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Exon Array Analysis to Identify Diethyl-nitrosamine Differentially Regulated and Alternately Spliced Genes in Early Liver Carcinogenesis in the Transgenic Mouse ATT-myc Model

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Abstract

Objectives: To identify the regulated genes or the spliced genes of diethylnitorsamine **(**NDEA) in ATT-myc mice versus control group. *Methods:* We analysed the 9 hybridizations on the MouseExon10ST array of NDEA treatments and control non- transgenic by application of a mixed model analysis of variance. *Results:* The 907 genes had regulated significantly between the groups and 916 genes had regulated with a significant exon-group interaction among of them 150 genes had regulated with both gene and possible splicing differences ($p<0.01$). The 7,618 genes had tested for the alternative gene up-regulation and splicing and compared to the gene-classifications. The genes functions, pathways and gene-classifications in the current study had presented in the contingency table analysis of the set of the regulated genes and alternatively spliced that regulated significantly in the ATT-myc mice treated by diethylnitorsamine versus control non-transgenic. The GOMolFn of gene-classification had 321 groups that had significantly regulated in the set of the regulated genes or differentially spliced. While the GOProcess of gene-classification had 330 groups that had significantly regulated in the set of differentially regulated genes or spliced. Additionally, the CELlLoc of gene-classification had 70 groups that had significantly regulated in the set of differentially regulated genes spliced. Finally, the Pathway gene-classification had 8 groups that had significantly regulated in the set of differentially regulated genes or spliced ($p<0.01$) in diethylnitorsamine when compared to control group. *Conclusion:* we summarized the toxicogenomics induced by diethylnitrosamine in early liver carcinogenesis in ATT-myc transgenic mice of liver cancer.

Keywords: N-Diethylnitrosamine (NDEA); Transgenic; Non-transgenic; Att-myc Mouse Model of Liver Cancer; Exon Array Gene Up-regulation; The Spliced Genes Genes.

1. Introduction

100,000 or more new substances, medications, and vaccines are introduced to our population each year, and the FDA and other safety organizations must allow their use for at least 18 months [1]. To protect more people and solve health issues, we need to reduce the time it takes for some vaccines and medicines to be approved in disasters, pandemics, and exotic diseases like corona.

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The use of transgenic models and exon array upregulation could provide quick information on chemical toxicity and carcinogenicity, but it is still expensive. Several experiments and techniques are used to determine the carcinogenicity of chemicals or drugs, which started with two years of testing and has progressed to more sophisticated technology such as microarray, exon array, and models that replicate human tumor genes. The differences between these tests are time consuming during the procedure of each test, and the accuracy of the data results from these projects is questionable. Several alternative research methods are being investigated, such as genetically engineered organisms, in vitro CELl-based assays, and computerized models to overcome the limitations in the cancer testing of chemicals [2-4]. Engineered laboratory animals can be useful in identifying liver carcinogens at an early stage, potentially shortening carcinogenicity tests.

To date, several valid transgenic mouse models and hepatocellular carcinoma knock-out mouse models have been published [5] for example the response to the genotoxic carcinogen NDEA was identified in a p53- p53-deficient mouse model [6] as well as in the rasH2 [7-9] and ATT-myc [10] transgenic mouse models.

Transgenic models and exon array upregulation could provide quick information on chemical toxicity and carcinogenicity, but the cost is still high for testing of chemical or drug carcinogenicity. To shorten the validation stage time or with higher-state analysis, it is critical to validate the transgenic model by testing the carcinogen such as diethylnitrosamine and comparing the date result with previously published dates of the same compound in old and classical tests [9, 11, 12].

The aim of this study was to use and analyze up-regulation of the exon array to identify DEN carcinogens using a meta-analysis of genomics function pathways in the ATT-myc transgenic mode

2. Materials and Methods

The experiment included 48 transgenic ATT-Myc mice of both sexes and 12 non-transgenic mice. All animals were housed on sawdust in groups of 1 to 4 mice per cage in a 12 hour light-dark cycle with 50% relative humidity and an ambient temperature of 22°C. The animals are fed a standardized diet and free access to water (Zucht, ssniff M-/, 10 mm, complete mice diet, ssniff Requirements GmbH, DE-59494, www.ssniff.de). All experiments were managed according to ethical guidelines.

2.1. Study Design and Treatment of Animals with NDEA

The mice were divided into three classes: the first and second groups of 48 transgenic mice each had 12 males and 12 females, with 24 non-transgenic mice of both sexes serving as a vehicle control. NDEA (99 percent purity, Sigma Aldrich, Germany) was given to transgenic mice at a dose of 100 mg once a week for 6 weeks, starting at the age of 2 months. Transgenic and non-transgenic control animals were both used to drive the car. Over the course of six weeks, transgenic mice were given a saline injection containing 100 mg/g NDEA once a week, while control mice received only saline injections [11].

2.2. Samples Collections and Preparation

Mice were anesthetized with CO2 and the thorax was opened using standard surgical procedures, with the liver being explanted with PBS, at the age of 4 (the end of treatment). Liver tissue was immediately frozen in liquid nitrogen and preserved at -80°C.

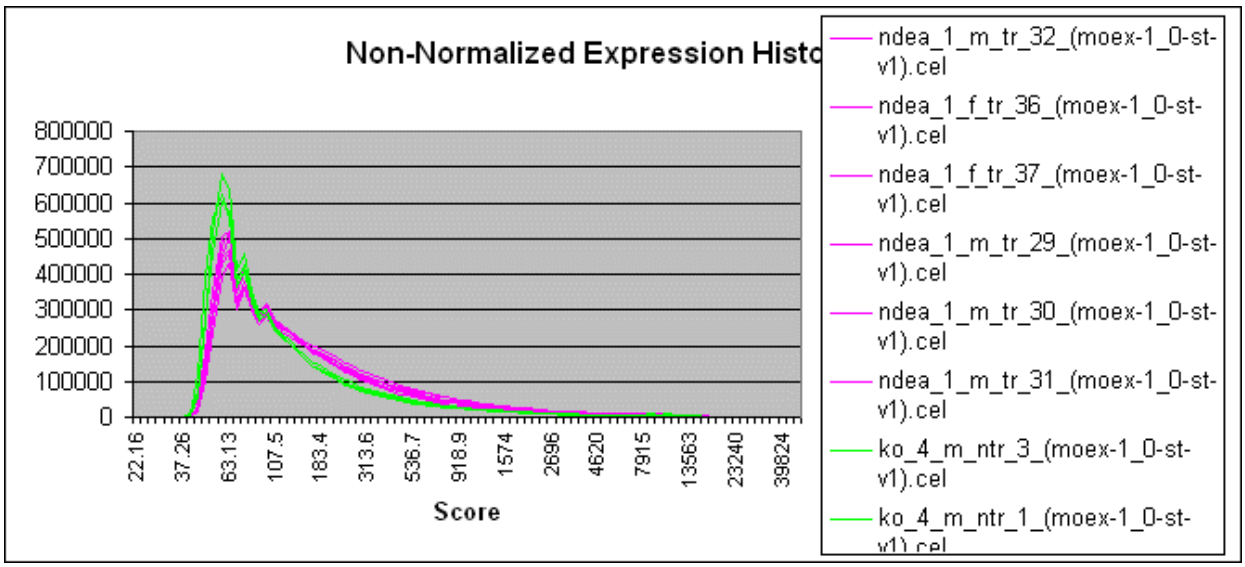
2.3. Isolation from Hybridization and RNA

Hybridization and RNA isolation

The RNeasy total RNA isolation protocol from QIAGEN was used to separate total RNA from frozen liver tissues. The Target Labelling Assay Manual had to be followed to the letter. Ribosomal RNA reduction, cDNA synthesis, cRNA hydrolysis, fragmentation, terminal labelling, hybridization, cleaning, chip staining, GeneChip scanning, and data interpretation were all part of the assay [13].

2.4. Data Analysis, Normalization and Comparison

The current work was performed using a mixed model analysis of variance on 6 hybridizations on the MouseExon10ST array of NDEA treatments and 3 hybridizations of control non-transgenic hybridizations on December 15, 2010 by installer with XRAY (version 3.2) software on 6 hybridizations on the MouseExon10ST array of NDEA treatments and 3 hybridizations of control non-transgenic hybridizations. Both probes' gene expression was normalized against the history (Figures 1 and 2). The fold changes were considered significant at p value ≤ 0.05 and statistical test was done by using student T test.



Group	CEL File
ndea_1s_mf	ndea_1_m_tr_32_(moex-1_0-st-v1). the CEL
ndea_1s_mf	ndea_1_f_tr_36_(moex-1_0-st-v) . the CEL
ndea_1s_mf	ndea_1_f_tr_37_(moex-1_0-st-v1). the CEL
ndea_1s_mf	ndea_1_m_tr_29_(moex-1_0-st-v1).the CEL
ndea_1s_mf	ndea_1_m_tr_30_(moex-1_0-st-v1) .the CEL
ndea_1s_mf	ndea_1_m_tr_31_(moex-1_0-st-v1) .the CEL
ntr_4s_mf	ko_4_m_ntr_3_(moex-1_0-st-v1) .the CEL
ntr_4s_mf	ko_4_m_ntr_1_(moex-1_0-st-v1) .the CEL
ntr_4s_mf	ko_4_m_ntr_2_(moex-1_0-st-v1). the CEL

Figure 1. For each array, the distribution of scores was summarized

The x axis represented score, and the y axis represented the number of probes with a score in the range divided by the total number of probes.

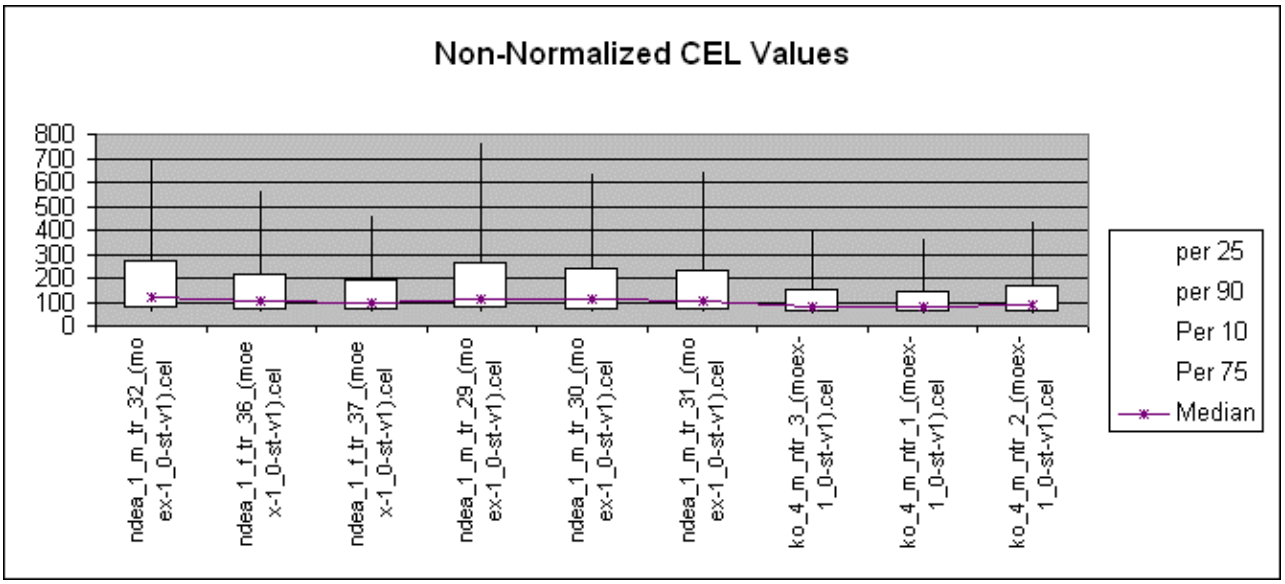


Figure 2. In the depicted graph, the distribution of scores was summarized for each array

A box plot was used to investigate each array, with the probe score median in the center (joined by lines to aid comparison). The highest and lowest lines in the box represented the 25th and 75th percentiles of probe score, respectively, and the highest and lowest lines represented the 10th and 90th percentiles of probe score, respectively.

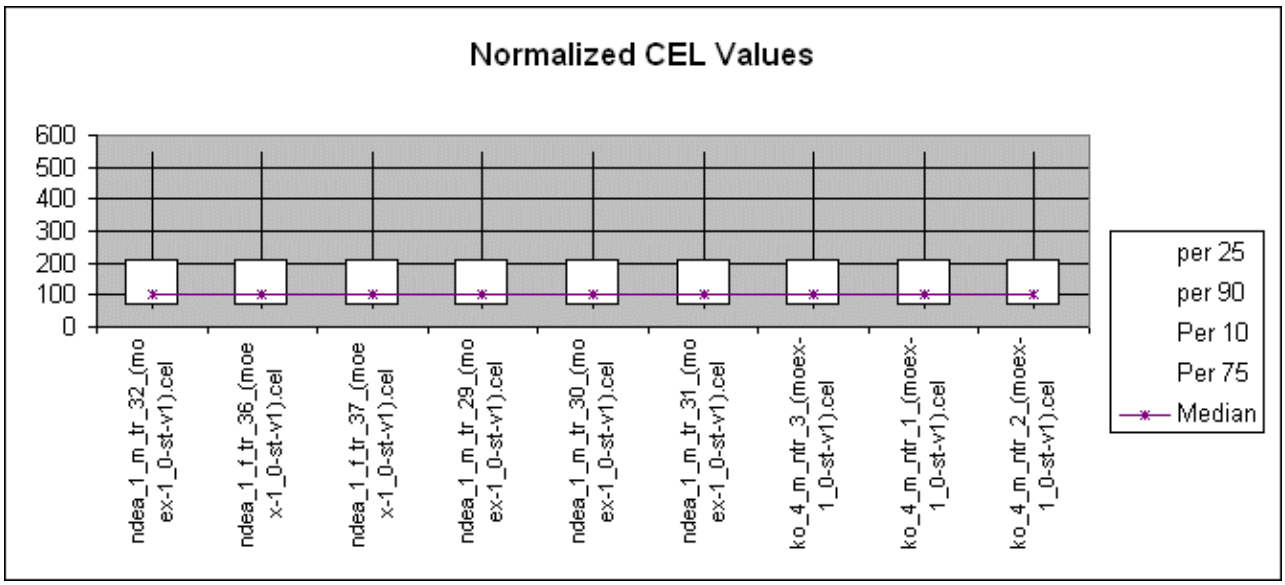


Figure 3. After normalization, the distribution of scores was summarized in this depicted graph for each array. Since complete quantile normalization required all input arrays to have similar "shapes," all box plots were identical

3. Results

3.1. Tissue Distribution of Genes Expression

There were 270,096 transcript clusters on the MouseExon10ST array. Following the above-mentioned filters, there were 7,618 applications ranging from 4 to 200 probe-sets. Statistical tests were used to identify gene up-regulation and spliced genes. The following table summarized the number of tested genes (transcript clusters) expressed in each category for the transcript clusters that were tested.

Group	**Number of transcript clusters with a significant up-regulation in group**
ndea_1s_mf	7,122 93.5% of genes tested
ntr_4s_mf	6,608 86.7% of genes tested

By application of the same test, the following table described all frequencies of pair-wise co-up-regulation between the study groups.

-	ndea_1s_mf	ntr_4s_mf
ndea_1s_mf	7,122(0,741)	6,381(6,381)
ntr_4s_mf	-	6,608(0,227)

The NDEA treatments 1s mf community had 7,122 genes that were substantially controlled above the background level. The following table lists all of the co-regulation trends. Frequency is a term that refers to the number of times

3.2. The Gene Upregulation and the Spliced Genes

The statistical analysis showed that 907 genes were significantly expressed with variations between groups, and 916 genes were significantly expressed with exon-group interaction (a symptom of spliced genes), with 150 genes having both gene differences and interaction.

The highest 10-fold changes in genes with significant gene expression are described in Table 1. The fold change was expressed in terms of normalized untransformed results, and Table 2 showed the top 10 spliced genes with a significant fold change.

Table 1. Genes with a major gene up-regulation showed the largest 10 fold shifts. The normalized untransformed data is used to calculate the fold shift

Gene Symbol	TCluster ID	Description	Fold Change	The Up-regulation p-value
4432416J03Rik	6995384	RIKEN cDNA 4432416J03 gene	5.51	3.53E-03
Ddx54	6933987	DEAD (Asp-Glu-Ala-Asp) box polypeptide 5	-1.31	1.92E-02
Mmp12	6986722	matrix metallopeptidase 12	-3.11	1.36E-02
Rpl4	6989868	ribosomal protein L4	-1.61	1.87E-02
Nol5a	6881172	nucleolar protein 5A	-2.61	2.04E-02
Nol11	6791992	nucleolar protein 11	-1.71	2.05E-02
Ga17	6889367	dendritic cell protein GA17	-1.41	2.01E-02
Josd1	6837122	Josephin domain containing 1	-1.51	2.06E-02
Snx5	6891675	sorting nexin 5	-1.41	1.92E-02
Vdac2	6817416	voltage-dependent anion channel 2	-1.91	1.91E-02

Table 2. Displayed the highest 10 with a significant the spliced genes

Gene Symbol	TCluster ID	Description	Exon-Tissue Interaction p-value
Dst	6748525	dystonin	2.93E-47
Ugt1a6a	6751362	UDP glucuronosyltransferase 1 family pol	2.54E-17
Cyp2c40	6873060	cytochrome P450 family 2 subfamily c pol	1.11E-16
Heatr1	6804849	HEAT repeat containing 1	6.95E-16
Abcb1a	6928740	ATP-binding cassette sub-family B (MDR/T	5.70E-13
Srpk1	6854616	serine/arginine-rich protein specific ki	4.96E-12
Lcn2	6885873	lipocalin 2	2.52E-11
Col3a1	6749142	procollagen type III alpha 1	4.51E-11
Gstm6	6908075	glutathione S-transferase mu 6	7.54E-11
Abcb1b	6928741	ATP-binding cassette sub-family B (MDR/T	1.72E-10

3.3. The Genes Expression According the GOMolFn of Ontology Classification

In the collection of differentially spliced or regulated genes, the GOMolFn gene-classification had 321 groups that had significantly increased (the splicing and gene up-regulation determined as described above). The top 30 groups are listed in Table 3 and Figure 1. The three columns include the number of tested genes found to have significant gene expression (with p-value of regulation), the number of tested genes performed to have significant splicing (with p-value of regulation), and the group name, respectively.

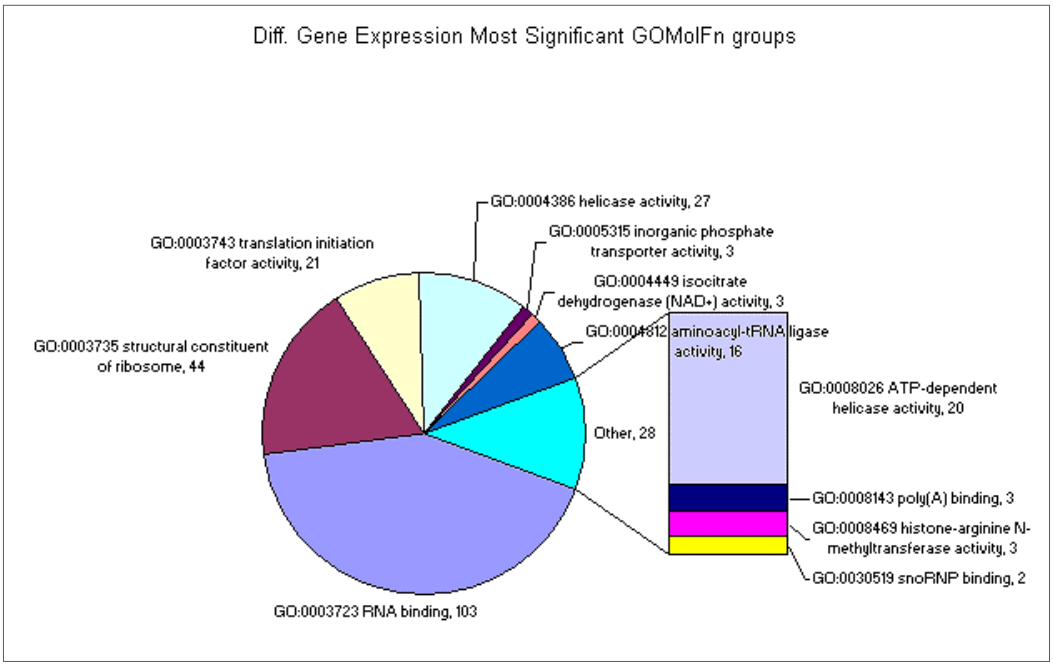


Figure 4. Displayed percentage of the GOMolFn of groups regulated due to NDEA treatment versus control non-transgenic

Table 3. Displayed the highest 30 GOMolFn of groups regulated due to NDEA treatment versus control non-transgenic

Number GE	Number AS	Group Name
103(0.00E+00)	67(1.65E-03)	GO:0003723 RNA binding
44(1.46E-12)	19(3.19E-01)	GO:0003735 structural constituent of of ribosome
21(4.35E-08)	6(7.14E-01)	GO:0003743 translation initiation factor
27(1.10E-06)	24(9.07E-05)	GO:0004386 helicase activity
3(1.19E-06)	1(1.28E-01)	GO:0005315 inorganic phosphate transport
3(1.19E-06)	0(1.00E+00)	GO:0004449 isocitrate dehydrogenase (NAD
0(1.00E+00)	3(1.38E-06)	GO:0017114 wide-spectrum protease inhibition
0(1.00E+00)	4(1.46E-06)	GO:0016717 oxidoreductase activity- activity
16(2.18E-06)	9(7.55E-02)	GO:0004812 aminoacyl-tRNA ligase activity
0(1.00E+00)	8(1.15E-05)	GO:0003995 acyl-CoA dehydrogenase activity
0(1.00E+00)	8(1.15E-05)	GO:0016627 oxidoreductase activity- activity
3(5.49E-01)	10(3.17E-05)	GO:0042626 transmembrane movement of substances
20(3.78E-05)	18(7.02E-04)	GO:0008026 ATP-dependent helicase activity
3(4.78E-05)	1(2.12E-01)	GO:0008143 poly(A) binding
3(4.78E-05)	1(2.12E-01)	GO:0008469 histone-arginine N-methyltransferase
0(1.00E+00)	3(5.33E-05)	GO:0004768 stearoyl-CoA 9-desaturase act
0(1.00E+00)	3(5.33E-05)	GO:0004718 Janus kinase activity
0(1.00E+00)	3(5.33E-05)	GO:0005504 fatty acid binding
2(5.87E-05)	1(4.92E-02)	GO:0030519 snoRNP binding
2(5.87E-05)	0(1.00E+00)	GO:0019863 IgE binding
2(5.87E-05)	0(1.00E+00)	GO:0003796 lysozyme activity
2(5.87E-05)	0(1.00E+00)	GO:0003730 mRNA 3'-UTR binding
2(5.87E-05)	0(1.00E+00)	GO:0004465 lipoprotein lipase activity
2(5.87E-05)	0(1.00E+00)	GO:0008175 tRNA methyltransferase activity
2(5.87E-05)	1(4.92E-02)	GO:0016005 phospholipase A2 activator ac
2(5.87E-05)	0(1.00E+00)	GO:0003883 CTP synthase activity
2(5.87E-05)	2(6.47E-05)	GO:0042007 interleukin-18 binding
2(5.87E-05)	2(6.47E-05)	GO:0048019 receptor antagonist activity
2(5.87E-05)	0(1.00E+00)	GO:0035241 protein-arginine omega-N mono
2(5.87E-05)	0(1.00E+00)	GO:0004576 oligosaccharyl transferase activity

3.4. The Genes Expression According the GOProcess of Ontology Classification

The way to go in the collection of differentially spliced or regulated genes, 330 groups were significantly regulated during the gene-classification process (the splicing and gene up-regulation determined as described above). The top 30 classes were listed in Table 4 and Figure 2. The number of tested genes revealed a significant the gene up-regulation (with p-value of regulation), the number of tested genes found to have a significant the splicing (with p-value of regulation), and the group name were all mentioned in the three columns.

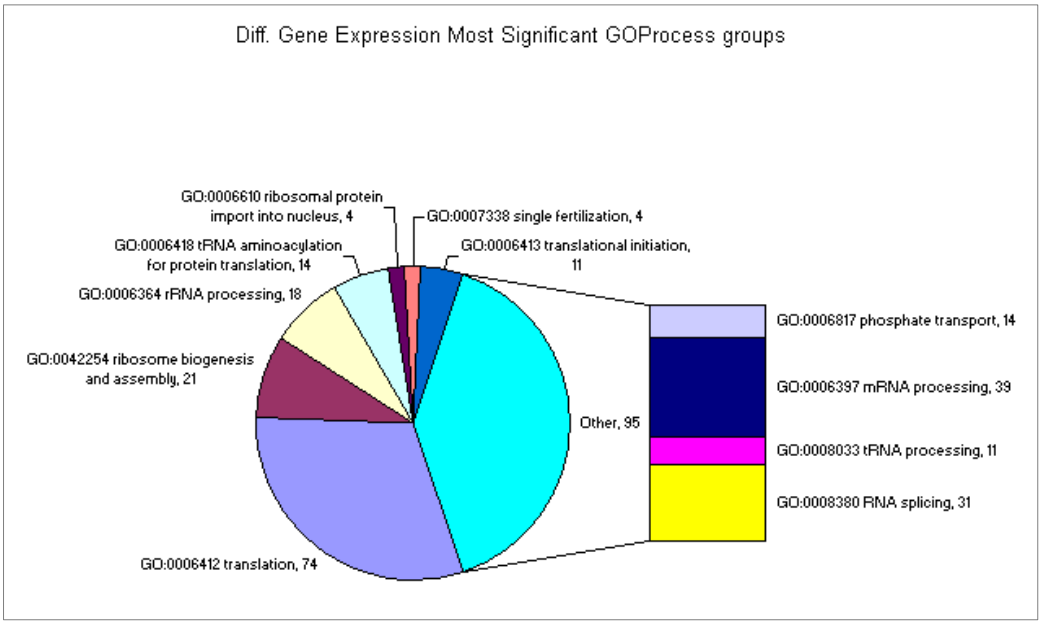


Figure 5. Displayed percentage of the GOProcess of groups regulated due to NDEA treatment versus control non-transgenic

Table 4. Showed the highest 30 GOProcess of groups regulated due to NDEA treatment versus control non-transgenic

Number GE	Number AS	Group Name
74(0.00E+00)	34(1.78E-01)	GO:0006412 translation
21(1.11E-16)	6(2.37E-01)	GO:0042254 ribosome biogenesis and *assembly*
18(2.74E-10)	6(3.25E-01)	GO:0006364 rRNA processing
14(9.88E-07)	6(2.37E-01)	GO:0006418 tRNA aminoacylation for protein
4(1.25E-06)	2(2.71E-02)	GO:0006610 ribosomal protein import into nucleus
4(1.25E-06)	0(1.00E+00)	GO:0007338 single fertilization
11(1.78E-06)	2(7.69E-01)	GO:0006413 translational initiation
14(3.10E-06)	8(6.01E-02)	GO:0006817 phosphate transport
39(4.15E-06)	25(1.40E-01)	GO:0006397 mRNA processing
11(7.26E-06)	5(1.93E-01)	GO:0008033 tRNA processing
4(4.57E-01)	11(4.98E-05)	GO:0006633 fatty acid biosynthetic process
31(5.05E-05)	21(1.16E-01)	GO:0008380 RNA splicing
2(5.87E-05)	1(4.92E-02)	GO:0006982 response to lipid *hydroperoxide*
2(5.87E-05)	0(1.00E+00)	GO:0048844 artery morphogenesis
2(5.87E-05)	1(4.92E-02)	GO:0006784 heme a biosynthetic process
2(5.87E-05)	1(4.92E-02)	GO:0008535 cytochrome c oxidase complex
2(5.87E-05)	0(1.00E+00)	GO:0045921 positive regulation of exocytosis
2(5.87E-05)	0(1.00E+00)	GO:0006101 citrate metabolic process
2(5.87E-05)	2(6.47E-05)	GO:0051170 nuclear import
2(5.87E-05)	0(1.00E+00)	GO:0051051 negative regulation of transport
2(5.87E-05)	1(4.92E-02)	GO:0030490 processing of 20S pre-rRNA
2(5.87E-05)	1(4.92E-02)	GO:0042274 ribosomal small subunit biogenesis
1(4.78E-02)	2(6.47E-05)	GO:0000266 mitochondrial fission
1(4.78E-02)	2(6.47E-05)	GO:0045010 actin nucleation
0(1.00E+00)	2(6.47E-05)	GO:0051084 posttranslational protein folding
0(1.00E+00)	2(6.47E-05)	GO:0006768 biotin metabolic process
0(1.00E+00)	2(6.47E-05)	GO:0030573 bile acid catabolic process
0(1.00E+00)	2(6.47E-05)	GO:0045329 carnitine biosynthetic process
0(1.00E+00)	2(6.47E-05)	GO:0043072 negative regulation of programmed cell death
6(6.69E-05)	6(7.63E-05)	GO:0000059 protein import into nucleus-docking

3.5. The Genes Expression According the CELlLoc of Ontology Classification

In the collection of differentially spliced or regulated genes, the CELlLoc gene-classification had 70 groups that were significantly expressed (the splicing and gene up-regulation determined as described above). The top 30 classes were shown in Table 5 and Figure 3. The number of tested genes found to have a significant the gene up-regulation (with p-value of regulation), the number of tested genes identified to have a significant the splicing (with p-value of regulation), and the group name were all mentioned in the three columns.

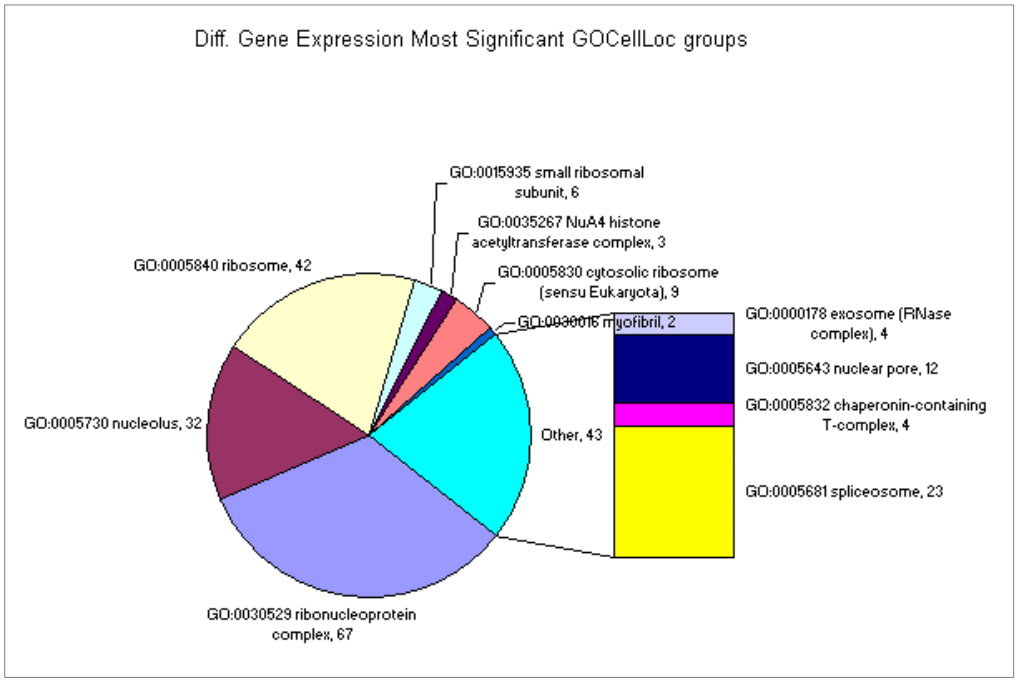


Figure 6. Displayed percentage of the GOCELlLoc of groups regulated due to NDEA treatment versus control non-transgenic

Table 5. Showed the highest 30 GOCELlLoc of groups regulated due to NDEA treatment versus control non-transgenic

Number GE	Number AS	Group Name
67(0.00E+00)	22(7.45E-01)	GO:0030529 ribonucleoprotein complex
32(1.51E-14)	15(4.01E-02)	GO:0005730 nucleolus
42(1.95E-13)	18(2.49E-01)	GO:0005840 ribosome
6(1.89E-07)	3(2.46E-02)	GO:0015935 small ribosomal subunit
2(8.61E-02)	5(6.63E-07)	GO:0046581 intercellular canaliculus
3(1.19E-06)	0(1.00E+00)	GO:0035267 NuA4 histone acetyltransferase
9(2.35E-06)	3(3.40E-01)	GO:0005830 cytosolic ribosome (sensu Euk
12(2.90E-04)	13(5.23E-05)	GO:0005643 nuclear pore
110(5.56E-02)	132(5.69E-05)	GO:0005737 cyhighestlasm
2(5.87E-05)	1(4.92E-02)	GO:0030016 myofibril
1(6.80E-01)	6(7.63E-05)	GO:0005913 the CELl-the CELl adherens junction
4(1.07E-04)	1(4.27E-01)	GO:0000178 exosome (RNase complex)
4(4.30E-04)	0(1.00E+00)	GO:0005832 chaperonin-containing T-complex
3(1.26E-02)	4(4.75E-04)	GO:0016604 nuclear body
23(1.06E-03)	15(2.61E-01)	GO:0005681 spliceosome
8(1.46E-03)	5(1.29E-01)	GO:0005925 focal adhesion
1(6.80E-01)	5(1.68E-03)	GO:0030173 integral to Golgi membrane
2(1.68E-03)	2(1.80E-03)	GO:0005588 collagen type V
2(1.68E-03)	0(1.00E+00)	GO:0035098 ESC/E(Z) complex

0(1.00E+00)	2(1.80E-03)	GO:0005971 small nucleolar ribonucleoprotein complex Gene Ontology
3(1.95E-03)	0(1.00E+00)	GO:0005851 eukaryotic translation initiation factor 2 complex
8(2.18E-03)	5(1.49E-01)	GO:0005654 nucleoplasm
0(1.00E+00)	6(2.70E-03)	GO:0015630 microtubule cytoskeleton
4(2.99E-03)	3(4.01E-02)	GO:0005732 small nucleolar ribonucleoprotein
1(3.23E-03)	0(1.00E+00)	GO:0032998 Fc-epsilon receptor I complex
1(3.23E-03)	0(1.00E+00)	GO:0005637 nuclear inner membrane
1(3.23E-03)	1(3.40E-03)	GO:0042564 NLS-dependent protein nuclear
1(3.23E-03)	0(1.00E+00)	GO:0005850 eukaryotic translation initiation factor 2
1(3.23E-03)	0(1.00E+00)	GO:0001650 fibrillar centre
1(3.23E-03)	0(1.00E+00)	GO:0005847 mRNA cleavage and polyadenylation specificity factor

3.6. The Genes Expression According the Pathway Ontology Classification

The collection of differentially spliced or regulated genes was divided into eight classes, each of which had a significant expression (the splicing and gene up-regulation determined as described above). The top eight classes are shown in Table 6 and Figure 4. The number of tested genes found to have a significant the gene up-regulation (with p-value of regulation), the number of tested genes revealed a significant the splicing (with p-value of regulation), and the group name were all mentioned in the three columns.

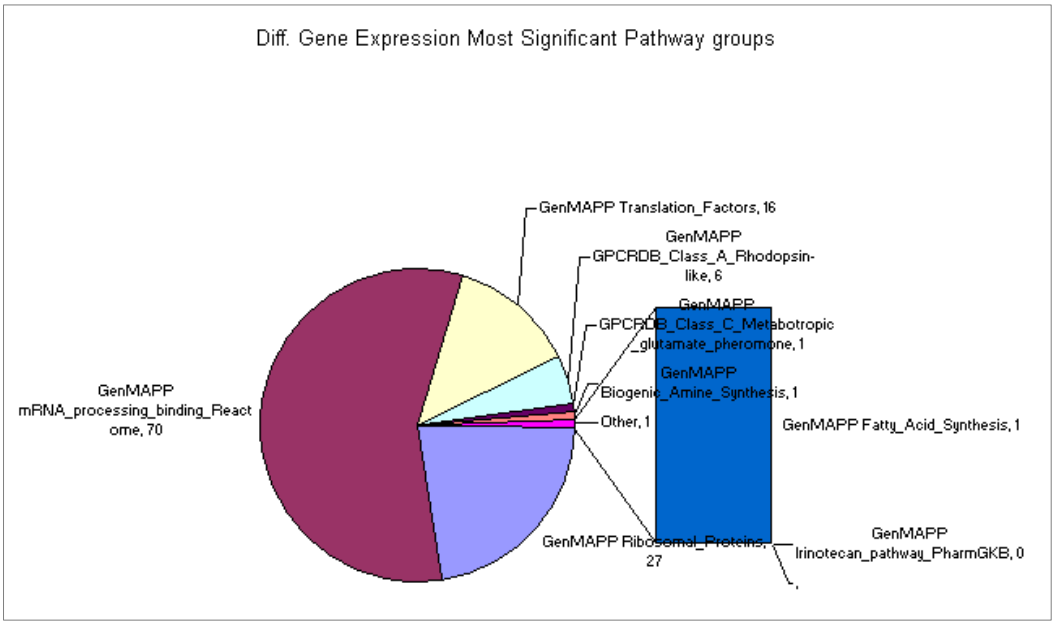


Figure 7. Displayed percentage of the groups of the Pathway regulated due to NDEA treatment versus control non-transgenic

Table 6. Showed the highest 8 groups of the Pathway regulated due to NDEA treatment versus control non-transgenic

Number GE	Number AS	Group Name
27(0.00E+00)	7(2.32E-01)	GenMAPP Ribosomal_Proteins
70(1.05E-10)	41(1.48E-01)	GenMAPP mRNA_processing_binding_Reactome
16(7.56E-08)	6(3.25E-01)	GenMAPP Translation_Factors
0(1.00E+00)	4(1.20E-04)	GenMAPP Irinotecan_pathway_PharmGKB
1(2.87E-01)	3(4.82E-04)	GenMAPP Biogenic_Amine_Synthesis
6(7.69E-04)	4(5.50E-02)	GenMAPP GPCRDB_Class_A_Rhodopsin-like
1(3.23E-03)	0(1.00E+00)	GenMAPP GPCRDB_Class_C_Metabotropic_glut
1(5.73E-01)	4(3.24E-03)	GenMAPP Fatty_Acid_Synthesis

4. Discussion

Hepatocellular carcinoma is one of the deadliest and most common cancers in the human population [14]. In comparison to many infrequently mutated genes that may also correlate to tumor biology, there are only a few studies that have well established driver genes that often have a mutation [14, 15]. So, there has been more interest in the developing pathway and network analysis methods about group of genes and illuminate the processes of each function group [16]. Animal models of hepato-carcinogenesis have provided reliable evidence for understanding the cellular production of HCC and further developing promising therapies [17-19]. The current study explored the function of liver genome at early carcinogenesis induced by NDEA in att-myc mouse model of hepatocellular carcinoma. In terms of gene groups regulated in GOProcess of the GOMolFn, GOProcess, CELlLoc, and Pathway classes, it was discovered that the collection of regulated genes and alternatively spliced genes was significantly regulated in groups of the GOMolFn, GOProcess, CELlLoc, and Pathway classes of NDEA treatment in ATT-myc transgenic model of liver cancer as well as similar study recorded earlier the molecular effect of NDEA in rat hepatocarcinogenesis [20]. Also, compared to usual livers, NDEA therapy increased the up-regulation and down-regulation of genes (DFGs) from progression dysplastic nodules, early tumor nodules, and liver tumor with lung metastasis. The gene ontology (GO) tree was used to categorize the DFGs into functional processes. The GO terms included metabolism, transport, hepatocellular proliferation, apoptosis, angiogenesis, adhesion, and others [15]. Additionally, when compared to liver tissue from normal rats and other one treated by NDEA, the above chosen tissues share 349 upregulated and 345 downregulated genes. Deregulated genes are involved in a variety of processes, including metabolism, transport, cell proliferation, apoptosis, cell adhesion, and angiogenesis. Inflammatory response, immune response, and oxidative stress are all represented by 41 upregulated and 27 downregulated genes [21].

The differentially spliced genes investigated previously in human liver cancer (pharmacological behaviour of berberine) and the possible functional cross-talking between the two sets of genes up-regulation and the spliced genes merits further investigation [22]. In this analysis, we looked at both gene up-regulation and spliced genes in the livers of NDEA-treated mice and found 321 controlled groups in the GOMolFn of early tumorigenesis in att-myc mice treated with NDEA, with the top 30 up-regulation groups discussed in Table 7.

Table 7. Showed the literature of similar to our finding according to the gene entomology in liver tumor

Highest 30 genes	Gene ontology in the GOMolFn	Gene upregulation explanations in relation to early liver carcinogenesis induced by NDEA
1	GO:0003723 RNA binding	The upregulation of RNA binding to protein involved in the regulation of protein synthesis to initiate biogenesis of the secondary tumor in hepatocellular carcinoma in mice [23].
2	GO:0003735 structural constituent of ribosome	The upregulation of structural constituent of ribosome, as mention in the highest 10 best associated gene sets with highest mean minimum Function-1 gene up-regulation in HCC [24].
3	GO:0003743 translation initiation factor	The upregulation of translation initiation factor, as recorded in GO functional enrichment analysis of DEGs when Aspergillus flavus was treated with, anti-aflatoxigenic mechanism, cinnamaldehyde [25].
4	GO:0004386 helicase activity	The up-regulation of helicase activity, as reported in the GO analysis of predicted target genes of LINC01296 pan-cancers and the molecular regulatory mechanism in hepatocellular carcinoma [26].
5	GO:0005315 inorganic phosphate transport	The upregulation of inorganic phosphate transport, as found in Recent studies of mice feed a diet with high in inorganic phosphate lead to increase tumorigenesis in the two-stage skin carcinogenesis model as well as the Kras lung cancer model [27, 28] and also the exposure of cancer cell to an environment with increased inorganic phosphate availability could increase the ability of these cells to induce an angiogenic response and/or attract endothelial cell [29].
6	GO:0004449 isocitrate dehydrogenase (NAD dependent)	The isocitrate dehydrogenase (NAD dependent) up-regulation in HCC as recorded that the abnormal up-regulation or mutations of human NAD-IDH (also called IDH3) are also found to be associated with the development of cancers and diseases [30].
7	GO:0017114 wide-spectrum protease inhibition	The up-regulation of wide-spectrum protease inhibition here may be a tool from the body defence against tumor growth as detected in particularly HIV protease inhibitors, for cancer treatment [31].
8	GO:0016717 oxidoreductase activity-activity	The oxidoreductase activity here could explained as regulated in ovarian and breast cancer regulated by Ets-1 and oxidative stress in tumor environment [32, 33].
9	GO:0004812 aminoacyl-tRNA ligase activity	The up-regulation aminoacyl-tRNA ligase here as reported that the ethology of specific diseases as cancer could connected to specific aminoacyl tRNA synthetases [34].

10	GO:0003995 acyl-CoA dehydrogenase activity	The Analysis of 158 liver cancer samples revealed that the decreased long-chain acyl-CoA dehydrogenases upregulation predicts patient mortality [35].
11	GO:0016627 oxidoreductase activity	Oxidoreductase constitute to one of the most important free radical scavenger systems such as catalase, superoxide dismutase and glutathione peroxidase and are down regulated in HepG2 cell line by 56% [36].
12	GO:0042626 ATPase activity- coupled to the transmembrane movement of substances	Sodium orthovanadate (SOV) is a phosphate analogue that had an anti-cancer activity. intriguingly, SOV inhibited ATPase activity, which was significantly increased in sorafenib-resistant HCC cells [37].
13	GO:0008026 ATP-dependent helicase activity	There were many numbers of ATP-dependent RNA helicases are important for constitutive RNA splicing and no helicases have been implicated in alternative RNA splicing [38].
14	GO:0008143 poly(A) binding	RNA polypurine tract (PPT) resists digestion by reverse transcriptase (RT) and primes plus-strand DNA synthesis [39].
15	GO:0008469 histone-arginine N-methyltransferase	Notably, the levels of protein arginine methyltransferase 5 (PRMT5) had increased more abundantly in the stem-like tumor spheres compared to other enzymes and the knockdown of PRMT5 dramatically decreased the up-regulation of stemness genes in HCC [40].
16	GO:0004768 stearoyl-CoA 9-desaturase act	stearoyl-CoA (*scd1*) had associated with a variety of diseases including cancers and miRNAs predicted to target the 3′-UTR region of *scd1* gene are associated with breast cancers, hepatoth cellular carcinoma [41].
17	GO:0004718 Janus kinase activity	Novel Azaspirane, a chemically novel and biological drug, targeting the Janus Kinase-Signal Transducer and Activator of Transcription (STAT) Pathway lead to decrease the tumor development in an orthohighestic HCC mouse model and in invite studies [42].
18	GO:0005504 fatty acid binding	The overup-regulation of fatty acid-binding protein 5 (FABP5) had a significant role in HCC progression and metastasis through the induction of epithelial-to-mesenchymal transition [43].
19	GO:0030519 snoRNP binding	SnoRNAs, small nucleolar RNA, are a class of non-coding RNAs divided into two classes: C/D box snoRNAs and H/ACA box snoRNAs. The Mutations and aberrant up-regulation of snoRNAs have been found in the Cell transformation, tumorigenesis, and metastasis, indicating to that its inhibition could be a therapeutic target of cancer [44].
20	GO:0019863 IgE binding	The blocking of IgE signalling not only reduces inflammatory cell infiltration mediated by the Th2 immune response but also inhibits other immune responses [45].
21	GO:0003796 lysozyme activity	The measurement of lysozyme in patients with chronic liver disease could be a helpful tool for detecting HCC [46].
22	GO:0003730 mRNA 3'-UTR binding	The miR-331-3p could inhibit VHL up-regulation by directly targeting its 3'-UTR in HCC cell and This data provided a useful tool in exploring the mechanism of HCC [47].
23	GO:0004465 lipoprotein lipase activity	*LPL* mRNA up-regulation is significantly up-regulated in HCC samples compared with the non-tumour liver samples [48].
24	GO:0008175 tRNA methyltransferase activity	tRNA methyltransferase (TRM) in translational decoding [49]. Also, the attenuating TRM-dependent translation in cancer cell may ablate the disease progression while leaving noncancerous cell unharmed [50].
25	GO:0016005 phospholipase A2 activator activity	Phospholipase-mediated calpain activation in the hepatocytes and HCC. Moreover, Melittin, a phospholipase A2 activator, elevated the calpain activity and cell necrosis while melittin-induced cell necrosis was ameliorated by the calpain protease inhibitor [51].
26	GO:0003883 CTP synthase activity	The presence of CTPS cytoophidia in various human cancers and some non-cancerous tissues. Moreover, among 203 tissue samples of hepato- cellular carcinoma, 28% of samples regulated many cytoophidia, whereas no cytoophidia had detected in the adjacent non-cancerous hepatocytes for all sample [52].
27	GO:0042007 interleukin-18 binding	The association between interleukin-18 (IL-18) polymorphisms and the susceptibility and prognosis of hepatocellular carcinoma [53].
28	GO:0048019 receptor antagonist activity	A Cholecystokinin Receptor prevented **hepatocellular** Carcinoma Antagonist as it Halts Nonalcoholic Steatohepatitis [54].
29	GO:0035241 protein-arginine *omega-N monomethyltransferase* activity	Three distinct types of methylated arginine residues occur in mammalian cell. The most prevalent is omega-N^G,N^G-dimethylarginine [55]. Protein arginine methyltransferases (PRMT1) is responsible for the 85% of total protein arginine methylation activity in cultured RAT1 fibroblast cell and also in the mouse liver [56].
30	GO:0004576 oligosaccharyl transferase activity	Inhibition the activity of Oligosaccharyltransferase can Overcome the therapeutic Resistance to EGFR Tyrosine Kinase Inhibitors [57].

5. Conclusion

In conclusions, we summarized the toxicogenomic effects of diethlynitrosamine in att-myc mouse model including 321 groups of GOMolFn of gene-classification that significantly regulated in the set of the regulated genes or differentially spliced. While the GOProcess of gene-classification had 330 groups that had significantly regulated in the set of differentially regulated genes or spliced. Additionally, the CELlLoc of gene-classification had 70 groups that had significantly regulated in the set of differentially regulated genes spliced and induced by diethlynitrosamine in att-myc transgenic mouse of liver cancer in order to understand the biology of liver cancer.

6. Abbreviations

NDEA: Diethyl nitrosamine	Att-myc: transgenic mice
1s: first sacrifice	4s: fourth sacrifice
F: female	M: male
Tr: transgenic	Ntr: non-transgenic

7. Declarations

7.1. Author Contributions

Conceptualization, M.M.E.; software, M.M.E.; formal analysis, M.M.E.; writing—review and editing, M.M.E. and J.B.; supervision, J.B. All authors have read and agreed to the published version of the manuscript.

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7.4. Ethical Approval

The study was conducted in accordance with the Declaration of Helsinki, and the research protocol was accepted by the animal welfare ethics committee of the city of Hannover, Germany (Tierversuchsvorhaben 33.9-42502-04-08/1619).

7.5. Data Availability Statement

The data presented in this study are available in article.

7.6. Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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