Differential Expression and Localization of Dicer1 and Ovarian Steroid Hormone Receptors in Human Fallopian Tubes

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Background

Tissue-specific *dicer1* knockout mice display severe, irreversible Fallopian tube damage and disruption of tubal transport. It is not known how Dicer1 affects human Fallopian tube function. Precise, controlled regulation of tubal estrogen receptor (ER) and progesterone receptor (PR) expression may be critical for normal tubal transport. However, the regulation of ER subtype and PR isoform levels in human Fallopian tubes during ovulation has not been fully elucidated.

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

Conclusion:

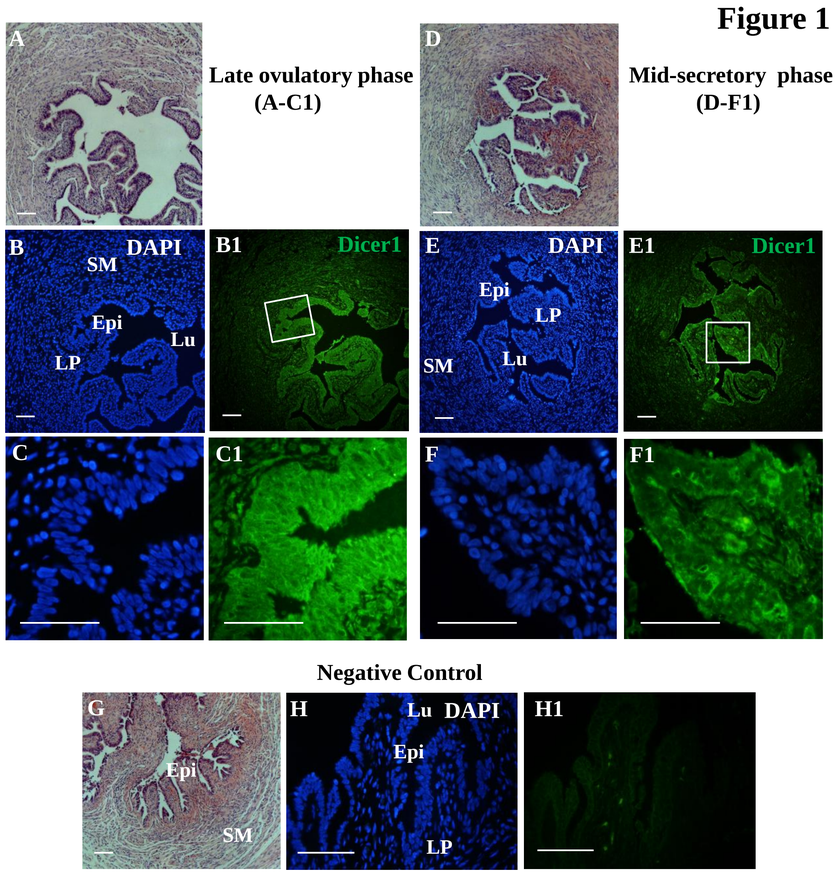
Dicer1 expression is upregulated in cell-specific fashion in human Fallopian tubes during ovulation. The stage-dependent expression of Dicer1 and its correlation with ERα, ERβ2, and PRB mRNA suggests that tubal Dicer1 helps regulate tubal expression of steroid hormone receptors in a cycle-dependent manner and may contribute to tubal transport in humans.

Results

Cellular location of Dicer1 Representative images of immunofluorescence staining of Dicer1 protein in human Fallopian tubes are shown in Figure 1 for the late ovulatory (Fig. 1A-C1 and G-H1) and mid-secretory (Fig. 1D-F1) phases. Dicer1 was found predominantly in the epithelial cell layer (Fig. 1B1 and E1), and the levels were higher in the late ovulatory phase (Fig. 1C1) than in the mid-secretory phase (Fig. 1F1). Lower expression was found in the lamina propria (Fig. 1C1 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. 1B1 and E1). The specificity of Dicer1 staining was based on negative controls in which the primary antibody (Fig. 1H1) or both primary and secondary antibodies were omitted or the primary antibody was gradually diluted, leading to disappearance of immunostaining (data not shown).

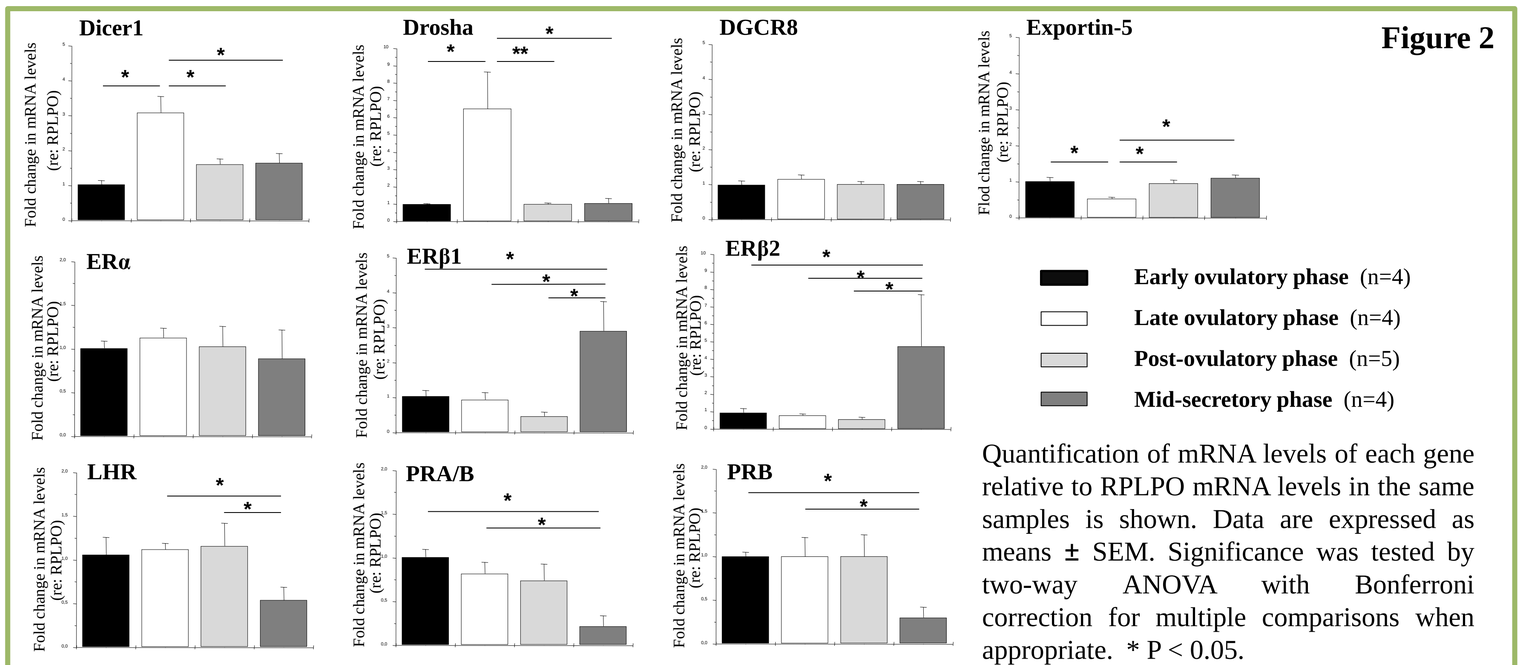
Dicer1, miRNA processors, and steroid hormone receptor mRNA expression mRNA levels of ERα, ERβ1, ERβ2, PRA/B, PRB, and LHR did not change significantly during ovulation; however, Dicer1 mRNA expression was significantly higher in the late ovulatory phase (Fig. 2). Since Drosha, DiGeorge syndrome critical region gene 8 (DGCR8), and exportin-5 act upstream of Dicer and are directly involved in miRNA generation, 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. 2). DGCR8 expression was unaffected. 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). Dicer1 mRNA expression correlated significantly with expression of ERα mRNA in the late ovulatory phase, ERβ2 mRNA in the mid-secretory phase, and PRB mRNA in the early ovulatory phase (Table). 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).

Figure 1



Abbreviation: Lu, lumen; Epi, epithelial cells; LP, lamina propria; SM, smooth muscle cells. *Scale bar*, 100 µm.

Figure 2



Quantification of mRNA levels of each gene relative to RPLPO mRNA levels in the same samples is shown. Data are expressed as means ± SEM. Significance was tested by two-way ANOVA with Bonferroni correction for multiple comparisons when appropriate. * P < 0.05.

Table Correlation of transcriptional alterations in Dicer1 and steroid hormone receptor mRNA in human Fallopian tubes

Receptor	Dicer1			
	Early ovulatory phase (n = 4)	**Late ovulatory phase** (n = 4)	**Postovulatory phase** (n = 5)	**Mid-secretory 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)

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

Methods

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

This work was supported by the Swedish Medical Research Council (Grant 5859), the Sahlgrenska Academy Research Council, the Göteborgs Läkaresällskap, Hjalmar Svensson Foundation, Fred G. and Emma E. Kanolds Foundation, Fredrik och Ingrid Thurings Foundation, Emil and Maria Palms Foundation, the Mary von Sydow Foundation, Anna Cederbergs Foundation and Tore Nilsons Foundation

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