Gene expression profiles and transcription factors involved in glucocorticoid signaling in Keratinocyte

Brian Lee, Constantinos Vouthounis, Olivera Stojadinovic, Marjana Tomic-Canic

Ronald O. Perelmen Department of Dermatology, New York University School of Medicine

Abstract

Glucocorticoid hormones exert profound effects to almost all organs and tissues in the body. GCs act through glucocorticoid receptors, which are versatile transcription factors. Using DNA microarray analysis, 173 genes were found to have at least 2 fold expression changes after 24h, 48h and 72 hr dexamethasone treatment on keratinocytes. Out of these 173 genes, 10 genes that were consistently upregulated or downregulated were chosen for further promoter analysis using web-based programs. Do genes that are regulated similarly by GCs have some commonality in their promoters? Here we present potential transcription factors and common framework of transcription factor binding sites (TFs) that may participate in GCs signaling.

Material and Methods

Keratinocyte were maintained in serum- free keratinocyte medium with epidermal growth factor and bovine pituitary extract (GIBCO) with or without Dexamethasone (Sigma). After each timepoint (24h, 48h, 72h) cells were trypsinized and stored in RNAlater(Ambion) and then total RNA was isolated using RNeasy (QIAGEN). 8µg of total RNA was reversed transcribed, amplified and labled according to the protocol. Labeled cRNA was hybridized to HGU95Av2 arrays (Affymetrix) and arrays were were washed and stained with anti-biotin streptavidin-phycoerythin labeled antibody using Affymetrix fluidics station and then scanned using the Agilent GeneArray Scanner system (Hewlett-Packard).

Microarray Suite 5.0 (Affymetrix) was used for data extraction and for further analysis, data mining tool 3.0 (Affmetrix) as well as GeneSpring[TM] software 5.1 (Silicon Genetics) was used for normalization, fold change calculations and clustering. To qualify as an up-regulated gene, a genes should meet the following criteria: (1) in each time point the signal in the DEX-treated sample should be greater than 2 fold of that in its untreated sample; (2) its absolute call should be P in all DEX-treated samples. With the same logic, a gene should meet the following criteria to be considered as down-regulated gene: (1) in each time point the signal in the untreated sample should be greater than 2-fold of that in the DEX-treated sample; (2) its absolute call should be P in all untreated samples.

Promoter analysis was done using Genomatix softwares (www.genomatix.de). Promoter sequences (500 upstream and 100 downstream of the transcription start site) of selected genes were extracted using Chip2Promoter (Genomatix). Using DiAlign (Genomatix) the promoter sequences were aligned to look for any coserved regions. To search for possible GRE binding sites in the sequences, two programs, findpatterns (GCG) and GEMS Launcher 3.5 (Genomatix) was used. Matinspector (Genomatix) was used to search for common transcription factor binding sites. To see if common framework of elements existed in a set of promoter sequences, Frame worker (Genomatix) was used.

Results

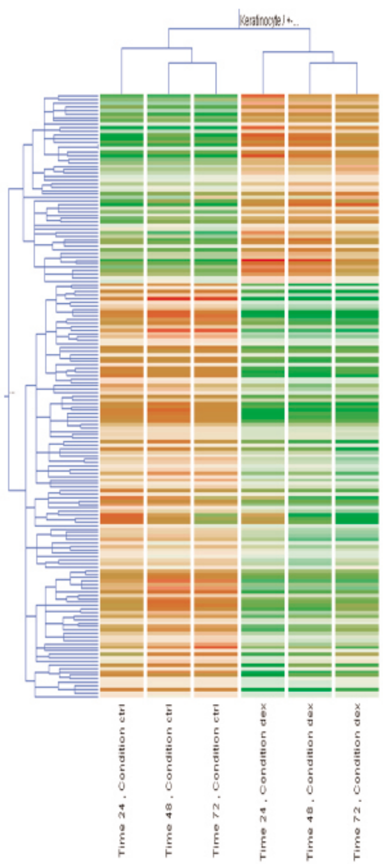

Figure 1. Gene expression of Keratinocyte with or without dexamethasone treatment. Genes correspond to rows and the time points of each experiment are the columns. Out of 12558 genes 173 genes that were consistently upregulated or downregulated in three different time points 2 folds or more were selected. Genes that share similar expression profiles as well as experiments that share similar gene profiles are grouped by a clustering algorithm.

Affymetrix Id	GENE NAME	Description	FUNCTION
31508_at	VDUP1, TXNIP	upregulated by 1,25-dihydroxyvitamin D-3	Unknown/Hypothetical
37701_at	RGS2	regulator of G-protein signalling 2, 24kD	G-regulated protein
39528_at	RRAD	Ras-related associated with diabetes	Ras pathway
36629_at	GILZ, DSIPI	glucocorticoid-induced leucine zipper	Transcription
40202_at	BTEB1	basic transcription element binding protein 1	Transcription factor
36203_at	ODC1	ornithine decarboxylase 1	Other metabolism
36543_at	F3	coagulation factor III (thromboplastin, tissue factor)	Proteolysis
37989_at	PTHLH	parathyroid hormone-like hormone	Secreted
33068_at	KBHF	cytokeratin type II	Cytoskeletal/keratin
34091_s_at	VIM	vimentin	Cytoskeletal

Table 1. Brief description of the genes that were selected for promoter analysis.

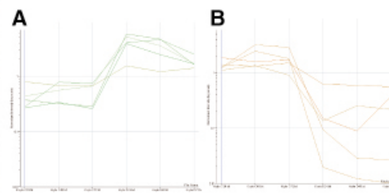

Figure 2. Normalized expression graphs showing expression levels of 5 genes that were consistently up-regulated (A) and 5 genes that were consistently down-regulated (B).

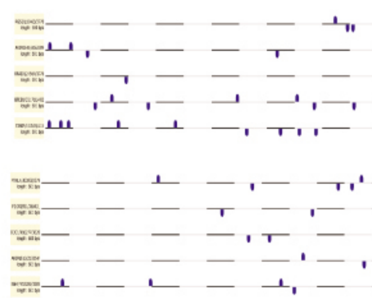

Figure 3. Possible GRE sites (AGAACA) were searched within the promoter sequences. The sequence "AGAACA" was searched on each promoter sequence allowing up to one mismatch. A perfect match was found in RGS2 and PTHLH. Possible active GRE site (homo-dimer) is present in the RGS2 promoter.

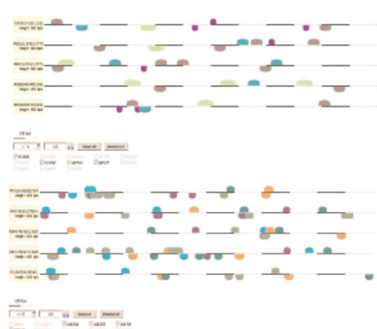

Figure 4. Possible common transcription factor binding sites revealed by the program GEMS Launcher 3.5 (Genomatix) on promoters with similar expression profiles based on microarray analysis. Only transcription factor binding sites that exist on all sequences are shown.

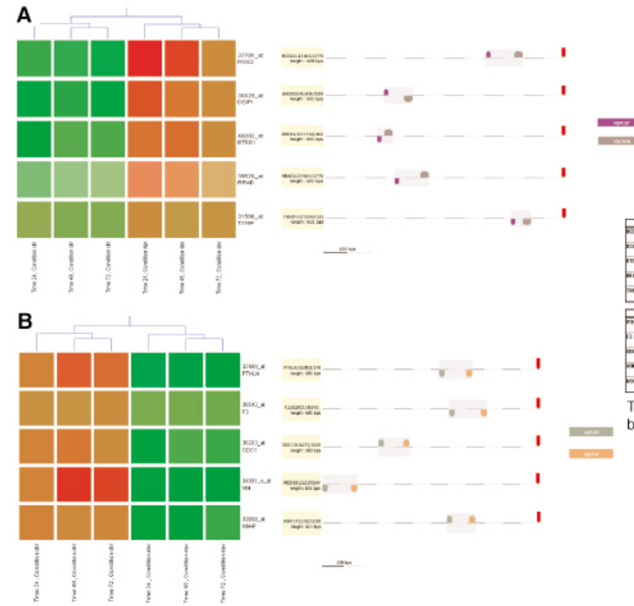

Figure 5. TFs that are within a 100bp distance in a consensus order is shown. **A** Up-regulated genes reveal two different transcription factors PCAT (Promoter CCAAT binding factors) and CREB (Camp-Responsive Element Binding proteins) as possible TFs that may participate in transcriptional regulation. **B** Down-regulated genes reveal two different transcription factors EGRF (EGR/nerve growth Factor Induced protein C & rel. fact.) and ETSF (Human and murine ETS1 Factors) as possible TFs that may participate in transcriptional regulation. **C** Genes that have different gene expression profiles were analyzed for common framework. No common framework was found. 5 random genomic sequence (600bp) was analyzed as well which did not yield any common framework (Data not shown).

GENES	PCAT	DISTANCE	CREB
RGS2 (500bps)	405 - 415 (410/0.92/+) [illegible]	76	478 - 496 (486/0.75/+) [illegible]
DSIPI (500bps)	146 - 158 (153/0.90/+) [illegible]	56	199 - 219 (209/0.75/+) [illegible]
BTEB1 (500bps)	131 - 141 (136/0.97/+) [illegible]	23	148 - 160 (155/0.92/+) [illegible]
RRAD (500bps)	175 - 185 (180/0.94/+) [illegible]	31	201 - 221 (211/0.75/+) [illegible]
TXNIP (500bps)	467 - 477 (472/0.90/+) [illegible]	35	497 - 517 (507/0.85/+) [illegible]

GENES	EGRF	DISTANCE	ETSF
PTHLH (500bps)	325 - 339 (332/0.89/+) [illegible]	76	408 - 416 (408/0.94/+) [illegible]
F3 (500bps)	353 - 367 (360/0.92/+) [illegible]	86	441 - 457 (449/0.95/+) [illegible]
ODC1 (500bps)	155 - 169 (162/0.77/+) [illegible]	70	224 - 240 (232/0.93/+) [illegible]
VIM (500bps)	1 - 15 (8/0.90/+) [illegible]	58	81 - 97 (89/0.92/+) [illegible]
KBHF (500bps)	345 - 359 (352/0.81/+) [illegible]	63	407 - 423 (415/0.94/+) [illegible]

Table 2. Detailed description of TF binding sites.

Conclusion

Our microarray analysis presents 173 genes that are regulated by dexamethasone in keratinocytes. Further analysis on promoters of genes that are similarly regulated, a common transcription factor framework has been found using web-based programs. Using a bioinformatics approach, we have analyzed possible transcription factors that may play a role in glucocorticoid signaling which provide some important hints for future GCs signaling mechanisms studies.

References

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