HHS Public Access

Author manuscript

Steroids. Author manuscript; available in PMC 2018 November 01.

Published in final edited form as:

Steroids. 2017 November ; 127: 56–61. doi:10.1016/j.steroids.2017.08.011.

Development of Monoclonal Antibodies Against the Human 3β-Hydroxysteroid Dehydrogenase/Isomerase Isozymes

Celso E. Gomez-Sanchez[1,2], **Mark Lewis**[2], **Kazutaka Nanba**[3], **William E. Rainey**[3], **Maniselvan Kuppusamy**[1], and **Elise P. Gomez-Sanchez**[4]

[1]Endocrine and Research Service, G.V. (Sonny) Montgomery VA Medical Center, Ann Arbor, MI

[2]Division of Endocrinology, University of Mississippi Medical Center, Ann Arbor, MI

[3]Jackson, MS, Departments of Molecular and Integrative Physiology & Internal Medicine, University of Michigan, Ann Arbor, MI

[4]Department of Pharmacology and Toxicology, University of Mississippi Medical Center, Jackson, MS

Abstract

The human 3β-hydroxysteroid dehydrogenase/isomerase (HSD3B) enzymes catalyze the conversion of 3β-hydroxy Δ 5-6 steroids into 3-keto Δ 4-5 steroids, which is required for the synthesis of the mature steroid hormones secreted by the adrenal and gonads. The human has 2 isozymes, the HSD3B1 that is traditionally located in placenta and extra-adrenal tissues and the HSD3B2 that is expressed in the adrenal and gonads. Mice with both *cryptochrome 1 and 2* genes deletion were recently found to have salt-sensitive hypertension and hyperaldosteronism. These deletions were also associated with overexpression of the Hsd3b6 enzyme, the homolog of the human HSD3B1, in the zona glomerulosa which was believed to explain the hyperaldosteronism. A report using antibodies against human HSD3B1 suggested that it was expressed in the zona glomerulosa of normal human adrenals and in patients with idiopathic hyperaldosteronism and the HSD3B2 expressed in both the zona fasciculata and glomerulosa.

We have developed specific monoclonal antibodies against the human HSD3B1 and HSD3B2 isozymes and found that the main enzyme expressed in the zona glomerulosa was the HSD3B2. Faint staining of the adrenal was also obtained using the anti-HSD3B1antibody only at high concentrations of antibody. This study fails to confirm that HSD3B1 expression in the human zona glomerulosa and double immunofluorescence clearly shows that the HSD3B2 is expressed in the zona glomerulosa and fasciculata and in the zona glomerulosa HSD3B2 is co-expressed with aldosterone synthase (CYP11B2).

Address Correspondence to: Celso E. Gomez-Sanchez, M.D., Research Service, G.V. (Sonny) Montgomery VA Medical Center, 1500 E. Woodrow Wilson Blvd, Jackson, MS 39216, USA, Tel 601 368 3844, Cgomez-sanchez@umc.edu.

Disclosures: The authors have nothing to disclose.

Keywords

Monoclonal antibodies; 3β-hydroxysteroid dehydrogenase/Δ4; 5 isomerase; Adrenal; Zona glomerulosa

Introduction

The biosynthesis of adrenal and gonadal steroids is produced from cholesterol by the consecutive action of multiple steroidogenic enzymes. Cholesterol is transported into mitochondria where it is converted by the enzyme CYP11A1 (side chain cleavage) into pregnenolone [1] and in the zona fasciculata-reticularis, pregnenolone is usually first hydroxylated in the 17α-position to 17α-hydroxy pregnenolone and then converted to 17α-hydroxyprogesterone by 3β-hydroxysteroid dehydrogenase/Δ4–5 isomerase (HSD3B) in endoplasmic reticulum and mitochondrial membranes [1, 2]. In the zona glomerulosa of the adrenal pregnenolone is converted to progesterone by the same enzyme. This enzyme oxidizes the 3β-hydroxy group to a 3-ketone and isomerizes the Δ5-6 in ring B to a Δ4-5 double bond in ring A. In the human there are two different isozymes, the HSD3B type I (HSD3B1) which consists of 372 amino acids (MW 42,251) and is mainly expressed in the placenta, mammary and, prostate glands, and other peripheral tissues including the skin and adipose tissue [3]. The second enzyme HSD3B type II (HSD3B2) consists of 371 amino acids (MW 42052) and is expressed primarily in the adrenal and gonads. The genes for both enzymes are located in chromosome 1p13.1 and are 93.5% homologous at the amino acid level [3]. The enzymes are located in the endoplasmic reticulum and mitochondria [1-3]. Most antibodies available for these enzymes have been unable to distinguish between the two, except for a recent report of isotype specific antibodies [4].

Mice with dual gene deletion of the clock genes *cryptochrome 1 and 2* were found to have salt-dependent hypertension and hyperaldosteronism with low plasma renin activity [5]. Searching for the cause of the hyperaldosteronism in these mice, the investigators identified increased expression of the Hsd3b6 isozyme only in the zona glomerulosa of the adrenal [5, 6]. The mouse has 6 different genes coding for Hsd3b enzymes, while the human has only two [3]. The mouse Hsd3b6 isozyme homolog in the human is the HSD3B1 isozyme. The group of Doi *et al* [4] used a commercial antibody (Abnova.com) to determine that the HSD3B1 was expressed in the ZG and its expression was increased in adrenals from patients with primary aldosteronism (PA) due to bilateral zona glomerulosa hyperplasia. The HSD3B2 enzyme was localized in both the ZF and ZG [4]. Patients with aldosterone-producing adenomas expressed much higher level of HSD3B2 enzyme than HSD3B1 in the adenoma, but the low level of expression of the *HSD3B1* at the mRNA level appeared to correlate with aldosterone secretion, the significance of which is unclear [7], as the presence of a mRNA does not necessarily is translated into the protein.

We have used peptides corresponding to amino acids to the area between 30-43 (HSD3B1) and 29-42 (HSD3B2) that differ in a single amino acid toward the C-terminal of the immunizing peptide and were able to generate specific monoclonal antibodies against both isozymes.

Experimental

The two human isozymes of the HSD3B are approximately 93% homologous. Peptides were synthesized by LifeTein LLC (Lifetein.com) to a selected area corresponding to amino acids 30-43 for the HSD3B1 isozyme (CLKIRVLDKAFGPEL amide, 88.29% pure) and amino acids 29-42 for the HSD3B2 (CLKIRALDKAFRPEL amide, 88.73% pure) differing by just 2 amino acids with an additional cysteine at the N-terminal end for conjugation. The peptides were then conjugated to both Imject™ Keyhole hemocyanin and Imject™ Blue Carrier™ Protein (hemocyanin from Concholepas concholepas) (ThermoFisher.com) using the reagent N-ε-malemidocaproyl-oxysuccinimide (molbio.com), and dialyzed against phosphate buffered saline. Ten micrograms of either conjugate mixed with complete Freund Adjuvant were initially injected subcutaneously into various sites of 4 mice each. Two and four weeks later similar injections were done using incomplete Freund Adjuvant. Two weeks later, the mice were injected intraperitoneally with conjugate without adjuvant and 3 days later the mice were anesthetized with isofluorane and blood obtained by intracardiac puncture and the spleen obtained under sterile conditions. Splenocytes were then obtained by mincing and triturating the spleen between two glass slides, collected and centrifuged for 5 min at 400 × G and dispersed in freezing media (DMEM with 20% Newborn Calf Serum, 10% Ficoll-70 and 5% DMSO) and stored in liquid nitrogen. All animal use was approved by the UMC/VA IACUC.

Production of lentivirus with the HSD3B1 and HSD3B2 and stable cell lines. The cDNA for HSD3B1 (Cat # HsCD00043178, accession # BC031999) and HSD3B2 (Cat # HsCD00043847, accession # BC038419) were obtained from DNASU (DNASU.org), were cloned unto the lentiviral plasmid pCDH-CMV-MCS-EF1-RFP+Puro (systembio.com), and confirmed by sequencing.

The generation of CHO cells expressing either pCDH-CMV-HSD3B1-EF1-RFP+Puro or pCDH-CMV-HSD3B2-EF1-RFP+Puro was done by transduction with the respective lentivirus in the presence of 8 μg of polybrene and spinoculation for 90 min at 3,000 × g. After culturing for 3 days, cells were selected with puromycin 2 μg/ml for 7 days. Surviving cells were grown in 4 × 155 mm dishes and when confluent were scrapped into RIPA buffer containing protease and phosphatase inhibitor solution (Thermofisher.com), mixed with Laemmli buffer, placed at 95 °C for 10 min, cooled, and the protein divided into aliquots.

JEG3 cells were grown in DME medium with 10% Fetalgro serum (rmbio.com), and H295R and HAC15 cells were grown with DME/F12 medium with 2% Fetalgro serum. Whole cells extract was prepared as above.

Fusion of spleen cells and selection of hybridomas expressing the monoclonal antibodies against HSD3B1 and HSD3B2. Initially sera from the mice were titered using the peptide conjugated as above with ovalbumin. Each serum was tested against both the HSD3B1 and HSD3B2 antigens and in each case the respective titer for the corresponding antigen was significantly higher than the other homologous peptide. The sera were then used to perform western using a single well 12% polyacrylamide gel, loaded with the corresponding extract from the CHO cells (400 μg/well), transferred to a PVDF membrane and screened using a

Mini-PROTEAN II Apparatus #1704017 (bio-rad.com). Spleen cells selected for fusion were from animals whose serum had a band at the appropriate molecular mass and no cross-reactivity for the homologous protein. Cells from half a spleen were fused with the myeloma cell line SP2-mIL6-hIL21-hTERT using polyethylene glycol 1450 (ATCC.org) 45% for one minute, diluted with ISCOVE media without serum, incubated for 30 min at 37 °C, centrifuged for 5 min at 400 × g and reconstituted with 100 ml of ISCOVE media with 15% Fetalclone I (Thermofisher.com), 20% of conditioned media (supernatant from CHO cells expressing mIL6 and hIL21), and HAT (sigmaldrich.com). Cells were then diluted and dispersed into 10 × 96-well plates (100 μl/well) and the plates were wrapped with Saran Wrap and incubated at 37 °C with 5% CO_2 for 7 days for hybridoma selection. The media was then replaced with 200 μl media + HT and 2-4 days later aliquots were taken for screening. Screening occurred when the clones were large enough and media started changing color by transferring 50 μl of media into plates that had been coated with 100 ng/100 μl of the inoculation peptide in a phosphate buffer, pH 6.2. After incubating for 1 h and washing, a second goat anti-mouse IgG light chain-HRP (Jacksonimmunoresearch.com) antibody was added for 30 min, washed, then the plates were developed using ABTS. Positive clones were then screened using PAGE as in the screening for serums. Specificity was tested initially by ELISA. Alternating rows in a plate were coated with 100 ng of HSD3B1-ovalbumin conjugate and HSD3B2-ovalbumin conjugate and media from each clone was titered as a 1:1 dilution in each of the different coated rows and processed as above. All of the clones tested reacted only to the corresponding immunized antigen; they were highly specific (Fig 1).

Selection of wells for subcloning. Cells from wells that secreted an antibody that showed a single band by western blot at the appropriate molecular mass were subcloned using approximately 300-500 cells dispersed in 14 ml high viscosity methylcellulose media and 1.4 ml were plated into each of 2 × 5 wells of 6-well plates (the additional well had 5 ml of sterile water to maintain humidity) [8]. After approximately 14 days clones were visible and 48 clones were picked using a 2 μl pipette and placed into 48-well plates with 0.5 ml of HT media as above. After a few days, antibody in the media was screened by ELISA and 6 wells with the highest titer were expanded and frozen. If a significant number of wells growing sub-cloned cells were negative during screening, the chosen positive clones for expansion were cloned again until all clones were positive as an indication of monoclonality.

Human tissue samples. The normal human adrenals were obtained from renal transplantation donors at the University of Michigan with informed consent from donors or their families. The placenta samples were obtained through the Cooperative Human Tissue Network. The use of these tissue samples was approved by the Institutional Review Boards of the University of Michigan and the G.V. (Sonny) Montgomery VA Medical Center.

Western blots. To determine specificity of the HSD3B1 monoclonal antibody, 12 % gels were loaded with 20 μg of protein from JEG-3 cells, HAC15, H293-HSD3B1 and H293-HSD3B2 (Fig 2A). A high protein concentration was used to determine potential other immunoreactive bands that might be present. Different concentrations of placenta protein homogenate (15, 10, 5, and 1 μg) and a higher fixed concentration of an adrenal protein (20 μg) were also used to examine expression of HSD3B1 protein in each tissue (Fig 3B). To

determine specificity for the HSD3B2 monoclonal antibody, a 12% gel was loaded with protein from the H295R (10 and 30 μg), with 30μg of H293-HSD3B1 and H293-HSD3B2 (Fig 2B). Different concentrations of adrenal protein homogenate (15, 10, 5, and 1 μg) and a higher fixed concentration of a placenta protein (20 μg) were also used to examine expression of HSD3B2 in each tissue (Fig 3C).

To determine if the expression of the HSD3B1 and HSD3B2 enzymes could be induced by stimulation of the HAC15 cell, cells were stimulated for 24 hrs. with angiotensin II (100 nM), Forskolin (10μM) or potassium chloride and the cells extracted as above.

Immunohistochemistry and immunofluorescence. Paraffin embedded formalin fixed normal human adrenal glands and placenta blocks and slides were used for staining.. Six micron samples in positive glass slides were heated at 56 °C for about 3-4 h. deparaffinized and then subjected to antigen retrieval (diethanolamine 0.02M, EDTA 5 mM pH 9) for 45 min in a steamer. Endogenous peroxidase activity was blocked with phenyl hydrazine 0.1% for 15 min. The tissues were blocked with a buffer containing 5% goat serum in TRIS buffer (0.05M pH 7.4) for 1 h. The tissues were then incubated with the different antibodies with the same blocking buffer overnight. After washing the slides were incubated with goat anti-mouse polymerized HRP (ImmPRESS HRP goat anti-mouse from Vectorlabs.com) for 1 h, washed, then developed using ImmPACT DAB peroxidase substrate (Vectorlabs.com). Samples were washed and then counterstained with Vector hematoxylin. After washing and dehydrating they were mounted using DPX Mountant (Sigmaaldrich.com). To more clearly define the zona fasciculata from zona reticularis, a different slide from the same adrenal was stained with a cytochrome B5 monoclonal antibody (AM31963PU-N acris-antibodies.com).

Double immunofluorescence was done in similar fashion, normal adrenal slides were incubated with the HSD3B2, HSD3B1 and CYP11B2 [9]. After the primary antibody, the secondary antibody used was an isotype specific goat Alexa Fluor 594-anti-mouse IgG2b (HSD3B1 or HSD3B2) (Jacksonimmunoresearch.com) and an isotype specific goat Alexa Fluor 488 anti-mouse IgG1 (CYP11B2) for detection. DAPI was then used to label nuclei as previously reported [9].

Quantitative real-time RT-PCR of adrenal and placenta. Total RNA extraction from whole tissues and cDNA generation was performed as previously described [10]. Quantitative realtime RT-PCR (qPCR) for *HSD3B1* and *HSD3B2* was done in the ABI StepOnePlus Real-Time PCR systems (Applied Biosystems) with following specific primers, HSD3B1-F: CGGCTAACGGGTGGAATCTG, HSD3B1-Probe: ACGGCGGCACCCT, HSD3B1-R: CCCCATAGATATACATGGGTCGTAAG, HSD3B2-F: GCGGCTAATGGGTGGAATCTA, HSD3B2-Probe: TGATACCTTGTACACTTGTGCGTTAAGACCCA, and HSD3B2-R: CATTGTTGTTCAGGGCCTCAT. Peptidylprolyl isomerase A (*PPIA*) transcript was used as a reference gene for sample normalization. Primer and probe mixture for the amplification of PPIA target sequence (Hs99999904_m1) was purchased from Thermo Fisher Scientific. The delta-delta threshold cycle (ΔΔCt) method was used to calculate fold changes in mRNA expression [11].

Results

HSD3B1

There were 32 positive clones by ELISA (out of 960 wells) in the initial screening, of which 12 gave a clear single band at the correct molecular mass in the western blot screening. Although all these clones also gave clear staining by IHC, a clone with the lowest non-specific staining and strongest staining in a placenta sample was selected and expanded (4C1, IgG2b isotype). Fig 1 shows the lack of binding of the HSD3B1-4C1 antibody to the wells coated with the HSD3B2 antigen. Fig 2A shows a western blot using this antibody in which a very strong band is observed with protein from whole homogenates from human choriocarcinoma cell line, JEG3 and H293TN cells transduced by a lentivirus with the HSD3B1. No bands could be observed from either the human adrenocortical carcinoma cell lines, HAC15 and H295R, or the H293TN cells transduced with a lentivirus with the HSD3B2. Fig 3B shows a western blot of different concentrations of placenta protein homogenate and a higher fixed concentration of an adrenal homogenate demonstrating expression in the placenta, but not in the adrenal. Stimulation of the adrenal carcinoma cell line HAC15 with the different secretagogues did not result in expression of the HSD3B1 (Fig 3A).

qPCR for the *HSD3B1* from adrenals (n=12) showed approximately 1/30,000 the expression of that of placentas (n=4) (Fig 2A). IHC of placenta showed very strong staining in intermediate trophoblast and syncytiotrophoblasts at a dilution of 1/4,000 dilution (Fig 5E) which remained clear at a 1/16,000 dilution (Fig 5F). In the adrenal there was no staining observed with 1/4000 antibody dilution (Fig 5C) that showed clear positive localization in the placenta. However, there was reactivity seen throughout the adrenal cortex, with significantly greater immunoreactivity in the ZG than the rest of the adrenal at dilutions of 1/500 (Fig 5A and 5B). Double IF staining for the HSD3B1 gave too low a signal to attempt to co-localize with the CYP11B2 (data not shown).

HSD3B2

There were 27 wells with media that were strongly positive by ELISA of which only one clone (clone 6, IgG2b isotype) detected a single band at the correct molecular mass on the screening western blot. There was no cross reactivity by ELISA using plates coated with the HSD3B1 antigen (Fig 1). There were 3 other clones that gave multiple bands with one of the bands at the correct molecular mass, but these were not pursued. Western blot (Fig 2B) shows a strong band at approximately 38 kDa mass for homogenates from the H295R cells and H293 cells transduced with the lentivirus with the cDNA for the HSD3B2 enzyme. The H293 transduced with the lentivirus for the cDNA for the HSD3B1 enzyme was negative. Western blot of different concentrations of adrenal homogenate gave clear bands that were clearly seen even at 5 μg of protein, while 20 μg of placenta homogenate did not give a signal (Fig 3C). Stimulation of the adrenal carcinoma cell line HAC15 with the different secretagogues did not modify the expression of the HSD3B2 (Fig 4B).

Immunohistochemistry exhibited strong staining in the zonas glomerulosa and fasciculata (Fig 6A and D), but not in the zona reticularis. As a positive control, the zona reticularis was

strongly stained by the cytochrome B5 antibody (Fig 6B). As expected, the CYP11B2 antibody stained some cells of the zona glomerulosa and aldosterone-producing cell clusters (APCC). Double immunofluorescence showed extensive staining of the zona glomerulosa and fasciculata for the HSD3B2 and co-localization in some areas of the zona glomerulosa with the CYP11B2 (Fig 6 E and 5F).

Discussion

Specific antibodies against the human HSD3B1 and HSD3B2 were developed from mice immunized against peptides that differed by two out of fourteen amino acids. In combination with qPCR, these antibodies were used to examine adrenal expression of protein and mRNA for both isozymes. Mice with double deletion of the cryptochrome 1 and 2 developed salt-sensitive hypertension and hyperaldosteronism [5, 6, 12] and a search for steroidogenic enzymes overexpressed in the adrenal showed that the Hsd3b6 was overexpressed in the zona glomerulosa. Of interest the Cyp11b2 enzyme, the last enzyme in the biosynthesis of aldosterone expression was unchanged and the group reached the conclusion that overexpression of the Hsd3b6 resulted in an increased in substrate leading to the increase in aldosterone biosynthesis [5, 6].

While the mouse has 6 *Hsd3b* genes, humans only have two *HSD3B* genes, *HSD3B1* and *HSD3B2* [3]. The mouse Hsd3b6 is the equivalent to the HSD3B1 and it was reported that the human zona glomerulosa expressed the HSD3B1 enzyme while both the zona glomerulosa and the zona fasciculata expressed the HSD3B2 [4]. Adrenals from patients with primary aldosteronism due to idiopathic hyperaldosteronism also exhibited increased immunoreactivity of the zona glomerulosa by the HSD3B1 antibody used in the earlier study [4]. However, as they reported and one can see in the immunohistochemistry of the HSD3B2 of their report, the HSD3B2 enzyme is strongly expressed in the zona glomerulosa and fasciculata in both the normal adrenal, idiopathic hyperaldosteronism and in aldosterone-producing adenomas [4]. Others have reported that human adrenals with aldosterone-producing adenomas express much higher amount of HSD3B2 enzyme compared to HSD3B1, in the adenoma and the adjacent adrenal [7]. The expression of the *HSDB1* mRNA was significantly lower than *HSD3B2*, but it correlated with the expression of the *CYP11B2* in adenomas [7]. Weak scattered staining of HSD3B1 was seen while stronger and more uniform staining was seen with the HSD3B2. The presence of the mRNA for the HSD3B1 does not necessarily mean that the protein is translated especially if the expression of the mRNA is low [7]. It is difficult to establish if the HSD3B1 plays a role in the zona glomerulosa considering the very low level of expression, but it remains possible as the affinity of the enzyme for its substrate is significantly higher than the HSD3B2 [13].

Our studies indicate that the mRNA expression of the *HSD3B1* in the adrenal is very low compared to placenta (Fig 3A); our antibody can only detect this isozyme in the adrenal using concentrations of the antibody at least 10 times greater than that required for clear immunoreactivity in the placenta (Fig 4 B-E). Our findings differ significantly from those of Doi *et al* [4] in that we did not find high expression of the HSD3B1 in the zona glomerulosa of the normal human adrenal.

In summary, we have developed very specific monoclonal antibodies against the two human HSD3B enzymes and have confirmed the classical concepts that the HSD3B2 is expressed in the adrenal and the HSD3B1 is expressed in the placenta with very low expression in the adrenal.

Acknowledgments

Research reported in this publication was supported by National Heart, Lung and Blood Institute grant R01 HL27255 (CEG), the National Institute of General Medical Sciences grant U54 GM115428 (CEG) and the National Institute of Diabetes and Digestive and Kidney grant R01 DK43140 and R01 DK06995 (WER). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

References

1. Miller WL, Auchus RJ. The molecular biology, biochemistry, and physiology of human steroidogenesis and its disorders. Endocr Rev. 2011; 32(1):81–151. [PubMed: 21051590]
2. Prasad M, Thomas JL, Whittal RM, Bose HS. Mitochondrial 3beta-hydroxysteroid dehydrogenase enzyme activity requires reversible pH-dependent conformational change at the intermembrane space. The Journal of biological chemistry. 2012; 287(12):9534–46. [PubMed: 22262841]
3. Simard J, Ricketts ML, Gingras S, Soucy P, Feltus FA, Melner MH. Molecular biology of the 3beta-hydroxysteroid dehydrogenase/delta5-delta4 isomerase gene family. Endocrine reviews. 2005; 26(4):525–82. [PubMed: 15632317]
4. Doi M, Satoh F, Maekawa T, Nakamura Y, Fustin JM, Tainaka M, Hotta Y, Takahashi Y, Morimoto R, Takase K, Ito S, Sasano H, Okamura H. Isoform-specific monoclonal antibodies against 3beta-hydroxysteroid dehydrogenase/isomerase family provide markers for subclassification of human primary aldosteronism. The Journal of clinical endocrinology and metabolism. 2014; 99(2):E257–62. [PubMed: 24423300]
5. Doi M, Takahashi Y, Komatsu R, Yamazaki F, Yamada H, Haraguchi S, Emoto N, Okuno Y, Tsujimoto G, Kanematsu A, Ogawa O, Todo T, Tsutsui K, van der Horst GT, Okamura H. Salt-sensitive hypertension in circadian clock-deficient Cry-null mice involves dysregulated adrenal Hsd3b6. Nat Med. 2010; 16(1):67–74. [PubMed: 20023637]
6. Okamura H, Doi M, Yamaguchi Y, Fustin JM. Hypertension due to loss of clock: novel insight from the molecular analysis of Cry1/Cry2-deleted mice. Current hypertension reports. 2011; 13(2):103–8. [PubMed: 21286865]
7. Konosu-Fukaya S, Nakamura Y, Satoh F, Felizola SJ, Maekawa T, Ono Y, Morimoto R, Ise K, Takeda K, Katsu K, Fujishima F, Kasajima A, Watanabe M, Arai Y, Gomez-Sanchez EP, Gomez-Sanchez CE, Doi M, Okamura H, Sasano H. 3beta-Hydroxysteroid dehydrogenase isoforms in human aldosterone-producing adenoma. Molecular and cellular endocrinology. 2015; 408:205–12. [PubMed: 25458695]
8. Davis JM. A single-step technique for selecting and cloning hybridomas for monoclonal antibody production. Meth in Enzymol. 1986; 121:322–327. [PubMed: 3724471]
9. Gomez-Sanchez CE, Qi X, Velarde-Miranda C, Plonczynski MW, Parker CR, Rainey W, Satoh F, Maekawa T, Nakamura Y, Sasano H, Gomez-Sanchez EP. Development of monoclonal antibodies against human CYP11B1 and CYP11B2. Molecular and cellular endocrinology. 2014; 383(1-2): 111–7. [PubMed: 24325867]
10. Xing Y, Nakamura Y, Rainey WE. G protein-coupled receptor expression in the adult and fetal adrenal glands. Molecular and cellular endocrinology. 2008
11. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods. 2001; 25(4):402–8. [PubMed: 11846609]
12. Nugrahaningsih DA, Emoto N, Vignon-Zellweger N, Purnomo E, Yagi K, Nakayama K, Doi M, Okamura H, Hirata K. Chronic hyperaldosteronism in cryptochrome-null mice induces high-salt- and blood pressure-independent kidney damage in mice. Hypertension research: official journal of the Japanese Society of Hypertension. 2014; 37(3):202–9. [PubMed: 24108235]

13. Thomas JL, Mason JI, Brandt S, Norris W. Differences in substrate and inhibitor kinetics of human type 1 and type 2 3beta-hydroxysteroid dehydrogenase are explained by the type 1 mutant, H156Y. Endocrine research. 2002; 28(4):471–5. [PubMed: 12530651]

14. Hattangady NG, Olala LO, Bollag WB, Rainey WE. Acute and chronic regulation of aldosterone production. Molecular and cellular endocrinology. 2012; 350(2):151–62. [PubMed: 21839803]

Highlights

- Monoclonal antibody against the human HSD3B1 enzyme was developed.
- Monoclonal antibody against the human HSD3B2 enzyme was developed.
- Each antibody was specific for the corresponding protein.
- Adrenal zona glomerulosa only expressed the HSD3B2 enzyme.

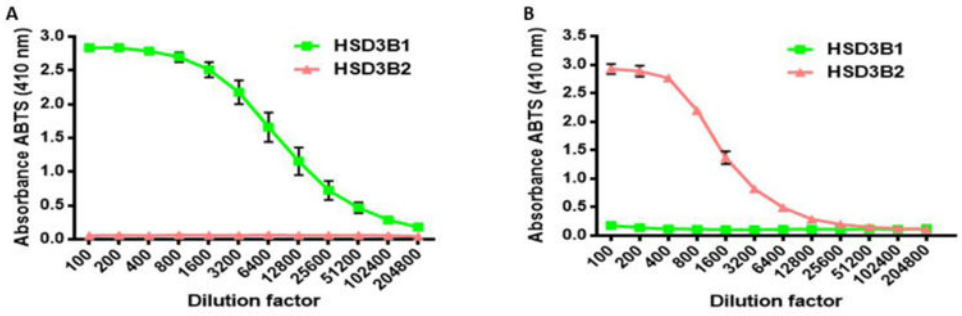

Figure 1.

A. Antibodies HSD3B1-4C1 and HSD3B2-6 dilutions were incubated in a microtiter plate coated with HSD3B1-BSA (A) and developed showing that only the HSD3B1-4C1 antibody was able to bind the immobilized peptide conjugate. B. Antibodies HSD3B1-4C1 and HSD3B2-6 dilutions were incubated in a microtiter plate coated with HSD3B2-BSA and developed showing that only the HSD3B2-6 antibody was able to bind the immobilized peptide conjugate.

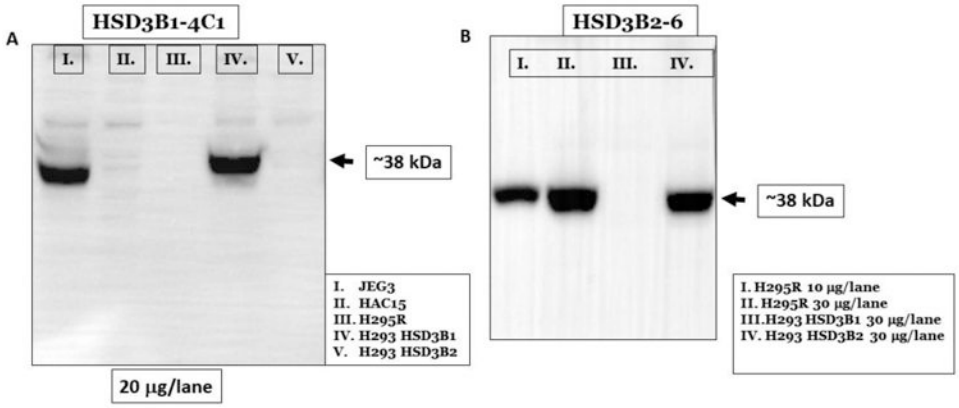

Figure 2.

A. Western blot using monoclonal antibody against HSD3B1-4C1. Protein extracted from cell homogenates. Lane I: JEG3 cells, Lane II: HAC15 cells. Lane III: H295R cells, Lane IV: H293-HSD3B1 expressing cells, Lane V: H293-HSD3B2 expressing cells. B. Western blot using monoclonal antibody against HSD3B2-6: Lane I and II: H295R cells (10µg and 30 µg). Lane III: H293-HSD3B1 expressing cells. Lane IV: H293-HSD3B2 expressing cells.

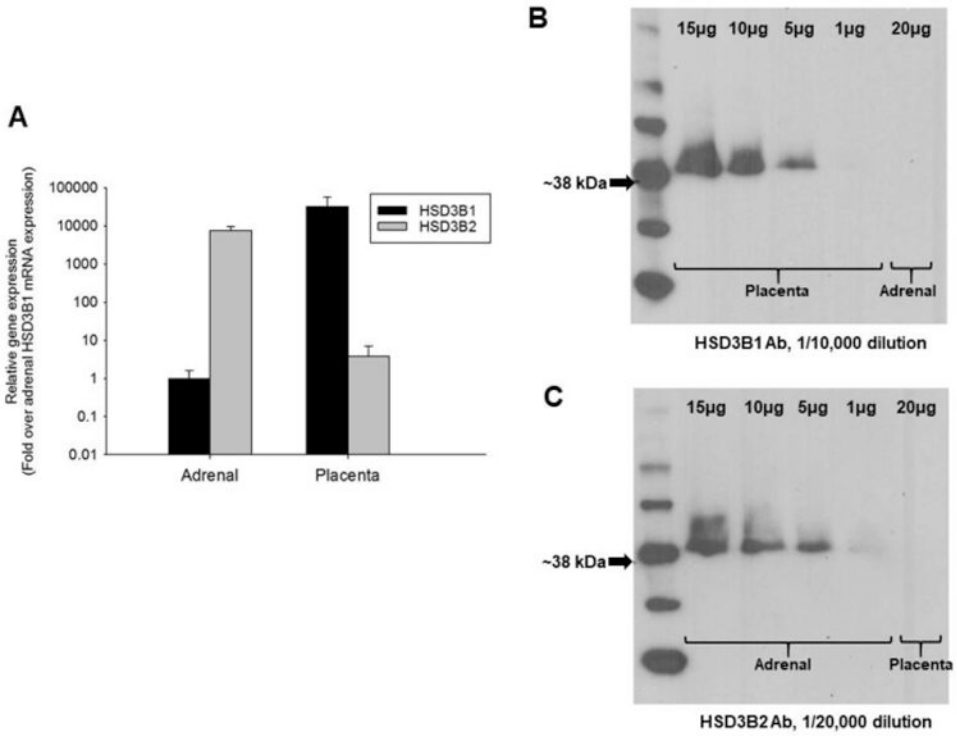

Figure 3.

A. qPCR of mRNA for *HSD3B1* and *HSD3B2* in human adrenals and placentas. B. Western blot of various protein amounts of placenta homogenate extract (15, 10, 5, 1 µg per lane) and a larger fixed amount adrenal homogenate (20 µg) reacted with the HSD3B1 4C1 antibody at 1/10,000 dilution. C. Western blot of various protein amounts of adrenal homogenate extract (15, 10, 5, 1 µg per lane) and a larger fixed amount placenta homogenate (20 µg) reacted with the HSD3B2-6 antibody at 1/20,000 dilution.

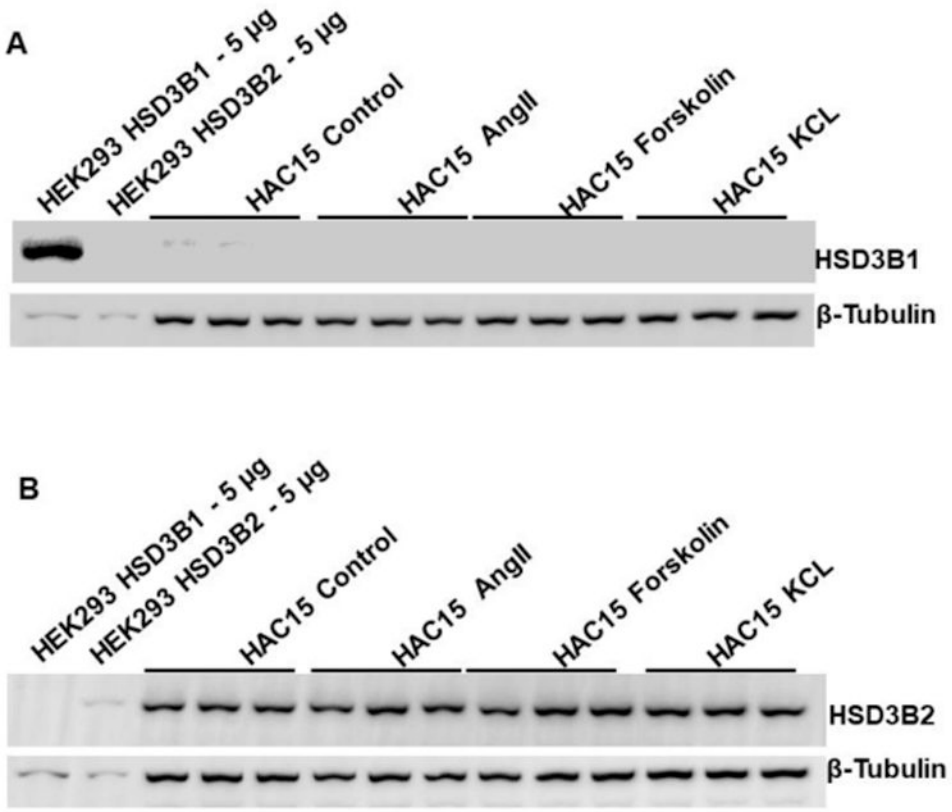

Fig 4.

Western blot of homogenate extracts of HEK293 HSD3B1, HEK HSD3B2 and HAC15 cells incubated with vehicle, angiotensin II (100 nM), Forskolin (10µg) and potassium chloride (12 mM) for 24 hrs. and probed with the HSD3B1 antibody (panel A) and the HSD3B2 antibody (panel B).

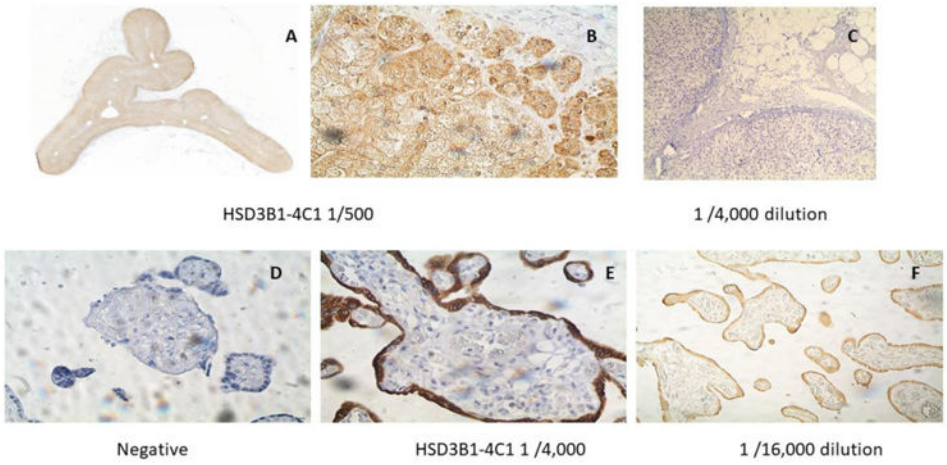

Figure 5.

A & B: Immunohistochemistry of a scanned whole adrenal section with IHC performed with an anti-HSD3B1-4C1 antibody at a 1/500 concentration. C: IHC performed with an anti-HSD3B1-4C1 antibody at a 1/4,000 concentration. D: Negative control with no primary antibody. E: Placenta incubated with an anti-HSD3B1-4C1 antibody at a 1/4,000 concentration. F: Placenta incubated with an anti-HSD3B1-4C1 antibody at a 1/16,000 concentration.

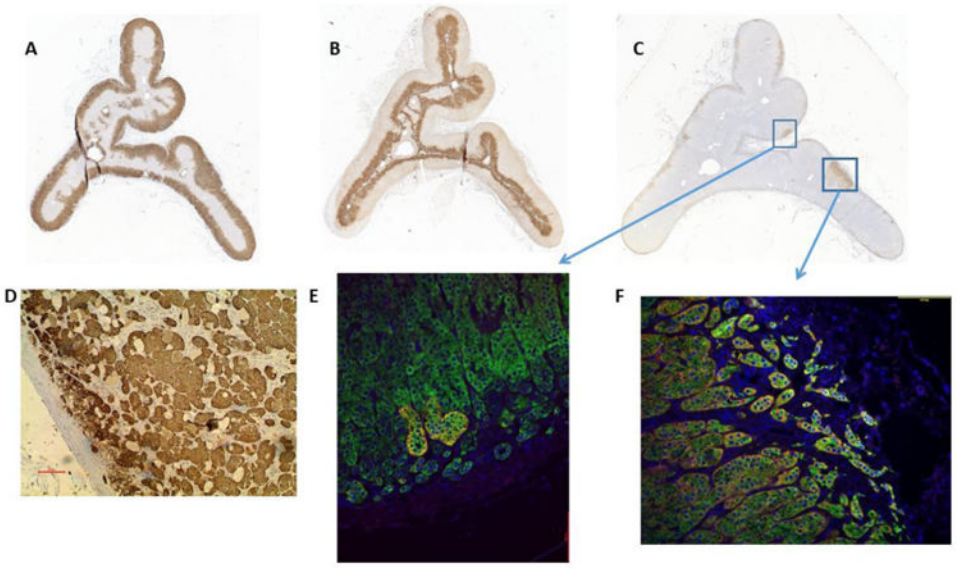

Figure 6.

A: Immunohistochemistry of a scanned whole adult adrenal section processed for IHC using an anti-HSD3B2-6 antibody at a 1/1,000 concentration. B: IHC of adrenal immunostained with anti-cytochrome B5 antibody at a 1/15,000 concentration. C: IHC of adrenal immunostained with anti-CYP11B2 (CYP11B2-41-13) antibody at a 1/30,000 concentration showing zona glomerulosa immunoreactivity. D. Adrenal immunostained with HSD3B2-6 antibody at a 1/1,000 concentration showing staining of the zona glomerulosa and fasciculata. E and F. Double immunofluorescence for CYP11B2 (red) and HSD3B2 (green) in two sections of the zona glomerulosa corresponding to the boxed sections of C.