Received: 20 November 2023 | Revised: 9 February 2024 | Accepted: 9 February 2024

DOI: 10.1002/pmic.202300616

Proteomics
Proteomics and Systems Biology

DATASET BRIEF

Prolonged exposure to dexamethasone alters the proteome and cellular phenotype of human testicular peritubular cells

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Funding information
Deutsche Forschungsgemeinschaft, Grant/Award Number: 427588170

Abstract

Human testicular peritubular cells (HTPCs) are smooth muscle cells, which in the testis form a small compartment surrounding the seminiferous tubules. Contractions of HTPCs are responsible for sperm transport, HTPCs contribute to spermatogenesis, have immunological roles and are a site of glucocorticoid receptor expression. Importantly, HTPCs maintain their characteristics in vitro, and thus can serve as an experimental window into the male gonad. Previously we reported consequences of 3-day treatment with Dexamethasone (Dex), a synthetic glucocorticoid and multipurpose anti-inflammatory drug. However, as glucocorticoid therapies in man often last longer, we now studied consequences of a prolonged 7-day exposure to 1 μM Dex. Combining live cell imaging with quantative proteomics of samples taken from men, we confirmed our recent findings but more importantly, found numerous novel proteomic alterations induced by prolonged Dex treatment. The comparison of the 7-day treatment with the 3-day treatment dataset revealed that extracellular matrix- and focal adhesion-related proteins become more prominent after 7 days of treatment. In contrast, extended stimulation is, for example, associated with a decrease of proteins related to cholesterol and steroid metabolism. Our dataset, which describes phenotypic and proteomic alterations, is a valuable resource for further research projects investigating effects of Dex on human testicular cells.

KEYWORDS
dexamethasone, human male fertility, human testis

Human testicular peritubular cells (HTPCs) are contractile smooth muscle cells [1], which together with extracellular matrix (ECM) form the peritubular compartment within the testis. This region is located between the compartment, in which spermatogenesis takes place, and the interstitial areas, which contain Leydig cells and blood vessels [2–4]. Their localization at the "interphase" between the testosterone-producing Leydig cells and Sertoli cells/germ cells enables them to interact with both, the tubular and the interstitial compartments [5]. Contractions of HTPCs cause sperm transport in the testis [6], but these cells also participate in testicular immune surveillance, are important for the spermatogonial stem cell niche and are involved in paracrine signaling [1, 7–9]. Of note, these human testicular cells can

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be isolated and studied in vitro, as they largely retain their in situ phenotype [1], thereby opening a valuable research opportunity of the male gonad [10]. Androgen and glucocorticoid receptor (GR, encoded by *NR3C1*) were unveiled in HTPCs in situ and in vitro [11–13] and their expression implies regulation by steroids. With particular respect to glucocorticoids (GC), clinical evidence points towards impaired testicular function upon exposure to excess or, to a less studied extent, decreased levels of GCs [14–17]. Recently, it was demonstrated that a clinically used synthetic GC, Dexamethasone (Dex), induced phenotypic changes in HTPCs, including increased expression of smooth muscle markers and altered composition of the ECM – a remodeling potentially associated with its stiffening due to increased accumulation of extracellular proteins, for example, the collagen- and laminin-family members [13, 18]. In this previous study, we investigated the effects of a 24 h and a 72 h Dex stimulation of HTPCs on proteome, secretome and cytokine levels, and evaluated the effects with respect to treatment time [18].

As patients frequently receive long-term treatment with Dex, a common drug for the treatment of rheumatoid and autoimmune diseases, allergies and ocular disorders, we now performed a further study to assess the impact of Dex on HTPCs for a longer, 7-day period. To assure comparability to our recently published work [18], experiments were carried out using the same analytical procedure. The overall workflow is displayed in Figure 1A.

HTPCs were prepared from small testicular biopsies of men (5–7 donors, 29–55 years) as previously established [18]. For proteomics each biological replicate was analyzed in triplicates ($n = 3$ Dex treated vs. $n = 3$ control samples). The study was carried out in accordance with the relevant guidelines and regulations. All participants granted written informed consent about use of their biological material for research purposes [18]. Treatment of HTPCs with 1 µM Dex (#1756; Sigma-Aldrich, Hamburg, Germany) occurred every second day for up to 7 days including controls, as outlined in detail previously [18]. Live cell images of cultured HTPCs at day 0 and day 7 of the experiment were collected (Leica DM IL LED microscope, Leica DFC3000G camera, Leica Microsystems GmbH, Wetzlar, Germany) and cell number and viability of HTPCs was assessed by an automated cell counting device as previously described [13]. Strikingly, when cultured in the presence of Dex for 7 days, HTPCs showed alterations of cellular morphology (Figure 1B). The HTPC-typical, elongated spindle-shaped form turned into a more bulky shape, as previously seen after a 3-day treatment and also in the context of replicative senescence [13]. In accordance with our 3-day treatment study, Dex neither influenced cell number after 7 days nor affected cell viability of HTPCs substantially. As found previously after 48 h, the levels of immunoreactive GR protein in whole cell lysates of HTPCs were reduced after 7 days (Supplementary figure).

Proteome analysis was performed as described in earlier: upon filtering for set significance criteria the proteins of lower and higher abundance were visualized by a 3 d- and a 7 d-Volcano plots (Figure 1C) [18]. Briefly, 10^6 cells per sample were lysed in a buffer consisting of 8 mol/L urea in 50 mmol/L NH_4HCO_3 and sonicated. Cysteines were reduced using 1,4-dithiothreitol and alkylation was performed with iodoacetamide. Protein digestion was performed using lysyl-endopeptidase C (1:100 enzyme/protein ratio, Lys C; Fujifilm Wako, Neuss, Germany) for 3 h at 37°C and overnight at 37°C with modified porcine trypsin (1:50 enzyme/protein ratio, Promega, Madison, WI, USA). For nano LC-MS/MS analysis 1.5 µg of peptides were loaded on an Ultimate 3000 RSLC instrument connected to a Q Exactive HF-X mass spectrometer (Thermo Scientific, Waltham, MA, USA) and separated on an EASY-spray column (Pepmap™ RSLC C18, 2 µm, 100 Å, 75 µm × 50 cm, Thermo Scientific, Waltham, MA, USA) at a flow rate of 250 nL/min. A two-step gradient from 6% B (0.1% (v/v) formic acid in acetonitrile) to 20% B in 80 min followed by a ramp to 40% B for 9 min was used. For data aquisition, a top 15 DDA method was used. For data analysis MaxQuant (1.6.11.0) using the *H. sapiens* subset of the Swiss-Prot (retrieval: 10/2020) was used. The analysis of the samples from the 7-day treatment experiment led to the identification of 52,838 unique peptides corresponding to 4301 proteins. In order to perform the "3-day vs 7-day Dex stimulation" comparison, our recently published dataset was used [18]. Statistics (Welch's *t*-test), volcano plots, principal component analysis (PCA) and heatmaps were performed with Perseus v1.6.5.0 [19] and R Statistical Software (v4.3.0; R Core Team 2023). For multiple testing correction and significance cut-off curves s0 = 0.1 and FDR <0.05 was applied. In total, 56 and 70 differentially abundant proteins (|log2 fold change| > 0.6, adjusted *p*-value (*q*-value) < 0.05) in the 3day- and 7day-experiment were detected, respectively (supplementary tables "Hits_3d_diffAbundance" and "Hits_7d_diffAbundance"). Of those, 19 entries were mutual ("Hits_3d_diffAbundance").

For the functional enrichment analysis (Figure 2), the "Database for Annotation, Visualization and Integrated Discovery" (DAVID) was used with the following parameters: BP_ALL, CC_ALL, MF_ALL, and KEGG and Reactome Pathways checked; stringency "high." Results with an enrichment score ≥1.3 and *p*-value ≤0.05 were plotted.

For the 3-day treated cells, proteins related to cellular motility, adhesive properties, as well as ECM structure were less abundant, whereas proteins related to cellular signaling and metabolism were more abundant (Figure 2A). Notably the functional enrichment analysis of the 7-day dataset (Figure 2B) displayed additional terms, that is, apoptosis-related and proteins associated to cholesterol and steroid metabolism/biosynthesis were decreased in abundance. Of particular note, the terms "ECM structural constituent conferring tensile strength (protein count: 3; *p*-value: 0.00348)" and "cell adhesion (protein count: 9; *p*-value: 0.00015)" appeared in the 7-day dataset for the more abundant proteins but in the 3-day dataset for the less

Statement of significance

This dataset extends the knowledge of glucocorticoid receptor mediated, dexamethasone-induced changes in human testicular peritubular cells, describes the phenotypic and proteomic changes, and thus provides a resource of novel protein targets for future research.

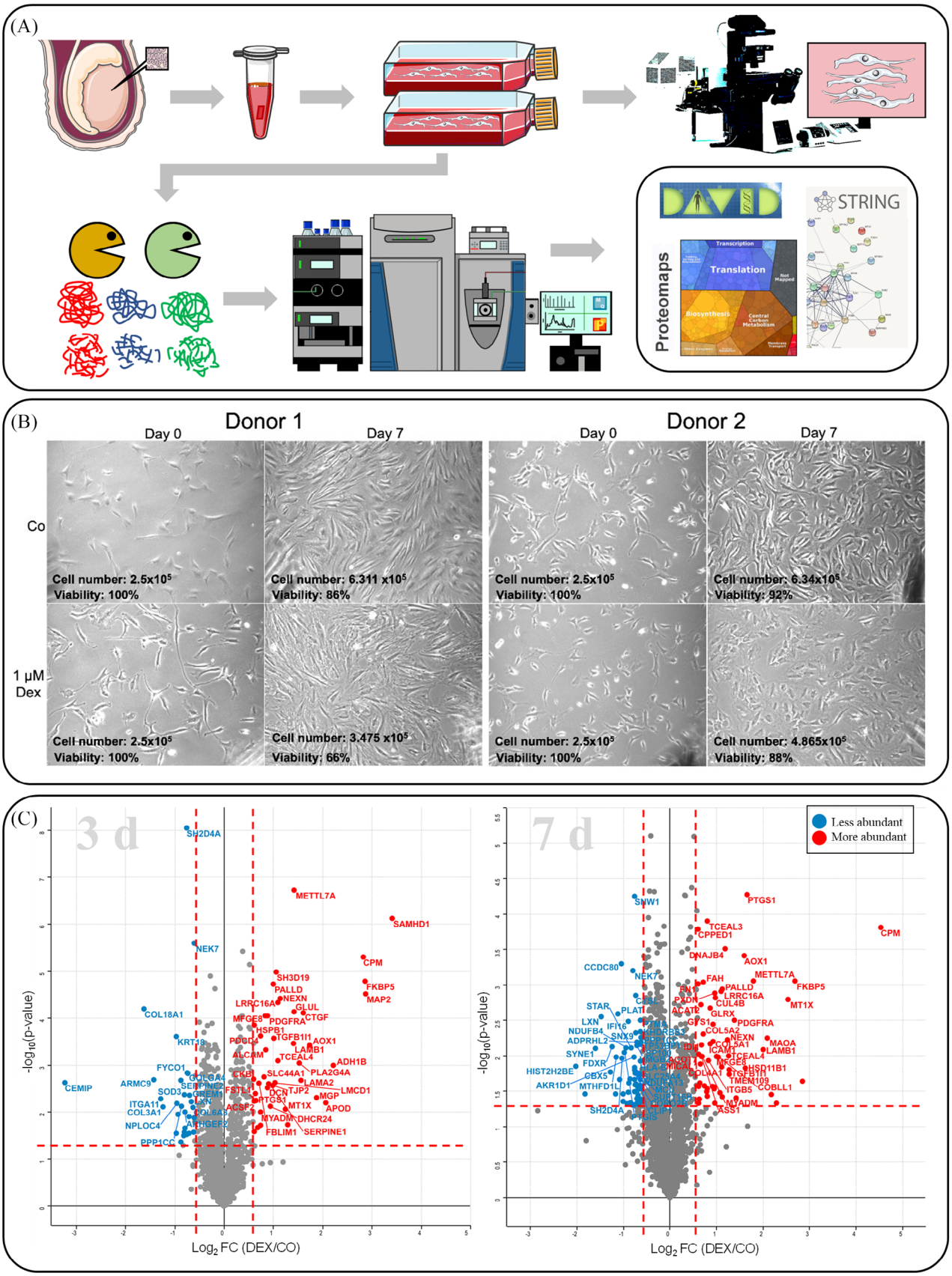

FIGURE 1 (A) Experimental design. (B) Live cell imaging of two donors. (C) Volcano plots: less (blue) and more (red) abundant proteins upon Dex stimulation for the 3 d—vs. 7 d experiments.

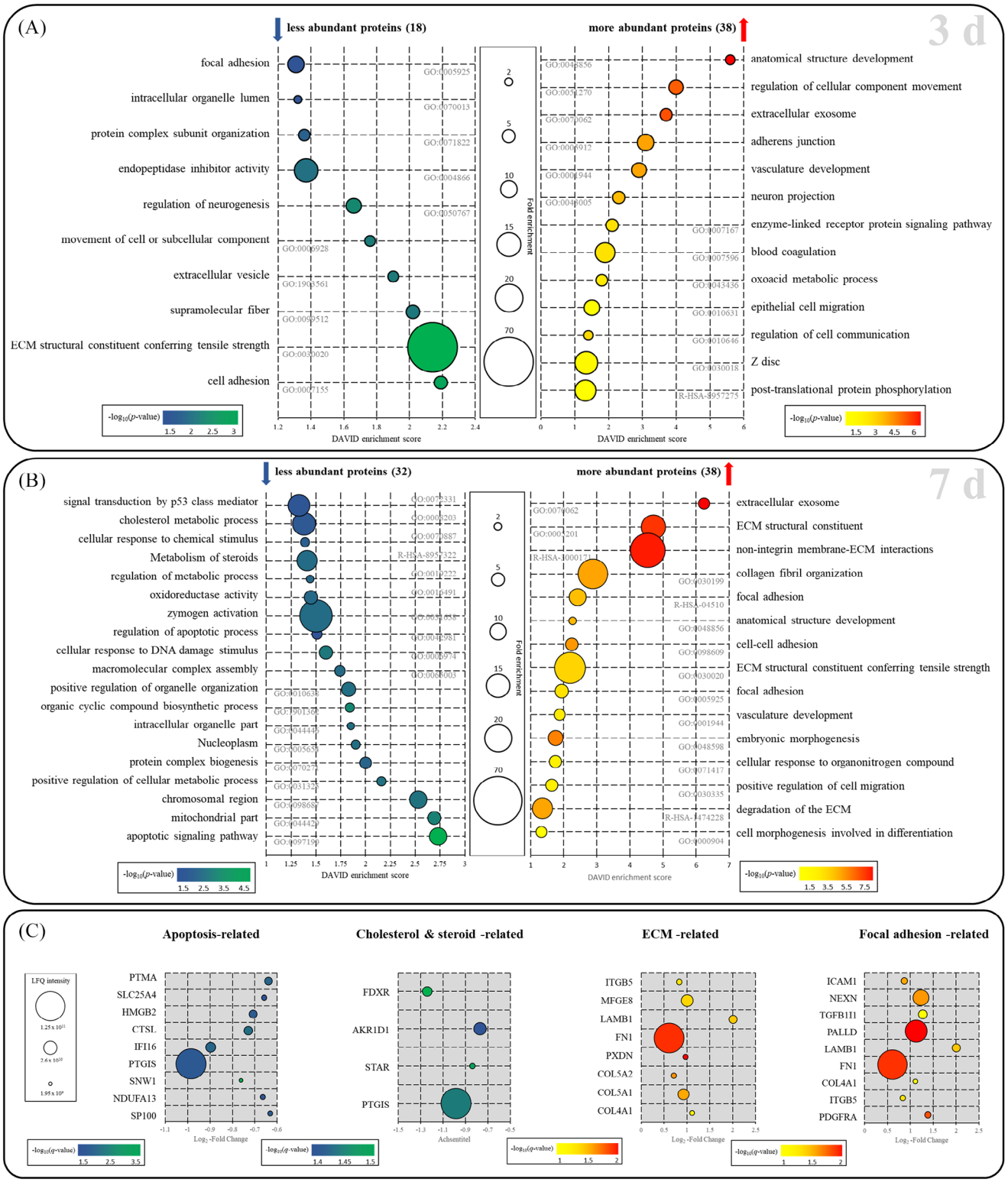

FIGURE 2 (A) Bubble plots for proteins less versus more abundant upon 3 days of stimulation with Dex. (B) Bubble plots for proteins less versus more abundant upon 7 days of stimulation with Dex. (C) GO-terms simultaneously picked out, grouped with relation to "Apoptosis," "Cholesterol + Steroid metabolism," "ECM," and "Focal Adhesion," and filtered for unique proteins.

abundant proteins (protein count: 7; *p*-value: 0.00119). Finally, decreased abundance of proteins associated with either "immune relevance" or involvement in "inflammatory response" became more obvious when Dex exposure was extended to 7 days.

Our previous studies investigated Dex effects on HTPCs for up to 3 days. We extended the time period to 7 days and observed numerous similarities including abundance alterations of the previously reported candidates FKBP5, NEXN, PALLD, LAMB1, and ICAM1 [18]. The consequences on the cellular shape were also comparable, with bulky and irregular morphological appearance upon Dex stimulation for 3 or 7 days. Importantly however, a number of distinct novel aspects became apparent. In contrast to our 3-day based findings, an increased abundance of several collagen-family proteins (COL4A1, -5A1, and -5A2), as well as fibronectin (FN1) were found together with increased levels of transforming growth factor beta-1-induced transcript 1 protein (TGFB1I1). TGFB1 was shown to promote accumulation of collagens and other extracellular proteins in vivo [20]. This situation may lead to the deposition of collagens into and stiffening of the ECM in situ, as reported for tumor initiation and cancer progression [20, 21] or fibrotic remodeling. In humans, fibrotic remodeling and thickening of the peritubular compartment is often seen in the testis of men with impairments of spermatogenesis [7, 22]. Also in contrast to our previously published findings, mitochondrial steroidogenic acute regulatory protein (StAR) attributed to steroid biosynthesis [23–25], displayed a decreased abundance in the 7-day dataset. This finding was additionally accompanied by reduced levels of steroid A-ring 5ß-reductase (AKR1D1), a protein involved in catabolism of steroids, but also reported to play a role in regulating GR activation [26, 27]. We further observed an increased abundance of 11-beta-hydroxysteroid dehydrogenase 1 (HSD11B1) important for conversion of biologically active GCs [28, 29], which is also required for testicular development [30].

Of further interest are decreased levels of proteins involved in cellular anti-inflammatory response. Among them were prothymosin alpha (PTMA) and gamma-interferon-inducible protein 16 (IFI16). The first mentioned possesses extracellular immunoenhancing roles [31] and was recently demonstrated to be expressed in the human testis, and is claimed to be involved in testicular cancer development [32]. The latter is a cytokine-family type protein crucially mediating anti-inflammatory actions carried out by type-I interferons [33, 34]. In humans IFI16 is supposed to be a functional modulator of GR function [35] associated with a possible role in testicular carcinoma development [36]. Though, the overall decrease of proteins possessing immune and/or inflammatory relevance is in line with the general mode of action by Dex in "streamlining" the reduction of inflammatory phenotype. Finally, regarding possible effects on cell death, among others, the high mobility group protein B2 (HMGB2) was identified. This protein is involved in apoptosis, DNA repair as well as cellular response to oxidative stress, and is expressed at rather high levels in the human testis [37, 38]. Another candidate worth mentioning in this regard is the mitochondrial protein NADH dehydrogenase (ubiquinone) 1 alpha subunit 13 (NDUFA13) also known as GRIM19, which is an apoptosis-inducing protein [39–41]. GC actions on apoptosis have been assessed as highly tissue-specific, ranging from pro- to anti-apoptotic. When observing the male reproductive system alone, numerous studies reported adverse, that is, apoptosis-inducing effects of Dex on male rat testicular germ cells and Leydig cells [42], while actions on peritubular cells have not been reported to our knowledge.

In conclusion, our results demonstrate that Dex alters the proteomic phenotype of HTPCs in a time-dependent way and entails profound changes in cellular homeostasis and the cellular phenotype. Compared to a 3-day exposure, prolonged 7-day Dex treatment induced additional distinct changes. If transferable to the in situ situation, long-term Dex therapies in men may thus have implications on testicular health. Remodeling of the cytoskeletal architecture of peritubular cells and alterations in ECM composition may have consequences for contractility. Also cellular homeostasis and metabolism and immune relevant factors could be affected. Further studies are required to examine, whether the changes described in vitro occur in situ and might be a possible risk factor for male fertility.

ACKNOWLEDGMENTS

The authors would like to thank Lena Wilde and Karolina Caban for their helpful advice. We further thank Miwako Kösters, Nicole Kreitmair, and Michaela Schneider for excellent technical support.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The mass spectrometry data were deposited to the ProteomeXchange Consortium (www.proteomexchange.org, accessed on 10.10.2023) via the Proteomics Identification Database (PRIDE) partner repository with the dataset identifiers PXD033504 for the 72 h dataset, and PXD046287 for the 7 days proteome dataset [43].

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How to cite this article: Stepanov, Y. K., Herrmann, C., Stöckl, J. B., Köhn, F.-M., Pickl, U., Trottmann, M., Fröhlich, T., Mayerhofer, A., & Welter, H. (2024). Prolonged exposure to dexamethasone alters the proteome and cellular phenotype of human testicular peritubular cells. *Proteomics*, e2300616. https://doi.org/10.1002/pmic.202300616