RESEARCH

Application of the cDNA macroarray technology aimed to detect gene expression changes following burn injury

✉ Isabel A Guillén, Freya Freire, Danay Cibrian, Jorge Berlanga-Acosta

Pharmaceutical Division and Cell Biology Department, Center for Genetic Engineering and Biotechnology
CP 10600, PO Box 6162, Cubanacan, Playa, Havana, Cuba
Fax: 53-7 271 4764; E-mail: isabel.guillen@cigb.edu.cu

ABSTRACT

Thermal injury is one of the most severe forms of trauma that affects every internal organ system. Therefore, the identification of the molecular mechanisms responsible for post-burn immune dysfunction, increased susceptibility to sepsis, and multiple organ failure under such conditions demand the development of improved pharmacological methods. Using a flexible system for gene expression profiling, we have investigated the gene expression of 1 152 unknown murine genes, obtained from mice exposed to a thermal insult. Genes with altered expression levels appeared both in the liver and jejunum of scalded mice in relation to the sham-scalded counterparts as early as 4 hours after the thermal injury. We identified 45 genes whose expression appeared up-regulated and 28 genes that were found to be down-regulated. To confirm five of the measurements detected, we re-examined the corresponding gene expression profile using the RT-PCR technique. The five genes selected, which represent about 3.33% of the overall positive signals, rendered similar expression profiles with the two techniques applied. Gene expression analysis reveled upregulation of Zinc Finger Protein 207 in the jejunum, the far upstream element binding protein and the Cadherin L genes in the liver, and down regulation of β Interferon, EST (AK015189) genes in the liver, and Cadherin L gene in the jejunum; none of them had ever been directly related with burn injuries in mice.

Keywords: DNA macroarrays, scalded mice, RT-PCR, burn

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RESUMEN

Aplicación de la tecnología de los macroarreglos de ADN al estudio de los cambios en la expresión génica inducidos por el daño por quemadura. El daño térmico es una de las formas de trauma más severas y complicadas, la cual afecta a cada sistema de órganos internos. Es así como, la identificación de los mecanismos moleculares responsables de la alteración de la respuesta inmune, el incremento de la sepsis, y el fallo múltiple de órganos en estas condiciones patológicas, ameritan del desarrollo de nuevas y más eficientes modalidades farmacológicas. Nosotros interrogamos con el ARN mensajero obtenido de ratones sometidos a un daño térmico, el perfil de expresión génico y los agrupamientos por niveles de expresión de 1 152 genes de ratón, punteados de forma duplicada sobre un soporte flexible. A las 4 horas del daño térmico se identificaron genes con niveles de expresión alterados tanto en el hígado como en el yeyuno de ratones quemados en relación con los controles no quemados. Con este estudio se identificaron 45 genes que aumentaron su expresión y 28 genes que la disminuyeron. Para confirmar los resultados obtenidos en cinco genes seleccionados por macroarreglos, se realizo la técnica de RT-PCR. Los 5 genes seleccionados, los cuales representan alrededor del 3.33% de todas las señales positivas obtenidas, mostraron un perfil de expresión similar entre las dos técnicas aplicadas. El análisis de la expresión génica reveló un aumento de los niveles de expresión de los genes *Zinc Finger Protein* 207 en el yeyuno, el *far upstream element binding protein* y la *Cadherin L* en el hígado, y disminución de la expresión de los genes Beta Interferon y el EST (AK015189) en el hígado, y la *Cadherin L* en el yeyuno. Ninguno de ellos antes relacionado directamente con el daño por quemadura en ratones.

Palabras claves: Macroarreglos de ADN, ratones quemados, RT-PCR

*I*ntroduction

Severe cutaneous thermal injuries remain a serious medical emergency, commonly associated with multiple organ dysfunctions, and demanding intensive and multidisciplinary medical interventions to ensure survival [1, 2]. While current procedures for burn injury management have improved; the patient's prognosis, morbidity and mortality remain major concerns. The identification of the molecular mechanisms responsible for post-burn immune dysfunction, increased susceptibility to sepsis, and multiple organ failure (MOF) under such conditions are therefore, demanding for the development of improved pharmacological methods [3].

The genetic background of the population suggests variability between individuals, particularly exemplified by differences in scar formation [4]. Clinical variability and unpredictability may be even larger when the cutaneous thermal injury, having different causal agents, is severe enough as to lead the victim to internal organs complications and to MOF.

Thermal injury is a universal trauma for animal economy and its molecular pathophysiology has remained largely entangled due to the complex interactions between cells and soluble mediators [5]. Although different technologies have been described for monitoring gene expression, complementary DNA (cDNA) microarray analysis has become increasingly important [6]. Modern advances in DNA array technology have allowed the characterization of physiologic and pathologic conditions from a global genomic viewpoint

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✉ Corresponding author

[7]. Indeed, the use of these genomic profiling techniques on diseased human tissue samples, will fuel the emergence of revolutionary and personalized therapeutic interventions, based on the rational understanding of the molecular pathophysiology of a process. Furthermore, it will also contribute to the understanding of the molecular bases of the therapeutic effects shown by growth factors and cytokine in humans [8, 9].

The main purpose of this paper is to provide evidence to scientists, committed with basic research in burn injury on the potential benefits of DNA macroarrays to determine the genes whose products may be critical for the patients' recovery.

Materials and methods

Animal model

Adult male Balb/c mice (22-25 g) were purchased from the National Center for Laboratory Animals (CENPALAB, Havana, Cuba) and maintained under controlled environmental conditions in a certified room of the animal facility at the Center for Genetic Engineering and Biotechnology (Havana, Cuba). Access to food and water was not restricted.

Ethics

This experiment was conducted according to current regulatory and ethical guidelines of the animal welfare committee of the animal facility at the Center for Genetic Engineering and Biotechnology, Havana, Cuba.

Experimental groups and scalding procedure

Two animals were randomly assigned to each of the following groups.

(A)- Mice receiving an intraperitoneal injection of normal saline solution (0.5 mL) and scalded.

(B)- Mice receiving an intraperitoneal injection of normal saline solution (0.5 mL) and sham scalded.

Diazepam was given as a pre-anesthetic medication, at 40 mg/kg, followed by ketamine at 50 mg/kg, both i.p. and a brief exposure to a diethylether mask before scalding. Dorsal scalds were induced [10] by immersing a pre-delimited and depilated dorsal area for 5 seconds in equilibrated water at 99-100 °C. All the mice were resuscitated with an intraperitoneal injection of 0.9% normal saline solution (40 mL/kg). Group B mice used as controls were not scalded but subjected to the same anesthetic medication procedures. Following actual or sham scalding maneuvers, mice were individually caged and left to evolve for 4 hours. A ketamine over-dose was used for termination. The liver and jejunum were selected as target tissues for gene expression, monitoring the effects of the acute impact of the scald.

Experimental design

Scalded mice were compared using a class comparison design [11], with sham-scalded counterparts in a direct relation (treated/control). The class comparison identified which genes were differentially expressed among the classes and the identification of patterns of differential expression within it.

RNA preparation

Fragments of liver and jejunum tissues were immediately frozen in liquid nitrogen and processed to assure the extraction of high quality mRNA. Total RNA was extracted from homogenized tissue samples using Tri Reagent (Sigma Chemical co., St. Louis, MO). RNA quality was verified by the visualization of 28S and 18S ribosomal bands in 1% non-denaturing agarose gel. A fixed amount of 25 µg of total RNA treated with DNase I, was used to extract the mRNA with the Quick Preparation Micro mRNA Purification Kit (Amersham Pharmacia Biotech, UK). Once the mRNA was extracted, the individual samples per organ (100 ng) and from each animal were pooled according to the corresponding experimental group. Liver and jejunum mRNA pools were generated. The mRNA mixture was reverse transcribed to first strand cDNA and labeled with the thermostable alkaline phosphatase enzyme according to the manufacturer's instructions using the AlkPhos kit (Amersham Pharmacia Biotech, UK).

cDNA library expansion

The commercial cDNA library of eleven day old mice (Catalog Number ML5015t, Clontech, Palo Alto, CA, USA) was transformed in DH5α cells by electroporation and grown overnight at 37 °C in solid Luria Broth medium with 50 µg/mL of ampicillin (LBA). Individual colonies were grown in the liquid LBA medium overnight at 37 °C.

Plasmid Purification

The R.E.A.L. Prep 96 Plasmid Kit (Quiagen, Germany) protocol was used as described by the manufacturer. A total of 1152 purified plasmid clones were organized in three plates of 384 wells and kept at an approximate concentration of 100 ng/µL in a 0.3 x SSC (NaCl, Sodium citrate, pH = 7) and 0.1% Sarcosyl and 0.25% bromophenol blue solution.

Macroarray construction

The macroarrays used in this report were produced at the Center for Genetic Engineering and Biotechnology, Cuba. The cDNA macroarray filter was designed using 2 filters of Nytran MS positively charged nylon membrane (Schleicher & Shuell, Germany) with 1 152 unknown mice genes in duplicated spots, yielding 2 304 spots per filter. The macroarray also contains 88 spots of known selected genes used as controls for filter design and performance. These included genes coding for growth factors, interleukins, beta amyloid protein, cell cycle kinases, transcription factors, erythroproietin, and housekeeping genes as glyceraldehyde 3-phosphate dehydrogenase and the β actin gene. Filter design included some areas where no DNA was spotted.

The pin printing head used (VP 386V) and its window frame alignment device (386VF) are commercially available (V & P Scientific Inc., San Diego CA, USA) [12]. DNA plasmid spotted on the membrane was left to dry for 30 minutes at room temperature (RT) and denatured with 0.4 N NaOH and 3 M NaCL for 5 minutes at RT. The membrane was later neutralized with 6 x SSC for 5 minutes. The filters were dried at RT and exposed to UV light at 200-mjoules/cm^2 using a Stratalinker 1 800 (Stratagene, La Jolla, CA, USA).

Macroarray hybridization

The macroarray filters were hybridized overnight with a chemiluminescent first strand cDNA probe (100 ng

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of labeled cDNA/filter), with 15 mL of the commercial hybridization solution AlkPhos (Amersham Pharmacia Biotech, UK) at 55 °C. Then, the membranes were washed and developed according to AlkPhos kit manufacturer's instructions. The blots were stripped at 60 °C in 0.5% SDS for one hour. Finally, blots were rinsed for 5 minutes at RT with Tris pH 8.0. The signal was detected using the CDP-Start system (Amersham Pharmacia Biotech, UK). In order to ensure the experimental consistency of the results, we used intra-filter hybridizations (hybridization of the same filter several times with different samples) at the conditions to be related in our experiments. The conditions related were A/B in liver and jejunum.

Image analysis

Chemiluminescence signal detection was performed by the exposure of the macroarray filter to Fuji Medical X-rays Films (Fuji Photographic Co, LTD, Japan) at different times to obtain the best image for the analysis. Films were scanned with a Hewlett Packard ScanJet 5100C Scanner for the quantification of the chemiluminescence signals. All the images were standardized by maximum image intensity as well as background intensity by the ScanJet 5100C and the Adobe Photoshop version 7 (Adobe system, USA, 1990-2002). The ArrayVision Software version 8 (Imaging research Inc., St. Catherine, Ontario, Canada) was used for signal quantification. A normalization step was applied in order to distinguish true biological changes from random noise or non-specific experimental variation [13]. Results on array quantification and normalization were expressed as the relative expression levels compared with the control.

Gene sequencing

The selected genes were sequenced with the pGAD10 primer: 5'-CCA AAC CCA AAA AAA GAG ATC TCT CG-3' at Tm: 66 °C, using the ALFexpress equipment, ReproGel High resolution and Thermo Sequenase Cy5 Dye Terminator Cycle Sequencing Kit (Amershan Biosciences, UK).

Semiquantitative reverse transcription-polymerase chain reaction (RT-PCR)

The same source of total RNA used in primary gene expression profiling was used in the RT-PCR assay. Approximately three micrograms (µg) of total RNA (mixture of 1.5 µg of total RNA per animal in the group), were reverse transcribed using an oligo-dT primer in 20 µL of the total volume of the reaction; approximately 0.3 µg of the resulting cDNA were used by the reaction of the PCR technique (we assume that total RNA has approximately 2% mRNA) [14]. The PCR was performed using the following primers for mice genes (*Mus musculus*):

Zinc finger protein 207 5'-ATA GTT CCT GGT ATG TGG GAA GAT-3' and 5'-CCA AGG TGG GCC TCC TCG AC-3', far upstream element binding protein (FBP) 5'-GTA ATC CTA AGA GGT TTA TCA G-3' and 5'-GGT CTT GTA TGC TAA CTG GAA CAC-3', beta interferon 5'-GCG TTC CTG CTG TGC TTC TCC AC-3' and 5'AAT AGC TCT TCA AGT GGA GAG CAG-3', cadherin L 5'-GGA GAAGTC CAC GGT GAA CTT ACA ATG-3' and 5'-GGG TGC TCC TCA CTG AAA CAA GAC AGT-3', b actin 5'-TGA CGG GGT CAC CCA CAC TGT GCC CAT CTA-3' and 5'-CTA GAA GCA TTG CGG TGG ACG ATG GAG GG-3', expression sequences tags (EST AK015189) 5'-CAT GAG TTG GGC CCA GGC CGT GGT AG-3' and 5'-CGT GGC ACC AGT CTC CTC ATA AAG T-3'

The design of the PCR was as follows: thirty cycles at 95 °C for 1 minute, 52 °C (for the zinc finger protein 207 gene), 50 °C (for the FBP gene), 55 °C (for the beta interferon gene), 53 °C (for the cadherin gene), 60 °C (for beta actin gene), 54 °C (for EST AK015189) for 1 minute, and 72 °C for one minute. A final primer extension at 72 °C for five minutes completed the reaction.

After amplification, the products were detected using 1% agarose gel in TAE buffer (50 x TAE is 242 g of the Tris base, 57.1 mL of glacial acetic acid, and 100 mL of 0.5 M EDTA, pH 8.0) with 10 µL of the PCR pro-duct and 2 µL of the loading buffer (0.25% bromophenol blue, 0.25% xylene cyanol FF, 30% glycerol) and the run was at 120 V for 30 min. The PCR products with a 207 bp for Zinc Finger Protein 207, 276 bp for FBP, 397 bp for beta interferon, 362bp for cadherin, 600 bp β actin, 438 bp for EST (AK015189) and negative controls were stained with ethidium bromide and visualized under UV light. The DNA concentration was determined by densitometry measurement of the electronic gel image using Kodak Digital Science 1D software (Eastman Kodak Co, Rochester NY).

Results

After obtaining liver and jejunum samples from mice with dorsal scalds involving 25-30% of body surface, the first step consisted of showing the optimal RNA integrity and quality (data not shown). The commercial RNA extraction kits rendered good results in terms of a pure and non-degraded RNA, compatible with the range of enzymatic reactions and detection systems used in gene expression analyses.

The following step was to use cDNA macroarrays containing gene sequences representative of 1 152 different mouse genes to analyze the gene expression profiles of two experimental groups. We observed an expression pattern obtained in the experiment using the pooled RNA from two mice that were scalded and undamaged. Two macroarray filters were hybridized twice to compare the gene expression patterns associated to each experimental setting. Filters were assigned as follows: filter 1 was hybridized with mRNA extracted from liver fragments of mice from groups A and B. Filter 2 was hybridized with mRNA harvested from the jejunum of groups A and B, representing its scalded and sham scalded mice groups respectively. Results from this combination would show gene expression changes produced by scalding inflicted in the liver and jejunum.

Computer analysis of the 1 152-cDNA elements on the array, provided information on the cDNA hybridization signals in filters 1 and 2. In total, 73 genes had changed the expression levels two fold or more from the base line (up or down regulated). The expression of 45 genes was increased two fold or more, whereas the expression of 28 genes exhibited decreased levels (Table 1).

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For five gene products whose expression met no change or differential expression, the criteria were selected from the macroarrays to analyze their behavior by the RT-PCR technique. Their sequence analysis revealed a high degree of homology (more than 98% homology) with mice genes reported in the gene bank database. The selected genes were the ZFP (zinc finger protein 207), FBP (Far upstream element binding protein), β Interferon, cadherin and the EST (expression sequence tags (accession number in the Gene Bank AK015189)) (Figure 1). The reliability of the RT-PCR assay is indicated by the lack of a specific signal for the H_2O control (data not shown). Mouse β actin was used as the housekeeping gene. According to DNA array hybridization, β actin transcript was detected at very similar levels in all the conditions studied (approximately Log_2 A/B: 0.01, very near the base line).

The relative changes in the expression of several genes as determined by the cDNA array and RT-PCR are presented in table 2. The semiquantitative conventional RT-PCR technique confirmed the majority of the cDNA array expression profile. Numerical differences between both assays were considered to be due to the intrinsic properties of each technique.

Discussion

Most tissue and organ responses to thermal injury are gene-mediated events that may range from the onset of cellular pro-survival mechanisms and inflammation, up to programmed cell death. These cellular and molecular processes are expressed in the clinical arena as immune response demise, prolonged and uncontrolled metabolism, as in multiple organ dysfunctions [8].

Methods to screen endogenous gene expression are important both for functional genomics as for potential medical applications. DNA macroarrays offer the opportunity to compare large numbers of samples to generate a more global view of a biological system. This technology may come to unravel the connection

Table 1. Filter quantification results using the arrayvision software.

Gene quantification	Filter 1 Ratio: A/B Organ: liver	Filter 2 Ratio: A/B Organ: jejunum
Number of genes with positive signals in the macroarray (percent referred to the total number of genes in the array)	149 (12.93%)	159 (13.80%)
Number of the up-regulated genes that increase the expression more than two fold from the base line (values=0) (log2 treated/untreated) (percent referred to the total number of genes with a positive signal in the macroarray).	30 (20.13%)	15 (9.43%)
Number of down regulated genes that decrease the expression more than two fold from the base line (log2 treated/untreated) (percent referred to the total number of genes with a positive signal in the macroarray).	23 (15.43%)	5 (3.14%)
Number of genes with a non-variable expression (±2 fold variation of the expression from the base line) (log2 treated/untreated) (percent referred to the total number of genes with a positive signal in the macroarray).	95 (63.75%)	139 (87.42)

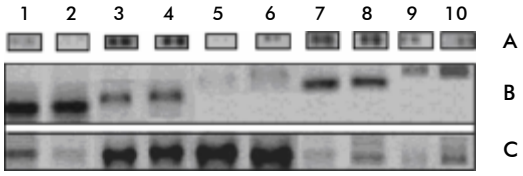

Figure 1. Macroarray and RT-PCR results. The RT-PCR technique was developed at different times for each gene analyzed; the figure shown a photographic composition of different agarose gels electrophoresis. Positions from 1 to 10 represent the gene expression profiles measured by macroarray (panel A) and by RT-PCR (panel B and C). Columns 1 and 2 represent the jejunum ZFP expression, columns 3 and 4 represent liver FBP expression, columns 5 and 6 represent liver β-interferon expression, columns 7 and 8 represent liver Cadherin expression, and columns 9 and 10 represent liver EST expression. Columns 1, 3, 5, 7, and 9 represent mice treated with saline and scalded and columns 2, 4, 6, 8, and 10 represent mice treated with saline and sham scalded. Panel C: RT-PCR results by using the β actin gene expression as the housekeeping gene.

Table 2. Comparison of the macroarray gene expression profile and the RT-PCR assay. The table shows the semiquantitative results of the five genes selected by macroarray and analyzed by RT-PCR

Gene name and access number in the Gene Bank	Tissue	DNA macroarrays The numbers are the ratios between sample/control	Semiquantitative RT-PCR The numbers are the ratios between sample/control	Gene function
Zinc Finger Protein 207 (ZFP) (BC003715)	Jejunum	Log2 A/B: 5.06	Log2A/B: 0.071	Nucleic acid binding; nucleus; regulation of transcription, DNA-dependent; transcription factor activity; zinc ion binding [16 and 17]
Far upstream element binding protein (FBP), (BC014763)	Liver	Log2 A/B: 0.31	Log2 A/B: 0.083	Activated gene expression. Contributes directly to c-myc transcription while bound to a single-strand site [18]
Beta Interferon (NM 010510)	Liver	Log 2 A/B: -3.33	Log 2 A/B: -0.27	Cytokine activity [19]
Cadherin L (X77557.1)	Liver	Log 2 A/B: 0.32	Log 2 A/B: 0.71	Calcium ion binding; cell adhesion; cytoplasm; homophilic cell adhesion; integral to membrane; membrane; plasma membrane Family of calcium-dependent adhesion molecules [20]
	Jejunum	Log 2 A/B: -1.12	Log 2 A/B: -0.25	
EST (AK015189)	Liver	Log 2 A/B:-0.021	Log 2 A/B:-0.05	DNA binding; nucleus; regulation of transcription, DNA-dependent; signal transducer activity; signal transduction [21]

between the injury and the ensued changes in cellular behavior, thus contributing to set up novel and comprehensive pathophysiological cascades, and therefore to introduce target aimed therapeutic strategies with a more rational approach [21].

We have developed a cDNA macroarray system by using nylon filters containing arrayed cDNA sequences derived from a commercial mouse library to test the capability of this technology to detect genes that appeared differentially expressed following a cutaneous thermal trauma using a validated model [23]. By means of comparative analyses performed between multiple hybridization using appropriate probes, this study revealed a diverse group of genes with altered expression levels in the liver and jejunum of mice subjected to scalding in relation to their uninjured counterparts. From a pool of 1 152 mice genes arrayed in nylon filters, DNA macroarrays allowed us to identify 45 genes that were up regulated and 28 genes that were down regulated. Other authors have analyzed the global changes in gene expression in injured murine skin and liver, following scalding [22, 23]. Similar to our studies in internal organs, theses studies revealed a prevalence of the up-regulation of gene expression after burn injury, also revealing in the gene ontology analysis an integrated up-regulation of genes involved in metabolic pathways, inflammatory processes and protease activity.

The liver and jejunum were the target organs selected to analyze gene expression changes as they are frequently affected in severely burned patients; the former is compromised by fulminant functional failure, and the latter because of its barrier function it is jeopardized following shock, thus contributing to toxic-septic translocation [24]. We have also aimed this genetic approach toward the acquisition of biologically relevant information within specific tissue niches. Indeed, the actual challenge of the macroarray system is to harvest biologically useful and applicable information [25].

The accuracy of the macroarray system was confirmed by RT-PCR. Specifically FBP gene expression was up regulated, whereas EST and beta interferon genes were down regulated in both analyses. The cadherin gene was up or down regulated in relation to the tissue analyzed. The ZFP gene expression was less confirmed due to large differences in expression levels revealed by the macroarray and the RT-PCR (71.26 fold). This discordance may be attributed to specific features of each technology (macroarrays and semiquantitative RT-PCR) as to their reliability to identify differentially expressed genes. Other factors are array production, RNA extraction, probe labeling, hybridization conditions and image analysis influence on macroarrays results. On the another hand, the conventional RT-PCR requires small amounts of RNA but its end-point analysis lacks precision for a quantitative determination. The later technique is affected by electrophoretic resolution, weak and differential priming, analysis of banding patterns, and procedures for band characterization. Thus, these differences may turn into numerical discordances between the two methods [26].

Post-burn multiple organ dysfunctions is one of the major causes of death in severely burned victims and its pathogenesis is complicated. Different studies highlight the relationship between apoptosis and multiple organ failure [27]. In our study, three of the genes analyzed are described as related with the apoptotic event. For example, the loss of function in the murine zinc finger protein (ZFP) family has been implicated in an increase in programmed cell death and a decrease in the mitotic index irrespective to the p53 function [28]. On the other hand, the FBP gene tended to increase its expression in the liver following the burn injury infliction, which may be simplistically interpreted as a signal toward either apoptosis or cell cycle arrest suppression. FBP binds to the single-stranded far upstream element that controls c-*myc* expression, while *myc* is a well-reputed regulator of the cell cycle and of apoptosis in many cell lineages [29, 30]. These observations suggest that the interpretation of the eventual outcome of any tissue seems to be complex, requires far more data as an integrative vision of the biological event. An appropriate knowledge of gene/protein physiology is also mandatory to prevent a misleading conclusion on the role of the genes whose expression is changing.

This experiment showed the tendency toward a reduction of β-interferon gene expression in the liver of burned animals. Altered cytokine induction profiles, including the suppression of the Th1 family members are believed to contribute to burn-associated immunosuppression and to the eventual development of sepsis [31]. Beta interferon has been shown to act as a pro-survival factor for T cells whereas its down-regulation leads to apoptosis, which may unquestionably contribute to the burned patient's immune dysfunction [32].

The cadherin gene exhibited a differential expression pattern in the liver and gut; up-regulated in the liver tissue and down regulated in the jejunum. Thus, gene expression in response to injury seems to be ruled in a tissue context and a time frame dependent manner, which demands caution while translating the data into a biological language. It is likely that the role played by the same gene/protein may change from one tissue to another even under the same triggering insult. The cadherin gene superfamily is particularly implicated in cell-to-cell adhesion and recognition within epithelial organs like the gut. Besides, cadherins are also involved in epithelial cell motility, growth, differentiation, and survival. Recent studies highlight the importance of cadherin gene expression up-regulation as a protective effect against apoptosis following burn injury [27, 33]. Indeed, this may suggest that liver cadherin expression enhancement may represent a successful genetic pro-rescue maneuver. Conversely, its down regulation in the jejunum may be indicative of a barrier failure not only because of apoptosis, but also due to cell junction breakdown.

The field of micro and macroarray expression analysis is today one of the most potent and sophisticated technologies for gene expression analysis. Literature is becoming plethoric with still more novel analytical methods each day. However, the gap between information technology derived data and an appropriate biomedical meaningful interpretation requires bridges made by a solid cell and tissue physiology understanding, knowledge of signaling pathways and molecular pathophysiology, as an integrative clinical and biological

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thought. Although the main purpose of this article is to encourage the application of this technology as a tool for the burn associated tissue damage research, we have attempted to use illustrative examples and hypothetical biological explanations on the bases of our findings. Certainly, in a non distant future this emerging technology will ensure novel therapeutic interventions for the critical burned victim.

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