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DIABETIC FOOT ULCER (DFU) GENE EXPRESSION IN HOMO SAPIENS SPECIES

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ABSTRACT
Diabetic foot ulcer (DFU) is a major problem in people with diabetes because more than 15% of patients have to treat DFU during their lifetime. 1 out of people with diabetes can experience a diabetic foot ulcer (DFU). At least a quarter of diabetic foot ulcer (DFU) sufferers cannot recover completely. The prevalence of diabetic foot ulcers (DFU) in Indonesia reaches 8.7%. The current DFU treatment approved by the FDA uses Becaplermin, a recombinant platelet-derived growth factor derivative. However, this treatment has a weakness in systemic bioavailability. FAgene expression analysis method is needed. to develop other therapies. This article aims to discover specific genes that play a role in diabetic foot ulcer (DFU) wound healing. In this systematic review, we searched the GEO Data Sets database to identify articles published from 2018 to 2023. The search results for DFU gene expression data for all species obtained 130 articles. Then, the DFU gene expression data series of Homo sapiens species was filtered to obtain ten related articles. This research has implications in providing better insight into the specific genes involved in the healing process of DFU wounds. This research also has the potential to contribute to early diagnosis of DFU injuries and better treatment.

Keywords: diabetic foot ulcer, wounds, homo sapiens.

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INTRODUCTION

Diabetic Foot Ulcer (DFU) is a major problem for people with diabetes. More than 15% of diabetic patients must treat DFU during their lifetime (Jaroenarpornwatana et al., 2023). DFU severely impairs patients' quality of life, causes long-term hospitalizations and causes more than 70,000 lower limb amputations annually in the United States. The prevalence of DFU in Indonesia is 8.7% (Yunir et al., 2022). Diabetic Foot Ulcer (DFU) is one of the most common complications of diabetes and chronic ulcers (Kurdi & Priyanti, 2020). This disease causes foot injuries and tissue damage in diabetic patients caused by abnormalities in the nerves in the legs (Hidayat & Nurhayati, 2014).

Studies have shown that complicating factors in DFU wound healing include microbial infections, epithelial cell damage, and decreased immunity (Maria Allen, 2022). Among all complications caused by type II diabetes, DFU is the main reason for the hospitalization of patients. Studies have shown that 25% of people with diabetes may develop foot ulcers (Rodrigues et al., 2023). Current treatments developed for Diabetic Foot Ulcer (DFU) include wound debridement, wound transport, regulation of blood sugar levels, and disease management (VITA, 2022). Currently, there are many technological developments for the treatment of Diabetic Foot Ulcer (DFU), including stem cells, the use of hyperbaric O2, and the use of Becaplermin. New technologies and drugs have been developed to treat DFU. However, the treatment effect is considered sub-optimal due to its strong pathophysiological processes. The annual cut-off rate of patients with DFU is 5.1%

(Theocharidis et al., 2022). From the research of PN Theocharidis G, it is possible to know the healing process of DFU by looking at gene expression. Several other studies have examined differences in gene expression in the DFU healing process using the Single-cell transcriptomic and Spatial transcriptomics methods using the RNA sequencing method. Gene expression analysis is an important technique used to compare differences between two or more groups of genes. This technique involves using methods such as microarrays or RNA sequencing (RNA-seq) which make it possible to examine gene activity under various conditions or situations (PN Theocharidis G, 2020). In addition, more specific target gene expression techniques, such as quantitative polymerase chain reaction (qPCR), are also used to perform this analysis.

Based on the above background, this study aimed to determine and analyze the expression of the diabetic foot ulcer (DFU) gene in the homo sapiens species.

METHODS

This review is based on research articles on the expression of the Diabetic Foot Ulcer gene in the Homo sapiens species. The data source for this review was obtained from the National Center for Biotechnology Information database; then, Geo Data Sets were selected. Geo Data Sets is a database with curated gene expression datasets and original series and platform records in the Gene Expression Omnibus (GEO) repository. After that, enter search keywords to find relevant research. Search keywords use a PICO strategy, such as: "Diabetic Foot Ulcer". 127 DFU gene expression studies were obtained with Homo sapiens species, 2 DFU gene expression studies with Mus musculus species, and 1 DFU gene expression study with Rattus norvegicus species. Articles will be analyzed and selected with the inclusion and exclusion criteria provisions.

The inclusion criteria are adjusted to the purpose of the review article, so the journal inclusion criteria used are research data that produce primary data that discusses the expression of the Diabetic Foot Ulcer gene, original articles, research subjects are humans, selected type of sample type series, year of publication of the journal at the period The last five years, namely 2018-2023, full-text articles.

The exclusion criteria that will be used to select articles are articles that do not specifically discuss the expression of the Diabetic Foot Ulcer gene in the Homo sapiens species, and the research subjects are Mus musculus or Rattus norvegicus. Then, the literature review of journals, short reports, case reports, and clinical trial reports.

Journals following the criteria for keywords, titles, and abstracts are reviewed in full text to find out the content and adjust it to the topic being studied. Journal search is used for review by conducting searches and paying attention to inclusion and exclusion criteria. The analysis carried out in this article review was carried out descriptively.

RESULTS AND DISCUSSION

One hundred thirty titles were identified for the initial review of search pages. The main search identified 130 journals, with 127 DFU gene expression studies with Homo sapiens species, 2 DFU gene expression studies with Mus musculus species, and 1 DFU gene expression study with Rattus norvegicus species. After being selected, 120 articles did not meet the inclusion criteria, and ten met the inclusion criteria. Articles that met the inclusion criteria were then reviewed. Articles were

studied based on the name of the researcher, year, name of the gene that plays a role in healing DFU, the methodology used, the results obtained and the outcome (Appendix).

The process of converting gene information into products that cells can recognise is called gene expression (Sandoval-Schaefer et al., 2023). The result of this gene expression can be a protein and an RNA product such as tRNA or snRNA (Ramirez-Acuña et al., 2019). Gene expression measurement is important in drug discovery, biomarker research, and gene pathway analysis. Gene expression analysis involves genome expression techniques such as microarrays or RNA sequencing (RNA-seq) and more specific target gene expression techniques such as qPCR (Kusnadi & Arumingtyas, 2020). Today, many publicly available gene expression data such as NCBI GEO or ArrayExpress. This data set contains valuable information for discovering and developing biomarkers and therapeutics.

Research on the expression of the Diabetic Foot Ulcer gene in the Homo sapiens species aims to look at gene expression that occurs in humans and to group genes that are influential in the healing process of DFU. This systematic review identified ten gene codes associated with DFU recovery. The following is a review of 10 articles that meet the inclusion criteria:

GSE223964 Gene Code

The gene code was obtained from a previous study entitled " Transcriptional heterogeneity in human diabetic foot wounds" (Santra et al., 2020). This study, using single-cell RNA sequencing in chronic foot ulcers of non-diabetics (NDFUs) and diabetes (DFUs), in the DFUs group showed transcriptional changes indicating reduced keratinocyte differentiation, fibroblast function and alteration, and defects in macrophage metabolism and ECM production compared to NDFUs. In addition, cellular interaction analysis revealed major changes in several altered signal pathways in DFUs. These data provide insight into the mechanism of wound healing in leg ulcers. They may provide new therapies for the treatment of DFUs. This study used single-cell RNA sequencing technology on cells taken from the foot wounds of a non-diabetic individual at different times and from five diabetic patients. The cells were isolated through mechanical and enzymatic breakdown, screening, and removal of red blood cells, then retrieved via single-cell RNA sequencing technology using the 10X Genomics platform (Sandoval-Schaefer et al., 2023).

GSE165816 Gene Code

Previous research with the article "Single-cell transcriptomic landscape of diabetic foot ulcers" used single-cell samples from skin cells of the feet and forearms and peripheral blood mononuclear cells (PBMC), ten samples from non-diabetic subjects and 17 patients. Diabetes, 11 with DFU, and six without DFU. Fifty-four samples were analyzed using the single-cell RNA sequencing technique and verified using the Immunohistochemistry and Spatial Transcriptomics techniques. The study demonstrated increased unique inflammatory fibroblast populations in DFU patients with healing wounds (Theocharidis et al., 2022). Patients with DFU who recovered also showed increased macrophages with M1 polarization. In contrast, DFU patients did not experience an increase in M2 macrophages.

GSE166120 Gene Code

Previous research used ulcer tissue samples from DFU patients who successfully healed and those who did not heal. Total sample 23. To understand the molecular mechanisms and cell types involved in DFU healing using the spatial transcriptome profiling method, with NanoString's GeoMx

digital spatial profiling platform on DFU tissue sections and comparing gene expression in the same area within the same ulcer, as well as between patients 12 weeks after surgery those who recovered from DFU (Talers, N=2) were compared to those who did not (Non-Healers, N=2) (VA Theocharidis G, 2021).

GSE134431 Gene Code

Previous research used skin tissue samples from DFU patients, oral wound tissue, and wound tissue, so 21 samples were obtained. The method used was RNA-sequencing on tissue biopsies from patients with DFU and compared with oral wounds and human skin to identify the mechanism. Molecular and transcriptional networks that are disrupted in DFU. As a result of this study, a unique inflammatory transcription exclusive to oral and skin wounds promotes cell proliferation and survival of immune cells lacking in DFU. In addition, identifying immune cell profiles shows the absence of macrophage and neutrophil activation and proliferation in DFU. These results indicate that an impaired immune response, including the activation, proliferation, and survival of immune cells, contributes to the pathogenesis of DFU.

GSE146028 Gene Code

In the previous study, seven types of immune cells were sampled, consisting of 1 reference (CD14) and six differentiated cell types: M0, M1, M2a, Mreg, Mreg_UKR, and PCMO. There are nine anonymized human donors, with at least three for each cell type. The donor served as a biological replication for statistical purposes of expression differences. The method used is the characterization of regulatory macrophages that have clinical relevance, namely Mreg and Mreg_UKR, programmable cells of monocyte origin (PCMO), as well as comparison macrophages (M1, M2a, and M0) using flow-cytometry, RT-qPCR, phagocytosis and secretome measurements, as well as RNA-Seq. The results obtained in macrophage production can produce reproducible cell phenotypes. At the same time, small changes introduced in a protocol can consistently affect the phenotype of the final product. In addition, we have identified new combinations of potential biomarkers specific to the process, which will support further clinical product development and lead to a better understanding of differentiation and activation of macrophage polarization (Gurvich et al., 2020).

GSE143735 Gene Code

Previous research was conducted using skin samples of DM sufferers, with as many as 13 patients isolated on the volar part of the forearm. A total of 5 patients recovered from DFU, four patients who did not recover from DFU and four patients who did not have ulcers. The total sample used was 14 samples. Multiplex array serum was used to detect systemic cytokines, chemokines, and growth factors correlating with DFU healing. In addition, forearm biopsies were used for histological analysis and transcriptome bulks to ensure DFU healing results were reflected in non-ulcerative skin sites. Analysis of RNA-seq bulks revealed extracellular matrix (ECM) related genes that are increased in recoverable DFU types, including MMP2 and the implication of IFNγ and IL13 as upstream regulators. Based on analysis of transcriptome data with an error rate of discovery (FDR) <0.05 and log2fold change (log2FC) >0.5, a total of 25 genes (3 up) were differentially expressed when comparing Non-Healer and Healer DFU types, 916 (530 up-regulated) in Healers compared to DM, and 160 (89 up-regulated) in Non-Healers compared to DM (PN Theocharidis G, 2020).

The results of this study show that the genes that increase in the "Healer" group include molecules related to inflammation, such as lymphoid chemokine ligand 19 (CCL19), complement

component 6 (C6), lipoprotein lipase (LPL), and beta-defensin 124 (DEFB124), as well as extracellular matrix-related proteins such as pigment-epithelium derived factor (SERPINF1), tenascin X (TNXB), biglycan (BGN), and matrix metalloproteinase-2 (MMP2). Whereas in the "Non-Healer" group, the genes that experienced an increase included members of the cytochrome P450 (CYP1A1), prostaglandin transporter (SLCO2A1), and metabolic regulator G0/G1 switch gene 2 (G0S2).

GSE114248 Gene Code

Previous studies using wound biopsy samples were divided into two groups: the Control Group, a group of patients who had wounds and were normal on the seventh day from healthy donors, and the Test Group, a group of patients with DFU (Diabetic Foot Ulcers). Circular RNA (circRNA) microarray analysis was performed to examine the expression profile of circRNA in diabetic foot ulcers (DFU) and human excision skin wounds seven days after injury. The result of this study is that there is a regulation of circular RNA expression in diabetic chronic wounds. Increased expression of hsa_circ_0084443 in diabetic chronic wounds. hsa_circ_0084443 localized in the cytoplasm of human epidermal keratinocytes. The hsa_circ_0084443 expression modulation does not affect PRKDC expression. hsa_circ_0084443 reduces keratinocyte motility and supports keratinocyte growth (Wang et al., 2020).

GSE114236 Gene Code

Previous studies studied samples of human primary epidermal keratinocytes transfected with 50 nM si-hsa_circ_0084443 or si-Control for 24 hours (biological triples in each group). A total of 6 samples were used. Global transcriptome analysis was performed on keratinocytes after decreasing hsa_circ_0084443 using the Affymetrix series. The results showed that the expression level of hsa_circ_0084443 decreased during normal skin wound healing. In contrast, a higher concentration level of hsa_circ_0084443 was found in non-healing chronic diabetic foot ulcers compared to normal wounds (Wang et al., 2020).

GSE132187 Gene Code

THP 1 cells under conditions of hyperglycemia and normoglycemia. A total of 6 samples. THP-1 cells were cultured under hyperglycemia or normoglycemia, and hypoxia and differentiated into macrophages by PMA. LPS activates macrophages. The effects of hyperglycemia and hypoxia on macrophage phenotypes were analyzed. For this purpose, microarray analysis was performed to study the gene expression profile of macrophages cultured in high glucose concentrations. The contribution of hypoxia and hyperglycemia to chronic inflammation and potential synergistic effects were evaluated in activated THP-1-derived macrophages. Long-term effects after activation (17 h) were only observed in increased expression of pro-inflammatory cytokines when hypoxia was combined with high glucose concentrations. This study showed that hyperglycemia increased the expression of pro-inflammatory cytokines such as TNF- α, IL-1, IL-6, and chemokines and decreased the expression of two receptors involved in phagocytosis (CD36 and class B collector type I receptors). Hyperglycemia and hypoxia do not affect wound-healing molecules such as TGF - β1. Overall, the study's results suggest that hyperglycemia acts synergistically with hypoxia to maintain a state of chronic inflammation in macrophages (Morey et al., 2019).

GSE114908 Gene Code

Previous studies used samples of human epidermal keratinocytes transfected with 20nM WAKMAR1 GapmeRs (GapmeR-WAKMAR1) and 20nM oligo control (GapmeR-Ctrl) for 24 hours

(biological triple in each group). In total, there are six samples. Global transcriptome analysis was performed on keratinocytes after WAKMAR1 inhibition using the Affymetrix sequence. Long noncoding RNAs (lncRNAs) are important regulators in cellular physiology and pathology, making them promising therapeutic and diagnostic entities. WAKMAR1 lncRNA was significantly decreased in wound edge keratinocytes in venous ulcers and diabetic foot ulcers compared with normal wounds. To study WAKMAR1-regulated genes, lncRNA transfection GapmeRs into human primary epidermal keratinocytes to inhibit their expression. The results of this study stated that WAKMAR1 is a lncRNA localized in the cell nucleus, transcribed by RNAPII, and has polyadenylation. WAKMAR1 expression was decreased in wound edge keratinocytes in chronic human wounds. WAKMAR1 expression is induced by TGF- β signalling in keratinocytes. WAKMAR1 regulates keratinocyte motility and re-epithelialization processes in ex vivo human wounds. WAKMAR1 regulates a network of genes that mediate pro-migrant functions in keratinocytes. WAKMAR1 activates E2F1 expression by inhibiting E2F1 promoter methylation (Li et al., 2019).

CONCLUSION

A total of 10 studies of the gene code associated with Diabetic Foot Ulcer concluded as follows: 1) Patients with DFUs (Diabetic Foot Ulcers) show transcriptional changes that indicate a decrease in keratinocyte differentiation, changes in function and fibroblast lines, as well as defects in macrophage metabolism, inflammation, and production ECM compared to NDFUs (Non-Diabetic Foot Ulcers). 2) There are major changes in several signalling pathways that change DFUs, as well as increased populations of inflammatory fibroblasts and M1-polarizing macrophages in recovered DFU patients. 3) There is a unique inflammatory transcription exclusive to oral wounds and skin wounds in DFU that plays a role in promoting cell proliferation and survival of deficient immune cells. 4) Identification of the immune cell profile showed no activation and proliferation of macrophages and neutrophils in DFU, indicating an impaired immune response. 5) The macrophage production process can produce reproducible cell phenotypes. However, small changes in the protocol can affect the phenotype of the final product. 6) Certain genes related to inflammation and extracellular matrix were increased in the "Healer" group, and certain genes related to metabolism were increased in the "Non-Healer" group. 7) Hsa_circ_0084443 is a lncRNA localized in the cytoplasm of human epidermal keratinocytes, and its expression is related to the movement and growth of keratinocytes. The expression level of hsa_circ_0084443 decreases during normal skin wound healing, whereas it increases in diabetic chronic wounds. 8) Hyperglycemia increases the expression of pro-inflammatory cytokines and reduces the expression of receptors involved in phagocytosis. 10) Hyperglycemia and hypoxia do not affect wound healing molecules such as TGF- β1. WAKMAR1 is a lncRNA localized in the cell nucleus, induced by TGF- β signals, and involved in regulating keratinocyte motility, re-epithelialization of wounds, and expression of migration genes. WAKMAR1 activates E2F1 expression by inhibiting E2F1 promoter methylation.

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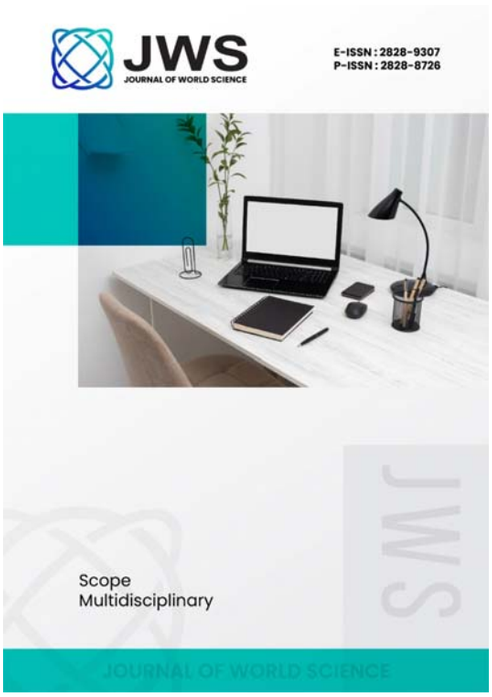

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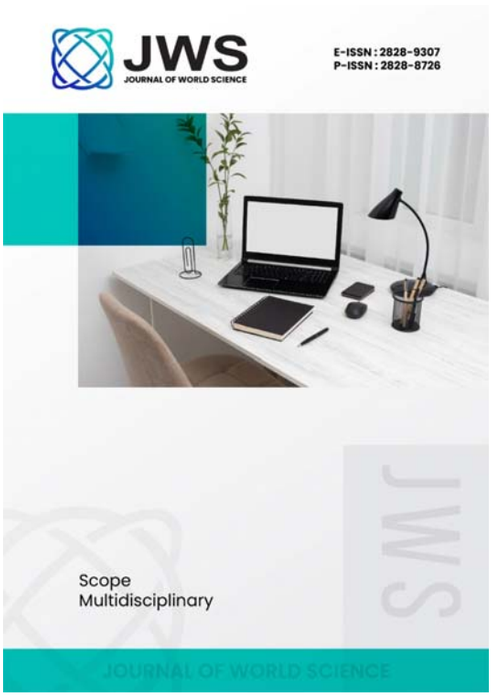

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