Retinoid signaling controlled by SRC-2 in decidualization revealed by transcriptomics

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Abstract

Establishment of a successful pregnancy requires not only implantation of a healthy embryo into a receptive uterus but also progesterone receptor (PGR)-dependent transformation of endometrial stromal cells (ESCs) into specialized decidual cells. Decidual cells support the developing embryo and are critical for placentation. We have previously shown that a known transcriptional coregulator of the PGR, steroid receptor coactivator-2 (SRC-2), is a critical driver of endometrial decidualization in both human and mouse endometrium. However, the full spectrum of genes transcriptionally controlled by SRC-2 in decidualizing ESCs has not been identified. Therefore, using an RNA- and chromatin immunoprecipitation-sequencing approach, we have identified the transcriptome of decidualizing human ESCs (hESCs) that requires SRC-2. We revealed that the majority of hESC genes regulated by SRC-2 are associated with decidualization. Over 50% of SRC-2-regulated genes are also controlled by the PGR. While ontology analysis showed that SRC-2-dependent genes are functionally linked to signaling processes known to underpin hESC decidualization, cell membrane processes were significantly enriched in this analysis. Follow-up studies showed that retinoid signaling is dependent on SRC-2 during hESC decidualization. Specifically, SRC-2 is required for full induction of the retinol transporter, stimulated by retinoic acid 6 (STRA6), which is essential for hESC decidualization. Together our findings show that a critical subset of genes transcriptionally reprogrammed by PGR during hESC decidualization requires SRC-2. Among the multiple genes, pathways and networks that are dependent on SRC-2 during hESC decidualization, first-line analysis supports a critical role for this coregulator in maintaining retinoid signaling during progesterone-driven decidualization.

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Introduction

For healthy couples, natural conception is remarkably inefficient with a success rate of only 30–40% per menstrual cycle (Wilcox *et al.* 1988, Zinaman *et al.* 1996, Macklon *et al.* 2002). While 30% of these early pregnancy failures are linked to impaired embryo development, 70% are associated with a dysfunctional uterus during the periimplantation period (Macklon *et al.* 2002). Periimplantation failure also compromises the full potential of assisted reproductive technologies, which rely on the transfer of healthy embryos into a receptive uterus (Blesa *et al.* 2014). In addition to the clinical challenges, such early pregnancy losses impose severe emotional distress (Greil *et al.* 2010) and economic

burdens on women and their families worldwide (Chambers *et al.* 2014).

Formation of the decidua, through a process termed decidualization, is essential for advancement of the implantation process toward placentation (Cha *et al.* 2012). Decidualization entails the rapid proliferation and differentiation of fibroblastic endometrial stromal cells into polygonal epithelioid decidual cells (Gellersen & Brosens 2014). While modulating trophoblastic invasion into the maternal compartment that leads to placentation, the decidua also supports the developing embryo by providing histotrophic nutrition and local immunosuppression (Carson *et al.* 2000, Gellersen *et al.* 2007). Importantly, inadequate decidualization not only leads to fetal demise at an early stage of gestation but

can potentially cause adverse pregnancy outcomes in subsequent trimesters (Wang & Dey 2006, Norwitz *et al.* 2015). Progesterone (P4), via its cognate nuclear receptor, the progesterone receptor (PGR), is essential for endometrial decidualization (Lydon *et al.* 1995). Using genome-wide analysis, a number of studies have recently shown that PGR-mediated transcriptional reprogramming of human endometrial stromal cells (hESCs) underpins P4-dependent decidualization of this cell type (Cloke *et al.* 2008, Mazur *et al.* 2015). However, little is known concerning the involvement of the transcriptional coregulators that modulate PGR action during this cellular transformation process.

The p160/steroid receptor coactivator (SRC) family consists of three evolutionary conserved coregulators of transcription, termed SRC-1, -2 and -3 (Xu *et al.* 2009). First discovered as primary coregulators of nuclear receptor (NR)-mediated transcription, SRCs are now known to serve as pleiotropic transcriptional coregulators of a diverse range of non-NR transcription factors (Onate *et al.* 1995, Voegel *et al.* 1996, Torchia *et al.* 1997, Xu *et al.* 2009). Because of the complex functional domain structure of SRCs, this coregulator class can integrate a myriad of signaling cues that control a broad spectrum of physiological and pathophysiological responses (Xu *et al.* 2009, Stashi *et al.* 2014). In the case of the uterus, we and others demonstrated that endometrial SRC-2 is required for early pregnancy establishment in the mouse (Gehin *et al.* 2002, Mukherjee *et al.* 2006). Using a conditional knockout mouse, we revealed that SRC-2 is indispensable for P4-dependent endometrial decidualization (Mukherjee *et al.* 2006). Importantly from a clinical perspective, we also showed that SRC-2 is essential for P4-driven hESC decidualization *in vitro* (Kommagani *et al.* 2013). Together, these studies underscored an evolutionary conserved role for SRC-2 in P4-dependent decidualization, a role not compensated by SRC-1 and/or SRC-3.

Given that P4-dependent hESC decidualization relies on transcriptional reprogramming and that SRC-2 as a transcriptional coregulator is critical for decidualization, we used RNA sequencing (RNA-seq) to identify the SRC-2-responsive transcriptome during this reprogramming event. To showcase the utility of this transcriptome dataset in elucidating new signaling connections with SRC-2 that are important for P4-dependent hESC decidualization, we reveal that full induction of stimulated by retinoic acid 6 (STRA6) expression is dependent on SRC-2 and required for hESC decidualization.

Materials and methods

Human subjects

Human protocols were prospectively approved by the Institutional Review Board at Baylor College of Medicine and were in accordance with the guidelines of the Declaration of Helsinki (WMA 1966) and written-informed consent was

obtained from all participating subjects. Isolation of hESCs from endometrial tissue biopsied from women of reproductive age during the proliferative phase of their menstrual cycle has been described in Kommagani *et al.* (2016).

Cell culture and siRNA transfection

Unless otherwise stated, cell culture media and reagents were purchased from Thermo Fisher Scientific. Human ESCs were maintained in DMEM/F12 medium containing 10% fetal bovine serum (FBS; Sigma-Aldrich), penicillin, streptomycin, an antimycotic, sodium bicarbonate and HEPES. Cells were transfected with siRNAs 48 h after plating into six-well plates at 8×10^4 cells per well. Following complexing of siRNAs with Lipofectamine RNAiMax (Thermo Fisher Scientific) in Opti-MEM 1 Reduced Serum Medium, cells were transfected with siRNAs at 60 pmol per well in MEM with 2% charcoal-stripped FBS (sFBS; Sigma-Aldrich).

In vitro decidualization

Following siRNA transfection, hESCs were decidualized with a hormone cocktail of 10^{-8} M 17β -estradiol (E2), 10^{-6} M medroxyprogesterone 17-acetate (MPA), and 5×10^{-5} M N⁶,2'-O-dibutyladenosine 3',5'-cyclic monophosphate sodium salt (cAMP) (the hormone decidualogenic stimulus; termed EPC (Sigma-Aldrich)) in Opti-MEM 1 reduced serum medium with 2% sFBS. Cells were collected for RNA and/or protein isolation at day 0 and day 3 of EPC treatment.

RNA isolation and sequencing

Total RNA was prepared with the RNeasy Plus kit (Qiagen). For RNA sequencing, RNA purity and integrity was assessed using a NanoDrop spectrophotometer (Thermo Fisher Scientific) and an Agilent Bioanalyzer 2100 with Agilent RNA Nano 6000 chips, respectively. Sequencing of RNA was performed as described in Szwarc *et al.* (2018). Briefly, libraries were prepared using TruSeq RNA library preparation kit v2 (Illumina, Inc.) and enriched by PCR. Adapter-ligated fragment concentration was measured by quantitative PCR assay with a KAPA library Quant kit (Kapabiosystems, Inc., Wilmington, MA, USA). Samples were then pooled and quantified again by qPCR. Bridge amplification using the cBot 2 system (Illumina, Inc.) was used for clonal cluster generation of library pools. Indexed paired-end sequencing was performed with the HiSeq 2500 sequencing system (Illumina, Inc.).

Analysis of RNA sequencing data

Raw reads of 101 nucleotides were trimmed by 11 nucleotides on their 5' ends to increase mappability. The resulting 90-nucleotide pair-ended reads were mapped to the human genome (UCSC hg19) using STAR (Dobin *et al.* 2013) with NCBI RefSeq genes as the reference. Read duplicates were removed using picard tools (<http://broadinstitute.github.io/picard/>) in order to reduce possible PCR biases. HTSeq (<http://www-huber.embl.de/users/anders/HTSeq>) was used to determine the number of reads falling within known genes

(Anders *et al.* 2015). The edgeR package (Robinson *et al.* 2010) was used to analyze the gene-based read counts to detect differentially expressed genes in the following comparisons: (1) EPC day 3 non-targeting (*NT*) siRNA vs EPC day 0 *NT* siRNA; (2) EPC day 0 SRC-2 siRNA vs EPC day 0 *NT* siRNA and (3) EPC day 3 SRC-2 siRNA vs EPC day 3 *NT* siRNA. The false discovery rate (FDR) of differentially expressed genes was calculated using the Benjamini and Hochberg method (Benjamini & Hochberg 1995) with FDR <0.05 considered to be statistically significant. Resulting gene sets were gated based on a FDR <0.05 and on absolute fold changes ($|FC|$) ≥ 1.5 . Gene ontology enrichment analysis was performed using the functional annotation clustering tools in DAVID (Database for Annotation, Visualization, and Integrated Discovery; <http://david.abcc.ncifcrf.gov/>) (Huang *et al.* 2009). Gene Set Enrichment Analysis (GSEA; <http://software.broadinstitute.org/gsea/>) was used to identify overlaps between our filtered gene subsets and hallmark gene set collection in the Molecular Signatures Database (Liberzon *et al.* 2015).

Chromatin immunoprecipitation followed by sequencing

Using a ChIP-grade antibody to human SRC-2 (Bethyl Laboratories, Montgomery, TX, USA; A300-346A), chromatin immunoprecipitation followed by sequencing (ChIP-seq) was performed as previously described on chromatin derived from hESCs treated with EPC for 3 days (Mazur *et al.* 2015, Kommagani *et al.* 2016).

Quantitative reverse transcriptase PCR

Reagents for quantitative reverse transcriptase PCR analysis were obtained from Applied Biosystems (AP) (Thermo Fisher Scientific). SuperScript VILO Master Mix was used to prepare cDNA according to the manufacturer's instructions. Resulting cDNAs of interest were quantified by real-time PCR using AP Biosystems QuantStudio 12K Flex Real-Time PCR System in reactions containing AP TaqMan Gene Expression Assays and AP TaqMan Universal Master Mix II. Taqman assays used in this study are listed in Supplementary Table 1 (see section on [supplementary data](#) given at the end of this article).

Protein isolation and Western blot

Protein was extracted from cells with NP40 buffer (150mM NaCl, 1.0% NP-40, 50mM Tris-Cl pH 8.0) with protease inhibitors (complete EDTA-free; Roche Diagnostics). Protein concentration was quantified using Pierce BCA Protein Assay Kit (Thermo Fisher Scientific). Protein (15–25 µg/lane) was resolved by SDS-PAGE (Biorad Laboratories) before transferring to a polyvinylidene fluoride membrane (Millipore). Non-specific IgG binding was blocked with 5% milk in Tris-buffered saline with 0.1% Tween. Immunoreactive bands were detected with the following antibodies: STRA6 (Novus Biologicals, Littleton, CO; H00064220-D01P, diluted 1:1250), β -actin (Sigma-Aldrich; A1978, diluted 1:10,000). Secondary horse radish peroxidase-conjugated antibodies were purchased from Santa Cruz Biotechnology.

Statistical analysis

Results are presented as averages $\pm$ standard deviation. Statistical analysis was conducted in R Studio (R Studio Inc., Boston, MA, USA). Normality of data was examined on quantile comparison plots. Calculations of *P* values were performed with ANOVA with *post hoc* analysis performed with Tukey's range test; $P > 0.05$ were considered as non-significant differences while $P \leq 0.05$ (*), $P \leq 0.01$ (**) and $P \leq 0.001$ (***) were considered significant.

Results

Identification of genes controlled by SRC-2 in hESCs during decidualization

To identify genes that are both important for hESC decidualization and transcriptionally dependent on SRC-2, RNA-seq was performed on the following hESC RNA samples in triplicate: (1) cells transfected with *NT* siRNA collected at day 0 of EPC treatment (*NT* siRNA day 0 EPC); (2) cells transfected with SRC-2 siRNA collected at day 0 of EPC treatment (SRC-2 siRNA day 0 EPC); (3) cells transfected with *NT* siRNA collected at day 3 of EPC treatment (*NT* siRNA day 3 EPC) and (4) cells transfected with SRC-2 siRNA collected at day 3 of EPC treatment (SRC-2 siRNA day 3 EPC). Principal component analysis (Supplementary Fig. 1A and B) revealed that individual experimental samples were primarily grouping based on the time-point of EPC treatment (day 0 and 3 of EPC treatment). From the resultant RNA-seq data, paired comparisons were performed to reveal the following gene expression changes: (1) *NT* siRNA day 3 EPC vs *NT* siRNA day 0 EPC: to reveal gene expression changes which occur during decidualization; (2) SRC-2 siRNA day 0 EPC vs *NT* siRNA day 0 EPC: to detect gene expression affected by SRC-2 knockdown before the onset of decidualization and (3) SRC-2 siRNA day 3 EPC vs *NT* siRNA day 3 EPC: to identify gene expression changes caused by SRC-2 knockdown at day 3 of EPC (decidualization). After applying analytical cut-offs of $|FC| \geq 1.5$ and FDR <0.05, the expression of over 5552 RefSeq genes was found to be significantly altered during decidualization (Fig. 1A; *NT* siRNA day 3 EPC vs *NT* siRNA day 0 EPC). The number of genes and the degree of gene expression changes with day 3 EPC (decidualization) treatment are in agreement with our previously published decidual control dataset (Szwarc *et al.* 2018), which served as a quality control for our current study (Supplementary Fig. 1C). The full list of gene expression changes in all three datasets is found in [Supplementary data 1](#).

Knockdown of SRC-2 caused significant changes in expression of 385 and 326 genes at day 0 and day 3 of EPC treatment, respectively (Fig. 1A). Over half of the genes transcriptionally regulated by SRC-2 are also induced or repressed during EPC-induced decidualization. While the most robust changes in gene expression occur during

Figure 1 Global gene expression changes that occur during hESC decidualization and with SRC-2 knockdown. (A) Venn diagram displaying the numbers of genes identified by RNA-seq for which expression is significantly changed ($|FC| \geq 1.5$, $FDR < 0.05$) by: (1) decidualization; (2) by SRC-2 knockdown before the onset of decidualization or (3) by SRC-2 knockdown at day 3 of decidualization/EPC treatment. These data also include the number of overlapping and non-overlapping genes between these three datasets. (B) Volcano plot of the FC and $-\log_{10} P$ -value of gene expression changes detected by RNA-seq as presented in (A). For clarity, $|FC|$ cut-off has been set at 50. Genes with the highest $|FC| > 100$ are represented in Supplementary Fig. 2. All data can be found in Supplementary data 1. (C) Quantitative reverse transcriptase PCR analysis of a select number of genes (highlighted in (B)) that confirms the gene expression changes identified by RNA-seq profiling.

decidualization with $|FC|$ s reaching as high as 8400, SRC-2 knockdown leads to gene expression changes found primarily in the -5 to $+5$ fold change (FC) range (Fig. 1B and Supplementary Fig. 2). The analysis of the expression changes of genes altered by both the EPC treatment (comparison 1 as shown in Fig. 1A) and the SRC-2 knockdown at either day 0 of EPC treatment (comparison 2, Fig. 1A) or day 3 of EPC treatment (comparison 3, Fig. 1A) revealed a very distinct pattern of expression change directionality. The majority of expression changes of genes altered both by knockdown of SRC-2 at day 3 of EPC treatment (comparison 3) and by EPC treatment (comparison 1) occurred in the opposite

direction (i.e. if a gene was upregulated in comparison 1, it would be downregulated in comparison 3 and vice versa) (Supplementary Fig. 3B). Conversely, SRC-2 knockdown at day 0 of EPC treatment (comparison 2) caused gene expression changes primarily in the same direction as occurs during decidualization (comparison 1) (Supplementary Fig. 3A).

As a first-line validation analysis to confirm the hESC gene expression changes due to SRC-2 knockdown as shown in the volcano plot (Fig. 1B), we used quantitative reverse transcriptase PCR analysis on an arbitrary chosen set of genes (Fig. 1C). As expected, SRC-2 knockdown resulted in the upregulation or downregulation of the following genes: ATP-binding cassette subfamily A member 6 (ABCA6), ABCA9, ADAM metalloproteinase with thrombospondin type 1 motif 15 (ADAMTS15), aldehyde dehydrogenase 1 family member A3 (ALDH1A3), cannabinoid receptor 1 (CNR1) and scavenger receptor class A member 5 (SCARA5).

Collectively, these results demonstrate that SRC-2 exerts potent coregulator control of genome-wide transcriptional reprogramming that occurs when hESCs undergo decidualization. These results provide a much needed molecular perspective to our previous studies, which demonstrated a critical functional role for this coactivator in hESC and murine uterine decidualization (Mukherjee *et al.* 2006, Kommagani *et al.* 2013).

Gene expression changes dependent on SRC-2 regulate cellular processes associated with hESC decidualization

To reveal the cellular and molecular processes controlled by SRC-2 at day 0 EPC and day 3 of EPC (the 385 and 326 genes altered by SRC-2 knockdown respectively as presented in Fig. 1A), Gene Ontology (GO) analysis with the DAVID Functional Annotation Clustering tool (Fig. 2) and Gene Set Enrichment Analysis (GSEA) (Supplementary Fig. 4) were performed. Bioinformatic analysis revealed that SRC-2 is primarily linked to the regulation of gene expression associated with cell membrane signaling and transport. Importantly, a subset of the molecular pathways and terms found to be enriched with SRC-2 knockdown have been previously implicated in the execution of the decidual progression program, such as extracellular matrix remodeling, immunomodulation and cell cycle regulation (Gellersen & Brosens 2014).

Expression of a subset of genes dependent on PGR requires SRC-2

To identify genes that are regulated during hESC decidualization by both SRC-2 and PGR, we integrated the lists of genes that are upregulated or downregulated when SRC-2 or PGR (Mazur *et al.* 2015) are knocked down at day 3 of EPC treatment. Although the number of hESC genes controlled by PGR is significantly

Figure 2 Signaling pathways and processes transcriptionally regulated by SRC-2 during hESC decidualization. Analysis with DAVID functional annotation clustering tool of genes for which expression is significantly changed by SRC-2 knockdown either at day 0 or day 3 of EPC treatment. Names for each cluster have been summarized based on the full lists of terms in each cluster. The numbers of genes are the total non-redundant number of genes per cluster. The complete list of gene clusters is in Supplementary data 2.

greater than the number regulated by SRC-2 at day 3 EPC treatment (2503 vs. 326 genes), almost 55% of the SRC-2 regulated genes (178 out of 326) are also regulated by PGR (Fig. 3A). Following DAVID functional annotation clustering analysis of genes that are (1) only regulated by SRC-2 (148 genes); (2) only regulated by PGR (2325 genes) or (3) regulated by both SRC-2 and PGR (178 genes), we found the overlap between SRC-2 and PGR altered genes was significantly enriched for genes involved in 'hormone activity/female pregnancy' (Fig. 3B; middle column (highlighted)). Validation of gene expression changes by qPCR (Fig. 3C) confirmed that these genes – adrenomedullin (ADM), chorionic gonadotropin alpha polypeptide (CGA), insulin like growth factor 1 (IGF1), parathyroid hormone like hormone (PTH1H), prolactin (PRL) and urocortin 2 (UCN2) – were not only upregulated or downregulated following SRC-2 knockdown, but the expression of these genes is significantly changed during hESC decidualization (Fig. 3C).

Induction of retinol transporters essential for hESC decidualization requires SRC-2

Integrating genes regulated by SRC-2 and PGR with gene expression changes resulting from EPC treatment for 3 days revealed that 89% of genes controlled by both SRC-2 and PGR are also responsive to EPC treatment (Fig. 4A). Moreover, integration of this RNA-seq dataset with a previously published ChIP-seq dataset for PGR (Mazur et al. 2015) and our ChIP-seq dataset for SRC-2 showed that from the 153 potential gene targets of SRC-2, PGR and EPC, 16 of these target genes are directly bound by both SRC-2 and PGR within 25 kb of the gene (Fig. 4B). Interestingly, one of these target genes is stimulated by retinoic acid 6 (STRA6; Fig. 3B), which is bound by both SRC-2 and PGR on its proximal promoter region (Fig. 4C). During hESC decidualization (day 3 vs day 0 EPC treatment), STRA6 transcript levels

are significantly increased, and this transcriptional induction is severely attenuated when SRC-2 levels are reduced (Fig. 4D). Apart from STRA6, we also showed that SRC-2 indirectly controls the induction of another important component of retinoid trafficking, retinol-binding protein 4 (RBP4) (Fig. 4E). Importantly, reduction of STRA6 protein levels (Supplementary Fig. 5) leads to a significant reduction in the induction of IGFBP1 and PRL (Fig. 4F), which are established molecular biomarkers of decidualization (Brosens et al. 1999). Together, our data reveal for the first time a signaling connection between SRC-2 and retinoid transport, which is required for full hESC decidualization.

Discussion

Notwithstanding the significant advances in delineating the pivotal effector signals that are critical for progesterone-dependent hESC decidualization, our knowledge of the key endometrial coregulators involved in these signaling processes remains incomplete. Using an advanced engineered mouse model, we previously demonstrated that SRC-2 abrogation results in a block in progesterone-driven endometrial decidualization, which results in pregnancy failure (Mukherjee et al. 2006, Mukherjee et al. 2007). Interestingly, forced SRC-2 overexpression in the murine uterus also blocks endometrial decidualization (Szwarc et al. 2014a), suggesting that critical controls on SRC-2 levels are necessary to maintain normal endometrial cell function (Szwarc et al. 2014b). Important from a clinical perspective, we demonstrated that SRC-2 is expressed in human endometrial tissue and essential for hESC decidualization *in vitro* (Mukherjee et al. 2007, Kommagani et al. 2013), providing critical translational support for observations made in the mouse. Because of the established role of SRC-2 as a pleiotropic coregulator of metabolism in other physiological systems (Stashi et al. 2014), we initially employed

Figure 3 Expression of PGR-controlled genes involved in reproductive processes in the human endometrium that require SRC-2. (A) Venn diagram showing the number of genes for which expression is significantly changed by knockdown of SRC-2, PGR, or both SRC-2 and PGR at day 3 of EPC treatment. (B) DAVID Functional Annotation clustering of genes for which expression is changed by knockdown of SRC-2 and/or PGR (as presented in (A)). (C) Validation by quantitative reverse transcriptase PCR of gene expression changes found in the ‘hormone activity/female pregnancy’ functional annotation cluster (highlighted in (B)).

metabolomic profiling to gain a firsthand mechanistic understanding of SRC-2’s role in hESC decidualization (Kommagani *et al.* 2013). This profiling method furnished essential metabolic insight into SRC-2’s role in hESC decidualization; however, SRC-2’s involvement in global transcriptional reprogramming that is known to occur with progesterone-driven decidualization could not be addressed by this approach.

Therefore, to annotate the genome-wide transcriptional responses that are modulated by SRC-2 during progesterone-dependent hESC decidualization, we used here a primary cell culture model, siRNA-mediated knockdown approaches and RNA-seq and ChIP-seq technologies followed by bioinformatic analysis and validation. With an integrative analytic strategy, we found that approximately 50% of genes regulated by SRC-2 are

also controlled by PGR; underscoring SRC-2’s dedicated coregulator role in PGR-mediated signaling during hESC decidualization. In keeping with its pleiotropic coregulator role, SRC-2 regulates a broad spectrum of target genes during hESC decidualization, many of which encode cell membrane-associated proteins. Importantly, functional gene enrichment analysis demonstrated a significant representation of genes regulated by SRC-2 along with PGR involved in the ‘hormone activity/reproduction/pregnancy’ category. Integration of RNA- and ChIP-seq datasets further stratified this category of genes in terms of direct targets of both PGR and SRC-2 to reveal STRA6 as a gene target directly regulated by this NR and coregulator.

This finding is significant as STRA6 is an integral cell membrane protein that transports retinol into the cell

Figure 4 Induction of STRA6 requires SRC-2 and is essential for hESC decidualization. (A) Venn diagram showing the numbers and overlaps of genes with significant expression changes induced by (1) decidualization (day 3 vs day 0 of EPC treatment); (2) knockdown of SRC-2 at day 3 of EPC treatment (decidualization) and (3) knockdown of PGR at day 3 of EPC (decidualization). (B) Genes (with associated expression changes) that overlap between all three datasets detailed in (A) and are direct targets of SRC-2 and PGR (Mazur *et al.* 2015) as assessed by ChIP-seq. (C) ChIP-seq analysis shows binding of SRC-2 and PGR to the STRA6 promoter in hESCs treated with EPC for three days. (D) Quantitative reverse transcriptase PCR shows that STRA6 transcript levels are induced with EPC and that SRC-2 is required for this induction. (E) Induction of RBP4 RNA levels in hESCs treated with EPC for 3 days is significantly diminished with SRC-2 knockdown. (F) Quantitative reverse transcriptase PCR shows that normal induction at the RNA level of IGFBP1 and PRL (decidual biomarkers) is significantly reduced with STRA6 knockdown in hESC treated with EPC for 3 days.

for conversion into retinoic acid, which in turn acts as the ligand for retinoic acid receptor subtypes (Leid *et al.* 1993). Even though *Strat6* knockout mice are fertile (Berry *et al.* 2013), the functional importance of STRA6 is strikingly different between human and rodent. In humans, STRA6 mutations leading to Matthew-Woods syndrome cause devastating phenotypes in human not seen in the mouse knockout, underscoring the functional divergence of STRA6 between human and rodent (Golzio *et al.* 2007, Pasutto *et al.* 2007). Retinoid signaling is known to control cell differentiation, proliferation and cell survival in numerous physiological and pathological contexts (Jiang *et al.* 2018). In the case of the endometrial stromal cell, a number of studies have shown that retinoid transport, metabolism and activity are essential for normal cellular function and that the observed perturbation of retinoid signaling in endometriotic cells may explain the decreased ability of these cells to decidualize (Pavone *et al.* 2011, Pierzchalski *et al.* 2014). In particular, STRA6 expression levels, previously shown to be dependent on PGR and responsible for retinoic acid transport in normal hESCs (Pavone *et al.* 2010), are strikingly reduced in endometriotic cells (Pavone *et al.* 2011). Our studies

also show that the normal induction of RBP4 expression during hESC decidualization is critically dependent on SRC-2. Compared to hESCs, levels of RBP4 have also been shown to be severely reduced in endometriotic stromal cells (Pavone *et al.* 2017). Interestingly, other studies have demonstrated that RBP4 acts as a paracrine factor when induced and secreted from hESCs during decidualization (Pavone *et al.* 2017). Secreted from hESCs, RBP4 is thought to act on adjacent epithelial cells to stimulate conversion of estradiol to estrone (Cheng *et al.* 2007), thereby reducing estradiol levels that can be detrimental to normal endometrial growth homeostasis and function (Cha *et al.* 2012). Apart from uncovering a novel convergence of SRC2, PGR and retinoid signaling that is critical for hESC decidualization, two important questions emerge for future investigation: (1) what other transcription factors (NR and/or non-NR) – apart from PGR – interact with SRC-2 to control the hESC transcriptome during decidualization? And (2) what is the makeup of the transcriptional interactome that enables SRC-2 to modulate transcriptional responses in decidualizing hESCs?

In sum, transcriptomic analysis described herein provides a much required global molecular perspective

on SRC-2's critical role in progesterone-dependent hESC decidualization and represents a powerful informational resource with which to uncover novel signaling paradigms for SRC-2 action in uterine function.

Supplementary data

This is linked to the online version of the paper at <https://doi.org/10.1530/REP-18-0282>.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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