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Exploring Transcriptional Relationships Within Fisher-344 Rats Exposed to 2-Aminoanthracene using VisANT Network Modeling and Gene Ontology Tools

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ABSTRACT

To understand the mechanism of arylamine toxicity, the effect of 2-Aminoanthracene (2-AA) on the pancreas of Fisher-344 rats was investigated. Twenty four post-weaning 3-4 week old F-344 male rats were exposed to 0 mg kg^{-1} diet (control), 50 mg kg^{-1} diet (low dose), 75 mg kg^{-1} diet (medium dose) and 100 mg kg^{-1} diet (high dose) 2-AA for 14 and 28 days. This was followed by analysis of the pancreas for global gene expression changes by Affymetrix Microarray GeneChips. More Gene Ontology (GO) categories were found to be significantly altered for the 14 day study than the 28 day study. In the 14 day study, 45, 57 and 237 cellular component, molecular function and biological process gene ontology categories were found to be significant, respectively. Similarly, 7, 5 and 25 cellular component, molecular function and biological process GO categories were altered, respectively. Some of these GO categories include; modification-dependent protein catabolic process, phospholipid biosynthetic process and glucose. VisANT was employed to analyze the dataset and to explore the biological network relationship between differentially expressed genes. This software was employed to analyze the dataset and to explore the relationships between differentially expressed genes. Three mRNA transcripts were identified from the network plot to have at least 50 links with other genes. These include SLC2A4 (glucose transporting gene), MAPK3 (a signal transduction pathway protein, mitogen activated protein kinase 3 and RAD23B (DNA repair protein and ubiquitin-like containing protein). The current study identified altered gene expression profiles associated with pancreatic carcinoma, pancreatitis and/or type 2 diabetes. This may be useful to identify and develop biomarkers as a diagnostic tool associated with the onset of these pathologies.

Key words: Pancreas, 2-aminoanthracene (2-AA), gene ontology (GO), SLC2A4 (glucose transporting gene, gene expression, fisher 344 (F-344)

INTRODUCTION

Aromatic amines such as 2-AA are employed in manufacturing dyes, drugs, inks, rubber antioxidants, plastics and agricultural chemicals (Boudreau *et al*., 2006). They are also used as curing agents in synthesizing epoxy resins and polyurethanes and can be found in road tars and synthetic fuels. Aromatic amines are known carcinogens that occur both naturally and synthetic form (Boudreau *et al*., 2006; Snyderwine *et al*., 1992; Zhu *et al*., 1995). The benchmark arylamine for toxicity studies is 2-amino anthracene. It is a model arylamine because, 2-amino

anthracene is a relatively potent direct-acting carcinogen and induces mutations in eukaryotic and prokaryotic cells (Boudreau *et al*., 2006; Snyderwine *et al*., 1992; Zhu *et al*., 1995).

Cigarette smoke is known to cause an estimated 2.4 million deaths annually from the production of toxicants that can disrupt normal metabolic processes (Adedeji and Etukudo, 2006). It is reported to be associated with various disease such as diabetes, pulmonary, cardio and cerebrovascular and cancers. When tobacco is combusted, more than 4700 chemicals are generated in the process. Some of these are large free radicals alkyl, alkoxyl, peroxyl and guinone/hydroquinone, Polycyclic Aromatic Hydrocarbons (PAH) (Benzo[a]Pyrene (BaP), N-nitrosamine, aldehydes, nicotine), arylamines and nitric oxide (Ramesh *et al*., 2007a, b; Tseng, 2009; Wang and Wang, 2005). Cigarette smoking has also been linked with several different cancers; lung, bladder, pancreatic and esophageal cancer. Pancreatic cancer is the fourth or fifth leading cause of cancer death but its mortality rate is at 98% in the US. Therefore, understanding its etiology and as well developing biomarkers for early detection will be crucial in overcoming it (Wang and Wang, 2005; Chipitsyna *et al*., 2009).

Also, insulin-dependent diabetes mellitus which results from specific disruption of the pancreatic islet insulin-secreting beta cells is induced in experimental animals by toxic agents including chemicals that are byproducts of cigarette smoking (Boudreau *et al*., 2006; Wang *et al*., 2007; Garelnabi, 2010). It has been previously shown that 2-AA directly suppresses Ins1 and Ins2 gene expression with prolonged exposure leading to symptomatology associated with insulin-dependent diabetes (Gato and Means, 2011b). Using Single Cell Gel Electrophoresis (SGCE) technique, also referred to as the comet assay, it was demonstrated that 2-AA can cause direct DNA damage (Gato and Means, 2011b).

2-AA is a promutagen known to cause mutations in both eukaryotic and prokaryotic cells, as a result, 2-AA exposure could increase the risk for cancer. Since pancreatic cancer is the most lethal form of human cancers with less than 3% survival rate, finding appropriate biomarkers for this disease is quite urgent (De Sa *et al*., 2007; Zhao *et al*., 2007; Domagk *et al*., 2007; Mazzolini *et al*., 2003). The focus has been on employing DNA microarray technology to analyze gene expression levels in order to determine differentially expression genes between normal and tumor tissues or among tumor tissues. For instance, Zhao *et al*. (2007) studied gene expression profiling of multidrug resistance in pancreatic cancer. They reported GO functions of 188 selected genes as binding, catalytic activity regulation of biological process, structural molecule activity, cell death and enzyme regulator activity. Similarly, Domagk *et al*. (2007) noted GO categories in their investigation of expression analysis of pancreatic cancer cell lines. Some of these include; calcium ion binding, cell motility, insulin-like growth factor, ribosome, protein binding, cytoplasm and alcohol metabolism. The objective of this study was to further explore the gene expression relationships of 2-AA exposed rats using VisANT network modeling and gene ontology tools.

MATERIALS AND METHODS

Experimental design: To measure the response of F-344 rats to 2-AA intoxication, the effects of 2-AA exposure on the pancreatic tissues was investigated. Twenty four post-weaning 3-4 week old Fisher 344 rats were purchased from Harlan Laboratories and randomly assigned to one of four dietary dose regimens of 0 mg kg^{-1} diet (control), 50 mg kg^{-1} diet (low dose), 75 mg kg^{-1} diet (medium dose) and 100 mg kg^{-1} diet (high dose) 2-aminoanthracene (2-AA) for either 14 or 28 days

Rats were provided distilled water *ad libitum*. Rats were housed at the Southern Illinois University Animal Facility. The animals were treated according to the principles outlined in the NIH and Southern Illinois University Guide for the Care and Use of Laboratory Animals. At the end of each exposure period (14 or 28 days), rats were euthanized with CO_2 and blood was collected by cardiac puncture. Feeding experiments were undertaken between 2009 and 2010 while the microarray experiments and data analysis were conducted between 2010 and 2011.

Diet preparation: 2-AA (CAS No. 613-13-8) [98+% Pure] was obtained from Sigma-Aldrich (St. Louis, MO) and used without further purification. A kilogram of rat diet supplied by PMI Nutrition International, LLC (Brentwood, MO) was immersed in 1 L molecular grade ethyl alcohol. This contained the mass of 2-AA necessary to yield the target dose concentrations in the diets. The ethyl alcohol was evaporated under the hood with periodic thorough mixing to assure homogeneity. The diet was stored in the freezer and protected from light until given to the animals.

Total RNA extraction: After animal sacrifice, excised pancreas was stored in RNA Later stabilization solution. Total RNA was isolated from rat pancreas using a Qiagen RNA Isolation kit (Qiagen, 2006). Less than 30 mg pancreas samples were homogenized in RNeasy Lysis buffer (RLT buffer). RLT buffer denatures and inactivates RNases. The RNA is then allowed to bind to a silica-gel membrane and finally eluted with RNase-free water. Total RNA was quantified using the UV-vis spectrophotometer at 260 and 280 nm absorbance and control electrophoretic gels were run for RNA quality assurance purposes.

Microarray gene expression analysis experiment: Double stranded cDNA was synthesized from total RNA samples using reverse transcriptase and oligo dT primer as described by Affymetrix (2008) Microarray manual. The synthesized cDNA served as a template for an *in vitro* Transcription (IVT) reaction in which aRNA is synthesized and involves the incorporation of biotin-conjugated nucleotide to produce cRNA molecules, also known as amplified RNA or aRNA. These aRNA molecules were purified to remove NTPs, salts, enzymes and inorganic phosphate and then fragmented and hybridized to GeneChip® Rat 230 2.0 Array and subsequently scanned as described in Affymetrix GeneChip™ Wash, Stain and Scan protocol (Affymetrix, 2008). The 230 2.0 chip contains over 31,000 probe sets analyzing more than 30,000 transcripts.

Data analysis: Significant differences in body weight gain and organ weight during the treatment period were analyzed by ANOVA and student t-test and were expressed as Mean±SE.

Affymetrix GeneChip Command Console software (AGCC) controlled the fluidic station and the scanner. Intensity data was generated from the image data acquired from the scanner. The image file is typically saved in format referred to as ".dat". Using the Affymetrix expression console, single intensity values were computed for every probe locus on the arrays from the image data. These intensity values were saved in ".cel" file format. Also, quality control data was generated using the plier algorithm in the expression console. The fluorescence intensity due to proper hybridization of each target was estimated by examining the difference in fluorescence intensities in perfect match and mismatch probe pairs present at each locus on the chip. Then, intensities were scaled for all valid probes using a default target signal threshold. Quantile normalization procedure of raw intensity data was implemented followed by using the plier algorithm.

The cel files were imported into Biometric Research Branch-Array Tools (BRB-ArrayTools version 3.8.1) software (Simon and Lam, 2009) for data collation, filtering and gene sub-setting. Genes which passed through the above quality assurance process were then employed in analyzing via gene ontology. Data collation involved importing data and aligning genes. BRB-ArrayTools converts either EXCEL or CHP files into a tab-delimited format. Transcripts that showed minimal variation across the sets of arrays were eliminated from the analysis. On the other hand, the expression of genes that differed by at least 1.5 fold from the median array in at least 20% of the arrays were retained.

Gene ontology: The Gene Ontology (GO) comparison tool provides the opportunity to show the relationship between and the association of genes with respect to each other in function and biochemical pathways. This analytical tool provides GO categories of differentially expressed genes among samples greater/lesser than would be expected by chance (Simon *et al*., 2003; Simon and Lam, 2009).

Network analysis by VisANT: VisANT, a web-based biological network modeling software (Hu *et al*., 2004) was utilized to generate connections of annotated differentially expressed pancreatic genes. The data employed in the construction of the biological network (Gato and Means, 2011a) involved F-344 rats that were fed 2-AA diet of varied doses.

RESULTS

The body weight gain and dietary intake levels between treated and untreated 2-AA rats were compared during studies lasting 14 or 28 days. Significant reductions in body weight gain were noted in animals fed adulterated diets of 2-AA at 75 mg kg^{-1} diet (medium dose) and 100 mg kg^{-1} diet (high dose). A significant decrease in body weight gain of medium and high dose animals compared with the untreated control rats exposed to 75 and 100 mg kg^{-1} diet 2-AA for 14 days (Fig. 1a). A similar trend was observed in 28-day group of rats exposed to 75 and 100 mg kg^{-1} diet 2-AA diet (Fig. 1b). When compared with the paired control diet groups, the body weight gain was significantly reduced. For the two week study, it decreased 39 and 23% in the high and medium dose, respectively, relative to controls. Similarly, the high and medium dose reduced 15 and 8%, respectively, relative to the controls as previously reported by Gato and Means (2011a). On the other hand, comparing the body weight gain of low dose (50 mg kg^{-1} diet) treated animals to the controls did not result in significant reductions for both the 14 and 28 days exposure time periods. However, a significant increase in dietary intake was observed for these animals exposed in both the 14 and 28 days study. This correspondingly produced significantly decreased "body weight gain per diet ingested" for the duration of the study. That is, the medium and high dose groups consumed more grams of diet while gaining less body weight in the process as shown in Fig. 1a and b.

The pancreatic islets of the Langerhans are involved in many major metabolic processes with regards to glucose metabolism. Pancreatic islets are unique and are involved in highly specialized processes (Cras-Meneur *et al*., 2004). A pancreatic tissue response to 2-AA adulterated diet consumption was investigated by GO analytical tool. This is an extension of an earlier study in which global quantitative microarray analysis was employed to examine gene expression patterns in pancreas tissues due to 2-AA exposure (Gato and Means, 2011a). Gene ontology categories found to be affected span the biological process, cellular component and molecular function classes. These

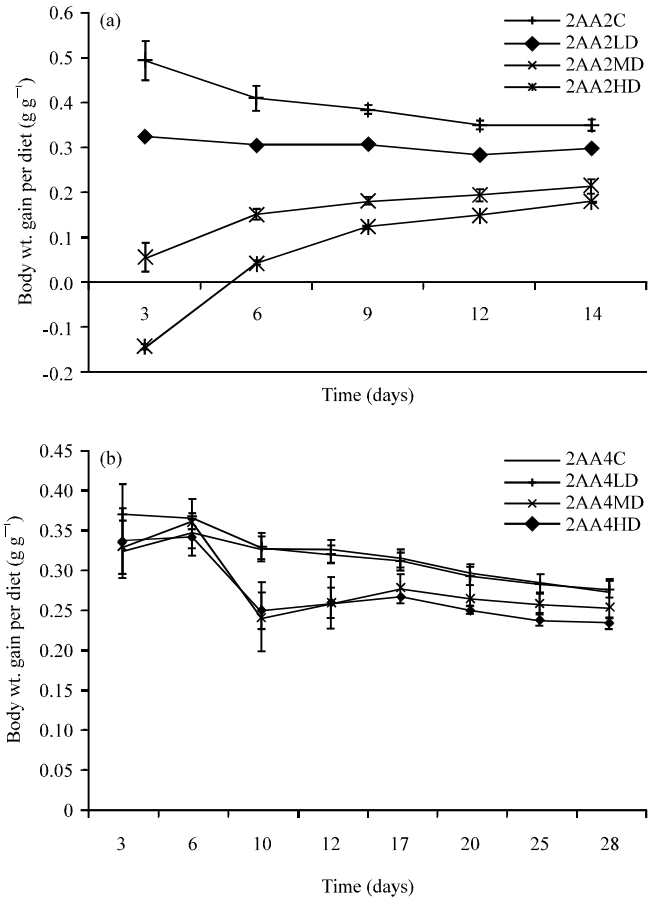

Fig. 1 (a-b): The effects of 2-AA consumption on F-344 rat dietary intake. Each data point is the mean of 3 F-344 male rats given untreated (0 mg kg^{-1} diet) and treated (50 mg kg^{-1} diet, 75 mg kg^{-1} diet and 100 mg kg^{-1} diet) 2-AA diet. Body weight gain per diet ingested was significantly reduced at $p<0.01$ for both time points in the medium and high dose treatments when compared with the controls. (a) 14-day treatment group and (b) 28-day time frame

GO categories group a set of genes by their unique influential role such as signal transduction in the case of biological process or being part of subcellular structures or much larger multisubunit proteins (not individuals) in the case of cellular component or such activities as binding or catalytic activity (molecular functions).

Selected GO categories are presented in Table 1 and 2. The complete set of data is available as extra materials (see http://opensiuc.lib.siu.edu). More GO categories were found to be significant for the two week study than for the four week study. With respect to the 14 day study, 45, 57 and 237 Cellular Component (CC), Molecular Function (MF) and Biological Process (BP) gene ontology categories were found to be significant, respectively. While a corresponding 7, 5 and 25 cellular component, molecular function and biological process GO categories, respectively, were significant for the 28-day experimental group as shown in Fig. 2. Some of the BP reported in the current study include: enzyme linked receptor protein signaling pathway, regulation of inflammatory response, signaling pathway, ubiquitin-dependent protein catabolic process, gluconeogenesis. Selected CC

GO categories were: ribonucleoprotein complex, small ribosomal subunit, ubiquitin ligase complex, proteasome complex and Golgi apparatus. Similarly, such MF categories as; lipase activity, carboxyl esterase activity, hydrolase activity, acting on ester bonds, ligase activity, cofactor binding, sequence-specific DNA binding were observed.

Table 1: Gene ontology categories significant at p<0.005 using LS or KS statistics for a 28-day 2-AA exposure. The categories investigated include biological process (BP), molecular function (MF) and cellular component (CC)

GO ontology	GO term	No. of genes	LS permutation p-value	KS permutation p-value	Efron-Tibshirani's GSA test p-value
BP	Enzyme linked receptor protein signaling pathway	11	0.00189	0.0006	0.055
MF	Ion channel activity	7	0.00235	0.10592	0.015
BP	Cellular response to chemical stimulus	20	0.00255	0.00046	0.01
CC	Extracellular region	73	0.00311	0.00026	0.31
BP	Developmental maturation	7	0.00494	0.04848	0.07
BP	Cell motion	13	0.00633	0.0002	0.08
BP	Localization of cell	13	0.00633	0.0002	0.08
MF	Structural molecule activity	81	0.00999	0.00003	0.31
BP	Translational elongation	70	0.01013	0.00002	0.28
CC	Cytosolic ribosome	43	0.01022	0.00001	0.205
BP	Response to hormone stimulus	42	0.01419	0.00248	0.255
BP	Positive regulation of macromolecule biosynthetic process	22	0.01588	0.00095	0.11
CC	Ribonucleoprotein complex	85	0.01613	0.00005	0.33
BP	Negative regulation of response to stimulus	6	0.01664	0.00023	< 0.005
BP	Transmembrane receptor protein tyrosine kinase signaling pathway	8	0.01731	0.00257	0.05
BP	Response to peptide hormone stimulus	23	0.01834	0.00386	0.265
BP	Positive regulation of macromolecule metabolic process	26	0.02471	0.0032	0.10
BP	Response to endoplasmic reticulum stress	6	0.0248	0.00054	0.08
CC	Ribosome	77	0.02548	0.00007	0.335
CC	Ribosomal subunit	46	0.02564	0.00003	0.27
BP	ER-nuclear signaling pathway	5	0.02788	0.00143	0.10
MF	DNA binding	47	0.02864	< 0.005	0.265
BP	Response to insulin stimulus	7	0.02929	0.00036	0.065
BP	Cell migration	10	0.03042	0.00137	0.115
BP	Cell motility	10	0.03042	0.00137	0.115
BP	Multicellular organism growth	7	0.03166	0.00062	0.025
BP	Negative regulation of hydrolase activity	5	0.03435	0.00469	0.18
BP	Cellular response to insulin stimulus	5	0.04376	0.00265	0.025
BP	Regulation of multicellular organism growth	6	0.04494	0.00163	0.06
BP	Regulation of cellular catabolic process	6	0.04958	0.0018	0.14
MF	Hydrolase activity, acting on ester bonds	11	0.05068	0.00048	0.175
BP	Extracellular matrix organization	5	0.07453	0.00469	0.25
BP	Regulation of inflammatory response	5	0.08077	0.00125	0.015
BP	Regulation of biological quality	58	0.1135	0.00275	0.47
BP	Neuron apoptosis	5	0.11922	0.00337	0.225
MF	Carboxyl esterase activity	5	0.14125	0.004	0.30
MF	Lipase activity	5	0.14125	0.004	0.30
BP	Lipid biosynthetic process	16	0.14438	0.00323	0.34
CC	Small ribosomal subunit	35	0.17296	0.00074	0.365

Table 2: Gene ontology categories significant at p<0.005 using LS or KS or Efron-Tibshirani's GSA statistics for a 14-day 2-AA exposure. The categories investigated include biological process (BP), molecular function (MF) and cellular component (CC)

GO ontology	GO term	No. of genes	LS permutation p-value	KS permutation p-value	Efron-Tibshirani's GSA test p-value
MF	Protein serine/threonine kinase activity	11	0.00076	0.07059	0.1
MF	Phosphotransferase activity, alcohol group as acceptor	16	0.00015	0.00688	0.1
BP	Cellular amino acid metabolic process	33	0.00486	0.00011	0.24
BP	Carboxylic acid metabolic process	82	0.01536	0.60711	< 0.005
BP	Regulation of transforming growth factor beta receptor signaling pathway	6	0.03657	0.0766	< 0.005
MF	Oxidoreductase activity, acting on the aldehyde or oxo group of donors, NAD or NADP as acceptor	6	0.04056	0.37095	< 0.005
BP	Protein modification process	93	0.06964	0.34579	< 0.005
BP	Post-translational protein modification	79	0.07458	0.59635	< 0.005
BP	Coenzyme catabolic process	14	0.08999	0.45436	< 0.005
BP	Hexose metabolic process	31	0.09548	0.45553	< 0.005
BP	Monosaccharide metabolic process	34	0.11923	0.3777	< 0.005
BP	Negative regulation of neuron apoptosis	5	0.13429	0.05024	< 0.005
BP	Cellular aromatic compound metabolic process	10	0.14294	0.79039	< 0.005
BP	Glucose metabolic process	26	0.14373	0.27974	< 0.005
BP	Lipid biosynthetic process	29	0.14719	0.57762	< 0.005
BP	Protein complex biogenesis	49	0.19646	0.78667	< 0.005
BP	Coenzyme metabolic process	38	0.25899	0.90453	< 0.005
BP	Gluconeogenesis	7	0.28235	0.45776	< 0.005
BP	Cellular response to stimulus	70	0.28235	0.45776	< 0.005
BP	Carbohydrate catabolic process	19	0.36946	0.61527	< 0.005
BP	Transcription initiation	5	0.50059	0.25586	< 0.005
BP	Transcription initiation from RNA polymerase II promoter	5	0.50059	0.25586	< 0.005
BP	Regulation of cell communication	61	0.50215	0.76572	< 0.005
CC	Transport vesicle	7	0.50286	0.25586	< 0.005
BP	Cell-cell signaling	26	0.50452	0.54177	< 0.005
BP	Glucose catabolic process	14	0.56972	0.66619	< 0.005
MF	Hormone receptor binding	5	0.56972	0.66619	< 0.005
MF	Adenyl nucleotide binding	77	0.00623	0.66619	< 0.005
BP	Synaptic transmission	19	0.65388	0.70229	< 0.005
BP	DNA repair	13	0.65569	0.65932	< 0.005
BP	Cell cycle	58	0.66226	0.83996	< 0.005
BP	Lipid modification	9	0.66895	0.80853	< 0.005
BP	Transcription	84	0.67237	0.90536	< 0.005
BP	Regulation of ligase activity	21	0.67378	0.34448	< 0.005
BP	Positive regulation of ligase activity	21	0.67378	0.34448	< 0.005
BP	Regulation of ubiquitin-protein ligase activity	21	0.67378	0.34448	< 0.005
BP	Proteasomal protein catabolic process	30	0.76417	0.48043	< 0.005
BP	Proteasomal ubiquitin-dependent protein catabolic process	30	0.76417	0.48043	< 0.005
CC	Centrosome	12	0.76886	0.78144	< 0.005
BP	Fatty acid oxidation	7	0.78549	0.82512	< 0.005
BP	Lipid oxidation	7	0.78549	0.82512	< 0.005
BP	Pentose-phosphate shunt	5	0.90102	0.49519	< 0.005
BP	NADP metabolic process	5	0.90102	0.49519	< 0.005
BP	Cellular response to stress	50	0.90349	0.77271	< 0.005
BP	Translational elongation	71	0.95052	0.00391	0.41

Figure 3 showed network displaying links between genes annotated from dietary exposure of F-344 rats to 2-AA for 14 and 28-days. An "-" sign indicates fully expanded nodes while "+" shows some connections that have not yet been displayed.

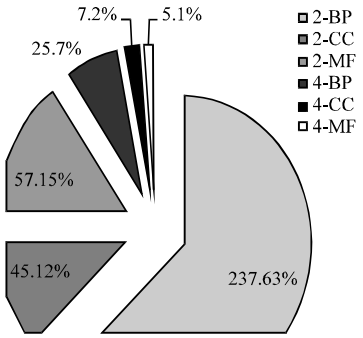

Fig. 2: Gene ontology category distribution of pancreatic gene expression analysis of 2-AA exposed male Fischer 344 rats for 14-and 28-days. The categories include biological process (BP), cellular component (CC) and molecular function (MF)

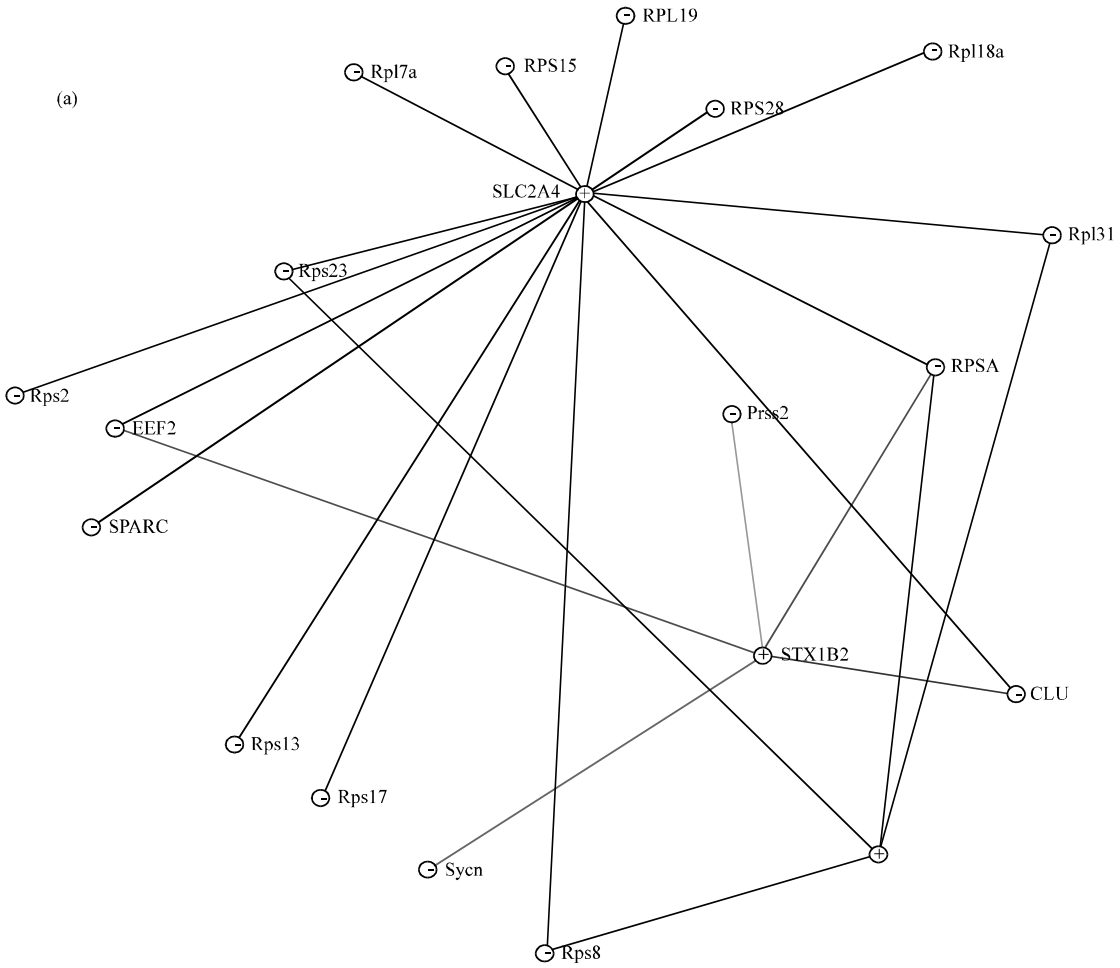

Fig. 3: Continued

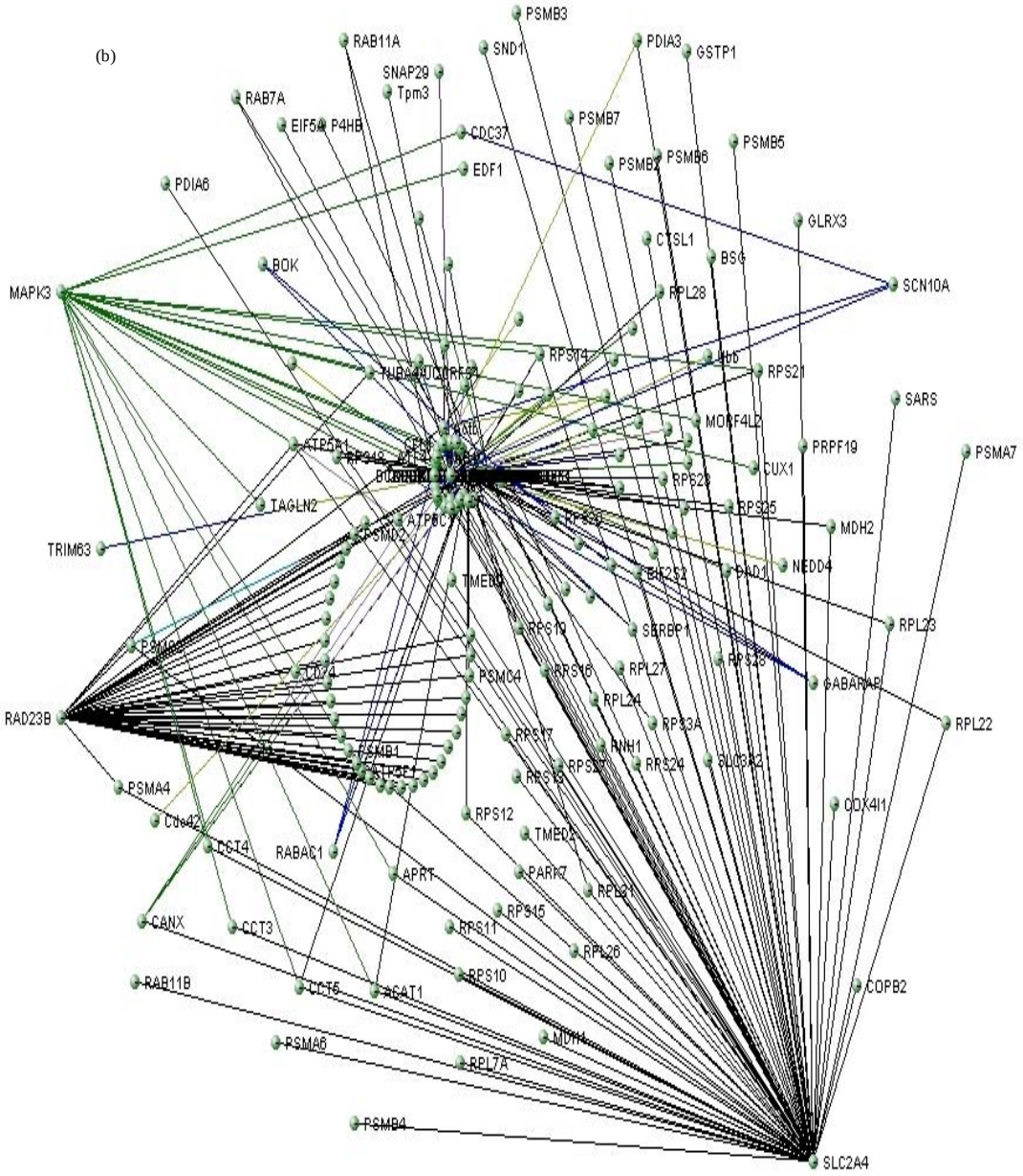

Fig. 3 (a-b): Network displaying links between genes annotated from dietary exposure of F-344 rats to 2-AA for 14 and 28-days. The links were generated using VisANT, an internet based tool that enables integration of biomolecular interactions data into graphical and cohesive display. (a) mRNA transcripts that are at least five fold altered up-or down-ward and (b) all genes that are at least two-fold expressed up-or down-ward

DISCUSSION

The effect of 2-AA ingestion was examined in F-344 male rats. The body weight gain data were similar to those previously reported by Boudreau *et al.* (2006). The present

study found animals fed 50 mg kg^{-1} diet treated animals did not show significant weight reductions when compared with the control animals. In contrast, significant decreases in body weight of rats that ingested 75 mg kg^{-1} diet and 100 mg kg^{-1} diet adulterated diet when compared with the control (0 mg kg^{-1} diet 2-AA). On the other hand, daily ingestion of 2-AA treated diets increased with increasing 2-AA concentration. As a result, weight gain per diet ingested reduced significantly. Animals on 75 mg kg^{-1} diet and 100 mg kg^{-1} diet 2-AA diets consumed significantly more diet than either the control or 50 mg kg^{-1} diet diets. This finding is in contrast to an earlier reported by Boudreau *et al.* (2006) which might be attributed to the differences in the length of study as well preparation of diet. These authors noted a significant decrease in dietary intake of rats exposed to 100 mg kg^{-1} diet of 2-AA at 2, 7 and 8 for 75 mg kg^{-1} diet treated animals while the rats on 50 mg kg^{-1} diet adulterated diet feeding habit were not significantly altered relative to the controls. The data in the present study suggest that the lack of weight gain was not due to food avoidance but rather due to altered metabolism.

Some of the GO categories reported in the current study were also found in a recent study that involved analysis of pancreatic gene expression profiling (Nishimura *et al.*, 2004). This study investigated molecules that participate in normal functions of pancreatic islet cells via the construction of recombination based cDNA library. From the sequence data, a mouse specific pancreatic islet sequence database was build and cDNA microarray membranes developed. Some of the GO categories in their study included: binding (nucleic acid binding, lipid binding), catalytic activity (ligase activity, hydrolase activity, oxidoreductase activity). These categories are important for the studying pancreatic islets and related diseases such as diabetes mellitus. Some of these such as hydrolase activity, ligase activity DNA binding were observed in our study. A similar investigation by Ohsugi *et al.* (2004) investigated glucose and insulin treatment of insulinoma cells reported molecular function GO categories; catalytic activity, binding activity, structural molecule activity and transcription and translation regulation that were found in our study. This insulinoma cell lines are widely employed to study the physiological and pathophysiological mechanisms of glucose metabolism (Baroni *et al.*, 1999). Present results showing GO categories typical to the ones observed from studying insulinoma cells may suggest 2-AA exposed pancreas undergoing similar transformations such as is observed by Ohsugi *et al.* (2004).

Boudreau *et al.* (2006) reported that the rat pancreas has been frequently employed as the animal model to investigate changes in the pancreas, including diseases such as, diabetes mellitus and pancreatic tumors. It has been noted that diabetes mellitus is a complex metabolic disorder that involves alterations in metabolism, cellular physiology and gene expression (Yechoor *et al.*, 2004). Transcriptional alterations were noted to be involved in the pathogenesis of diabetes (Sreekumar *et al.*, 2002; Yechoor *et al.*, 2002; Van Lunteren and Moyer, 2009; Rome *et al.*, 2009), including biosynthesis, oxidoreductase activity, the extracellular matrix and induction of apoptosis among others. These gene expression changes could be due to decreased insulin action as a result of receptor-mediated signaling or indirect metabolic and humoral changes linked with the disease (Yechoor *et al.*, 2004). Sreekumar *et al.* (2002) studied gene expression profiling in skeletal muscle of type 2 diabetes and the effect of insulin treatment. The results suggest transcripts (GLUT4, Rad genes, insulin receptor substrate-1, mitogen-activated protein kinase, serine threonine kinase) are important in insulin signaling, transcription factors and mitochondrial maintenance, play significant roles in preventing chronic. Also, reported were candidate genes involved in signal transduction, structural and contractile functions, growth and tissue development, protein, fat and energy metabolism which may represent transcripts that are involved in the pathogenesis of type

2 diabetes. Other researchers employed streptozotocin-induced diabetes in the rat heart and diaphragm (Van Lunteren and Moyer, 2009). Following gene expression analysis, they reported GO biological process, some of which include: lipid metabolism, protein ubiquitination, cellular metabolism, glucose metabolism, hexose catabolism. Selected molecular function GO categories were catalytic activity, oxidoreductase activity, ligase activity, protein binding, binding and calcium ion binding. With respect to cellular component, extracellular region, intracellular, cytoplasm and cytosol were cited (Van Lunteren and Moyer, 2009, 2007). Although, the models employed in these studies are quite different from the current one, we found similar GO categories as well as the genes responsible for them being altered by 2-AA exposure.

To study the relationships between differentially expressed genes of the F-344 exposed to 2-AA via diet, VisANT, interactive software platform that enables biological network modeling was employed to further analyze the dataset. Three mRNA transcripts were identified from the network plot to have at least 30 links with other genes. One of these proteins, SLC2A4, was the central link between the others when we examined the annotation for genes at least five-fold differentially expressed. The other two transcripts were RAD23B and MAPK3. SLC2A4 (solute carrier family 2 (facilitated glucose transporter), also referred to as glucose transporter type 4 or insulin-responsive plays a critical role in glucose homeostasis and diabetes pathogenesis (Tarazona-Santos *et al*., 2010). Glucose Transporters (GLUT-4) respond to insulin stimulation by transporting glucose from the cell surface into the cell thereby reducing blood glucose and making it possible for the synthesis of glycogen and triglycerides. It is known that the trafficking pathway of GLUT4 involves multiple insulin regulation (Tarazona-Santos *et al*., 2010; Huang and Czech, 2007). Mitogen Activated Protein Kinase (MAPK) on the other hand is involved in signal transduction pathways that regulate gene expression and other important cellular processes (Cuadrado and Nebreda, 2010) in response to extracellular stimuli. Altered MAPK3 gene expression is reported to be implicated in various cancers including melanoma, breast cancer and myeloid leukemias (Yang *et al*., 2004; Gout *et al*., 2006). When mitogens, hormones or neurotransmitters bind to receptor tyrosine kinases, MAP kinases signaling cascade is triggered which consequently activates oncogenic RAS (Inamdar *et al*., 2010). Finally, the yeast protein Rad23 is reported to play an important role in Nucleotide Excision Repair (NER) (Sugasawa *et al*., 1997; Dantuma *et al*., 2009; Clement *et al*., 2010; Thoma and Vasquez, 2003). 2-AA has been documented to form DNA adducts which require excision repair or they will cause gene mutations. DNA lesions are typically determined via xeroderma pigmentosum group C (XPC), a protein that detects different kinds of helix-distorting DNA adducts. Subsequently, the XPC molecular sensor initiates Global Genome Repair (GGR) pathway. The XPC was found to be associated with Rad23B. Other researchers also report similar findings in which XPC is tightly complexed with hHR23B (human homolog of Rad23) *in vivo* (Dantuma *et al*., 2009; Clement *et al*., 2010; Thoma and Vasquez, 2003; Fousteri and Mullenders, 2008). Although XPC possesses DNA binding activity, it is subject to proteasomal degradation. However, when XPC is complexed with Rad23, the initiator complex is protected from degradation and its DNA repair activity is stimulated (Sugasawa *et al*., 1997; Dantuma *et al*., 2009; Clement *et al*., 2010; Thoma and Vasquez, 2003; Fousteri and Mullenders, 2008).

The dataset employed for the current analysis had been previously validated via Quantitative Real-Time Polymerase Chain Reaction (QRT-PCR) using Pancreatic Lipase (Pnlip), carboxypeptidase A2 (pancreatic) (Cpa2), insulin 1 (Ins1), chymotrypsin C (caldecrin) (Ctrc), Carboxyl Ester Lipase (Cel), Colipase, Pancreatic (Clps) Relative to beta-Actin (Actb) genes (Gato and Means, 2011a). This QRT-PCR technique has become the most used method for

validating and quantifying gene expression (Chen *et al*., 2011; Baatartsogt *et al*., 2011; Endo *et al*., 2007). Briefly, when the mRNA expression values were compared to the controls, they ranged from 0.99-fold to 5-fold change. These gene expression values were less than those reported from the microarray analysis though they follow similar patterns.

CONCLUSIONS

The effects of 2-AA on the male Fischer 344 rats seems to be pleiotropic in nature. This is confirmed by the number of GO categories observed to be significantly altered by 2-AA. Although, gene ontology categories from the current study showed GO categories related to insulin processing pathways and pancreatic carcinoma, some of which seem to be involved in both, many of categories were also identified that do not necessarily fall within these classes of disease situations. The current study is the first of its kind in employing DNA microarray to analyze global gene expression in response to dietary 2-AA consumption. Clearly, the protein SLC2A4 plays important roles in diabetogenesis induction in the study via its downward expression. When the expression of SLC24A gene is reduced, the ability of GLUT4 to transport glucose and thereby enable glucose metabolism is greatly hampered, consequently, creating the conditions for glucose intolerance. The current and previous experiments examining 2-AA toxicity effects did not find any direct evidence for *ras* oncogenes expression, however, the role of MAPK3 as an indirect cascading intermediate seems plausible. RAD23B, a DNA repair protein and ubiquitin-like containing protein is shown to regulate several proteins including PSMC3, ATP1B1, SLC25A4, HSPA8 and TUFM. The expression of rad23b may be indicative of ongoing pancreatic pre-cancerous processes that the cell seems to try to repair. The data from the current study identified altered gene expression profiles that may be associated with pancreatic carcinoma, pancreatitis and/or type 2 diabetes. This will be useful to identify and develop biomarkers as a diagnostic tool associated with the onset of these pathologies.

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REFERENCES

Adedeji, O.A. and M.H. Etukudo, 2006. Lipid profile of cigarette smokers in calabar municipality. Pak. J. Nutr., 5: 237-238.

Affymetrix, 2008. Affymetrix GeneChip® expression analysis. Technical Manual. http://www.stat.duke.edu/~mw/ABS04/RefInfo/expression_ever_manual.pdf.

Baatartsogt, O., P.K. Mandal, H. Lim, C.H. Lee, J. Lee and K. Choi, 2011. Differential expression of immuno-inflammatory genes in synovial cells from knee after inducing post-traumatic arthritis in swine. Asian J. Anim. Vet. Adv., 6: 333-343.

Baroni, M.G., M.G. Cavallo, M. Mark, L. Monetini, B. Stoehrer and P. Pozzilli, 1999. Beta-cell gene expression and functional characterization of the human insulinoma cell line CM. J. Endocrinol., 161: 59-68.

Boudreau, M.D., H.W. Taylor, D.G. Baker and J.C. Means, 2006. Dietary exposure to 2-aminoanthracene induces morphological and immunocytochemical changes in pancreatic tissues of Fisher-344 rats. Toxicol. Sci., 93: 50-61.

Chen, Y., S.L. Yang, W.D. Deng and H.M. Mao, 2011. Differential gene expression in different tissues of black-bone sheep and normal sheep. Asian J. Anim. Vet. Adv., 5: 379-384.

Chipitsyna, G., Q. Gong, R. Anandanadesan, A. Alnajar and S.K. Batra *et al.*, 2009. Induction of osteopontin expression by nicotine and cigarette smoke in the pancreas and pancreatic ductal adenocarcinoma cells. Int. J. Cancer, 125: 276-285.

Clement, F.C., U. Camenisch, J. Fei, N. Kaczmarek, N. Mathieu and H. Naegeli, 2010. Dynamic two-stage mechanism of versatile DNA damage recognition by xeroderma pigmentosum group C protein. Mutat. Res./Fundam. Mol. Mech. Mutagenesis, 685: 21-28.

Cras-Meneur C., H. Inoue, Y. Zhou, M. Ohsugi and E. Bernal-Mizrachi *et al.*, 2004. An expression profile of human pancreatic islets mRNAs by serial analysis of gene expression (SAGE). Diabetology, 47: 284-299.

Cuadrado, A. and A.R. Nebreda, 2010. Mechanisms and functions of p38 MAPK signaling. Biochem. J., 429: 403-417.

Dantuma, N.P., C. Heinen and D. Hoogstraten, 2009. The ubiquitin receptor Rad23: At the crossroads of nucleotide excision repair and proteasomal degradation. DNA Repair. DNA Repair, 8: 449-460.

De Sa, S.V., M.L. Correa-Gianella, M.C. Machado, K. Krogh and M.Q. de Almeida *et al.*, 2007. Serpin peptidase inhibitor clade A member 1 as a potential marker for malignancy in insulinomas. Clin. Cancer Res., 13: 5322-5330.

Domagk, D., K.L. Shaefer, M. Eisenacher, Y. Braun and D.H. Wai *et al.*, 2007. Expression analysis of pancreatic cancer cell lines reveals association of enhanced gene transcription and genomic amplifications at the 8q22.1 and 8q24.22 loci. Oncol. Reports, 17: 399-407.

Endo, C., S. Zhifu, R.J. Molina, R.J. Attewell and Y. Ping, 2007. Evaluation of reference genes for normalization of quantitative real time PCR in Non-small cell lung cancer. Int. J. Cancer Res., 3: 1-12.

Fousteri, M. and L.H. Mullenders, 2008. Transcription-coupled nucleotide excision repair in mammalian cells: molecular mechanisms and biological effects. Cell Res., 18: 73-84.

Garelnabi, M., 2010. Emerging evidences from the contribution of the traditional and new risk factors to the atherosclerosis pathogenesis. J. Med. Sci., 10: 153-161.

Gato, W.E. and J.C. Means, 2011a. Pancreatic gene expression altered following dietary exposure to 2-aminoanthracene: Links to diabetogenic activity. J. Pharmacol. Toxicol., 6: 234-248.

Gato, W.E. and J.C. Means, 2011b. Single cell gel electrophoretic analysis of 2-aminoanthracene exposed F-344 rats. Am. J. Biochem. Mol. Biol., 1: 275-283.

Gout, S., C. Morin, F. Houle and J. Huot, 2006. Death receptor-3, a new e-selection counter-receptor that confers migration and survival advantages to colon carcinoma cells by triggering p38 and ERK MAPK activation. Cancer Res., 66: 9117-9124.

Hu Z., J. Mellor, J. Wu and C. Delisi, 2004. VisANT: An online visualization and analysis tool for biological interaction data. BMC Bioinformatics, 5: 1-7.

Huang, S. and M.P. Czech, 2007. The GLUT4 glucose transporter. Cell Metab., 5: 237-252.

Inamdar, G.S., S.V. Madhunapantula and G.P. Robertson, 2010. Targeting the MAPK pathway in melanoma: Why some approaches succeed and other fail. Biochem. Pharmacol., 80: 624-637.

Mazzolini, G., I. Narvaiza, L.A. Martinez-Cruz, A. Arina and M. Barajas *et al.*, 2003. Pancreatic cancer escape variants that evade immunogene therapy through loss of sensitivity to IFNγ-induced apoptosis. Gene Therapy, 10: 1067-1078.

Nishimura, M., N. Yokoi, T. Miki, Y. Horikawa and H. Yoshioka *et al.*, 2004. Construction of a multi-functional cDNA library specific for mouse pancreatic islets and its application to microarray. DNA Res., 11: 315-323.

Ohsugi, M., C. Cras-Meneur, Y. Zhou, W. Warren, E. Bernal-Mizrachi and M.A. Permutt, 2004. Glucose and insulin treatment of insulinoma cells results in transcriptional regulation of a common set of genes. Diabetes, 53: 1496-1508.

Qiagen, 2006. Rneasy® Mini Handbook. 4th Edn., Qiagen, Valencia, CA.

Ramesh, T., R. Mahesh and B.V. Hazeena, 2007a. Effect of Sesbania grandiflora on lung antioxidant defense system in cigarette smoke exposed rats. Int. J. Biol. Chem., 1: 141-148.

Ramesh, T., R. Mahesh and V.H. Begum, 2007b. Effect of sesbania grandiflora on membrane-bound ATPases in cigarette smoke exposed rats. J. Pharmacol. Toxicol., 2: 559-566.

Rome, S., E. Meugnier, V. Lecomte, V. Berbe and J. Besson *et al.*, 2009. Microarray analysis of genes with impaired insulin regulation in the skeletal muscle of type 2 diabetic patients indicates the involvement of basic helix-loop-helix domain-containing, class B, 2 protein (BHLHB2). Diabetol., 52: 1899-1912.

Simon, R.M., E.L. Korn, L.M. McShane, M.D. Radmacher, G.W. Wright and Y. Zhao, 2003. Design and Analysis of DNA Microarray Investigations. Springer-Verlag, New York..

Simon, R. and P. Lam, 2009. BRB-array tools version 3.8: User's guide. http://linus.nci.nih.gov/techreport/Manual_v3_8_1.doc

Snyderwine, E.G., H.A.J. Schut, R.H. Adamson, U.P. Thorgeirsson and S.S. Thorgeirsson, 1992. Metabolic activation and genotoxicity of heterocyclic arylamines. Cancer Res., 52: 2099s-2102s.

Sreekumar, R., P. Halvatsiotis, J.C. Schimke and K.S. Nair, 2002. Gene expression profile in skeletal muscle of type 2 diabetes and the effect of insulin treatment. Diabetes, 51: 1913-1920.

Sugasawa, K., J.M.Y. Ng, C. Masutani, T. Maekawa and A. Uchida *et al.*, 1997. Two homologs of Rad23 are functionally interchangeable in complex formation and stimulation of XPC repair activity. Mol. Cell. Biol., 17: 6924-6931.

Tarazona-Santos, E., C. Fabbri, M. Yeager, W.C. Magalhaes and L. Burdett *et al.*, 2010. Diversity in the glucose transporter-4 gene (SLC2A4) in humans reflects the action of natural selection along the old-world primates evolution. PLoS One, 5: e9827-e9827.

Thoma, B.S. and K.M. Vasquez, 2003. Critical DNA damage recognition functions of XPC-hHR23B and XPA-RPA in nucleotide excision repair. Mol. Carcinog., 38: 1-13.

Tseng, P.J., 2009. Human exposure to perfluorinated compounds via smoking and second-hand smoke. J. Applied Sci., 9: 2475-2479.

Van Lunteren, E. and M. Moyer, 2007. Oxidoreductase, morphogenesis extracellular matrix and calcium ion-binding gene expression in streptozotocin-induced diabetic rat heart. Am. J. Physiol. Endrocrinol. Metabol., 293: E759-E768.

Van Lunteren, E. and M. Moyer, 2009. Gene expression profiling in the type 1 diabetes rat diaphragm. PLoS One, 4: e7832-e7832.

Wang, X.L. and J. Wang, 2005. Smoking-gene interaction and disease development: Relevance to pancreatic cancer and atherosclerosis. World J. Surg., 29: 344-353.

Wang, T., K. Shankar, M.J. Ronis and H.M. Mehendale, 2007. Mechanisms and outcomes of drug-and toxicant-induced liver toxicity in diabetes. Crit. Rev. Toxicol., 37: 413-459.

Yang, J., M.A. Price, C.L. Neudauer, C. Wilson and S. Ferrone *et al.*, 2004. Melanoma chondroitin sulfate proteoglycan enhances FAK and ERK activation by distinct mechanisms. J. Cell. Biol., 165: 881-891.

Yechoor, V.K., M.E. Patti, R. Saccone and C.R. Kahn, 2002. Coordinated patterns of gene expression for substrate and energy metabolism in skeletal muscle of diabetic mice. Proc. Nat. Acad. Sci., 99: 10587-10592.

Yechoor, V.K., M.E. Patti, K. Ueki, P.G. Laustsen, R. Saccone, R. Rauniyar and R. Kahn, 2004. Distinct pathways of insulin-regulated versus diabetes-regulated gene expression: An *in vivo* analysis in MIRKO mice. Proc. Natl. Acad. Sci. USA., 47: 16525-16530.

Zhao, Y.P., G. Chen, B. Feng, T.P. Zhang, E.L. Ma and Y.D. Wu, 2007. Microarray analysis of gene expression profile of multidrug resistance in pancreatic cancer. Chin. Med. J., 120: 1743-1752.

Zhu, Y., S. Bye, P.J. Stambrook and J.A. Tischfield, 1995. Aflatoxin b1 2-aminoanthracene and 7,12-dimethylbenz[a]anthracene-induced frameshift mutations in human APRT. Mol. Mutagen., 26: 234-239.