Estradiol and follicle stimulating hormone increase cell viability in adult-type ovarian granulosa cell tumor cells – implications for hormonal therapy

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Introduction

Adult-type granulosa cell tumors (AGCTs) are sex-cord derived ovarian malignant tumors. In advanced disease new treatments are needed, as traditional chemotherapeutics have only limited efficacy. Hormonal treatments have been empirically used in treatment of recurrent AGCTs, even though the biological basis for this remains unclear. We aimed to characterize the hormone receptor expression levels in AGCTs and study the effects of estradiol and FSH on AGCT cell growth.

Material and methods

The expressions for FSH receptor (FSHR), aromatase (CYP19A1) and estrogen receptors (ER α , ER β and GPER) were studied in AGCT tissue microarray (175 samples) using immunohistochemistry and RNA *in situ* method. The effects of FSH and E2 stimulation were assessed in two cell models, established AGCT cell line KGN and primary cultured AGCT cells from six AGCTs. The expression of CYP19A1 in cell lysates were analyzed by RT-PCR. Cell culture medium was collected and analyzed for E2 concentration using mass spectrophotometer. Cell viability was assessed by WST-1.

A Patients n=138		n (% of total)
Age at diagnosis, years ^a		53 (26-81)
Tumor stage at diagnosis		
	I	126 (93)
	II	8 (6)
	III	1 (1)
Follow-up time, years ^a		15.0 (0.7-42.3)
Recurrence		
	Yes	51 (37)
	No	87 (63)
B Tumor characteristics (n=175)		n (% of total)
	Primary	121 (69)
	Recurrent	54 (31)
Subtype		
	Sarcomatose	72 (42)
	Differentiated	98 (58)
Tumor size		
	<10 cm	103 (60)
	≥10 cm	70 (40)

^aMedian (range)

Table 1: Patient data in tumor microarray used in expression analyses. All tumors are tested positive for FOXL2-mutation.

Results

Figure 1:

A) FSHR is widely expressed in AGCTs. Expression was detected in 90% of AGCTs, in 60% of tumors staining was strong or moderate.

B-C) CYP19A1 is expressed in 48% of AGCTs. (B: RNA expression, C: protein expression)

Figure 2:

A-C) FSH increases CYP19A1 mRNA expression and E2 production in AGCT cells *in vitro* and increases cell viability in subset of AGCTs.

Figure 3:

A-C) ER β is the main estrogen receptor in AGCTs, while ER α and GPER are expressed in only minority.
ER α in 35% of AGCTs
ER β in 94% of AGCTs
GPER in 14% of AGCTs

Figure 4:

Intensity of ER β expression is increased in recurrent tumors.

Figure 5:

E2 increases cell viability in primary cultured AGCT cells.

Conclusions

FSH induces CYP19A1 in AGCTs and increases estradiol production and cell viability in AGCTs. ER β is the main estrogen receptor in AGCT and its expression is increased in recurrent tumors. Estradiol increases cell viability in AGCTs.