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The Epithelial Cell in Lung Health and Emphysema Pathogenesis

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Abstract

Cigarette smoking is the primary cause of the irreversible lung disease emphysema. Historically, inflammatory cells such as macrophages and neutrophils have been studied for their role in emphysema pathology. However, recent studies indicate that the lung epithelium is an active participant in emphysema pathogenesis and plays a critical role in the lung's response to cigarette smoke. Tobacco smoke increases protease production and alters cytokine expression in isolated epithelial cells, suggesting that these cells respond potently even in the absence of a complete inflammatory program. Tobacco smoke also acts as an immunosuppressant, reducing the defense function of airway epithelial cells and enhancing colonization of the lower airways. Thus, the paradigm that emphysema is strictly an inflammatory-cell based disease is shifting to consider the involvement of resident epithelial cells. Here we review the role of epithelial cells in lung development and emphysema. To better understand tobacco-epithelial interactions we performed microarray analyses of RNA from human airway epithelial cells exposed to smoke extract for 24 hours. These studies identified differential regulation of 425 genes involved in diverse biological processes, such as apoptosis, immune function, cell cycle, signal transduction, proliferation, and antioxidants. Some of these genes, including VEGF, glutathione peroxidase, IL-13 receptor, and cytochrome P450, have been previously reported to be altered in the lungs of smokers. Others, such as pirin, cathepsin L, STAT1, and BMP2, are shown here for the first time to have a potential role in smoke-associated injury. These data broaden our understanding of the importance of epithelial cells in lung health and cigarette smoke-induced emphysema.

Keywords

Epithelium; cytokine; inflammation; tobacco; microarray; apoptosis; alveolar; development; vitamins; antioxidants

DISTINCT LINEAGES OF LUNG EPITHELIAL CELLS

Large air-breathing mammals must efficiently extract oxygen from the environment to provide fuel for metabolic needs. This physiologic requirement is met through the development of highly branched respiratory units which comprise the large alveolar surface area in the lungs essential for gas exchange (70m^2 in adult humans). The distinct functions of the upper and

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lower airways and alveoli include gas exchange and mucus production, while prohibiting entry of water, small particulates, and microbes. As a result, the epithelium of the lung consists of cells of a variety of lineages, organized by location along the pulmonary tree, allowing each cell to be influenced by the level of oxygenation, inflammatory cell milieu, and proximity to the vasculature. The uppermost portion of the respiratory tree consists of the larynx, trachea, and upper airways; the former is lined with squamous epithelium and the latter is lined with ciliated columnar and mucus-secreting epithelial cells [1] Goblet cells are located on the surface epithelium of upper and lower airways, and produce mucus to coat the airways and trap particulates to be cleared [2]. The more distal airways are lined with non-ciliated Clara cells, which secrete mature surfactant proteins A, B, D, and several detoxifying enzymes. Several reports have shown that Clara cells can be induced to generate ciliated cells, implicating this cell type, as has been shown for basal cells in large airways [3], as airway “stem cells” [4,5]. Moving further down the lung, the alveoli are covered by types 1 and 2 epithelial cells [1]. The flat type I epithelial cells cover 95% of the peripheral lung surface. This cell type resides in close proximity to capillary beds and thus is the site of gas exchange. Importantly, expansion of the lung epithelium during alveolar branching occurs concurrently with the development of the pulmonary vasculature (during the late embryonic and postnatal stage in rodents and humans). Type II epithelial cells (type II pneumocytes) are precursors of type I epithelial cells. These cells secrete components of surfactant proteins and regulate lung fluid balance. In addition, type II cells play important repair and anti-inflammatory roles by phagocytosing apoptotic neighbor cells [6,7].

The epithelial cell types of the lung are varied and numerous. Together they provide structural integrity, are a physical barrier against environmental insults, allow gas exchange, enhance ion and fluid transport, secrete growth factors, chemoattractants, antimicrobials, and express adhesion receptors, oxidant species, and lipid mediators for neighboring cell communication and matrix attachment [1,8,9]. Interruption of the creation of the specialized epithelial cell types negatively impacts on lung morphogenesis. For example, infant rhesus monkeys exposed to allergen or ozone demonstrate impaired formation of the tracheal epithelium and basement membrane [10]. Similarly, genetically modified mouse models reveal key roles for growth factors, transcription factors, and morphogenic molecules in determining the branching pattern and cellular composition of the lung [1,11,12]. Of particular interest is the importance of early lung patterning to the proper function of the adult lung. During fetal lung branching, signaling between epithelial and mesenchymal cells stimulate epithelial cell proliferation and differentiation, and creation of an epithelial-mesenchyme trophic unit is reported to drive aberrant repair of epithelial injury and the subsequent mesenchymal response [1,13]. These interactions may play a role in the pathology of chronic obstructive pulmonary disease (COPD). For instance, damaged epithelial cells secrete repair molecules, including growth factors such as EGF, PDGF, endothelin, FGF2, TGF-β, and cytokines such as TGF-α which stimulate lung myofibroblasts under the epithelial layer in the lamina reticularis to migrate, proliferate, differentiate, and alter matrix production, particularly fibronectin and collagens type I and III [9].

CIGARETTE SMOKING AND THE OXIDANT BURDEN ON LUNG EPITHELIUM

Cigarette smoking is the most common cause of preventable death in the U.S. [14], and even occasional tobacco use and sidestream smoke exposure impact negatively on health [15–18]. Further, tobacco smoke exposes the immune system to numerous primary and secondary insults [19], and causes significant financial, societal, and public health losses as a result of hospitalization, premature death, and reduced work productivity [20]. A leading disorder caused by smoking is COPD [21]. COPD, which includes emphysema and chronic bronchitis, is a major contributor to morbidity and mortality in all countries, and is the fourth largest cause of death in the United States [21,22]. As rates of cigarette smoking increase in world populations, morbidity and mortality rates for COPD will also increase [23].

The airway epithelium is exposed to high levels of environmental oxidants. In addition, the airway epithelial cell must quench endogenous intracellular oxidants produced through normal mitochondrial electron transport and oxidants produced by inflammatory cells. As approximately 10 reactive oxygen species are present in each puff of a cigarette [19,24,25], the oxidant burden on the airways and lung tissue of a chronic smoker is significant (Fig. 1). Epithelial cell injury and oxidative stress may influence disease risk. For instance, smoke-derived oxidants can inhibit α_1-antitrypsin (α_1-AT) [26–28], and chronic cigarette smoke exposure induces formation of DNA adducts in lung tissue of rats [29]. We hypothesized that exposure of cultured human small airway epithelial cells (SAECs) to cigarette smoke extract (CSE) would induce genes relevant to smoking-associated lung disease, particularly emphysema. Here, using Affymetrix Hu95Av2 microarrays we examined expression of over 12,000 genes in SAECs after 24 hours of exposure to 5% CSE, prepared as previously reported [30]. Significant alterations in expression (t-test $p < 0.05$) were detected for over 400 genes, listed according to Gene Ontology (GO) Biological Process/Molecular Function (Table 1). These analyses suggest that CSE exposure increases expression of 210 genes and decreases expression of 215 genes in these cells.

i. Production of Mucus by Lung Epithelial Cells

Epithelial goblet cells are more numerous in lung tissue from COPD patients compared to normal subsets [8], and airway goblet cells become hyerplastic following cigarette smoke exposure [31,32]. Metaplasia of goblet cells and smooth muscle cell hypertrophy are thought to result from the increased inflammatory cell numbers in the lung tissue following smoke exposure. One often overlooked feature of inflammation is the central role of resident epithelial cells in recruiting circulating neutrophils and macrophages into the tissue. The mechanism in part involves cytokine production, since epithelial cells produce various cytokines which are chemoattractant for neutrophils.

The enhanced goblet cell population stimulates mucin gene synthesis and hypersecretion, resulting in thickened mucus [2,33]. In fact, cigarette smoke alone induces MUC5A expression [34]. The result of this series of events impairs mucociliary function and increases susceptibility to viral and bacterial infection.

The molecular mechanisms for increased mucin expression have been examined. Elevations in EGF receptor (EGFR) signaling are detected in the lungs of chronic smokers [35]. EGFR is a transmembrane receptor tyrosine kinase activated by extracellular binding of ligands such as EGF and TNF-α [36]. It is unclear whether cigarette smoke enhances ligand-receptor binding per se, or whether smoke itself directly modifies the ligand outside the cell. However, increased levels of downstream targets of EGFR signaling are found in the lungs of smokers, and in the lungs of patients with emphysema [30,35]. Our laboratory demonstrated elevated phosphorylation of one EGFR downstream effector, ERK1/2 MAP kinase, in the lung tissue of emphysema patients [30]. Cigarette smoke activates airway epithelial MUC5AC gene transcription, *via* an EGFR kinase dependent pathway, and TNF-α has also been shown to induce mucin gene expression [37]. In COPD and asthma, excess mucin production contributes to airway obstruction and infection. Other important contributors to airway mucin regulation are TGF-β2 [38], VEGF [39], IL-16 and IL-17 (through ERK) [40], and IL-13. IL-13 and VEGF have been studied extensively, and are produced by epithelial cells in response to inflammatory stimuli, but their induction by smoke in lung epithelial cells is less clear. Our microarray data suggest that 5% CSE induces both VEGF (Table 2) and interleukin-13 receptor (Table 3) in SAECs. Directed transgenic overexpression of VEGF in the lung induces an asthma-like phenotype, with mucus metaplasia through IL-13–dependent and IL-13–

independent mechanisms [39]. However, depending on the system and concentrations, IL-13 has been shown to stimulate [41,42] or abrogate [43] mucin expression, often by a MAPK dependent pathway [42]. Many studies have examined the role of IL-13 in the asthmatic airway and will not be reviewed here.

ii. Vitamins, Antioxidants and Epithelial Lining Fluid

A key protective measure against oxidant injury is the expression of antioxidant and detoxification genes. The bronchial epithelial cells and epithelial lining fluid provide a first line of defense during tobacco smoke exposure. One important contribution is the rich network of antioxidants that these cells produce, including superoxide dismutase (SOD), catalase, glutathione peroxidase, thioredoxin, and glutaredoxin [44]. Specific markers of oxidant-induced cell damage, such as DNA modification, lipid peroxidation and adduct accumulation have been demonstrated in lungs of patients with COPD [45,46]. In fact, several studies suggest that antioxidants may have a positive impact on lung function [47–49].

The antioxidant vitamins A, C, E, and selenium protect cells from the burden of free radicals. There are numerous studies on the effects of vitamin A on the lung. These reports likely arose as a result of an understanding of the importance of vitamin A on developing lung epithelium [50], and of the demonstration that vitamin A deficiency causes emphysema in rats [51,52]. The vitamin A metabolite, *all trans* retinoic acid (atRA), was found by several investigators to restore alveolar tissue destroyed by elastase instillation in rats [52,53] and in dexamethasone-treated mice [52]. Other groups, however, reported that atRA could not restore alveolar damage in elastase-treated or TNF-alpha transgenic mouse models [54]. Importantly, a three month trial of atRA in human COPD patients demonstrated no reversal of emphysema [55]. Large scale human studies such as the Beta-Carotene and Retinol Efficacy Trial (CARET) [56] and the Alpha-Tocopherol, Beta-Carotene (ATBC) Lung Cancer Prevention Study [57], have brought to light the potential effects of retinoids on lung cell proliferation and tumorigenesis [58]. The evidence supports a procarcinogenic biological activity of these micronutrients. An ATBC follow-up study found that 5–8 years of supplementation with either alpha-tocopherol (vitamin E) or beta carotene did not prevent cough, phlegm, or dyspnea in male smokers [59]. However, one follow-up study to the CARET trial reported that an increase in serum beta-carotene levels was associated with lung function protection in a cohort of 816 heavy smokers exposed to asbestos [60].

Vitamin C (ascorbic acid) is required in the diet of humans and guinea pigs, and is essential for the synthesis of collagen [61,62], the major structural matrix protein of the lung. Vitamin C is able to prevent oxidation of lung lipids induced by cigarette smoke [61]. Although serum levels of vitamin C are lower in patients with stable and exacerbations of COPD [63], few studies have examined the role of vitamin C in emphysema. The Dutch MORGEN study found a positive association between high dietary intakes of either beta carotene or vitamin C with FEV_1 and FVC [49]. Vitamin C intake was inversely correlated with cough in this study [49]. Vitamin E is a potent antioxidant shown to prevent DNA damage induced in human umbilical vein endothelial cells following cigarette smoke exposure [64], and can prevent the oxidation of lipids in the lung following smoke exposure [61]. However, studies have demonstrated that although vitamin E plasma levels are low in patients with COPD, dietary supplementation has no effect on lung function [65]. The MORGEN study reported that high vitamin E intakes were positively associated with cough, with no effect on lung function [49]. Vitamin E also failed to reduce the cytotoxicity of alveolar macrophages from smokers [66]. Studies on vitamin antioxidants must be pursued with caution, as these micronutrients can produce secondary radicals and additional injury when interacting with tissue oxidants [67,68]. Importantly, although epidemiologic studies report positive associations between lung function with serum antioxidant levels and intakes of antioxidant-containing fruits and vegetables [69], studies using individual supplements have not consistently demonstrated significant lung function improvements. The greatest benefit from antioxidants may best be obtained from a nutrient-rich, balanced diet and not from individual vitamin supplements [70,71], since fruits and vegetables provide a variety of antioxidant polyphenols, flavenoids, catechins, and other micronutrients. Nonetheless, airway epithelial cells and the epithelial lining fluid are essential for airway antioxidant production and transport.

Cellular antioxidants include not only the vitamins, but also thiols and enzymes such as dismutase, glutathione, glutathione peroxidase, and catalase [72]. Increased levels of manganese SOD are detected in the bronchial epithelium of smokers with COPD [73], but not in healthy smokers [74], suggesting that the lung's defense against cigarette smoke-derived oxidants may influence the COPD development. Animal models provide direct evidence for antioxidants' role in COPD. Recently, it was shown in mice that genetic ablation of the oxidant responsive transcription factor Nuclear factor, erythroid-derived 2, like 2 (Nrf2) leads to enhanced inflammation, lung cell apoptosis, and development of smoke-induced emphysema [75]. The mechanism for emphysema susceptibility, as suggested by the authors, is that loss of Nrf2 transcriptional action leads to failure to induce expression of cytoprotective, antioxidant genes [75]. Further evidence for the protective role of antioxidants in emphysema susceptibility is suggested by differences in antioxidant gene expression between airway epithelial brushings from smokers and nonsmokers [74]. This study showed that many critical antioxidants, including SOD, were not induced in healthy smokers. Although it is remains unclear how expression differences ultimately affect disease susceptibility, failure to induce expression of these enzymes may be a potential marker of disease risk. Our laboratory recently determined that overexpression of EC-SOD prevents smoke-induced inflammation and emphysema in mice [76], suggesting that there is a role for antioxidants in the early pathogenesis of inflammation and tissue destruction of emphysema.

In addition, the cytochrome P450 family genes CYP1A1 and CYP1B1 were all suggested to be increased by CSE (Table 4). Cytochrome P450 genes have been shown to be induced by cigarette smoke [77]. One epidemiologic study suggested that a genetic deletion polymorphism of CYP2A6, the main gene responsible for nicotine metabolism, may influence nicotine dependence, smoking habit, and emphysema development [78]. A role for glutathione in epithelial cytoprotection is shown in Clara cells, which develop resistance to injury from naphthalene, a component in cigarette smoke, through continued glutathione production [79]. In correlation with these data, our microarray analyses suggest increased expression of glutathione S transferase ($p= 0.039$) and glutathione peroxidase ($p = 0.013$) in SAECs. The microarray data also suggest that CSE induces a different xenobiotic enzyme, epoxide hydrolase 1 (Table 4), which hydrolyzes anti-inflammatory epoxyeicosatrienoic acids [80]. We further found potentially reduced expression of nicotinamide nucleotide transhydrogenase (Table 5), which has been shown using C. elegans mutants to be involved in defense against mitochondrial oxidant stress [81].

Glutathione is part of an enzymatic metabolic pathway that protects cellular proteins and DNA from oxidation caused by cigarette smoking. Glutathione levels in epithelial lining fluid are typically higher than levels in plasma [44]. Our array suggests that smoke extract increased airway epithelial cell expression of glutathione peroxidases-2 and −4 (Table 4). Several earlier studies have measured antioxidant expression or activity in lung tissue from COPD patients, and have compared how airway epithelial cells from never-smokers, normal smokers, and patients with COPD respond to oxidant stress [74,82–84]. Our microarray data suggest that expression of SAEC genes involved in electron transport (Table 5) and DNA repair (Table 6) is changed by CSE exposure.

Cigarette smoke has been shown to induce senescence of cultured A549 cells, a rapidly growing type II epithelial cell isolated from lung adenocarcinoma [85]. Treating these cells with antioxidants, such as catalase, ascorbic acid, or N-acetylcysteine (a cellular antioxidant that is a glutathione precursor) has distinct effects on the senescence associated with smoke exposure. Catalase has little effect on the induction of the senescence-associated marker β-galactosidase by smoke exposure, while reductions are observed in the presence of either ascorbic acid or N-acetylcysteine [85]. The specificity of the response to antioxidants in these studies hints that the particular antioxidant expressed by epithelial cells may be critical in modulating pulmonary damage resulting from smoke and oxidant exposure. Additionally, it suggests that interactions among individual antioxidants are critical: an isolated increase in one antioxidant may actually augment oxidative injury if there is not an increase in downstream antioxidants/enzymes to detoxify reactive intermediates.

iii. Apoptosis and Epithelial Cells of the Lung

During lung development populations of epithelial and mesenchymal cells participate in reciprocal interactions that result in the formation of functioning respiratory units. The mesenchymal tissue is required for proper lung branching morphogenesis, as it has been shown that isolated lung epithelium fails to branch when cultured in the absence of mesenchyme [86]. As the early lung grows the ratio of epithelial to mesenchymal cells increases due to loss of mesenchymal cells through apoptosis [87]. Although apoptosis is generally difficult to detect because of efficient clearance mechanisms, mesenchymal cell loss is observed in early lung development, including embryonic, pseudo-glandular, and canalicular stages [88]. After this period of development, apoptosis of both mesenchymal and epithelial cell types occurs [89–92].

Apoptosis occurs throughout normal organ development and branching morphogenesis, and is an important component of controlling inflammation, but it is uncommon in healthy adult lung [88,90]. However, several studies demonstrate ongoing apoptosis in human COPD and emphysema [93–97], and in animal models of airspace enlargement (emphysema) [98,99]. While animal studies have detected emphysema- or smoke-associated apoptosis in the range of 1% to 16%, data from human lung tissue appears to be much lower [93,97]. Segura-Valdez *et al.* demonstrated apoptosis of alveolar epithelial, endothelial and inflammatory cells in human COPD lungs [93]. Yokohori found increased apoptosis of alveolar epithelial cells in COPD lung compared to normal lung tissue [96]. Our laboratory reported an inverse correlation between pulmonary cell apoptosis in human COPD and lung tissue surface area [97]. The SAEC microarray data propose that changes in expression of several key apoptosis-related genes may occur during CSE exposure (Table 7). The mechanisms are not known, but work in mice and humans suggest that various factors, such as circulating TNF-α [98], placental growth factor [100], and loss of vascular endothelial growth factor (VEGF) signaling [99, 101] may each be involved [102]. Importantly, the microarray data suggest that SAEC VEGFb expression is increased by CSE exposure at 24 hours (Table 2). Increased VEGFb expression so soon following smoke exposure suggests that VEGF ligand induction may be a protective response.

Direct induction of lung cell apoptosis using intratracheal instillation of active caspase-3 led to rapid onset of airspace enlargement in mice [103]. Although apoptosis is increased in the lung in COPD, there is evidence that proliferation is also enhanced [96,97], perhaps in an attempt to replace cells that have been lost. Depending on the cell type involved, the result of even modest cell loss can be significant, and may contribute to reduced surfactant production, loss of surface area for gas exchange, tissue necrosis, vascular effects and inflammation [102,103]. Cigarette smoke has been shown to increase lung epithelial cell proliferation *in vivo* [104,105] and to induce apoptosis in a variety of lung cell types *in vitro*, including macrophages [106], fibroblasts (reduced proliferation) [107], and neutrophils. *In vitro* cigarette smoke exposure leads to loss of glutathione and subsequent apoptosis of fibroblasts [24]. In addition, apoptosis of cultured bronchial cells is influenced by cell adhesion [108], and not all epithelial cells demonstrate the same apoptotic response to smoke. For example, although normal human bronchial epithelial cells (NHBEs) can undergo significant (20%) apoptosis upon smoke exposure, A549 adenocarcinoma cells are resistant to apoptosis after a CSE exposure time of 30 minutes [109]. Others have found that very high levels of CSE do lead to 20% apoptosis of A549 cells [110]. We found that exposure to 24–48 hours of CSE had little effect on total numbers of A549 and SAEC in culture (Fig. 2).

In vivo data suggest that cigarette smoke induces lung cell apoptosis in mice [32,75,111], but strain differences have a potent influence on the extent of cell loss and resulting emphysema pathology [32,75,104,111–113]. A net increase in apoptotic cell counts in emphysema has been proposed to result in part from insufficient phagocytosis, which can contribute to local inflammation. Lavage macrophages from COPD patients demonstrate a reduced capacity to phagocytose apoptotic epithelial cells [114]. Oxidants may be indirectly involved, as macrophages exposed to matrix proteins that have been modified by smoke extract demonstrate impaired phagocytosis of apoptotic neutrophils [115]. Cultured human SAECs can specifically phagocytose apoptotic eosinophils *in vitro* using an integrin-dependent mechanism [116]. Further, epithelial cells produce anti-bacterial compounds which help to reduce inflammation, and are capable of selective phagocytosis, as demonstrated by phagocytosis of apoptotic eosinophils but not neutrophils [117]. *In vivo* evidence for the importance of phagocytosis of apoptotic cells in the lung has been demonstrated using mice with genetic ablation of the phosphatidylserine receptor (PSR) [118]. This cell surface receptor recognizes exposed phosphatidylserine residues on the plasma membrane of apoptotic cells, leading to engagement of the dying cell and phagocyte, and removal of the apoptotic cell remnant. These PSR knockout mice have severe lung defects and die at birth due to cyanotic respiratory failure. Levels of surfactant are normal. There is currently no evidence for altered PSR expression or function in COPD.

iv. Signal Transduction Pathways and Transcription Factors Activated by Tobacco Smoke

Cigarette smoke impairs epithelial and fibroblast repair processes [107,119], and alters the phosphorylation state and expression of various signal transduction mediators. Our laboratory previously demonstrated rapid and lasting cigarette smoke-induced phosphorylation of ERK-1/2 MAP kinase in cultured SAECs, with no detectable activation of p38 or JNK kinases [30]. The microarray data here suggest increased expression of STAT1 (Table 8), a molecule recently shown to be activated by carbon monoxide, a component of tobacco smoke [120]. STAT1 is involved in diverse signaling pathways and changes in its expression could have broader cellular effects, including IFNγ -mediated growth arrest. Similar smoke-induced changes in other transcriptional regulators are reported not only in cultured cells, but also in lung tissue of human subjects and animal models. We detected elevated pulmonary ERK-1/2 phosphorylation in mice smoke-exposed and in airway and alveolar epithelial cells of patients with COPD, compared to non-emphysematous controls [30]. *In vitro* studies of A549 cells and *in vivo* studies with rats demonstrate tobacco smoke induction of c-Fos, MEK1, and ERK2 MAP kinase [109,121]. In contrast, there appears to be no effect of cigarette smoke treatment on oxidant-sensitive NFκB signaling in A549 cells [122], although activation does occur in NHBE cells [109], revealing the importance of cell type and treatment conditions such as duration of smoke exposure and extract concentration when interpreting *in vitro* studies. These changes in the phosphorylation state of signal transduction molecules can activate pro-proliferative or proinflammatory transcription factors such as c-fos and c-myc, AP-1, and Elk-1 [123], which translocate to the nucleus and enhance gene expression. Targets include several genes involved in emphysema pathogenesis, such as *MMP1*, *MMP9*, and *MUC5*. Interestingly, our microarray data suggest that smoke increases histone deacetylase-3 (HDAC3), an enzyme involved in chromatin remodeling and, typically, silencing of gene expression (Table 7). Previous *in vitro* studies with A549 cells have demonstrated a CSE-mediated decrease in HDAC activity after CSE treatment, contributing to unwinding of nuclear chromatin and enhanced gene expression [122].

v. Growth Factors and Inflammation Production by Lung Epithelial Cells

Many researchers prepare their own primary cell cultures from airway explants of guinea pigs, rats, or humans who have undergone lobectomy or pneumonectomy [124,125]. These *in vitro* studies provide valuable information with regard to the response of epithelial cells to cigarette smoke apart from the complex inflammatory milieu of the lung, under controlled treatment conditions. Even in culture isolated epithelial cells produce inflammatory and remodeling molecules, including IL-1 [125], IL-6, IL-8 [126], GM-CSF [126], as well as IL-1 β, RANTES, MIP1-α [127], MCP-1 [127], and TNF-α [128]. Importantly, following cigarette smoke exposure NHBE cells can rapidly increase expression of IL-1β, RANTES, IL-6, IL-8, and GM-CSF [109].

The ability of epithelial cells to recruit neutrophils (by secretion of the aforementioned cytokines) has significance to COPD pathology, as neutrophil numbers correlate with airflow limitation [129]. Lung epithelial cells express a variety of cytokines, and can be stimulated to increase cytokine production [83,127,129]. HBE cells from COPD patients have lower levels of basal and smoke-inducible IL-8 and TNF-α than normal smokers [130]. However, the response to TNF-a was the opposite, with HBEs from COPD patients exhibiting higher levels of IL-8 [130]. These data suggest that bronchial epithelial cells from individuals with COPD are altered in their ability to be activated and recruit neutrophils. These changes impair epithelial adhesion and permit neutrophils to transit into the airway lumen [131]. The epithelial cell also produces anti-inflammatory cytokines such as IL-11 [132,133]. Our array data suggest that various several inflammatory molecules may be directly affected by smoke exposure. These include increased expression of the gene for IL-13 receptor, and decreased expression of IL-7 receptor and IL-6 genes (Table 3). Together, these data propose a role for the airway epithelium in lung remodeling, signaling, and repair during smoke exposure.

Various growth factors are critical for lung morphogenesis or epithelial differentiation [1,6, 134]. These include EGF [135] and KGF [136], which promote branching; sonic hedgehog, FGF1, FGF2, FGF7, FGF9, FGF10, and FGF11, noncanonical Wnt5a [137], epithelial Wnt7b [138], Wnt-10b, mesenchymal Wnt-2, −2b and −11 [139]; TGFβ (which limits branching) [136], BMP2 and BMP4, VEGF, PDGF, and IGF. The microarray data suggest that BMP2 expression is increased by smoke extract exposure (Table 9). In addition, several Wnt pathway genes are potentially affected by smoke extract exposure, including increased expression of casein kinase 1 (Table 6), amino-terminal enhancer of split (Table 10), and LRP6 (Table 10), and reduced expression of frizzled receptor homolog 6 (Table 12). The data also suggest a smoke-induced increase in expression of pirin (Table 10), a recently identified metal-binding protein believed to be involved in redox reactions. Pirin interacts with nuclear Bcl-3 and regulates NFκB signaling [140].

Recent research has identified impaired VEGF signaling in emphysema. VEGF levels are lower in COPD lung tissue than normal lung, and mice who have lost VEGF signaling develop rapid emphysema-like lesions due to apoptosis of both endothelial and epithelial cells [141]. The microarray data suggest that VEGFb expression is increased by smoke exposure (Table 2), which may indicate that VEGF induction is an acute response to smoke injury, for survival of not only endothelial cells, but also epithelial cells. In fact, type II epithelial cells are a predominant source of VEGF in the lung, and the epithelial lining fluid levels of VEGF are higher than levels in plasma [142].

vi. Proteolytic Enzyme Production by Airway Epithelium

The tissue destruction in emphysema is proposed to result from a net imbalance in the activities of proteases and their inhibitors. The production of proteolytic enzymes, such as MMPs, by epithelial cells of the lung parenchyma and airways is normally low. However, exposure to tobacco smoke increases several proteases implicated in COPD pathogenesis. In addition, inflammation induces secondary increases in MMPs [143]. Primary type II epithelial cells from rat lung explants increase production of MMP-2 and MMP-9 after LPS stimulation [144]. We have shown expression of MMP-1, MMP-2, and MMP-9 in human SAECs, with significant increases in MMP-1 expression and activity following smoke exposure [30]. MMP-12 expression has recently been detected in human COPD [145,146]. *In vitro* studies demonstrate production of MMP-12 by cultured bronchial epithelial cells [147] and its induction following cigarette smoke exposure [148]. Further, MMP-12 knockout mice are protected from smoke-induced airspace enlargement [149].

Direct evidence of MMP-1, MMP-2, and MMP-9 production by airway and parenchymal epithelial cells has been demonstrated in several human COPD studies [93,146,150]. In addition to cleavage of lung collagen, elastin, and fibronectin matrix proteins, MMPs cleave cell surface receptors, activate growth factors, chemoattractants molecules, and other MMP proenzymes [151]. Therefore, the increase in MMP activity can contribute to altered epithelial repair, reduced adhesion to provisional matrix, and enlarged alveolar airspaces. Although several MMPs were elevated in the initial microarray analysis, none were significant altered after the statistical normalization. The arrays did reveal the potential for CSE to induce expression of cathepsin L (Table 11), a cysteine proteinase with elastolytic activity previously detected in alveolar macrophages of smokers [152], and in patients with emphysema [153]. We also detected putative changes in expression of less well-studied proteases during SAEC exposure to CSE (Table 11), including decreased expression of an ubiquitin-specific protease. The involvement of this particular enzyme in emphysema is not yet known. However, the creation of databases of relevant smoke-associated and COPD-associated gene expression changes in lung epithelial cells of smokers, never-smokers, and former smokers [82,83] will enhance our understanding of the distinct epithelial responses induced by acute and chronic smoke exposures.

CONCLUSION

Historically, the airway epithelium was considered to be a tight localization of cells whose main purpose was to act as a physical barrier from inhaled pollutants and microbes. However, research has confirmed that the epithelial cell is an active participant in the defense of the lung. The airway epithelium contributes to the integrity of the structure of the lung through production of inflammatory mediators, mucus, defensins, matrix proteins, lipid molecules, antioxidants, and cell surface receptors. In particular, the airway epithelial cells have immediate responses to tobacco smoke. For example, the protective barrier of the epithelium is disrupted by cigarette smoke exposure, increasing permeability [155]. This type of injury leads to increased inflammatory cell influx into the epithelial layer of the airway [156].

In summary, the potential of epithelial cells to be damaged by cigarette smoke exposure results from their repeated exposures to the insult. Once exposed, these varied cells initiate repair mechanisms to reduce oxidant injury and prevent extensive inflammation [72], [156]. Chronic exposure to cigarette smoke, however, may overwhelm some of these early defense mechanisms, and actually enhance injury through activation of signal transduction pathways and induction of cytokines, growth factors, and chemoattractant molecules [157], [158]. The previous paradigm of COPD pathology centered on the increased inflammatory cell influx is evolving to consider the potent role of the epithelial cell. A role for the epithelial cell in alveolar destruction and airspace enlargement is demonstrated by several reports which show increased epithelial expression of MMPs in lung tissue from patients with COPD [93,146,150]. Thus the epithelial cell should be considered an important mediator of the destructive process. Cells of the bronchial epithelium can signal to inflammatory cells through expression of cell surface adhesion molecules, and by production of chemokines that stimulate the adaptive and innate immune systems. Included in this collection of chemoattractants are MCP-1 and MIP-1α [127]. Our microarray studies suggest that extensive numbers of SAEC genes are affected by CSE. These were not specifically discussed due to space limitations, but are listed in various tables based on their primary function. These genes belong to diverse Gene Ontology (GO) biological process groups, including cell cycle regulation (Table 9), G protein signaling (Table 12), lipid metabolism (Table 13), ATP binding (Table 14) , GTPase/GTP binding (Table 15), RNA processing (Table 16) , DNA replication (Table 17), carbohydrate metabolism (Table 18), cell-cell signaling (Table 19), protein modification (Table 20), cell adhesion/motility (Table 21), and genes with presently unclassified function (Table 22). Together, impaired oxidant metabolism and cell signaling, accompanied by elevated mucus, cytokine, and protease production, contribute to cigarette smoke-induced epithelial injury. Evidence for the diverse functions of airway epithelial cells reveals that these cells are involved in the recruitment of macrophages and neutrophils, and in the clearance of damage cells and inhaled particulates. These functions are critical for lung health, and may play a direct role in COPD, where the epithelial cell itself is affected.

MATERIALS AND METHODS

In Vitro Smoke Exposure Studies

Two independent smoke exposure studies were performed and analyzed in triplicate. Human SAECs were purchased from Clonetics (Cambrex Bio Sciences, Baltimore, MD) and cultured according to manufacturer’s instructions. A549 human lung adenocarcinoma cells from American Type Culture Collection (Manassas, VA) were cultured in DMEM containing 10% FBS with penicillin and streptomycin. Fresh CSE was prepared as previously reported [30]. The apoptosis inducer staurosporine was from Biomol (Plymouth Meeting, PA) and bacterial LPS was from Sigma (St. Louis, MO). Human A549 cells were treated for 40 hours and primary SAECs for 24 hours with CSE at the indicated concentrations. Cells were washed with PBS, collected by trypsinization, and aliquots were counted in triplicate using a hemocytomer.

RNA Isolation and Microarray Analyses

A separate series of CSE-exposure experiments was performed using SAECs for microarray studies. Preparation of cRNA for microarray analyses was conducted following the Affymetrix (Santa Clara, CA) GeneChip expression analysis technical manual. Total RNA from SAECs was isolated with Trizol (Invitrogen). Five micrograms of total RNA were used to synthesize first strand cDNA, using a T7-$(dT)_{24}$ oligonucleotide primer (Genset) and Superscript II reverse transcriptase (Invitrogen). Second strand cDNA was synthesized using DNA polymerase I, and the resulting double-stranded cDNA was purified by phenol/chloroform extraction and ethanol precipitation. The BioArray RNA transcript labeling kit (ENZO, New York, NY) was used to synthesize biotinylated cRNA using the double-stranded cDNA as a template, following manufacturer’s instructions. The cRNA was cleaned up with QIAGEN RNeasy columns, ethanol-precipitated, fragmented, and used for Affymetrix GeneChip analyses. Each sample was hybridized onto U95Av2 DNA arrays containing probes for approximately 12,600 human genes, and scanned following the Affymetrix protocol as described [159].

Statistical Analyses

Probe-level analyses were performed according to the robust multiarray algorithm [16] (Gene-Traffic; Iobion, La Jolla, CA). Differentially expressed genes were identified using a paired

T-test within BRB ArrayTools (version 3.01; R. Simon and A. P. Lam, National Cancer Institute, Bethesda, MD) [17,18]. These criteria identified a final list of 425 genes. Of these, 210 were increased and 215 were decreased by 24-hours exposure to 5% CSE.

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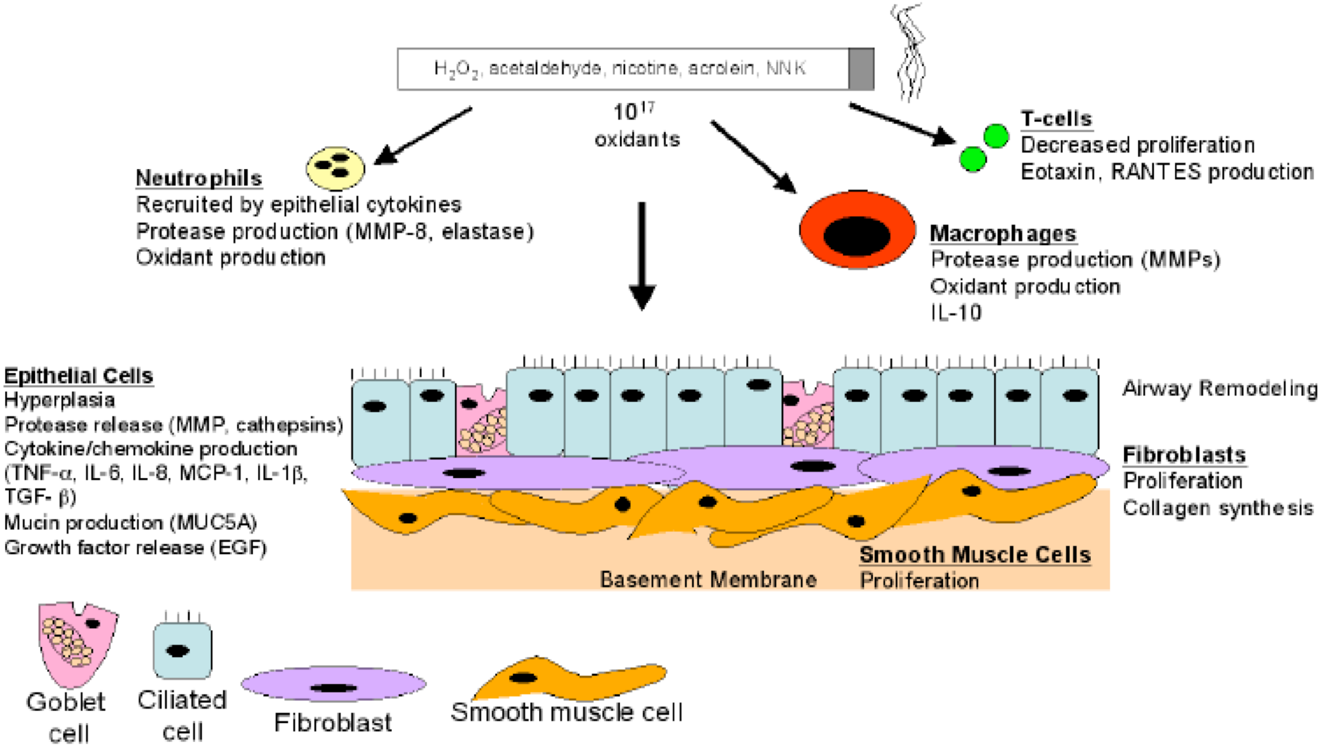

Fig. (1).

Exposure to cigarette smoke induces distinct responses by the varied cells of the airways. The bronchial airway epithelial cells are the first to encounter toxins and oxidants in smoke, and influence, through the production of cytokines such as IL-8 and TNF-α, neighboring cells such as fibroblasts and smooth muscle cells. Epithelial and inflammatory cells produce proteolytic matrix metalloproteinases (MMPs) leading to damage to the airway and more distal lung matrix. (NNK, nitrosamine 4-methylnitrosamino-1-(3-pyridyl)-1-butanone). See text for details.

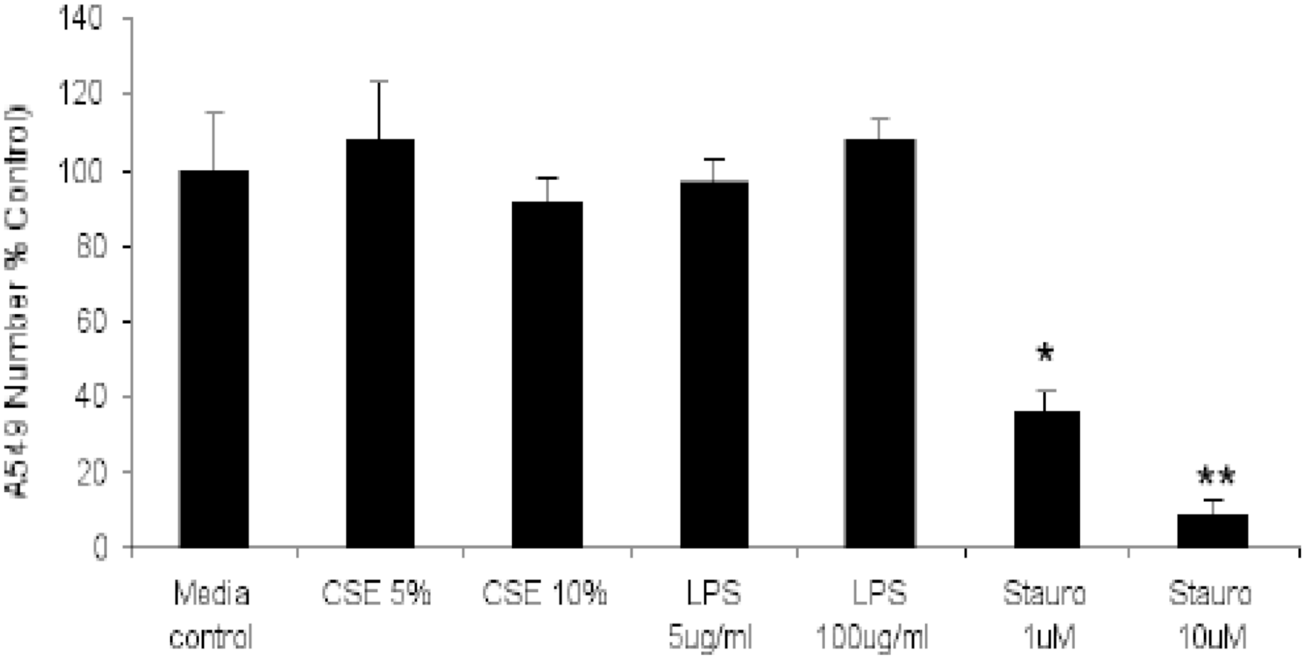
A549 Number % Control)
0
20
40
60
80
100
120
140
Media control
CSE 5%
CSE 10%
LPS 5ug/ml
LPS 100ug/ml
Stauro 1uM
Stauro 10uM
*
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Fig. (2A)

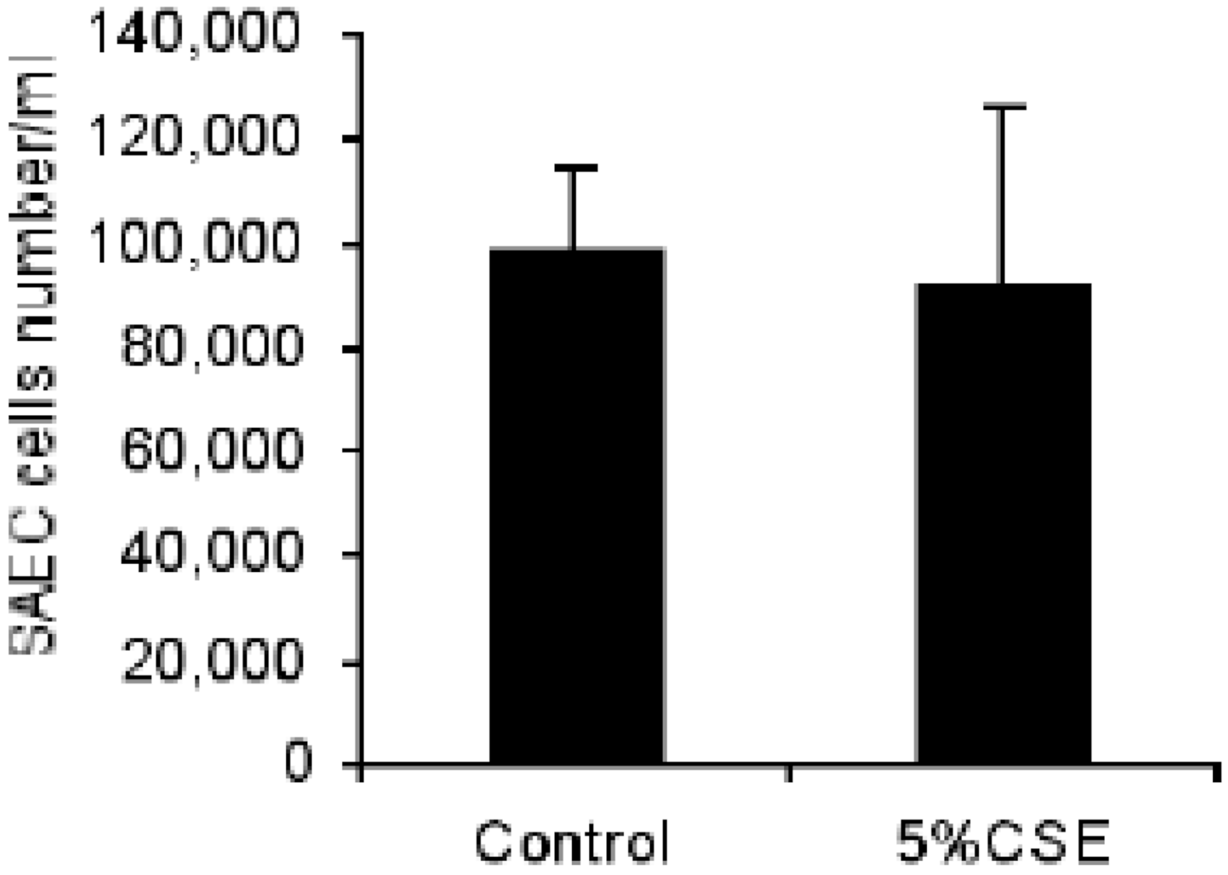

Fig. (2B)

Fig. (2).

Fig. (2A). A549 lung adenocarcinoma cell numbers are not reduced by 48 hours of exposure to CSE or lipopolysaccharide (LPS). However, treatment with increasing doses of the known apoptosis inducer staurosporine (stauro) significantly reduces cell numbers. $*p < 0.05$ or $**p < 0.01$, using Student's t test from two independent experiments.

Fig. (2B). SAEC numbers are not reduced by 24 hours of exposure to 5% CSE. The p value for Student's t test is 0.82, control versus 5% CSE.

Table 1

SAEC Genes Differentially Expressed Following 24 Hours Exposure to Cigarette Smoke Extract (5% CSE)

GO Biological Process/ Molecular Function	% of Total Genes Detected	# Genes	# Increased (%)	# Decreased (%)
Protein Modification	12.7	54	29 (53.7)	25 (46.3)
Transport (Electron, Ion, Protein)	8.2	35	20 (57.1)	15 (42.9)
Transcription/DNA Binding	6.6	28	19 (67.9)	9 (32.1)
Adhesion/Motility	6.4	27	9 (33.3)	18 (66.7)
Cell Cycle	5.2	22	7 (31.8)	15 (68.2)
Inflammation/Cell Defense	4.7	20	8 (40.0)	12 (60.0)
ATP Binding	4.2	18	11 (61.1)	7 (38.9)
Lipid Metabolism	4.2	18	5 (27.8)	13 (72.2)
Carbohydrate Metabolism	4.2	18	9 (50.0)	9 (50.0)
DNA Replication/Nucleotide Metabolism	4.0	17	10 (58.8)	7 (41.2)
Xenobiotic/Antioxidant	4.0	17	9 (52.9)	8 (47.1)
Signal Transduction	3.1	13	9 (69.2)	4 (30.8)
RNA Processing	3.1	13	8 (61.5)	5 (38.5)
DNA Repair	2.8	12	7 (58.3)	5 (41.7)
Apoptosis	2.6	11	9 (81.8)	2 (18.2)
Cell Proliferation	2.1	9	4 (44.4)	5 (55.6)
Proteolysis	1.9	8	2 (25.0)	6 (75.0)
G protein Related	1.6	7	2 (28.6)	5 (71.4)
Cell-Cell Signaling	0.9	4	1 (25.0)	3 (75.0)
GTPase/GTP Binding	0.9	4	2 (50.0)	2 (50.0)
Unclassified	16.5	70	30(42.9)	40 (57.1)
TOTAL		**425**	**210 (49.4)**	**215 (50.6)**

Table 2

Genes involved in Cell Proliferation

Gene symbol	Probe set	Ratio CSE/Con	p-value	Description	GO Biological Process	GO Molecular Function	GO Cellular Component	Pathway
AKR1C3	37399_at	2.5	0.04	aldo-keto reductase family 1, member C3 (3-alpha hydroxysteroid dehydrogenase, type II)	cell proliferation / lipid metabolism / prostaglandin metabolism	aldo-keto reductase activity / electron transporter activity / prostaglandin-F synthase activity / trans-1,2-dihydrobenzene-1,2-diol dehydrogenase activity	---	Prostaglandin and leukotriene metabolism
VEGFB	37268_at	1.11	0.01	vascular endothelial growth factor B	cell proliferation / positive regulation of cell proliferation / regulation of cell cycle / regulation of cell growth / signal transduction	growth factor activity / heparin binding / vascular endothelial growth factor receptor binding	extracellular / membrane	---
RFP	40176_at	1.11	0.02	ret finger protein	cell proliferation / protein ubiquitination / regulation of transcription, DNA-dependent / spermatogenesis / transcription	DNA binding / metal ion binding / transmembrane receptor protein tyrosine kinase activity / ubiquitin-protein ligase activity / zinc ion binding	integral to plasma membrane / membrane fraction / nucleus / ubiquitin ligase complex	---
FGFR1OP	38571_at	1.11	0.03	FGFR1 oncogene partner	positive regulation of cell proliferation	---	---	---
OSMR	39277_at	0.91	0.004	oncostatin M receptor	cell proliferation / cell surface receptor linked signal transduction	oncostatin-M receptor activity / receptor activity	oncostatin-M receptor complex	---
CTBP2	40780_at	0.83	0.02	C-terminal binding protein 2	L-serine biosynthesis / negative regulation of cell proliferation / viral genome replication	oxidoreductase activity / oxidoreductase activity, acting on the CH-OH group of donors, NAD or NADP as acceptor	nucleus	---
TPD52L2	40076_at	0.83	0.01	tumor protein D52-like 2	cell proliferation	---	---	---
DD5	39036_g_at	0.77	0.01	progestin induced protein	cell proliferation / ubiquitin cycle / ubiquitin-dependent protein catabolism	ligase activity / ubiquitin conjugating enzyme activity / ubiquitin-protein ligase activity	nucleus / soluble fraction	---
LAMP3	37168_at	0.71	0.04	lysosomal-associated membrane protein 3	cell proliferation	---	lysosomal membrane	---

Table 3

Genes Associated with Inflammation or Cellular Defense

Gene symbol	Probe set	Ratio CSE/Con	p-value	Description	GO Biological Process	GO Molecular Function	GO Cellular Component	Pathway
SPP1	34342_s_at	3.33	0.04	secreted phosphoprotein 1 (osteopontin, bone sialoprotein I, early T-lymphocyte activation 1)	T-helper 1 type immune response / anti-apoptosis / cell-cell signaling / cell-matrix adhesion / immune cell chemotaxis / induction of positive chemotaxis / negative regulation of bone mineralization / ossification / positive regulation of T-cell proliferation / regulation of myeloid blood cell differentiation	cytokine activity / growth factor activity / integrin binding / protein binding	Extracellular matrix (sensu Metazoa)	TGF_Beta_Signa_ling_Pathway
SPP1	2092_s_at	2.50	0.03	secreted phosphoprotein 1 (osteopontin, bone sialoprotein I, early T-lymphocyte activation 1)	T-helper 1 type immune response / anti-apoptosis / cell-cell signaling / cell-matrix adhesion / immune cell chemotaxis / induction of positive chemotaxis / negative regulation of bone mineralization / ossification / positive regulation of T-cell proliferation / regulation of myeloid blood cell differentiation	cytokine activity / growth factor activity / integrin binding / protein binding	Extracellular matrix (sensu Metazoa)	TGF_Beta_Signa_lingvPathway
IL13RA2	1016_s_at	1.67	0.02	interleukin 13 receptor, alpha 2	---	interleukin receptor activity / receptor activity	extracellular space / integral to membrane / soluble fraction	---
FCGR2A	37687_i_at	1.11	0.004	Fc fragment of IgG, low affinity IIa, receptor for (CD32)	immune response	IgG binding / receptor activity / receptor signaling protein activity	integral to membrane / plasma membrane	---
MYD88	38369_at	1.11	0.004	myeloid differentiation primary response gene (88)	cell surface receptor linked signal transduction / immune response / inflammatory response / positive regulation of I-kappaB kinase/NF-kappaB cascade	death receptor binding / protein binding / signal transducer activity / transmembrane receptor activity	membrane	---
APBA2B P	41306_at	1.11	0.03	amyloid beta (A4) precursor protein-binding, family A, member 2 binding protein	antibiotic biosynthesis / protein metabolism / protein secretion / regulation of amyloid	calcium ion binding / oxidoreductase activity / protein binding	Golgi cis cisterna / cytoplasm / endoplasmic reticulum membrane	---

Gene symbol	Probe set	Ratio CSE/Con	p-value	Description	GO Biological Process	GO Molecular Function	GO Cellular Component	Pathway
					precursor protein biosynthesis			
KCNN4	41106_at	1.11	0.04	potassium intermediate/small conductance calcium-activated channel, subfamily N, member 4	defense response / ion transport / potassium ion transport	calmodulin binding / ion channel activity / small conductance calcium-activated potassium channel activity	integral to membrane / membrane fraction / voltage-gated potassium channel complex	---
MAPK11	40033_at	1.11	0.05	mitogen-activated protein kinase 11	antimicrobial humoral response (sensu Vertebrata) / protein amino acid phosphorylation / protein kinase cascade / response to stress / signal transduction	ATP binding / MAP kinase activity / MP kinase activity / protein serine/threonine kinase activity / transferase activity	---	---
LTA4H	38081_at	0.91	0.04	leukotriene A4 hydrolase	inflammatory response / leukotriene biosynthesis / proteolysis and peptidolysis	epoxide hydrolase activity / membrane alanyl aminopeptidase activity / metallopeptidase activity / zinc ion binding	---	Eicosanoid_Sy nthesis / Prostaglandin and leukotriene metabolism
ALOX5A P	37099_at	0.83	0.001	arachidonate 5-lipoxygenase-activating protein	inflammatory response / leukotriene biosynthesis	binding / enzyme activator activity	integral to membrane / membrane fraction	Eicosanoid_Sy nthesis
PROC	39255_at	0.83	0.03	protein C (inactivator of coagulation factors Va and VIIIa)	anti-inflammatory response / blood coagulation / negative regulation of apoptosis / negative regulation of blood coagulation / proteolysis and peptidolysis	calcium ion binding / chymotrypsin activity / hydrolase activity / protein C (activated) activity / trypsin activity	extracellular	---
B2M	34644_at	0.83	0.03	beta-2-microglobulin	antigen presentation, endogenous antigen / antigen processing, endogenous antigen *via* MHC class I / immune response	MHC class I receptor activity	extracellular	---
IL7R	36227_at	0.83	0.03	interleukin 7 receptor	antimicrobial humoral response (sensu Vertebrata) / cell surface receptor linked signal transduction / immune response / regulation of DNA recombination	antigen binding / hematopoietin/ interferon -class (D200-domain) cytokine receptor activity / interleukin-7 receptor activity / receptor activity	integral to membrane	---
CLECSF2	40698_at	0.83	0.04	C-type (calcium dependent, carbohydrate-recognition domain) lectin, superfamily member 2 (activation-induced)	antimicrobial humoral response (sensu Vertebrata)	sugar binding	integral to plasma membrane	---

Gene symbol	Probe set	Ratio CSE/Con	p-value	Description	GO Biological Process	GO Molecular Function	GO Cellular Component	Pathway
CD59	39351_at	0.77	0.03	CD59 antigen p18-20 (antigen identified by monoclonal antibodies 16.3A5, EJ16, EJ30, EL32 and G344)	blood coagulation / cell surface receptor linked signal transduction / immune response	---	membrane fraction / plasma membrane	---
ZNF148	41465_at	0.77	0.04	zinc finger protein 148 (pHZ-52)	cellular defense response / negative regulation of transcription from Pol II promoter / regulation of transcription, DNA-dependent	DNA binding / nucleic acid binding / specific RNA polymerase II transcription factor activity / transcriptional activator activity / zinc ion binding	DNA-directed RNA polymerase II, core complex / nucleus	---
MCP	38441_s_at	0.77	0.05	membrane cofactor protein (CD46, trophoblast-lymphocyte cross-reactive antigen)	complement activation, classical pathway	receptor activity	integral to plasma membrane	---
ANKRD1 5	37225_at	0.71	0.04	ankyrin repeat domain 15	immune response / negative regulation of cell cycle	GTP binding / GTPase activity	---	---
CXCL11	35061_at	0.67	0.04	chemokine (C-X-C motif) ligand 11	cell-cell signaling / chemotaxis / immune response / inflammatory response / response to pathogenic fungi / signal transduction	chemokine activity	extracellular	---
IL6	38299_at	0.59	0.02	interleukin 6 (interferon, beta 2)	acute-phase response / cell surface receptor linked signal transduction / cell-cell signaling / humoral immune response / negative regulation of cell proliferation / positive regulation of cell proliferation	cytokine activity / interleukin-6 receptor binding	extracellular space	---

Table 4

Genes with Antioxidant/ Xenobiotic Metabolism Function

Gene symbol	Probe set	Ratio CSE/Con	p-value	Description	GO Biological Process	GO Molecular Function	GO Cellular Component	Pathway
GPX2	35194_at	3.33	0.01	glutathione peroxidase 2 (gastrointestinal)	response to oxidative stress	electron transporter activity / glutathione peroxidase activity / oxidoreductase activity	cytoplasm	Glutathione metabolism
GCLC	31850_at	2.50	0.02	glutamate-cysteine ligase, catalytic subunit	circulation / cysteine metabolism / glutamate metabolism / glutathione biosynthesis	glutamate-cysteine ligase activity / ligase activity / nucleic acid binding	---	Glutamate metabolism / Glutathione metabolism
EPHX1	38790_at	2.00	0.00001	epoxide hydrolase 1, microsomal (xenobiotic)	aromatic compound catabolism / response to toxin / xenobiotic metabolism	epoxide hydrolase activity / hydrolase activity	endoplasmic reticulum / integral to membrane / microsome	Tetrachloroethene degradation
UGT1A10	32392_s_at	2.00	0.05	UDP glycosyltransferas e 1 family, polypeptide A10	metabolism / metabolism / xenobiotic metabolism / bilirubin conjugation / digestion / estrogen metabolism / xenobiotic metabolism	glucuronosyltransferase activity / transferase activity, transferring hexosyl groups / transferase activity / UDP-glycosyltransferase activity	integral to membrane / microsome / microsome / endoplasmic reticulum	Pentose and glucuronate interconversions / Androgen and estrogen metabolism / Starch and sucrose metabolism / Porphyrin and chlorophyll metabolism
ALDH3A2	40409_at	1.67	0.01	aldehyde dehydrogenase 3 family, member A2	central nervous system development / epidermis development / lipid metabolism / peripheral nervous system development	aldehyde dehydrogenase (NAD) activity / oxidoreductase activity	endoplasmic reticulum / integral to membrane / microsome	Glycolysis / Gluconeogenesis / Ascorbate and aldarate metabolism / Fatty acid metabolism / Bile acid biosynthesis / Valine, leucine and isoleucine degradation / Lysine degradation / Arginine and proline metabolism / Histidine metabolism / Tryptophan metabolism / beta-Alanine metabolism / Glycerolipid metabolism / Pyruvate metabolism / 1,2-Dichloroethane degradation / Propanoate metabolism / Butanoate metabolism / Limonene and pinene degradation
GPX4	33931_at	1.43	0.03	glutathione peroxidase 4 (phospholipid hydroperoxidase)	development / phospholipid metabolism / response to oxidative stress	electron transporter activity / glutathione peroxidase activity / oxidoreductase activity / phospholipid-hydroperoxide glutathione peroxidase activity	mitochondrion	Glutathione metabolism
CBR1	38773_at	1.43	0.04	carbonyl reductase 1	metabolism	15-hydroxyprostaglandin dehydrogenase (NADP+) activity / carbonyl	cytosol	Prostaglandin and leukotriene metabolism / Prostaglandin and leukotriene metabolism

Gene symbol	Probe set	Ratio CSE/Con	p-value	Description	GO Biological Process	GO Molecular Function	GO Cellular Component	Pathway
						reductase (NADPH) activity / oxidoreductase activity / prostaglandin-E2 9-reductase activity		
MPST	36124_at	1.11	0.01	mercaptopyruvate sulfurtransferase	cyanate catabolism / response to toxin / sulfate transport	3-mercaptopyruvate sulfurtransferase activity / thiosulfate sulfurtransferase activity / transferase activity	mitochondrial matrix	Cysteine metabolism
GSTM1	556_s_at	1.11	0.04	glutathione S-transferase M1	metabolism	glutathione transferase activity / transferase activity	cytoplasm	Glutathione metabolism
GSTZ1	1212_at	0.91	0.04	glutathione transferase zeta 1 (maleylacetoacet ate isomerase)	L-phenylalanine catabolism / aromatic amino acid family metabolism / tyrosine catabolism	catalytic activity / glutathione peroxidase activity / glutathione transferase activity / isomerase activity / maleylacetoacetate isomerase activity / transferase activity	cytoplasm / mitochondrion	Tyrosine metabolism / Styrene degradation / Glutathione metabolism
SMS	38792_at	0.91	0.05	spermine synthase	methionine metabolism / polyamine metabolism	spermidine synthase activity / spermine synthase activity / transferase activity	---	Urea cycle and metabolism of amino groups / Arginine and proline metabolism / beta-Alanine metabolism
PRNP	36159_s_at	0.83	0.00	prion protein (p27-30) (Creutzfeld-Jakob disease, Gerstmann-Strausler-Scheinker syndrome, fatal familial insomnia)	metabolism	---	---	---
MUT	40105_at	0.83	0.03	methylmalonyl Coenzyme A mutase	metabolism	cobalt ion binding / isomerase activity / methylmalonyl-CoA mutase activity	mitochondrion	Valine, leucine and isoleucine degradation / Propanoate metabolism
DECR1	38104_at	0.77	0.00	2,4-dienoyl CoA reductase 1, mitochondrial	metabolism	2,4-dienoyl-CoA reductase (NADPH) activity / oxidoreductase activity	mitochondrion	---
PON2	40504_at	0.77	0.02	paraoxonase 2	---	aryldialkylphosphatase activity / arylesterase activity / hydrolase activity	extracellular / membrane	---
P4HA1	37037_at	0.77	0.04	procollagen-proline, 2-oxoglutarate 4-dioxygenase (proline 4-hydroxylase), alpha polypeptide I	---	oxidoreductase activity / oxidoreductase activity, acting on single donors with incorporation of molecular oxygen, incorporation of two atoms of oxygen / procollagen-proline 4-dioxygenase activity	endoplasmic reticulum	Arginine and proline metabolism

Gene symbol	Probe set	Ratio CSE/Con	p-value	Description	GO Biological Process	GO Molecular Function	GO Cellular Component	Pathway
GGH	37263_at	0.77	0.03	gamma-glutamyl hydrolase (conjugase, folylpolygamma glutamyl hydrolase)	---	exopeptidase activity / gamma-glutamyl hydrolase activity / hydrolase activity	lysosome	Folate biosynthesis

Table 5

Genes Involved in Electron, Ion, or Protein Transport

ymbol	Probe set	Ratio CSE/Con	p-value	Description	GO Biological Process	GO Molecular Function	GO Cellular Component	Pathway
1C1	32805_at	5.00	0.002	aldo-keto reductase family 1, member C1 (dihydrodiol dehydrogenase 1; 20-alpha (3-alpha)-hydroxysteroid dehydrogenase)	canalicular bile acid transport / digestion / lipid metabolism / transport / xenobiotic metabolism	bile acid transporter activity / binding / electron transporter activity / oxidoreductase activity / trans-1,2-dihydrobenzene-1,2-diol dehydrogenase activity / aldo-keto reductase activity / electron transporter activity	cytoplasm	---
D1	38066_at	5.00	0.003	NAD(P)H dehydrogenase, quinone 1	electron transport / nitric oxide biosynthesis / response to toxin / synaptic transmission, cholinergic / xenobiotic metabolism	NAD(P)H dehydrogenase (quinone) activity / cytochrome-b5 reductase activity / oxidoreductase activity	cytoplasm	---
RD1	39425_at	3.33	0.02	thioredoxin reductase 1	electron transport / signal transduction	disulfide oxidoreductase activity / metal ion binding / thioredoxin-disulfide reductase activity	cytoplasm	Pyrimidine metabolism
B1	40071_at	3.33	0.03	cytochrome P450, family 1, subfamily B, polypeptide 1	electron transport / eye morphogenesis (sensu Mammalia) / visual perception	electron transporter activity / monooxygenase activity / oxygen binding	endoplasmic reticulum / membrane / microsome	---
A1	1025_g_at	2.50	0.01	cytochrome P450, family 1, subfamily A, polypeptide 1	electron transport	monooxygenase activity / oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen, reduced flavin or flavoprotein as one donor, and incorporation of one atom of oxygen / oxygen binding	endoplasmic reticulum / membrane / microsome	Fatty acid metabolism / gamma-Hexachlorocyclohexane degradation / Tryptophan metabolism
B1	859_at	2.50	0.03	cytochrome P450, family 1, subfamily B, polypeptide 1	electron transport / eye morphogenesis (sensu Mammalia) / visual perception	electron transporter activity / monooxygenase activity / oxygen binding	endoplasmic reticulum / membrane / microsome	---
D	36963_at	2.00	0.003	phosphogluconate dehydrogenase	pentose-phosphate shunt, oxidative branch	electron transporter activity / oxidoreductase activity / phosphogluconate dehydrogenase (decarboxylating) activity	---	Pentose_Phosphate_Pathwa y / Pentose phosphate pathway
A1	36767_at	2.00	0.01	cytochrome P450, family 1, subfamily A, polypeptide 1	electron transport	monooxygenase activity / oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen, reduced flavin or flavoprotein as one donor, and incorporation of one atom of oxygen / oxygen binding	endoplasmic reticulum / membrane / microsome	Fatty acid metabolism / gamma-Hexachlorocyclohexane degradation / Tryptophan metabolism
M1	40898_at	2.00	0.05	sequestosome 1	endosome transport / intracellular signaling cascade / positive regulation of transcription from Pol II promoter / protein localization / regulation of I-kappaB kinase/NF-	SH2 domain binding / protein kinase binding / ubiquitin binding / zinc ion binding	cytosol	---

mbol	Probe set	Ratio CSE/Con	p-value	Description	GO Biological Process	GO Molecular Function	GO Cellular Component	Pathway
					kappaB cascade / response to stress			
B	36881_at	1.43	0.02	electron-transfer-flavoprotein, beta polypeptide	electron transport	electron carrier activity	mitochondrial matrix	---
O2	36880_at	1.25	0.01	NAD(P)H dehydrogenase, quinone 2	electron transport	NAD(P)H dehydrogenase (quinone) activity / NADPH dehydrogenase (quinone) activity / electron transporter activity / oxidoreductase activity	---	Biosynthesis of steroids
X5	40396_at	1.25	0.01	purinergic receptor P2X, ligand-gated ion channel, 5	ion transport	ATP binding / ion channel activity / receptor activity	membrane	---
B1	37669_s_at	1.25	0.02	ATPase, Na+/K+ transporting, beta 1 polypeptide	potassium ion transport / sodium ion transport / transport	sodium:potassium-exchanging ATPase activity	integral to membrane / sodium:potassi um-exchanging ATPase complex	---
M1	39134_at	1.25	0.02	target of myb1 (chicken)	endocytosis / endosome transport / intra-Golgi transport / intracellular protein transport	protein binding / protein transporter activity	Golgi stack / cytosol / early endosome / endosome / lysosome / membrane	---
G2	38390_at	1.25	0.02	component of oligomeric golgi complex 2	Golgi organization and biogenesis / intra-Golgi transport / intracellular protein transport / oligosaccharide biosynthesis / protein amino acid glycosylation	protein transporter activity	Golgi membrane / Golgi transport complex / membrane	---
A1	1024_at	1.25	0.04	cytochrome P450, family 1, subfamily A, polypeptide 1	electron transport	monooxygenase activity / oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen, reduced flavin or flavoprotein as one donor, and incorporation of one atom of oxygen / oxygen binding	endoplasmic reticulum / membrane / microsome	Fatty acid metabolism / gamma-Hexachlorocy clohexane degradation / Tryptophan metabolism
PA	36977_at	1.25	0.04	N-ethylmaleimide-sensitive factor attachment protein, alpha	intra-Golgi transport / intracellular protein transport / membrane fusion	intracellular transporter activity	Golgi apparatus / endoplasmic reticulum	---
A2	32228_at	1.11	0.0005	adaptor-related protein complex 2, alpha 2 subunit	endocytosis / intracellular protein transport / protein complex assembly	lipid binding / structural molecule activity	AP-2 adaptor complex / Golgi apparatus / clathrin coat of trans-Golgi network vesicle / coated pit	---
3IP	34896_at	1.11	0.003	SEC23 interacting protein	Golgi organization and biogenesis / intracellular protein transport	metal ion binding / protein binding	ER-Golgi intermediate compartment	---
FS8	38257_at	1.11	0.004	NADH dehydrogenase (ubiquinone) Fe-S protein 8, 23kDa (NADH-coenzyme Q reductase)	electron transport / mitochondrial electron transport, NADH to ubiquinone	NADH dehydrogenase (ubiquinone) activity / NADH dehydrogenase activity / electron carrier activity / iron ion binding	membrane fraction / mitochondrion	Electron_Tran sport_Chain

ymbol	Probe set	Ratio CSE/Con	p-value	Description	GO Biological Process	GO Molecular Function	GO Cellular Component	Pathway
A16	33262_at	0.91	0.003	solute carrier family 25 (mitochondrial carrier; Graves disease autoantigen), member 16	transport	binding / solute:solute antiporter activity	integral to membrane / mitochondrial inner membrane / mitochondrion	---
0882	38254_at	0.91	0.02	KIAA0882 protein	---	calcium ion binding	---	---
21	36128_at	0.91	0.02	transmembrane trafficking protein	ER to Golgi transport / protein transport	---	Golgi apparatus / integral to plasma membrane / membrane fraction / microsome	---
L5	36488_at	0.91	0.02	EGF-like-domain, multiple 5	---	calcium ion binding / structural molecule activity	integral to membrane	---
D	37342_s_at	0.91	0.03	isovaleryl Coenzyme A dehydrogenase	electron transport	isovaleryl-CoA dehydrogenase activity / oxidoreductase activity	mitochondrial matrix	Valine, leucine and isoleucine degradation
0A3	34457_at	0.91	0.05	solute carrier family 30 (zinc transporter), member 3	cation transport / transport / zinc ion transport	cation transporter activity / zinc porter activity	endosome / integral to plasma membrane / membrane / membrane fraction / synaptic vesicle	---
0A1	34759_at	0.83	0.005	solute carrier family 30 (zinc transporter), member 1	cation transport / transport / zinc ion transport	cation transporter activity	integral to membrane	---
R1	32778_at	0.83	0.01	inositol 1,4,5-triphosphate receptor, type 1	calcium ion transport / cation transport / signal transduction	calcium channel activity / inositol 1,4,5-triphosphate-sensitive calcium-release channel activity / inositol-1,4,5-triphosphate receptor activity	Endoplasmic reticulum membrane / integral to membrane	Calcium_Channels / G_Protein_Si gnaling
SF2	34307_at	0.83	0.02	transmembrane 9 superfamily member 2	transport	transporter activity	endosome / integral to plasma membrane	---
K1	37552_at	0.83	0.04	potassium channel, subfamily K, member 1	ion transport / potassium ion transport	inward rectifier potassium channel activity / potassium channel activity / voltage-gated ion channel activity	integral to membrane / membrane fraction / voltage-gated potassium channel complex	---
T	41722_at	0.77	0.003	nicotinamide nucleotide transhydrogenase	electron transport / energy pathways / proton transport	NAD(P)+ transhydrogenase (AB-specific) activity / NAD(P)+ transhydrogenase (B-specific) activity / electron transporter activity / oxidoreductase activity	integral to membrane / mitochondrial electron transport chain / mitochondrion	---
C3	36851_g_at	0.77	0.01	tumor suppressor candidate 3	electron transport	electron transporter activity	integral to membrane	---
R1	755_at	0.77	0.01	inositol 1,4,5-triphosphate receptor, type 1	calcium ion transport / cation transport / signal transduction	calcium channel activity / inositol 1,4,5-triphosphate-sensitive calcium-release channel activity / inositol-1,4,5-triphosphate receptor activity	endoplasmic reticulum membrane / integral to membrane	Calcium_Cha nnels / G_Protein_Si gnaling
6IP	36572_r_at	0.71	0.002	ADP-ribosylation factor-like 6 interacting protein	---	---	integral to membrane	---
P2	36069_at	0.63	0.02	formin binding protein 2	metal ion transport	GTPase activator activity / metal ion binding	---	---

Table 6

DNA Repair-Associated Genes

Gene symbol	Probe set	Ratio CSE/Con	p-value	Description	GO Biological Process	GO Molecular Function	GO Cellular Component	Pathway
FLJ22028	34685_at	1.43	0.04	hypothetical protein FLJ22028	DNA repair	ATP binding / ATP-dependent DNA helicase activity / DNA binding / hydrolase activity	nucleus	---
MPG	37768_at	1.25	0.01	N-methylpurine-DNA glycosylase	DNA dealkylation / base-excision repair	alkylbase DNA N-glycosylase activity / damaged DNA binding / hydrolase activity	nucleoplasm	---
CSNK1D	493_at	1.25	0.02	casein kinase 1, delta	DNA repair / Wnt receptor signaling pathway / protein amino acid phosphorylation / signal transduction	ATP binding / casein kinase I activity / protein serine/threonine kinase activity / transferase activity	---	---
MAPK12	984_g_at	1.25	0.02	mitogen-activated protein kinase 12	DNA damage induced protein phosphorylation / MAPKKK cascade / cell cycle / cell cycle arrest / muscle development / myoblast differentiation / negative regulation of cell cycle / signal transduction	ATP binding / MAP kinase activity / SAP kinase 3 activity / magnesium ion binding / protein serine/ threonine kinase activity / transferase activity	cytoplasm	MAPK_Cascade / S1P_Signaling / Integrin-mediated_cell_adhesion
ADPRTL1	37303_at	1.11	0.004	ADP-ribosyltransferase (NAD+; poly (ADP-ribose) polymerase)-like 1	DNA repair / inflammatory response / necrosis / protein amino acid ADP-ribosylation / response to drug / transport	NAD+ ADP-ribosyltransferase activity / transferase activity, transferring glycosyl groups	nucleus / ribonucleoprotein complex	---
TSN	36177_at	1.11	0.01	translin	DNA recombination	DNA binding	nucleus	---
DDB1	1641_s_at	1.11	0.02	damage-specific DNA binding protein 1, 127kDa	nucleotide-excision repair	damaged DNA binding	nucleus	---
HMGB1	32220_at	0.91	0.0002	high-mobility group box 1	DNA recombination / DNA repair / DNA unwinding / base-excision repair, DNA ligation / establishment and/or maintenance of chromatin architecture / negative regulation of transcriptional preinitiation complex formation / regulation of transcription from Pol II promoter	DNA bending activity / transcription factor binding	chromatin / condensed chromosome / nucleus	---
XRCC5	38733_at	0.83	0.002	X-ray repair complementing defective repair in Chinese hamster cells 5 (double-strand-break rejoining; Ku autoantigen, 80kDa)	DNA recombination / double-strand break repair *via* nonhomologous end-joining / regulation of DNA repair	ATP-dependent DNA helicase activity / double-stranded DNA binding / helicase activity	DNA-dependent protein kinase complex / nucleus	---

Gene symbol	Probe set	Ratio CSE/Con	p-value	Description	GO Biological Process	GO Molecular Function	GO Cellular Component	Pathway
CHEK2	37887_at	0.83	0.03	CHK2 checkpoint homolog (S. pombe)	DNA damage checkpoint / cell cycle / cell growth and/or maintenance / protein amino acid phosphorylation / response to DNA damage stimulus	ATP binding / protein kinase activity / protein serine/threonine kinase activity / transferase activity	nucleus	Cell_cycle
MBD4	34386_at	0.83	0.05	methyl-CpG binding domain protein 4	base-excision repair	endodeoxyribonuclease activity / hydrolase activity / satellite DNA binding	nucleus	---
HMGB2	38065_at	0.53	0.02	high-mobility group box 2	DNA repair / DNA replication / DNA unwinding / base-excision repair, DNA ligation / establishment and/or maintenance of chromatin architecture / nucleosome assembly / regulation of transcription from Pol II promoter	DNA bending activity / double-stranded DNA binding / single-stranded DNA binding / transcription factor activity	chromatin / condensed chromosome / nuclear chromosome / perinuclear region	---

Table 7

Apoptosis-Related Genes

Gene symbol	Probe set	Ratio CSE/Con	p-value	Description	GO Biological Process	GO Molecular Function	GO Cellular Component	Pathway
TP53I3	36079_at	1.43	0.02	tumor protein p53 inducible protein 3	induction of apoptosis by oxidative stress	alcohol dehydrogenase activity, zinc-dependent / zinc ion binding	---	---
IER3	1237_at	1.25	0.03	immediate early response 3	anti-apoptosis / apoptosis / cell growth and/or maintenance / morphogenesis	---	integral to membrane	---
MAEA	32832_at	1.11	0.02	macrophage erythroblast attacher	apoptosis / cell adhesion / development	---	integral to plasma membrane / membrane fraction	---
PTK2B	33804_at	1.11	0.02	PTK2B protein tyrosine kinase 2 beta	apoptosis / cell adhesion / positive regulation of cell proliferation / protein amino acid phosphorylation / protein complex assembly / response to stress / signal complex formation / signal transduction	ATP binding / non-membrane spanning protein tyrosine kinase activity / signal transducer activity / transferase activity	cytoskeleton	---
MAP2K4	1845_at	1.11	0.01	mitogen-activated protein kinase kinase 4	JNK cascade / protein amino acid phosphorylation / signal transduction	ATP binding / protein serine/threonine kinase activity / protein-tyrosine kinase activity / transferase activity	---	Apoptosis / MAPK_Cascad e
DFFA	32047_at	1.11	0.04	DNA fragmentation factor, 45kDa, alpha polypeptide	DNA fragmentation during apoptosis / apoptosis / intracellular signaling cascade	caspase-activated deoxyribonuclease activity / protein binding	cytosol / nucleus	Apoptosis
TRADD	1729_at	1.11	0.005	TNFRSF1A–associated *via* death domain	apoptosis / induction of apoptosis / positive regulation of I-kappaB kinase/NF-kappaB cascade / signal transduction	protein binding / signal transducer activity	---	Apoptosis
HDAC3	35821_at	1.11	0.01	histone deacetylase 3	anti-apoptosis / chromatin modification / histone deacetylation / regulation of cell cycle / regulation of transcription, DNA-dependent	histone deacetylase activity / hydrolase activity / transcription factor binding	cytoplasm / histone deacetylase complex / nucleus	Cell_cycle
DAXX	1754_at	1.11	0.02	death-associated protein 6	apoptosis / regulation of transcription, DNA-dependent	calcium ion binding	nucleus	Apoptosis
RTN4	31536_at	0.91	0.02	reticulon 4	negative regulation of anti-apoptosis / negative regulation of axon	protein binding	endoplasmic reticulum / integral to endoplasmic	---

Gene symbol	Probe set	Ratio CSE/Con	p-value	Description	GO Biological Process	GO Molecular Function	GO Cellular Component	Pathway
					extension / regulation of apoptosis		reticulum membrane / nuclear membrane	
PORIMIN	40803_at	0.71	0.002	pro-oncosis receptor inducing membrane injury gene	---	receptor activity	integral to membrane	---

Table 8

Signal Transduction Related Genes

Gene symbol	Probe set	Ratio CSE/Con	p-value	Description	GO Biological Process	GO Molecular Function	GO Cellular Component	Pathway
MPP1	32207_at	1.25	0.01	membrane protein, palmitoylated 1, 55kDa	signal transduction	guanylate kinase activity / protein binding	integral to plasma membrane / membrane / membrane fraction	---
SECTM1	41045_at	1.25	0.02	secreted and transmembrane 1	positive regulation of I-kappaB kinase/NF-kappaB cascade	signal transducer activity	integral to membrane	---
SH2D2A	34432_at	1.25	0.02	SH2 domain protein 2A	angiogenesis / intracellular signaling cascade	---	---	---
MTVR1	32209_at	1.25	0.04	Mouse Mammary Tumor Virus Receptor homolog 1	---	receptor activity	---	---
STAT1	33338_at	1.25	0.05	signal transducer and activator of transcription 1, 91kDa	I-kappaB kinase/NF-kappaB cascade / STAT protein nuclear translocation / caspase activation / intracellular signaling cascade / regulation of cell cycle / regulation of transcription, DNA-dependent / response to pest, pathogen or parasite / transcription from Pol II promoter / tyrosine phosphorylation of STAT protein	hematopoietin/interferon-class (D200-domain) cytokine receptor signal transducer activity / signal transducer activity / transcription factor activity	cytoplasm / nucleus	TGF_Beta_ Signaling_ Pathway
SHB	40614_at	1.11	0.02	SHB (Src homology 2 domain containing) adaptor protein B	intracellular signaling cascade	SH3/SH2 adaptor protein activity	---	---
RHOC	1395_at	1.11	0.03	ras homolog gene family, member C	positive regulation of I-kappaB kinase/NF-kappaB cascade / small GTPase mediated signal transduction	GTP binding / GTPase activity / catalytic activity / signal transducer activity	---	---
NFKBIE	38276_at	1.11	0.03	nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, epsilon	cytoplasmic sequestering of transcription factor / positive regulation of I-kappaB kinase/ NF-kappaB cascade	signal transducer activity	cytoplasm	---
GNB2	38831_f_at	1.11	0.05	guanine nucleotide binding protein (G protein), beta polypeptide 2	signal transduction	signal transducer activity	---	G_Protein_Signal ing
RSU1	32544_s_at	0.83	0.01	Ras suppressor protein 1	signal transduction	---	---	---
HIP14	35973_at	0.77	0.03	huntingtin interacting protein 14	positive regulation of I-kappaB kinase/NF-kappaB cascade	metal ion binding / signal transducer activity	Golgi apparatus / integral to membrane	---
RAPGEF2	32026_s_at	0.77	0.04	Rap guanine nucleotide exchange factor (GEF) 2	MAPKKK cascade / cAMP-mediated signaling / small	Rap GTPase activator activity / Rap guanyl-nucleotide exchange	integral to plasma membrane / membrane	---

Gene symbol	Probe set	Ratio CSE/Con	p-value	Description	GO Biological Process	GO Molecular Function	GO Cellular Component	Pathway
					GTPase mediated signal transduction	factor activity / calcium ion binding / diacylglycerol binding / guanyl-nucleotide exchange factor activity / protein binding / signal transducer activity		
DPYSL2	40607_at	0.53	0.04	dihydropyrimidinase-like 2	neurogenesis / nucleobase, nucleoside, nucleotide and nucleic acid metabolism / signal transduction	dihydropyrimidinase activity / hydrolase activity	---	---

Table 9

Genes involved in Cell Cycle Regulation

Gene symbol	Probe set	Ratio CSE/Con	p-value	Description	GO Biological Process	GO Molecular Function	GO Cellular Component	Pathway
BMP2	40367_at	1.25	0.04	bone morphogenetic protein 2	cell growth and/or maintenance / cell-cell signaling / growth / skeletal development	cytokine activity / growth factor activity	extracellular	---
CDK8	1189_at	1.25	0.05	cyclin-dependent kinase 8	cytokinesis / protein amino acid phosphorylation / regulation of cell cycle / regulation of transcription, DNA-dependent	ATP binding / protein serine/threonine kinase activity / transferase activity	---	---
CCNE1	41060_at	1.11	0.01	cyclin E1	G1/S transition of mitotic cell cycle / cytokinesis / regulation of cell cycle	---	nucleus	Cell_cycle
MSF	41220_at	1.11	0.03	MLL septin-like fusion	cell cycle	GTP binding	---	---
PMS2	38556_at	1.11	0.03	PMS2 postmeiotic segregation increased 2 (S.cerevisiae)	mismatch repair / negative regulation of cell cycle	ATP binding / DNA binding	nucleus	---
MDK	38124_at	1.11	0.03	midkine (neurite growth-promoting factor 2)	cell differentiation / cell proliferation / cell-cell signaling / neurogenesis / regulation of cell cycle / signal transduction	cytokine activity / growth factor activity / heparin binding	extracellular space	---
RASSF1	39601_at	0.91	0.005	Ras association (RalGDS/AF-6) domain family 1	Ras protein signal transduction / cell cycle / cell cycle arrest / intracellular signaling cascade / negative regulation of cell cycle	diacylglycerol binding / protein binding / zinc ion binding	microtubule cytoskeleton / nucleus	---
CDKN2C	36053_at	0.91	0.01	cyclin-dependent kinase inhibitor 2C (p18, inhibits CDK4)	cell cycle / cell cycle arrest / negative regulation of cell proliferation	cyclin-dependent protein kinase inhibitor activity	cytoplasm / nucleus	---
MTM1	36920_at	0.91	0.02	myotubular myopathy 1	cell growth and/or maintenance / muscle development / protein amino acid dephosphorylation	hydrolase activity / protein serine/threonine phosphatase activity / protein tyrosine phosphatase activity	---	---
MLLT10	33773_at	0.91	0.03	myeloid/lymphoid or mixed-lineage leukemia (trithorax homolog, Drosophila); translocated to, 10	cell growth and/or maintenance / regulation of transcription, DNA-dependent	transcription factor activity	nucleus	---

Gene symbol	Probe set	Ratio CSE/Con	p-value	Description	GO Biological Process	GO Molecular Function	GO Cellular Component	Pathway
FLJ14001	40530_at	0.91	0.03	hypothetical protein FLJ14001	G2/M transition of mitotic cell cycle / cytokinesis / mitosis / regulation of cell cycle	---	nucleus	---
YES1	1674_at	0.91	0.04	v-yes-1 Yamaguchi sarcoma viral oncogene homolog 1	cell growth and/or maintenance / intracellular signaling cascade / protein amino acid phosphorylation	ATP binding / protein-tyrosine kinase activity / transferase activity	---	---
FHIT	1992_at	0.91	0.04	fragile histidine triad gene	cell cycle / negative regulation of cell cycle / nucleotide metabolism	bis(5'-adenosyl)-triphosphatase activity / hydrolase activity / magnesium ion binding / manganese ion binding	cytoplasm	Purine metabolism
JUN	1895_at	0.83	0.004	v-jun sarcoma virus 17 oncogene homolog (avian)	cell growth and/or maintenance / regulation of transcription, DNA-dependent	RNA polymerase II transcription factor activity / transcription factor activity	nuclear chromosome	Apoptosis / MAPK_Cascade / TGF_Beta_Signaling_Pathway / Wnt_signaling
KATNA1	32708_g_at	0.83	0.01	katanin p60 (ATPase-containing) subunit A 1	mitosis	ATP binding / nucleotide binding	cytoskeleton	---
STMN1	1782_s_at	0.83	0.02	stathmin 1/oncoprotein 18	cell growth and/or maintenance / intracellular signaling cascade	signal transducer activity	cytosol	---
CCND2	36650_at	0.77	0.01	cyclin D2	cytokinesis / regulation of cell cycle	---	nucleus	Ovarian_Infertilit y_Genes / Wnt_signaling / Cell_cycle
CDK2	1792_g_at	0.77	0.02	cyclin-dependent kinase 2	G2/M transition of mitotic cell cycle / cell cycle / cytokinesis / mitosis / positive regulation of cell proliferation / protein amino acid phosphorylation / regulation of DNA replication / traversing start control point of mitotic cell cycle	ATP binding / cyclin-dependent protein kinase activity / protein serine/ threonine kinase activity / transferase activity	cytoplasm / nucleus	DNA_replication / Cell_cycle
KRAS2	1940_at	0.77	0.03	v-Ki-ras2 Kirsten rat sarcoma 2 viral oncogene homolog	cell growth and/or maintenance / regulation of cell cycle / small GTPase mediated signal transduction	GTP binding / GTPase activity	---	G_Protein_Signal ing / MAPK_Cascade
WEE1	36909_at	0.63	0.01	WEE1 homolog (S. pombe)	mitosis / protein amino acid phosphorylation / regulation of cell cycle	ATP binding / protein serine/threonine kinase activity / protein-tyrosine kinase activity / transferase activity	nucleus	Cell_cycle

Gene symbol	Probe set	Ratio CSE/Con	p-value	Description	GO Biological Process	GO Molecular Function	GO Cellular Component	Pathway
TTK	572_at	0.63	0.04	TTK protein kinase	mitotic spindle assembly / mitotic spindle checkpoint / positive regulation of cell proliferation / protein amino acid phosphorylation / regulation of cell cycle	ATP binding / protein serine/threonine kinase activity / protein-tyrosine kinase activity / transferase activity	spindle	---
KNTC2	40041_at	0.53	0.05	kinetochore associated 2	mitosis / mitotic sister chromatid segregation	---	chromosome, pericentric region / nucleus	---

Table 10

Genes involved in Transcription Regulation

Gene symbol	Probe set	Ratio CSE/Con	p-value	Description	GO Biological Process	GO Molecular Function	GO Cellular Component	Pathway
PIR	35724_at	2.50	0.01	Pirin	transcription from Pol II promoter	transcription cofactor activity	nucleus	---
AES	41337_at	1.43	0.01	amino-terminal enhancer of split	Wnt receptor signaling pathway / development / organogenesis / regulation of transcription, DNA-dependent	---	nucleus	---
DRAP1	39076_s_at	1.43	0.02	DR1-associated protein 1 (negative cofactor 2 alpha)	negative regulation of transcription from Pol II promoter	transcription corepressor activity / transcription factor activity	---	---
NR1H2	518_at	1.43	0.03	nuclear receptor subfamily 1, group H, member 2	regulation of transcription, DNA-dependent	steroid hormone receptor activity / transcription factor activity	nucleus	Nuclear_ Receptors
IRLB	32961_at	1.25	0.003	c-myc promoter-binding protein	regulation of transcription, DNA-dependent	DNA binding	nucleus	---
KEAP1	35322_at	1.25	0.003	kelch-like ECH-associated protein 1	regulation of transcription, DNA-dependent	protein binding	---	---
TAF10	868_at	1.25	0.01	TAF10 RNA polymerase II, TATA box binding protein (TBP)-associated factor, 30kDa	regulation of transcription, DNA-dependent / transcription initiation	RNA polymerase II transcription factor activity / transcription factor activity	nucleus / transcription factor TFIID complex	---
TSNAX	41051_at	1.25	0.01	translin-associated factor X	---	DNA binding / protein transporter activity	nucleus	---
SMAD3	1433_g_at	1.25	0.02	SMAD, mothers against DPP homolog 3 (Drosophila)	regulation of transcription, DNA-dependent / transcription from Pol II promoter / transforming growth factor beta receptor signaling pathway	transcription factor activity	intracellular	---
ACYP2	36221_at	1.25	0.03	acylphosphatase 2, muscle type	phosphate metabolism / regulation of transcription, DNA-dependent	acylphosphatase activity / hydrolase activity / nucleic acid binding	intracellular	---
NR1H2	519_g_at	1.25	0.04	nuclear receptor subfamily 1, group H, member 2	regulation of transcription, DNA-dependent	steroid hormone receptor activity / transcription factor activity	nucleus	Nuclear_Recepto rs
THAP11	33123_at	1.25	0.04	THAP domain containing 11				

Gene symbol	Probe set	Ratio CSE/Con	p-value	Description	GO Biological Process	GO Molecular Function	GO Cellular Component	Pathway
SETBP1	34990_at	1.25	0.04	SET binding protein 1	regulation of transcription, DNA-dependent	DNA binding	nucleus	---
SMAD3	38944_at	1.25	0.04	SMAD, mothers against DPP homolog 3 (Drosophila)	regulation of transcription, DNA-dependent / transcription from Pol II promoter / transforming growth factor beta receptor signaling pathway	transcription factor activity	intracellular	---
ELK1	33275_at	1.11	0.01	ELK1, member of ETS oncogene family	regulation of transcription, DNA-dependent	transcription factor activity	nucleus	MAPK_Cascade
ING3	31808_at	1.11	0.02	inhibitor of growth family, member 3	regulation of transcription, DNA-dependent	DNA binding / structural molecule activity	viral capsid	---
ZNF410	35838_at	1.11	0.03	zinc finger protein 410	regulation of transcription, DNA-dependent	DNA binding / zinc ion binding	nucleus	---
TCFL4	32578_at	1.11	0.04	transcription factor-like 4	regulation of transcription, DNA-dependent	transcription factor activity	cytoplasm / nucleus	---
TAX1BP1	498_at	1.11	0.04	Tax1 (human T-cell leukemia virus type I) binding protein 1	---	nucleic acid binding / zinc ion binding	nucleus	---
PRDM2	315_at	0.91	0.02	PR domain containing 2, with ZNF domain	regulation of transcription, DNA-dependent	metal ion binding / transcription factor activity / zinc ion binding	nucleus	---
LRP6	34697_at	0.91	0.03	low density lipoprotein receptor-related protein 6	Wnt receptor signaling pathway / development / endocytosis	protein binding / receptor activity	integral to membrane / plasma membrane	---
HNRPK	39415_at	0.83	0.02	heterogeneous nuclear ribonucleoprotein K	---	DNA binding / RNA binding	nucleus / ribonucleoprotein complex	---
SRF	40109_at	0.83	0.00	serum response factor (c-fos serum response element-binding transcription factor)	regulation of transcription from Pol II promoter / signal transduction	RNA polymerase II transcription factor activity / transcription factor activity	nucleus	---
HIS1	40220_at	0.83	0.05	HMBA-inducible	negative regulation of cyclin dependent protein kinase activity / negative regulation of transcription from Pol II promoter	cyclin-dependent protein kinase inhibitor activity / protein binding / snRNA binding / transcriptional repressor activity	cytoplasm / nucleus	---
SCML2	38518_at	0.83	0.03	sex comb on midleg-like 2 (Drosophila)	morphogenesis / regulation of transcription, DNA-dependent	transcription factor activity	nucleus	---

Gene symbol	Probe set	Ratio CSE/Con	p-value	Description	GO Biological Process	GO Molecular Function	GO Cellular Component	Pathway
SSA2	35294_at	0.83	0.05	Sjogren syndrome antigen A2 (60kDa, ribonucleoprotein autoantigen SS-A/Ro)	transcription from Pol III promoter	RNA binding	ribonucleoprotein complex	---
CREB1	37535_at	0.77	0.03	cAMP responsive element binding protein 1	regulation of transcription, DNA-dependent / signal transduction	protein binding / transcription cofactor activity / transcription factor activity	nucleus	---
RCOR1	37651_at	0.71	0.04	REST corepressor 1	---	DNA binding	nucleus	---

Table 11

Genes involved in Proteolysis

Gene symbol	Probe set	Ratio CSE/Con	p-value	Description	GO Biological Process	GO Molecular Function	GO Cellular Component	Pathway
CTSL	37391_at	1.43	0.05	cathepsin L	proteolysis and peptidolysis	cathepsin L activity / hydrolase activity	extracellular / lysosome	---
KLK8	37131_at	1.11	0.02	kallikrein 8 (neuropsin/ovasin)	neurogenesis / proteolysis and peptidolysis	chymotrypsin activity / hydrolase activity / peptidase activity / trypsin activity	---	---
YME1L1	40988_at	0.91	0.03	YME1-like 1 (S. cerevisiae)	proteolysis and peptidolysis	ATP binding / hydrolase activity / metalloendopeptidase activity / nucleotide binding	membrane / mitochondrion	---
CPZ	37248_at	0.91	0.05	carboxypeptidase Z	development / proteolysis and peptidolysis	carboxypeptidase A activity / carboxypeptidase activity / transmembrane receptor activity	membrane	---
LAMP2	38403_at	0.83	0.03	lysosomal-associated membrane protein 2	---	---	integral to plasma membrane / lysosomal membrane	---
ADAM9	34761_r_at	0.83	0.01	a disintegrin and metalloproteinase domain 9 (meltrin gamma)	protein kinase cascade / proteolysis and peptidolysis	SH3 domain binding / hydrolase activity / integrin binding / metalloendopeptidase activity / protein binding / protein kinase binding / zinc ion binding	integral to plasma membrane	---
CTSB	32372_at	0.67	0.04	cathepsin B	proteolysis and peptidolysis	cathepsin B activity / hydrolase activity	intracellular / lysosome	---
SPUVE	40078_at	0.67	0.01	protease, serine, 23	proteolysis and peptidolysis	chymotrypsin activity / hydrolase activity / trypsin activity	---	---

Table 12

Genes involved in G protein activity

Gene symbol	Probe set	Ratio CSE/Con	p-value	Description	GO Biological Process	GO Molecular Function	GO Cellular Component	Pathway
RGS19	34268_at	1.11	0.04	regulator of G-protein signalling 19	G-protein coupled receptor protein signaling pathway / autophagy / signal transduction / small GTPase mediated signal transduction	GTPase activator activity / protein binding / signal transducer activity	Golgi apparatus / heterotrimeric G-protein complex / membrane / membrane fraction	---
GNA11	40562_at	1.11	0.01	guanine nucleotide binding protein (G protein), alpha 11 (Gq class) / guanine nucleotide binding protein (G protein), alpha 11 (Gq class)	G-protein coupled receptor protein signaling pathway / protein amino acid ADP-ribosylation / signal transduction	GTP binding / GTPase activity / signal transducer activity	cytoplasm / plasma membrane	G_Protein_ Signaling
MC5R	1141_at	0.91	0.04	melanocortin 5 receptor	G-protein signaling, coupled to cyclic nucleotide second messenger	melanocortin receptor activity / rhodopsin-like receptor activity	integral to plasma membrane	GPCRs_Class_ A_Rhodopsin-like / Peptide_GPCRs
RGS7	40653_at	0.91	0.02	regulator of G-protein signalling 7	intracellular signaling cascade / regulation of G-protein coupled receptor protein signaling pathway	regulator of G-protein signaling activity / signal transducer activity	heterotrimeric G-protein complex	---
FZD6	34472_at	0.83	0.05	frizzled homolog 6 (Drosophila)	G-protein coupled receptor protein signaling pathway / development / establishment of tissue polarity / frizzled signaling pathway	G-protein coupled receptor activity / Wnt receptor activity	integral to plasma membrane	Wnt_signaling
ADRB2	610_at	0.83	0.02	adrenergic, beta-2-, receptor, surface	G-protein coupled receptor protein signaling pathway / G-protein signaling, coupled to cAMP nucleotide second messenger / activation of MAPK / adenylate cyclase activation / endosome to lysosome transport / protein kinase cascade / receptor mediated endocytosis / transmembrane receptor protein tyrosine kinase activation (dimerization)	beta2-adrenergic receptor activity / rhodopsin-like receptor activity	endosome / integral to plasma membrane / lysosome	GPCRs_Class_ A_Rhodopsin-like / Monoamine_GP CRs

Table 13

Genes involved in Lipid Metabolism

Gene symbol	Probe set	Ratio CSE/Con	p-value	Description	GO Biological Process	GO Molecular Function	GO Cellular Component	Pathway
DHRS3	40782_at	2.50	0.04	dehydrogenase/reduct ase (SDR family) member 3	fatty acid metabolism / metabolism / visual perception	electron transporter activity / nucleotide binding / oxidoreductase activity	integral to membrane	---
FADS1	41719_i_at	1.43	0.003	fatty acid desaturase 1	fatty acid biosynthesis / fatty acid desaturation	C-5 sterol desaturase activity / oxidoreductase activity	integral to membrane	---
HSD17B2	38178_at	1.43	0.05	hydroxysteroid (17-beta) dehydrogenase 2	estrogen biosynthesis / metabolism	estradiol 17-beta-dehydrogenase activity / oxidoreductase activity	Endoplasmic reticulum membrane / integral to membrane	Steroid_Biosynthesis / Androgen and estrogen metabolism
FADS1	41720_r_at	1.25	0.003	fatty acid desaturase 1	fatty acid biosynthesis / fatty acid desaturation	C-5 sterol desaturase activity / oxidoreductase activity	integral to membrane	---
CAMK2G	32104_i_at	1.11	0.01	calcium/calmodulin-dependent protein kinase (CaM kinase) II gamma	insulin secretion / protein amino acid phosphorylation / signal transduction	ATP binding / calcium- and calmodulin-dependent protein kinase activity / calcium-dependent protein serine/threonine phosphatase activity / calmodulin binding / kinase activity / protein serine/threonine kinase activity / protein-tyrosine kinase activity / transferase activity	---	---
FDFT1	34848_at	0.83	0.03	farnesyl-diphosphate farnesyltransferase 1	biosynthesis / cholesterol biosynthesis / isoprenoid biosynthesis / steroid biosynthesis	farnesyl-diphosphate farnesyltransferase activity / magnesium ion binding / oxidoreductase activity / transferase activity	endoplasmic reticulum / integral to membrane	Cholesterol_Biosynthesis / Biosynthesis of steroids / Terpenoid biosynthesis
SAMD4	40855_at	0.67	0.04	sterile alpha motif domain containing 4	---	---	---	---
XTP2	32509_at	0.67	0.01	HBxAg transactivated protein 2	---	---	---	---
LAMP2	38402_at	0.59	0.02	lysosomal-associated membrane protein 2	---	---	integral to plasma membrane / lysosomal membrane	---

Table 14

Genes involved in ATP binding

Gene symbol	Probe set	Ratio CSE/Con	p-value	Description	GO Biological Process	GO Molecular Function	GO Cellular Component	Pathway
ATP6V1H	33741_at	1.25	0.01	ATPase, H+ transporting, lysosomal 50/57kDa, V1 subunit H	ATP synthesis coupled proton transport / proton transport	ATP binding / hydrogen-transporting ATP synthase activity, rotational mechanism / hydrogen-transporting ATPase activity, rotational mechanism / hydrolase activity	proton-transporting two-sector ATPase complex	Oxidative phosphorylation / ATP synthesis / Photosynthesis / Flagellar assembly / Type III secretion system
IKBKB	35960_at	1.25	0.03	inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase beta	protein amino acid phosphorylation	ATP binding / protein serine/threonine kinase activity / transcriptional activator activity / transferase activity	cytoplasm	---
ABCC1	1896_s_at	1.25	0.04	ATP-binding cassette, sub-family C (CFTR/MRP), member 1	protein amino acid phosphorylation / response to drug / transport	ATP binding / ATPase activity, coupled to transmembrane movement of substances / nucleotide binding / protein kinase activity / transporter activity	integral to membrane / integral to plasma membrane / membrane fraction	---
PDK2	38844_at	1.25	0.04	pyruvate dehydrogenase kinase, isoenzyme 2	glucose metabolism / protein amino acid phosphorylation	ATP binding / [pyruvate dehydrogenase (lipoamide)] kinase activity / protein kinase activity / transferase activity	mitochondrion	Krebs-TCA_Cycle
ADRBK1	38447_at	1.11	0.002	adrenergic, beta, receptor kinase 1	protein amino acid phosphorylation / signal transduction	ATP binding / G-protein coupled receptor kinase activity / beta-adrenergic-receptor kinase activity / signal transducer activity / transferase activity	cytoplasm / soluble fraction	---
PRKACA	438_at	1.11	0.004	protein kinase, cAMP-dependent, catalytic, alpha	protein amino acid phosphorylation	ATP binding / cAMP-dependent protein kinase activity / protein serine/threonine kinase activity / transferase activity	cAMP-dependent protein kinase complex / nucleus	G_Protein_Signaling / Phosphatidylinositol signaling system
	39456_at	1.11	0.02	Clone IMAGE 23915	protein amino acid phosphorylation	ATP binding / protein serine/threonine kinase activity / protein-tyrosine kinase activity / transferase activity	---	---
PCTK1	1225_g_at	1.11	0.02	PCTAIRE protein kinase 1	protein amino acid phosphorylation / regulation of cell cycle	ATP binding / protein serine/threonine kinase activity / transferase activity	---	Phosphatidylinositol signaling system

Gene symbol	Probe set	Ratio CSE/Con	p-value	Description	GO Biological Process	GO Molecular Function	GO Cellular Component	Pathway
DYRK4	101_at	1.11	0.04	dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 4	protein amino acid phosphorylation	ATP binding / protein serine/threonine kinase activity / protein-tyrosine kinase activity / transferase activity	---	---
STK16	31868_at	1.11	0.04	serine/threonine kinase 16	protein amino acid phosphorylation / protein complex assembly	ATP binding / protein serine/threonine kinase activity / protein-tyrosine kinase activity / transferase activity	---	---
FARS1	36504_at	1.11	0.05	phenylalanine-tRNA synthetase 1 (mitochondrial)	phenylalanyl-tRNA aminoacylation / tRNA processing	ATP binding / phenylalanine-tRNA ligase activity / tRNA binding	mitochondrion / soluble fraction	---
KRTHA3 B	34568_at	0.91	0.03	keratin, hair, acidic, 3B	---	structural molecule activity	intermediate filament	---
ERBB3	1742_at	0.91	0.03	v-erb-b2 erythroblastic leukemia viral oncogene homolog 3 (avian)	protein amino acid phosphorylation / transmembrane receptor protein tyrosine kinase signaling pathway	ATP binding / epidermal growth factor receptor activity / receptor activity / transferase activity	integral to plasma membrane	---
MAP3K7	36905_at	0.83	0.02	mitogen-activated protein kinase kinase kinase 7	protein amino acid phosphorylation / transforming growth factor beta receptor signaling pathway	ATP binding / MAP kinase kinase kinase activity / magnesium ion binding / protein serine/threonine kinase activity / protein-tyrosine kinase activity / transferase activity	---	---
DDX42	38762_at	0.77	0.04	DEAD (Asp-Glu-Ala-Asp) box polypeptide 42	---	ATP binding / ATP-dependent helicase activity / hydrolase activity / nucleic acid binding	---	---
FGFR2	1363_at	0.71	0.03	fibroblast growth factor receptor 2 (bacteria-expressed kinase, keratinocyte growth factor receptor, craniofacial dysostosis 1, Crouzon syndrome, Pfeiffer syndrome, Jackson-Weiss syndrome)	protein amino acid phosphorylation	ATP binding / fibroblast growth factor receptor activity / protein serine/threonine kinase activity / protein-tyrosine kinase activity / receptor activity / transferase activity	integral to membrane	---
BAT1	35292_at	0.71	0.04	HLA-B associated transcript 1	---	ATP binding / ATP-dependent RNA helicase activity / RNA binding	nucleus	---
SMC5L1	41379_at	0.67	0.02	SMC5 structural maintenance of chromosomes 5-like 1 (yeast)	chromosome segregation	ATP binding	nucleus	---

Table 15

Genes with GTPase or GTP Binding Activity

Gene symbol	Probe set	Ratio CSE/Con	p-value	Description	GO Biological Process	GO Molecular Function	GO Cellular Component	Pathway
MAPRE3	40825_at	1.43	0.05	microtubule-associated protein, RP/EB family, member 3	---	small GTPase regulatory/interacting protein activity	---	---
TBC1D1	32506_at	1.25	0.01	TBC1 (tre-2/USP6, BUB2, cdc16) domain family, member 1	---	GTPase activator activity	nucleus	---
ARHGAP12	39923_at	0.77	0.04	Rho GTPase activating protein 12	---	GTPase activator activity	---	---
8-Sep	38067_at	0.77	0.03	septin 8	---	---	---	---

Table 16

Genes involved in RNA processing

Gene symbol	Probe set	Ratio CSE/Con	p-value	Description	GO Biological Process	GO Molecular Function	GO Cellular Component	Pathway
LSM2	41375_at	1.43	0.001	LSM2 homolog, U6 small nuclear RNA associated (S. cerevisiae)	nuclear mRNA splicing, *via* spliceosome	RNA binding / U6 snRNA binding / pre-mRNA splicing factor activity	nucleus / small nucleolar ribonucleoprotein complex	---
BFSP1	32999_at	1.25	0.002	beaded filament structural protein 1, filensin	RNA processing	3'-5'-exoribonuclease activity / RNA binding / protein binding / structural constituent of cytoskeleton / structural constituent of eye lens	cytoskeleton / intermediate filament / membrane	---
RBPMS	34163_g_at	1.25	0.03	RNA binding protein with multiple splicing	RNA processing	RNA binding	---	Circadian_Exercis e
POLDIP3	33868_at	1.11	0.03	polymerase (DNA-directed), delta interacting protein 3	---	RNA binding	nucleus	---
RBPMS	38047_at	1.11	0.03	RNA binding protein with multiple splicing	RNA processing	RNA binding	---	Circadian_Exercis e
DHX8	744_at	1.11	0.05	DEAH (Asp-Glu-Ala-His) box polypeptide 8	RNA splicing / nuclear mRNA splicing, *via* spliceosome	ATP binding / ATP-dependent RNA helicase activity / RNA binding / pre-mRNA splicing factor activity	spliceosome complex	---
RBM16	34274_at	0.91	0.03	RNA binding motif protein 16	---	RNA binding	---	---
PUM1	40048_at	0.83	0.01	pumilio homolog 1 (Drosophila)	mRNA metabolism / regulation of translation	RNA binding	---	---
DCP2	34191_at	0.83	0.02	decapping enzyme hDcp2	---	hydrolase activity	---	---
SFRS2	36112_r_at	0.77	0.03	splicing factor, arginine/ serine-rich 2	RNA splicing / nuclear mRNA splicing, *via* spliceosome	RNA binding / pre-mRNA splicing factor activity	nucleus	---
SFRS2IP	35259_s_at	0.77	0.05	splicing factor, arginine/ serine-rich 2, interacting protein	RNA splicing / mRNA processing	pre-mRNA splicing factor activity	DNA-directed RNA polymerase II, core complex	Apoptosis
IVNS1ABP	33752_at	0.71	0.04	influenza virus NS1A binding protein	RNA splicing / response to virus / transcription from Pol III promoter	protein binding	spliceosome complex / transcription factor complex	---
PCF11	41665_at	0.67	0.05	pre-mRNA cleavage complex II protein Pcf11	mRNA cleavage	pre-mRNA cleavage factor activity	mRNA cleavage factor complex / nucleus	---

Table 17

Genes involved in DNA Replication

Gene symbol	Probe set	Ratio CSE/Con	p-value	Description	GO Biological Process	GO Molecular Function	GO Cellular Component	Pathway
HMGA1	39704_s_at	1.67	0.03	high mobility group AT-hook 1	DNA unwinding / chromosome organization and biogenesis (sensu Eukaryota) / loss of chromatin silencing / nucleosome disassembly / positive regulation of transcription / protein complex assembly / regulation of transcription, DNA-dependent	AT DNA binding / DNA binding / ligand-dependent nuclear receptor transcription coactivator activity / peroxisome proliferator activated receptor binding / retinoic acid receptor binding / retinoid X receptor binding / transcription factor activity	chromatin / nucleus / transcription factor complex	---
HIST1H1C	37018_at	1.67	0.04	histone 1, H1c	chromosome organization and biogenesis (sensu Eukaryota) / nucleosome assembly	DNA binding	chromosome / nucleosome / nucleus	---
CDC34	1274_s_at	1.25	0.02	cell division cycle 34	DNA replication initiation / G1/S transition of mitotic cell cycle / ubiquitin cycle	ligase activity / ubiquitin conjugating enzyme activity / ubiquitin-protein ligase activity	nucleus	Ubiquitin mediated proteolysis
ITPA	35801_at	1.25	0.03	inosine triphosphatase (nucleoside triphosphate pyrophosphatase)	nucleotide metabolism	hydrolase activity / nucleoside-triphosphate diphosphatase activity	---	Purine metabolism / Pyrimidine metabolism
IMPDH1	40695_at	1.25	0.03	IMP (inosine monophosphate) dehydrogenase 1	de novo' pyrimidine base biosynthesis / GMP biosynthesis / GTP biosynthesis / purine nucleotide biosynthesis / visual perception	IMP dehydrogenase activity / catalytic activity / dihydroorotate dehydrogenase activity / oxidoreductase activity	---	Nucleotide_Me tabolism / Purine metabolism
APRT	34310_at	1.25	0.04	adenine phosphoribosyltra nsferase	adenine salvage / nucleoside metabolism / purine ribonucleoside salvage	adenine phosphoribosyltransferase activity / transferase activity, transferring glycosyl groups	---	Purine metabolism
UPP1	37351_at	1.25	0.05	uridine phosphorylase 1	nucleoside metabolism	transferase activity, transferring glycosyl groups / uridine phosphorylase activity	---	---
POLD4	38397_at	1.11	0.03	polymerase (DNA-directed), delta 4	DNA replication	delta DNA polymerase activity	nucleus	DNA_replication
CENPB	37931_at	1.11	0.03	centromere protein B, 80kDa	centromere and kinetochore complex maturation	chromatin binding / satellite DNA binding	chromosome, pericentric region / nucleus	---

Gene symbol	Probe set	Ratio CSE/Con	p-value	Description	GO Biological Process	GO Molecular Function	GO Cellular Component	Pathway
SMARCD1	37753_at	1.11	0.05	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily d, member 1	chromatin modification / chromatin remodeling	---	chromatin remodeling complex / nucleus	---
CHD1	39231_at	0.91	0.03	chromodomain helicase DNA binding protein 1	chromatin assembly or disassembly / chromosome organization and biogenesis (sensu Eukaryota) / regulation of transcription from Pol II promoter	ATP binding / ATP-dependent DNA helicase activity / chromatin binding / helicase activity / hydrolase activity	chromatin / nucleus	---
CAP2	33404_at	0.91	0.03	CAP, adenylate cyclase-associated protein, 2 (yeast)	adenylate cyclase activation / establishment and/or maintenance of cell polarity / signal transduction	---	membrane	---
PURA	35221_at	0.83	0.03	purine-rich element binding protein A	DNA replication initiation / regulation of transcription, DNA-dependent	RNA polymerase II transcription factor activity, enhancer binding / single-stranded DNA binding	nucleus	Circadian_Exercise
DUT	38368_at	0.77	0.05	dUTP pyrophosphatase	DNA replication / dUTP metabolism / nucleotide metabolism	dUTP diphosphatase activity / hydrolase activity / magnesium ion binding	mitochondrion / nucleus	Pyrimidine metabolism
CAP2	33405_at	0.77	0.01	CAP, adenylate cyclase-associated protein, 2 (yeast)	adenylate cyclase activation / establishment and/or maintenance of cell polarity / signal transduction	---	membrane	---
TSPYL4	33835_at	0.67	0.01	TSPY-like 4	nucleosome assembly	---	nucleus	---

Table 18

Genes involved in Carbohydrate Metabolism

Gene symbol	Probe set	Ratio CSE/Con	p-value	Description	GO Biological Process	GO Molecular Function	GO Cellular Component	Pathway
AKR1B10	37482_at	5.00	0.03	aldo-keto reductase family 1, member B10 (aldose reductase)	---	aldo-keto reductase activity	---	---
ALDH3A1	40031_at	3.33	0.01	aldehyde dehydrogenase 3 family, memberA1	aldehyde metabolism / carbohydrate metabolism / metabolism	aldehyde dehydrogenase [NAD(P)+] activity / electron transporter activity / oxidoreductase activity	cytosol	Glycolysis / Gluconeogenesis / Histidine metabolism / Tyrosine metabolism / Phenylalanine metabolism
AKR1B1	36589_at	1.67	0.04	aldo-keto reductase family 1, member B1 (aldose reductase)	carbohydrate metabolism	aldehyde reductase activity / electron transporter activity / oxidoreductase activity	extracellular space	Pentose and glucuronate interconversions / Fructose and mannose metabolism / Galactose metabolism / Glycerolipid metabolism / Pyruvate metabolism
TALDO1	37311_at	1.43	0.01	transaldolase 1	carbohydrate metabolism / pentose-phosphate shunt	transaldolase activity / transferase activity	cytoplasm	Pentose_Phosphate_Pathway / Pentose phosphate pathway
ALDOC	40107_at	1.11	0.002	aldolase C, fructose-bisphosphate	fructose metabolism / glycolysis	fructose-bisphosphate aldolase activity / lyase activity	---	Glycolysis_and_Gluconeogenesis / Glycolysis / Gluconeogenesis / Pentose phosphate pathway / Inositol metabolism / Fructose and mannose metabolism / Carbon fixation
HS3ST1	41556_s_at	1.11	0.005	heparan sulfate (glucosamine) 3-O-sulfotransferase 1	---	sulfotransferase activity / transferase activity	Golgi lumen / integral to membrane	Chondroitin / Heparan sulfate biosynthesis
PGM1	32210_at	1.11	0.01	phosphoglucomutase 1	carbohydrate metabolism / glucose metabolism	isomerase activity / magnesium ion binding / phosphoglucomutase activity	cytoplasm	Glycogen_Metabolism / Glycolysis / Gluconeogenesis / Pentose phosphate pathway / Galactose metabolism / Starch and sucrose metabolism / Streptomycin biosynthesis
GALE	31598_s_at	1.11	0.01	galactose-4-epimerase, UDP-	carbohydrate metabolism / galactose metabolism / nucleotide-sugar metabolism	UDP-glucose 4-epimerase activity / isomerase activity	---	Galactose metabolism / Nucleotide sugars metabolism
PPP2R2A	1383_at	1.11	0.05	protein phosphatase 2 (formerly 2A), regulatory subunit B (PR 52), alpha isoform	---	---	---	Glycogen_Metabolism
TGDS	41667_s_at	0.91	0.04	TDP-glucose 4,6-dehydratase	nucleotide-sugar metabolism	dTDP-glucose 4,6-dehydratase activity / lyase activity	---	---
WDHD1	40355_at	0.91	0.03	WD repeat and HMG-box DNA binding protein 1	main pathways of carbohydrate metabolism / regulation of transcription, DNA-dependent	DNA binding / transferase activity, transferring acyl groups, acyl groups converted into alkyl on transfer	cytoplasm / nucleoplasm	---

Gene symbol	Probe set	Ratio CSE/Con	p-value	Description	GO Biological Process	GO Molecular Function	GO Cellular Component	Pathway
ZNF141	34058_at	0.91	0.01	zinc finger protein 141 (clone pHZ-44)	carbohydrate metabolism / morphogenesis / regulation of transcription, DNA-dependent	DNA binding / hydrolase activity, hydrolyzing O-glycosyl compounds / specific RNA polymerase II transcription factor activity / zinc ion binding	extracellular / nucleus	---
MDH1	36608_at	0.83	0.03	malate dehydrogenase 1, NAD (soluble)	tricarboxylic acid cycle	L-malate dehydrogenase activity / malic enzyme activity / oxidoreductase activity	cytosol	Glycolysis_and_Glucone ogenesis / Krebs-TCA_Cycle / Citrate cycle (TCA cycle) / Pyruvate metabolism / Glyoxylate and dicarboxylate metabolism / Carbon fixation / Reductive carboxylate cycle (CO2 fixation)
ACYP1	33334_at	0.83	0.04	acylphosphatase 1, erythrocyte (common) type	phosphate metabolism	acylphosphatase activity / hydrolase activity	---	Glycolysis / Gluconeogenesis / Pyruvate metabolism / Benzoate degradation *via* CoA ligation
AGL	38253_at	0.83	0.05	amylo-1, 6-glucosidase, 4-alpha-glucanotransferase (glycogen debranching enzyme, glycogen storage disease type III)	carbohydrate metabolism / glycogen biosynthesis	4-alpha-glucanotransferase activity / amylo-alpha-1,6-glucosidase activity / hydrolase activity, acting on glycosyl bonds / transferase activity, transferring glycosyl groups	isoamylase complex	Glycogen_Metabolism / Starch and sucrose metabolism / Starch and sucrose metabolism
AIM1	32112_s_at	0.77	0.02	absent in melanoma 1	---	sugar binding	---	---
CHSY1	41447_at	0.71	0.01	carbohydrate (chondroitin) synthase 1	---	---	---	---

Table 19

Genes involved in Cell-Cell Signaling

Gene symbol	Probe set	Ratio CSE/Con	p-value	Description	GO Biological Process	GO Molecular Function	GO Cellular Component	Pathway
PBEF1	33849_at	1.25	0.04	pre-B-cell colony enhancing factor 1	cell-cell signaling / positive regulation of cell proliferation / pyridine nucleotide biosynthesis / signal transduction	cytokine activity / nicotinate phosphoribosyltransferase activity	---	---
PTHLH	37989_at	0.83	0.02	parathyroid hormone-like hormone	cAMP metabolism / cell-cell signaling / epidermis development / lactation / negative regulation of cell proliferation / positive regulation of cell proliferation / pregnancy	hormone activity	cytoplasm / extracellular space / nucleus	---
EFNB2	34334_at	0.71	0.01	ephrin-B2	cell-cell signaling / morphogenesis / neurogenesis	ephrin receptor binding	integral to plasma membrane	---
GJA1	32531_at	0.67	0.04	gap junction protein, alpha 1, 43kDa (connexin 43)	cell-cell signaling / heart development / muscle contraction / perception of sound / positive regulation of I-kappaB kinase/NF-kappaB cascade / transport	connexon channel activity / ion transporter activity / signal transducer activity	connexon complex / integral to plasma membrane	Gap_Junction_ Proteins-Connexins

Table 20

Description	GO Biological Process	GO Molecular Function	GO Cellular Component	Pathway
rane 4 superfamily member 2	N-linked glycosylation	---	integral to plasma membrane	---
monooxygenase/tryptopha n 5- ctivation protein, epsilon polypeptide	---	protein domain specific binding	---	---
ase (L-kynurenine hydrolase)	tryptophan catabolism	kynureninase activity / peptidase activity	---	Tryptophan metabolism
se 1, regulatory (inhibitor) subunit 11	---	protein phosphatase inhibitor activity	soluble fraction	---
tate hydrolase (fumarylacetoacetase)	L-phenylalanine catabolism / aromatic amino acid family metabolism / metabolism / tyrosine catabolism	fumarylacetoacetase activity / hydrolase activity	---	Tyrosine metabolism / Styrene degradation
ol 4-kinase, catalytic, beta polypeptide	---	phosphotransferase activity, alcohol group as acceptor	---	---
AN binding protein 3	protein transport / small GTPase mediated signal transduction	RAN protein binding	nuclear pore / nucleus	---
geranyltransferase, alpha subunit	protein amino acid prenylation / protein modification / visual perception	Rab-protein geranylgeranyltransfer ase activity / protein prenyltransferase activity / transferase activity	---	---
nily 6 (neurotransmitter transporter, taurine), member 6	amino acid metabolism / neurotransmitter transport	symporter activity / taurine:sodium symporter activity	integral to plasma membrane	---
E3 ubiquitin protein ligase (mouse)	ubiquitin cycle / viral entry	ligase activity / protein binding / ubiquitin-protein ligase activity	nucleus	---
ng finger protein 126	protein ubiquitination	ubiquitin-protein ligase activity / zinc ion binding	ubiquitin ligase complex	---
meotic-like 2 (Drosophila)	---	protein binding	nucleus	---
ell CLL/lymphoma 7B	---	actin binding	---	---
me 20 open reading frame 18	protein ubiquitination	ubiquitin-protein ligase activity / zinc ion binding	ubiquitin ligase complex	---
translation elongation factor 2	protein biosynthesis / translational elongation	GTP binding / translation elongation factor activity	---	Translation_Fa ctors
MUF1 protein	ubiquitin cycle	---	---	---
4 homolog (Drosophila)	protein ubiquitination	ubiquitin-protein ligase activity / zinc ion binding	ubiquitin ligase complex	---
etastasis associated 1	protein biosynthesis / regulation of transcription, DNA-dependent / signal transduction	structural constituent of ribosome / transcription factor activity	nucleus / ribosome	---
ulinum toxin substrate 3 (rho family, GTP binding protein Rac3)	protein transport / small GTPase mediated signal transduction	GTP binding	---	---

Description	GO Biological Process	GO Molecular Function	GO Cellular Component	Pathway
KIAA0153 protein	protein modification	ligase activity / tubulin-tyrosine ligase activity	---	---
ke 1, mitochondrial (E. coli)	mitochondrial matrix protein import / protein folding	adenyl-nucleotide exchange factor activity / chaperone binding / protein homodimerization activity / unfolded protein binding	mitochondrial matrix	---
ine ligase-like family, member 1	protein modification	ligase activity / tubulin-tyrosine ligase activity	---	---
ting enzyme E2M (UBC12 homolog, yeast)	ubiquitin cycle	ligase activity / ubiquitin conjugating enzyme activity / ubiquitin-protein ligase activity	---	Ubiquitin mediated proteolysis
nd BTB (POZ) domain containing 2	---	protein binding	---	---
TcD37 homolog	---	pyrophosphatase activity	cytoplasm	---
se 2A, regulatory subunit B' (PR 53)	protein amino acid dephosphorylation	phosphatase activator activity / protein phosphatase type 2A regulator activity / protein tyrosine phosphatase activator activity	soluble fraction	Glycogen_Meta bolism
7D ((alpha-N-acetylneuraminyl-2,3-)-N-acetyl galactosaminide alpha-2,6-sialyltransferase)	protein amino acid glycosylation	(alpha-N-acetylneuraminyl-2,3-beta-galactosyl-1,3)-N-acetyl-galactosaminide 6-alpha-sialyltransferase activity	Golgi apparatus / integral to membrane	Ganglioside biosynthesis
tidylinositol glycan, class F	GPI anchor biosynthesis	ethanolaminephospho transferase activity	endoplasmic reticulum membrane / integral to membrane	Circadian_Exercise
rin alpha 1 (importin alpha 5)	NLS-bearing substrate-nucleus import / intracellular protein transport / regulation of DNA recombination	nuclear localization sequence binding / protein binding / protein transporter activity	cytoplasm / nuclear pore / nucleus	---
recognition particle 54kDa	SRP-dependent cotranslational protein-membrane targeting / protein targeting	GTP binding / RNA binding / nucleotide binding	signal recognition particle (sensu Eukaryota)	---
se 7 (alpha (1,3) fucosyltransferase)	L-fucose catabolism / protein amino acid glycosylation	alpha(1,3)-fucosyltransferase activity / transferase activity, transferring glycosyl groups	Golgi apparatus / integral to membrane	---
e (PRKA) anchor protein 10	protein localization / signal transduction	kinase activity / protein binding / signal transducer activity	mitochondrion	---
ng finger protein 110	protein ubiquitination / regulation of transcription, DNA-dependent	transcription factor activity / ubiquitin-protein ligase activity / zinc ion binding	nucleus / ubiquitin ligase complex	---
KIAA1354 protein	---	protein binding	---	---
carrier membrane protein 1	post-Golgi transport / protein transport	---	integral to membrane / membrane fraction	---
aptophysin-like protein	synaptic transmission / transport	transporter activity	integral to plasma membrane / synaptic vesicle	---
uitin specific protease 33	protein deubiquitination / ubiquitin-dependent protein catabolism	cysteine-type endopeptidase activity / hydrolase activity / protein binding / ubiquitin thiolesterase activity	VCB complex / cytoplasm	---

Description	GO Biological Process	GO Molecular Function	GO Cellular Component	Pathway
e (PRKA) anchor protein 11	protein kinase cascade	protein kinase A binding / protein phosphatase 1 binding	---	---
nonsense transcripts homolog (yeast)	protein biosynthesis	RNA binding	---	---
KIAA0433 protein	---	acid phosphatase activity	---	---
lobin, family 2A, member 1	---	androgen binding	---	---
ate (D-aspartate) O-methyltransferase	protein amino acid methylation / protein modification / protein repair	methyltransferase activity / protein-L-isoaspartate (D-aspartate) O-methyltransferase activity / transferase activity	endoplasmic reticulum	---
se 3 (formerly 2B), catalytic subunit, oform (calcineurin A beta)	protein amino acid dephosphorylation / regulation of cell cycle / signal transduction / transcription, DNA-dependent	calcium ion binding / calmodulin binding / hydrolase activity / phosphoprotein phosphatase activity / protein serine/threonine phosphatase activity	calcineurin complex	---
antigen, golgin subfamily a, 4	vesicle-mediated transport	---	Golgi trans face / membrane fraction	---
, 2-oxoglutarate 5-dioxygenase (lysine hydroxylase) 2	protein metabolism / protein modification	oxidoreductase activity / oxidoreductase activity, acting on single donors with incorporation of molecular oxygen, incorporation of two atoms of oxygen / procollagen-lysine 5-dioxygenase activity	endoplasmic reticulum / membrane	---
translation initiation factor 5B	protein biosynthesis / regulation of translational initiation	GTP binding / translation initiation factor activity	---	Translation_Factors
cetyl) transferase 1, core 2 (beta-1,6-N-glucosaminyltransferase)	O-linked glycosylation	beta-1,3-galactosyl-O-glycosyl-glycoprotein beta-1,6-N-acetylglucosaminyltransferase activity / transferase activity, transferring glycosyl groups	Golgi apparatus / integral to membrane	O-Glycan biosynthesis
partylglucosaminidase	protein deglycosylation	N4-(beta-N-acetylglucosaminyl)-L-asparaginase activity / hydrolase activity	lysosome	N-Glycan degradation
ock protein (hsp110 family)	protein folding / response to unfolded protein	ATP binding / unfolded protein binding	cytoplasm / nucleus	---
nc finger protein 294	protein ubiquitination	ubiquitin-protein ligase activity / zinc ion binding	ubiquitin ligase complex	---
adjacent to zinc finger domain, 1A	protein ubiquitination / regulation of transcription, DNA-dependent	DNA binding / ubiquitin-protein ligase activity / zinc ion binding	nucleus / ubiquitin ligase complex	---
somal biogenesis factor 3	peroxisome organization and biogenesis	---	integral to peroxisomal membrane / integral to plasma membrane / peroxisome	---
obutyrate aminotransferase	aminobutyrate metabolism / neurotransmitter catabolism / synaptic transmission	(S)-3-amino-2-methylpropionate transaminase activity / 4-aminobutyrate transaminase activity / pyridoxal phosphate binding / transferase activity	mitochondrial matrix	Glutamate metabolism / Alanine and aspartate metabolism / beta-Alanine metabolism / Propanoate metabolism / Butanoate metabolism

Table 21

Genes involved in Adhesion and Cell Motility

Gene symbol	Probe set	Ratio CSE/Con	p-value	Description	GO Biological Process	GO Molecular Function	GO Cellular Component	Pathway
MAP2	1972_s_at	1.43	0.02	microtubule-associated protein 2	microtubule stabilization	calmodulin binding / structural molecule activity	cytoskeleton / microtubule associated complex	MAPK_Cascade
DNAL4	33292_at	1.25	0.02	dynein, axonemal, light polypeptide 4	microtubule-based movement	ATPase activity, coupled / microtubule motor activity	axonemal dynein complex	---
FEZ1	37743_at	1.25	0.03	fasciculation and elongation protein zeta 1 (zygin I)	axon guidance / cell adhesion / neurogenesis	---	---	---
EPB49	37192_at	1.11	0.01	erythrocyte membrane protein band 4.9 (dematin)	actin filament bundle formation / cytoskeleton organization and biogenesis	actin binding	actin cytoskeleton	---
KRTHA1	36310_at	1.11	0.03	keratin, hair, acidic, 1	epidermis development	structural constituent of cytoskeleton / structural molecule activity	intermediate filament	---
SNTA1	35627_at	1.11	0.03	syntrophin, alpha 1 (dystrophin-associated protein A1, 59kDa, acidic component)	muscle contraction	actin binding / calcium ion binding / calmodulin binding	cytoskeleton / membrane	---
SYNJ2	36532_at	1.11	0.04	synaptojanin 2	---	inositol or phosphatidylinosito l phosphatase activity	---	---
ITGB3	852_at	1.11	0.05	integrin, beta 3 (platelet glycoprotein IIIa, antigen CD61)	blood coagulation / cell-matrix adhesion / integrin-mediated signaling pathway	protein binding / receptor activity	integrin complex	Integrin-mediated_cell_a dhesion
KIF1C	35941_f_a t	1.11	0.05	kinesin family member 1C	retrograde transport, Golgi to ER	ATP binding / motor activity	Golgi apparatus / cytoskeleton / endoplasmic reticulum / microtubule associated complex	---
THBS1	115_at	0.91	0.02	thrombospondin 1	blood coagulation / cell adhesion / cell motility / development / neurogenesis	calcium ion binding / endopeptidase inhibitor activity / heparin binding / protein binding / signal transducer activity / structural molecule activity	extracellular	Inflammatory_R esponse_Pathwa y / TGF_Beta Sign_aling Pathway
TNPO1	33349_at	0.91	0.02	transportin 1	cell adhesion / protein amino acid dephosphorylation	hydrolase activity / protein binding / protein tyrosine phosphatase activity / receptor activity / transmembrane receptor protein tyrosine phosphatase activity	integral to plasma membrane	---
ADAM1 2	38769_at	0.91	0.05	a disintegrin and metalloproteinase domain 12 (meltrin alpha)	cell adhesion / myoblast fusion / proteolysis and peptidolysis	hydrolase activity / metalloendopeptida se activity / protein binding / zinc ion binding	integral to membrane / plasma membrane	---

Gene symbol	Probe set	Ratio CSE/Con	p-value	Description	GO Biological Process	GO Molecular Function	GO Cellular Component	Pathway
CD164	34819_at	0.83	0.02	CD164 antigen, sialomucin	cell adhesion / development / hemopoiesis / immune response / negative regulation of cell adhesion / negative regulation of cell proliferation / signal transduction	---	endosome / integral to plasma membrane / membrane fraction / soluble fraction	---
TSPAN-1	34775_at	0.83	0.02	tetraspan 1	cell adhesion / cell motility / cell proliferation	---	integral to membrane	---
MAP7	39732_at	0.83	0.03	microtubule-associated protein 7	establishment and/or maintenance of cell polarity / microtubule cytoskeleton organization and biogenesis	structural molecule activity	microtubule associated complex	---
CD44	2036_s_at	0.83	0.04	CD44 antigen (homing function and Indian blood group system)	cell adhesion / cell-cell adhesion / cell-matrix adhesion	collagen binding / hyaluronic acid binding / receptor activity	integral to plasma membrane / membrane	---
PAFAH1B1	32569_at	0.83	0.04	platelet-activating factor acetylhydrolase, isoform Ib, alpha subunit 45kDa	cell motility / cytokinesis / lipid metabolism / mitosis / neurogenesis / signal transduction	---	cytoskeleton	Wnt_signaling / Glycerolipid metabolism
AMOTL2	38842_at	0.77	0.0005	angiomotin like 2	---	---	---	---
ITGA1	37484_at	0.77	0.02	integrin, alpha 1	cell-matrix adhesion / integrin-mediated signaling pathway	collagen binding / magnesium ion binding / receptor activity	integral to membrane / integrin complex	---
ITGA1	120_at	0.71	0.01	integrin, alpha 1	cell-matrix adhesion / integrin-mediated signaling pathway	collagen binding / magnesium ion binding / receptor activity	integral to membrane / integrin complex	---
LAP1B	40832_s_at	0.71	0.01	lamina-associated polypeptide 1B	---	---	---	---
SPARC	671_at	0.71	0.02	secreted protein, acidic, cysteine-rich (osteonectin)	ossification	calcium ion binding / collagen binding	basement membrane	---
PLS3	34794_r_at	0.71	0.04	plastin 3 (T isoform)	---	actin binding / calcium ion binding	actin cytoskeleton	---
PPP1R12A	40438_at	0.67	0.01	protein phosphatase 1, regulatory (inhibitor) subunit 12A	regulation of muscle contraction	signal transducer activity	actin cytoskeleton	---
CALD1	41739_s_at	0.63	0.02	caldesmon 1	muscle contraction / muscle development	actin binding / calmodulin binding / myosin binding / tropomyosin binding	cytoskeleton	---
KIF14	34563_at	0.59	0.05	kinesin family member 14	---	---	---	---
CALD1	41738_at	0.53	0.03	caldesmon 1	muscle contraction / muscle development	actin binding / calmodulin binding / myosin binding / tropomyosin binding	cytoskeleton	---

Table 22

Genes with Unclassified Function

Gene symbol	Probe set	Ratio CSE/Con	p-value	Description	GO Biological Process Description	GO Molecular Function Description	GO Cellular Component Description	Pathway
D15Wsu75e	41670_at	1.4	0.03	DNA segment, Chr 15, Wayne State University 75, expressed	---	---	---	---
C6orf74	34360_s_at	1.4	0.03	chromosome 6 open reading frame 74	---	---	---	---
	38850_at	1.4	0.04		---	---	---	---
HCG4	33940_at	1.3	0.01	HLA complex group 4	---	---	---	---
LOC126208	32608_at	1.3	0.01	hypothetical protein LOC126208	---	---	---	---
	1843_at	1.3	0.01		---	---	---	---
ZFPL1	40263_at	1.3	0.01	zinc finger protein-like 1	---	---	integral to membrane	---
MGC10433	34880_at	1.3	0.02	hypothetical protein MGC10433	---	---	---	---
DEXI	41637_at	1.3	0.03	dexamethasone-induced transcript	---	---	---	---
ATP5G2	38776_at	1.3	0.03	ATP synthase, H+ transporting, mitochondrial F0 complex, subunit c (subunit 9), isoform 2	---	---	---	---
DKFZP434K046	34810_at	1.3	0.03	hypothetical protein DKFZp434K046	---	---	---	---
MGC24381	41116_at	1.3	0.04	hypothetical protein MGC24381	---	---	---	---
E46L	39685_at	1.3	0.04	like mouse brain protein E46	---	---	---	---
ZP3	39720_g_at	1.3	0.04	zona pellucida glycoprotein 3 (sperm receptor)	---	---	---	---
PHTF2	34910_s_at	1.3	0.04	putative homeodomain transcription factor 2	---	---	---	---
GLE1L	35441_at	1.3	0.05	GLE1 RNA export mediator-like (yeast)	---	---	---	---
MEA	39067_at	1.3	0.05	male-enhanced antigen	development / male gonad development / spermatogenesis	---	---	---
HUMAGCGB	38476_at	1.3	0.05	chromosome 3p21.1 gene sequence	---	---	---	---
COPS7A	34404_at	1.3	0.05	COP9 constitutive photomorphogenic homolog subunit 7A (Arabidopsis)	---	---	---	---
MGC11308	40934_at	1.1	0.002	hypothetical protein MGC11308	---	---	---	---
PTEN	39552_at	1.1	0.005	phosphatase and tensin homolog (mutated in multiple advanced cancers 1)	---	---	---	---
B7	40280_at	1.1	0.01	B7 gene	---	---	---	---
MCRS1	33898_at	1.1	0.02	microspherule protein 1	---	---	nucleus	---
PMF1	39126_at	1.1	0.03	polyamine-modulated factor 1	---	---	---	---
MUTYH	489_at	1.1	0.04	mutY homolog (E. coli)	---	---	---	---

Gene symbol	Probe set	Ratio CSE/Con	p-value	Description	GO Biological Process Description	GO Molecular Function Description	GO Cellular Component Description	Pathway
FCHO1	41054_at	1.1	0.04	FCH domain only 1	---	---	---	---
	1280_i_at	1.1	0.04		---	---	---	---
SDBCAG84	40514_at	1.1	0.04	serologically defined breast cancer antigen 84	---	---	integral to membrane	---
PP784	35357_at	1.1	0.04	PP784 protein	---	---	---	---
ARHGEF16	1277_at	1.1	0.05	Rho guanine exchange factor (GEF) 16	---	---	---	---
	41575_at	0.9	0.02	Clone 24739 mRNA sequence	---	---	---	---
PSG11	33758_f_at	0.9	0.02	pregnancy specific beta-1-glycoprotein 11	---	---	---	---
ARHGEF9	35288_at	0.9	0.02	Cdc42 guanine nucleotide exchange factor (GEF) 9	---	---	---	---
KIAA0266	39405_at	0.9	0.02	KIAA0266	---	---	---	---
	34141_at	0.9	0.02	MRNA full length insert cDNA clone EUROIMAGE 112333	---	---	---	---
C9orf97	40577_at	0.9	0.03	chromosome 9 open reading frame 97	---	---	---	---
HCCR1	36961_at	0.9	0.03	cervical cancer 1 protooncogene	---	---	---	---
LOC54499	39116_at	0.9	0.04	putative membrane protein	---	---	---	---
BPHL	40912_s_at	0.9	0.04	biphenyl hydrolase-like (serine hydrolase; breast epithelial mucin-associated antigen)	---	---	---	---
B4GALT5	39889_at	0.9	0.04	UDP-Gal:betaGlcNAc beta 1,4-galactosyltransferase, polypeptide 5	---	---	---	---
FLJ46603	41273_at	0.9	0.04	FLJ46603 protein	---	---	---	---
KIAA0599	40959_at	0.9	0.05	KIAA0599	---	---	---	---
PLAGL1	36944_f_at	0.9	0.05	pleiomorphic adenoma genelike 1	---	---	---	---
ENTH	40128_at	0.8	0.001	enthoprotin	---	---	---	---
FLJ21174	32251_at	0.8	0.001	hypothetical protein FLJ21174	---	---	---	---
SLC39A6	1798_at	0.8	0.002	solute carrier family 39 (zinc transporter), member 6	---	---	---	---
	32672_at	0.8	0.01	MRNA; cDNA DKFZp586N1918 (from clone DKFZp586N1918)	---	---	---	---
OBRGRP	33829_at	0.8	0.01	leptin receptor gene-related protein	---	---	integral to plasma membrane	---
MGC8721	36975_at	0.8	0.01	hypothetical protein MGC8721	---	---	---	---
KIAA0102	37359_at	0.8	0.01	KIAA0102 gene product	---	---	---	---
CLASP2	38711_at	0.8	0.01	cytoplasmic linker associated protein 2	---	---	---	---
KIAA0893	35720_at	0.8	0.02	KIAA0893 protein	---	---	---	---

Gene symbol	Probe set	Ratio CSE/Con	p-value	Description	GO Biological Process Description	GO Molecular Function Description	GO Cellular Component Description	Pathway
NPC2	39345_at	0.8	0.04	Niemann-Pick disease, type C2	---	---	---	---
SDCCAG1	39228_at	0.8	0.04	serologically defined colon cancer antigen 1	---	---	nucleus	---
NUMB	37693_at	0.8	0.04	numb homolog (Drosophila)	development	---	integral to plasma membrane / membrane	---
HSHIN1	38778_at	0.8	0.04	HIV-1 induced protein HIN-1		---	---	---
KIBRA	34213_at	0.8	0.04	KIBRA protein	---	---	---	---
KIAA0582	40191_s_at	0.8	0.05	KIAA0582	---	---	---	---
KAB	33893_r_at	0.8	0.01	KARP-1-binding protein	---	---	---	---
INSIG2	35833_at	0.8	0.02	insulin induced gene 2	---	---	---	---
MGC14376	35224_at	0.8	0.03	hypothetical protein MGC14376	---	---	---	---
	1629_s_at	0.8	0.04		---	---	---	---
HNRPDL	32393_s_at	0.8	0.04	heterogeneous nuclear ribonucleoprotein D-like	---	---	---	---
DKFZP564C152	32338_at	0.8	0.04	DKFZP564C152 protein	---	---	---	---
COBLL1	41755_at	0.8	0.04	COBL-like 1	---	---	---	---
LOC339005	33186_i_at	0.7	0.03	hypothetical protein LOC339005	---	---	---	---
MDM1	37819_at	0.7	0.03	nuclear protein double minute 1	---	---	nucleus	---
	38510_at	0.7	0.03	MRNA; cDNA DKFZp586B0220 (from clone DKFZp586B0220)	---	---	---	---
PTPLB	35342_at	0.7	0.02	protein tyrosine phosphatase-like (proline instead of catalytic arginine), member b	---	---	---	---