Souhir M. M'farrej, PhD, Sameh D. Bergaoui, PhD (Student),

Nejet Z. Ghariani, MD, Aghleb B. Bartegi, PhD,

Nidhal N. Ben Amor, PhD, Rafia B. Nouira, MD.

Skin cancer is one of the most common human malignancies. It includes melanomas and non-melanoma cancers, such as basal cell carcinomas, and squamous cell carcinomas (SCC). Epidermal squamous cells (or keratinocytes) of the skin's upper layer are among the most frequent sites of cutaneous SCC (CSCC) metastases.¹ Non-melanoma cancer cells are characterized by their resistance to apoptosis. The extrinsic apoptotic pathway is mediated through the death-ligands including tumor necrosis factor-alpha (TNF- α) TNF-receptors (TNF-R). The TNF-R superfamily (TNFRSF) is a large superfamily including different members such as TNF-R2, and TROY ("TNFRSF expressed on the mouse embryo", TNFRSF19, Taj "Toxicity and JNK inducer" in mouse). Even though TNF-R2 and TROY genes are involved in carcinogenesis, few reports showed their altered expression in cancers. The objective of our research was to evaluate TNF-R2 and TROY genes expression in Tunisian patients with CSCC.

Six patients with CSCC were included in the study. These patients were followed up in the Dermatology Service in Farhat Hached Hospital, Sousse, Tunisia. Height biopsies were collected from January to May 2009 with authorization of the Dermatology Service. Six biopsies were from the tumor center, and 2 others from the adjacent healthy skin site. Healthy control biopsies were collected at approximately 2 cm far from the tumor border. All specimens were prepared for histological examination to confirm the absence or presence of CSCC. Total ribonucleic acid (RNA) was isolated from biopsies with the SV total RNA Isolation System Kit (PROMEGA Corp., Madison, WI, Dane County, USA) following the manufacturer's instructions, and a semi-quantitative reverse transcription polymerase chain reaction (RT-PCR) was performed. Sense and antisense primer sets are as follow: 5'ACTCTCCTATCCTGCCTGCT3' and 5'TTCTGGAGTTGGCTGCGTGT3' for a 242 base pair (bp) TNF-R2 product; 5'GATGCACAGTGTGTGACGTG3' and 5'CATGTCTTGAAAGCCGACAA3'

for a 194 bp TROY product; and 5'GTTGGTATAAGCCAGACTTTGTTG3' and 5'CAGATTTTCCAAACTCAACTTGAA3' for a 174 bp hypoxanthine phosphoribosyl transferase (HPRT) product as internal control. Experiments showed a high amount of TNF-R2 messenger ribonucleic acid (mRNA) in 66% of CSCC compared to controls (mean of increase percentage=120%). Interestingly, TNF-R2 expression level decreased in 33% of CSCC. It showed also increased TROY mRNA level expression in all CSCC samples (mean of increase percentage=160%). As TNF-R2 mRNA levels were enhanced in CSCC by 66%, as well as TROY mRNA levels by 100%, we concluded to a concomitant expression of both TNF-receptors, TNF-R2 and TROY. This co-expression has been previously detected in ovarian carcinoma and melanoma. Besides, the amount of soluble TNF-R1 and TNF-R2 measured in patients' sera was enhanced in actinic keratoses, SCC, and basal cell carcinomas.² The authors suggested that these soluble receptors, resulting probably from proteolytic cleavage are capable to bind circulating TNF- α , and also to compete with TNF-R leading to TNF- α responsiveness decrease, thus probably functioning as TNF- α inhibitors.

The TNF-R2 transcripts overexpression highlighted in this study might lead to unfavourable prognosis in patients with CSCC. This expression may be associated to a high protein levels at the CSCC cell surface conducting probably to a more enhanced response to TNF- α cytokine majorly produced in cancer microenvironment in favor of CSCC progression. Moreover, high levels of soluble TNF-R in patients with CSCC may promote metastatic potential.³ As it is overexpressed in melanoma, Spanjaard et al⁴ have proposed TROY as a useful biomarker, diagnosing the disease, as well as a potential target to inhibitors and immunotherapies. In addition to these functions, we propose TROY as a valuable CSCC biomarker. The rationale of this speculation is, in one hand, the implication of TROY in tissues proliferation, and on the other hand, the expression of TROY in skin cancers, such as melanoma. Indeed, TROY has no death domain (DD) and shares 55% sequence homology in its TNF-R domain, with X-linked ectodermic dysplasia receptor (XEDAR), a nuclear factor-kB (NF)-kappa B activator.⁵ In addition, like most TNFRSF members, TROY might lead to cell survival after activator protein-1 (AP-1) pathway activation. In fact, AP-1, involved in both skin tumor promotion and progression is constitutively activated in malignant cells. Further studies of the TROY's role as a CSCC biomarker are warranted.

In conclusion, we analyzed TNF-R2 and TROY gene expression in CSCC patients. We have demonstrated that TNF-R2 and TROY mRNA levels is enhanced

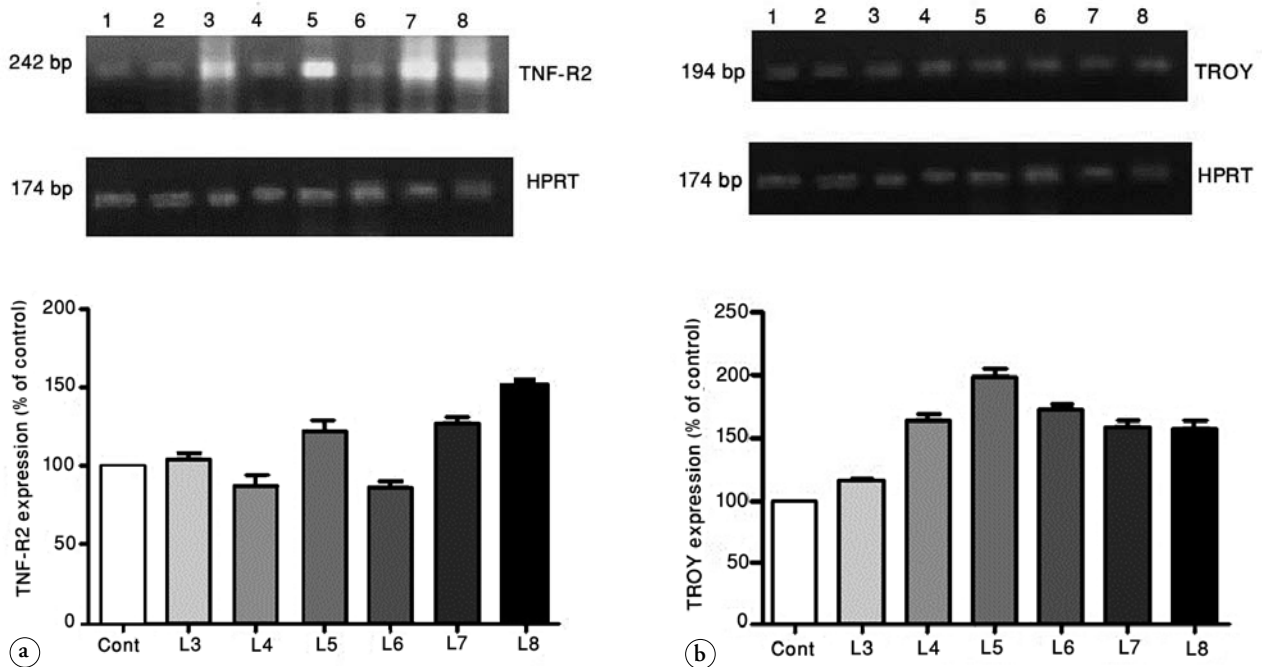


Figure 1 - Semi-quantitative reverse transcription polymerase chain reaction assay readily detects a) tumor necrosis factor-receptors (TNF-R2), and b) TROY expression in cutaneous squamous cell carcinoma in healthy (Lines 1 and 2, Cont) and tumor (Lines 3-8) tissues. Hypoxanthine phosphoribosyl transferase (HPRT) serves as loading control. A representative experiment from quadruplets is shown. Histograms show TNF-R2 messenger ribonucleic acid (mRNA) or TROY mRNA quantification presented as percentage of the controls. Data are expressed as mean \pm standard error of the mean.

in tumor CSCC cells compared to healthy ones. The concomitant overexpression noticed with TNF-R2 and TROY is not surprising because these latter TNFRSF members share their major ligands, such as TNF- α and lymphotoxin- α . Further studies with a large cohort of CSCC patients are still needed to conclude with certainty on TNF-R2, and TROY involvement on CSCC carcinogenesis process. This may have great implications for the use of TNF-R2 and TROY as prognostic indicators, as well as potential targets in CSCC therapy.

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From the Department of Life Sciences (Zidi, Bartegi, Ben Amor), Sciences College, King Faisal University, Al-Ahsa, Kingdom of Saudi Arabia, the Biochemistry and Molecular Interaction Research Unit (Zidi, M'farrej, Bergaoui, Bartegi, Ben Amor), Higher Institute of Biotechnology of Monastir, Monastir, and the Dermatology Service (Ghariani, Nouina), Farhat Hached Hospital, Sousse, Tunisia. Address correspondence and reprints request to: Dr. Ines Zidi, Biochemistry and Molecular Interaction Research Unit (02/UR/09-01), Higher Institute of Biotechnology of Monastir, Avenue Tabar Haddad BP 74, Monastir, 5000, Tunisia. Fax: +216 (73) 461404. E-mail: ines.zidi@techemail.com

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