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Human steroidogenic factor-1 (hSF-1) regulates progesterone biosynthesis and growth of ovarian surface epithelial cancer cells

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ABSTRACT

The majority of cancers derived from ovarian surface epithelial (OSE) cells are lethal. Estrogens promote proliferation of OSE cells, whereas progesterone inhibits proliferation and promotes apoptosis of OSE cells. Human steroidogenic factor-1 (hSF-1) induction of the steroidogenic acute regulatory protein (*StAR*) gene, and the steroidogenic enzymes CYP11A1 and HSD3B2 is central to progesterone biosynthesis. Whereas hSF-1 and StAR are expressed in human ovarian surface epithelial (HOSE) cells, hSF-1 and StAR protein were not expressed in a panel of malignant ovarian cancer cell lines (SKOV-3, BG-1, and Caov-3), and in human OSE cells immortalized by SV40 large T antigen (IOSE-121). Transient expression of hSF-1 in SKOV-3 cells activated the expression of StAR, p450scc and 3βHSD-II mRNAs, and induced progesterone biosynthesis. Additionally, hSF-1 suppressed proliferation and promoted apoptosis of SKOV-3 cells and suppressed SKOV-3 cell growth induced by ERα and estradiol. These findings suggest that hSF-1 is central to progesterone biosynthesis in OSE cells. Human SF-1 may decrease OSE cancer cell numbers directly by apoptosis, and indirectly by opposing estradiol-induced proliferation. These findings are consistent with the hypothesis, that down-regulation of hSF-1 contributes to progression of ovarian epithelial cancers.

1. Introduction

Ovarian cancer is the leading cause of death in women with pelvic malignancies, ranking second among new gynecological cancers diagnosed each year [1]. The majority of lethal ovarian tumors (90%) originate from the epithelial compartment of the ovary [2]. The lack of early and specific symptoms combined with insidious onset frequently leads to late diagnosis, high recurrence rate and poor 5-year patient survival [1,2].

Studies in ovarian cancer have shown that the nuclear receptor human steroidogenic factor 1 (hSF-1) and estrogen receptor α (ERα) regulate the signal transduction pathways crucial to ovarian steroidogenesis and cell proliferation [3–6]. We therefore hypothesized that hSF-1-regulated progesterone biosynthesis may down-regulate signal transduction networks that govern the transformation of normal ovarian surface epithelial (OSE) cells to the malignant phenotype and control the proliferation of epithelial cancer cells. Steroidogenic factor-1 is a global regulator of steroid hydroxylase enzymes including cytochrome P450 (CYP11A1) and 3β-hydroxysteroid dehydrogenase (HSD3B2), and these enzymes are crucial to biosynthesis of progesterone from cholesterol [7–9]. SF-1 also regulates CYP19 (aromatase p450) gene promoter PII, to promote cAMP stimulated estrogen biosynthesis in the ovarian follicle [10,11]. Furthermore, hSF-1 activates steroidogenic acute regulatory (StAR) protein through multiple hSF-1 response elements (SFREs), and the hSF-1-mediated *StAR* gene expression is enhanced by cyclic adenosine monophosphate (cAMP) [12]. Steroidogenic acute regulatory protein expression is pivotal to the initial rate-limiting step of acute steroid biosynthesis, including progesterone production, as illustrated by undetectable progesterone levels in two females with mutations of the *StAR* gene, resulting in nonfunctional StAR protein and lipoid congenital adrenal hyperplasia [13].

We previously isolated and characterized hSF-1 and determined its expression in human tissues [14,15]. Our Northern blot and in situ hybridization studies have shown that hSF-1 is expressed in both steroidogenic (adrenal cortex, granulosa and theca cells of the ovaries and the testes) and non-steroidogenic (atretic folli-

Abbreviations: hSF-1, human steroidogenic factor-1; StAR, steroidogenic acute regulatory protein; OSE, ovarian surface epithelial cells; ERα, estrogen receptor alpha; SFRE, steroidogenic factor-1 response element; IOSE, human ovarian surface epithelial cells immortalized by SV40 virus; FACS, fluorescence activated cell sorter; PARP-1, poly(ADP-ribose) polymerase-1.

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cles of the ovary and spleen) human tissues. Other investigators have reported expression of immunoreactive hSF-1 in both the steroidogenic cells of the normal and malignant ovaries [16–18]. Immunohistochemical and real-time PCR analysis of human epithelial ovarian carcinoma demonstrate a positive correlation between StAR, CYP11A1, and HSD3B2 expression in tumor cells and patient survival [19]. It is noteworthy that all these genes are activated by hSF-1. Furthermore, a recent cDNA microarray study found that late-stage ovarian cancers consistently demonstrate a greater than 10-fold decrease in StAR gene expression compared to early-stage ovarian cancers [20]. A growing body of experimental evidence shows that normal OSE cells are capable of steroidogenesis *in vitro* [21], and intratumoral steroid biosynthesis is important in ovarian epithelial cell carcinogenesis [22–24]. Whereas estrogen promotes ovarian epithelial cancer cell proliferation, progesterone inhibits OSE cell proliferation and promotes apoptosis [25,26]. Cell culture and epidemiological studies support the protective role of progesterone against ovarian cancer [27,28]. Moreover, a loss of heterozygosity at 11q23.3-24.3, the location of progesterone receptor (PR) gene locus, is frequently (75%) observed in ovarian carcinomas and is associated with poor prognosis [26]. Collectively, these studies suggest that intratumoral *StAR* gene expression and progesterone biosynthesis may inhibit OSE cancer cell proliferation.

We determined expression of hSF-1 and StAR in HOSE cells of normal ovaries by immunohistochemistry and performed Western blot analysis, real-time RT-PCR studies, transient co-transfection studies, cell proliferation, apoptosis and progesterone biosynthesis assays using ovarian cancer cell lines to determine whether hSF-1 regulates *StAR*, *CYP11A1* and *HSD3B2* gene expression to modulate progesterone biosynthesis, inhibits cell proliferation, and promotes apoptosis in ovarian epithelial cancer cells. Data from these experiments suggest that *hSF-1* and *StAR* are expressed in HOSE cells. In contrast, their expression is lost in transformed OSE cells and that re-expression of hSF-1 reconstitutes the progesterone biosynthesis pathway in ovarian cancer cell lines. Furthermore, hSF-1 initiates apoptosis and opposes the mitogenic effects of estradiol on SKOV-3 cells.

2. Materials and methods

2.1. Plasmid constructs

Directional cloning of hSF-1 cDNA generated hSF-1 expression vector GFP•hSF-1. Specifically, a 3.1 kb hSF-1 insert was digested with the EcoRI and Xba1 restriction endonucleases to obtain a 2355 bp fragment containing the entire open reading frame. This 2355 bp fragment of hSF-1 was sub-cloned into identical cleavage sites of GFP expression vector (Invitrogen), to generate GFP•hSF-1. Estrogen receptor α expression vector, pSG5•ER (HEGO) was kindly provided to us by Dr. Pierre Chambon (Strasbourg, France). ERα expression vector was generated by subcloning a 1817 bp fragment of the ER (HEGO) into the BamHI and EcoRI cloning sites of pSG5 vector containing a SV40 promoter. The hSF-1-mutant constructs, Bpu-delSF-1 [29] and R92QSF-1 [30] were generous gifts from Dr. Li of National Health Research Institute, Taiwan and Dr. Jameson of Northwestern University Medical School, Chicago, respectively.

2.2. Cell lines

BG-1 human ovarian cancer cells were a kind gift from Dr. Matthew Burow of Tulane University School of Medicine. HEK-293, H295R, SKOV-3 and Caov-3 cells were purchased from American Type Culture Collection (ATCC Manassas, Virginia). Dr. Nelly Auersperg kindly provided us the human immortalized ovarian surface epithelial (IOSE-121) cells. These are normal human ovarian surface epithelial cells immortalized with SV40 large T antigen. We thank Dr. Nelly Auersperg for access to the Canadian Ovarian Tissue Bank.

2.3. Immunohistochemistry

Following approval of a research protocol by Tulane University Institutional Review Board Committee as part of an ongoing study to determine expression of hSF-1 and StAR expression by IHC in normal and malignant ovaries, we obtained 6 archived paraffin tissue blocks of normal ovaries from the Department of Pathology, Tulane University. IHC studies were performed on paraffin embedded normal ovarian tissue and adrenal cortex. Human adrenal cortical tissue was used as positive control. Deparaffinized tissue sections of normal human ovarian tissue were rehydrated by ethanol and incubated in 0.3% hydrogen peroxide to inactivate endogenous peroxidases. Slides were washed with PBS, blocked in blocking serum for 0.5 h at room temperature and incubated with primary anti-hSF-1 (1: 200) antibody or anti-StAR antibody (1:800) in blocking buffer overnight at 4 °C. Slides were then incubated with biotinylated secondary antibody goat-anti-mouse-IgG (1:200) in blocking buffer. Paraffin embedded normal human ovarian follicles in the ovarian sections was used as internal positive controls. Negative control sections were incubated in blocking buffer only. Sections were incubated with AB enzyme reagent containing avidin and biotinylated horseradish peroxidase for 30 min and then covered with peroxidase substrate until desired stain developed. Sections were counterstained with hematoxylin. Finally, sections were dehydrated in ethanol and xylene, mounted on slides with permanent mounting medium, and covered with glass coverslips.

2.4. Western blot

The SF-1 antibody was kind gift from Dr. Ken Morohashi of the National Institutes of Basic Biology, Okazaki, Japan, and the StAR antibody was kindly given to us by Dr. Douglas Stocco of Texas Tech University Health Sciences Center, Lubbock, Texas. The secondary antibody used for hSF-1 and StAR western blots was goat-anti-rabbit-IgG linked to horseradish peroxidase (Jackson ImmunoResearch Laboratories Inc.).

Total cellular protein was extracted from H295R, HEK-293, and human ovarian cancer cell lines SKOV-3, BG-1, Caov-3, and IOSE-121 cells, and used for Western blot analysis of hSF-1 and StAR expression. In Western blots for hSF-1 and StAR, H295R cells that express hSF-1 and StAR were used as a positive control, and human embryonic kidney cell line HEK-293 cells which do not express hSF-1 and StAR were used as negative control. To determine StAR expression, cells were treated for 6 h with 0.5 mM cAMP or vehicle. Seventy-five micrograms of each total protein extract was boiled in SDS sample buffer. The hSF-1 and StAR protein samples and pre-stained protein markers were separated by electrophoresis in 12% SDS-polyacrylamide gel. The separated proteins were transferred onto a nitrocellulose membrane (Hybond-ECL, Amersham Pharmacia Biotech), and immunodetection was performed after blocking the membrane for 1 h at room temperature in nonfat dry milk with 0.05% Tween 20 in 1× Tris buffered saline. The hSF-1 and StAR antibodies were diluted to 1:1000 and 1:800, respectively and incubated for 1.5 h at room temperature. Following incubation with the primary antibody, the membrane was rinsed and incubated with secondary antibody (Santa Cruz Biotechnologies, Inc.) for 1 h at room temperature. For an internal loading control, Western blot for β-actin or GAPDH was performed on all the samples using β-actin monoclonal antibody (Sigma) or GAPDH antibody (Chemicon). Chemiluminescence reagents were used to detect the specifically bound primary antibody and secondary antibody

Table 1
Primer sequences for real-time RT-PCR.

Target gene	Sequence 5′–3′	Amplicon size
StAR	Fwd: TTA GCA ACC AAG AGG GCT GGA AGA Rev: TTG CCC ACA TCT GGG ACC ACT TTA	85
P450scc	Fwd: GCT GAG CAA AGA CAA GA Rev: GAA TGA GGT TGA ATG TGG TG	182
3βHSD-II	Fwd: ATC CAC ACC GCC TGT ATC AT Rev: TCT GGA TGA TTT CCT TGT AGG AG	181
GAPDH	Fwd: CAA CTA CAT GGT TTA CAT GTT C Rev: CTC GCT CCT GGA AGA TG	121

complex (ECL Amersham). Immunoreactive bands were developed using an enhanced chemiluminescence detection system (Amersham Pharmacia Biotech), the bands were visualized on a Kodak Image Station, and printed on high-density thermal paper (Mitsubishi K65HM-CE).

2.5. Real-time PCR

Induction of StAR, and steroid hydroxylase enzymes CYP11A1 and HSD3B2 mRNA by hSF-1 in SKOV-3 cells was determined by real-time RT-PCR. Human ovarian adenocarcinoma SKOV-3 cells were grown in RPMI-1640 medium supplemented with 10% FBS, and transfected with plasmid constructs GFP or GFP•hSF-1, 20 mg DNA per 150 mm^2 flask using transfection reagent FuGENE 6 Transfection Reagent and were cultured for 48 h. To obtain greater than 90% transfection efficiency, the transfected cells were sorted for GFP using fluorescence activated cell sorter (FACS) 48 h post-transfection. Total RNA from the sorted cells was isolated by Trizol Reagent (Invitrogen, Carlsbad, California) and dissolved in DEPC-treated water. DNase I (Invitrogen, Carlsbad, California) was used to remove the contaminating DNA and purify the RNA sample. Complementary DNA then was synthesized using the SuperScript III FirstStrand Synthesis System for RT-PCR (Invitrogen, Carlsbad, California) according to the manufacturer's protocol. Primers for *StAR* gene were designed using IDT PrimerQuest (Integrated DNA Technologies). Published primer sequences were used for CYP11A1, HSD3B2 and *GAPDH* genes [19,31]. Primer sequences are given in Table 1. Invitrogen, Carlsbad, CA, manufactured all the primers.

Real-time PCR reactions were performed using Bio-Rad iCYCLER iQ5 and the real-time PCR reactions were carried out using IQ SYBR-Green Bio-Rad Super mix (Bio-Rad, catalogue #18068-015). The real-time PCR reaction mixture contained SYBER Green Supermix, forward and reverse primers and 1 μl of 10-fold diluted template cDNA for StAR, CYP11A1, HSD3B2, and GAPDH mRNA quantification. The PCR reaction conditions are as follows: samples were heated at 95 °C for 3 min and subjected to 40 cycles of denaturing at 95 °C for 10 s, annealing and extension at 61 °C for 30 s for, StAR, and GAPDH and for *CYP11A1* and *HSD3B2* genes the reaction was subjected to 40 cycles of denaturing at 95 °C for 10 s, annealing and extension at 56 °C for 30 s. GAPDH mRNA was used as an internal control. Values were quantified using published comparative CT method [32]. $2^{-\Delta\Delta C_T}$ gave the amount of target gene, where $\Delta\Delta C_T$ is the threshold C_T value of the gene of interest normalized to GAPDH. The data is presented as a fold-increase of mRNA levels of gene of interest in SKOV-3 cells transfected with hSF-1 over mRNA levels in SKOV-3 cells transfected with control (GFP) vector alone. The bars represent mean and SEM, of three experiments, each performed in triplicate.

2.6. Progesterone biosynthesis assay

SKOV-3 cells were grown in OPTI-MEM culture medium supplemented with 1% charcoal-stripped FBS. Cells were transfected with expression vectors, GFP, GFP•SF-1, or mutDBDhSF-1. Cells were seeded in 12-well plates at a density of 2×10^4 cells/well and incubated at 37 °C in 5% CO_2. Three days post-transfection progesterone assay was performed in the culture medium using an ELISA kit (Progesterone EIA Kit. Cat# is 582601, Cayman Chemical Company, Ann Arbor, MI), according to the manufacturer's protocol. Standard curve was generated and progesterone levels were measured in pg/ml. Each experiment was done in triplicate and repeated more than three times.

2.7. Cell counts

SKOV-3 cells were grown in RPMI-1640 culture medium supplemented with 5% FBS. Cells were co-transfected with expression vectors (GFP, GFP and hSF-1, or GFP and mutant hSF-1 vectors: Bpu-delhSF-1 or R92QhSF-1) using FuGENE 6 Transfection Reagent. To obtain greater than 90% transfection efficiency, the transfected cells were sorted by FACS and counted on day 4 post-transfection. Specifically, SKOV-3 cells were detached with trypsin/EDTA and split into five 75 mm^2 flask at a density of 1×10^6 per flask and incubated for 24 h at 37 °C in 5% CO_2. After the 24 h incubation, the cells were transfected using FuGENE 6 (ratio of FuGENE 6 to DNA was 3:1). Control plasmid GFP or GFP•SF-1 concentration was 12 mg/flask. Following transfection, cells were incubated at 37 °C for 6 h, after which the culture medium was changed to RPMI-1640 supplemented with 10% FBS. Cells were further incubated at 37 °C for 18 h. Cells were then harvested with trypsin/EDTA, pelleted, washed with PBS, and re-suspended in 1 ml PBS. The cells were filtered with a 40 mm cell strainer (BD Biosciences cat #352340) and sorted for GFP expression by FACS. Sorted green fluorescent protein expressing cells were washed with PBS, re-suspended in 1 ml of RPMI-1640 supplemented with 10% FBS, and plated in triplicate for each group in 6-well plates at a density of 10^4 cells/well and incubated at 37 °C in 5% CO_2. Live and dead cells were counted on a hemacytometer using the Trypan blue stain on the fourth day after transfection and the ratio of dead cell count to live cell count for each treatment group was calculated. Each experiment was done in triplicate, and repeated more than three times.

SKOV-3 cells were grown in RPMI-1640 culture medium supplemented with 10% FBS. After 24 h the culture medium was changed to OPTI-MEM supplemented with 1% charcoal-stripped FBS. Cells were then plated, in triplicate for each of the two treatment groups, in 6-well plates at a density of 10^4 cells/well and incubated at 37 °C in 5% CO_2. The cells were treated for 3 days with either progesterone concentration of 10^{-7} M or vehicle (ethanol). After treatment with progesterone for 3 days, live and dead cells were counted on a hemacytometer using the Trypan blue stain and the ratio of dead cell count to live cell count for each treatment group was calculated. Each experiment was done in triplicate, and repeated three times.

SKOV-3 cells were grown in RPMI-1640 culture medium supplemented with 10% FBS. Cells were transfected with expression vectors GFP or GFP•hSF-1 using FuGENE 6 transfection reagent (Roche). After 24 h cells were plated, in triplicate for each of the three treatment groups, in 12-well plates at a density of 10^4 cells/well and incubated at 37 °C in 5% CO_2 for 6 h. The culture medium was changed to OPTI-MEM supplemented with 1% charcoal-stripped FBS and the cells were treated for 3 days with either progesterone receptor (PR) antagonist RU486 (Sigma) at a concentration of 0.1 μM or vehicle (ethanol). Subsequently live and dead cells were counted on a hemacytometer using the Trypan blue stain and the ratio of dead cell count to live cell count for each treatment group was calculated. Each experiment was done in triplicate, and repeated three times.

Cells transfected with ERα or co-transfected with GFP•hSF-1 and ERα were incubated post-transfection in 5% CO_2 at 37 °C for 48 h

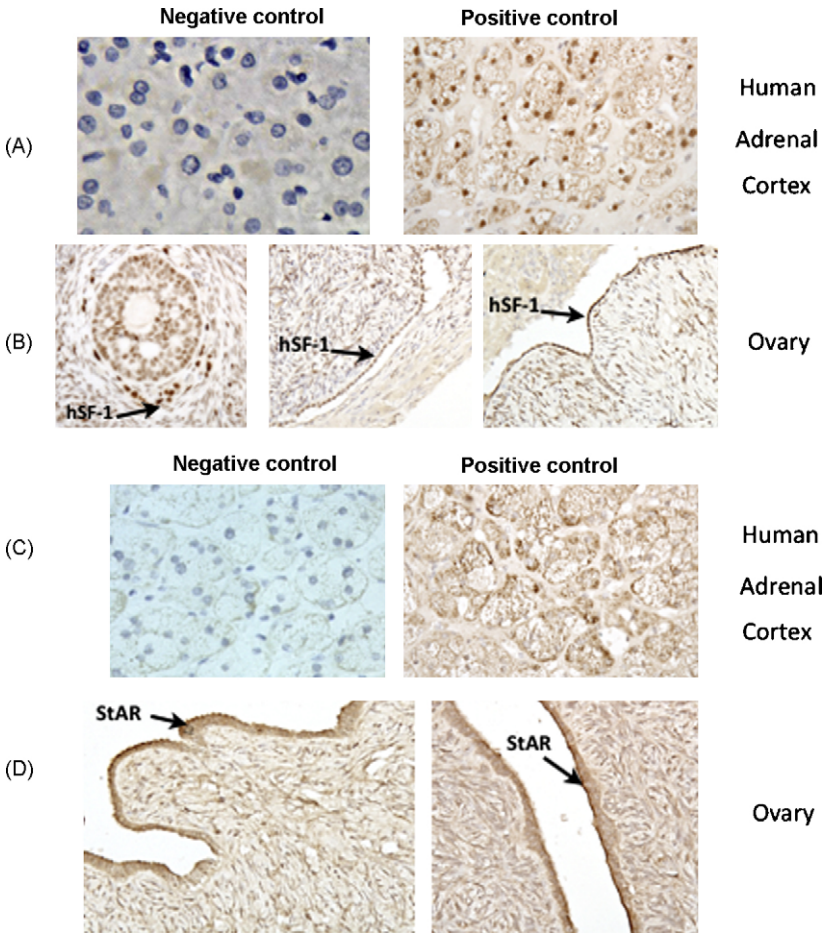

Fig. 1. (A–D) hSF-1 and StAR are expressed in HOSE cells. IHC studies were performed on paraffin embedded normal human ovarian and human adrenal cortical tissue. Slides were incubated with primary antibody human SF-1 or anti-StAR antibody. Paraffin embedded normal human ovarian follicles in the ovarian sections were used as positive controls. Negative control sections were incubated in blocking buffer only. Horseradish peroxidase and peroxidase substrate was used until desired stain developed. Sections were counterstained with hematoxylin. Finally, sections were dehydrated in ethanol and xylene, mounted on slides with permanent mounting medium, and covered with glass coverslips. (A) hSF-1 expression in human adrenal cortex. (B) hSF-1 expression in ovarian surface epithelial cells and granulosa and theca cells of the graffian follicle follicle. (C) StAR expression in human adrenal cortex. (D) StAR expression in ovarian surface epithelial cells.

in RPMI-1640 supplemented with charcoal-stripped 10% FBS. Subsequently cells were treated with 17β-estradiol (E_2) concentration of 8 nM or vehicle (ethanol) for 48 h prior to harvesting. Cells were counted 4 days post-transfection on a hemacytometer. Trypan blue exclusion test was used to identify living cells.

2.8. DNA laddering

SKOV-3 cells were gown to 50–60% confluence in 75 mm^2 flasks in RPMI-1640 with 10% FBS. SKOV-3 cells were transfected using FuGENE 6 with GFP vector, GFP•SF-1 or co-transfected with hSF-1and ERα. Cells co-transfected with GFP•hSF-1 and ERα were incubated post-transfection in 5% CO_2 at 37 C for 48 h in RPMI-1640 supplemented with charcoal-stripped 10% FBS. Subsequently cells were treated with E_2 (8 nM) or vehicle (ethanol) for 48 h prior to harvesting. Four days post-transfection the cells were harvested, washed with PBS, and pelleted by centrifugation. Cell pellets were treated with lysis buffer containing 1% Igepal CA630 (Sigma), 20 mM EDTA, and 50 mM Tris (pH 7.5). Apoptotic DNA fragments were extracted by centrifugation at 1600 × *g* for 5 min. Supernatants were collected and treated with 0.2 mg/ml RNase A (Sigma) in 1% SDS incubated for 2 h at 56 °C. Samples were then digested with 1.2 mg/ml proteinase K (Sigma) for at least 2 h at 37 °C, and DNA fragments were precipitated by adding 0.67 volume of 7.5 M ammonium acetate and 2.5× volume of 100% ethanol. DNA was pelleted by centrifugation at 12,000 rpm for 15 min. DNA pellets were dissolved in TE buffer (pH 8.0) and loading buffer was added. DNA fragments were separated by electrophoresis in 1% agarose gels containing 0.5 mg/ml ethidium bromide.

2.9. PARP-1 cleavage assay

SKOV-3 cells were grown in RPMI-1640 with charcoal-stripped 10% FBS to 60% confluence and 2×10^6 cells were seeded in 150 mm^2 flasks. Cells were transfected with GFP vector, GFP•hSF-1 or co-transfected with GFP•hSF-1 and ERα. FuGENE 6 transfection reagent was used for all transfections. The total plasmid concentration was 1 mg/ml. SKOV-3 cells transfected with ERα were treated with vehicle (ethanol) or E_2 (concentration of 8 nM) for 48 h prior to harvesting the cells. Four days post-transfection, the cells were detached with Trypsin/EDTA and washed with cold PBS twice. Cell lysates were prepared in radioimmunoprecipitation assay (RIPA) buffer supplemented with protease inhibitor cocktail and phosphatase inhibitors sodium fluoride (NaF) and sodium vanadate (NA3VO4). The total protein concentration was determined using the protein assay dye reagent (Bio-Rad Laboratories, Hercules, CA). Forty micrograms of total protein from each cell lysate was analyzed by 10% SDS-polyacrylamide gel-electrophoresis and transferred to Hybond membrane (Amersham Biosciences, Piscataway, NJ). The membrane was washed

Table 2
IHC studies for hSF-1 StAR expression in ovaran surface epithelial cells and cortical inclusion cyst epithelium. Deparaffinized tissue sections of normal human ovarian tissue were rehydrated by ethanol and stained for hSF-1 and StAR by immunohistochemistry. Paraffin embedded normal human ovarian follicles in the ovarian sections were used as positive controls. Whereas, hSF-1 expression was positive in OSE cells in 5 out of the 6 normal ovarian tissues studied only 2/6 samples showed positive hSF-1 staining in the OSE of the inclusion cysts. StAR expression was positive in OSE cells in all the tissue samples and in 5/6 samples, OSE cells of the inclusion cysts stained positive for StAR.

Ovarian tissue	hSF-1 expression IHC		StAR expression IHC	
	Surface epithelium	Inclusion cyst epithelium	1.405 surface epithelium	Inclusion cyst epithelium
1	+	–	+	+
2	+	+	+	+
3	–	–	+	+
4	+	–	+	–
5	+	+	+	+
6	+	–	+	+

and blocked by TBS-Tween-20 and 5% nonfat milk. Mouse anti-poly(ADP-ribose) polymerase (PARP) monoclonal antibody (BD Biosciences, San Jose, CA) was used as primary antibody in 1:2000 dilution buffer TBS-Tween-20 and 5% nonfat milk. Secondary goat-anti-mouse antibody coupled to horseradish peroxidase (Santa Cruz Biotechnology, Santa Cruz, CA) was used as 1:3000 dilutions in TBS-Tween-20 and 5% nonfat milk. Proteins were detected using the ECL reagents (Amersham Biosciences, Piscataway, NJ). The chemiluminescent bands of PARP, uncleaved 116 kDa and cleaved 85 kDa were visualized by the Kodak Image Station and printed on high-density thermal paper (Mitsubishi K65HM-CE). Membranes were stripped with ECL stripping buffer and probed with hSF-1 antibody (HEK-293 cells) or sequentially probed with hSF-1and ERα antibodies (SKOV-3 cells). For an internal loading control, Western blot for β-actin was performed on all the samples.

2.10. Statistical analysis

Paired Student's *t*-test and one-way ANOVA with Tukey's post hoc test was performed using GraphPad Prism version 4.0c for Macintosh, GraphPad Software, San Diego, California, USA.

3. Results

3.1. Human SF-1 and StAR expression in normal ovarian surface epithelial cells

We examined the expression of hSF-1 and StAR in ovarian surface epithelial cells in paraffin embedded sections of six normal ovarian tissues by immunohistochemistry. Both hSF-1 and StAR expression was observed in the surface epithelial cells (Fig. 1B and D). Whereas hSF-1 was expressed in ovarian surface epithelial cells of five out of the six ovaries, in four out of the six ovaries, hSF-1 expression was absent in the cortical inclusion cyst (CIC) epithelium (Table 2). StAR expression was observed in all the OSE cells of all the six ovaries and in only one of the six ovaries expression of StAR was absent in the CIC epithelium (Table 2).

3.2. Human SF-1 and StAR expression in ovarian cancer cell lines

We examined the expression of hSF-1 and StAR in ovarian cancer cell lines as well as IHOSE cells using Western blot analysis. Human steroidogenic factor-1 (Fig. 2A) expression was undetectable in the ovarian cancer cell lines SKOV-3, BG-1 and Caov-3, and in SV40 immortalized human ovarian surface epithelial cells (IHOSE121 cells). StAR expression was also undetectable in these cell lines (Fig. 2B). Human adrenocortical carcinoma H295R cells were used as a positive control for hSF-1 and StAR expression. Further induction of StAR protein in H295R cells occurred by treatment of cells with 0.5 mM of cAMP (Fig. 2B). StAR protein was not induced by cAMP in any of the ovarian cancer cells nor in IHOSE121 cells.

3.3. Human SF-1 induces StAR, CYP11A1 and HSD3B2 mRNA expression in ovarian cancer cells

Human steroidogenic factor-1 induces StAR, CYP11A1 and HSD3B2 expression in normal OSE cells to promote progesterone biosynthesis (Fig. 3A). To determine whether hSF-1 could induce endogenous StAR, CYP11A1 and HSD3B2 expression in ovarian cancer cells, we performed transient transfections in hSF-1-negative SKOV-3 cells and confirmed hSF-1 protein expression by Western blot analysis (Fig. 3B). Following transfection of hSF-1 into SKOV-3 cells, we examined StAR, p450scc and HSD3B2 expressions by RT-PCR analysis. StAR mRNA and HSD3B2 mRNA expression normalized to GAPDH expression was increased 65-fold in SKOV-3 cells transfected with SF-1 compared to vector-transfected cells (Fig. 3C, bar 1 and 3). Likewise, CYP11A1 expression normalized to GAPDH expression was increased more than 150-fold (Fig. 3C, bar 2 and Table 3) over the vector-transfected SKOV-3 cells.

3.4. Human SF-1 induces progesterone biosynthesis in SKOV-3 cells

To determine whether transient re-expression of hSF-1 in ovarian cancer cells induces progesterone biosynthesis, we first

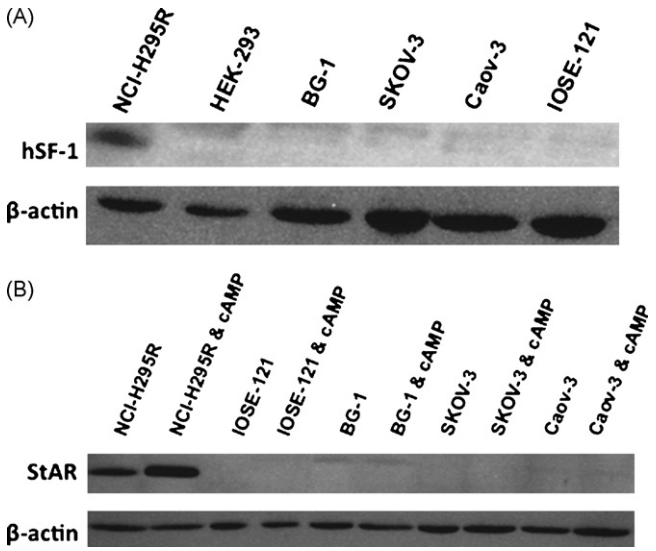

Fig. 2. (A and B) hSF-1 and StAR are not expressed in human ovarian cancer cell lines and StAR expression is not induced by cAMP. Western blot analysis was performed to determine expression of hSF-1 and StAR protein in ovarian cancer cell lines. H295R cells were used for positive control and HEK-293 cells were used for negative control. 75 mg of each total protein extract was resolved by 12% SDS PAGE and prepared for Western blotting with hSF-1 or StAR antibody. (A) Human SF-1 protein expression in ovarian cancer cell lines SKOV-3, BG1, Caov-3, and in human OSE cells immortalized by SV40 large T antigen (IOSE-121). (B) Human StAR expression in ovarian cancer cell lines incubated with vehicle or cAMP (0.5 mM concentration) to induce StAR.

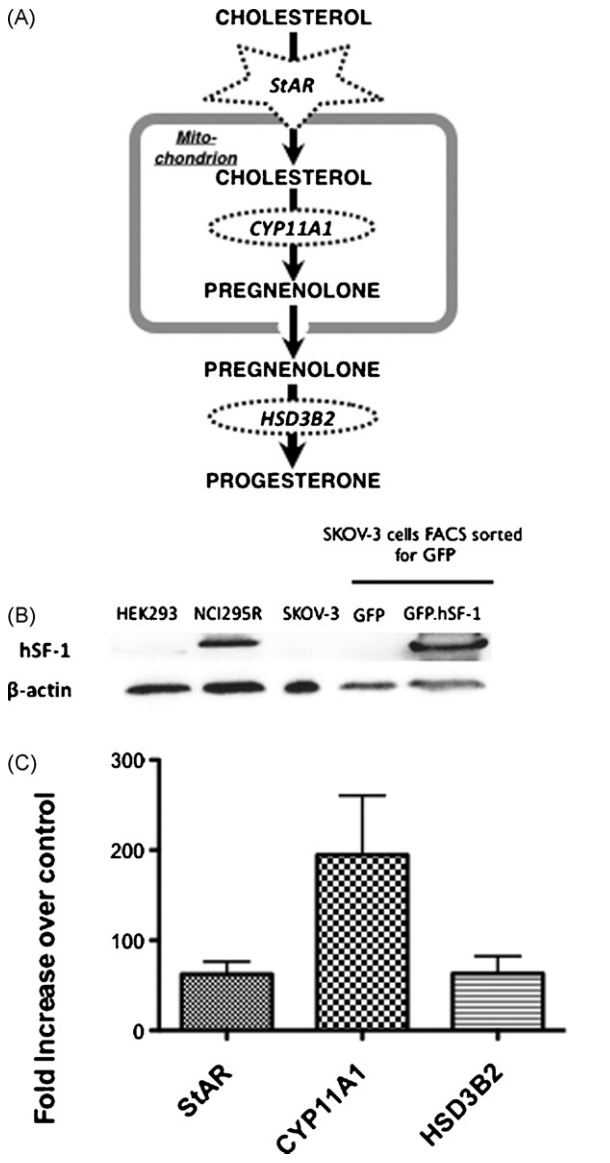

Fig. 3. (A–C) hSF-1 induces StAR mRNA CYP11A1 and HSD3B2 mRNA expression in SKOV-3 cells. SKOV-3 cells were transfected with GFP expression vector alone or with GFP•hSF-1 expression vector (GFP•SF-1) and the cells were FACS sorted for GFP. Real-time RT-PCR was performed to quantify StAR, CYP11A1 and HSD3B2 mRNA induction by hSF-1. StAR, CYP11A1 and HSD3B2 mRNA levels were normalized to endogenous human GAPDH levels, which was used as an internal control. (A) Progesterone biosynthetic pathway in normal ovarian surface epithelial cells. (B) Western blot of hSF-1 expression in transfected SKOV-3 cells. (C) Fold-increase over control in mRNA levels of gene of interest in SKOV-3 cells transfected with hSF-1 compared to mRNA levels in SKOV-3 cells transfected with GFP (vector) alone. The bars represent mean and SEM, of three experiments, each performed in triplicate. All the genes (StAR, CYP11A1 and HSD3B2) showed a significant fold-increase in mRNA expression in hSF-1-transfected cells compared to GFP (vector) transfected cells. Paired Student's *t*-test was used to compare normalized gene of interest mRNA expression between hSF-1 and GFP (vector) transfected cells. $^{*}p < 0.05$ and $^{**}p < 0.005$.

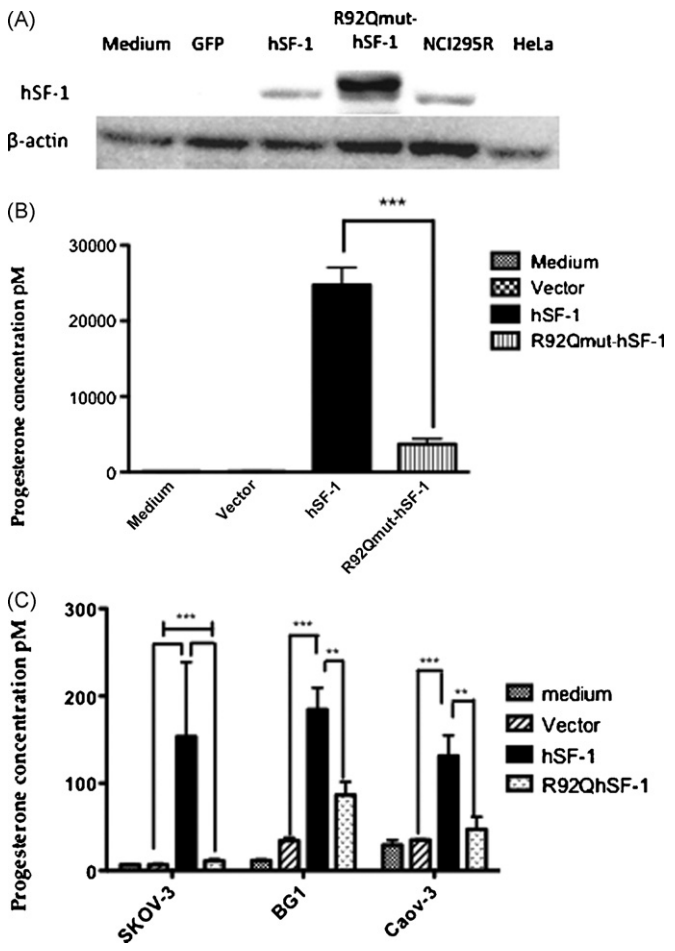

Fig. 4. (A–C) hSF-1 induces progesterone biosynthesis in ovarian cancel cells. Ovarian cancer cells (SKOV-3, BG1 and Caov-3) and immortalized OSE cells (IOSE-121) were transfected with expression vectors, GFP, GFP•SF-1, or mutDBDSF-1. Three days post-transfection progesterone assay was performed in the culture medium. The progesterone concentration is represented as picomoles. Each experiment was done in triplicate and repeated more than three times. Results are shown as the mean and ±SEM of nine samples in three independent experiments. (A) WB of SKOV-3 cells transfected with hSF-1, non-transfected NCI295R cells and non-transfected HeLa cells. (B) Progesterone biosynthesis in immortalized HOSE 121 cells. (C) Progesterone biosynthesis in ovarian cancer cell lines. One-way ANOVA was used to compare the progesterone concentration in each treatment group. $^{*}p < 0.05$, $^{**}p < 0.005$, and $^{***}p < 0.0005$.

ascertained that the progesterone concentration in serum free culture medium (OPTI-MEM) was similar to OPTI-MEM supplemented with 1% CS-FBS. Subsequently, ovarian cancer cells grown in OPTI-MEM supplemented with 1% CS-FBS were transfected with GFP vector, hSF-1 or mutant hSF-1 expression vector (R92QhSF-1). Three days post-transfection, the culture medium was collected for progesterone assays and cells were harvested for Western blot analysis to confirm hSF-1 expression (Fig. 4A). A standard curve was generated and the progesterone assay was performed with an ELISA kit according to the manufacturer's protocol. Results from these experiments demonstrate that hSF-1 induced significant progesterone biosynthesis in immortalized HOSE121 cells (Fig. 4B, bar 3) and ovarian cancer cell lines (Fig. 4C, bar 3) compared to GFP vector and the mutant hSF-1 (Fig. 4B and C, bars 2 and 4, Table 4).

3.5. Human SF-1 inhibits proliferation of SKOV-3 cells

To determine whether hSF-1 and progesterone inhibit proliferation of SKOV-3 cells, we performed cell counts on (1) SKOV-3 cells transfected with hSF-1, (2) SKOV-3 cells treated with either vehicle or progesterone and (3) SKOV-3 cells transfected with hSF-1 and treated with vehicle or PR antagonist RU486. SKOV-3 cells were transfected with vector (GFP), wild type hSF-1, or with the mutant SF-1 vectors. The mutant hSF-1 vector, mutAF2-hSF-1 is a truncated hSF-1 construct where the AF2 domain is deleted. The hSF-1-mutant construct, mutDBDhSF-1 has a mutation in the DNA binding domain (Fig. 5A). Transfected cells were sorted for GFP expression by FACS, replated and cultured for 4 days. Cell counts performed 4 days post-transfection demonstrated that SF-1 trans-

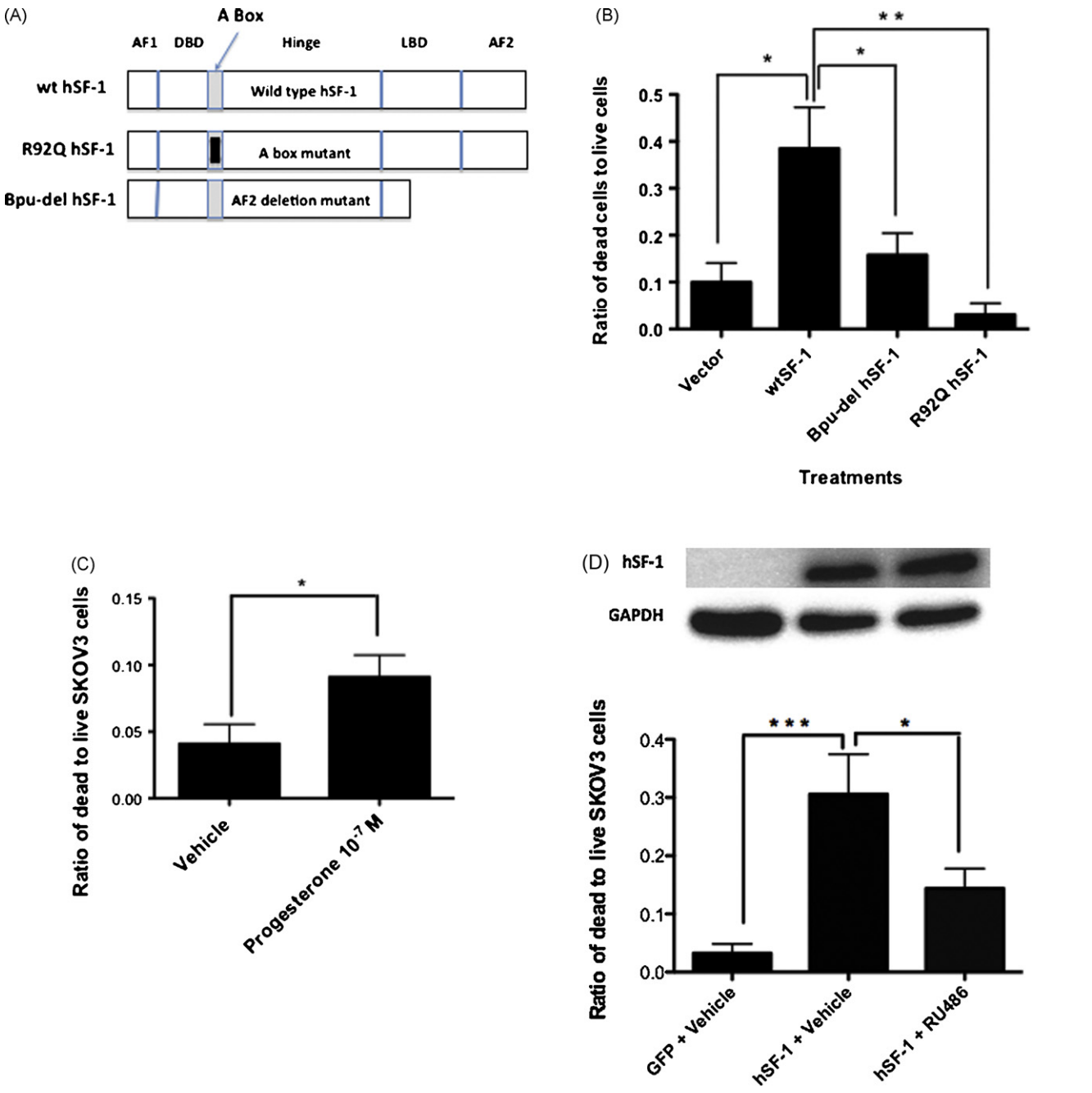

Fig. 5. (A–E) hSF-1 inhibits proliferation of SKOV-3 ovarian cancer cells. SKOV-3 were transfected with expression vectors, GFP, GFP•SF-1, or mutant hSF-1 vectors mutAF2SF-1 or mutDBDSF-1. The SKOV-3 cells were sorted by FACS for GFP. Live and dead cells were counted on a hemacytometer using the Trypan blue stain on the fourth day after transfection. (A) Expression vectors. (B) Ratio of dead cell count to live cell count for each treatment group. (C) Progesterone induced increase in ratio of dead to live cells. (D) Ratio of dead cell count to live cell count for cells transfected with control (GFP) vector or hSF-1 and treated with PR antagonist RU 486 or vehicle. Each experiment was done in triplicate and repeated more than three times. Results are shown as the mean and ±SEM of nine samples in three independent experiments. SKOV-3 cells. One-way ANOVA was used to compare the ratio of dead cells to live cells for each treatment group. $^{*}p < 0.05$, $^{**}p < 0.005$, and $^{***}p < 0.0005$.

fection induced apoptosis of SKOV-3 cells, and hSF-1 increased the ratio of dead cell to live cell numbers by 3–4-fold (Fig. 5B, bar 2) compared to the vector and the mutant hSF-1 constructs (Fig. 5B, bars 1, 3, and 4). Cells treated with progesterone showed a statistically significant increase in the ratio of dead to live cells (Fig. 5C, bar 2) as compared to cells treated with vehicle (Fig. 5C, bar 1). Also, SKOV-3 cells transfected with hSF-1 and treated with vehicle showed a significant increase in the ratio of dead to live cells (Fig. 5D, bar 2) compared to cells transfected with control (GFP) vector (Fig. 5D, bar 1). Treatment of hSF-1-transfected cells with RU486 showed a significant decrease in the ratio of dead to live cells (Fig. 5D, bar 3) compared to cells transfected with hSF-1 and treated with vehicle (Fig. 5D, bar 2).

3.6. Human SF-1 inhibits ERα mediated growth of SKOV-3 cells

To determine whether hSF-1 down-regulates functional ERα mediated proliferation of SKOV-3 cells, we transfected GFP vector, GFP•hSF-1, and ERα into SKOV-3 cells that were cultured in estrogen-free medium supplemented with charcoal-stripped FBS. The transfected cells were sorted for GFP expression by FACS, replated in 6-well plates, and incubated with vehicle (ethanol

Table 3
Activation of StAR, CYP11A1 and HSD3B2 mRNA by hSF-1 in SKOV-3 cells. Mean and SEM ΔC_T values by real-time RT-PCR of StAR, CYP11A1 and HSD3B2 mRNA in SKOV-3 cells transfected with control (GFP) vector and hSF-1 are shown. The C_T value of each gene of interest has been normalised to C_T value of GAPDH. The numerical C_T value is inversely proportional to the amount of amplicon in the reaction, i.e., the lower the C_T value, the greater the amount of amplicon. Each experiment was repeated three times.

Gene	ΔC_T hSF-1		ΔC_T control	
	Mean	SEM	Mean	SEM
StAR	1.64	1.40	7.52	1.76
CYP11A1	2.97	0.33	10.31	0.98
HSD3B2	4.09	1.00	9.94	1.37

0.001%) or E_2 (10 nM) for 48 h. Cell extracts were prepared to confirm protein expression by Western blot (Fig. 6). Cell counts that were performed on day 4 post-transfection demonstrated that hSF-1 decreased estradiol-induced proliferation of SKOV-3 cells by approximately 50% ($p < 0.05$) (Fig. 6, bar 6). Furthermore, hSF-1 also significantly ($p < 0.05$) decreased unliganded ERα-mediated SKOV-3 cell proliferation (Fig. 6, bar 5). Unlike the experiment in Fig. 5 in which SKOV-3 cells were cultured in medium with full FBS containing estradiol, the present experiments Fig. 6 were conducted in SKOV-3 cells that were cultured in estrogen-free medium (charcoal-stripped FBS). The absence of estradiol in the medium likely explains why there was no significant decrease in the growth of SKOV-3 cells transfected with GFP•hSF-1 alone.

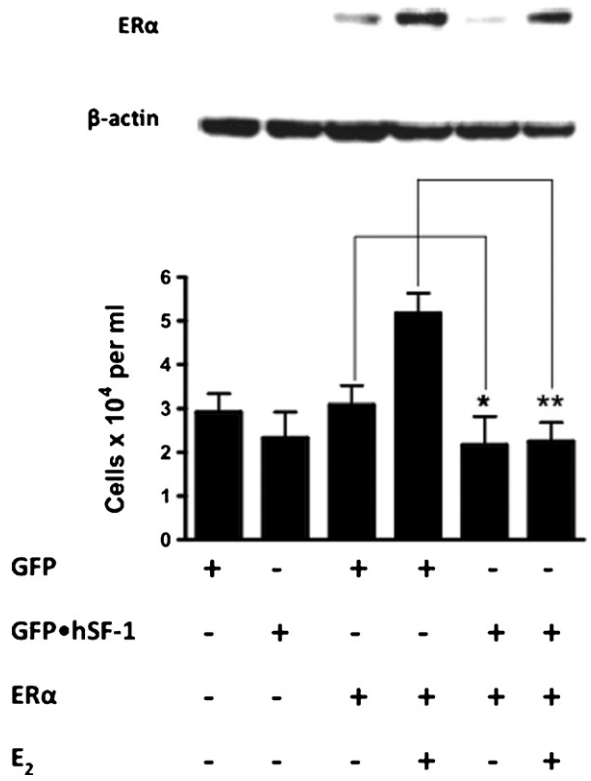

Fig. 6. hSF-1 down-regulates ER-mediated cell proliferation. SKOV-3 cells were transfected with GFP expression vector alone, GFP•SF-1 or ERα. The transfected cells were FACS sorted for GFP. Sorted cells were seeded in 6-well plates at a density of 10^4 cells/well. Prior to harvesting, the cells were treated with vehicle (ethanol) or 8 nM concentration of 17β-estradiol (E_2) for 48 h. Four days after transfection, cell counts were performed on a hemacytometer using the Trypan blue stain. Each experiment was done in triplicate and repeated more than three times. Results are shown as the mean and ±SEM of nine samples in three independent experiments. One-way ANOVA was used to compare the treatment groups. $^*p < 0.05$ and $^{**}p < 0.005$.

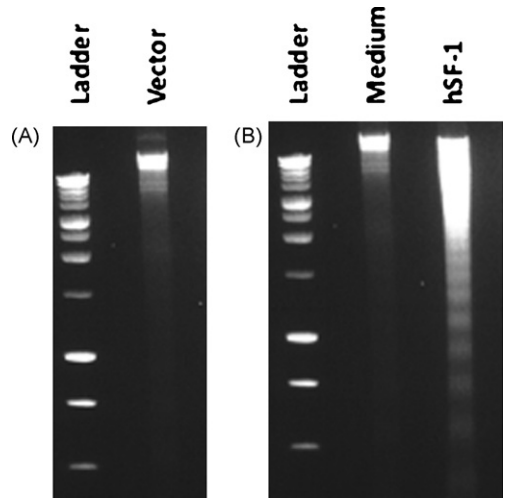

Fig. 7. (A and B) hSF-1 induces apoptosis of SKOV-3 ovarian cancer cells. SKOV-3 cells were transiently transfected with expression vectors alone GFP (A), or with GFP•SF-1 (B). Four days post-transfection, the cells were harvested and cell pellets were treated with lysis buffer containing 1% Igepal CA630, 20 mM EDTA, and 50 mM Tris. Apoptotic DNA fragments were treated with 0.2 mg/ml RNase A in 1% SDS, digested with 1.2 mg/ml proteinase K and DNA fragments were precipitated. DNA fragments were separated by electrophoresis in 1% agarose gels containing 0.5 mg/ml ethidium bromide.

3.7. Human SF-1 promotes apoptosis of human ovarian cancer cells

To determine whether hSF-1 promoted apoptosis of SKOV-3 cells, we transfected cells with GFP or GFP•hSF-1, and measured apoptosis 4 days following transfection using the DNA laddering assay. Expression of hSF-1 induced apoptosis of SKOV-3 cells (Fig. 7B, lane 3). To confirm these results, we performed the PARP-1 cleavage assay in SKOV-3 cells transfected with GFP, GFP•hSF-1 or with GFP•hSF-1 plus ERα and incubated with vehicle or E_2 (Fig. 8). Cells transfected with hSF-1 showed an increased amount of PARP-1 cleavage (Fig. 8, lanes 6 and 7) as compared to cells transfected

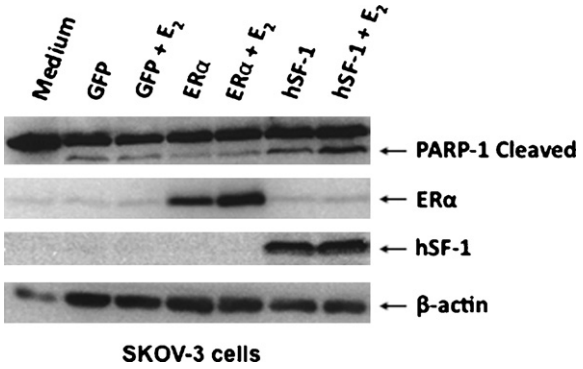

Fig. 8. Effect of hSF-1 on PARP-1 cleavage of SKOV-3 cells. SKOV-3 cells were grown in RPMI-1640 with 10% FBS to 60% confluence and 2×10^6 cells were seeded in 150 mm^2 flasks. SKOV-3 cells were transfected with GFP, hSF-1 and ERα or co-transfected with hSF-1 and ERα. SKOV-3 cells transfected with ERα were treated with vehicle (ethanol) or 17β-estradiol (E_2) concentration of 8 nM for 48 h. Four days post-transfection the cells were harvested. Forty micrograms of total protein from each cell lysate was analyzed by 10% SDS-polyacrylamide gel-electrophoresis. Mouse anti-poly(ADP-ribose) polymerase (PARP) monoclonal antibody (1:2000 dilution) was used as primary antibody. Goat-anti-mouse antibody (1:3000 dilution) coupled to horseradish peroxidase was used as secondary antibody. Proteins were detected using the ECL reagents. The chemiluminescent bands of PARP-1, uncleaved 116 kDa and cleaved 89 kDa were visualized. Membranes was stripped with ECL stripping buffer and sequentially probed with hSF-1 and ERα antibodies. For an internal loading control, Western blot for β-actin was performed on all the samples.

Table 4
Induction of progesterone biosynthesis in immortalised human ovarian surface epithelial (IOSE-121) cells and ovarian cancer cells by hSF-1. Mean and SEM values of progesterone concentration (picomoles) in each cell line as well as progesterone concentration in cells transfected with control vector (GFP), wild type hSF-1 and mutant hSF-1 vector are shown. IOSE-121 cells had a much higher induction of progesterone biosynthesis by hSF-1 as compared to ovarian cancer cell lines.

Plasmid	None		Vector		hSF-1		R92Omut-hSF-1	
Progesterone pM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
IOSE-121	55.48	15.97	69.16	3.45	24753.10	2310.23	3688.39	733.19
SKOV-3	6.69	0.67	6.54	1.50	153.36	85.52	11.09	1.96
BQ-1	11.6	1.18	34.19	3.52	184.44	25.00	86.65	15.10
Caov-3	29.36	5.62	34.97	0.63	131.19	23.81	47.28	14.29

with GFP vector and incubated with E_2 or with ERα and incubated with E_2 (Fig. 8, lanes 3 and 5).

4. Discussion

Human steroidogenic factor-1 (hSF-1) regulates ovarian steroidogenesis by activating StAR expression [12,33]. Cultured normal human OSE cells are reported to be capable of steroidogenesis, and immortalized OSE cells express hSF-1, p450 aromatase and 17β-HSD [4,22]. Because progesterone inhibits proliferation and promotes apoptosis of OSE cells and hSF-1 is pivotal to steroidogenesis, we hypothesized that hSF-1 may be a potential key inhibitor of OSE cell growth. And, loss of hSF-1 expression in malignant OSE cells may promote cell growth.

To determine the expression of hSF-1 and StAR in normal OSE cells, we examined paraffin embedded sections of normal ovarian tissues by immunohistochemistry. Both hSF-1 and StAR expression was observed in the surface epithelial cells (Fig. 1). In contrast, the ovarian cancer cells BG1, SKOV-3 and Caov-3, and immortalized but not malignant IOSE-121 cells, lacked hSF-1 and StAR expression (Fig. 2). Immunohistochemical studies of hSF-1 expression in ovarian sex cord stromal tumors showed that hSF-1 is expressed only in steroidogenic tumor cells [18]. Sasano et al. studied hSF-1 expression in epithelial and metastatic tumors of the ovary, and demonstrated that although stromal cells in the carcinomas and metastatic carcinomas expressed hSF-1, neither the adenoma nor the carcinoma cells exhibited hSF-1 immunoreactivity [17]. The Western blot data obtained in this study showing a lack of hSF-1 expression in the ovarian cancer cell lines is in agreement with the studies by Sasano et al. in ovarian epithelial cancer cells.

In addition to hSF-1, DAX-1, a transcription factor, regulates steroidogenesis in the ovary [34]. DAX-1 crosstalks with hSF-1 to down-regulate hSF-1 mediated transcriptional activity and repress *StAR* gene expression [35–37]. Immunohistochemical analysis of DAX-1 expression in OSE cancers showed that DAX-1 immunoreactivity was an independent marker of adverse outcome in patients with OSE [38]. In a follow up study by Abd-Elaziz et al. on the immunoreactivity of hSF-1, StAR, steroidogenic enzymes, and DAX-1 in epithelial ovarian carcinomas, a positive statistical correlation was found between hSF-1 and StAR immunoreactivity [39]. Furthermore, a significant inverse correlation was found between hSF-1 immunoreactivity and tumor size and tumor grade. In addition to a decreased hSF-1 expression in carcinomas as compared to benign or borderline tumors, a strong positive correlation between StAR and hSF-1 expression, and a negative correlation between StAR and DAX-1 an established repressor of hSF-1 expression, was observed. A significant inverse correlation was observed between StAR, HSD3B2, CYP11A1 expression and residual tumor size as well as international federation of obstetrics and gynecology (FIGO) tumor stage, both of which are clinical markers of poor outcome and survival. In addition, StAR expression positively correlated with PR expression. A recent clinico-pathalogical study of 322 tissue microarray samples of primary ovarian carcinoma showed that over-expression of progesterone receptor was associated with a favorable prognosis [40]. Our Western blot data demonstrating a lack of hSF-1 and StAR expression in ovarian cancer cell lines are in agreement with the immunohistochemistry studies by Abd-Elaziz et al. and tissue microarray studies by Lee et al. [39,40].

Hyperplastic and metaplastic changes have been frequently observed in epithelial cells lining the cortical inclusion cysts of the ovary [41–44]. In a study of grossly normal ovaries retrieved from 215 patients, including 33 women with contralateral ovarian tumor, mucinous metaplasia of CIC epithelium was more frequently observed in patients with contralateral ovarian tumor compared to those without ovarian tumor (12.1% vs. 1.9%; $p < 0.05$) [45]. Also, IHC studies have demonstrated that various ovarian carcinoma antigens are more frequently expressed in CIC epithelium compared to ovarian surface epithelium [46]. It has been suggested that OSE of cortical inclusion cysts may be precursors of ovarian epithelial cancers [41–44,47]. Interestingly, although 5 out of the 6 ovarian tissues examined showed hSF-1 expression in normal OSE cells, 4/6 ovaries did not show hSF-1 expression in epithelial cells of cortical inclusion cysts (CIC) (Table 2). StAR expression was observed in the OSE cells of all the 6 normal ovaries examined and in CIC epithelium of 5/6 ovaries (Table 2). Although the sample size is small, the lack of hSF-1 expression in OSE of CIC in 4/6 ovaries (Table 2) is supportive of the role hSF-1 as a putative tumor suppressor (Fig. 9) and is in agreement with above studies on CIC epithelium as a precursor of ovarian.

In OSE cells, impaired progesterone biosynthesis may be a key first step that initiates transformation to the pre-malignant and malignant phenotype. A recent study suggests that in ovarian epithelial tumors progesterone inhibits cell cycle activity partly by down-regulating cdk 1/cyclin B complex [48]. After confirming the validity of GFP•hSF-1 construct by StAR promoter-luciferase reporter assay in HeLa cells, we performed real-time PCR studies in ovarian cancer SKOV-3 cells transiently transfected with GFP or GFP•hSF-1. These real-time PCR studies support the role of hSF-1 in stimulating *StAR*, *CYP11A1* and *HSD3B2* gene expression, in ovarian cancer cells (Table 3) since mRNA expression of these genes was induced approximately 60–200-fold by wild type hSF-1 (Fig. 3C). Additionally in contrast to mutant hSF-1, wild type hSF-1 was able to induce progesterone biosynthesis in immortalized OSE cells and in ovarian cancer cell lines (Table 4), demonstrating that hSF-1 is crucial to the activation of *StAR*, *CYP11A1*and *HSD3B2* gene expressions to promote progesterone biosynthesis in OSE cells (Fig. 4, panel B–D). The absence of SF-1 expression in ovarian cancer cells may lead to a lack of progesterone biosynthesis and cancer cell proliferation. In essence, *hSF-1*, *StAR*, *CYP11A1* and *HSD3B2* expressions, and ultimately steroid biosynthesis may be a marker of tumors with low malignancy potential and improved prognosis.

The role of hSF-1 in the human ovary may extend beyond steroidogenesis to cell growth, senescence and death. Several lines of evidence support our data that hSF-1 can function as a suppressor of ovarian cancer cell proliferation. Ectopic expression of SF-1 inhibited the proliferation of rat ovarian epithelial cell proliferation, causing G1 cell cycle arrest and promoting apoptosis [49]. Studies in rat OSE cells also suggest that SF-1 expression was suppressed when cells enter the cell cycle; SF-1 expression was higher in the G0/G1 phase as compared to the S-G2/M of

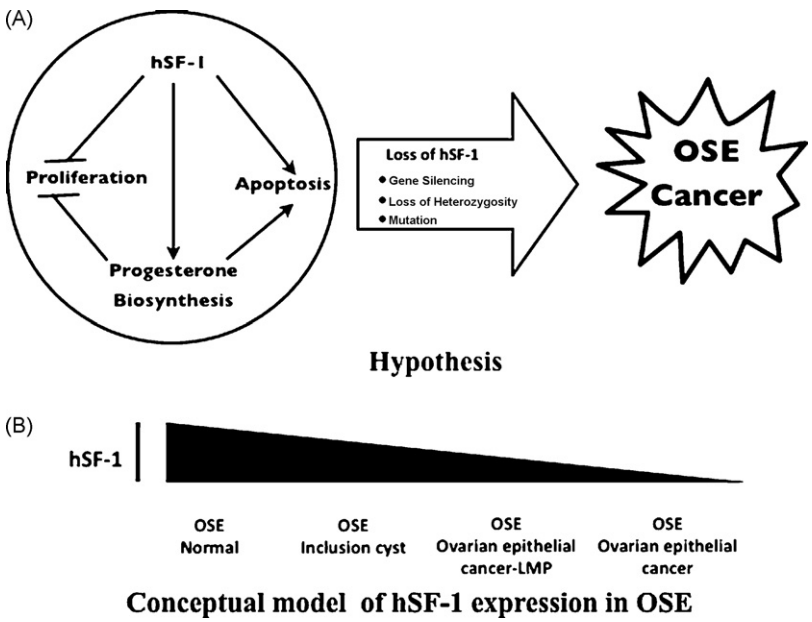

Fig. 9. (A and B) Hypothesis and conceptual model of human steroidogenic factor-1 as a tumor suppressor in ovarian epithelial cancer.

the cell cycle [49]. Our cell proliferation data demonstrate that hSF-1 inhibits growth of SKOV-3 cells (Fig. 5B). Wild type hSF-1 significantly increased the ratio of dead cells to live cells as compared to vector and mutant hSF-1-transfected cells (Fig. 5B, bar 2). These data suggest that the presence of hSF-1 was important for apoptosis of SKOV-3 cells. Recent studies have demonstrated PR expression in some ovarian cancer cell lines and ovarian epithelial cancer tissues by IHC [50–54]. In a study of 86 women, 40 years of age or younger with ovarian cancer, 57% of the patients showed expression of PR [55]. And PR expression was associated with favorable survival [53,55]. PR polymorphisms (PROGINS) and mutations have been observed in association with increased risk of ovarian cancer [56]. In women with ovarian cancers positive for PR, progesterone mediated activation of PR may down-regulate cell proliferation. Our cell-proliferation studies demonstrate that as compared to vehicle treated SKOV-3 cells, progesterone treated cells have a significantly increased ratio of dead to live cells (Fig. 5C, bar 2). Furthermore treatment of SF-1-transfected SKOV-3 cells with PR antagonist RU486 decreases hSF-1 mediated inhibition of cell growth (Fig. 5D, bar 3) suggesting that hSF-1 induced progesterone actions mediated through PR inhibit OSE cancer cell growth. Therefore, re-constitution of hSF-1 mediated progesterone biosynthetic pathway, by reversal of hSF-1 gene silencing, may have a potential therapeutic role in PR positive ovarian cancers.

Previous studies suggest that SKOV-3 cells are growth-resistant to estradiol [57,58]. It has been suggested that over-expression of HER-2/neu and cathepsin D in SKOV-3 cells interferes with the mitogenic pathways, resulting in unresponsiveness of SKOV-3 cells to estradiol [57]. A nonfunctional ERα transcript with a 32 bp deletion in exon 1 detected in SKOV-3 cells may explain the unresponsiveness to E_2 [58]. As we were interested in determining whether hSF-1 is able to down-regulate ERα-mediated proliferation of SKOV-3 cells, we co-transfected SKOV-3 cells with hSF-1 and functional ERα expression plasmids. The mitogenic effect of E_2 on SKOV-3 cells was demonstrated by an increase in estradiol-mediated growth of SKOV-3 cells transfected with functional ERα (Fig. 6, bar 4). Our findings show that hSF-1 significantly ($p < 0.05$) down-regulates ERα and E_2/ERα-mediated mitogenic effects on SKOV-3 cells (Fig. 6, bars 5 and 6). The altered growth conditions in this experiment, in which SKOV-3 cells cultured in estrogen-free medium (charcoal-stripped FBS) likely explains the lack of a significant decrease in growth of SKOV-3 cells transfected with GFP•hSF-1 alone.

Our DNA laddering (Fig. 7A and B) and PARP-1 cleavage (Fig. 8) assays demonstrated that hSF-1 promotes apoptosis of SKOV-3 cells. These data suggest that hSF-1 induced apoptosis contributes to growth suppression of SKOV-3. Our data suggest that hSF-1 may utilize several mechanisms to regulate OSE cancer growth. First, hSF-1 mediated activation of *StAR*, *CYP11A1* and *HSD3B2* gene expression induces progesterone biosynthesis. Second, a direct regulation of genes promoting apoptosis by hSF-1 may also lead to a decrease in cell growth. Finally, hSF-1 down-regulates ERα and E_2/ERα -mediated ovarian cancer cell growth. Estrogens are known to be mitogenic and increase the proliferation of ovarian cancer cells [59]. It is possible that in OSE-derived cancers of the ovary, expression of hSF-1 may have an inhibitory effect on cell proliferation driven by intratumoral E_2 levels. Human SF-1 mediated induction of apoptotic pathways or crosstalk between hSF-1 and ERα, resulting in down-regulation of ERα-target genes involved in ovarian cancer cell proliferation, may be responsible for the suppression of ERα-mediated proliferation of SKOV-3 cells by hSF-1. Thus, hSF-1 may act as a suppressor of ovarian epithelial cell-derived cancers (Fig. 9). Studies to address these possibilities are currently underway.

Human steroidogenic factor-1-mediated induction of progesterone biosynthesis in SKOV-3 cells is particularly noteworthy, as these cells are intrinsically cisplatin-resistant [60]. Recent *in vitro* studies on cisplatin-resistant SKOV-3 and OVCAR-3 ovarian cancer cells and in vivo studies on SKOV-3 cell-xenografts in athymic mice demonstrated that the addition of high-dose progesterone to cisplatin therapy enhanced platinum accretion, resulted in apoptosis of cancer cells, and suppression of tumorigenesis [61]. Thus, re-expression of hSF-1 to induce intratumoral-progesterone biosynthesis may be useful as a therapeutic modality in cisplatin-resistant ovarian epithelial cancers.

5. Conclusions

In summary, the data presented in this paper demonstrate that hSF-1 and steroidogenic acute regulatory (StAR) protein are expressed in normal ovarian surface epithelial cells. In contrast, hSF-1 and StAR expression is absent in a panel of ovarian cancer

cell lines. Transient expression of hSF-1 in SKOV-3 cells in culture, activated StAR, CYP11A1and HSD3B2 mRNA and induced progesterone biosynthesis. Furthermore, hSF-1 promotes apoptosis and down-regulates estradiol-mediated proliferation of SKOV-3 cells. Thus, these results support the novel role of human steroidogenic factor-1 (hSF-1) as a putative tumor suppressor of ovarian epithelial cancer. Understanding the molecular mechanisms governing hSF-1-mediated effects on ovarian cancer cell growth and apoptosis may be useful in the development of effective therapies for chemotherapy-resistant ovarian cancers and thus, reduce recurrence and improve survival.

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