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Regulation of kidney development by histone deacetylases

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Abstract

There is accumulating evidence that gene expression can be regulated independently of DNA sequence changes, also called epigenetic modifications. Histone deacetylases (HDACs), a specific epigenetic group of enzymes, dynamically and reversibly removes acetyl groups from histone tails projecting from the nucleosome. Clinically, valproic acid fetopathy sheds some insight on the effects of altered HDACs on human embryonic development since valproic acid is an antiepileptic drug and an HDAC inhibitor. The fetal anomalies include severe renal dysgenesis supporting a role of HDACs in human kidney development. Our recent studies have shown that HDACs regulate the transcriptional networks required for controlling the cell cycle, Wnt signaling, and the pathway upstream of the GDNF/RET signaling pathway in the developing kidney. Here, we describe novel HDAC target genes not previously implicated in renal development based on studies using genome-wide microarrays. These genes can be divided into transcription factors, modulators of matrix biology, chromatin remodelers, and DNA repair genes. We also report that HDACs are requisite for tissue specific gene expression.

Keywords

Kidney development; Histone deacetylases; Histone acetylation; Transcriptional profiling

Introduction

Chromatin modifications occurring independently of DNA sequence changes introduce another layer of understanding cell function, regulation, and identity. Acetylation, methylation, phosphorylation and ubiquitination are all epigenetic modifications that occur on specific residues of histone tails and lead to alterations in the coiling of DNA and affect protein-protein interactions [1, 2]. These epigenetic combinations generate a “histone code” which research increasingly demonstrates is vital for regulation of gene expression. Histone acetylation is a dynamic and reversible alteration on histone tails mediated by histone acetyltransferases (HATs) and histone deacetylases (HDACs). HDACs are a conserved group of enzymes that remove acetyl groups from histone tails that project from the nucleosome, causing condensed chromatin architecture. Initially, HDACs were described as forming heterochromatin and repressors of gene expression. This generalization has been modified after studies have recently shown activation of various genes [3, 4]. HDACs are divided into four classes based on sequence homology to yeast HDAC genes: Class I (HDAC 1–3, and 8), Class II (HDAC 4–7, 9, and 10), Class III (Sir2/SIRT 1–7), and Class IV (11) [4]. Studies show evidence of elevated HDAC activity in propagating cells, with dysregulation of HDACs implicated in causing cancer, cardiovascular, and immune disorders [5]. HDACs role in embryogenesis and organogenesis, however, is just beginning

to be explored. Studies thus far have supported individual roles for specific HDACs. For example, HDAC 1 is imperative for regulating cell proliferation and is essential for embryo survival [6]. Also, HDAC 1 and 2 perform redundant yet vital functions in cardiac growth and development and in the differentiation of oligodendrocytes [7, 8]. HDAC 4, 5, and 9 exhibit anti-hypertrophic properties in chondrocytes and the heart, and HDAC 7 null mice have loss of vascular integrity causing lethality by E11.0 [9–11].

Normal metanephric kidney development in vertebrates begins with the differentiation of the metanephric mesenchyme (MM), from the caudal portion of the intermediate mesoderm. Then, reciprocal inductive interactions occur between the MM and the adjacent ureteric bud (UB), an outgrowth from the medial mesonephric (or Wolffian) duct resulting in formation of the collecting duct system, the calyces, the ureter, and functional nephrons [12, 13]. Recent work from our laboratory demonstrated that HDACs are involved in several signaling pathways which are vital for normal metanephric kidney development [Chen S and El-Dahr SS, manuscript submitted]. These include the Wnt-catenin pathway, the phosphatidylinositol 3-kinase (PI3K)/AKT pathway, and the TGF- β signaling pathway. All three signaling cascades display significant dysregulation with histone deacetylase inhibitor (HDACi) treatment, thereby revealing an essential function for HDACs.

Although it has not been established that genetic mutations in HDAC genes cause abnormal embryogenesis in humans, there is evidence that HDAC genes are required in human embryonic development. Valproic acid, an antiepileptic drug with properties of Class I and Class II HDAC inhibition, is associated with congenital anomalies after first trimester fetal exposure [14]. These malformations include extensive kidney pathology and sequelae, which will be discussed as a patient case, and suggest a function of HDACs in human kidney development.

To reconstruct this model, we examined the role of HDACs in metanephric kidney development through the use of a pharmacologic HDACi. Scriptaid, belonging to the hydroxamic acid class of HDACi, inhibits Class I and II HDACs [15]. Nullscript, used as the control, is a structural analogue of Scriptaid, with no HDACi ability [16]. Day 13.5 embryonic kidneys were cultured with either Scriptaid or Nullscript for 6 hours and a genome-wide microarray analysis was done from extracted RNA followed by Ingenuity Pathway software analysis (IPA). The results revealed that HDACs regulate the major transcriptional networks known to regulate renal development [Chen S and El-Dahr SS, unpublished data]. However, a great number of significantly affected genes have no known function in kidney development. The aim here is to recognize and discuss common themes of the functions of these novel genes to help decipher the role of HDACs.

Methods

Genome-wide microarray experiment

Microarray analysis was performed according to established protocols [17]. Total RNA was extracted in three independent experiments from CD1 mouse kidney explants treated with Scriptaid (2 μ g/ml) or Nullscript (2 μ g/ml) for 6 hours. Cyanine-labeled cDNA from both the Scriptaid and Nullscript treated kidneys was mixed with the same amount of reverse color cyanine from the oppositely treated kidney samples. The cDNA was hybridized to Agilent 44K Whole Mouse Genome Oligonucleotide Microarray (containing ~41,000 probes), the arrays were scanned using a dual-laser DNA microarray scanner (Agilent), data was extracted from images by the Feature Extraction software 6.1 (Agilent), and network analysis was accomplished through IPA software. The data was RMA normalized followed by dye-swap correction for the experiments and filtering by statistical significance (t test p value=0.05, using multiple test correction – the Benjamini and Hochberg false discovery

rate). We report here the top 20 upregulated and 20 down-regulated genes with the most significant changes and that, to our knowledge, have not been previously reported to be involved in regulation of kidney development.

Ex vivo metanephric organ culture

Ex vivo culture protocol was performed as previously described [18].

IPA and Data Analysis

Novel genes are selected from the significantly upregulated and downregulated transcripts (change ≥ 1.5 fold; $p < 0.05$; $n = 3$ independent experiments). The top 250 transcripts are further selected based upon known function and expression patterns. Of these, genes are further designated if they have similar gene function.

Case Report

A 6 year old female presents with altered mental status. She has had episodes of screaming, irritability, head banging, hair pulling, and biting over the past week. Her past medical history is significant for valproic acid fetopathy, patent ductus arteriosus, atrial septal defect, dilation of the third and lateral brain ventricles, hypertension, gastro-esophageal reflux, swallowing dysfunction, and developmental delay. The patient has facial dysmorphism with puffiness. Physical exam is otherwise negative. Pertinent laboratory values include a BUN 126, Creatinine of 6.6, and a Potassium level of 6.3. Urinalysis has a specific gravity of 1.011, pH of 7.5, with 4+ protein, large hemoglobin, 50–100 red blood cells, and 10–20 white blood cells. Kidney ultrasound demonstrates the presence of hypoplastic kidneys (right length 3.1 cm, left length 3.0 cm) with increased parenchymal echogenicity and mild hydronephrosis. Voiding cystogram shows the presence of grade III vesicoureteral reflux (VUR) in the right and of grade II VUR in the left kidney. She was treated with hemodialysis with a slow return to baseline mentation. A diagnosis of end-stage renal disease is established secondary to renal dysplasia from gestational valproic acid use. As noted earlier, valproic acid is a Class I and II HDAC inhibitor.

Results

In a recent study using genome-wide microarray analysis results, our laboratory found that ~4,800 of 41,000 (~12%) probes were significantly altered from HDACi treatment by ≥ 1.5 fold ($p \leq 0.05$, $n = 3$) with 5% of gene transcripts upregulated (range 1.5 to 14 fold increase, $n = 2,085$) and 7% of gene transcripts down-regulated (range 1.5 to 26 fold decrease, $n = 2,865$) [Chen S, and El-Dahr SS, unpublished data]. Furthermore, we reported that the networks controlling the GDNF/RET signaling pathway are significantly altered by HDACi treatment. In addition, IPA network analysis identified other renal developmental pathways where numerous genes were dysregulated with HDAC inhibition. Figure 1A, B, and C highlights these pathways: the Wnt/ β -catenin, the PI3K/AKT, and the TGF- β pathway. Genes are colored green to demonstrate significantly decreased expression and are red to show increased expression. Also, the brighter the color means a more extreme fold change. Figure 2 depicts confirmation of the gene array results using real-time quantitative RT-PCR of 5 randomly selected genes.

Genes with an unknown renal function have been organized into a table that tells the fold change found with HDACi treatment and describes the known function (Table 1 and 2). When evaluating these altered factors, common themes began to take shape. The validity of making conclusions about the functions of HDACs became clearer as we realized that multiple genes with similar functions were either being turned off or on. Using that information, we are able to postulate conclusions about innate functions of HDACs.

Discussion

Major findings discovered by IPA network analysis include several signaling cascades with numerous altered genes following HDAC treatment that are recognized to be intimately involved in renal morphogenesis. The Wnt/ β -catenin pathway is fundamental in nephrogenesis as well as UB outgrowth and branching [19, 20]. The PI3K/AKT signaling pathway is downstream from the GDNF/RET pathway and mediates localized cell proliferation and directed cell movement in the prospective UB tip [21]. The TGF- β pathway delays branching while permitting elongation in UB development. This function was determined because kidneys cultured with TGF- β led to an unusual anomaly of branching, where the UB elongated without branching until reaching the periphery of the kidney [22].

By evaluating these three pathways, it is apparent that HDAC inhibition causes severe dysregulation. As stated above, the genes colored green have significantly decreased fold expression, while the red genes have significantly increased expression. In the Wnt-catenin network, Wnt family members 2, 4, and 5 and Frizzled receptors have decreased expression. Continuing downstream, nuclear *LEF/TCF* is repressed with the downstream effectors *Myc*, *Ccnd1*, *Gja1*, and *Axin2* all having significantly decreased expression. *SFRP1*, a known Wnt inhibitor, has increased expression, also contributing to the severe repression of this pathway. The PI3K/AKT pathway reveals downregulation of the P110 subunit of *PI3K* with downstream decreased expression of *AKT*. The effectors further downstream also display repression, as can be seen with *CCd1*, *P53*, and *Bcl-2*. The TGF- β network continues to show repression beginning with the BMP family to its receptor. Then, the Smad family downstream exhibits consistent downregulation. Although *GCS* and *PITX2* show increased expression, it is likely due to secondary factors since the pathway is repressed until that point. In summary, close assessment of these key renal developmental pathways reveal that they are significantly repressed following HDAC inhibition by direct inhibition of genes in these pathways or by secondary effects.

Other *novel* genes also show decreased expression after HDACi treatment, which are not part of a known nephric embryonic network. These genes can be grouped into the following functional classes: transcription factors, chromatin remodelers, genes that control the extracellular matrix, and DNA repair genes (Table 1 and 2).

Transcription factors

A variety of transcription factors have decreased expression after HDAC inhibition. Among them are Pre-B cell leukemia transcription factor 1 (*Pbx1*), transcription factor AP4 (*Tcfap4*), E74-like factor 5 (*Elf5*), SRY-box containing gene 7 (*Sox7*), basic helix-loop-helix family member e22 (*Bhlhb5*), myelocytomatosis oncogene (*Myc*), and zinc finger protein X-linked (*Zfx*), which all regulate when genes are turned on and off. *Pbx1*, *Tcfap4*, and *Elf5* function as transcriptional activators. *Sox7*, *Bhlhb5*, *Myc*, and *Zfx* determine cell fate. Also, general transcription factor II I (*Gtf2i*) functions in cell proliferation. The cell cycle regulator cyclin D1 (*Ccnd1*) is vital for the G1/S progression. Inhibition of the transition through the G1 to S phase of the cell cycle forces cells to become quiescent and enter the G₀ phase. Collectively, the function of all these inhibited genes strongly suggests that decreased proliferation is a major mechanism mediating the negative effects of HDACi on renal growth.

Chromatin modifiers/remodelers

Genes such as male-specific lethal 1 homolog (*Msl1*), SET domain containing 1B (*Set1b*), chromodomain helicase DNA binding protein 3 (*Chd3*), male-specific lethal 2-like 2

(*Msl3l2*), protein arginine N-methyltransferase 1 (*Prmt1*), forkhead A1 (*Foxa1*), DNA methyltransferase 3a (*Dnmt3a*), and sirtuin 1 (*Sirt1*) are all chromatin remodelers and their expression is decreased in response to HDACi. Msl1 is a part of a HAT complex. Set1b and Prmt1 are both histone methyltransferases, known to add a methyl group to histone tails and cause repression. Foxa1 is a pioneer factor acting by opening chromatin architecture, forming euchromatin, and allowing protein interactions with DNA [23]. Dnmt3a is a DNA methyltransferase which mediates de novo DNA methylation, and is a key developmental regulator. Sirt1 is a class III HDAC, which acts in a complex to recruit HDAC enzymes leading to transcriptional repression and also deacetylates the tumor suppressor p53. It is interesting to note that while HDAC inhibition is an identified chromatin remodeler itself, it appears to have wide ranging effects on other epigenetic modifiers.

Genes controlling the extracellular matrix

The cellular environment is altered by decreased synthesis of genes important for generating and maintaining the extracellular matrix. Scleraxis (*Scx*), a regulator of type I collagen, showed a -5.4 decrease in expression with HDACi treatment. Also, matrix metalloproteinase 14 (*Mmp14*) showed a -4.5 fold decrease in expression. Mmp14 functions to breakdown the extracellular matrix in embryonic development. In the kidney, it may be necessary to clear the extracellular environment to allow branching morphogenesis to occur. Versican (*Vcan* or *Cspg2*) encodes a chondroitin sulfate proteoglycan, a major extracellular component known to be involved in cell-to-cell interactions, proliferation, and tissue morphogenesis and maintenance. Having decreased expression of all these genes can be interpreted to have a deleterious effect on the developing renal system.

DNA repair genes

DNA repair mechanisms also appear to be inhibited with HDACi treatment. Nibrin (*Nbn*) is a factor in the MRE11/RAD50/NBN (MRN complex) with decreased expression of -3.5 fold. It is vital in the double-stranded DNA break repair pathway, in the maintenance of telomere activity, cell cycle checkpoint regulation, and meiosis. Nucleoside diphosphate linked moiety- type motif 15 (*Nudt15*) binds to and helps prevent breakdown of proliferating nuclear cell antigen (*PNCA*). PNCA functions in DNA replication and in DNA repair through the RAD6-dependent pathway. When DNA repair mechanisms are disrupted, DNA mutations accumulate and force the cell to undergo cell death.

When investigating the genes with upregulated expression, many genes that are induced are not normally expressed in the developing kidney. These genes are known to have expression in a variety of organ systems, including the gonadal system, the nervous system, and the musculoskeletal system. Desert hedgehog (*Dhh*), extraembryonic spermatogenesis homeobox 1 (*Esx1*), fibronectin type 3 and ankyrin repeat domains 1 (*Fank1*), heat shock protein 2 (*Hspa2*), Ly6/Plaur domain containing 3 (*Lypd3*), transcription factor-like 5 (*TCFL5*), and pre-B cell leukemia homeobox 4 (*Pbx4*) function in early spermatogenesis and testis development. None of them have ever been described as having expression in the developing kidney but have *de novo* expression with HDACi treatment. Likewise, Rho family GTPase 1 (*Rnd1*), homeobox A1 (*Hoxa1*), neuronal PAS domain protein 1 (*Npas1*), zinc finger, CCHC domain containing 12 (*Zcchc12*), forkhead box 6 (*Foxo6*), LIM homeobox protein 5 (*Lhx5*), NK6 homeobox 2 (*Nkx6-2*), oligodendrocyte transcription factor 1 (*Olig1*), deltex 1 homolog (*Dtx1*), synapsin II (*Syn2*), and serine peptidase inhibitor, clade I, member 1 (*Serpini1*) have a role in brain differentiation, neuronal, and central nervous system cellular growth. Bone morphogenetic protein subunits a and b (*Bmp8a* and *Bmp8b*), FBJ osteosarcoma oncogene (*Fos*), and osteopetrosis associated transmembrane protein 1 (*Ostm1*) are factors involved in bone formation, skeleton maintenance, and osteoclastic maturation. It can be deduced that HDACs function to keep the chromatin

compact thus maintaining tissue-specific gene expression. This de novo expression impairs differentiation and can alter development since the tissues are dysregulated.

Metanephric kidney culture experiments performed in our laboratory have demonstrated that HDACi treatment not only affects the key transcriptional networks of kidney development, but also results in renal hypoplasia due to decreased UB branching and impaired nephrogenesis [Chen S and El-Dahr, unpublished data]. HDACi treatment alters various gene pathways and it is likely the combined effect of these mechanisms that involves global kidney development.

When evaluating the functions of genes whose expression is dysregulated significantly, there is a clear connection that these impaired genes play a role in growth and proliferation. These manifestations are consistent with what was demonstrated in our patient with valproic acid fetopathy. Also, recent evidence investigating the developmental pathways effected with gestational valproic acid exposure demonstrate the same developmental pathways are dysregulated [24] as with our study with Scriptaid, confirming that valproic acid fetopathy is very likely due to HDAC inhibition.

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Figure 1. Ingenuity Pathway analysis of 3 major pathways affected by HDAC inhibition. Green indicates downregulated genes; pink, indicates upregulated genes.

Figure 2.

Quantitative real-time RT-PCR confirming the changes in gene expression observed in the microarray analysis in E13.5 embryonic kidneys treated with Scriptaid (HDAC inhibitor) or its inactive structural analogue, Nullscript. Nullscript values are normalized to 1 and data are presented as relative fold difference.

Table 1
Upregulated Novel Genes (≥ 1.5 -fold, $p < 0.05$)

CM, condensing mesenchyme; UB: ureteric bud.

Gene	Fold change (+)	Expression Pattern	Function
Dhh	13.9	male reproductive organs	May function as a spermatocyte survival factor in the testes. Essential for testes development
Isl2	12.0	CM, UB, tubule, renal interstitium, and renal vasculature	A Transcription factor which defines subclasses of motoneurons that segregate into columns in the spinal cord and select distinct axon pathways
Hoxa1	6.0	ureter, bladder, and testis.	Transcription factor which acts in the maintenance and/or generation of hindbrain segments
Gbx2	5.7	N/A	A transcription factor for cell pluripotency and differentiation genes
Zcchc12	5.4	N/A	Transcriptional coactivator in the bone morphogenetic protein (BMP)-signaling pathway.
Foxo6	4.8	N/A	A transcription factor with restricted expression in the developing and adult brain, particularly in cortical and hippocampal structures.
Onecut2	4.5, 3.2	CM, ureteric tip, early and late tubule, ureteric trunk, renal interstitium	A transcription factor which stimulates expression of genes involved in melanocyte and hepatocyte differentiation (e.g., HNF3B)
Gadd45b	4.4, 3.0	renal vasculature.	Involved in the regulation of growth and apoptosis.
Bmp8a	4.3	N/A	Induces cartilage and bone formation.
Myf1	4.1	No expression detected	Involved in lineage commitment of primary hemopoietic progenitors. Suppresses RFW2/COP1 activity via CSN3, which activates p53 and induces cell cycle arrest.
Atp6v1c2	3.8	metanephros	H ⁺ -ATPase (also known as vacuolar ATPase, V-ATPase) is a enzyme transporter that functions to acidify intracellular compartments. Linked to regulation of Wnt signaling.
Cbx7	3.5	N/A	Transcriptional repressor and a chromatin modifier.
Foxj1	3.4	CM, UB, tubule, renal vasculature	Regulates transcription of genes that control production of motile cilia and determination of left-right asymmetry.
Lhx5	3.3	No expression detected in E15.5 tissues	Regulation of precursor cell proliferation and control of neuronal differentiation and migration during hippocampal development.
Cdkn2b	3.2	N/A	A cyclin-dependent kinase inhibitor which forms a complex with and inhibits CDK4 or CDK6. Expression of this gene is induced by TGF beta.
Nkx6-2	3.1	bladder and testis.	Important for differentiated oligodendrocyte function and in the regulation of myelin gene expression.
Dkk1	3.1	N/A	The dickkopf protein family interacts with and inhibits the Wnt signaling pathway.
Hoxd1	2.8	N/A	A transcription factor involved in limb development
Olig1	2.7	No expression detected at E15.5	Promotes formation and maturation of oligodendrocytes.
Pmaip1	2.7	unspecified strength in E13.5 mesonephros.	Promotes activation of caspases and apoptosis.
Ostm1	2.6	N/A	Degradation of G proteins via the ubiquitin-dependent proteasome pathway. Required for osteoclast and melanocyte maturation and function
Pbx4	2.5	testes and ureter	

Note: Expression pattern information was found in www.gudmap.org and relevant literature. Gene function was found in www.genecards.org and relevant literature.

Table 2
Downregulated Novel Genes (≥ 1.5 -fold change, $p < 0.05$)

CM, condensing mesenchyme; UB: ureteric bud.

Gene	Fold decrease	Expression Pattern	Function
Scx	-5.4	CM, ureteric tip, early and late tubule group, the renal interstitium, UB, and renal vasculature	Scleraxis is a basic helix-loop-helix type transcription factor involved in mesoderm and heart valve formation. Regulates collagen type I gene expression in cardiac fibroblasts and myofibroblasts, and it may play a role in myocardial remodeling.
Mmp14	-4.5	N/A	Breakdown of extracellular matrix in normal physiological processes, such as embryonic development, reproduction, and tissue remodeling.
Pbx1	-4.0	renal interstitium	Pbx1 $-/-$ mice die by embryonic day 15 or 16 with severe hypoplasia of multiple organs and widespread patterning defects of the axial and appendicular skeleton.
Ccnd1	-3.7	Tubule precursors	This cyclin forms a complex with and functions as a regulatory subunit of CDK4 or CDK6, whose activity is required for cell cycle G1/S transition. A known target of the Wnt-b-catenin pathway.
Notch4	-3.4		Functions as a receptor for membrane-bound ligands Jagged1, Jagged2 and Delta1 to regulate cell-fate determination.
Sema3a	-3.4, -2.8	metanephros and in the renal/urinary system	A member of the semaphorin family and is vital for normal neuronal pattern development
Zfhx1b	-3.3, -3.1, -3.1	reproductive system, mesonephros, and mesonephric mesenchyme.	functions as a DNA-binding transcriptional repressor that interacts with activated SMADs. Mutations in this gene are associated with Hirschsprung disease/Mowat-Wilson syndrome.
Ms11	-3.3, -3.0	N/A	Component of histone acetyltransferase complex responsible for the majority of histone H4 acetylation at 'Lys-16' which is implicated in the formation of higher-order chromatin structure
Setd1b	-3.2	N/A	SET1B is a component of a histone methyltransferase complex that produces trimethylated histone H3 at Lys4. H3 'Lys-4' methylation represents a specific tag for epigenetic transcriptional activation
Chd3	-3.2	renal interstitium, renal vasculature	A component of a histone deacetylase complex referred to as the Mi-2/NuRD complex which participates in the remodeling of chromatin by deacetylating histones.
Kif26b	-3.2	N/A	Kif26b, a kinesin family gene, is a downstream target of Sall1 and disruption of this gene causes kidney agenesis.
Smo	-2.9	CM and renal interstitium	Smoothered (Smo) receptors are non-classical G-protein-coupled receptors that transmit hedgehog signaling.
Elf5	-2.9	ureteric trunk	A member of an epithelium-specific subclass of the Ets transcription factor family. May play a role in regulating terminal differentiation
Kif12	-2.9	N/A	KIF12 is a member of the kinesin superfamily of microtubule-associated molecular motors that play important roles in intracellular transport and cell division (component of the ciliary apparatus)
Sall2	-2.8	CM	Sall2-deficient mice show no apparent abnormal phenotypes. Mice lacking both Sall1 and Sall2 show kidney phenotypes comparable to those of Sall1 knockout, thereby demonstrating the dispensable roles of Sall2 in embryonic and kidney development
Prmt1	-2.8	N/A	A protein arginine methyltransferase that functions as a histone methyltransferase
Foxa1	-2.7	renal medulla and ureteric trunk	A Transcription factor that acts as a 'pioneer' factor opening the compacted chromatin for other proteins through interactions with nucleosomal core histones
Dnmt3a	-2.7, -2.9	N/A	This gene encodes a DNA methyltransferase that is functions in de novo methylation. Homozygotes for a targeted null mutation become runted and die around 4 weeks of age

Gene	Fold decrease	Expression Pattern	Function
Sirt1	-1.5	N/A	NAD-dependent protein (histone) deacetylase, which regulates processes such as apoptosis and muscle differentiation by deacetylating key proteins, e.g., p53.

Note: Expression pattern information was found in www.gudmap.org and relevant literature. Gene function was found in www.genecards.org and relevant literature.