Distinct Expression Pattern of Dicer1 Correlates with Ovarian-Derived Steroid Hormone Receptor Expression in Human Fallopian Tubes during Ovulation and the Midsecretory Phase

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Context: Tissue-specific *dicer1* knockout mice display severe, irreversible Fallopian tube damage and disrupted tubal transport. It is not known how Dicer1 affects human Fallopian tube function.

Objective: The aim of the study was to investigate the regulation of tubal Dicer1 expression during ovulation and the midsecretory phase and to assess Dicer1-associated alterations in estrogen receptor (ER) subtype and progesterone receptor (PR) expression.

Design: Fallopian tissue was obtained from patients at early (n = 4), late (n = 4), and postovulatory (n = 5) phases and the midsecretory phase (n = 4). Serum was obtained immediately before surgery (sterilization or hysterectomy) to confirm the phases. The localization and regulation of Dicer1, ER subtypes, and PR isoforms were determined by immunofluorescence, confocal microscopy, and quantitative RT-PCR.

Results: Dicer1 protein was expressed most abundantly in Fallopian epithelial cells; mRNA and protein levels peaked in the late ovulatory phase. ER subtype and PR isoform mRNA levels were not related to ovulatory stages; however, ERβ1 and ERβ2 mRNA/protein levels were highest and PRA/B and PRB mRNA/protein levels were lowest in the midsecretory phase. Dicer1 mRNA expression correlated positively with ERα mRNA expression in the late ovulatory phase and negatively with ERβ2 mRNA expression in the midsecretory phase and PRB mRNA in the early ovulatory phase.

Conclusion: Dicer1 expression is up-regulated in cell-specific fashion in human Fallopian tubes during ovulation. The stage-dependent expression of Dicer1 and its correlation with ERα, ERβ2, and PRB mRNA suggests that tubal Dicer1 helps regulate tubal expression of steroid hormone receptors in a cycle-dependent manner and may contribute to tubal transport in humans. **(*J Clin Endocrinol Metab* 96: E869–E877, 2011)**

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Abbreviations: DGCR8, DiGeorge syndrome critical region gene 8; E2, 17β-estradiol; ER, estrogen receptor; LHR, LH receptor; miRNA, microRNA; P4, progesterone; PR, P4 receptor; rhCG, recombinant human choriogonadotropin; RPLPO, ribosomal protein; SM, smooth muscle.

Female reproductive function appears to require intricate spatiotemporal expression of microRNAs (miRNAs) that are important for gene regulation (1, 2). Aberrant expression of miRNAs contributes to infertility (2) and other reproductive diseases (2–4). miRNAs are differentially expressed in normal human Fallopian tubes (3), and some are misexpressed in women with Fallopian tube carcinoma, allowing identification of tissue-specific miRNA expression signatures (3). Dicer (encoded by *dicer1*) is the key ribonuclease III enzyme required for miRNA maturation and function (5, 6). In mice, loss of *dicer1* inhibits miRNA maturation, resulting in early embryonic lethality (7). The significance of miRNAs in mammalian reproduction has been dissected by tissue-specific expression of mutant *dicer1* in mice. Adult female mice with a floxed *dicer1* allele under the control of Cre recombinase driven by the anti-Müllerian hormone receptor 2 promoter are infertile and have Fallopian tubal hypotrophy and prominent tubal cysts at the isthmus near the uterotubal junction, leading to disruption of tubal transport (8–10). These findings suggest that Dicer1 is important in the regulation of Fallopian tube development and function. However, the expression, regulation, and function of Dicer1 in human Fallopian tube during ovulation and the normal reproductive cycle are unknown.

Coordinated, synchronized actions of 17β-estradiol (E2) and progesterone (P4) are essential for the regulation of diverse physiological processes in human Fallopian tube (11). E2 and P4 are specific ligands of the estrogen receptor (ER) and P4 receptor (PR), respectively (12). Their levels affect transport of gametes and the early embryo and other dynamic functions of the Fallopian tube (13–15). The ER subtypes, ERα and ERβ, are encoded by separate genes (16), whereas the PRA and PRB isoforms result from alternative start codons on the same gene (12). The ER and PR are both expressed in human and rodent Fallopian tubes (17–20). We showed that ER subtypes and PR isoforms in rodent Fallopian tube are regulated in response to E2 or P4 (18–20). Thus, tubal expression of ER subtypes and PR isoforms may vary during the normal reproductive cycle in relation to changes in E2 and P4 levels.

An important function of the Fallopian tube in ovum transport is its ability to respond to steroid hormone released from the ovary after ovulation. Precise, controlled regulation of tubal ER and PR expression may be critical for normal tubal transport (21). However, the regulation of ER subtype and PR isoform levels in human Fallopian tubes during ovulation has not been fully elucidated. Interestingly, in human ovarian cancer cells *in vivo* and *in vitro*, there is evidence of an association between ER and Dicer1 expression (22). Moreover, treatment with E2 increases *dicer1* expression in ER-positive breast cancer cells *in vitro* (23), suggesting an interaction between ER-mediated E2 signaling events and Dicer regulation.

To test the hypothesis that Dicer is a physiological regulator of Fallopian tube function, we investigated the expression and regulation of Dicer1 in human Fallopian tubes at different stages of ovulation and in the midsecretory phase of the reproductive cycle. We also determined whether tubal Dicer1 regulation is connected to the expression of ovarian steroid hormone receptors (ERα, ERβ1, ERβ2, PRA, and PRB).

Subjects and Methods

Patients and tubal biopsies

Fallopian tube samples were obtained from 17 women admitted for sterilization or hysterectomy. The inclusion criteria were: regular menstrual cycles (25–29 d), age 28–37 yr (mean, 34.8), with proven fertility (para $>$ 1; mean, 2.9), no chronic systemic diseases (*e.g.* diabetic and inflammatory conditions), and no hormonal therapy for at least 3 months before surgery. All patients were monitored by serial transvaginal ultrasound (Aloka SSD-900/2000; Aloka, Tokyo, Japan) for at least one menstrual cycle (mean, two cycles) before surgery to determine whether follicular development occurred. Women received a sc injection of recombinant human choriogonadotropin (rhCG) (250 μg Ovitrelle; Serono International, Geneva, Switzerland) to mimic the natural LH peak when the dominant follicles were at least 14 mm and no more than 20 mm (mean ± SEM, 17.1 ± 0.3

TABLE 1. Clinical characteristics and biochemical evaluation of patients

Variable	Phase of ovulation			Midsecretory phase
	Early	Late	Post	
n	4	4	5	4
Age (yr)	34.0 ± 1.8	35.8 ± 0.6	35.2 ± 0.6	34.0 ± 2.0
Historical cycle length (d)	27.3 ± 0.8	28.0 ± 0.0	27.0 ± 0.7	26.0 ± 0.9
Cycle day of surgery	12.3 ± 0.8	13.3 ± 0.5	12.2 ± 0.4	20.0 ± 3.0
Follicle size (mm)	17.0 ± 0.4	17.6 ± 0.9	17.5 ± 0.5	16.3 ± 0.5
Hours after rhCG	16.5 ± 2.4	23.3 ± 0.9	38.8 ± 2.9	0
E2 (nmol/liter)	0.8 ± 0.1	0.6 ± 0.2	0.5 ± 0.1	0.6 ± 0.1
P4 (nmol/liter)	1.4 ± 0.4	1.7 ± 0.4	2.4 ± 0.7	11.1 ± 9.9

Values are expressed as mean ± SEM.

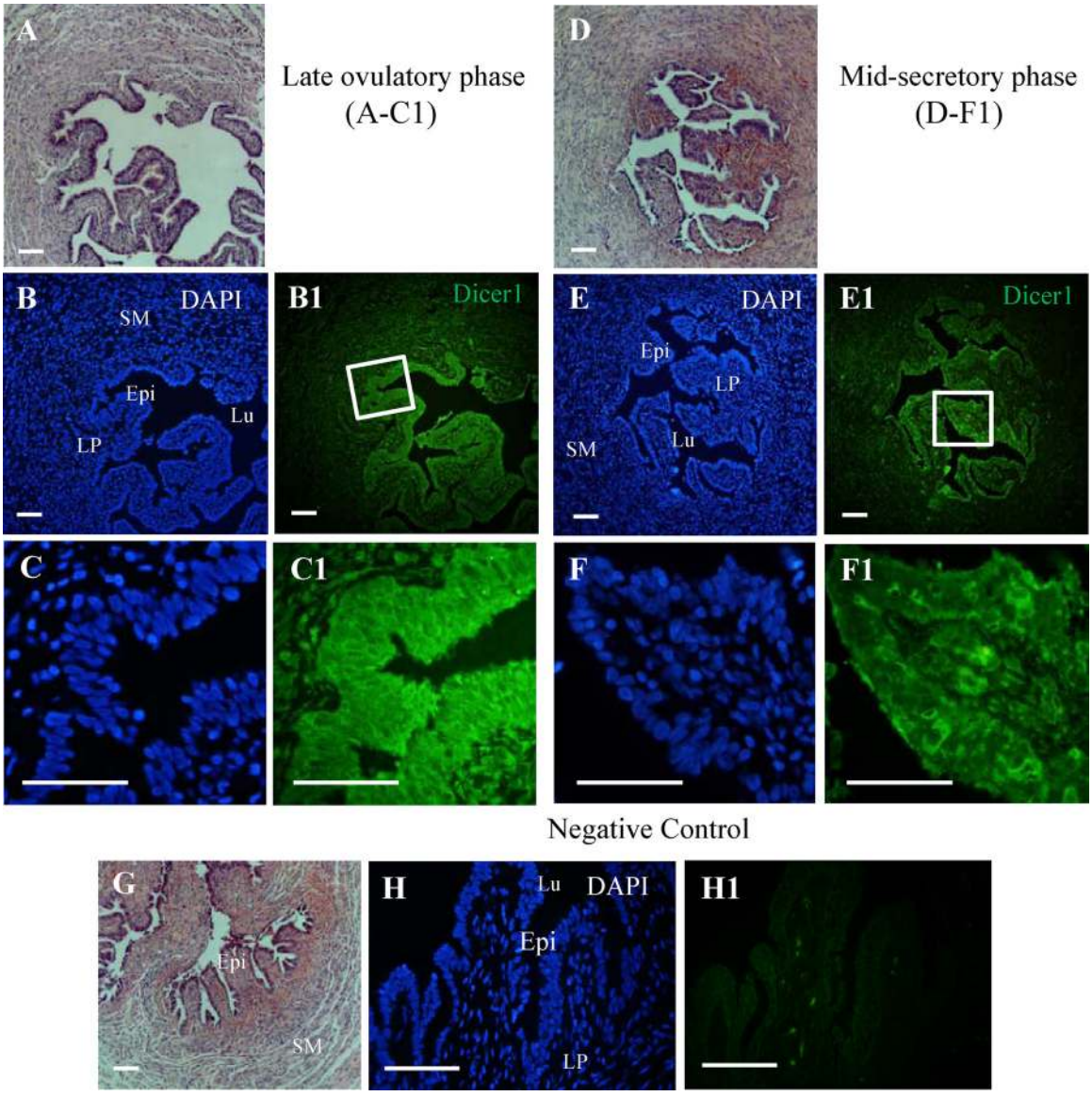

FIG. 1. Immunofluorescence detection of Dicer1 in formalin-fixed sections of human Fallopian tubes. Histology of hematoxylin and eosin-stained human tubal biopsy samples (A, D, and G). Single labeling for Dicer1 (*green*) shows extensive expression in epithelial and stromal cells and a significant difference in expression between the late ovulatory (B1 and C1) and midsecretory (E1 and F1) phases. Areas in *white boxes* are shown at higher magnification in C1 and F1 to reveal the epithelial cell layer in more detail. All sections were counterstained with 4′,6-diamidino-2-phenylindole (DAPI) (*blue*, B, C, E, and F). Negative immunological control with DAPI (H) and secondary antibody (H1) shows little staining in the lamina propria. Representative photomicrographs are shown. Lu, Lumen; Epi, epithelial cells; LP, lamina propria; SM, smooth muscle cells. *Scale bars*, 100 μm.

mm). The ovulatory stage was determined as described (24); the early phase was defined as 12 to 18 h or less after rhCG (n = 4), the late phase as more than 18 to no more than 24 h after rhCG (n = 4), and the postovulatory phase as more than 24 to 45 h (n = 5) after rhCG. The midsecretory phase (n = 4) was determined from the last menstrual period and endometrial histology. Serum was obtained immediately before surgery to confirm the ovulatory and midsecretory phases.

During laparoscopic surgery for sterilization (three ovulatory stages), the procedure was continued by clamping and excising the proximal parts (isthmus) of the Fallopian tubes, followed by diathermia of the tube ends (24). The biopsy sample (~0.5 cm) was removed from the abdomen in a laparoscopic sac. All hysterectomy patients were in the midsecretory phase. The hysterectomies were performed for heavy menstrual bleeding in patients who suffered from abdominal pain or vaginal bleeding but had no uterine pathology. The samples were washed with ice-cold RNase-free PBS and either snap-frozen in liquid nitrogen and stored at −70 C for RNA extraction or quantitative RT-PCR analysis or fixed in 4% formaldehyde and embedded for hematoxylin and eosin and immunofluorescence staining.

Written informed consent was obtained from all patients. The study was approved by the Research Ethics Committee at Gothenburg University and was conducted at the Sahlgrenska Academy at the University of Gothenburg in accord with the Declaration of Helsinki for medical research involving human subjects.

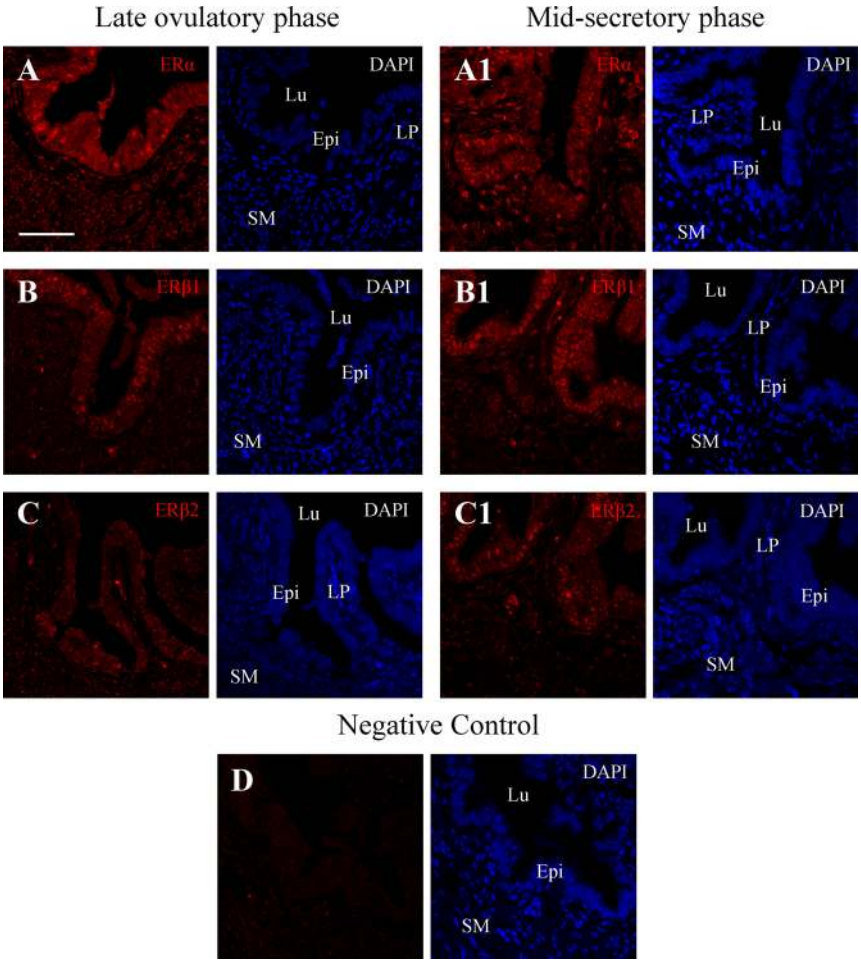

FIG. 2. Immunofluorescence detection of ER subtypes in formalin-fixed sections of human Fallopian tubes. Single labeling for ERα (*red*) shows expression in epithelial and stromal cells, with no significant difference between late ovulatory (A) and midsecretory phases (A1). Single labeling for ERβ1 (B and B1, *red*) and ERβ2 (C and C1, *red*) shows higher nuclear expression in epithelial cells in the midsecretory phase (B1 and C1) than in the late ovulatory phase (B and C). In all panels, nuclei are stained with 4′,6-diamidino-2-phenylindole (DAPI; *blue*). Negative immunological control with DAPI and secondary antibody (D) shows no staining in the Fallopian tube. Lu, Lumen; Epi, epithelial cells; LP, lamina propria; SM, smooth muscle cells. *Scale bar*, 50 μm.

Experimental procedures

Immunofluorescence and quantitative real-time PCR analyses were performed as described previously (14, 15, 19) and in the Supplemental Data (published on The Endocrine Society's Journals Online web site at http://jcem.endojournals.org). The antibodies, primers, and reagents used are also described in the Supplemental Data.

Serum hormone assays

Serum LH, E2, and P4 were measured with human-specific chemiluminescent microparticle immunoassay kits (LH-6C25, E2–7K72, and P4–7K77; Abbott Scandinavia, Solna, Sweden) using the Architect system (Abbott Scandinavia). Values were obtained with serial dilutions of human serum paralleling the standard curve in these assays.

Statistical analysis

Results are expressed as mean ± SEM. Significance was tested by two-way ANOVA followed by Bonferroni correction for multiple comparisons as appropriate. $P < 0.05$ was considered significant. Matrixes were constructed to calculate r values (two-tailed bivariate Pearson's correlation coefficients); the significance of r coefficients was calculated on the basis of correlation values and sample sizes. SPSS (v. 13.0; SPSS, Chicago, IL) was used for all statistical analyses.

Results

The age, historical cycle length, cycle days of surgery, follicle size, hours after rhCG, and E2 and P4 levels for all patients are given in Table 1.

Cellular location of Dicer1

Representative images of immunofluorescence staining of Dicer1 protein in human Fallopian tubes are shown in Fig. 1 for the late ovulatory (Fig. 1, A–C1 and G–H1) and midsecretory (Fig. 1, D–F1) phases. Dicer1 was found predominantly in the epithelial cell layer (Fig. 1, B1 and E1), and the levels were higher in the late ovulatory phase (Fig. 1, C1) than in the midsecretory phase (Fig. 1, F1). Lower expression was found in the lamina propria (Fig. 1, C1 and F1), which contained blood vessels and free gland blending with the submucosa. Little or no Dicer1 staining was present in smooth muscle (SM) cells (Fig. 1, B1 and E1). The specificity of Dicer1 staining was based on negative controls in which the primary antibody (Fig. 1, H1) or both primary and secondary antibodies were omitted or the primary antibody was gradually diluted, leading to disappearance of immunostaining (data not shown).

Cellular location of ER subtypes and PR isoforms

Representative images of immunofluorescence staining for ERα, ERβ1, ERβ2, PRA, and PRB protein and cell type-specific marker proteins, including cytokeratin (epithelial cells) and α-SM actin (SM cells), are shown in Figs. 2 and 3 for the late ovulatory (Fig. 2, A–C, and Fig. 3, A–C) and midsecretory (Fig. 2, A1–C1, and Fig. 3, A1–C1) phases. In epithelial cells, ERα protein was more abundant in the nucleus than in the cytoplasm (Fig. 2, A and A1). ERα protein was concentrated in epithelial cells and lamina propria but was also detected at low levels in SM cells

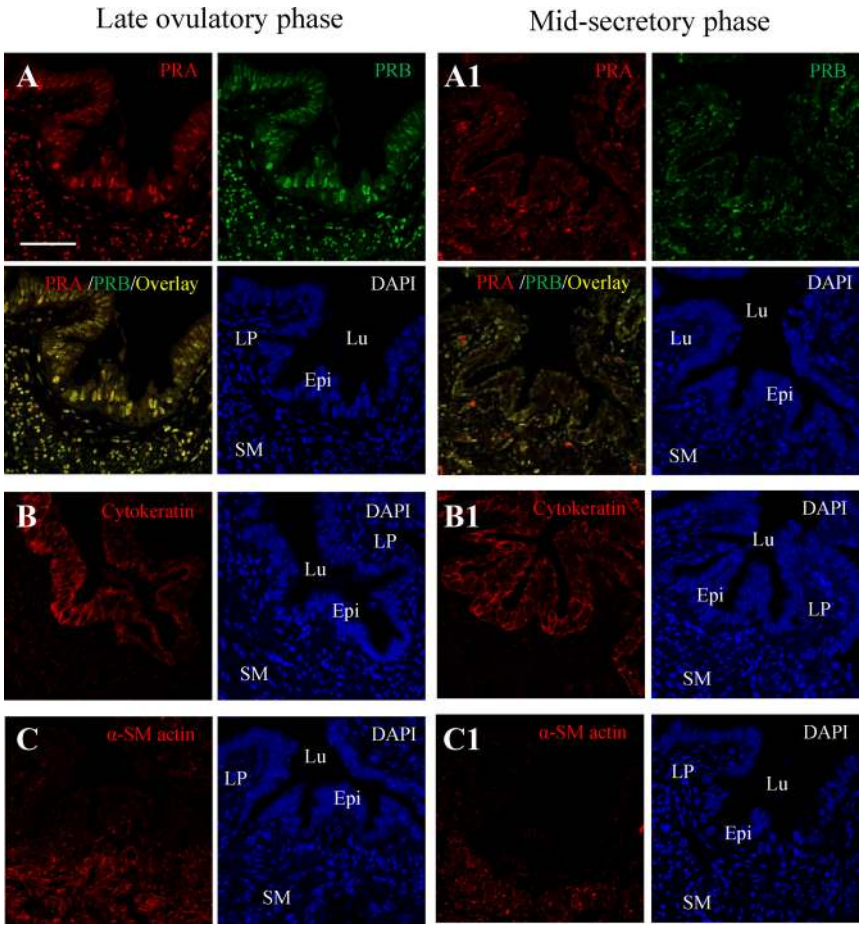

FIG. 3. Immunofluorescence detection of PR isoforms and cell markers in formalin-fixed sections of human Fallopian tubes. Double labeling for PRA (*red*) and PRB (*green*) shows colocalization in epithelial cells (A, *bottom left*) and stromal cells (A and A1, *bottom left*). In contrast to the late ovulatory phase (A), nuclear localization of PRA and PRB is barely detectable in the epithelial cells in the midsecretory phase (A1). Colocalization of PRA (*red*) and PRB (*green*) proteins results in a *yellow color*. Single labeling for cytokeratin (B and B1, *red*) and α-SM actin (C and C1, *red*) shows that epithelial cells are predominantly stained for cytokeratin (B and B1), whereas muscle cell structures are stained exclusively with α-SM actin (C and C1). In all panels, nuclei are stained with 4′,6-diamidino-2-phenylindole (DAPI; *blue*). Lu, Lumen; Epi, epithelial cells; LP, lamina propria; SM, smooth muscle cells. *Scale bar*, 50 μm.

in the late ovulatory and the midsecretory phases (Fig. 2, A and A1). ERβ1 and ERβ2 proteins were also present in the nucleus of epithelial cells at a distinctly higher level in the midsecretory phase (Fig. 2, B1 and C1) than in the late ovulatory phase (Fig. 2, B and C). However, little or no ERβ1 and ERβ2 protein expression was detected in lamina propria and SM cells at any phase. Colocalization of ERα with ERβ1 or ERβ2 or of ERβ1 with ERβ2 could not be assessed because all antibodies were monoclonal mouse antibodies.

Dual immunofluorescence staining with PRA- and PRB-specific antibodies (Fig. 3, A and A1) showed colocalization of PRA and PRB in the nucleus. These results were confirmed with anti-PR antibody (sc-538; Santa Cruz Biotechnology, Santa Cruz, CA), which is specific for PRA in human cells (25) and also colocalized with PRA (data not shown) and PRB (Fig. 3A). PRA or PRB immunoreactivity was detected in all cell types of the Fallopian tube (epithelial cells and SM cells) but differed between the late ovulatory and midsecretory phases (Fig. 3, A and A1); in particular, epithelial cell nuclei were negative for PRA and PRB in the midsecretory phase (Fig. 3A1). Cytokeratin immunoreactivity was evident in all epithelial cells (Fig. 3, B and B1), but α-SM actin immunoreactivity was most prominent in the stromal and muscle cell layers (Fig. 3, C and C1), as expected. Incubating sections with nonimmune IgG from the same Fallopian tube tissue did not produce specific immunoreactivity (Fig. 2D).

Dicer1, miRNA processors, and steroid hormone receptor mRNA expression

Expression of Dicer1, ERα, ERβ1, ERβ2, PRA, and PRB was determined by real-time RT-PCR. Expression of the LH receptor (LHR), which is differentially expressed in human Fallopian tubes, was also examined (26). mRNA levels of ERα, ERβ1, ERβ2, PRA/B, PRB, and LHR did not change significantly during ovulation; however, Dicer1 mRNA expression was significantly higher in the late ovulatory phase (Fig. 4).

To identify correlations of ER subtype and PR isoform genes to Dicer1, which could imply potential regulation of ER subtypes and PR isoforms by Dicer1, gene expression was normalized to expression of human ribosomal protein (RPLPO). No significant differences were detected in ERα mRNA levels during ovulation and the midsecretory phase. However, ERβ1 and ERβ2 mRNA levels were higher in the midsecretory phase than in the early, late, or postovulatory phase. Conversely, PRA/B, PRB, and LHR mRNA levels were lower in the midsecretory phase than during ovulatory phases. Dicer1 mRNA expression correlated significantly with expression of ERα mRNA in the late ovulatory phase, ERβ2 mRNA in the midsecretory phase, and PRB mRNA in the early ovulatory phase (Table 2). Expression of Dicer1 mRNA did not correlate with expression of ERβ1 or PRA/B mRNA or with circulating LH, E2, and P4 levels in any of the phases (data not shown).

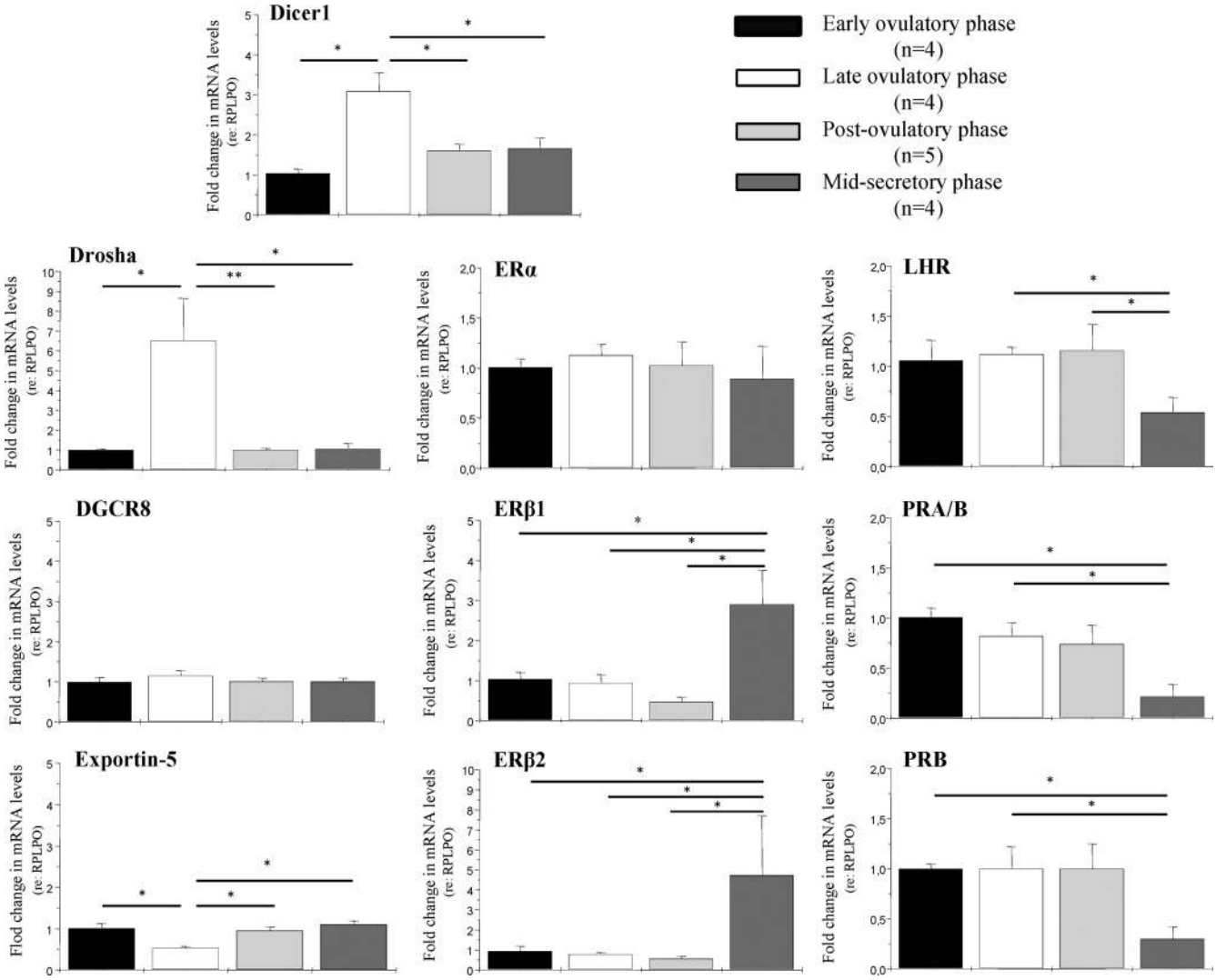

FIG. 4. Change in mRNA levels of Dicer1, miRNA processors, steroid hormone receptors, and LHR in the human Fallopian tube during ovulation and in the midsecretory phase. Fallopian tubes from the early, late, and postovulatory phases as well as the midsecretory phase were analyzed for Dicer1, Drosha, DGCR8, exportin-5, ERα, ERβ1, ERβ2, PRA/B, PRB, and LHR mRNA levels by quantitative RT-PCR. n, Number of patients from whom samples were obtained. mRNA levels of each gene are relative to RPLPO mRNA levels in the same samples. Values are expressed as mean ± SEM. Significance was tested by one-way ANOVA with Bonferroni correction for multiple comparisons when appropriate. *, $P < 0.05$; **, $P < 0.01$.

Because Drosha, DiGeorge syndrome critical region gene 8 (DGCR8), and exportin-5 act upstream of Dicer and are directly involved in miRNA generation (6), we examined whether their expression levels are also regulated in the same samples. Real-time RT-PCR demonstrated that Drosha mRNA levels were significantly increased and exportin-5 mRNA levels were significantly decreased in the late ovulatory phase (Fig. 4). DGCR8 expression was unaffected.

Discussion

This study shows that Dicer1 is expressed in cell-specific fashion in human Fallopian tubes during precise stages of the menstrual cycle. Dicer1 was highly expressed in epithelial cells and the lamina propria, and its mRNA expression peaked in the late ovulatory phase. Several studies suggest that Dicer-dependent regulation of miRNA is highly tissue- and stage-specific (4, 27) and that LH/hCG selectively regulates miRNAs in periovulatory mouse granulosa cells (28). We collected Fallopian tube tissue from women treated with rhCG to mimic the natural LH peak. Thus, samples were obtained at precise stages. The distinct expression of Dicer1 in human Fallopian tubes during ovulation is likely to be regulated, directly or indirectly, by LH/hCG. Human tubal epithelial cells express LHRs (26) and are responsive to hCG *in vitro*. Alternatively, indirect steroid hormonal regulation might be involved because systemic E2 and P4 concentrations vary considerably during the menstrual cycle (11). Although we

TABLE 2. Correlation of transcriptional alterations in Dicer1 and steroid hormone receptor mRNA in human Fallopian tubes

	Dicer1			
Receptor	**Early ovulatory phase (n = 4)**	**Late ovulatory phase (n = 4)**	**Postovulatory phase (n = 5)**	**Midsecretory phase (n = 4)**
ERα	−0.05 (0.94)	**0.94** (0.05)	0.44 (0.45)	0.82 (0.17)
ERβ1	−0.13 (0.86)	0.05 (0.94)	0.77 (0.12)	0.47 (0.52)
ERβ2	−0.37 (0.62)	−0.17 (0.82)	0.78 (0.11)	−**0.98** (0.01)
PRA/B	0.18 (0.81)	0.93 (0.07)	0.48 (0.41)	0.56 (0.43)
PRB	−**0.98** (0.01)	0.91 (0.85)	0.35 (0.55)	0.68 (0.31)
Drosha	−0.10 (0.90)	−0.61 (0.39)	0.51 (0.38)	0.58 (0.42)
DGCR8	0.10 (0.90)	0.29 (0.71)	0.55 (0.34)	0.27 (0.73)
Exportin-5	−0.95 (0.054)	**0.99** (0.02)	−0.54 (0.35)	−0.60 (0.40)

Values represent Pearson correlation coefficients. The significance of the correlation is in *parentheses*. *Boldface* values are statistically significant.

did not determine whether E2 or P4 directly regulates Dicer1 expression in human Fallopian tubes, treatment with E2 increases *dicer1* expression in ER-positive breast cancer cells *in vitro* (23), and P4 synthesis is impaired in *dicer1* knockout mice (8, 29).

Previously, we showed that the differential expression of ER subtypes and PR isoforms in rodent Fallopian tubes reflects their responses to E2 or P4 (18–20). Here, we provide evidence that ERβ1, ERβ2, PRA, and PRB expression in epithelial cells in human Fallopian tube is regulated in a stage-dependent manner during the menstrual cycle. Steroid hormone receptor (ER and PR) activity can be regulated by modulation of receptor gene expression, ligand binding to receptors, receptor protein stability, and transactivation (12). Dicer-dependent biogenesis of miRNAs is involved in regulating multiple genes through post-transcriptional blockade of the translation and degradation of target mRNAs (6). Tissue-specific inactivation of *Dicer* in mice led to the conclusion that Dicer is essential for generation of many miRNAs (8–10). Although the miRNA expression signature in human Fallopian tubes during the ovulatory process remains to be identified, we found significant changes in mRNA levels of Drosha and exportin-5 miRNA that paralleled increased Dicer1 mRNA levels in the late ovulatory phase, reflecting a basic mechanism for the induction of miRNAs to diversify miRNA target selection (6). It is therefore reasonable to hypothesize that multiple miRNAs can be differentially expressed in human Fallopian tubes during ovulation. E2-dependent activation of ER signaling may selectively regulate miRNA expression. Nothnick and Healy (30) and Yamagata *et al.* (31) reported that E2 treatment either increases or decreases expression of a set of miRNAs in mouse uterus *in vivo* and human breast cancer cell lines *in vitro*. Conversely, treatment with the ER antagonist ICI 182,780 reverses the estrogenic effect (30). Furthermore, there is an association between Dicer1 and ER expression in human ovarian cancer cells *in vivo* and *in vitro* (22), and ovarian ERβ mRNA expression is down-regulated in Dicer1-deficient mice (32).

In the present study, tubal Dicer1 mRNA expression correlated positively with ERα mRNA expression in the late ovulatory phase and negatively with ERβ2 mRNA expression in the midsecretory phase and with PRB mRNA expression in the early ovulatory phase. Moreover, a connection between Dicer1 and these receptors is reflected in the contribution of Dicer1 and ERα/ERβ1/ERβ2/PRA/PRB expression in epithelial cells in the Fallopian tube. Dicer (6) and steroid hormone receptors (33) are essential for female reproduction, and ER subtypes and PR isoforms have both been experimentally validated as miRNA targets in human endometrial epithelial cells and breast cancer cell lines (34, 35). Therefore, the Dicer-dependent miRNA pathway may be a novel mechanism for selective regulation of steroid hormone receptor expression in human Fallopian tubes during the menstrual cycle. Further exploration is necessary to investigate the relation between ER/PR and miRNA in the Fallopian tube.

Humans have more than 700 miRNAs, and most are expressed in highly tissue-specific patterns (6), indicating their importance in many biological function. Although miRNA-based regulation has direct implications for the pathogenesis of reproductive diseases (3, 4), the importance of miRNA activity in the human Fallopian tube is highlighted by the finding that some miRNA genes are expressed differently in tubal tumors than under normal conditions (3). A principal function of the Fallopian tube is to transport gametes and the early embryo into the uterus to establish pregnancy (11). This process is regulated by steroid hormones through steroid receptors (13–15) on epithelial and SM cells in mammals (11).

Fallopian tube epithelium consists of differentiated ciliated and secretory cells that are responsible for ciliary activity and tubal fluid synthesis and secretion (11). Alterations in these functions may deregulate tubal transport, leading to human tubal ectopic pregnancy, a poten-

tially life-threatening condition (21). Although the absence of Dicer does not appear to impair cilia function (8, 10), Dicer knockout female mice exhibit epithelial cell pathology and distended tubal cysts filled with fluid (8, 10), suggesting that Dicer regulates secretion from epithelial cells in the Fallopian tube. Our understanding of how Dicer controls female reproduction is steadily expanding (6). However, the molecular mechanism of Dicer-dependent tubal cell dysfunction leading to human tubal ectopic pregnancy is mostly unknown (21). Significant changes in Dicer expression in epithelial cells of the isthmus in human Fallopian tubes are interesting because aberrant epithelial cells in the isthmus contribute to the tubal transport failure after loss of Dicer function in mice (8–10) and possibly also in humans. Ongoing studies are examining whether cellular expression of Dicer1 can be regulated in women with tubal ectopic pregnancy, and if so, which miRNAs regulated by Dicer1 contribute to tubal transport under normal conditions and in tubal diseases.

In tubal epithelial cells, but not stromal cells, the intensity of immunostaining for ERβ1, ERβ2, PRA/B, and PRB varied significantly between the late ovulatory and midsecretory phases. These findings suggest fundamental differences in the mechanisms by which ER subtypes and PR isoforms are regulated in these cells. However, others have found no menstrual cycle-related changes in ERβ1, ERβ2, and PRA/B localization in different cellular components of human Fallopian tubes, as judged by immunohistochemistry (17). This discrepancy may reflect the use of different techniques. For example, in one study, one in five immunohistochemical analyses in human tissues was overinterpreted compared with the same test using *in situ* fluorescence (36). Importantly, our conclusions are based on manipulating each antibody under nonsaturating concentration and on using well-studied human cycle-endometrial tissues as controls (37–40).

In conclusion, our findings show that cell-specific, stage-dependent regulation of Dicer1 expression correlates with multiple changes in steroid hormone receptor gene and protein expression in human Fallopian tubes. The current challenge is to understand the complex process by which steroid hormones regulate Dicer1 expression and function in humans during tubal transport. It will also be important to determine whether Dicer-dependent regulation of miRNAs contributes to Fallopian tube dysfunction, such as tubal ectopic pregnancy.

Acknowledgments

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