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Angiotensin II Regulation of Adrenocortical Gene Transcription

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Abstract

Angiotensin II (Ang II) is the key peptide hormone in the renin-angiotensin-aldosterone system (RAAS). Its ability to regulate levels of circulating aldosterone relies on actions on adrenal glomerulosa cells. Many of the Ang II effects on glomerulosa cells involve a precisely coordinated regulation of signaling cascades and gene expression. The development of genome-wide gene arrays has allowed the definition of transcriptome-wide effects of Ang II in adrenocortical cells. Analysis of the Ang II gene targets reveals broad effects on cellular gene expression, particularly the rapid induction of numerous transcription factors that may regulate long-term steroid metabolism and cell growth/proliferation. Herein we discuss the Ang II-induced genes in adrenocortical cells and review the progress in defining the role of these genes in zona glomerulosa function.

Keywords

angiotensin; microarray; aldosterone; signaling; transcription factors; adrenal

1. Introduction

The RAAS plays a very important role in regulating blood volume and systemic vascular resistance. Renin, primarily released by the kidneys, converts circulating angiotensinogen into angiotensin I, which is further converted to the potent octapeptide Ang II by angiotensin-converting enzyme (ACE). Ang II is a major player in blood pressure regulation. Ang II elevates blood pressure by direct vasoconstrictor action and also by increasing sodium retention and blood volume through stimulation of aldosterone production from the adrenal cortex (Weir and Dzau, 1999). Aldosterone biosynthesis is also physiologically regulated by adrenocorticotropin (ACTH) and potassium (K⁺) (Williams, 2005). In addition to salt and water regulation, aldosterone is linked to the pathogenesis of several conditions, including inflammation and cardiovascular remodeling (Brown, 2005; Marney and Brown, 2007; Mehta and Griendling, 2007; Brown, 2008). Thus, the mechanisms regulating aldosterone production have recently acquired additional importance due to the pathologic implications of inappropriate aldosterone secretion.

Ang II influences aldosterone production through its capacity to directly activate signaling pathways, many of which are coupled to the expression of genes that chronically control adrenal differentiation and cell growth (Spat and Hunyady, 2004). The two types of Ang II receptors, AT1R (type 1) and AT2R (type 2), were identified by selective ligands and

characterized as seven transmembrane receptors by molecular cloning. The G protein-coupled AT1R is the primary receptor for Ang II-regulated aldosterone production (Hajnoczky et al., 1992; Tsuchida et al., 1998) (Fig. 1). In the human adrenal cortex there is only one type of the AT1R, and it is expressed at much higher levels in the glomerulosa (aldosterone-producing zone) than in the fasciculata (cortisol-producing zone) (Bergsma et al., 1992). In rodents there are two genes that encode two isoforms of AT1R (AT1AR and AT1BR). The AT1AR is found in both the glomerulosa and medulla while the AT1BR is found only in the adrenal glomerulosa (Burson et al., 1994; Gasc et al., 1994; Wakamiya et al., 1994). Adrenal glomerulosa expression of the AT1R is enhanced on a low-sodium diet, suggesting that Ang II has the ability to enhance expression of its own receptor and, as a result, increase chronic aldosterone production (Lehoux et al., 1994). The actions of Ang II on the adrenal glomerulosa are often divided into acute effects, which relate to the rapid induction of aldosterone production, and the chronic effects that relate to increasing the capacity to produce aldosterone through both enlargement of the glomerulosa and increased expression of enzymes needed for aldosterone synthesis. It is likely that both acute and chronic actions of Ang II relate to the activation of several signaling cascades through the AT1R (Fig. 1).

2. Ang II intracellular signaling

Activation of AT1R, predominantly via G_q , stimulates phospholipases A_2 (PLA_2), C, and D, thereby activating protein kinase C (PKC) isoforms and inositol trisphosphate (IP_3)/ Ca^{2+} signaling (Bollag et al., 2002; Hunyady et al., 2004; Spat and Hunyady, 2004). G protein stimulation also leads to activation of mitogen-activated protein kinase pathways (MAPK), such as mitogen-activated protein kinase kinase - extracellular signal-regulated kinase 1 and 2 (MEK-ERK1/2), and calcium/calmodulin-dependent kinases (CaMK) (Chabre et al., 1995; Cote et al., 1998) (Fig. 1). Ang II can also act through G protein-independent pathways including β -arrestins, tyrosine kinases, and Janus kinase (JAK)/signal transduction and activators of transcription (STAT) pathways (Seta et al., 2002; Thomas and Qian, 2003; Wei et al., 2003; Miura et al., 2004). Studies in a variety of Ang II target cell types have shown that the Ang II activation of different pathways is time dependent. For example, activation of the G-protein-dependent pathway and generation of IP_3 occurs in seconds, while MAPK and JAK/STAT activation occurs in minutes to hours after initial AT1R activation (Ishida et al., 1995; Schmitz et al., 1998). While many of the Ang II signaling cascades have been defined, an understanding of the downstream changes in gene expression has moved ahead very slowly, often through the definition of one gene-target at a time. Recent use of high-density microarray analysis has provided the methodology to define, in large numbers, the Ang II gene-targets in adrenocortical cell models. Over time these genes will be linked to function as well as the specific signaling pathway(s) that increase their expression.

3. Early Ang II response genes

It has long been known that adrenal cells *in vitro* and *in vivo* respond to Ang II treatment with the rapid induction of new gene expression (Viard et al., 1992b; Viard et al., 1992a; Viard et al., 1993). Several studies have recently made use of microarray analysis to identify a large numbers of transcripts that are acutely up-regulated following Ang II stimulation (Romero et al., 2004; Nogueira et al., 2007; Romero et al., 2007). Four adrenal model systems from three species have been used to define Ang II-responsive genes. We have defined a set of Ang II early response genes in primary cultures of rat and bovine glomerulosa cells as well in the human H295R adrenal cell line (Nogueira et al., 2007). In this study, adrenocortical cells were stimulated with Ang II followed by RNA isolation and analysis using species-specific microarrays. The microarrays used in this study covered a wide range of genes; approximately 40, 30, and 20 thousand independent genes from the

human, rat, and bovine transcriptome, respectively. In comparison Romero *et al.*, 2004 and 2007 used similar human and rat arrays for their study of H295R adrenal cells and freshly isolated rat glomerulosa cells (Romero *et al.*, 2004; Romero *et al.*, 2007). However, there were temporal differences between these studies that should be noted. We used a single 1 h Ang II-incubation with the goal of determining direct gene-targets that resulted from activation of cell signaling cascades. Romero *et al.* determined the Ang II-responsive genes following a 3 h treatment period of the H295R cell line and of freshly isolated rat glomerulosa cells. In addition, we used a concentration of 10 nM Ang II while Romero and colleagues used 100 nM Ang II. Based on data from these studies a view of the acute effects of Ang II on adrenal cell gene expression is now available.

Based on comparison of the microarray data from the cells in monolayer culture, there were a number of common genes that were increased with Ang II treatment. Interestingly eight of the most elevated transcripts were transcription factors. It is also interesting that, of the eight transcription factors, six had been shown, prior to microarray technology, to be increased in adrenocortical cells following Ang II-stimulation (Clark *et al.*, 1992; Viard *et al.*, 1992a; Enyeart *et al.*, 1996; Fernandez *et al.*, 2000). Two of the families of transcription factors, the activator protein 1 (AP-1) and nerve growth factor-induced B (NGFI-B) families, have been studied with regard to their expression and function within the adrenal cells (Table 1).

Members of the NGFI-B family, including the nuclear receptor subfamily 4 group A member 1 (NR4A1), NR4A2, and NR4A3, have been shown to be highly expressed within the mouse, rat and human adrenal cortex (Wilson *et al.*, 1993; Davis and Lau, 1994; Rainey *et al.*, 2001). NR4A2 is primarily expressed in the adrenal glomerulosa, and *in vitro* studies suggest that it is a target for both Ang II and K⁺ treatment (Bassett *et al.*, 2004b; Romero *et al.*, 2004). NR4A1 is expressed in both the rat and human adrenal glomerulosa and fasciculata and is a target-gene for both Ang II and ACTH signaling (Bassett *et al.*, 2004a; Lu *et al.*, 2004). Both NR4A1 and NR4A2 can increase transcription of three genes that encode the enzymes responsible for the last steps in aldosterone synthesis: HSD3B2 (3 β -hydroxysteroid dehydrogenase type 2), CYP21 (21-hydroxylase), and CYP11B2 (aldosterone synthase) (Wilson *et al.*, 1993; Bassett *et al.*, 2004c; Bassett *et al.*, 2004b; Kelly *et al.*, 2004). Thus the NGFI-B family likely plays an important role in the expression of the enzymes needed for production of both mineralocorticoids and glucocorticoids.

Ang II also enhances expression of the members of the AP-1 complex [v-fos FBJ murine osteosarcoma viral oncogene homolog (FOS), FBJ murine osteosarcoma viral oncogene homolog B (FOSB), jun B proto-oncogene (JUNB), and v-jun sarcoma virus 17 oncogene homolog (JUN)]. These genes have long been recognized as Ang II target genes in bovine, rat and human adrenocortical cells (Viard *et al.*, 1992b; Vinson *et al.*, 1998; Romero *et al.*, 2004; Nogueira *et al.*, 2007). Adrenal cell induction of the AP-1 proteins is regulated, not only by Ang II, but also by ACTH, basic fibroblast growth factor (b-FGF), insulin-like growth factor (IGF-I), and transforming growth factor beta (TGF- β) (Viard *et al.*, 1993; Lehoux *et al.*, 1998; Naville *et al.*, 2001). AP-1 proteins increase the expression of 11 β -hydroxylase (CYP11B1) in rat and human adrenocortical cells (Mukai *et al.*, 1995; Romero *et al.*, 2007). It should be noted that members of the AP-1 complex are able to homo- and hetero-dimerize, and the subunit composition of AP-1 can often influence its effects on the expression of particular genes (Mukai *et al.*, 1998). Thus, the ratios of FOS, FOSB, JUNB and JUN may be important in determining the ultimate cellular phenotype. Indeed we find that FOS heterodimers with members of the JUN family can repress transcription of the 17 α -hydroxylase (CYP17) expression (Rainey and Sirianni, 2007). This repression may help explain the absence of CYP17 in the human and bovine adrenal glomerulosa cells.

Four members of the early growth response (EGR) transcription factor family, including EGR-1, EGR-2, EGR3, and EGR-4 are increased by acute Ang II treatment in adrenocortical cells (Nogueira et al., 2007; Romero et al., 2007) (Table 1). Romero *et al.* demonstrated that CYP11B2 transcription was increased by EGR2, while CYP11B1 transcription was stimulated by both EGR1 and EGR2 in H295R adrenocortical cells (Romero et al., 2007). The expression of EGR-1 is induced by diverse signals that initiate growth and differentiation in PC-12 pheochromocytoma cell line (Cao et al., 1990), as well as in vascular smooth muscle cells (VSMC) (Zhu et al., 2007). Therefore, EGR family members may be involved in several aspects of the Ang II regulation of the adrenal cortex.

There are many non-transcription factor genes that are rapidly induced following Ang II treatment. Several have recently been studied, including RGS2, a regulator of G protein signaling, that is rapidly induced by Ang II in H295R cells. RGS2 decreases aldosterone synthesis, presumably by modulating G protein coupling of AT1R to downstream pathways through its ability to accelerate the GTPase activity of G α subunits of heterotrimeric G proteins (Romero et al., 2006). RGS2 overexpression decreases CYP11B2 expression and aldosterone production without affecting cortisol secretion, suggesting that RGS2 levels are increased by Ang II as a negative feedback mechanism (Romero et al., 2006). Mitogen-activated protein kinase phosphatase-1 (MKP-1), also named dual-specificity phosphatase 1 (DUSP-1), appears to be another such negative feedback pathway that is increased following Ang II treatment. MKP-1 is an early response gene that dephosphorylates and inactivates ERK-1/2 and has been linked to an inhibitory effect on mineralocorticoid synthesis in the adrenal cortex (Casal et al., 2007).

Taken together, these results suggest that signaling molecules activated by Ang II and the AT1R rapidly stimulate transcription of genes that play diverse role in the adrenal cortex, including cell growth and proliferation, and regulation of steroid production. Thus, this early cascade of events that happens shortly after Ang II stimulation is likely involved in many of the chronic changes in gene expression.

4. Chronic Ang II response genes

The chronic effects of Ang II on the adrenal glomerulosa are indeed dramatic. Rats and mice on a low sodium diet respond with increased renin and Ang II, which drives the expansion of the adrenal glomerulosa and its capacity to produce aldosterone. Studies examining the chronic effects of Ang II on glomerulosa cell gene expression have been limited mainly to genes that are directly involved in the regulation and/or production of steroid hormones. However, we recently examined the effects of chronic treatment on H295R cells using microarray analysis (Table 2). Below we discuss both the recent transcriptome analysis as well as the studies examining selective genes involved in steroidogenesis.

Importantly, Ang II is able to regulate transcription and membrane expression of its own receptor in adrenocortical cells. *In vivo* studies using rats provided a low sodium diet demonstrate that these animals increase adrenal expression of both AT1AR and AT1BR mRNA; and this effect is blocked by AT1R pharmacological blockage (Wang and Du, 1995; Du et al., 1996) and by ACE inhibitors (Lehoux et al., 1994; Wakamiya et al., 1994). In spontaneous hypertensive rats (SHR), which have increased RAAS activity, both ACE inhibition and a calcium channel blockade decrease AT1R mRNA in the adrenal gland (Kitami et al., 1992). Conversely, in human adrenal fasciculata/reticularis (inner zones of the adrenal cortex) cells, Ang II decreases expression and binding of AT1R (Naville et al., 1993); and similar effects are obtained using bovine adrenal glomerulosa cells (l'Allemand et al., 1996). In addition, in H295R cells, AT1R expression is rapidly decreased after treatment with Ang II (through PKC and Ca²⁺ signaling activation), K⁺ (through Ca²⁺

signaling activation) and forskolin/dibutyryl-cAMP (through activation of protein kinase A) (Bird et al., 1994; Bird et al., 1995a; Bird et al., 1995b). However this inhibitory effect decreases over time such that by 48 h of Ang II-treatment levels are similar to those in untreated cells (Bird et al., 1995a). The results of our microarray analysis of H295R cells treated with Ang II for 72 h agrees with this last study, and AT1R is not differentially expressed between treated and control samples after long-term Ang II incubation. Taken together the chronic effects of Ang II on its own receptor appear to differ depending on the models system. *In vivo* studies appear to support the ability of Ang II to up-regulate its expression, while cell culture studies do not show a chronic effect on AT1R mRNA or binding.

Glomerulosa cells like all steroid producing cells produce steroid using cholesterol as precursor. Adrenocortical cells produce steroids from both cholesterol synthesized *de novo* and that taken up from the circulation bound to HDL via the scavenger receptor B type I (SR-BI) and to LDL via the LDL receptor pathway (Gwynne et al., 1976; Rainey et al., 1992; Cherradi et al., 2003; Pilon et al., 2003). Ang II has been shown to up-regulate expression of both of these receptors (Pilon et al., 2003). Interestingly, the increased transcription of genes involved in the regulation of cholesterol uptake by adrenocortical cells, such as low density lipoprotein receptor-related protein binding protein (LRP2PB) occurs shortly after Ang II-stimulation (Nogueira et al., 2007). After a longer Ang II incubation, additional cholesterol synthesis/uptake genes, such as 3-hydroxy-3-methylglutaryl-coenzyme A synthase 1 (HMGCS1), mevalonate kinase (MVK), CCAAT/enhancer-binding protein (C/EPB), and scavenger receptor class B, member 1 (SCARB1) are up-regulated (Liang et al., 2007). The next step in steroidogenesis, the transport of cholesterol into the mitochondria by StAR protein, has also been demonstrated to be up-regulated by Ang II stimulation in a number of studies (Clark et al., 1995; Cherradi et al., 1998). Microarray analysis of primary bovine adrenal glomerulosa cells shows that up-regulation of StAR is initiated as early as 1 h after Ang II stimulation, while in human H295R cells StAR levels (mRNA and protein) are significantly elevated only after a 6 h incubation with Ang II (Clark et al., 1995).

Part of the chronic response of glomerulosa cells to activation of the RAAS is to increase the capacity to produce aldosterone. To that end, Ang II also regulates the expression of the enzymes responsible for aldosterone and cortisol production, namely: cholesterol side-chain cleavage (CYP11A1), HSD3B2, CYP21, CYP11B1 (the last step in cortisol biosynthesis), and CYP11B2 (Rainey et al., 1991; Tremblay et al., 1992; Adler et al., 1993; Holland and Carr, 1993; Bird et al., 1998; Spat and Hunyady, 2004). Not all steroidogenic enzymes increase in response to Ang II. Several reports using bovine, sheep and human adrenal cell cultures document the ability of Ang II through PKC signaling to decrease expression of CYP17 (Naseeruddin and Hornsby, 1990; Rainey et al., 1991; Bird et al., 1992; Bird et al., 1996). This agrees with the fact that CYP17 is not present in the glomerulosa of adrenals from cortisol secreting species. Our microarray analysis of H295R cells treated for 72 h with Ang II demonstrates changes in several of the gene mentioned above including CYP11B2 as the most up-regulated gene, followed by other genes involved in cholesterol uptake and metabolism such as the HDL receptor SCARB1 and a steroid binding protein SCGB1C1 (Table 2). Thus, Ang II chronically increases the capacity for steroidogenesis by increasing cholesterol uptake and synthesis as well as increasing steroid biosynthetic enzymes.

Contrasting with the presence of more than three hundred up-regulated transcription factors in the 1 h study, 72 h of Ang II treatment only increased 5 transcription factors, namely: paternally expressed 3(PEG3), estrogen-related receptor gamma (ERRG), RAR-related orphan receptor A (RORA), ocular development-associated gene (ODAG), transcriptional coactivator tubedown-100 (TDBN100). PEG3 expression has been described to activate

tumor necrosis factor (TNF)/ nuclear factor- κ B (NF- κ B) pathway in human embryonic kidney cells, and therefore may participate in cell proliferation and differentiation, although the function of PEG3, as well as ERRG, ODAG, RORA, and TDBN100 in the adrenal cortex has not been described.

In addition to the chronic effects on secretion of aldosterone, Ang II also stimulates proliferation of adrenocortical cells *in vivo* (Tian et al., 1995; McEwan et al., 1999) and primary bovine adrenal glomerulosa cells *in vitro* (Tian et al., 1995), although rat adrenal glomerulosa cells *in vitro* exhibit Ang II-elicited hypertrophy rather than proliferation (Otis et al., 2008). The mitogenic effects of Ang II in adrenocortical cells have also been shown in terms of the Ang II-induced induction of the expression of cyclin D1 (Watanabe et al., 1996), an important molecule involved in the G1 phase progression of the cell cycle. This increase has been shown to be partially mediated by EGR1 (Guillemot et al., 2001) which is one of the Ang II rapid response genes (Table 1). Ang II exerts proliferating and hypertrophic effects in other targets, including epithelial cells, VSMC, fibroblasts and cardiomyocytes. While expansion of the adrenal glomerulosa is part of the physiologic mechanisms of increasing aldosterone biosynthesis, chronic effects of Ang II on cell growth and deposition of extracellular matrix under pathological conditions ultimately can lead to vascular dysfunction and cardiac insufficiency.

5. Conclusions

Microarray analysis and real-time RT-PCR have become common-place in the examination of gene expression. Using adrenocortical cells, we have shown that large numbers of genes are increased within a short time of Ang II stimulation; followed by a group of genes that continue to show differences in expression days later. Acutely, Ang II increases the expression of hundreds of genes, most of which have not been studied with regard to adrenal function. Previous research has focused on the role of several transcription factors, including the NGFI-B family that appear to increase expression of the enzymes involved in aldosterone biosynthesis. However, at present, the numbers of Ang II response genes that have not been studied far outnumber the genes which have been examined. Therefore, further characterization of the Ang II activated signaling cascades and their association with specific gene induction is needed and should provide a clearer understanding of how Ang II regulates adrenocortical aldosterone production.

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Fig. 1. Schematic of Ang II-induced gene-transcription through AT1R in adrenocortical cells and its effects on adrenal glomerulosa function. Ang II binds to Ang II type 1 receptors (AT1R) activating phospholipase C (PLC) through G protein subunit q/11 ($G_{q/11}$), and also activating several other signaling molecules, including protein kinase C (PKC), calcium/calmodulin-dependent kinases (CaMK), mitogen-activated protein kinase kinase - extracellular signal-regulated kinase 1 and 2 (MEK1/2-ERK1/2), src-family kinases (SRC), ras/raf kinases (RAS/RAF), and Janus kinase (JAK)/signal transduction and activators of transcription (STAT). Ang II-activation of such wide variety of intracellular signaling pathways culminates with phosphorylation/activation of transcription factors, leading to subsequent modulation of gene transcription, and therefore to changes in cell function.

Table 1
Ang II-responsive genes in three glomerulosa cell models following 1 h of treatment

Gene Name	Gene Function	Symbol	Fold Change
Human adrenocortical cell line – H295R			
Nuclear receptor subfamily 4, group A, member 2	Transcription factor	NR4A2	30.9
v-106 FBJ murine osteosarcoma viral oncogene homolog	Transcription factor	FOS	25.4
FBJ murine osteosarc, viral oncogene homolog B	Transcription Factor	FOSB	15.5
Nuclear receptor subfamily 4, group A, member 3	Transcription Factor	NR4A3	12.1
Early growth response 4	Transcription Factor	EGR4	10.3
Early growth response 1	Transcription Factor	EGR1	9.3
Cysteine-rich, angiogenic inducer, 61	Angiogenesis factor	CYR51	7.7
Phosphoprotein regulated by mitogenic pathways	Signal transducer	TRIB1	7.6
Dual specificity phosphatase 1 (MKP-1)	Protein phosphatase	DUSP1	7.2
Early growth response 2	Transcription Factor	EGR2	7.0
Bovine – primary adrenal glomerulosa cells			
Nuclear receptor subfamily 4, group A, member 3	Transcription Factor	NR4A3	27.4
Nuclear receptor subfamily 4, group A, member 2	Transcription Factor	NR4A3	10.6
Murine FBU osteosarcoma viral (v-fos) oncogene homolog	Transcription Factor	FOS	8.1
Nuclear receptor subfamily 4, group A, member 1	Transcription Factor	NR4A1	7.1
Highly similar to <i>H. sapiens</i> SNF1-like kinase (SNF1LK)	Protein Kinase	SNF1LK	6.2
Coagulation factor III (thromboplastin, tissue factor)	Coagulation factor	F3	5.1
Similar to DUSP-1 (MKP-1)	Protein Phosphatase	MKP-1	4.9
Steroidogenic acute regulatory protein	Cholesterol transport	STAR	4.2
Synaptonemal complex protein 3	Transcription Factor	SYCP3	4.0
GDNF family receptor alpha 2 pre-pro-protein	Receptor activity	GFRA2	4.0
Rat – primary adrenal glomerulosa cells			
Early growth response 3	Transcription Factor	EGR3	24.7
FBJ murine osteosarcoma viral oncogene homolog B	Transcription Factor	FOSB	20.6
Nuclear receptor subfamily 4, group A, member 1	Transcription Factor	NR4A1	16.0
Nuclear receptor subfamily 4, group A, member 3	Transcription Factor	NR4A3	10.8
B-cell translocation gene 2 anti-proliferative	Transcription Factor	BTG2	5.6
Striated muscle activator of Rho-dependent signaling	Signal transducer	STARS	5.5
FBJ murine osteosarcoma viral oncogene homolog	Transcription Factor	FOS	4.6
Nuclear receptor subfamily 4, group, member 2	Transcription Factor	NR4A2	4.5
Carbon catabolite repression 4 protein homolog (noctumin)	Transcription Factor	CCR4	4.4
Inhibitor of DNA binding 4	Transcription repressor	IDB4	3.8

H295R cells were treated for 1 h followed by RNA isolation and microarray analysis. This table depicts the 10 genes with the highest fold induction at the end of treatment. Values represent the fold change as compared to untreated cells. (Nogueira et al., 2007)

Table 2
Ang II-responsive genes following treatment with H295R for 72 h

Gene Name	Gene Function	Symbol	Fold Change
Human adrenocortical cell line – H295R			
Cytochrome P450, family 11, subfamily B, polypeptide 2	Catalytic activity	CYP11B2	6.7
Scavenger recetor class B, member 1	Receptor activity	SCARB1	6.0
Matastasis associated lung adenocarcinoma transcript 1	Non-coding sequence	MALAT-1	4.7
Secretoglobin, family 1C, member 1 (RYD5)	Steroid binding	SCGB1C1	4.4
Secreted frizzled-related protein 1	Signal transducer	SFRP1	4.3
Rho GTPase activating protein 1B	Signal transducer	ARHGAP18	4.0
Maternaaly expressed 3	Tumor suppressor	MEG3	3.8
UPD-glucose ceramide glucosyltransferase	Transferase activity	UGCC	3.8
Chromobox homolog 3 (HP1 gamma homolog)	Chromatin remodeling	CBX3	3.6
Wiskott-Aldrich syndrome like	GTPase regulator activity	WASL	3.5

H295R cells were treated for 72 h followed by RNA isolation and microarray analysis. This table depicts the 10 genes with the highest fold induction at the end of treatment. Values represent the fold change as compared to untreated cells