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A microarray analysis of temporal gene expression profiles in thermally injured human skin

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ABSTRACT

Partial-thickness burns incite a multitude of responses which eventually culminate in cutaneous wound repair. We hypothesized that these events would evoke extensive alterations in gene expression thereby orchestrating the complexity of spatial and temporal events that characterize "normal" human wound healing. In the present study, gene expression from partial-thickness areas at defined temporal periods (1–3 days, 4–6 days, and 7–18 days) after injury were compared to normal non-wounded skin. Gene alterations proved extensive (2286 genes). Statistically significant alterations were noted among increased and decreased genes expressed in the three different temporal groupings. Our foundational data (based on samples from 45 individuals) provide a comprehensive molecular gene expression portrait of the cutaneous reparative responses that are initiated during the first 17 days after injury. Our efforts also represent an initial endeavor to move beyond the historically defined "morphological phases" of wound repair toward reporting molecular clues that define the temporal sequence of healing in human subjects. Further analysis of genes that are either affected or remain not affected following injury to normal skin is expected to identify potential targets for therapeutic augmentation or silencing.

1. Introduction

Thermal injury to the skin can induce local and systemic perturbations that are costly in terms of human suffering as well as in strains on the health care system. While these unexpected cutaneous injuries are neither as prevalent as chronic wounds nor as well studied, these acute wounds are nevertheless substantial in terms of their numbers. According to the 2005 estimates by the American Burn Association, burn injuries in the United States exceeded 1.25 million. Approximately 600,000 burn patients per annum require emergency treatment while 50,000 victims sustain burn injuries severe enough to warrant admission to specialized burn centers. Deep partial-thickness and full-thickness skin damage that encompass large body surface areas create significant therapeutic challenges and measurably increase morbidity and mortality [1,2]. Recently, a multi-centered, NIH funded microarray gene analysis was initiated to address the systemic inflammatory changes that occur after burn injury [3]. This top-down approach was designed to focus on data derived from blood samples and muscle biopsies while utilizing a diverse population to define simultaneous molecular derangements that occur in burn and trauma patients [3]. Molecular events within the cutaneous wound itself were not targeted for microarray analysis and have remained unexplored, a situation we sought to remedy in the present study.

Thermal injury to the skin evokes a cascade of events resulting in progressive deepening of the zone of injury during

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the first 24–48 h after injury [4–6]. In the days following injury, extensive gene expression alterations impart a host of derangements that can exert an overwhelmingly negative impact on the reparative capacity of human skin. While the literature is filled with postulated mediators of this progressive inflammation such as neuropeptides [7], pro-inflammatory interleukins-1, 6, and 8 [8–10], arachidonic acid pathway products [11], and tumor necrosis factors [12], no interventional therapies have emerged as standards of care to counteract the inevitable progression in the depth and extent of burn injury [5]. Deeper injuries usually require surgical excision with skin replacement through autografts, allografts, temporary dressings or permanent skin substitutes. Regardless of the treatment plan, most healing scenarios give way to extensive hypertrophic scarring and contracture, an undesirable scenario that develops in 30–60% of burn wounds [13,14].

The initial genomic approach aimed to describe the local events within a burn appeared in 2003 [15]. This early microarray study defined 35 over-expressed or under-expressed genes in hypertrophic scars and served as a valuable outcome study but was not designed to uncover evidence as to why burn wounds have the propensity to scar and undergo excessive fibrosis [16]. A potentially more gainful means to study hypertrophic scar lies in uncovering events occurring earlier during the acute wound phase that eventually lead to unsightly aesthetic results and functional impairments typical of hypertrophic scars [5].

To date, the more acute responses of human skin to injury and the sequential early events of human wound healing have not yet been examined through a functional genomic approach. The present study is based on our hypothesis that the perturbations within wounded skin during the acute period after injury and later during the subsequent processes of wound repair are best identified using a comprehensive method to analyze diverse patterns of genetic expression. To achieve our primary goal, a microarray experiment was devised to monitor changes in gene expression within the target organ—injured skin from 45 burn patients as compared to normal skin from 15 healthy patients (Table 1). As a secondary goal, our study was designed to establish a foundational time-course aimed toward elucidating the sequential molecular events during wound healing that define the first 17 days after injury. Our complex dataset offers a screening approach that can contribute statistical precision in defining the temporal sequence of after cutaneous injury in previously normal skin that presumably has the capacity to heal. All parameters for this initial examination were deliberately designed to be broadly inclusive. The goal of our discovery process was to reflect universal molecular alterations (confirmed with statistical confidence) across a diverse population of patients with acute wounds.

2. Material and methods

2.1. Study design

A total of 60 patients were recruited for this study according to a protocol approved by the Institutional Review Board at Vanderbilt University. The control group consisted of normal skin specimens from 15 patients undergoing elective cosmetic surgery procedures in which excess skin was removed as a part of the operative plan (e.g. reduction mammaplasty, abdominoplasty, and blepharoplasty). Exclusion criteria included specimens that might have poor skin quality due to extensive stretch, sun exposure, or recurring skin lesions; medical comorbidity; or history of thwarted healing or recurrent skin infections. Exclusions were made based on findings of the investigators after a thorough history and physical examination was taken on each patient. Normal skin specimens in the control group were provided by a predominance of female patients (13/15 patents) that ranged in age from 26 to 63 years. For these 15 control patients, the mean age was 39.5 years (median = 43).

The 45 patients who provided specimens for the burn group were identified by daily inspection of the Vanderbilt University Burn Center census. All human subjects in this study were inpatients with cutaneous injuries severe enough to warrant operative excision for deep partial-thickness or full-thickness burn. The demographics of this group were varied in regards to age (16–80 years), total body surface area burned (TBSA), depth of burn, and underlying comorbid conditions. Grossly infected specimens were excluded from this study. The mean age of the burn group was 39.7 years with a median of 37. Table 1 contains further demographic information. Vanderbilt's standards of care for burn victims such as standard wound management with topical antimicrobials and antibiotics and nutritional supplementation were consistently applied to this population that consented to supply injured skin tissues.

Skin specimens from burn patients were arbitrarily placed into three groups based on time from burn insult to operative excision (post-burn day = PBD), which loosely corresponds to the phases of wound healing. We have previously published our unsupervised multivariate principal component analysis of protein expression patterns from similar burn wound samples that appropriately clustered dataset into their correct temporal healing periods [17]. This earlier study indicated a unique protein signature within each of three time periods after burn injury. Thus patient samples for microarray analysis were clustered similarly into early (PBD 0–3), middle (PBD 4–7), and late (PBD 7–17) time groupings.

2.2. Sample pooling

Since the goal of this study was to derive data that would be broadly representative of the general burn population, the burn patients recruited for this microarray analysis represented a wide demographic spectrum. In order to limit the possibility that the effects of any one patient's individual-specific genetic response to trauma would skew the results, equal quantities of RNA from five patients in each group were combined to create a pooled RNA sample for microarray hybridization [18–20]. The impact of individual genetic alterations that might represent extremes in comparison to the population is blunted when the RNA containing those "outlier" changes is combined with RNA corresponding to more expected (in terms of population-wide) responses to burn. Pooling of samples provided a cost-effective means to increase the power of the microarray study while minimizing the financial impact. For example, without pooling, the budget