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Identification of genes differentially regulated by transcription factor, AP-2Delta

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1. ABSTRACT

In an effort to better understand the transcriptional program regulated by transcription factor, AP-2delta, we used cDNA microarray to evaluate the relative expression of human genes in AD293 cells by exogenous expression of AP-2delta. Microarry showed 29 genes that were up-regulated and 39 genes with a down-regulated expression pattern. Among the identified genes were those encoding transcription factors, signal transduction molecules, kinases, as well as genes regulating cell growth, differentiation, and cell adhesion, a set of genes suggested to be regulated by AP-2. The results of microarray was valided for some regulated genes by real-time PCR analysis.These observations shed novel insight into the mechanism of AP-2delta action and provide a range of target genes for further investigation.

2. INTRODUCTION

AP-2 transcription factors play pivotal roles in orchestrating embryonic development by influencing the differentiation, proliferation, and survival of cells (1). Furthermore, AP-2 transcription factors have been implicated in carcinogenesis, a process where the normal growth and differentiation program of cells is disturbed (2). The mammalian AP-2 family of transcription factors consists of five members, AP-2alpha, AP-2belta, AP-2gamma, AP-2delta and AP-2epsilon. The defining feature of the AP-2 family is a highly conserved carboxy-terminal basic helix-turn-helix domain that is involved in dimerization and sequence-specific DNA-binding (3). A number of functional AP-2 binding sites, consensus to a palindromic core sequence 5' -$GCCN_3GGC$-3', have been identified in cellular and viral enhancers, and preferred binding to the sequence motifs $GCCN_3GGC$, $GCCN_4GGC$,

and $GCCN_{3/4}GGG$ was observed in an in-vitro binding site selection assay (4, 5). At the N-terminus, AP-2 proteins show weak sequence similarities and contain proline- and glutamine-rich domains and a PY motif (XPPXY) that harbors most of their transcriptional activation properties (1). Interestingly, AP-2delta lacks the PY motif and several others conserved residues that are important for the transcriptional activity of AP-2 proteins, yet functions as an equally strong activator (6).

Transcription factor AP-2 regulates the genes involved in a spectrum of important biologeical functions. Some of the AP-2-activated genes are tyrosine hydroxylase (7), p21WAF/CIP (8), transforming growth factor-a (9), estrogen receptor (10), tyrosine kinase receptor gene c-KIT (11), HIV type-1 (12), HTLV-I (13,14), HER-2/neu (15,16), insulin-like growth-factor binding-5 (17), the dopamine beta-hydroxylase gene (18), keratinocyte-specific genes (19), type IV collagenase (20), and SV40 enhancer region (21),. AP-2 also negatively regulates a number of genes, including KLF-4 (22), MCAM/MUC18 (23); c/EBP-a, during adipogenesis (24); and c-myc (25).

Despite the considerable sequence similarities and overlap in the expression of AP-2 family members, it has been described that each of the five AP-2 genes displays a distinct expression pattern in mouse embryos, suggesting that each of the family members play a different role during development. And the knockout of individual AP-2 members in mice results in specific developmental defects. Consequently identification of targets of each member is the most important to aid in further understanding AP-2 function.

In this report, a variety of genes have been proposed as targets for transcriptional regulation of AP-2delta by gene chip. Ap-2delta is the domain different member of the AP-2 family. It lacks the PY motif and several others conserved residues that are important for the transcriptional activity of AP-2 proteins, yet functions as an equally strong activator as other members. In an effort to better understand the transcriptional program regulated by transcription factor AP-2delta, we used cDNA microarray to evaluate the relative expression of human genes by AP-2delta in AD293 cells.

3. MATERIALS AND METHODS

3.1. Plasmid constructs

To construct pcDNA4C-AP-2delta, oliginucleotide primers 5'-GGGGTACCATGTCAACTACCTTTCCGGGAC-3' and 5'-CCCTCGAGGGTCTGTCTTTTCTGTTTTGCCCTC-3' were used to amplify the AP-2delta open reading frame (ORF) by PCR. The PCR fragment was the subcloned into pcDNA4C vector with kpnI and XhoI restriction enzymes. An empty vector, pcDNA4C, was used as a negative control for tranfection assays.

3.2. Cell culture and transient transfection

AD293 cells obtained from Shanghai Institute of Cell Biology and Biochemistry (Shanghai, China), were cultured in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum. The cells were split on 60-mm dishes at 1X106/dish. After 24 h, the cells were transfected using lipofectamine 2000 (Invitrogen) according to the manufacturer's protocol. Each 60-mm dish was transfected with 10μg of pcDNA4C-AP-2delta. An empty vector, pcDNA4C, was used as a negative control for tranfection assays.

3.3. RNA isolation

Total cellular RNA was isolated using Trizol (Biodev) according to the manufacturer's protocol. One μg of total RNA from cells was used to generate first strand cDNA after an initial annealing reaction to 0.1 μg of random hexamers at 70 °C for 10 min. Following equilibration to ambient temperatures, a buffer containing 50 mM Tris-HCl, pH 8.3, 75 mM KCl, 3 mM $MgCl_2$, 10 mM dithiothreitol, 500 μM of each of four dNTPs, and 200 units/μg of Superscript reverse transcriptase was added, and the mix was incubated at 37 °C for 1 h. The reaction was terminated by placing the tubes on ice, and the cDNA was recovered by ethanol precipitation. The pellet was washed with 70% ethanol and resuspended in 100 μl of 5 mM Tris, 0.5 mM EDTA mix.

3.4. Construction of microarray and probe preparations

The construction of the microarray used in this study (14400 chip) was carried out following Brown's method (26). The 14400 microarray consisted of 14,400 sequences including full-length and partial cDNAs representing known, novel, and control genes provided by United Gene Holdings. All the sequences were verified. The known genes were selected from the NCBI Unigene set and cloned into a plasmid vector. The novel genes were obtained through systematic full-length cloning efforts carried out at United Gene Holding. The control spots of non-human origin in the 14400 chip included the rice U2 RNA gene (8 spots), the hepatitis c virus (HCV) coat protein gene (8 spots), and spotting solution alone without DNA (32 spots). The cDNA inserts were amplified by use of the polymerase chain reaction (PCR) using universal primers to plasmid vector sequences and were then purified (27). All PCR products were examined by agarose gel electrophoresis to ensure the quality and the identity of the amplified clones as expected. Then the amplified PCR products were dissolved in a buffer containing 3· SSC solution. The solution with amplified PCR products were spotted onto silylated slides (CEL Associates, Houston, TX., USA) using a Cartesian PixSys 7500 motion control robot (Cartesian Technologies, Irvine, CA., USA) fitted with ChipMaker Micro-Spotting Technology (TeleChem International, Sunnyvale, CA., USA). The glass slides were then hydrated for 2 hrs in 70% humidity, dried for 0.5 hrs at room temperature, and UV crosslinked (65 mj/cm). They were further processed at room temperature by soaking in0.2% sodium dodecyl sulfate (SDS) for 10 min, distilled H_2O for 10 min, and 0.2% sodium borohydride (NaBH4) for 10 min. The slides were dried again and ready for use. The fluorescent cDNA probes were prepared through reverse transcription of the isolated mRNAs and then purified according to the methods of Schena et al (28, 29). The RNA samples from empty pcDNA4C transfected AD293 cells were labeled with Cy3-dUTP and those from

Table 1. Primer pairs used for real-time RT-PCR

Primer name	Forward primer (5'-3')	Reverse primer (5'-3')
HMOX1	CAGGAGCTGCTGACCCATGA	AGCAACTGTCGCCACCAGAA
ITSN1	TTTGCACAGTGACCTTGTAGCC	AAACCCCAAACAAAACACCAAC
LDOC1L	AGGTAGGCACAAGGTGGGTCTC	ATCTGCGCTGTGCCAACTG
RPL29	AACCACACCACACACAACCA	AACCTCCTTGGGCTTTACGA
hMIS12	CCAGTGACTGTTCAAACCAACCATA	CATACCTCAGCAAAGTGGACCAA
G3PDH	GCACCGTCAAGGCTGAGAAC	ATGGTGGTGAAGACGCCAGT
AP-2delta	TGGAATGCACCCAGATCAAAGA	TCCACCTTGGCCATTGAGAAC

pcDNA4C-AP-2delta transfected AD293 cells with Cy5-dUTP.

3.5. Hybridization

The probe was dissolved in 20 µl of Hybridization Solution (5X SSC (0.75M NaCl and 0.075M sodium citrate), 0.4% SDS, 50% formamide). Microarray was pre-hybridized with a hybridization solution containing 0.5 mg/ml denatured salmon sperm DNA at 42ºC for 6 hrs. Fluorescent probe mixtures were denatured at 95ºC for 5 minutes, and then applied onto the pre-hybridized chip under a cover glass. Chips were hybridized at 42ºC for 15-17 hours. Next, the hybridized chips were each washed at 60°C for 10 min in solutions of 2X SSC and 0.2% SDS, 0.1X SSC and 0.2% SDS, and 0.1X SSC, then dried at room temperature.

3.6. Detection and Analysis

The chips were scanned with a ScanArray 4000 (GSI Lumonics, Bellerica, MA) at two wavelengths, 635nm and 532 nm, to detect emission from both Cy5 and Cy3 respectively. The acquired images were analyzed using GenePix Pro 3.0 software. The intensities of each spot at the two wavelengths represent the quantity of Cy3-dUTP and Cy5-dUTP. Ratios of Cy5 to Cy3 were computed using the GenePix Pro 3.0 median of ratio method. Overall intensities were normalized using the corresponding GenePix default normalization factor. All spots flagged "Bad" or "Not Found" by GenePix software was removed from the final data. Only genes with raw intensity values for both Cy3 and Cy5 of >200 counts were chosen for differential analysis. Genes were identified as differentially expressed if the ratio was >2.0 or <0.5.

3.7. Real Time RT-PCR

To confirm some interesting genes regulated by AP-2delta, real-Time PCR was performed by using pcDNA4C-AP-2delta and empty pcDNA4C vector transient transfection AD293 cells preparations isolated in independent experiments according to the protocol described by TaKaRa RT Reagents (TaKaRa Biotechnology, Dalian, China). Briefly, AD293 cells were transfected with pcDNA4C-AP-2delta or empty pcDNA4C vector as described in cell culture and transient transfection, total RNA samples were then extracted and reverse-transcribed. The resulting cDNA was used in subsequent real-time PCR reaction after five times dilution. Real-time PCR was carried out using an ABI 7900HT sequence Detection System in 384-well reaction plates using ABSOLUTE™ QPCR SYBR GREEN LOW ROX MIXES (ABgene UK). All primers were designed using Prime Primer 5 software (PREMIER Biosoft International) (Table 1). Samples were analyzed in triplicate; the threshold cycle (Ct) method, as described in the manufacturer's protocol, was used to generate relative expression values. Results were expressed relative to G3PDH control.

4. RESULTS

4.1. Identification of genes differentially regulated by AP-2delta

We used cDNA microarray analysis to identify genes that were differentially regulated by exogenous expression of AP-2delta in AD293 cells. We had proved that AP-2delta expressed in AD293 cells. Since the regulation of genes by transcriptional factors involves several co-operators, we used AD293 cells which could express AP-2delta in case that there were proteins co-operated with it in these cells. Of the 14400 cDNAs present on the array, we found that approximately 29 genes had become up-regulateds and 39 genes with a down-regulated expression pattern were identified (Table 2). The regulated genes were related functionally to transcription factors, kinases, and signal transduction molecules, as well as regulating cell growth, differentiation, and cell adhesion.

4.2. Prediction of AP-2delta binding sites

In our study, we used the computational approach to the identification of promoters and transcription factor binding sites of AP-2delta target genes. It is reported that four consensus AP-2 binding sites have been identified: $GCCN_3GGC$, $GCCN_4GGC$, $GCCN_3GGG$ and $GCCN_4GGG$. We have analyzed all the promoters of genes screened by microararay according the four identified AP-2 binding sites to search the potential AP-2delta binding motifs in the 2000bp upstream promoter of these genes, respectively (Table 3). Most of the screened genes have more than one AP-2 binding sites. We also found some of the genes do not contain the consensus AP-2 binding motif. However, these genes may still be regulated by AP-2delta in two possible ways. One is that these genes may be regulated by AP-2delta in an indirect way. The other is that the AP-2delta consensus sequences exit in other regulate region of these genes beyond the 2000bp promoters or there are other AP-2 binding sites which have not been identified yet.

4.3. Confirmation of gene changes by real-time RT-PCR

Five regulated genes involved in several pathways were selected and subjected to real-time PCR analysis to confirm our microarray results. AP-2delta expression was used as a positive control for real-time PCR. As shown in Figure 1, the expression of HMOX1, ITSN1, LDOC1L and RPL29 was up-regulated and hMIS12 was down-regulated after AP-2delta transfection in AD293 cells, which was consistent with the microarray results. The difference of expression levels between real time PCR analysis and microarray signal intensity analysis might come from different data normalization method (G3PDH control vs global normalization).

Table 2. Identification of genes differentially regulated by exogenous expression of AP-2delta

Gene Name	Definition	Ratio (Cy5/Cy3)
Signal transduction		
GPR124	G protein-coupled receptor	2.227
RGS20	regulator of G-protein signalling 20	0.405
TSSK-3	testis-specific serine/threonine kinase 3	2.487
ITSN1	intersectin 1 (SH3 domain protein)	2.674
CAPNS1	calpain, small subunit 1	3.568
RSPO3	R-spondin 3 homolog	0.321
OR13A1	olfactory receptor, family 13, subfamily A, member 1	0.411
XPR1	xenotropic and polytropic retrovirus receptor	2.563
ACE2	angiotensin I converting enzyme (peptidyl-dipeptidase A)	0.407
SORCS3	sortilin-related VPS10 domain containing receptor 3	0.390
PDGFC	platelet derived growth factor C	2.307
DUSP10	dual specificity phosphatase 10	0.406
SSTR1	somatostatin receptor 1	3.064
Transcription-related		
PRDM10	PR domain containing 10	0.439
BAT2D1	BAT2 domain containing 1	0.475
NFIB	nuclear factor I/B	2.381
AFF4	AF4/FMR2 family, member 4	2.972
ZNF395	zinc finger protein 395	3.768
ZNF250	zinc finger protein 250	0.482
ZNF741	Kruppel-like factor 8	0.377
LDOC1L	leucine zipper, down-regulated in cancer 1-like	2.294
SUHW2	suppressor of hairy wing homolog 2	0.454
MTPN	myotrophin	2.266
MMS19	MMS19-like protein	2.675
NR1D2	nuclear receptor subfamily 1, group D, member 2	0.466
Metabolism		
ATP13A4	ATPase activity	0.331
MVK	mevalonate kinase (mevalonic aciduria	2.196
PDP2	pyruvate dehydrogenase phosphatase isoenzyme 2	2.628
ATP2C1	ATPase, Ca++ transporting, type 2C, member 1 transcript variant 1	0.365
AGPAT4	1-acylglycerol-3-phosphate O-acyltransferase 4	0.490
ALDH1A2	aldehyde dehydrogenase 1 family, member A2	2.786
PFKFB1	6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 1	2.188
HMOX1	heme oxygenase (decyclizing) 1	2.804
PCYT1B	phosphate cytidylyltransferase 1, choline, beta	0.391
CDS1	CDP-diacylglycerol synthase (phosphatidate cytidylyltransferase) 1	3.335
Response to stimulus		
IFI44	interferon-induced protein 44	0.386
IFIT1	interferon-induced protein 1	0.388
IFITM2	interferon induced transmembrane protein 2	0.261
IFITM1	interferon induced transmembrane protein 1	0.384
STATIP1	signal transducer and activator of transcription 3 interacting protein 1	0.470
GDAP1	ganglioside-induced differentiation-associated protein 1	0.428
TRIM22	tripartite motif-containing 22	0.320
OAS3	2'-5'-oligoadenylate synthetase 3	0.360
Cell movement and Metastasis		
FMN2	formin 2	0.413
MSN	moesin	0.413
CNTN5	contactin 5	0.210
Translation and ER-related		
PS1D	putative S1 RNA binding domain protein	0.358
RPL29	ribosomal protein L29	2.498
EIF4B	eukaryotic translation initiation factor 4B	5.434
VPS29	vacuolar protein sorting 29 (yeast) transcript variant 2	2.747
Mitosis		
RCC2	regulator of chromosome condensation 2	0.422
hMIS12	MIS12 homolog (yeast)	2.062
FANCF	Fanconi anemia, complementation group F	2.293
RIPX	rap2 interacting protein x	0.362
Cep290	centrosome protein cep290	0.462
Cell adhesion		
PCDHA	protocadherin alpha	2.220
CELSR2	cadherin, EGF LAG seven-pass G-type receptor 2	0.449
Unknown		
CR619847	cDNA clone CS0DI058YC13	0.411
AK098354	cDNA FLJ25488 fis, clone CBR00232	0.403
NM_152399	hypothetical protein FLJ30834	0.438
NM_005128	chromosome 21 open reading frame 5 (C21orf5)	0.391
AL137291	cDNA DKFZp434K1316	0.452
NM_024675	hypothetical protein FLJ21816	2.455
BC022859	cDNA clone MGC:22822 IMAGE:3826071	2.627
BC016374	DNA clone MGC:27259 IMAGE:4618034	2.202
XM_371267	hypothetical protein LOC164045	0.404
NM_015544	DKFZP564K1964 protein	0.436

Ratio >2.0 was up-regulation, ratio <0.5 was down-regulation. Gene expression data for 29 genes that were found to be up-regulated and 39 were found to be up-regulated by AP-2delta expression are shown

Table 3. Summary of the putative AP-2delta binding sites

Gene Name	Promoter			
	GCCN$_3$GGC 5'-3'	GCCN4GGC 5'-3'	GCCN3GGG 5'-3'	GCCN4GGG 5'-3'
Signal transduction				
GPR124	2	3	4	2
RGS20	0	1	0	2
TSSK-3	4	5	9	7
ITSN1	0	0	2	1
CAPNS1	0	1	2	4
RSPO3	2	0	2	3
OR13A1	0	0	0	1
XPR1	0	0	0	1
ACE2	0	0	0	0
SORCS3	2	1	2	5
PDGFC	6	8	8	4
DUSP10	3	1	3	6
SSTR1	2	0	0	0
Transcription-related				
PRDM10	4	2	3	3
BAT2D1	0	0	0	0
NFIB	9	6	7	4
AFF4	0	2	2	2
ZNF395	0	0	1	2
ZNF250	4	3	1	5
ZNF741	3	1	2	1
LDOC1L	2	4	3	3
SUHW2	1	0	0	1
MTPN	0	0	1	0
MMS19	10	10	4	8
NR1D2	2	2	0	1
Metabolism				
ATP13A4	0	0	0	0
MVK	1	1	1	5
PDP2	1	2	3	1
ATP2C1	1	0	2	1
AGPAT4	3	0	1	1
ALDH1A2	2	6	6	2
PFKFB1	2	1	1	1
HMOX1	2	1	1	1
PCYT1B	1	1	4	5
CDS1	1	1	1	0
Response to stimulus				
IFI44	0	0	1	1
IFIT1	3	4	4	6
IFITM2	1	2	4	4
IFITM1	0	0	0	0
STATIP1	5	2	4	4
GDAP1	2	0	0	3
TRIM22	0	1	0	0
OAS3	0	0	0	0
Cell movement and Metastasis				
FMN2	0	0	0	1
MSN	1	0	1	4
CNTN5	1	1	1	1
Translation and ER-related				
PSID	3	1	0	1
RPL29	2	1	0	1
EIF4B	1	0	0	1
VPS29	0	1	0	1
Mitosis				
RCC2	0	0	0	1
hMIS12	1	3	3	2
FANCF	1	0	0	1
RIPX	2	2	4	1
Cep290	1	2	1	0
Cell adhesion				
PCDHA	4	1	1	1
CELSR2	8	4	6	7
Unknown				
CR619847	1	1	2	0
AK098354	2	1	2	5
NM_152399	2	0	0	1
NM_005128	0	0	0	2
AL137291	2	2	4	4
NM_024675	0	1	2	2
BC022859	0	0	1	0
BC016374	0	1	1	0
XM_371267	0	0	0	1
NM_015544	3	3	4	4

AP-2delta binding sites: GCCN$_3$GGC5'-3', GCCN$_4$GGC5'-3' GCCN$_3$GGG5'-3', GCCN$_4$GGG5'-3', in the 2000bp upstream promoter of genes screened from the microassay

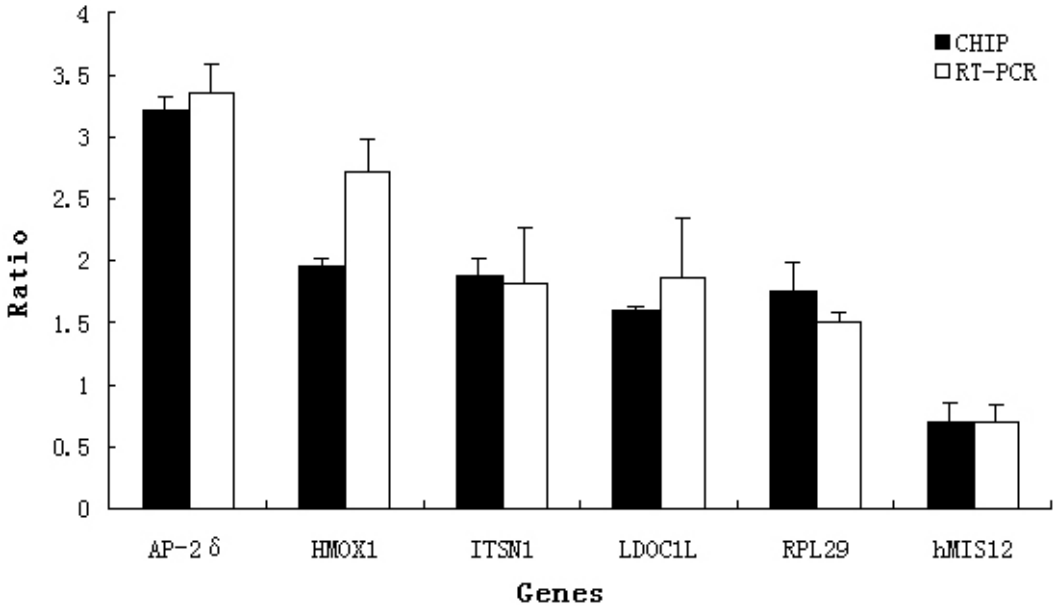

Figure 1.. Effects of exogenous AP-2delta on expression of HMOX1, ITSN1, LDOC1L, RPL29 and hMIS12 in AD293 cells. AD193 cells were transfected with pcDNA4C-AP-2delta and subjected to cDNA microarray and real time PCR as described in "Materials and Methods". Data are expressed as average ratio (n=3) relative to control. An empty vector, pcDNA4C, was used as a negative control for tranfection assays.

5. DISCUSSION

Transcription factor AP-2 has been shown to activate different signal transduction pathways, ultimately leading to gene transcription, but thus far relatively few genes regulated by AP-2delta have been reported. In the study presented here we used cDNA microarray analysis and real time RT-PCR to identify target genes that were regulated by overexpression of AP-2delta in AD293 cells. Of the 14400 genes present on the array, 68 genes had become regulated were identified.

Initial analysis indicated that a number of genes were regulated by exogenous expression of AP-2delta. In the genes encoding transcription factors, we found a series of zinc finger gene, ZNF250, ZNF741 (Kruppel-like factor 8) and ZNF395. ZNF250 and ZNF741 were down-regulated and ZNF395 was up-regulated. While some member of zinc finger family has been shown to activate transcription, most members of the family have been implicated in the repression of gene expression (30). In some instances, it appears that repressing and activating ZNF family proteins co-exist within the same cell and it is possible that their competing activities may serve to balance transcriptional output (31). The members of zinc finger proteins are broadly expressed in many different tissues throughout development and despite considerable work; the precise biological roles of these proteins have been difficult to define. It was supposed that some members of the zinc finger family were involved in maintenance of the nucleolus, cell differentiation, cell proliferation, apoptosis, and neoplastic transformation (30).

In the genes encoding signal transduction molecules, we found an interesting gene, TSSk-3, which was found up-regulation by the exogenous expression of AP-2delta. TSSK-3 is a member of TSSk family. The TSSk family members have been found expressed postmeiotically in germ cell (32). The TSSK(s) expression patterns suggest that TSSk might play a role in mammalian germ cell differentiation and/or sperm function, although the precise pathways are unclear (33). Although human AP-2delta is a widely expressed gene, it has high expression level in adult prostate and testis tissues (34). One mechanism can be postulated to explain the observed induction of TSSK-3 gene in our exogenous Ap-2delta-expressing AD293 cells. The regulation of gene expression involves a complex regulatory network. AP-2delta may be one of the important components of this network. AP-2delta may regulate mammalian germ cell differentiation and sperm function by transactivated the TSSK-3.

Genes down-regulated by Ap-2delta include members with any of a wide variety of functions. We focused our attention on a series of interferon-induced proteins negatively regulated by AP-2delta in AD293 cells. Altogether, interferon(IFN)-responsive genes comprised 4 genes and included typical members such as IFITM1, IFITM2, IFIT1 and IFI44, as well as the transcription factor STATIP1, which activate IFN-responsive genes (35). IFN activate multiple signaling pathways, regulate gene transcription, and are involved in viral protection, inhibition of cell growth, and modulation of differentiation (36). A possible explanation of the observed down-regulation of IFN-responsive genes in exogenous Ap-2delta-expressing AD293 cells would be an AP-2delta-repressed production of various IFNs to reduce expression of IFN-responsive genes. Yet another possibility is that Ap-2delta would be a competitor of IFN to IFN-responsive genes. Exogenous expression of AP-2delta inhibits the IFN

induction by combining to the promoters of IFN-responsive genes.

Apart from the regulating genes mentioned above, AP-2delta also was found to regulate the expression level of a range of other genes (Table. 2). These genes showed a similar expression pattern but did not belong to any specific transcriptional activation program. When attempting to obtain a global overview of the transcriptional program activated by AP-2delta, it was clear that a number of genes of different classes or with common functional properties were affected. Hence, genes encoding transcription factors (e.g., NFIB, PRDM10, BAT2D1, AFF4 and LDOC1L), translation and ER-related gene (e.g., PS1D, RPL29, EIF4B and VPS29), and signal transduction molecules (e.g., GPR124, RGS20, PDGFC, ITSN1 and STATIP1) were identified. Based on common functional properties, genes involved in the regulation of cell metabolism (e.g., ATP13A4, MVK, PDP2, ATP2C1 and AGPAT4), cell movement and adhesion (e.g., PCDHA, CELSR2, FMN2, MSN and CNTN5) and mitosis (e.g., RCC2, hMIS12, FANCF, RIPX and Cep290) could be discriminated, features all known to be affected by AP-2delta.

In this study, we used microarray to screen Ap-2delta regulating genes. It is clear, as well as from previous reports, that expression of AP-2 results in activation/repression of a multitude of signaling pathways, several of them subject to complex feedback regulatory signaling. Further dissection and experimental interference with these pathways likely will shed important insights into AP-2delta-mediated genes regulation.

6. ACKNOWLEDGEMENT

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Abbreviations: AP-2delta: activate protein, G3PDH: glyceraldehyde-3-phosphate dehydrogenase, HIV: human immunodeficiency virus, HTLV-I: human t-cell leukemia virus-I, SV40: sarcoma virus, MUC18: mucabo virus18, EBP: estradiol-binding protein

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