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Construction of an immunorelated protein–protein interaction network for clarifying the mechanism of burn

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ABSTRACT

Background and aim: Severe burn is known to induce a series of pathological responses resulting in increased susceptibility to systemic inflammatory response and multiple organ failure, but the underlying molecular mechanism remains unclear at present. The main aim of this study was to expand our understanding of the events leading to circulating leukocyte response after burn by subjecting the gene expression profiles to a bioinformatic analysis.

Materials and methods: Comprehensive gene expression analysis was performed to identify differentially expressed genes (DEGs) using the expression profile GSE7404 (*Mus musculus*, circulating leukocyte, 25% of total body surface area (TBSA), full thickness) downloaded from the Gene Expression Omnibus, followed by the Gene Ontology and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses. In addition, a postburn protein–protein interaction (PPI) network was constructed to identify potential biomarkers.

Results: Maximum changes in the gene expression profile were detected 1 day post burn. Separate Gene Ontology (GO) functional enrichment analysis for upregulated and downregulated DEGs revealed significant alterations of genes related to biological process such as ''response to stimuli,'' ''metabolic,'' ''cellular and immune system processes,'' ''biological regulation,'' and ''death'' in the leukocyte transcriptome after the burn. The KEGG pathway enrichment analysis showed that the upregulated DEGs were significantly enriched in the nodes of immunorelated and signal transduction-related pathways, and the downregulated genes were significantly enriched for the immunorelated pathways. The PPI network and module analysis revealed that, 1 day after the burn, lymphocyte-specific protein tyrosine kinase (Lck) (downregulated), Jun (upregulated), Cd19 (downregulated), Stat1 (downregulated), and Cdk1 (upregulated) were located centrally in both the PPI network and modules.

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Conclusions: Based on an integrated bioinformatic analysis, we concluded that Lck, Jun, Cd19, Stat1, and Cdk1 may be critical 1 day after the burn. These findings expand our understanding of the molecular mechanisms of this important pathological process. Further studies are needed to support our work, focused on identifying candidate biomarkers with sufficient predictive power to act as prognostic and therapeutic biomarkers for burn injury.

1. Introduction

Burn is a complex traumatic event with various local and systemic effects on several organ systems in addition to the skin [1]. Major burns induce a pathophysiological response with a marked inflammatory component [2]. As a consequence, an undesirable inflammatory response is triggered, resulting in excess production of various inflammatory cells and release of cytokines, such as tumor necrosis factor alpha (TNF-α), interleukin (IL)-6, nitric oxide and prostaglandin E2 (PGE2). This then leads to the development of systemic inflammatory response, immune dysfunction, and multiple organ failure [3–5]. Thus, the mechanisms responsible for burn must be elucidated to improve treatment modalities [6].

Time-course microarray experiments are successfully used to obtain the temporal profiles for thousands of genes simultaneously for information on dynamic changes in gene expression [7]. Gene expression profiling provides a means of developing potential new therapeutic options after thermal injury, in which datasets obtained from global transcriptional patterns can help identify new targets and options for the intervention and prevention of burn immune dysfunction [1]. Therefore, current research on immune dysfunction burn injury should be implemented in a global context. Previously, Lederer et al. and Xiao et al. successively applied time-course microarray experiments to perform genome-wide expression analysis for comparing the circulating leukocyte transcriptome after severe trauma and burn between mouse and human models [8,9]. Based on the transcriptional data, researchers have provided new insights into immunological responses to severe burn. However, they mainly compared the gene expression changes between burns and trauma, without analyzing the dynamic changes in gene expression after burn.

To elucidate the molecular mechanism underlying circulating leukocyte activity after burn, an integrated bioinformatic approach integrating gene expression profile data was used to screen differentially expressed genes (DEGs), key biological functions, and related pathways. Further, a burn protein–protein interaction (PPI) network was constructed to identify potential biomarkers for burn. The integrated findings further expand our understanding of the molecular regulatory mechanisms of leukocyte response to burn injury, possibly providing prospective targets for developing novel therapeutic strategies.

2. Methods

2.1. Description of datasets

The GSE7404 microarray dataset was downloaded from the Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo/) database through approved access [8]. The microarray dataset is based on the Affymetrix Mouse Genome 430 2.0 Array. Raw files on thermal injury (*Mus musculus*, circulating leukocyte, 25% total body surface area (TBSA), full thickness) were extracted for further analysis. In total, 32 samples were available, including 16 samples of thermal injury and 16 controls. The samples were divided into four groups according to the labels: (1) 2 h after burn versus control, (2) 1 day after burn versus control, (3) 3 days after burn versus control, and (4) 7 days after burn versus control.

2.2. Identification of DEGs

Statistical analyses were performed using open-source statistical software R version 3.02 [10]. The gene expression profile data were recalculated and normalized using the Robust Multi-array Average (RMA) algorithm [11]. Student's *t*-test and the fold change method were used to select DEGs between burn and sham burn controls. All genes with *P*-values <0.01 and $|\log FC| > 1$ were set as the cutoff values to identify DEGs for further analysis.

2.3. Functional enrichment analysis

Gene Ontology (GO) aims to obtain information on gene function by producing a controlled vocabulary that can be applied to all organisms. GO consists of three hierarchically structured vocabulary sets that describe gene products in terms of their associated biological processes, cellular components, and molecular functions [12].

The GO function [13] extracts biologically relevant terms from statistically significant GO terms for diseases with DEGs as input. It efficiently applies the hierarchical relationships among GO terms, and it prevents the dilution of potentially important biological concepts by reducing global and local redundancy. Significantly enriched GO terms are identified using a hypergeometric test and finally selected with an adjusted *P*-value <0.01 calculated using the Benjamini–Hochberg false discovery rate (FDR) method.

The Kyoto Encyclopedia of Genes and Genomes (KEGG) database is a collection of manually drawn pathway maps