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Letter to the Editors

Cluster analysis of genes with significant change in expression in cells conditioned to survive TBOOH

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Abstract

Immortal murine lens epithelial cells, αTN4-1 have been conditioned to survive H_2O_2, H cells, or TBOOH, T cells, at concentrations that will cause cataract in vitro. Since H cells are killed by TBOOH but T cells survive H_2O_2, it is of interest to examine the gene expression of these cell lines. We now report the results of cluster analysis of genes whose expression is significantly changed by TBOOH. The analysis has revealed a small group of antioxidative defense genes that contribute to the survival of T and H cells when exposed to oxidative stress.

Keywords: microarrays; gene expression; oxidative stress; cataract; GST

It is well established that oxidative stress is associated with maturity onset cataract formation and that peroxides are major oxidants contributing to the oxidative damage found in cataract (Augusteyn, 1981; Spector and Garner, 1981; Bhuyan and Bhuyan, 1984; Ramachandran et al., 1991; Spector, 1995; Giblin, 2000; Reddy, 2000). Peroxides and their oxidation products have also been shown to cause opacification of the lens in organ culture (Zigler and Hess, 1985; Babizhayev and Deyev, 1989; Spector et al., 1993; Ansari et al., 1996). Such observations have led to efforts to define antioxidative defense (AOD) systems, which would protect the lens from peroxide attack. Specific inhibitors of suspected key defenses have been examined (Bhuyan et al., 1973), lens systems have been established which are deficient or enriched in a suspected key AOD gene (Tumminia et al., 1993; Spector et al., 1996, 2001; Reddy et al., 2001), changes in gene expression and protein patterns in lens epithelial cells in response to peroxide stress have been examined (Garland et al., 1996; Kantorow et al., 1998; Carper et al., 2001; Spector et al., 2002a; Goswami et al., 2003) and potent antioxidants have been added to lenses in culture to determine protection against H_2O_2 stress (Zigler et al., 1996; Reddan et al., 2003).

This laboratory has developed conditioned immortal murine epithelial cells, which are resistant to 125 μM H_2O_2 (H) or 130 μM TBOOH (T), a lipid peroxide prototype (Spector et al., 2000, 2002b). Such peroxide concentrations will cause cataract in vitro. It was found that the H cells were killed by low concentrations of TBOOH but the T cells were resistant to either H_2O_2 or TBOOH even though they were not exposed to H_2O_2. Such experiments indicate that the AOD defense against one peroxide may not be sufficient to protect against another type of peroxide. Since it is believed that lipid peroxides as well as H_2O_2 may be involved in causing the development of cataract, it is important to differentiate the defense systems which are specific for each peroxide type. We now report that using cluster analysis of genes shown to have significant change in expression in T cells in relation to control (C) cells, a small group of AOD genes can be defined which appear to protect the lens from TBOOH, H_2O_2 or both peroxides.

αTN4-1 cells, kindly provided by Paul Russell (National Eye Institute, NIH), were conditioned to survive TBOOH stress essentially as described previously for other cell lines (Spector et al., 2000, 2002a,b). Enzyme assays, isolation of RNA, preparation of cRNA, microarray analysis, RT-PCR and statistical analysis have all been previously described (Spector et al., 2000, 2002b).

To determine the population of genes which may contribute to the survival of the T cells, the gene expression

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of the conditioned and control cell lines were analyzed utilizing Affymetrix murine arrays, MG-U74A, Version 2. These arrays can detect 12 422 genes and ESTs. For each cell line, five independently isolated RNA samples were utilized. Data was analyzed utilizing Affymetrix Microarray Suite 5.0 and Microsoft Excel software. Standard linear one way ANOVA ($P \leq 0.001$) and Tukey test for contrasts ($\alpha = 0.001$) was used to analyze the data. Gene selection was also based on expression increase or decrease of at least 1·5 fold and at least a 40% 'increase' or 'decrease' change call in the same direction as well as at least one 'present' detection call assigned by the Affymetrix software for a given gene in all experiments.

An average of approximately 52% of the genes and ESTs were expressed and about 22% (1415) had a significant change in T/C gene expression. They are listed in electronic File 1. These genes potentially may be contributing to the T cells' resistance to TBOOH. They have been examined by comparing their gene expression in T cell preparations with H cell as well as control samples utilizing the same statistical criteria described above. Thus, T/C, H/C and T/H comparisons have been made and the genes have then been clustered into 10 categories (Fig. 1). The number of genes in each category is indicated by the first number in the lower left-hand corner of each square. The second number indicates the distribution of the 41 AOD genes found in this population. These genes are listed in Table 1.

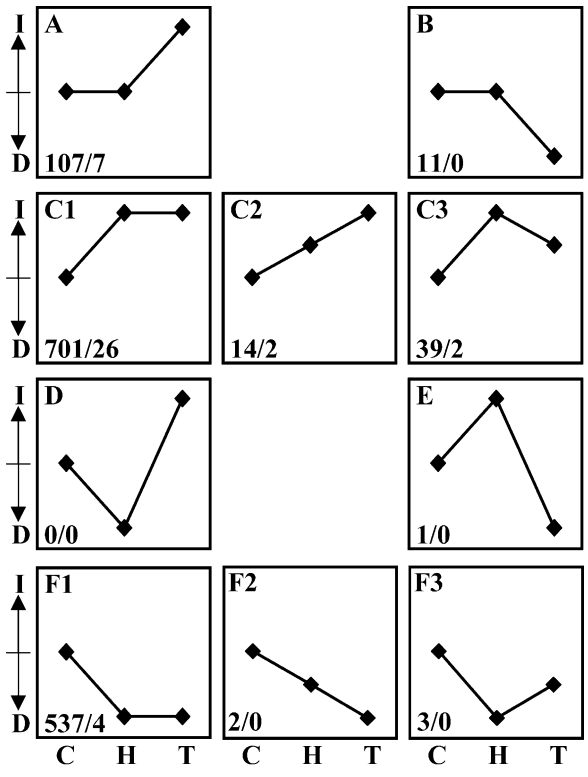

Fig. 1. Cluster analysis of genes with significant change in expression in T cells with respect to H and C cells. The first number in the lower left-hand corner of each panel indicates the total number of genes with the indicated pattern, the second number is the AOD genes in that population.

Of particular interest are those genes, which have a significant change in expression in T cells with respect to both H or C cells. Only 135 genes meet this criteria. Their behavior is represented by panels A, B, C2, D, E and F2. They are listed in Table 2. Since the H cells are killed by TBOOH, genes in panels C3 and F3 are excluded. Nine AOD genes potentially important for TBOOH resistance are represented in this group, seven with a pattern shown by panel A have an exclusive increase in gene expression in T cells and two shown in C2 have a significant increase in H cells and then a further significant augmentation of expression in T cells. Included in the panel A group is GST alpha 3, a member of a large family containing more than 50 genes (Hayes and Pulford, 1995) that is well represented among these 41 AOD genes. These enzymes use GSH to detoxify a wide variety of oxidized components and xenobiotics. Different classes of GSTs have different substrate specificities and different locations within the cell. GST alpha 3 is a member of one of five cytosolic gene families. These GSTs vary markedly in their substrate specificity (Hayes and Pulford, 1995). Recently, a GST, alpha was shown to protect human lens epithelium from H_2O_2 induced lipid peroxidation (Yang et al., 2002). Other AOD members of panel A include hephaestin, a multi-copper transmembrane oxidase involved in iron transport; thioredoxin interacting protein, an important regulator of cellular redox; aldehyde dehydrogenase 1A7, a modifier of toxic aldehydes using NADP as a cofactor; acyl protein thioesterase 1, which can degrade toxic phospholipids; GARG 16, a glucocorticoid attenuated response gene with potent anti-inflammatory characteristics and zeta crystallin, an NADPH dependent quinone oxidoreductase. The C2 group includes alcohol dehydrogenase 7 class 4, an enzyme with broad specificity which metabolizes xenobiotics and carbonyls as well as lipid peroxidation products and PAF acetylhydrolase which is similar to phospholipase A2 and hydrolyses phospholipids and eliminates inflammatory properties of PAF. The overall group represents genes with varied functions targeting toxic molecules resulting from oxidative processes and components that can catalyze the formation of oxidized components acting on both lipid and aqueous environments.

Examination of the overall group of TBOOH responsive genes shown in Table 2 does not explicitly explain why their expression was significantly modified although a few of them may be involved in AOD reactions. However, most of these genes do not appear to be involved in AOD. Some genes have not had their function defined or have poorly defined functions or may be involved in more than one area. This is particularly true of transcription factors and proteins that bind to a variety of biologically active components. Possibly the expression of some of these genes has inadvertently been modified by changes in chromatin

Table 1
AOD genes with significant change in expression in T cells found in various clusters on the basis of T/C, H/C and T/H gene expression comparisons

Cluster	Gene	Probe set	ANOVA	Tukey			Average signal		
				H/C	T/C	T/H	C	H	T
A	GST, alpha 3	93015_at	3.9×10^{-7}	–	***	***	3	11	48
	Hephaestin	104194_at	1.1×10^{-7}	–	***	***	2	4	160
	Thioredoxin interacting protein	160547_s_at	9.2×10^{-7}	–	***	***	17	48	160
	Aldehyde dehydrogenase 1A7	94778_at	9.7×10^{-7}	–	***	***	8	5	62
	Acyl Protein Thioesterase 1	100880_at	8.5×10^{-7}	–	***	***	223	173	788
	GARG 16	100981_at	1.3×10^{-11}	–	***	***	203	106	1682
	Crystallin, zeta	98131_at	2.0×10^{-5}	–	***	***	525	371	917
C1	Catalase	160479_at	2.2×10^{-9}	***	***	–	290	2344	2233
	GST, pi 2	99583_at	1.5×10^{-5}	***	***	–	3904	9067	8479
	GST 3, microsomal	96258_at	1.5×10^{-6}	***	***	–	81	292	395
	GST, mu 5	100629_at	6.1×10^{-5}	–	***	–	178	235	344
	GST, alpha 2	101872_at	2.0×10^{-12}	***	***	–	499	9372	8593
	GST 2, microsomal	104742_at	7.4×10^{-8}	***	***	–	13	781	776
	GST, mu 2	93009_at	1.1×10^{-9}	***	***	***	273	1093	1516
	Glucose-6-phosphate dehydrogenase 2	101294_g_at	6.4×10^{-6}	***	***	–	473	2427	1791
	Thioredoxin reductase 2	160437_at	2.7×10^{-5}	***	***	–	50	217	174
	Lysosomal thiol reductase	97444_at	8.1×10^{-8}	***	***	–	48	302	395
	Peroxiredoxin 5	100332_s_at	3.0×10^{-7}	***	***	–	409	2622	2669
	Phospholipase A2-V	94665_at	2.6×10^{-7}	***	***	–	32	124	135
	Ferredoxin 1	92587_at	5.7×10^{-6}	***	***	–	715	1378	1326
	Aldo-Keto reductase 1C13	95015_at	2.1×10^{-8}	***	***	–	249	1249	1120
	Leukotriene B4 12-hydroxydehydrogenase	98440_at	1.1×10^{-11}	***	***	***	814	7677	5229
	Copper chaperone for superoxide dismutase	103909_at	2.0×10^{-6}	***	***	–	115	616	428
	sulfide quinone reductase-like	94515_at	9.7×10^{-5}	***	***	–	241	394	382
	Reticulocalbin	160896_at	1.4×10^{-5}	***	***	–	1101	2622	2411
	Crystallin, alpha C	160139_at	1.0×10^{-5}	***	***	–	702	1210	1177
	Heat shock protein	161030_at	5.3×10^{-9}	***	***	***	878	2953	1930
	Macrosialin (CD 68)	103016_s_at	1.9×10^{-6}	***	***	–	27	285	428
	SOD1, soluble	100538_at	7.6×10^{-6}	***	***	–	1099	2089	1842
	P450 (cytochrome) oxidoreductase	99019_at	3.4×10^{-4}	–	***	–	212	567	590
	Epoxide hydrolase 2, cytoplasmic	93051_at	2.6×10^{-4}	–	***	–	7	46	66
	Aldehyde dehydrogenase 2, mitochondrial	96058_s_at	1.4×10^{-5}	–	***	–	277	341	407
	Heat shock protein 2	103214_at	2.0×10^{-5}	–	***	–	105	327	459
C2	Alcohol dehydrogenase 7 class 4	93695_at	1.7×10^{-7}	***	***	***	111	1025	2004
	PAF acetylhydrolase	101923_at	2.4×10^{-11}	***	***	***	38	78	206
C3	GST, alpha 4	96085_at	1.9×10^{-14}	***	***	***	289	3953	1775
	Secretory leukocyte protease inhibitor	92858_at	3.6×10^{-11}	***	***	***	5	1591	547
F1	Peroxiredoxin 2	99608_at	2.5×10^{-5}	***	***	–	61	17	8
	Ceruloplasmin	92851_at	1.9×10^{-4}	***	***	–	3251	44	7
	Glutathione reductase 1	160646_at	1.3×10^{-4}	–	***	–	1448	831	699
	Paraoxonase 2	104378_at	7.5×10^{-4}	–	***	–	638	455	389

***, Significant change, –, insignificant change.

structure to facilitate the increased expression of a spatially related important AOD gene. Changes in the concentrations of regulatory factors also undoubtedly contribute to the overall modification of the cell biology. Only 14 genes were found, which have their expression inhibited and most of them have profile B, i.e. expression only affected by TBOOH. It is not apparent why their expression was suppressed by TBOOH stress. It may be pertinent to also mention that at least 18 of the genes with increased expression only in response to TBOOH have their expression modulated by interferon. It is of particular interest that the modifications required to make these cell lines resistant to H_2O_2 are permanent but the resistance to TBOOH requires continuous TBOOH exposure.

The 135 TBOOH responsive genes have also been examined utilizing the gene ontology (GO) (The Gene Ontology Consortium, 2000) data base where genes have been categorized on the basis of biological process or molecular function, as shown in Fig. 2. Some genes are involved in more than one biological process or molecular function and, thus, may be indicated more than one time.

Of the 135 genes, 85 have been annotated for a GO biological process resulting in 126 assignments. The AODs contribute 5·1%. It is interesting that most aspects of cell

Table 2
Genes with significant change in expression in T cells relative to H and C cells expression

Cluster	Gene	Probe set	ANOVA	Tukey			Average signal		
				H/C	T/C	T/H	C	H	T
Genes with increased expression in T cells relative to H and C cells									
A	Interferon-stimulated protein	98822_at	2.3×10^{-13}	–	***	***	531	938	5652
	Interferon-stimulated protein	161511_f_at	3.4×10^{-13}	–	***	***	93	147	1356
	Ubiquitin specific protease 18	95024_at	4.2×10^{-12}	–	***	***	240	52	2116
	Interferon-induced protein with tetratricopeptide repeats 1 (GARG 16)	100981_at	1.3×10^{-11}	–	***	***	203	106	1682
	Unknown	93185_at	8.7×10^{-11}	–	***	***	28	28	89
	Unknown	92718_at	9.3×10^{-11}	–	***	***	116	24	864
	Unknown	103446_at	1.6×10^{-10}	–	***	***	47	44	489
	Programmed cell death 5	93968_at	2.2×10^{-10}	***	***	***	743	1359	2183
	Guanylate nucleotide binding protein 3	103202_at	7.6×10^{-10}	–	***	***	128	184	1255
	Interferon-g induced GTPase	98410_at	8.6×10^{-10}	–	***	***	372	169	1406
	Unknown	103517_at	1.6×10^{-9}	–	***	***	15	12	72
	Unknown	96789_i_at	1.9×10^{-9}	–	***	***	1	13	64
	Nucleosome binding protein 1	103654_at	2.7×10^{-9}	–	***	***	145	223	491
	Interferon gamma induced GTPase	160933_at	4.6×10^{-9}	–	***	***	91	67	804
	Kidney-derived aspartic protease-like protein	101972_at	6.5×10^{-9}	–	***	***	9	221	828
	Xlr-related, meiosis regulated	102818_at	7.8×10^{-9}	–	***	***	375	354	1357
	Interferon-inducible GTPase	96764_at	1.2×10^{-8}	–	***	***	27	10	589
	Adenosine deaminase, RNA-specific	96188_at	1.2×10^{-8}	–	***	***	84	132	512
	Interferon dependent positive acting transcription factor 3 gamma	103634_at	1.4×10^{-8}	–	***	***	416	329	1039
	Transforming growth factor beta regulated gene 1	98937_at	1.5×10^{-8}	–	***	***	535	666	1335
	Signal transducer and activator of transcription 1	101465_at	1.9×10^{-8}	–	***	***	76	21	197
	Troponin T2, cardiac	100593_at	1.9×10^{-8}	–	***	***	19	19	58
	Guanylate nucleotide binding protein 2	104597_at	3.0×10^{-8}	–	***	***	36	150	579
	Histocompatibility 2, T region locus 10	93865_s_at	3.3×10^{-8}	–	***	***	348	252	859
	T-cell specific GTPase	102906_at	3.3×10^{-8}	–	***	***	8	9	277
	Unknown	95447_at	4.8×10^{-8}	–	***	***	125	147	255
	Histocompatibility 2, T region locus 17	101876_s_at	5.8×10^{-8}	–	***	***	492	384	1215
	Component of Sp100-rs	101845_s_at	8.7×10^{-8}	–	***	***	10	5	306
	Tripartite motif protein 21	102678_at	8.7×10^{-8}	–	***	***	98	163	339
	Annexin A8	97529_at	1.0×10^{-7}	–	***	***	297	233	830
	Hephaestin	104194_at	1.1×10^{-7}	–	***	***	2	4	160
	Component of Sp100-rs	101846_r_at	1.1×10^{-7}	–	***	***	17	12	239
	Unknown	93427_at	1.2×10^{-7}	–	***	***	9	17	52
	Transporter 1, ATP-binding cassette, sub-family B (MDR/TAP)	103035_at	1.3×10^{-7}	–	***	***	147	168	322
	G1 to phase transition 2	103261_at	1.6×10^{-7}	–	***	***	44	86	150
	Unknown	96533_at	2.0×10^{-7}	–	***	***	439	278	684
	Myxovirus (influenza virus) resistance 2	102699_at	2.0×10^{-7}	–	***	***	33	17	186
	2-cell-stage, variable group, member 1	102254_f_at	2.1×10^{-7}	–	***	***	4	13	47
	Interferon-induced protein with tetratricopeptide repeats 3	93956_at	2.5×10^{-7}	–	***	***	30	21	545
	Unknown	93138_at	2.8×10^{-7}	–	***	***	88	112	261
	Insulin-like growth factor 2, binding protein 1	102627_at	2.9×10^{-7}	–	***	***	140	63	439
	Interferon-inducible GTPase	103963_f_at	3.1×10^{-7}	–	***	***	19	11	244
	Rhophilin, Rho GTPase binding protein 2	98485_at	3.2×10^{-7}	–	***	***	155	249	394
	Hypothetical protein MGC18837	95442_at	3.6×10^{-7}	–	***	***	340	353	565
	Nuclear antigen Sp100	101847_at	3.8×10^{-7}	–	***	***	10	14	75
	Glutathione S-transferase, alpha 3	93015_at	3.9×10^{-7}	–	***	***	3	11	48
	Interferon inducible protein 1	97409_at	4.7×10^{-7}	–	***	***	105	119	428
	Viral hemorrhagic septicemia virus (VHSV) induced gene 1	104177_at	5.6×10^{-7}	–	***	***	5	84	316
	Hypothetical protein	96387_at	6.0×10^{-7}	–	***	***	10	35	66
	Integrin alpha 3	104210_at	6.4×10^{-7}	–	***	***	279	673	1328
	Protein kinase, interferon-inducible double stranded RNA dependent	93672_at	6.6×10^{-7}	–	***	***	40	65	124
	Galactosidase, alpha	102341_at	6.7×10^{-7}	–	***	***	10	21	73
	Inhibitor of DNA binding 2	93013_at	7.0×10^{-7}	–	***	***	343	874	1649
	Interferon gamma inducible protein	104750_at	7.9×10^{-7}	–	***	***	15	4	48
	Diabetic nephropathy-related gene 1 (Acyl Protein Thioesterase 1)	100880_at	8.5×10^{-7}	–	***	***	223	173	788
	Myosin VIIa	94713_at	8.6×10^{-7}	–	***	***	437	422	760
	Thioredoxin interacting protein	160547_s_at	9.2×10^{-7}	–	***	***	17	48	160
	Aldehyde dehydrogenase family 1, subfamily A7	94778_at	9.7×10^{-7}	–	***	***	8	5	62

(*continued on next page*)

Table 2 (*continued*)

Cluster	Gene	Probe set	ANOVA	Tukey			Average signal		
				H/C	T/C	T/H	C	H	T
	Unknown	98037_at	1.1×10^{-6}	–	***	***	247	328	523
	Growth arrest specific 2	94338_g_at	1.1×10^{-6}	–	***	***	29	73	123
	Moloney leukemia virus 10	103025_at	1.2×10^{-6}	–	***	***	138	293	505
	Lectin, galactose binding, soluble 9	103335_at	1.6×10^{-6}	–	***	***	659	1010	2139
	Neutrophil cytosolic factor 4	103662_at	1.7×10^{-6}	–	***	***	5	31	60
	Integrin alpha 3	104211_at	1.7×10^{-6}	–	***	***	167	624	1177
	Interferon regulatory factor 7	162202_f_at	1.9×10^{-6}	–	***	***	33	59	169
	Histocompatibility 2, class II, locus Mb1	98034_at	2.1×10^{-6}	–	***	***	15	18	81
	Unknown	97798_at	2.3×10^{-6}	–	***	***	71	89	192
	Interferon regulatory factor 7	104669_at	2.3×10^{-6}	–	***	***	27	112	943
	ATP-binding cassette, sub-family B (MDR/TAP), member 1A	102910_at	2.4×10^{-6}	–	***	***	4	2	15
	Death associated protein 3	94524_at	2.4×10^{-6}	–	***	***	270	323	606
	Nuclear antigen Sp100	101848_g_at	2.5×10^{-6}	–	***	***	14	13	103
	Deubiquitinating enzyme 1	100680_at	2.8×10^{-6}	–	***	***	11	17	101
	Melanoma inhibitory activity	101453_at	2.9×10^{-6}	–	***	***	7	13	509
	Adrenomedullin	102798_at	3.2×10^{-6}	–	***	***	6	456	1045
	Prostate stem cell antigen	160508_at	3.2×10^{-6}	–	***	***	34	53	945
	Myxovirus (influenza virus) resistance 1	98417_at	3.3×10^{-6}	–	***	***	11	2	33
	Interferon activated gene 205	94224_s_at	3.8×10^{-6}	–	***	***	65	34	135
	Mitochondrial ribosomal protein L23	92646_at	4.4×10^{-6}	–	***	***	296	520	858
	Scotin gene	95102_at	4.5×10^{-6}	–	***	***	391	708	1301
	2-cell-stage, variable group, member 1	96584_f_at	5.0×10^{-6}	–	***	***	13	37	89
	Thrombospondin 3	103869_at	5.3×10^{-6}	–	***	***	74	142	279
	Renin binding protein	96154_at	5.3×10^{-6}	–	***	***	594	433	1494
	Tripartite motif protein 30	98030_at	5.6×10^{-6}	–	***	***	80	31	199
	Histocompatibility 2, K region	99379_f_at	6.6×10^{-6}	–	***	***	257	406	802
	Junction cell adhesion molecule 3	98957_at	6.7×10^{-6}	–	***	***	21	9	50
	Nitrilase 1	160075_at	8.7×10^{-6}	–	***	***	111	112	193
	DNA for intragenic sequence including B2 element	AFFX-MUR_b2_at	9.2×10^{-6}	–	***	***	214	247	429
	Mitogen-activated protein kinase 12	92323_at	1.2×10^{-5}	–	***	***	133	174	307
	Chemokine (C-C motif) ligand 5	98406_at	1.3×10^{-5}	–	***	***	4	73	424
	Serine (or cysteine) proteinase inhibitor, clade I, member 1	99494_at	1.4×10^{-5}	–	***	***	6	23	55
	Distal-less homeobox 2	92332_at	1.5×10^{-5}	–	***	***	166	204	338
	Endothelial cell growth factor 1 (platelet-derived)	160292_at	1.5×10^{-5}	–	***	***	76	135	250
	Crystallin, zeta	98131_at	2.0×10^{-5}	–	***	***	525	371	917
	Hypothetical protein 9630029F15	95905_at	2.3×10^{-5}	–	***	***	6	20	48
	Metaxin 1	94277_at	2.3×10^{-5}	–	***	***	120	189	411
	Histocompatibility 2, class II, locus Mb1	98035_g_at	2.3×10^{-5}	–	***	***	124	155	401
	Prostaglandin-endoperoxide synthase 2	104647_at	2.6×10^{-5}	–	***	***	220	222	698
	Proline-rich Gla (G-carboxyglutamic acid) polypeptide 2	94924_at	2.6×10^{-5}	–	***	***	87	153	331
	Unknown	101507_at	4.4×10^{-5}	–	***	***	59	78	139
	Unknown	93004_r_at	5.9×10^{-5}	–	***	***	121	147	247
	Peptidylprolyl isomerase C-associated protein	97507_at	9.1×10^{-5}	–	***	***	250	193	716
	Immunoglobulin superfamily, member 8	160820_at	1.6×10^{-4}	–	***	***	344	326	604
	Glycoprotein 49 A	100325_at	2.0×10^{-4}	–	***	***	2	1	15
	Lymphocyte cytosolic protein 1	94278_at	2.1×10^{-4}	–	***	***	11	8	85
	Adenosine deaminase, RNA-specific	102741_at	2.2×10^{-4}	–	***	***	30	30	87
	Lectin, galactose binding, soluble 9	161301_f_at	2.8×10^{-4}	–	***	***	50	51	117
	High mobility group AT-hook 2	99058_at	3.3×10^{-4}	–	***	***	311	305	773
C2	Fragilis	160253_at	1.4×10^{-12}	***	***	***	497	1187	4284
	Clone L8 variable group of 2-cell-stage gene family	94749_f_at	8.3×10^{-12}	***	***	***	21	79	161
	Phospholipase A2, group VII (PAF acetylhydrolase, plasma)	101923_at	2.4×10^{-11}	***	***	***	38	78	206
	B-cell linker	100772_g_at	3.1×10^{-10}	***	***	***	206	1463	2386
	Retinoic acid early transcript gamma	102649_s_at	8.8×10^{-10}	***	***	***	20	86	200
	Unknown	96790_f_at	9.2×10^{-10}	***	***	***	15	46	105
	LPS-responsive beige-like anchor	104264_at	1.6×10^{-9}	***	***	***	205	440	700
	Similar to variable group of 2-cell-stage gene family, member 1	95532_at	1.8×10^{-9}	***	***	***	4	57	99
	B-cell linker	100771_at	2.3×10^{-9}	***	***	***	139	1170	1896
	Folate receptor 1 (adult)	93785_at	2.9×10^{-9}	***	***	***	38	168	261
	FXYD domain-containing ion transport regulator 5	103394_at	1.1×10^{-8}	***	***	***	73	252	406

(*continued on next page*)

Table 2 (*continued*)

Cluster	Gene	Probe set	ANOVA	Tukey			Average signal		
				H/C	T/C	T/H	C	H	T
	Tripartite motif protein 25	100475_at	1.4×10^{-8}	***	***	***	204	402	741
	Cell division cycle 25 homolog C (S. cerevisiae)	102934_s_at	4.1×10^{-8}	***	***	***	186	414	647
	Alcohol dehydrogenase 7 (class IV), mu or sigma polypeptide	93695_at	1.7×10^{-7}	***	***	***	111	1025	2004
Genes with decreased expression in T cells relative to H and C cells									
B	Ubiquitin-associated protein 2	104715_at	5.2×10^{-7}	–	***	***	1547	1317	692
	Placentae and embryos oncofetal gene	101368_at	6.8×10^{-7}	–	***	***	130	90	16
	Lanosterol synthase	160737_at	5.1×10^{-6}	–	***	***	424	362	204
	Lipin 1	98892_at	6.4×10^{-6}	–	***	***	167	195	95
	Unknown	97425_at	9.2×10^{-6}	–	***	***	301	333	141
	Tubulin, beta 2	94835_f_at	9.3×10^{-6}	–	***	***	1951	2203	1222
	Solute carrier family 11, member 2	104451_at	1.5×10^{-5}	–	***	***	150	138	76
	Unknown	160177_at	2.1×10^{-5}	–	***	***	877	958	470
	Solute carrier family 31, member 1	103845_at	7.5×10^{-5}	–	***	***	683	612	341
	Calcium modulating ligand	104529_at	9.7×10^{-5}	–	***	***	235	230	118
	Unknown	94549_at	1.5×10^{-4}	–	***	***	556	555	341
E	Profilin 2	93567_at	1.0×10^{-10}	***	***	***	175	367	2
F2	Cyclin-dependent kinase inhibitor 2B (p15, inhibits CDK4)	101900_at	1.9×10^{-9}	***	***	***	331	213	79
	Squalene epoxidase	94322_at	7.9×10^{-8}	***	***	***	1748	1281	586

***, Significant change, –, insignificant change.

biology are included and it is not possible to get a sense of oxidative stress defense from the distribution of genes in these categories. A similar conclusion can be made from the delineation of GO molecular functions where 90 genes have been annotated and 148 assignments have been made. Twenty-nine of the genes were not assigned a GO biological process or molecular function. The biological basis for the cell's selection of these genes is unclear. While some of them may be contributing to the AOD of the cell, it is probable that in many cases, the change in gene expression is due to alteration of chromatin structure, the sharing of similar regulatory elements and/or response to changes in

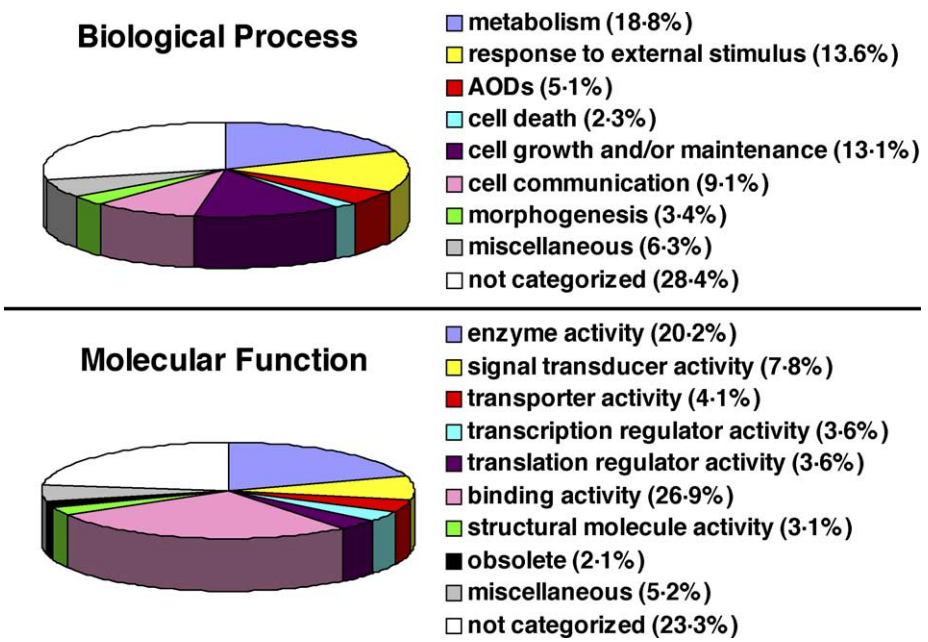

Fig. 2. Categorization of genes shown to have significant change in expression in T cells relative to both H and C cells. The gene ontology consortium database for biological processes and molecular function was utilized.

cellular biochemistry caused by the stress or the balance in metabolic activity. In any case, this work defines a small group of key genes that may protect the cell from specific and general oxidative attack.

The largest group of AOD genes are in the group with comparable increased or decreased expression in response to either H_2O_2 or TBOOH. There are 26 AOD genes with increased expression and four with decreased expression. Among the genes with enhanced expression are six GSTs, including GST, alpha 2 and GST, pi 2, which have the highest average signals of all the AOD genes; catalase, a H_2O_2 degrading enzyme previously shown to have a very large increase in enzyme activity in H cells; peroxiredoxin 5, another peroxidase which uses thioredoxin as a cofactor; thioredoxin reductase 2, involved in thioredoxin redox; ferredoxin 1, a powerful low molecular weight reductant; SOD 1, which degrades superoxide; the copper chaperone for SOD, a protein which delivers copper to the copper/zinc SOD 1; NADPH dependent leukotriene B4 12-hydroxydehydrogenase, a lipid oxidoreductase, which reduces α, β unsaturated aldehydes and ketones; aldo-keto reductase 1C13, which binds NADPH and reduces a broad group of compounds; reticulocalbin, an endoplasmic reticulum protein which binds calcium and controls cellular calcium concentration; macrosialin, a transmembrane glycoprotein, which scavenges LDL; a number of HSPs including crystallin alpha C, a member of the HSP 20 family containing an alpha crystallin domain.

The AOD genes with comparable decreased expression in T and H cells include peroxiredoxin 2, which has only minimal expression in the C cells; ceruloplasmin, which is involved in copper transport and scavenges H_2O_2, a gene which has strong expression in C cells and is essentially turned off by the peroxides; and paraoxonase 2, an ester hydrolase associated with HDL which has been shown to decrease intracellular oxidation of cells exposed to H_2O_2. All of these enzymes appear to be effective AOD genes and, yet, they have been turned off or markedly suppressed by peroxide. This demonstrates the complex response of the cell to oxidative stress.

It should be emphasized that a number of classical AOD genes have not been significantly affected by peroxide stress. Many of them are expressed in control cells and may contribute to the overall AOD of the cell. Furthermore, genes that are increased with both peroxides may be necessary for the defense of either peroxide. Catalase, for example, is required for the cell to survive TBOOH attack (unpublished results). Thus, the vulnerability of aging systems to oxidative attack may be a result of not only the increased presence of oxidants, but also the decrease in baseline gene expression and inability to respond swiftly to the stress by degrading the oxidants and producing AODs. The enrichment of the lens with some of the genes revealed by this study to be major contributors to AOD against peroxides may prevent or retard the development of cataract in the aging lens.

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