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Molecular finds of pressure ulcer: A bioinformatics approach in pressure ulcer

Eloa Mangabeira Santos [a], Lucyana Conceição Farias [a], Sérgio Henrique Sousa Santos [b], Alfredo Maurício Batista de Paula [a], Carla Silvana de Oliveira e Silva [c], André Luiz Sena Guimarães [a, *]

[a] Department of Dentistry, Universidade Estadual de Montes Claros, Montes Claros, Minas Gerais, Brazil
[b] Institute of Agricultural Sciences, Food Engineering College, Universidade Federal de Minas Gerais (UFMG), Montes Claros, Minas Gerais, Brazil
[c] Department of Nursery, Universidade Estadual de Montes Claros, Montes Claros, Minas Gerais, Brazil

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ABSTRACT

Background: Understanding the biological processes underlying Pressure Ulcer (PU) is an important strategy to identify new molecular targets. Bioinformatics has emerged as an important screening tool for a broad range of diseases.
Objective: This study aim of the current study is to investigate the protein-protein interaction in the PU context by bioinformatics.
Methods: We performed a search in gene databases, and bioinformatics algorithms were used to generate molecular targets for PU based in silico investigation. Interactions networks between protein-coding genes were built and compared to skin.
Results: *TNFA*, *MMP9*, and *IL10* genes have higher disease-related connectivity than a connectivity general global. *MAGOH*, *UBC*, and *PTCH1* as were leader genes related to skin. Ontological analysis demonstrated different mechanisms associated, such as response to oxidase stress.
Conclusion: *TNFA*, *MMP9*, and *IL10* are possible therapeutic targets for pressure ulcer. Additional investigation of cell post-transcriptional machinery should be investigated in PU.

1. Introduction

Pressure ulcers (PU) are also known as decubitus ulcers, or bedsores lesions. PU develops in consequence of prolonged periods of continuous pressure on the skin leading to areas of tissue necrosis [1]. Pressure ulcers lead to significant health problems, such as pain, functional and aesthetic, leading to disfigurement area [2,3]. Also contributing to the increased risk of medical complications [4]. The main factors for PU development are intensity and duration of pressure [5]. Elderly, victims of stroke, diabetics, those with dementia boards and people who use wheelchairs or bedridden with any change in mobility or sensitivity are the most committed by PU [6,7]. The majority of patients affected with PU are those having health conditions that lead to immobility for prolonged periods of time [1,8]. On the other hand, even thirty years after the publication of the first clinical guidelines for prevention of this type of injury incidence rates remain high [9], PU treatment still complex and challenging [10].

Recently, molecular biology has been impacted the understanding of the pathogenesis of several diseases including PU [11–13]. Specifically, inflammatory cytokines play a major role in the etiology of pressure ulcers, relating to increased expression of genes related to them and tissue damage in areas subjected to pressure [14]. The risk to develop PU may be attributable to the individual differences in response to inflammatory stressors [15]. Inflammatory mediators [16], such as interleukin 1A (IL1A), IL1B, IL1 receptor antagonist (IL1RA), IL-6, IL10, and tumor necrosis factor-α (TNFA) are associated with the PU development [17–20].

Bioinformatics has emerged as an important screening tool for a broad range of diseases [21–23]. Specifically, Bioinformatics is useful for analysis of genomic and proteomic using web-based databases [24,25]. For example, Bioinformatics was recently used to characterize two distinct inflammatory diseases that present the

* Corresponding author. Universidade Estadual de Montes Claros, Hospital Universitário Clemente Faria, Laboratório de Pesquisa em Saúde, 562 Av. Cula Mangabeira Santo Expedito, Montes Claros, Minas Gerais 39401-001, Brazil.
E-mail address: andreluizguimaraes@gmail.com (A.L.S. Guimarães).

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same etiological factors [21]. Considering the complex nature of PU pathogenesis, the purpose of the current study was to perform a Bioinformatics analyze of PU.

2. Material and methods

2.1. Genes selection

The Bioinformatics approach was described previously [21,24]. Briefly, the first step has identified the genes associated with PU according to MEDLINE database based on the Medical Subject Headings (Ulcer pressure, Decubitus Ulcer, bedsore and Pressure Ulcer). Search criteria were the inclusion of each Medical Subject Headings and the keyword gene to generate a list of genes associated to PU. We also searched in Genecards database (www.genecards.org) [26]. A search for the term skin only in Genecards (www.genecards.org) [26] to create a control group to compare with PU. As a second step, first gene lists for PU and control were expanded. Network expansion was performed using STRING (version 10) [27–29]. STRING (version 10) [27–29] is a biological web resource database to predicted protein–protein interactions. Only predicted associations with a higher level of confidence (results with a score ≥0.9) were selected. The Weighted Number of Links (WNL) represents the gene interactions in a specific network. WNL is obtained by the sum of all interactions on the specific network multiplying to 1000 [21,23,30]. On the other hand, Total Interaction Score (TIS) represents all gene interactions in the entire STRING database. To obtained TIS value, all interactions of a gene in the whole STRING database were summed and adjusted by multiplying to 1000.

2.2. Statistical analysis

Analyses were performed using SPSS carried out in SPSS (Version 18.0, IBM, New York, NY, USA). Kolmogorov-Smirnov and the Shapiro-Wilk Tests were conducted to evaluate data distribution. Samples presented as a normal distribution. Statistical significance was accepted at $p < 0.05$. According to WNL and TIS, all genes were clustered, using K-means Clustering [23,30]. Genes with no interactions were orphan genes [21,24]. After K-Means Clustering, ANOVA, and Tukey- Kramer post hoc tests were applied to certify the results. Genes with no interactions were defined as orphan genes [22,30]. The category with higher WNL and lower TIS was considered as therapeutical targets. All other classes apart from leader genes were not taken into account for analyses purpose.

2.3. Topological and ontological analysis

Cytoscape [31] is a software platform was used for visualization of molecular interaction networks. All topological analyses were carried out with Cytoscape [31] to evaluate both networks. Biological Networks Gene Ontology (BiNGO) tool is Cytoscape plugin

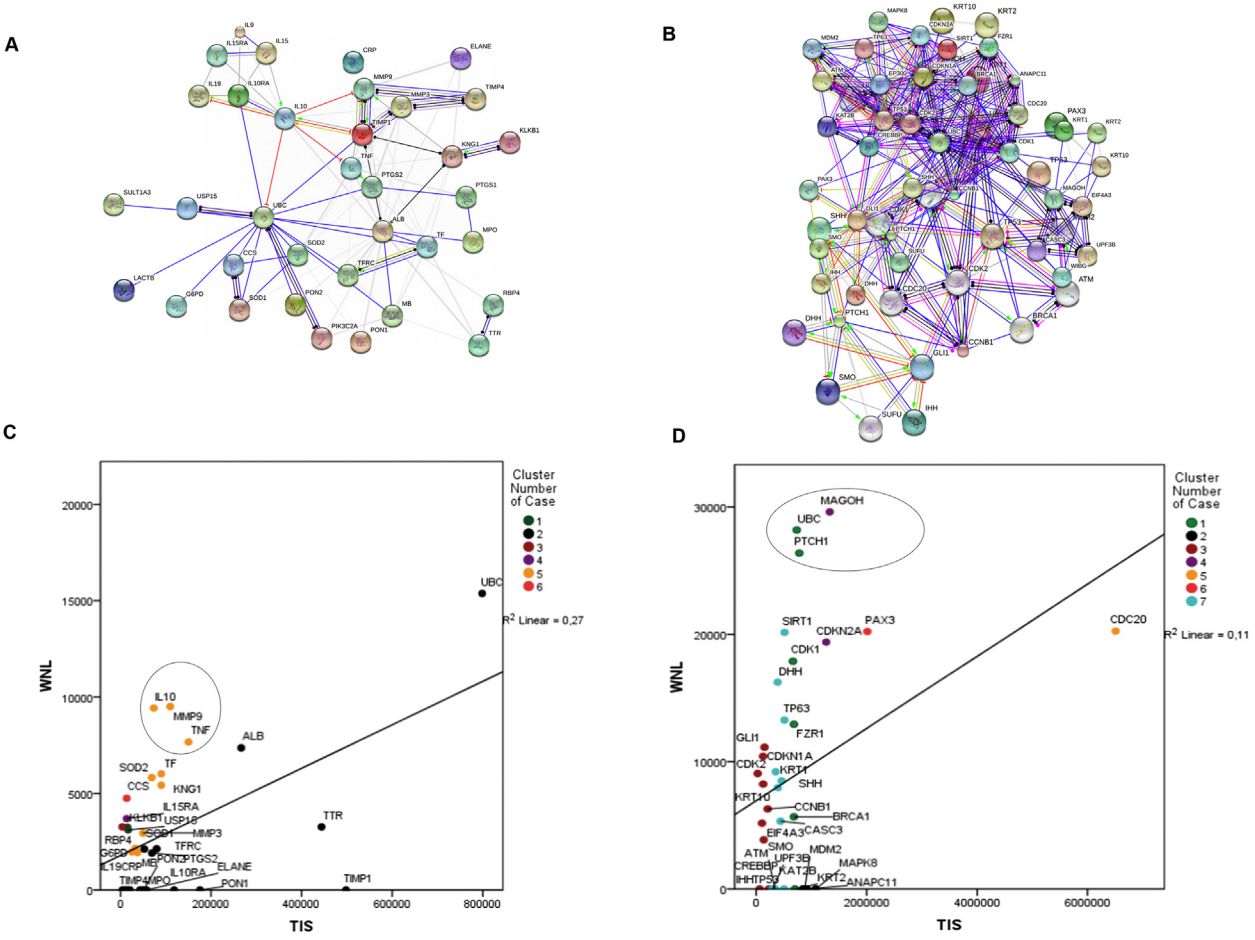

Fig. 1. Comparative networks of Pressure Ulcer and Skin. STRING networks of in A Pressure Ulcer and B Skin. In C and D scatter diagrams are showing condition-related connectivities (WNL, weighted number of links) versus the global connectivities (TIS Interactions Total Score). The leader genes and clusters Pressure Ulcer and Skin were presented in C and D respectively. *TNFA, MMP9* and *IL10* genes presented higher WNL and lower TIS in PU. On the other hand, *MAGOH*, *UBC*, and *PTCH1* were associated with skin.

to assess overrepresentation of Gene Ontology categories in Biological Networks [32]. All ontology analyses were performed with BiNGO [32] as described before [21,23,30]. To build heat map WNL and TIS values were plotted in Microsoft Excel (Version 2016, Microsoft, Redmond, WA, USA).

3. Results

A comparison of final networks demonstrated differences between PU and skin (Figs. 1 and 2). Both Skin and PU networks were composed of 35 genes (Figs. 1 and 2). Considering genes that

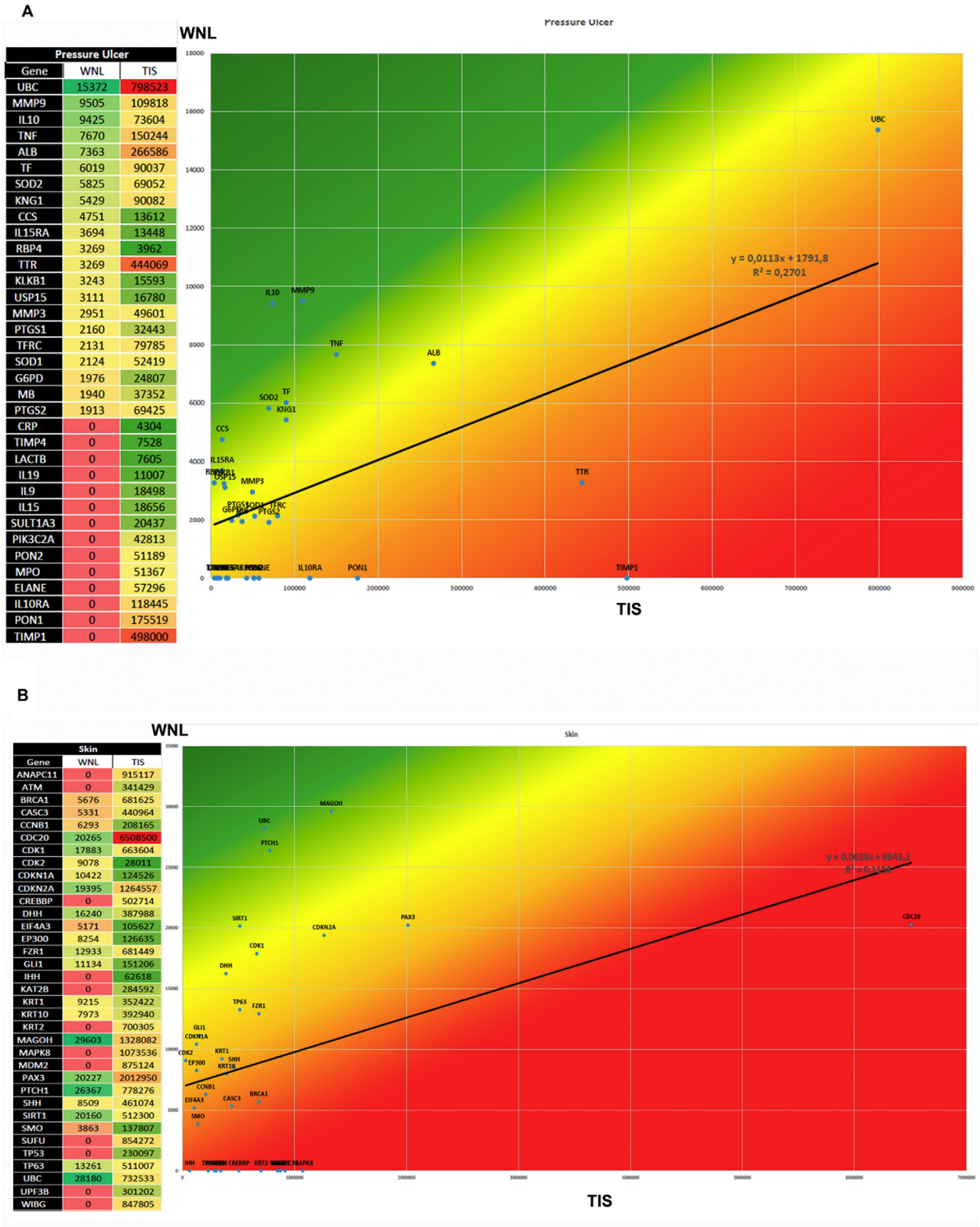

Fig. 2. Comparative heat maps of Pressure Ulcer and Skin. In An, a heat map for pressure ulcer and B represents skin.The green area represents higher WNL and lower TIS. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

presented a combination of higher WNL and lower TIS were *TNFA*, matrix metallopeptidase 9 (*MMP9*) and Interleukin 10 (*IL10*) were considered the main therapeutic target for PU (Fig. 1C). 35 genes were selected for skin analyses (Fig. 1B). Analyses revealed that the gene mago homolog (*MAGOH*), ubiquitin (*UBC*), and patched 1 (*PTCH1*) were leader genes associated to skin (Fig. 1D). The heat map was built for PU and Skin based on WNL and TIS values to illustrate leader genes (Fig. 2A and B respectively). Green represents potential therapeutical targets. Red represents targets which might be associated with exacerbated side effects.

PU network exhibits a power law behavior (correlation: 0,966; R2:0,946, Fig. 3A) in agreement with the scale-free theory of network. The relation between WNL and TIS are disposed of in the (Fig. 3B). The ontological analysis demonstrated that regulation of biological quality, response to oxidative stress and response to stress are the main biological processes associated with PU. (Fig. 3C and Table 1).

4. Discussion

PU is a localized injury with presents many systemic consequences such as deep vein thrombosis, diabetes mellitus and rheumatoid arthritis [33,34]. The challenge of understanding molecular mechanisms related to PU is critical to the development of new therapies. Current knowledge originated mostly from analysis of scarce human tissue, wound fluid, and a few animal models [1,12,35,36]. Systemic manifestations such as increases in inflammatory cells, excessive neutrophils, as well as changes in inflammatory mediators are found to have been associated with PU formation [33]. Specifically, it was demonstrated that IL1, TNFA, and MMP9 are increased in PU with patients [35,37–40]. Additionally, reduction of TNFA was observed to be related to wound healing in PU context [41]. Interestingly enough TNFA inhibitors have benefit in inflammatory conditions autoimmune diseases inflammatory signals induced by this cytokine [42,43]. The current study pointed to *TNFA*, *MMP9*, and *IL10* as the main targets for the treatment of PU. Our results corroborate with a previous study [12] which demonstrated the decrease of anti-inflammatory IL10 and the increasing TNFA in a study model of PU. Additionally, High expression of TNF in local skin tissue under pressure might be substantial for MMP9 increasing [48] and consequently PU development. MMP9 levels are influenced by TNF [44–46]. MMP9 is involved in the breakdown of extracellular matrix with is related to physiological or pathological process [47]. IL10 is an important anti-inflammatory cytokine that could control both TNFA and MMP9 levels [12,48]. High expression of MMP9 might cause the loss of the skin and subcutaneous tissue [55]. As a result of MMP9 increasing levels, the skin, and the subcutaneous tissue could be thickened [55]. Moreover, it was observed that the receptors of inflammatory cytokines such as GM-CSF, IL1, IL-6, and TNFA were up-regulated during PU development. On the contrary, receptors for anti-inflammatory cytokines such as IL-2, IL10, IL11, and TGF-β were not changed [12].

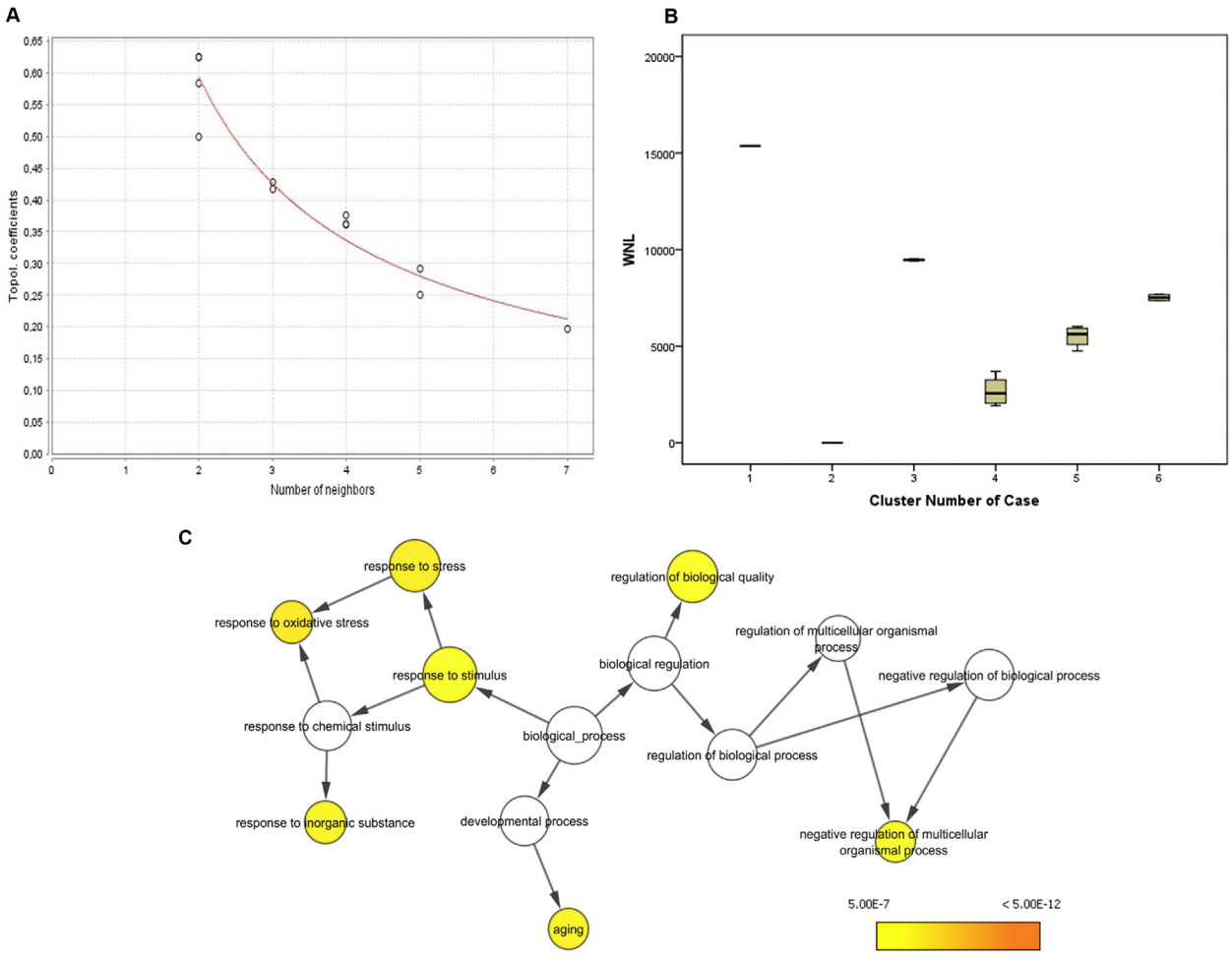

Fig. 3. Pressure Ulcer Topological and Ontological Analyses. In A, topological coefficients of the network. In B, Clusters number disposed of by (WNL, weighted number of links) for genes involved in the phenomenon. In C, Ontological analysis demonstrated different mechanisms associated, such as response to oxidase stress.

Table 1
Ontological analysis of Pressure Ulcer.

GO-ID	Description	p-val	corr p-val	Genes
65008	regulation of biological quality	2,64E-07	3,42E-04	IL10,G6PD,TFRC,IL10RA,PTGS2,SOD2,TNF,KNG1,PTGS1,SOD1, TF,RBP4,MB,ALB,TIMP1,KLKB1,ELANE
6979	response to oxidase stress	7,11E-07	4,62E-04	G6PD,TNF,MB,SOD2,PTGS2,MPO,PTGS1,SOD1,MMP9
6950	response to stress	2,00E-06	8,67E-04	IL10,G6PD,TFRC,IL15,IL10RA,PTGS2,SOD2,TNF,MPO,KNG1,PTGS1, SOD1,TF,MB,ALB,KLKB1,ELANE
10035	response to inorganic substance	5,54E-06	1,72E-03	TF,TFRC,NOS3,MB,SOD2,PTGS2,MPO,SOD1
7568	aging	6,61E-06	1,72E-03	IL10,TF,TFRC,IL15,IL10RA,SOD2,TNF,KLKB1,KNG1,SOD1,MMP9
50896	response to stimulus	2,06E-05	4,40E-03	IL10,TF,PTGS2,TNF,KLKB1,KNG1,ELANE
51241	negative regulation of multicellular organismal process	2,37E-05	4,40E-03	RBP4,NOS3,PTGS2,TNF,KLKB1,KNG1,SOD1

The significant pathways represented as: cellular components, molecular functions and biological processes, pathologic phenomenon and genes.

UBC gene presented both TIS and WNL higher in PU network. An alternative explanation could be attributed to the dysregulation of *TNFA* gene expression by the absence of linear ubiquitin chain assembly complex (LUBAC) [49–51]. LUBAC components or a cIAP would unbalance TNF signaling output of pro-inflammatory cell death [49,50]. Additionally, *UBC* plays an important part in several innate and adaptive immune signaling pathways, including the one triggered by TNFA [52–54]. Intriguingly, this cell death is not only apoptotic but also necrosis [54]. TNFA inhibition would exert its therapeutic effect by reduction of inflammation levels, reduction of apoptosis, inhibition of metalloproteinases such as MMP2 and MMP9 [44–46].

The analysis of skin was performed to find constitutive leader genes. *MAGOH*, *UBC*, and *PTCH1* were considered the leaders genes in skin. Our findings point towards the complex network of interactions between proteins modified by UBC and UBC recruited proteins. The UBC performs an important role in the regulation of proteins [52]. It marks unwanted proteins with poorly folded proteins, to be degraded by a multiprotein complex called the proteasome [52,56]. The presence of certain linkage types, such as linear ubiquitin chains, may promote recruitment of factors such MAGOH, a core component of the splicing-dependent multiprotein exon junction complex (EJC) deposited at splice junctions on mRNAs [57]. The EJC is related to mRNA metabolism [57]. PTCH1 codifies a 12pass-transmembrane receptor for Sonic Hedgehog (SHH) and other Hedgehog proteins [58]. PTCH1 has been associated not only with Basal Cell Carcinomas but also with Odontogenic keratocyst [59] and Gorlin Syndrome [58]. It is important to highlight that the expression of *SHH* gene was associated with wounds in diabetic patients [60]. Inhibition of the SHH pathway disrupted multiple parameters in wound healing such as wound closure, granulation tissue formation, vascularization and follicular regeneration [41]. Keratinocytes participate in innate immune response secreting proinflammatory cytokines such as IL1β [18]. Moreover, IL1β, interferon gamma (INFG), transforming growth factor beta 1 (TGFB1), and TIMP-1 are increased after pressure stimulus [61–63]. IL1β are expressed in human keratinocytes both in vitro [20] and in vivo [64,65]. IL1β is also activated by several molecular patterns released from stressed and damaged cells [66].

Bioinformatics analyses of a specific phenomenon can potentially disclose knowledge about protein-protein, direct or indirect, interactions, cellular processes and molecular mechanisms. Using this newly obtained information, that is a detailed gene interaction map considering both direct and indirect physical functional protein linkages, further experimental design and target therapies may be planned. In the present study, genes with a potential role in PU were searched, described and grouped for disclosure of their functions and significance, through gene ranking following a clustering method that combined experimental and theoretical results. Findings outlined in this study should contribute to our understanding to the function of inflammation and their role in PU biology.

In conclusion, *TNFA, MMP9,* and *IL10* are possible therapeutic targets for PU. Additional investigation of cell post-transcriptional machinery should be investigated in PU.

Conflicts of interest statement

There is no conflict of interest.

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