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Dynamics of Short-Term Gene Expression Profiling in Liver Following Thermal Injury

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Abstract

Background—Severe trauma, including burns, triggers a systemic response that significantly impacts on the liver, which plays a key role in the metabolic and immune responses aimed at restoring homeostasis. While many of these changes are likely regulated at the gene expression level, there is a need to better understand the dynamics and expression patterns of burn injury-induced genes in order to identify potential regulatory targets in the liver. Herein we characterized the response within the first 24 h in a standard animal model of burn injury using a time series of microarray gene expression data.

Methods—Rats were subjected to a full thickness dorsal scald burn injury covering 20% of their total body surface area while under general anesthesia. Animals were saline resuscitated and sacrificed at defined time points (0, 2, 4, 8, 16, and 24 h). Liver tissues were explanted and analyzed for their gene expression profiles using microarray technology. Sham controls consisted of animals handled similarly but not burned. After identifying differentially expressed probesets between sham and burn conditions over time, the concatenated data sets corresponding to these differentially expressed probesets in burn and sham groups were combined and analyzed using a "consensus clustering" approach.

Results—The clustering method of expression data identified 621 burn-responsive probesets in 4 different co-expressed clusters. Functional characterization revealed that these 4 clusters are mainly associated with pro-inflammatory response, anti-inflammatory response, lipid biosynthesis, and insulin-regulated metabolism. Cluster 1 pro-inflammatory response is rapidly up-regulated (within the first 2 h) following burn injury, while Cluster 2 anti-inflammatory response is activated later on (around 8 h post burn). Cluster 3 lipid biosynthesis is downregulated rapidly following burn, possibly indicating a shift in the utilization of energy sources to produce acute phase proteins which serve the anti-inflammatory response. Cluster 4 insulin-regulated metabolism was down-regulated late in the observation window (around 16 h postburn), which suggests a potential mechanism to explain the onset of hypermetabolism, a delayed but well-known response that is characteristic of severe burns and trauma with potential adverse outcome.

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Conclusions—Simultaneous analysis and comparison of gene expression profiles for both burn and sham control groups provided a more accurate estimation of the activation time, expression patterns, and characteristics of a certain burn-induced response based on which the cause-effect relationship among responses were revealed.

Keywords

Burn; gene expression; microarray; inflammation; liver

Introduction

Thermal injury, one of the most severe forms of trauma, triggers a number of physiological responses including local and systemic inflammation, hyper-metabolism, immune-suppression, and eventually organ dysfunction (1). Clinical studies have shown that an uncontrolled and prolonged action of inflammatory cytokines, which is evidenced by a sustained release of acute phase proteins, may contribute to detrimental complications (2). Liver is an important player in the modulation of the inflammatory response since it largely controls circulating levels of metabolites and the production of acute phase proteins. It is known that inflammatory mediators as well as metabolic changes in the circulation result in alterations in gene expression levels in the liver (1, 3). Therefore, understanding the liver response at the molecular level is critical to understanding the systemic inflammatory disease, as well as its potential as a target for therapeutic approaches.

Prior studies using classical RT-PCR to analyze gene expression in liver have shown that inflammation upregulated specific receptors (such as CD14 receptors, protease activated receptors, histamine H-1 and H-2 receptors), transcription factors (NF-κβ, Stat3, and C/EBP-β) and other proteins or kinases (such as ERK, JNK, and p38) involved in the MAPK, Jac/STAT, and Ik-B/NF-kB signaling pathways (4–12). Recently, microarray technology and transcriptional profiling have been used to elucidate genome-wide changes in the liver following the burn injury (1, 3, 13). Vemula and co-workers (3) analyzed gene expression changes in rat livers during the first 24 h following the burn injury. Functional analysis of differentially expressed genes revealed that metabolism and inflammation accounted for the majority of the differentially expressed genes. Altered inflammatory genes included several classic acute phase response markers, and other genes involved in the complement, kinin, clotting, and fibrinolytic protein systems. On the other hand, metabolic genes showed that fatty acid oxidation increased after burn presumably to meet the enhanced energy demands. Dasu et al. (1) also analyzed the gene expression profiles in rat livers at different time points (2h, 6h, 24h, 240h) after a more severe burn than in the prior studies.

In general, unsupervised hierarchical clustering was applied in order to identify specific patterns of gene expression in the liver associated with burn injury. A limitation of the aforementioned studies is that the control sham-burn group was defined as the initial pre-burn condition corresponding to the 0 h time point. However, gene expression in a healthy animal liver naturally fluctuates over time due to circadian rhythms (14). In order to obtain a better resolution of the dynamics of the injury response, it is therefore necessary to account for the dynamics of the sham group as well.

In this study, we used a standard burn injury model of the rat to compare the dynamics of gene expression in liver in burn vs. sham conditions during the first 24 h. The differentially expressed genes between sham and burn condition over time whose expression patterns were significantly altered following burn were identified and clustered. Simultaneous analysis of both burn and sham-burn groups' expression profiles enabled to characterize the dynamic patterns of both groups and reveal a comprehensive picture regarding the

temporally coordinated inflammatory and metabolic changes in the liver following burn injury.

Material and Methods

Animal Model

Male Sprague-Dawley rats (Charles River Labs, Wilmington, MA) weighing between 150 and 200 g were used. The animals were housed in a temperature-controlled environment (25°C) with a 12-hour light-dark cycle and provided water and standard chow ad libitum. All experimental procedures were carried out in accordance with National Research Council guidelines and approved by the Rutgers University Animal Care and Facilities Committee.

A systemic hypermetabolic response was induced by applying a full-thickness burn on an area of the dorsal skin corresponding to 20% of the total body surface area (TBSA) as described elsewhere (15). This model was chosen because it has nearly 100% long-term survival, no evidence of systemic hypoperfusion, and no significant alterations on feeding patterns (13). Rats were first randomized into two groups: burn and sham burn (control group). Rats were anesthetized by intraperitoneal injection of 80 to 100 mg/kg ketamine + 12 to 10 mg/kg xylazine, and all hair removed from the dorsal abdominal area using electric clippers. The animal's back was immersed in water at 100°C for 10 s to produce a full-thickness scald injury covering 20% TBSA. Immediately after burns, the animals were resuscitated with 50 mL/kg of saline injected intraperitoneally. Negative controls (sham burn) consisted of animals treated identically but immersed in lukewarm water (37°C). Rats were single caged after burn or sham burn and given standard rat chow and water ad libitum until sacrifice. No post-burn analgesics were administered, consistent with other studies with this full thickness burn model since the nerve endings in the skin are destroyed and the skin becomes insensate (16). Furthermore, after animals woke up, they ate, drank and moved freely around the cage, responded to external stimuli, and did not show clinical signs of pain or distress. Animal body weights were monitored daily and found to increase at the same rate in both groups.

Microarray experiments to generate liver gene expression data have been explained elsewhere (3). Briefly, animals were sacrificed (starting at 9am) at different time points (0, 2, 4, 8, 16 and 24hr post-treatment, i.e., sham burn and burn) and liver tissues were collected, snap frozen in liquid nitrogen and stored at −80°C (n=3 per time point per group). The tissues were lysed and homogenized using Trizol, and the RNAs were further purified and treated with DNase using RNeasy columns (Qiagen). Then cRNAs prepared from the RNAs of liver tissues using protocols provided by Affymetrix were utilized to hybridize Rat Genome 230 2.0 Array (GeneChip, Affymetrix) comprised of more than 31,000 probe sets.

Data Analysis

In this study gene expression data analysis includes data preprocessing, filtering for “between class temporal differential expressions”, combining the datasets and clustering as seen in Figure 1. First, DNA chip analyzer (dChip) software (17) was used with invariant-set normalization and perfect match (PM) model to generate expression values. Microarray outlier filter analysis (18) identified that there were approximately 10% outliers in sham and burn gene expression data, which is typically observed in a microarray data. The outliers were replaced by the mean of the replicates (19). Then the data sets corresponding to burn and sham groups were investigated to identify the differentially expressed probesets by using the method (EDGE) proposed by Storey et al. (20). The statistical test used is analogue to an F statistics which compares the goodness of fit of the model under the null hypothesis to that under the alternative hypothesis. The null hypothesis model is obtained by fitting a

time-dependent curve to the two or more groups combined, and the alternative hypothesis model is obtained by fitting a separate curve to each group. The significance threshold for this analysis was set as *q-value* <0.01 and *p-value*<0.01. This step determined a set of probesets whose expression patterns were significantly altered following the treatment considering the temporal differences between the control and treatment groups. Finally the data sets corresponding to those differentially expressed probesets in either burn and/ or sham groups were combined to form one single matrix, which was then clustered using the a approach "consensus clustering" (21), in an unsupervised manner. This provided a set of burn responsive genes, which is significantly different than that of control group. We further applied one-way ANOVA test ($p<0.01$) independently for each gene in each cluster and animal group in order to verify if the gene has been differentially expressed across the time only. Moreover, t-test was used additionally for pair-wise comparison of burn and sham genes identified in the clusters at each time point in order to estimate the activation time of a certain response related to burn injury. We characterized the biological relevance of the intrinsic responses by evaluating the enrichment of the corresponding gene subsets using the KEGG database through ARRAYTRACK (22) as well as analyzing the functions of each individual gene (23).

Results and Discussion

We examined liver-specific gene expression levels at 0, 2, 4, 8, 16, and 24 h post-treatment consisting of a 20% total body surface area (TBSA) burn or sham-burn. 1534 temporally differentially expressed, i.e., burn responsive, were identified and subsequently clustered, using a consensus clustering approach, leading to identification of sub-set of genes assigned to 4 dominant expression patterns composed of 62, 82, 404, and 73 probe sets respectively. The average expression patterns of each dominant cluster are depicted in Figure 2 (right panel) while a heat map of all probe sets is shown in the left panel. A subset of critical genes is listed in Table 1, while a complete description for all 621 probe sets can be found in "Supplementary Materials". ArrayTrack, as well as single gene ontology analysis, was used to further elaborate the functional annotations of burn injury responsive genes.

Functional Characterization of major clusters

Cluster 1 (Figure 2) exhibits an early up-regulation during the first two hours following thermal injury. One-way ANOVA ($p<0.01$) indicates that the majority of the probesets in this cluster are not differentially expressed in sham-burn, clearly indicating that their activity is the result of the burn injury. Burn injury induces a rapid, but transient, up-regulation of the genes in this cluster which resolves within 8 hrs post-burn. Functional annotation and characterization of cluster 1 identifies cytokines, chemokines and chemokine receptors as well as genes related to the modulation of innate and adaptive immune responses, including: IL-1α, a pro-inflammatory cytokine playing a central role in the regulation of the immune response by binding to the IL-1 receptor (25) known to exhibit increased activity in the early stage of inflammatory response (26); chemokine (C-X-C motif) ligand 16 (CXCL16), a chemoattractant belonging to the CXC chemokine family whose expression is induced by inflammatory cytokines, such as IFN-γ and TNF-γ (27); chemokine (C-C motif) ligand 11 (CCL11), an inflammatory mediator belonging to the CC chemokine family that is known as Eotaxin-1; chemokine (C-C motif) ligand 9 known as macrophage inflammatory protein (MIP)-1γ which is constitutively expressed in macrophages (28). The release of the pro-inflammatory cytokines of Cluster 1 postburn are hypothesized to trigger and enhance the inflammatory response and to mediate catabolic effects (24). A list of representative genes is depicted in Table 1, while a detailed account of the gene information is provided in the supplementary material accompanying the manuscript.

Cluster 2 (Figure 2) exhibits characteristics of a persistent down regulation following burn injury, beginning at about 2hr post-burn, compared to the temporal differential expression under sham (ANOVA, $p<0.01$), thus indicating suppression of expression in burn. A Student t-test ($p<0.01$) reveals that the most significant suppression occurred at 8 h and 16 h post-burn. The functional characterization of Cluster 2 revealed down-regulation of genes involved primarily in unsaturated fatty acid biosynthesis, fatty acid metabolism, synthesis of ketone bodies and lipid metabolism and transport, consistent with earlier indications. The decrease in fatty acid biosynthesis as well as increase in fatty acid oxidation during the first 24h suggests that fatty acid is utilized in the liver during the first 24h postburn as the early energy source (3). Thus, down-regulation of fatty acid biosynthesis associated enzymes is possibly implying an enhanced energy demand. Prior studies elucidating the circadian rhythmicity of gene expression in rat indicated that fatty acid biosynthesis is up-regulated in the late afternoon and early evening hours (32). Our sham results, consistent with this observation, indicate the possibility of circadian rhythmicity in Cluster 2 with a return to base-line values within 24 hr. This observation leads us to speculate the possibility of circadian disruption following burn injury. Furthermore, genes related to cell-cell junctions are also identified in Cluster 2 including: ABLIM3, a molecular component of adherence junctions (AJs) and possibly a novel component of adherens junctions with actin-binding activity (33); alkaline ceramidase 2 encoded by A*cer2* playing an important role in regulating β1 maturation and cell adhesion mediated by β1 integrins (34); cadherin 17 is a Ca(2+)-dependent cell-cell adhesion molecule expressed in liver and intestine which plays a role in the morphological organization of liver and intestine (35). The products of those genes are associated with the integrity of the barrier function of hepatocytes linings. Given the many studies revealing that intestinal permeability is increased in burn patient shortly after the injury possibly due to the junction integrity alterations (36), the suppression of cell-cell junctions and membrane structural integrity may be indication of the liver damage caused by burn injury. Representative genes are depicted in Table 1, while a detailed account of the gene information is provided in the supplementary material accompanying the manuscript.

Cluster 3 (Figure 2) captures a dynamics response which, under burn, exhibits deviation from sham around 8 hr post injury. Beyond this point, the burn responses remain effectively suppressed (ANOVA, $p<0.01$). Functional annotation of Cluster 3 reveals gene products involved in complement and coagulation cascade, N-Glycan biosynthesis, ribosome and Jak-STAT signaling as well as involved in transcription/translation, protein synthesis/folding and targeting. All the above constitute processes critical in the production of acute phase proteins (APP) which are diffusible anti-inflammatory mediators (38). Furthermore, the anti-inflammatory response is induced by the suppressor of cytokine signaling proteins (SOCS) activated by Jak/STAT signaling pathway. Thus these gene encompassing Cluster 3 point to the activation of anti-inflammatory mechanisms resulting in an increase in the synthesis of the acute phase proteins and important anti-inflammatory cytokines. APPs produced by the liver is a prominent characteristic of the acute phase response following thermal injury, which is believed to be critical for the adaptation of the body to stress (39). In addition, the transcription of APPs is activated in the late phase starting around 8 h post-burn, consistent with previous observations that the level of amyloid A, a APP, is not increased until the concentration of IL-6, a late phase cytokine, increases (40). The requirement of the energy and amino acids (AA) to produce large amount of APP in liver are satisfied by the increased flux of amino acids from the periphery to the liver, especially from the accelerated breakdown of muscle proteins (41). The alterations in nitrogen and protein metabolism represent a major threat for the organism, as it leads to a debilitating loss of lean body mass (42). Thus, a sustained or exaggerated acute phase response has been shown to be an indicator of a potentially life threatening uncontrolled and prolonged action of pro-inflammatory cytokines leading to multiple-organ failure.

Critical cytokines in this cluster are well known anti-inflammatory cytokines such as IL-13 and IL-4. IL-13 inhibits the ability of host immune cells to destroy intracellular pathogens by recruiting a large number of Th2 cells while IL-4 induces differentiation of naive helper T cells (Th0 cells) to Th2 cells. IL-4 promotes the activation of macrophages into repair macrophages which is coupled with secretion of IL-10 and TGF-beta that result in the diminution of pathological inflammation. Anti-inflammatory cytokines, such as IL-4, are released later on in an attempt to counter-regulate the effects of the pro-inflammatory cytokines (43). Following the burn injury, a state of immunosuppression occurs whose intensity and duration is closely related to morbidity and mortality in burn patients (44). The inflammatory response after burn injury may play a role in the induction of adaptive immunosuppression. Both in *vivo* and *in vitro* studies manifest the altered adaptive immunity after burn which have shown that there is a decreased production of Th1- type cytokines (IL-2 and IFN-γ) and an increased production of Th2- type cytokines (IL-4 and IL-10) (45). In the current study, the gene expression of Th2- type cytokines, IL-4 and IL-13, is enhanced starting from 8 h post burn, which may imply the onset of the host immunosuppression. Our results reveal that the gene expression of critical proteins (IRAK1, LBP, and TRAF3) in the TLR4 signaling pathway is upregulated at 8 h post burn injury (Figure2, cluster 3). The TLR4 signaling pathway is critical for Gram-negative bacterial infections. It is well known that patients with severe burn injury are exceedingly susceptible to bacterial infections. Not only bacterial infection from the injured area but also bacterial translocation from the gut cause septic complications in the hosts. Mesenteric lymph nodes and liver indeed contain bacteria after burn injury in mice (46). It is generally accented that the decreased resistance to infection and enhanced secondary inflammatory response following serious injury is associated with abnormalities of both natural and adaptive immunity. Fang et al. (47) observed that thermal injury can markedly up-regulate lipopolysaccharide-binding protein (LBP) gene expression in various organs. LBP, a soluble acute-phase protein, binds to bacterial LPS to facilitate the immune response. Excessive LBP mRNA expression may be associated with enhanced synthesis and release of TNF-α stimulated by burn induced-endotoxin. Paterson et al. demonstrated that burn injury significantly increased TLR2- and TLR4-induced IL-1, IL-6, and TNF- α production by liver cells as early as 1 day after injury and they were found to be persistent for at least 7 days (48). Thus, the alteration of the TLR4 signaling pathway may imply that burn injury primes the innate immune system for enhanced TLR4-mediated responses to subsequent infection and provides evidence to suggest that an augmented Toll-like receptor signaling pathway might contribute to the development of increased systemic inflammation following severe burn injury.

Finally, a number of bile acid production related genes were also identified in this cluster. Bile acids are end products of cholesterol and the major driving force for bile formation, and the major excretory products of cholesterol. Bile acid production is expected to increase following burn injury (3). The main function of bile acids is to promote the formation of micelles, which facilitate fat digesting and absorption. Therefore, the enhanced production of bile acids may also reflect the demand of the energy from food intake. In fact, nutritional therapy is commonly used with burn patients (52) in an attempt to compensate for burn injury-induced metabolic abnormalities although it is limited given that it does not address the underlying mechanisms that are responsible for hypermetabolic and catabolic induction. Although nutritional supplements partially alleviate the hyper-catabolic condition, they seldom can cannot reverse or completely restore the nitrogen balance. Representative genes are listed in Table 1, and detailed information is provided in the supplementary material accompanying the manuscript.

Cluster 4 is encompasses genes which are downregulated long after the injury is induced (16 h post burn) (Figure 2, cluster 4). The probesets of this cluster in both sham and burn groups

exhibit an early down-regulation. Although the control group to recover their expression within 24 h, persistent downregulation is observed in the burn group. The maximum deviation between the sham-burn and burn groups occurs at 24 h postburn (two sample t-test, $p<0.01$). The genes in this cluster are involved in the insulin signaling pathway, glycine, serine and threonine metabolism, and galactose metabolism. Consistent with prior observations, our data point to the possibility of impaired insulin signaling (55). Insulin is an anabolic hormone which promotes the storage of substrates in liver by stimulating lipogenesis, glycogen and protein synthesis (56). Thus, downregulation of the genes involved in the insulin signaling pathway suggests a potential mechanism to explain the onset of a hypercatabolic state which is characteristic of hypermetabolism. Furthermore, the expression of genes associated with amino acid metabolism are known to be under circadian regulation in rat liver (32). Consistent with this observation, the insulin and amino acid metabolism-related genes in the sham-burn group also exhibited characteristics reminiscent of daily oscillation reaching a nadir at the interface of the light and dark phases. However, this daily oscillation was disrupted and suppressed maximally 24 h postburn, as demonstrated by the dynamic gene expression profile of the burn group pointing again to the possibility of circadian rhythms disruption. Representative genes are listed in Table 1, and detailed information is provided in the supplementary material accompanying the manuscript.

Assessing and interpreting the differences in transcriptional dynamics between sham and burn

The richness of our data and the fact that we analyze in tandem the dynamics of sham and burn injuries allowed to identify not only the differentially expressed responses but also the critical turning points where deviations induced by the burn injury manifest themselves. We identify, therefore, that the release of pro-inflammatory cytokines (Cluster 1) is almost instantaneous, whereas the synthesis of APP is delayed (Cluster 3), fatty acid biosynthesis (Cluster 2) precedes impairment of insulin signaling (Cluster 4). In a manner analogous to (57) we hypothesize that the time dependence among the profiles, may imply putative causal relations, which are succinctly summarized in the putative network structure of Figure 3, where arrows indicate possibly activation and/or induction, and circles indicate inhibition. The relations are derived based on the time lag elucidated from the temporal dynamics of individual responses.

The early upregulation of pro-inflammatory cytokines and chemokines, and their corresponding receptors in Cluster 1 indicates the activation of the immune system and a pro-inflammatory response, whereas the suppression of fatty acid biosynthesis associated genes in Cluster 2 implies an enhanced energy demand. In Cluster 3, the downregulation of the genes functioning as cell-cell junctions and providing membrane structural integrity indicate possible damage caused by the injury. Later activation of the expression of well-known anti-inflammatory cytokines may suggest the upcoming immune suppression. The activation of the Toll-like receptor signaling pathway, also in the Cluster 3, is possibly indicative of a priming effect to a subsequent secondary stimulus, i.e., infection. The most significant feature of Cluster 3 is the enhanced production of positive APPs, which is correlated to hyper-catabolism in muscle. In the same cluster, the enhanced expression of bile acid synthesis related genes may also be an indication of enhanced energy demand from nutrition supply. Finally, the late downregulation of the insulin signaling pathway-associated genes in Cluster 4 leads to the catabolism and insulin resistance. The dynamic picture which is assembled is indicative of the fact that once a pro-inflammatory response has been mounted there is a subsequent release of signals stimulating an anti-inflammatory response that inhibits the pro-inflammatory response, which drives the system back to homeostasis. The burn-induced response in Cluster 4, representing insulin-mediated metabolism, was

characterized by an early and persistent downregulation. While prior work (58), indicated the possibility of an early downregulation the anabolic response in liver, our results indicate that the down-regulation is in fact delayed in time, given that the nature progression of the sham responses also points to an early down-regulation, although it recovers, pointing to the possibility that the burn-specific down-regulation occurs only later in time. Therefore, we argue that the onset of insulin resistance and the putative associated catabolic response (which is regulated by insulin) is not as immediate as previously thought (58). A delayed response is in fact more consistent with the observed dynamics of cytokine and chemokine activation, which presumably drive the molecular mechanisms leading to insulin resistance (59), because impaired insulin signaling should occur after and not before the release of cytokines. In addition, insulin is known to suppress bile acid synthesis in cultured rat hepatocytes by down-regulating the key enzymes in the synthesis of bile acids from cholesterol. Therefore, the impaired insulin signaling can also explain the increased bile acid excretion observed in humans with untreated diabetes mellitus and in experimental animals with insulin deficiency (60). However, in our study, the insulin signaling pathway was suppressed around 16 h postburn, which was later than the enhanced production of bile acids. Thus, our results suggest that the increased bile acid production in inflammation is more likely caused by mechanisms other than the impaired insulin signaling pathway. Moreover, insulin is a well-known critical anabolic hormone which promotes the storage of substrates in liver such as lipids (56). However, since the decreased synthesis of fatty acids (~2 h postburn) occurs earlier than the impaired insulin signaling (~16 h postburn), the suppression of fatty acid biosynthesis herein may not be caused by the insulin resistance either.

We observed that a significant number of positive APP genes were up-regulated, which requires an increased energy utilization. Thus, the biosynthesis of unsaturated fatty acid starts to be suppressed around 2 h post burn which may imply the preservation of the energy sources for the synthesis of positive APP activated later - around 8h post burn. This suggests that FA is utilized in the liver during the first 16 h after the burn injury. However, new energy sources and AA pool to produce positive APP are required after the exhaustion of available energy and AAs in the liver. It is well known that burn injury results in accelerated breakdown of muscle proteins which increase the AA concentrations in the circulation thus AA uptake-rates in the liver (41). We observed that the impaired insulin signaling pathway occurs later, starting around 16h postburn consistent with previous reports indicating that muscle protein breakdown is exacerbated in burn-injured patients as a result of insulin resistance (61). Thus, our results suggest the possibility that the impaired insulin signaling pathway may further intensify the catabolic response which has already been driven by the uncontrolled positive APP production. In addition, due to the suppression of fatty acid biosynthesis, it seems that the fatty acid oxidation might serve as the main source of energy in the liver following the burn injury.

Conclusions

We have shown a short-term liver gene expression profiling in response to thermal injury. This analysis characterizing the dynamic patterns of both burn and sham groups elucidated that temporal changes in the expression levels after the injury are mainly associated with the pro-inflammatory response, fatty acid biosynthesis, the anti-inflammatory response, and insulin-regulated metabolic responses. The network of dynamic changes in gene expression observed in this study revealed the possible links between the diverse burn-induced responses. Based on our results, the pro-inflammatory response is activated immediately around 2 h following burn treatment which triggers the anti-inflammatory response starting around 8 h postburn. The biosynthesis of unsaturated fatty acid starts to be suppressed around 2 h which may imply the preservation of the energy sources for the synthesis of

APPs whose genes were activated later around 8 h post burn. In addition, the impaired insulin signaling pathway, starting from around 16 h postburn and putatively as a result of the alterations in inflammatory gene expression, is expected to further strengthen the catabolic response. A Suppression of fatty acid synthesis and enhanced production of bile acids were also observed, but were not likely due to the impaired insulin signaling because of the discrepancy in the dynamics of these responses. In conclusion, simultaneous analysis of both burn and sham-burn groups' expression profiles enables to characterize the dynamic patterns of both groups. Our results reveal critical gene expression pattern changes triggered by burn injury which reflects host physiological and biological alterations and provides a more comprehensive understanding of the pathophysiology of the disease state.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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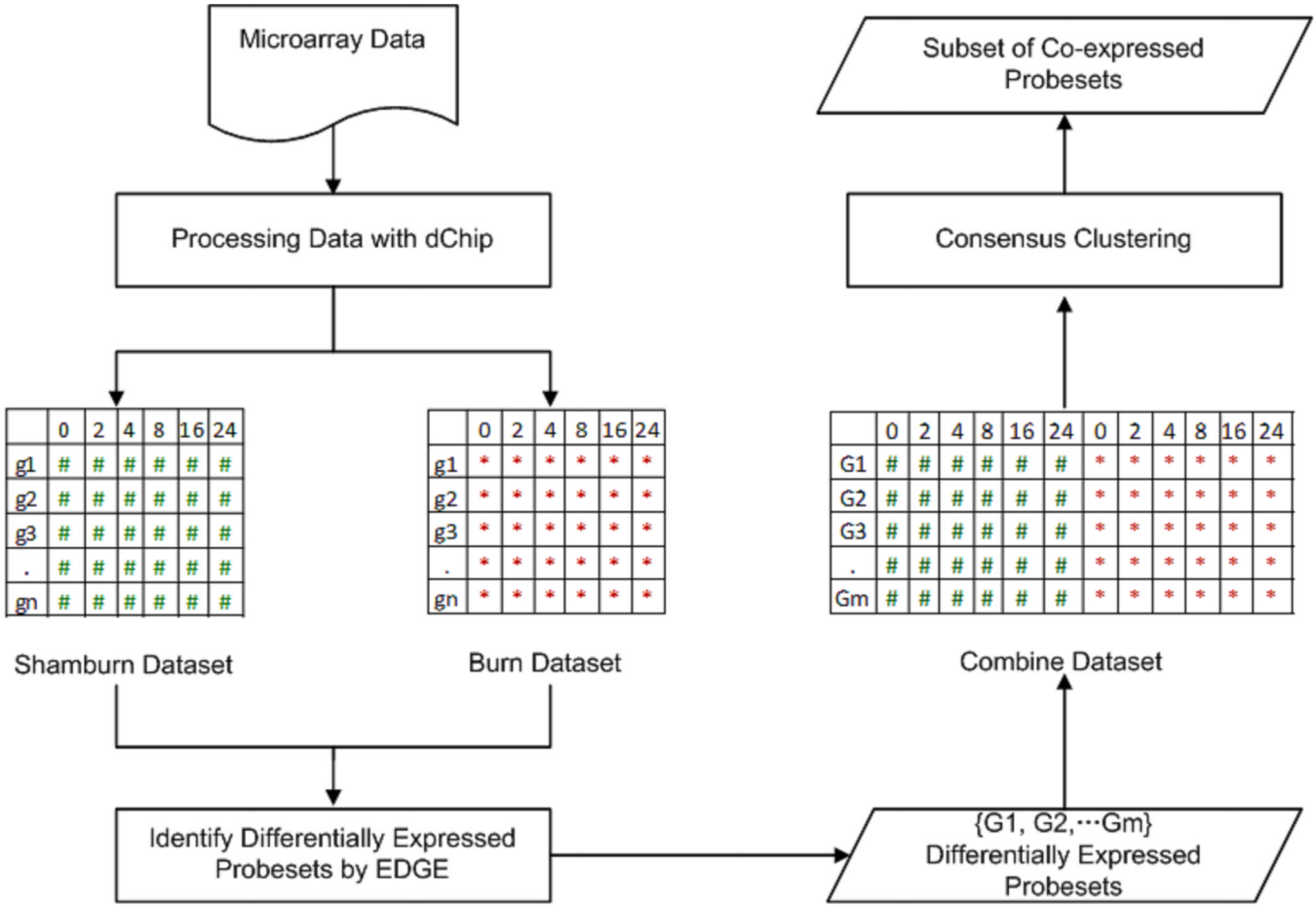

Figure 1. Schematic overview of the microarray data analysis

Microarray data was preprocessed by using dChip. Then, two data sets corresponding to burn and sham groups, respectively, were analyzed to identify the differentially expressed probesets by using EDGE with 'between classes' option under the statistical threshold $q<0.01$, $p<0.01$. Finally, the data sets corresponding to those differentially expressed probesets in burn and sham groups were combined to form one single matrix, which was then clustered using the approach of "consensus clustering" with threshold $p<0.01$.

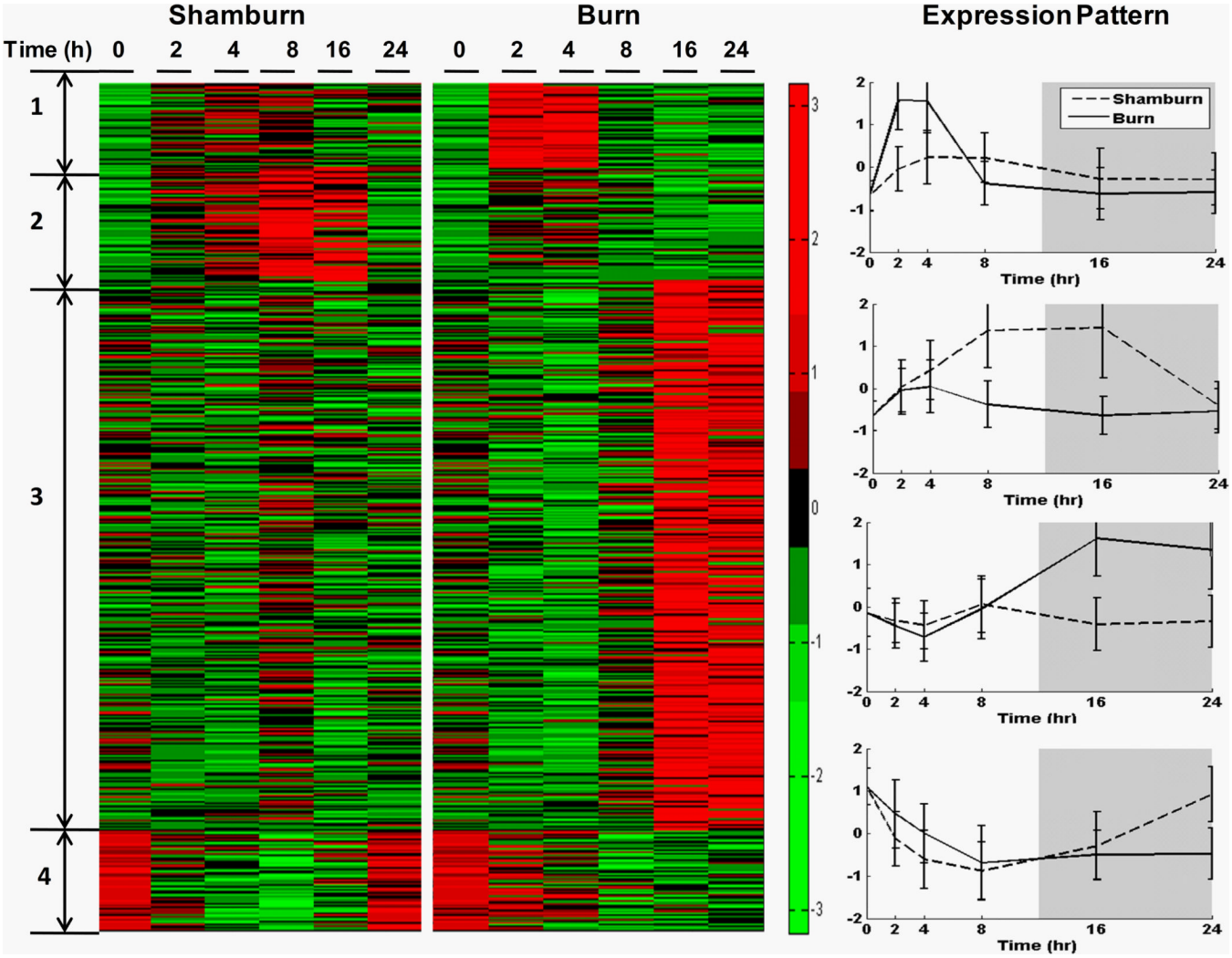

Figure 2. Gene expression profiles of rat livers in response to sham-burn or burn injury

Left Panel, expressions of 62, 82, 404 and 73 probesets in 4 clusters in sham-burn rats and burn rats at 0, 2, 4, 8, 16, 24 h post-treatment are exhibited in a heatmap.

Right Panel, the expression patterns are shown by plotting the average normalized (z-score) expression values of 62, 82, 404 and 73 probe sets in 4 clusters in sham-burn and burn groups (displayed as the means ± SEM).

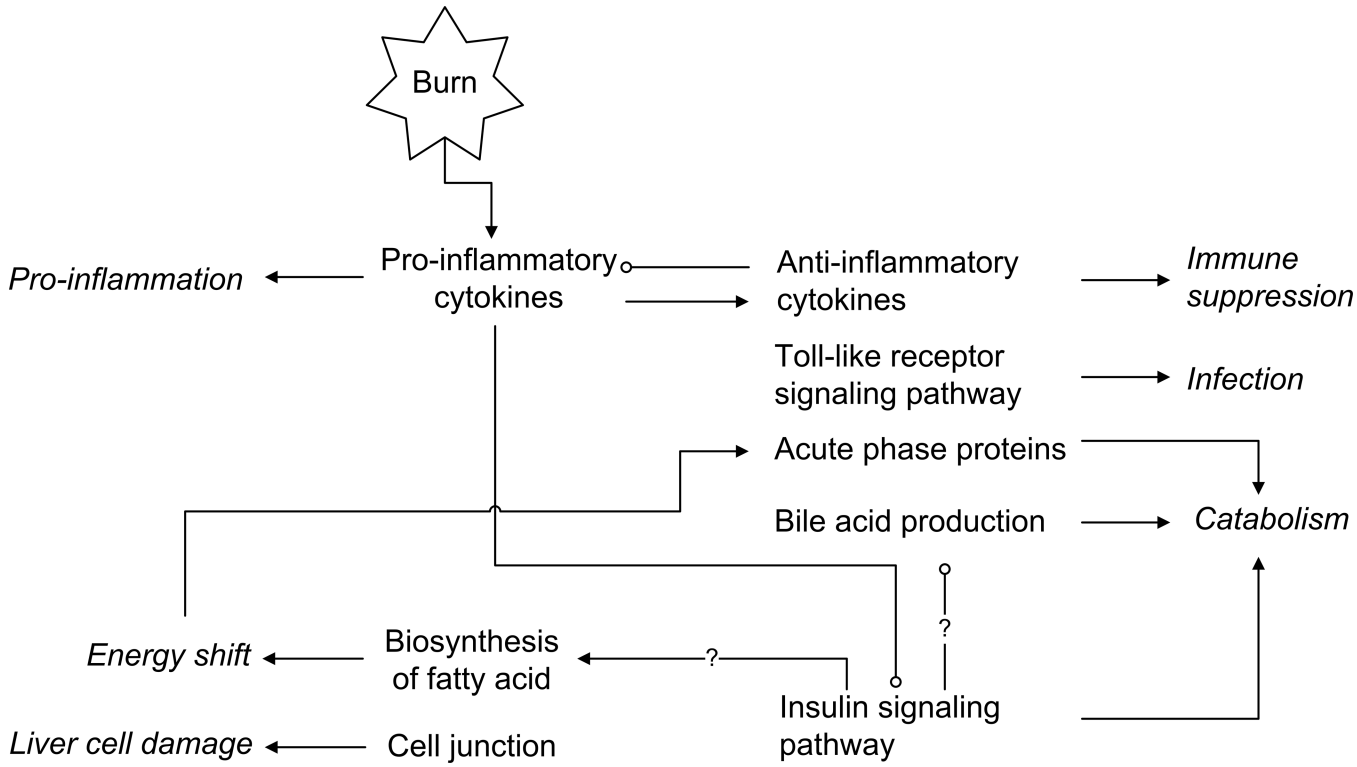

Figure 3. Proposed network of changes in the liver following burn injury

Italics represent outcomes following burn-induced gene expression alterations. Arrows indicate activation and/or induction, and circles indicate inhibition.

Table 1

Information of critical genes in each of the four clusters

Cluster number	Function	Gene name
1	Pro-inflammatory Cytokine	*IL1a*
	Chemokine	*Cxcl16, Ccl11, Ccl9*
	Adaptive immune response regulation	*Ceacam1*
2	Unsaturated fatty acid biosynthesis	*Acot1, Acot2, Acot3*
	Fatty acid metabolism	*Acaa2, Cpt1a, Dci*
	Synthesis of ketone bodies	*Hmgcs2*
	Lipid metabolism and lipid transport	*Eci1, Pigo, cyp4b1, Adfp, Pnpla8, Pank4, Crot, Etfdh*
	Cell-cell junctions	*Ablim3, Acer2, Cdh17*
3	Complement and coagulation cascade	*C2, C4bpa, C8a, Cfh, Masp1, Serping1*
	N-glycan biosynthesis	*B4galt1, Dad1, Ddost, Dpagt1, Ganab, Man1b1*
	Ribosome	*Rps25, Rps2*
	Jak-STAT signaling	*Il13, Il4, Il7, Jak3*
	TLR4 signaling	*IRAK1, LBP, TRAF3*
	Anti-inflammatory cytokine	*Il13, Il4*
	Transcription	*Brca1, Mcm7, Tcf25, Kdm1, Nfyb, Tef, Cited4, Otx1, Sox4, Acvr1, Tbx2, Zfhx2, Dmrt2, Tsc22d1, Ccdc 101*
	Translation	*Atpif1, Mrps21, Rps25, Rps2, Mrps11*
	Protein folding	*Dnajb11, Ppib, Hyou1, Edem2, Sep15, Pdia6, Dnajc3, Pdia3, Ugcgl1, Sec63, Mlec, Mecp2*
	Protein degradation	*Pcolce, Cpn1, caspase 12, Cdc34, Spink3, Hspa5, RGD1306508, Erlec1, Aph1a, Derl2, Os9, Rnf20, Ppp2r5c, Aurkaip1, Prss32, Prepl, Tbl1xr1, St14, P4hb*
	Protein target	*Tmed3, Ssr4, Tram1, Sec61a1, Rrbp1, Arfgap2, Gabarap, Erp29, Ssr3, Rrbp1, Sec61a1, Derl1, Gosr2, Cope, Tmed2, Copz1, Copg, Sec13, Abcb10, Kdelr1, Kdelr2, Tram1, Serp1, Ssr3, Atp6v0d1, Rabac1, Vps28*
	Bile acid production	*Idi1, tmem97, Npc2, Hsd17b4*
4	Insulin signaling pathway	*Gck, Irs1, Mknk2, Trip10*
	Phenylalanine metabolism	*Ddc*
	Glycine, serine and threonine metabolism	*Bhmt*
	Galactose metabolism	*Gck*