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Rejuvenation of Gene Expression Pattern of Aged Human Skin by Broadband Light Treatment: A Pilot Study

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Studies in model organisms suggest that aged cells can be functionally rejuvenated, but whether this concept applies to human skin is unclear. Here we apply 3′-end sequencing for expression quantification ("3-seq") to discover the gene expression program associated with human photoaging and intrinsic skin aging (collectively termed "skin aging"), and the impact of broadband light (BBL) treatment. We find that skin aging was associated with a significantly altered expression level of 2,265 coding and noncoding RNAs, of which 1,293 became "rejuvenated" after BBL treatment; i.e., they became more similar to their expression level in youthful skin. Rejuvenated genes (RGs) included several known key regulators of organismal longevity and their proximal long noncoding RNAs. Skin aging is not associated with systematic changes in 3′-end mRNA processing. Hence, BBL treatment can restore gene expression pattern of photoaged and intrinsically aged human skin to resemble young skin. In addition, our data reveal, to our knowledge, a previously unreported set of targets that may lead to new insights into the human skin aging process.

Journal of Investigative Dermatology (2013) **133,** 394–402; doi:10.1038/jid.2012.287; published online 30 August 2012

INTRODUCTION

Aging is under complex genetic and environmental control. Aging is associated with large-scale changes in gene expression, and how such changes may be modulated for healthful benefits in human beings is not clear. Numerous single-gene mutations have been identified that can extend the lifespan of model organisms (Partridge, 2010; de Magalhaes *et al.*, 2012), and dietary restriction can slow the rate of aging, even if applied late in life (Partridge, 2010). More recently, several interventions have been shown to confer features of youthfulness to aged cells or tissues, demonstrating a remarkable plasticity of the aging process. For instance, heterochronic parabiosis between young and old mice enables circulatory factors to restore the functions of aged muscle stem cells (Liu and Rando, 2011). Similarly, inducible blockade of the transcription factor NF-κB in aged murine epidermis can abrogate cellular senescence and restore the global gene expression program of old skin to resemble that of young skin (Adler *et al.*, 2007). An important question is whether similar plasticity exists in human skin, where aging occurs over decades rather than over months or years as seen in model organisms. Defining clinically viable strategies to unlock the plasticity of human aging is a critical challenge.

An ideal technology to test this concept is broadband light (BBL), also known as intense pulse light, a commonly available and popular treatment to ''rejuvenate'' the skin. According to the American Society for Aesthetic Plastic Surgery, over $215 million dollars were spent in the United States in 2009 on these procedures. Unlike ablative light-based treatments that improve the overall appearance of aged skin through thermal destruction and regrowth of the epidermis and superficial dermis, BBL uses a broad band of noncoherent light waves, ranging from 560 to 1,200 nm, that are absorbed by a number of components in the skin. Currently, BBL procedures are used to decrease the appearance of fine rhytides, dyspigmentation, erythema, and elastosis (Bitter Jr, 2000; Negishi *et al.*, 2001). Nevertheless, the molecular changes that are induced by this treatment are unclear.

''Rejuvenation'' is a term that has been used by many investigators and the lay public with different meanings, and thus needs to be carefully defined. Here we define ''rejuvenation'' as the restoration of characteristics of youthfulness to aged cells and tissues. After BBL treatment, is the skin truly ''rejuvenated'' at a molecular level, i.e., more closely resembles younger skin, or is the treatment merely inducing a wounding or scarring response that differs fundamentally from uninjured youthful skin?

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This study was accepted as a poster presentation at the 2012 Society of Investigative Dermatology Annual Meeting

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Abbreviations: BBL, broadband light; GO, Gene Ontology; lncRNA, long noncoding RNA; polyA, polyadenylated; qRT–PCR, quantitative reverse transcription–PCR; RG, rejuvenated gene; 3-seq, 3′-end sequencing for expression quantification

Received 19 February 2012; revised 19 June 2012; accepted 11 July 2012; published online 30 August 2012

Histologically, BBL has been reported to diminish melanin deposition in the dermis and reduce telangiectasias (Bitter Jr, 2000; Prieto *et al.*, 2002), with some reports also reporting an increase in new upper papillary dermal collagen formation at 3 weeks after treatment (Negishi *et al.*, 2001). However, this neocollagen formation may be a more variable or short-term effect, as ultrastructural analyses of skin 3 months after treatment have not shown any collagen or elastin fiber effects (Prieto *et al.*, 2002). We examine the molecular basis of the BBL treatment response by defining the global gene expression programs of photoaged and intrinsically aged human skin and response to BBL. The intent is to capture the broadest spectrum of changes in RNA induced by aging and BBL, including alterations in gene expression (coding and noncoding) and gene regulation.

RESULTS

Clinical and histologic changes after BBL treatment

To gain insights into the gene expression program associated with skin aging and BBL treatment, we used skin biopsies from young female volunteers (age <30 years, $n=5$) and site-matched untreated and treated skin of aged female volunteers (age >50 years, $n=5$), the latter after three courses of monthly BBL treatment ($n=5$; Figure 1a). The treated subjects were healthy *older* females with moderate to severe photodamage on the forearms, and resided in the Santa Clara or San Jose, California metropolitan area, where on average there are 257 sunny days out of 365 days, with the average UV Index being 5.1 (average UV Index in the United States is 4.3; source: www.bestplaces.net, accessed 25 April 2012). Tanning beds, topical retinoids, or any other skin treatments on the arms were prohibited for 1 month before enrollment and during the study. During the study, the participants were instructed to sun-protect their arms with a broad-spectrum sunscreen and long-sleeved clothing, as well as avoid prolonged sun exposure. The untreated young subjects had the same inclusion criteria, but did not have evidence of photoaging on the arms.

After three BBL treatments, arm skin showed improvements in clinical ratings of intrinsic and extrinsic skin aging parameters: fine wrinkling ($P=0.03$), abnormal pigmentation ($P=0.02$), and global skin aging assessment ($P=0.01$; Figure 1a–c). On histologic examination, the elastotic fibers in the treated aged samples were found to be diminished and less distinct compared with those in untreated aged samples (Figure 1d–g). The periodic acid–Schiff stain showed no obvious changes in collagen quantity in the dermis between treated and untreated aged samples, although they did appear less disordered after treatment (Figure 1h and i). The treated aged samples also displayed subjective increases in epidermal thickness (Figure 1e, g and i) compared with untreated aged samples (Figure 1d, f and h).

Expression program of coding and noncoding RNAs in aging skin

Although gene expression programs of aging in several tissues have been previously examined by microarray hybridization, we used 3′-end sequencing for expression quantification (3-seq), an efficient strategy of deep sequencing of RNA 3′ ends (Tariq *et al.*, 2011). The potential advantages of 3-seq include accurate quantification of transcript levels not obscured by cross-hybridization, an ability to determine alterations in RNA termination and processing, and the ability to discover previously unannotated genes, such as long noncoding RNAs (lncRNAs). We generated 6.5–12.4 million uniquely mappable reads for each sample, and identified differentially expressed transcripts using DESeq algorithm (see Materials and Methods).

To rigorously define aging in molecular terms, we first identified transcript alterations associated with aging by comparing untreated young with untreated aged samples, and then tested how BBL treatment to aged skin affected these parameters.

Comparison of mRNA transcript levels in untreated young versus untreated aged, as well as untreated aged versus treated aged, samples revealed a consistent significant change in the expression level in 3,530 genes (Figure 2a). The directionality of the gene expression change with BBL treatment is shown in Figure 2a, with blue indicating a 2-fold decrease and yellow indicating a 2-fold increase. Genes whose transcript levels changed significantly between untreated young and untreated aged ($n=2,265$) are shown in Supplementary Table S1 online.

To visually display the locations of significant genes on the large heat map (Figure 2a), we have provided columns (in magenta) to the right of the large heat map that represent biological themes, according to Gene Ontology (GO) terms. For instance, the ''rejuvenated genes'' (RGs) and lncRNAs are distributed on both the upper and lower parts of the large heat map. In contrast, the ''immune response'' genes and ''translation'' genes are located on the lower half of the heat map. The ''cell adhesion'' genes are located on the upper half of the large heat map and are decreased in the untreated young group, increased in the untreated aged group, and intermediate in the treated aged group. The magenta columns hence provide a general sense of what biological function is altered and in what direction (increased (yellow) or decreased (blue)), enabling comparison between untreated aged, treated aged, and untreated young in the large heat map. For instance, both the treated older samples and the young untreated samples show increased transcript levels in ''immune response'' and ''translation,'' as both these groups are ''up'' (yellow). In contrast, the untreated aged group shows decreased transcript levels, or ''down'' (blue) in ''immune response'' and ''translation'' genes compared with the other two groups.

The gene programs associated with aging are multifaceted, and are enriched for several biological themes. The top five most significant GO terms that are *increased* in the aged untreated compared with young untreated group included *translation* ($P=4.7\times10^{-12}$), *translational elongation* ($P=5.1\times10^{-7}$), *macromolecular complex assembly* ($P=7.5\times10^{-6}$), *ncRNA metabolic processing* ($P=6.2\times10^{-6}$), and *RNA processing* ($P=2.5\times10^{-6}$). The top five GO terms that *decreased* in the aged untreated group compared with the young untreated group were genes encoding functions related to *cell adhesion* ($P=1.5\times10^{-17}$),