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Genomics Analysis of the Reduced Anti-oxidant Capacity of Aging Skin

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INTRODUCTION

Reactive oxygen species (ROS) are considered to play important roles in the process of UV radiation-induced skin damage, skin photo-aging and melanogenesis (1). Much less is known about the role of ROS in intrinsic skin aging, although ROS have long been implicated in the aging process in other organ systems.

The antioxidant response element (ARE) is a transcriptional control element that meditates a family of Phase 2 enzymes and anti-oxidant proteins, under control of the transcription factor NRF2. Induction of ARE-dependent genes plays an important role in protection of cells against oxidative damage. The up-regulated family of proteins can protect against this damage not only by increasing endogenous antioxidant levels in cells but also by up-regulating proteins that monitor for and repair the damage caused by ROS. Transcription of this family of enzymes, therefore, provides a powerful protection and repair mechanism for our skin against the continual assault of UV and environmentally induced ROS.

OBJECTIVE

To analyze gene expression in young versus old sun-protected and sun-damaged skin for differences related to response to oxidative stress.

METHODS

Ten young (18-20) and ten aged (60-67) female subjects were recruited. Three 4 mm punch biopsies were obtained from each of two skin sites: sun-protected (buttocks) and sun-exposed (outer forearm). The older subjects had moderate to severe photo-damage. RNA was extracted and purified from the biopsies. Labeled target cRNA was synthesized and analyzed using Affymetrix HG-U133 Plus 2.0 microarrays, containing 54613 probe sets covering the entire human genome. The data were subjected to rigorous quality control, statistical and bioinformatic analysis.

To identify regulated genes related to antioxidant defenses or oxidative stress the following steps were used.

1) Functional annotation for genes on the HG-U133 Plus 2.0 GeneChip from the Gene Ontology, EntrezGene and SwissProt databases was searched for the following terms: oxidative stress OR antioxidant OR free radical OR hydrogen peroxide OR organic peroxide OR superoxide OR reactive oxygen species. A total of 245 unique annotated genes were identified.

2) The list of oxidative stress-related genes was subjected to the following filtering to identify genes regulated in either intrinsic or photoaging.
- At least 10% Present calls across all samples (>= 4 Present calls)
- *t*-test p value <0.05 for the Arm OR Buttock Older to Younger comparisons
- Fold change<-1.5 OR >1.5 for the Arm OR Buttock Older to Younger comparisons

A total of 27 genes met the filtering criteria (see table).

RESULTS (27 genes regulated in aging skin related to oxidative stress)

	Gene Title	Gene Symbol	Old to Young Fold Change		Old to Young *t*-test p value		Function
			Arm	Buttock	Arm	Buttock	
Key transcription factors	nuclear factor (erythroid-derived 2)-like 2	NRF2 (NFE2L2)	-2.04	-1.52	7.81E-03	5.00E-02	Transcription activator that binds to antioxidant response elements (ARE). Important for the coordinated up-regulation of genes in response to oxidative stress.
	metal-regulatory transcription factor 1	MTF1	-1.6	-1.19	1.03E-05	9.57E-03	Transcription factor that induces expression of metallothioneins. Involved in protection against metal toxicity.
Markers of oxidative stress	ceruloplasmin (ferroxidase)	CP	3.49	1.53	1.38E-06	6.04E-03	Plays a role in iron metabolism and homeostasis; indicator of oxidative stress.
	clusterin	CLU	1.64	1.41	8.34E-05	2.48E-03	Appears to be an indicator of stress including oxidative stress.
	toll-like receptor 4	TLR4	2.29	1.33	1.36E-03	3.00E-02	Functions in innate immune activated by reactive oxygen and bacterial LPS.
H_2O_2 Production	superoxide dismutase 3, extracellular	SOD3	2.48	1.52	4.12E-10	2.11E-05	Catalyzes conversion of superoxide to hydrogen peroxide.
	aldehyde oxidase 1	AOX1	2.72	1.54	5.97E-06	6.66E-04	Produces hydrogen peroxide and, under certain conditions, can catalyze the formation of superoxide.
H_2O_2 Detoxification	glutathione peroxidase 2	GPX2	-1	-1.51	9.00E-01	1.88E-03	ARE-regulated gene. Detoxification of hydrogen peroxide.
Anti-oxidant related genes	aldehyde dehydrogenase 3 family, member A2	ALDH3A2	-1.63	-1.2	1.92E-04	5.00E-02	Detoxification of aldehydes generated by alcohol metabolism and lipid peroxidation.
	24-dehydrocholesterol reductase	DHCR24	-1.71	-1.69	1.93E-03	4.67E-04	Catalyzes the reduction of the delta-24 double bond of sterols. Protects from oxidative stress by reducing caspase 3 activity during apoptosis induced by oxidative stress.
	microsomal glutathione S-transferase 1	MGST1	-1.33	-2	2.80E-01	1.00E-02	ARE-regulated gene. Catalyzes the conjugation of glutathione to electrophiles and the reduction of lipid hydroperoxides.
	solute carrier family 23 (nucleobase transporters), member 2	SLC23A2	-1.75	-1.2	7.95E-05	1.00E-01	High affinity vitamin C transporter.
Skin Structure Associated	apolipoprotein C-I	APOC1	-2.86	-2.56	8.80E-03	1.98E-03	Lipid transport. Involved in regulation of epidermal barrier lipids. Mice over expressing APOC1 develop atopic dermatitis.
	ceroid-lipofuscinosis, neuronal 8 (epilepsy, progressive with mental retardation)	CLN8	-2.44	-1.37	2.37E-04	1.00E-02	Postulated to function in lipid synthesis, transport, or sensing. Oxidative stress associated with defects in this gene.
	S100 calcium binding protein A7	S100A7	-1.8	-5.09	3.50E-01	1.32E-03	Exact function unknown, highly up-regulated in hyperkeratoses such as psoriasis. Induced in response to reactive oxygen species.
	apolipoprotein E	APOE	-1.56	-1.17	2.39E-03	6.00E-02	Lipid transport. Specific alleles associated with Alzheimer disease.
	collagen, type I, alpha 1	COL1A1	1.03	-1.7	7.60E-01	1.00E-02	Major dermal collagen. Expression can be up-regulated by hydrogen peroxide.
Apoptosis	uveal autoantigen with coiled-coil domains and ankyrin repeats	UACA	1.62	1.35	4.51 E-03	2.00E-02	May modulate cell shape and motility after injury. Positive regulator of apoptosis.
	serine/threonine kinase 4	STK4	-1.53	-1.19	1.00E-02	1.80E-01	Stress-activated, pro-apoptotic kinase activated by oxidative stress.
Cellular Health & Metabolism	heat shock 60kDa protein 1 (chaperonin)	HSPD1	-2.1	-1.02	7.48E-06	8.00E-01	Member chaperonin family. Essential for the folding and assembly of newly imported proteins in the mitochondria.
	cytochrome c, somatic	CYCS	-1.93	-1.22	1.61E-04	2.20E-01	Component of the electron transport chain in mitochondria, involved in initiation of apoptosis.
	NADH dehydrogenase (ubiquinone) Fe-S protein 1, 75kDa (NADH-coenzyme Q reductase)	NDUFS1	-1.9	-1.23	2.42E-05	5.00E-02	Part of mitochondrial electron transport chain. Transfers electrons from NADH to the respiratory chain.
	ATPase, Cu++ transporting, alpha polypeptide (Menkes syndrome)	ATP7A	-1.79	-1.39	3.68E-03	8.00E-02	Under conditions of elevated extracellular copper, functions in the efflux of copper.
	phosphoinositide-3-kinase, class 3	PIK3C3	-1.74	-1.13	5.96E-04	2.10E-01	Activation and intracellular signaling mediates leukotriene B4-induced chemotaxis, generation of reactive oxygen species, and degranulation.
	serum/glucocorticoid regulated kinase 2	SGK2	-1.62	-1.72	0.0019	2.18E-03	Involved in the activation of potassium channels. Activated by hydrogen peroxide.
	arachidonate 12-lipoxygenase	ALOX12	-1.39	-1.62	1.15E-03	1.00E-02	Involved in leukotriene biosynthesis. Can release superoxide.
	ectodermal-neural cortex (with BTB-like domain)	ENC1	1.01	-1.63	8.40E-01	2.94E-03	p53-inducible actin binding protein.

Cells colored if p ≤ 0.05 and fold change ≤ -1.2	
Cells colored if p ≤ 0.05 and fold change ≥ 1.2	
Highlighting: key genes indicative of oxidative stress in aging skin	

SUMMARY

- In general, the patterns of expression of oxidative stress-related genes in aging skin were similar in photo-protected (buttock) and photo-damaged (arm) sites with regard to directionality.
- Key transcription factors that regulate protective mechanisms were down-regulated with aging:
  - **NRF2** regulates genes with antioxidant response elements
  - **MTF1** regulates metallothioneins
- Markers of oxidative stress (**ceruloplasmin** and **clusterin**) were substantially up-regulated in aging skin.
- There was substantial up-regulation of genes related to H_2O_2 production (**SOD3** and **AOX1**) without increases in necessary detoxifying enzymes (catalase and glutathione peroxidase), which may contribute to oxidative stress in aging skin.
  - **GPX2** was significantly down-regulated in intrinsically aged skin
  - H_2O_2 can induce **MMP2** (gelatinase A) (2,3), which was significantly up-regulated in photoaged skin in this study. Increased MMP activity induced by oxidative stress will contribute to the loss of dermal matrix associated with aging.
- The pattern of expression observed in aging skin, with increased **ceruloplasmin** and **SOD** and decreased **glutathione peroxidase,** resembles that reported in rheumatoid arthritis (5).
  - Up-regulation of **TLR4** suggests activation of the innate immune response in aging skin, possibly associated with oxidative stress.
- Other antioxidant-related genes were down-regulated in aging skin: **ALDH3A2, DHCR24, MGST1, SLC23A2.**

CONCLUSIONS

- Global gene expression profiling of intrinsically aged and photoaged skin reveals evidence of oxidative stress in both cases and decreased capacity to detoxify reactive oxygen species. These changes likely contribute to the gradual deterioration of skin with intrinsic and extrinsic aging.

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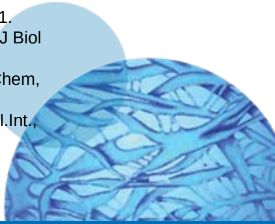
Reference List

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