Reproductive BioMedicine Online (2010) 21, 109–117

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Expression of growth-differentiating factor 9 and its type 1 receptor in human ovaries

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Dr Oron recently completed her residency in obstetrics and gynecology at Helen Schneider Hospital for Women, Rabin Medical Center, Israel. Dr Oron published four original articles in the field of obstetrics. Her study 'Pregnancy outcome in women with heart disease undergoing induction of labor' is cited in the 22nd edition of the Williams Obstetrics textbook as reference guidance. Her study on the serum soluble CD40 ligand in relation to preeclampsia was presented in 14th World Congress of the International society for the Study of Hypertension in Pregnancy and was awarded a young investigator travel award.

Abstract The expression of growth-differentiating factor 9 (GDF9) has not been studied in ovaries from girls and human fetuses nor has its receptor transforming growth factor-β1 receptor (TGFβR1) been investigated in ovaries of girls/women. The aim of this study was to fill these gaps. Ovarian samples were obtained from 16 human fetuses at 21–35 gestational weeks and from 34 girls/women aged 5–39 years. These specimens were prepared for immunohistochemical staining of the GDF9 and TGFβR1 proteins. Reverse transcription polymerase chain reaction was used to detect GDF9 mRNA transcripts and in-situ hybridization to localize TGFβR1 mRNA transcripts. Positive staining for the GDF9 protein was identified in oocytes and granulosa cells in all samples tested. GDF9 mRNA transcripts were present in all samples. Protein expression of TGFβR1 was identified in granulosa cells in all samples. Oocyte staining was identified in samples from girls/women but in only one fetal sample. TGFβR1 mRNA transcripts were identified in granulosa cells and oocytes in 50% of the samples from fetuses aged over 22 gestational weeks and in samples from girls/women. The detection of GDF9 and TGFβR1 at both at the protein and mRNA levels suggests that GDF9 may have functions in human preantral follicles.

KEYWORDS: GDF9, human ovaries, immunohistochemistry, in-situ hybridization, reverse transcription polymerase chain reaction, TGFβR1

1472-6483/$ - see front matter
doi:10.1016/j.rbmo.2010.03.011

Introduction

The options for fertility preservation in cancer patients consist mainly of cryopreservation of ovarian tissue containing primordial follicles (Abir et al., 2006, 2008a; Feigin et al., 2008) A successful in-vitro maturation system for primordial follicles would eliminate the risk of reseeding malignancy with frozen–thawed ovarian grafts (Abir et al., 2006; Feigin et al., 2008). The development of such a technology is currently hindered by uncertainties regarding the factors that activate primordial follicular growth. One of the possible candidates for this role is growth-differentiating factor 9 (GDF9) (Abir et al., 2006).

GDF9 belongs to the transforming growth factor β (TGFβ) superfamily (McPherron and Lee, 1993). TGFβ members have two major types of membrane-bound receptors (Cheifetz et al., 1987, 1990). The type-I receptor for GDF9 has been identified as TGFβR1 or activin-like receptor 5 (ALK5) (Mazebourg et al., 2004) and its type-II receptor bone morphogenetic protein type-II receptor (BMPRII; Abir et al., 2008b; Shimasaki et al., 1996). GDF9 binding to its type-II receptor results in transphosphorylation of the type-I receptor, which in turn activates the intracellular-signal-mediated (Smad) proteins 2 and 3.

GDF9 protein and/or GDF9 mRNA transcripts have previously been identified in oocytes from the unilaminar stages in mice (Dong et al., 1996; Laitinen et al., 1998; McGrath et al., 1995), rats (Hayashi et al., 1999; Jaatinen et al., 1999), possums (Eckery et al., 2002), goats (Silva et al., 2004), sheep, cows (Bodensteiner et al., 1999) and women (Aaltonen et al., 1999; Teixeira Filho et al., 2002). A previous study from this laboratory reported the identification of the mRNA transcripts and protein of BMPRII in oocytes and granulosa cells from fetuses and girls/women (Abir et al., 2008b). Another group identified the mRNA transcripts and protein of TGFβR1 in human fetal ovaries up to the age of 24 gestational weeks, with protein expression in oogonia/oocytes (Schilling and Yeh, 1999). However, the mRNA transcripts have not been localized to the specific ovarian sites.

To date, no information is available on the presence of GDF9 in human ovaries from fetuses and girls or localization of the protein and mRNA transcripts of TGFβR1 in human ovaries from older fetuses, girls and women. The present study sought to investigate the expression of GDF9 and its receptor in ovaries from fetuses and girls/women at the protein and mRNA levels. Immunohistochemistry was used to identify the protein for GDF9 and its receptor TGFβR1; reverse transcription polymerase chain reaction (RT-PCR) to detect GDF9 mRNA transcripts; and in-situ hybridization to localize the mRNA transcripts for TGFβR1.

Materials and methods

Human ovaries from fetuses, girls and women

Ovarian samples were obtained from 16 aborted human fetuses aged 21–35 gestational weeks (GW): nine fetuses had anatomical abnormalities, five had chromosomal aberrations, one had a genetic disorder (achondroplasia) and one was normal (Abir et al., 2009; Pinkas et al., 2008). In addition, small ovarian biopsy samples were donated by 34 girls/women aged 5–39 years or their guardians. All had undergone gynaecological laparoscopies. Twenty-three had various forms of cancer and their operation was performed for cryopreservation of ovarian tissue before commencement of chemotherapy (Abir et al., 2008a). The ethics committee of Rabin Medical Centre approved the study protocol and every woman or minor's parents signed an informed consent form. The samples were cut into a uniform size and fixed immediately in Bouin's solution (BDH Chemicals, Poole, England; Sigma, St. Louis, MO, USA) for immunohistochemistry and in-situ hybridization studies (Abir et al., 2009; Pinkas et al., 2008). The remaining sample material was frozen for subsequent RNA extraction.

Cryopreservation of ovarian tissue

Tissue slices were placed in cryogenic vials (Nalge Nunc International, Roskilde, Denmark) filled with a solution of 1.5 mol/l dimethylsulphoxide (Sigma) (Abir et al., 2009; Pinkas et al., 2008). Prior to freezing, the samples were kept on ice for 30 min to achieve equilibrium. All samples were frozen in a programmable freezer (Kryo 360-1/7; Planer Biomed, Sunbury on Thames, UK) and immediately placed into liquid nitrogen. The slices were cryopreserved for between 3 months and 2 years until RNA extraction.

Histological preparation

The histological preparation method has been described in detail elsewhere (Abir et al., 2009; Pinkas et al., 2008). The fixed specimens were dehydrated in a graded series of ethanol followed by paraffin embedding and sectioning. Unstained sections were placed on OptiPlus positive-charged microscope slides (BioGenex Laboratories, San Ramon, CA, USA) for immunohistochemistry and in-situ hybridization.

Immunohistochemistry for GDF9 and TGFβR1

The immunohistochemistry method has been employed in several earlier studies (Abir et al., 2009; Pinkas et al., 2008). Two sections per sample were utilized to identify the proteins for GDF9 and TGFβR1. As the expression of GDF9 was previously reported in ovaries of women (Aaltonen et al., 1999), it was tested only in samples from fetuses and girls (13 girls, age 5–18 years). The protein for TGFβR1 was tested in all the samples from the three tissue sources. Unfortunately, there are no commercially available positive controls for either GDF9 or TGFβR1. To enhance antigen retrieval, all the slides were microwaved with citrate buffer (CheMate buffer; DakoCytomation, Glostrup, Denmark), and to block endogenous peroxidase activity, the slides were quenched in 3% hydrogen peroxide (Vitamed, Binyamina, Israel).

Goat polyclonal antibodies were used as the primary antibodies against GDF9 and TGFβR1, as they were reported to be suitable for immunohistochemistry by the manufacturer (sc12244 and sc33933, respectively; Santa Cruz Biotechnology, Santa Cruz, CA, USA). The samples were incubated with the primary antibodies, diluted 1:10 and 1:30. Negative control solution was prepared by the absorption of the primary antibodies against GDF9 and TGFβR1 with their corresponding blocking peptides (sc12244P and