

Supplemental Methods

cDNA microarray construction, Array hybridization, Data Analysis

Nylon filters were spotted with individual cDNA clones as previously described (Chiang et al., 2001). Experiments 1 and 2 used a 30,000 clone array (30K) while experiment three was analyzed on a “complete genome” (CG) array containing 56,844 clones; 36,999 I.M.A.G.E. clones from Research Genetics, 19,414 Incyte clones and 36 Millennium clones. Controls included genomic DNA from human, monkey, rat, mouse, yeast, and *B. Subtilis*. Competitor (cot) DNA was prepared from human and mouse genomic DNA, pBlueScript vector, GAPDH, tubulin, as well as human and mouse ribosomal RNA.

DNA templates prepared by PCR amplification of individual clones were arrayed onto nylon filters at a density of 81/cm² (30K) and 121/cm² (CG) on three (30K) or five (CG) 3 x 5 cm filters. After drying, the arrayed DNA was denatured in 0.4 M NaOH, neutralized and dried to completion.

Total RNA, isolated using the Qiagen RNeasy kit, was reverse transcribed and labeled as previously described (Chiang et al., 2001). Briefly, RNA (1 μg) and oligo-dT₃₀ (1μg) were incubated with SuperScript II Reverse transcriptase (Life Technology) in the presence of dNTP and ³³P-dCTP. Radiolabeled cDNA was purified over Chroma Spin™ +TE-30 columns (Clontech) and annealed at 65°C for 1 h in the presence of poly (dA) >200 (Amersham Pharmacia) and 10 μg rat Cot 10 DNA. The annealed cDNA at 2 x 10⁶ mixture was hybridized at 2 x 10⁶ cpm/ml to array filters at 65°C overnight in sodium phosphate buffer containing 10% formamide. Filters were washed, dried and exposed to phosphoimager screens for 60h. Hybridization signals were captured by phosphoimaging (Fuji) and digitized to give an intensity value using ARRAY VISION software (Imaging Research). All array hybridizations were

performed in duplicates. Array intensities were normalized to the median array for each experiment independently.

The detection threshold for experiment one was found by graphing the average coefficient of variation (CV) versus mean gene expression and was defined as the intensity below which the average CV was greater than 0.4. Data were analyzed using GeneSpring software (Silicon Genetics). Scatter plots of replicate arrays performed after normalization showed highly reproducible data above absolute expression intensities of 0.8. After this normalization, based upon scatter plots of replicate arrays

Clones whose expression did not change more than 1.5 fold (for example 31,763 clones for CG array) or whose hybridization intensity did not reach a value of 0.8 in at least one sample were filtered). (9033 clones on the CG array) leaving 16,548 clones out of 56,844 clones for further analysis. Expression data from these cDNA probes were analyzed by K-means clustering as described in the results.

Real Time PCR

Total RNA was isolated using the RNeasy method (Qiagen) and samples were treated with DNaseI. For reverse transcription (RT), 10 μ g of total RNA was annealed to 3 μ l of oligo dT primer at 70° C for 10 min in 33 μ l. As a no amplification control (NAC), 1 μ g of total RNA was annealed to 1 μ l oligo dT primer in 11 μ l. 10X PCR buffer (Applied Biosystems), MgCl₂, dNTP, DTT was added and incubated at 42° C for 5 min. 5 μ l of Superscript II Reverse transcriptase (Life Technologies) was added to the RT sample. Both the samples were incubated for 50 min at 42° C and for 15 min at 70°C. The samples were then treated with 3 μ l (RT) and 1 μ l (NAC) of RNase H for 20 min at 37°C. After adding 140 μ l of DEPC water samples were stored at -70° C. The expression of 3 control genes was determined in samples (diluted 1:5) and controls (5 μ l

NAC or 5 μ l H₂O) in triplicate and β 2Microglobulin was selected as the most stable control. cDNA samples where the NAC controls had a Ct>38-40 and the Ct for β 2Microglobulin uniform were aliquoted to master 96-well plates along with two NTC per gene. cDNAs were then amplified using the primer sets indicated below. The cycle threshold for each gene in each samples was determined by measuring the liberated fluorescent probe in a ABI7700 Real-Time PCR machine. The control probe and the gene of interest probe contain different fluorescent probes at their 5'end. The data were analyzed using the software.

The sequence of the primers and probe are:

Gene	5' Primer	3' Primer	Probe
β 2Microglobulin	gtctcgctccgtggcctta	gagtacgctggatagcctcca	tgctcgcgctactctctctttctggc
IGFBP1	atctgatggccccttctgaa	agccttcgagccatcatagga	tcattccatcctttgggacgccatc
DEPP	ccaggaaaagcagccaagc	atctgtctaagtggaccccagaag	caaggaggaaactggcccctctgctg
Cyclin G2	agaagttggtttggaatcgttca	ttggcagctcaggaactatagct	cacagcccagaacctccacaacagt
SLC5A3	tcacttggctcaaaggatcca	tccctaagagctatggctacatga	tgtatTTTggcacaagagcctggcc
IGFBP3	tgctgggtgtggataagtatgg	agtgcacgtcctccttccc	cagcctgtgggaggctacaccacca

Supplemental Figure Legends

Supplemental Figure 1. Additional validation of Class I FKHR targets.

A and B) Q-PCR was used to measure mRNA levels produced 24 hours after infection with the indicated adenoviruses. C) Northern blot analysis of DEPP mRNA from 786-O cells infected with the indicated adenovirus and then treated with or without serum starvation.

Supplemental Figure 2. Expression pattern of other putative FKHR target genes

The level of mRNA expression, at the indicated time points after infection with the indicated adenovirus, detected on the 60K array in experiment three is shown for the indicated genes. The x-axis represents mRNA level in arbitrary units. The y-axis is time in hours.

Supplemental Table I: Transcripts found in Class I

<u>Gene Name</u>	<u>GB Acc.#</u>	<u>Unigene ID</u>	<u>FKHR; AAA</u>		<u>FKHR; HRAAA</u>	
			<u>Exp1</u>	<u>Exp2</u>	<u>Exp3</u>	<u>Exp3</u>
Semaphorin E	AB000220	Hs.171921	4.8	3.5	17.7	1.3
Ceruloplasmin	M13669	Hs.296634	9.2	13.2	16.4	1.4
IGFBP1	M59316	Hs.102122	19.8	21	15.75	0.78
HT2A	BC003154	Hs.236218			14.4	0.66
STK17A	AB011420	Hs.9075			14.3	0.6
Lumican	U21128	Hs.79914	6.9	7.8	14.3	0.6
DEPP	AB022718	Hs.93675	16.7	23	11.1	2.1
ALDH8A1	AF303134	Hs.18443	4	2.8	7.86	0.6
Est	AF038169	Hs.145567	5	6.6	7.3	2.5
C-type lectin	X96719	Hs.85201	5.1	6.9	7.2	1
DAF/CD55	M31516	Hs.1369	3.2	2.5	6.9	3.4
Vigilin	M64098	Hs.177516			6.4	0.6
ISOT-3	U75362	Hs.85482			6.4	1.9
MAPKKK6	AF100318	Hs.194694	4.9	4.3	6.3	1.3
DHODH	B1253747	Hs.94925			6	1
TRIP8	L40411	Hs.6685			5.9	2.6
FBXO30	AF248640	Hs.95667			5.7	1.1
RTN3	AH007509	Hs.252831			5.6	2
BTG1	X61123	Hs.77054	3.7	3.6	5.3	3.3
FOX P1	AF146696	Hs.274344			5.3	1.2
KIAA1373	AB037794	Hs.16229			5.2	1.2
HG38	AF062006	Hs.285529			5.2	0.7
C8orf1/hT41	AF061326	Hs.40539	2.7	3	5.1	0.7
MyosinVI	AB002387	Hs.22564			4.9	0.8
ATP6A1	L09235	Hs.281866			4.9	1.8
Gab1	U43855	Hs.239706			4.7	1.6
Grancalcin	M81637	Hs.79381			4.4	1.1
Eps8	U12535	Hs.2132	3	2.7	4.1	0.8
GUCY1A3	X66534	Hs.75295			3.7	1
TIP120	AF157326	Hs.184786			3.6	0.9
SRY	X70683	Hs.83484			3.3	1.1
PAR4	U63809	Hs.176090			3.1	1.1
SRE-ZBP	Z11773	Hs.237786			2.9	1.1

Supplemental Table II: Transcripts found in Class IIa

<u>Gene Name</u>	<u>GB Acc.#</u>	<u>Unigene ID</u>	FKHR; AAA		FKHR; HRAAA	
			<u>Exp1</u>	<u>Exp2</u>	<u>Exp3</u>	<u>Exp3</u>
Dyrk2	Y13493	Hs.173135			16.8	11.3
DDB1	U32986	Hs.108327	5.7	6.2	10.6	7
CNR1	X54937	Hs.75110			9.8	11.2
Mn SOD2	X07834	Hs.318885	8.8	5.6	9.3	3.5
Cbl-b	U26712	Hs.3144	10.3	2.8	7.1	2.8
ITF2 (TCF4)	M74719	Hs.326198			6.8	2.8
TEM7R	AF378757	Hs.33033			6.3	6.6
Kelch like 4	AB051474	Hs.49075			6.1	5.7
FZD4	AB032417	Hs.19545			5.8	3.5
Est	AK026237	Hs.4947	4.4	7.7	4.2	5.6
IGFBP7	S75725	Hs.119206			4	5.3
SMARCB1	U04847	Hs.159971			3.9	3.9
PRO2032	AF116683	Hs.283609			3.9	2.2
KIAA1696	AF208848	Hs.106826			3.6	2.9
CCNDBP1	AF082569	Hs.36794			3.3	2.8
PA26	AF033122	Hs.14125			3.3	2.7
BCL6	U00115	Hs.155024			3.1	3.4
EFEMP1	U03877	Hs.76224			3.1	3.1
TGFBR3	L07594	Hs.342874			2.1	1.9
TPST1	AF038009	Hs.17279			2.1	2.4

Supplemental Table III: Transcripts found in Class IIb

Gene Name	GB Acc.#	Unigene ID	FKHR; AAA		FKHR; HRAAA	
			Exp1	Exp2	Exp3	Exp3
SYT13	AB037848	Hs.12365			2.6	53
GCLC	M90656	Hs.151393			5.6	16.9
SLC5A3	L38500	Hs.324787	4.9	2.6	1.9	16.2
ARG2	U75667	Hs.172851			1.7	12.6
MMP7	AI675414	Hs.2256	4.9	4.2	5.8	12.4
BCMP1	AL136550	Hs.8769			1.4	10.3
Est	AB037765	Hs.30652			3	10
ID1	X77956	Hs.75424			1.5	8.4
STAT2	U18671	Hs.72988			4.8	7.1
MGC2541	BC009906	Hs.173103			1.3	7
MxiI	AH006633	Hs.118630	2.1	4.4	3.7	7
RNF24	AL079313	Hs.30524			1.3	7
SRPX	U78093	Hs.15154			1.5	7
Est	AK000790	Hs.246885			2.3	6.4
RPGR	U57629	Hs.153614			2.3	6
MSL3L1	AK025642	Hs.88764			3.9	5.8
TPD52L1	U44427	Hs.16611			0.6	5.6
FKBP12	BC002614	Hs.77643			1.5	5.4
NCOA1	U40396	Hs.74002			2.7	5.1
FKBP51	AL122066	Hs.7557			1.2	5
TUBB1	AL109840	Hs.303023			1.6	4.6
TSPAN-5	AF053455	Hs.20709			1.6	4.5
MID1/opitz/BBB	Y13667	Hs.27695			1.1	4.4
LOC83693	AF237684	Hs.171937			2.6	4.1
MRPS6	BC010076	Hs.6945			1.1	4.1
ATRN	AF034957	Hs.194019			1.1	4.1
IRLB	X63417	Hs.135202			2.2	4
RNASE3L	AF116910	Hs.49163			2.2	3.8
3-PAP	AK000483	Hs.93872			1.1	3.7
ATP10D	AB040920	Hs.173540			2	3.6
PTPRC	A00062	Hs.170121			0.8	3.4
DNALC8	AF083190	Hs.74711			2.2	3.4
GLUL	Y00387	Hs.170171			1.7	3.4
OSBPL3	AY008372	Hs.197955			1.7	3.3
SLC11A2	AB004857	Hs.57435			1.1	3.2
CGL-44	AF042284	Hs.8185			0.6	3.2
TACC1	AF049910	Hs.173159			1.2	3
OXR1	AK000987	Hs.169111			1.1	2.8
TP53INP1	AB017926	Hs.75497			1.8	2.

Supplemental Table IV: Transcripts found in Class III

Gene Name	GB Acc.#	Unigene ID	FKHR; AAA		FKHR; HRAAA	
			Exp1	Exp2	Exp3	Exp3
Epiregulin	D30783	Hs.115263	0.28	0.16	0.1	0.05
FN14	AB035480	Hs.10086			0.1	0.06
TNFSF7	S69339	Hs.99899			0.14	0.14
FSTL3	U76702	Hs.25348			0.2	0.14
FHOD1	AF113615	Hs.95231			0.23	0.24
Est	AB028927	Hs.274453			0.24	0.18
SLC16A3	U81800	Hs.85838			0.24	0.22
PCNA	M15796	Hs.78996			0.24	0.22
IGFBP3	M35878	Hs.77326	0.17	0.39	0.24	0.13
Cyclin D2	D13639	Hs.75586	0.4	0.22	0.27	0.25
SLC21A12	AB031051	Hs.235782			0.28	0.48
KIAA0664	AB014564	Hs.22616			0.29	0.25
IFRD2	U73167	Hs.315177			0.3	0.27
PPP2R5A	L42373	Hs.155079			0.31	0.30
LRR	U32907	Hs.155545			0.34	0.28
Apo-CIV	U32576	Hs.110675			0.36	0.29
JTV-1	U24169	Hs.301613			0.36	0.44
STK12	AB011450	Hs.180655			0.39	0.33
OAZ1	D89870	Hs.125078			0.40	0.20
Mcm6	D84557	Hs.155462			0.41	0.26
FREQ	AL161981	Hs.301760			0.43	0.38
NCL	M60858	Hs.79110			0.43	0.38
SCAND1	AF204271	Hs.274411			0.44	0.33
Cyclin E2	AA830205	Hs.30464			0.45	0.39
DDX31	AF427339	Hs.69331			0.45	0.4
Cdk4	BC015669	Hs.95577			0.49	0.26
CDC45L	AF053074	Hs.114311			0.50	0.48
Cyclin D1	X59798	Hs.82932	0.2	0.25	0.57	0.65