Expression and regulation of Sprouty-2 in the granulosa-lutein cells of the corpus luteum

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Growth factor signalling has important modulatory roles in the process of human follicular growth, oocyte maturation and corpus luteum (CL) formation. Recently, Sprouty-2, an inhibitor of receptor tyrosine kinase (RTK) signalling pathway was advocated as a marker of oocyte competence in the bovine ovary. We sought to study Sprouty-2 expression and regulation in the human ovary. RT–PCR was used to detect Sprouty-2 mRNA in human granulosa-lutein cells (GLC) collected from follicular aspiration of IVF patients. The effect of epidermal and fibroblast growth factors (EGF and FGF) on Sprouty-2 mRNA expression in GLC was studied using quantitative real-time PCR. Immunohistochemistry was performed on cultured GLC, human CL and stimulated rat ovary sections. Sprouty-2 mRNA was expressed in human GLC. EGF and basic FGF, but not FGF4 and FGF10, increased Sprouty-2 mRNA expression in GLC. The Sprouty protein was localized to GLC of early and late human CL but not to the theca cell layer. Immunostaining of developing rat CL confirmed the temporal and spatial expression of Sprouty in humans. The detection of Sprouty-2 mRNA and protein in human GLC may suggest a role for Sprouty-2 during the final stages of follicle maturation and CL formation.

Key words: corpus luteum/follicle/granulosa/ovary/Sprouty

Introduction

Transformation of a fluid-filled preovulatory follicle into a solid, progesterone-producing corpus luteum (CL) occurs after ovulation. The preovulatory follicle is compartmentalized into a highly vascular theca layer and a non-vascular granulosa layer separated by a basement membrane (BM) and independently regulated by the gonadotrophin hormones (Redmer and Reynolds, 1996; Fraser and Wulff, 2001). Definitive structural and functional changes take place in these two compartments around the time of ovulation. Shortly before ovulation, the BM breaks down, and the capillaries at the inner capillary plexus of the theca interna rapidly sprout and invade the inner granulosa cell layer (Redmer and Reynolds, 1996; Fraser and Wulff, 2001). During ovulation, the follicular wall ruptures, and the follicle collapses to form folds which interdigitate with the granulosa-lutein cells (GLC). A complex capillary network is formed bringing each GLC into close approximation to the vascular bed. Approximately 50% of the cells of the mature CL are endothelial cells (Fraser and Wulff, 2001). The vascular development of the CL is regulated by angiogenic and cell differentiation factors, such as vascular endothelial growth factor (VEGF) (Berisha et al., 2000; Geva and Jaffe, 2000), epidermal growth factor (EGF) (Serta and Seibel, 1993; Khan et al., 2005) and basic fibroblast growth factor (bFGF) (Stirling et al., 1991; Redmer and Reynolds, 1996; Yamamoto et al., 1991). Basic FGF mRNA synthesis was shown to be stimulated by LH in bovine CL and in cultured luteal cells (Stirling et al., 1991; Berisha et al., 2000). During human CL formation, bFGF was localized in granulosa and theca lutein cells (Redmer and Reynolds, 1996; Yamamoto et al., 1997).

The Sprouty proteins are novel negative modulators of growth factors that participate in signal transduction, morphogenesis and angiogenesis. Sprouty was first described in receptor tyrosine kinase (RTK) signalling inhibition of FGF receptor-mediated branching of epithelial cells during tracheal formation in Drosophila (Hacohen et al., 1998). Three human genes were identified with sequence similarity to Drosophila Sprouty. In most tissues, Sprouty appears to be co-regulated by FGFs. Interactions between the EGF receptor pathway and Sprouty in eye development have suggested a central role for Sprouty in restricting the activity of EGF receptor pathway as well (Casci et al., 1999; Kramer et al., 1999; Cabrita and Christofori, 2003). Sprouty also inhibit VEGF-induced mitogen activated protein kinase (MAPK) phosphorylation in mouse endothelial cells (Lee et al., 2001; Impagnatiello et al., 2001). In the mouse, Sprouty-1, Sprouty-2 (Impagnatiello et al., 2001) and Sprouty-4 (Lee et al., 2001) inhibited FGF- and VEGF-induced endothelial cell proliferation and differentiation by repressing pathways leading to MAPK activation.

The precise mechanism of inhibition of Sprouty in the RTK pathway is controversial: inhibition of Ras activation was suggested by Gross et al. (2001) through Spred (Wakioka et al., 2001), whereas Reich et al. (1999) proposed interaction with Raf or downstream to Raf. RTK signalling, on the other hand, has been shown to induce Sprouty gene expression by the extracellular signal-regulated kinase (ERK) pathway downstream of RTK (Ozaki et al., 2001).
The CL is one of a few adult human organs that exhibit cyclic physiological angiogenesis and dramatic cell proliferation. These processes are tightly controlled and finely orchestrated. Still the control mechanisms of luteal angiogenesis and tissue remodelling are not well understood. Based on the data that (i) FGF-induced Sprouty blocked signalling in distant stalk cells of apical branches of the Drosophila airways (Hacohen et al., 1998), (ii) Sprouty-2 mRNA was associated with bovine oocyte developmental competence (Robert et al., 2001) and (iii) EGF and bFGF were major differentiation factors of folliculogenesis and luteal transformation, we postulate a role for Sprouty in the control of follicular and luteal function of the human ovary.

Materials and methods

Animals

Intact, immature female Wistar rats were obtained from Harlan (Jerusalem, Israel) and maintained in 16 h light, 8 h dark schedule with food and water ad libitum. Animals were treated in accordance with the National Research Council (NRC) publication Guide for Care and Use of Laboratory Animals (copyright 1996, National Academy of Science). All protocols had the approval of the Institutional Committee on Animal Care and Use, The Medical School of Medicine, The Hebrew University of Jerusalem.

Hormone-induced ovulating rats

Twenty-four prepubertal 24- to 27-day-old female Wistar rat participated in the study. The animals were stimulated by intraperitoneal injection of 5 IU pregnant mare’s serum gonadotrophins (PMSG) at noon, and 4 IU HCG was administered 48 h later. Four animals were killed by cervical dislocation at each specific time point: 0 and 24 h after PMSG, 48 h after PMSG, 12 h after HCG, 24 h after HCG and 48 h after HCG, and their ovaries were removed for further evaluation.

Human tissue preparation

The study was approved by the institutional human research committee. Tissue specimens from 10 premenopausal regularly cycling, non-pregnant subjects aged 16–52 years old, undergoing either elective or urgent operations were retrospectively collected. The patients had no known previous ovarian pathologies or history of infertility. They were admitted for either cystectomy or oophorectomy for various diagnoses, such as complicated ovarian cystic mass, or as an accompanying procedure to total abdominal hysterectomy for a variety of reasons excluding malignancies. Ten to fifteen tissue sections from each subject were formalin fixed and paraffin embedded. Sections of 6 μm were either stained with hematoxylin and eosin or prepared for immunohistochemistry studies. The ovarian CL were staged by a pathologist with an expertise in gynecological pathology and classified according to basic morphological criteria. The follicles/corpus lutea were examined for the following parameters: evidence of ovulation or subsequent haemorrhage as well as size, colour and tissue consistency. Early CL is characterized by central haemorrhage, mild fibroblastic reaction and non-convoluted CL wall. In contrast, the mature CL wall has developed into well-defined lobules. According to these histological criteria, follicles were grouped into early and mature mid-stage CL (Clement, 1992). The follicles/corpora lutea were examined for the following parameters: evidence of ovulation or subsequent haemorrhage as well as size, colour and tissue consistency and non-convoluted CL wall. In contrast, the mature CL wall has developed into well-defined lobules. According to these histological criteria, follicles were grouped into early and mature mid-stage CL (Clement, 1992). The clinical information including the date of the last menstrual period was confirmatory of the morphological criteria in assessing menstrual cycle phase.

GLC retrieval and isolation

Use of discarded material was approved by the Hadassah hospital review board. Granulosa cells were retrieved from large follicles obtained from 10 normal ovulatory women undergoing ovulation induction IVF, as previously described (Hurwitz et al., 1995). Briefly, these patients underwent a long mid-luteal phase suppression protocol utilizing gonadotrophin-releasing hormone agonist (Decapeptil; Ferring Pharmaceutical, Malmo, Sweden). Two weeks later, after achieving pituitary suppression, gonadotrophin administration was commenced. Recombinant FSH (Serono SA, Aubonne, Switzerland) was started at a dose of 3 ampoules per day for 5 days and subsequently adjusted according to the individual response. When at least three follicles reached 18 mm in diameter, HCG (5000 IU) was administered. All accessible follicles, larger than 14 mm were harvested by transvaginal ultrasonographic aspiration, 36 h following HCG administration, and oocytes were removed from the follicular aspires. Overall 30 follicles were aspirated and studied. The follicular aspires were layered on 10 ml of histopaque columns (Sigma-Aldrich, St. Louis, MO, USA) and centrifuged at 700 x g for 30 min to remove the red blood cells (RBCs). Thereafter, the cells were aspirated from the interface, gently pipetted, resuspended and counted in a hemocytometer. Viability, tested by trypan blue, was more than 90% (Hurwitz et al., 1995).

Cell sorting of follicular aspires

White blood cells (WBCs) contaminate follicular aspires (Loukides et al., 1990). To remove WBCs, follicular aspires were centrifuged at 900 x g for 4 min, the supernatant was discarded and the pellet dissolved in Roswell Park Memorial Institute 1640 (RPMI 1640) medium, containing 2% fetal calf serum (FCS) (Biological Industries, Kibbutz Beit Haemeek, Israel). The cells were incubated for 20 min at room temperature with tetrameric antibody complexes (TAC) cocktail with monoclonal antibodies to human CD45 and CD66b cell surface antigens (RosetteSep, Vancouver, BC, USA). In keeping with the manufacturer’s recommendations, leukocytes were resorted to RBCs with tetrameric antibody complexes. Incubates were then placed into a Ficoll-Paque (Amersham Pharmacia Biotech AB, Uppsala, Sweden) and centrifuged at 1200 x g for 20 min at room temperature. Purified granulosa cells were aspirated from the interface, gently pipetted, resuspended with RPMI and respan at 900 x g for 4 min. The wash was repeated twice to ensure complete removal of leukocytes. The resultant preparation was subjected to RNA extraction, as described below.

Human granulosa cell culture

The viable cells were counted and 5 x 10^5 cells were plated onto 12-well tissue culture plastic dishes (Nunc, Roskilde, Denmark) precoated with 1-ml RPMI 1640 medium with 10% FCS (Biological Industries) supplemented with 1-glutamine, penicillin and streptomycin. Cells were cultured for 24–48 h at 37°C humidified, 5% CO2 and 95% air before each growth factor challenge. The time in culture before growth factor challenge was identical for all growth factors tested in each experiment. The attached cells were gently rinsed with serum-free media and incubated with or without recombinant human EGF (Sigma, St Louis, MI, USA) (10 and 20 ng/ml), recombinant human bFGF (Sigma) (10 and 50 ng/ml), recombinant human FGF4 (R&D Systems, Minneapolis, MN, USA) (25 and 50 ng/ml) or recombinant human FGF10 (R&D Systems) (500 and 1000 ng/ml) for 1–2 h. The concentration of each growth factor was determined according to E_{50} reported by the manufacturer. At the end of the experiment, 0.5 ml of Trinit Reagent (Molecular Research Center, Cincinnati, OH, USA) was added and the cell lysate was collected and stored at –70°C until RNA isolation was performed and assayed for Sprouty expression. The experiments were repeated four times for each growth factor concentration.

For immunohistochemistry, human granulosa cells (2 x 10^5) were cultured on precoated (with heat-inactivated FCS) plastic Labtek slides (Nunc, Rochester, NY, USA) containing RPMI 1640 and 10% FCS supplemented with L-glutamine, penicillin and streptomycin for 48 h. Adherent cells were gently rinsed with serum-free media, fixed for 10 min in 95% ethanol and stored at –70°C until used.

RT–PCR

Total RNA from GLC was isolated using Tri Reagent (Molecular Research Center). Complementary DNA was reverse transcribed from total RNA using random primers (Promega, Madison, WI, USA) according to the manufacturer instructions with the addition of an RNAse inhibitor (Promega). PCR amplification of Sprouty-2 cDNA fragments employed the following oligonucleotide primers: 5’-GGATCCCATGGTGTGGATATTCTGAGA-3’ (forward) and 5’-AACCTTGGTCTGTTGGGCTTCTGG-3’ (reverse). The PCR conditions were initial denaturation at 94°C for 30 s, annealing at 60°C for 30 s, extension at 72°C for 30 s for 30 cycles, followed by a final extension at 72°C for 5 min. Aliquots of 10 μl of the amplification products were separated by 1.5% agarose gel electrophoresis and visualized by ethidium bromide staining.

Quantitative PCR

Real-time quantitative PCR was performed using the GeneAmp 5700 system (PE-Biosystems, USA) with fluorescent detection of SYBR green dye (PE-Biosystems, Drive Foster City, CA, USA), according to the manufacturer’s instructions.
The following primer sequences (Metabion, MBC, Martinsried, Germany) were used: 18S 5’-CGCCGCTAGAGTGAATTC-3’ forward, 18S 5’-CGACCTCCGACCTTTGCTCT-3’ reverse, QHSPR2 5’-CATGGGTGT- CATGTCCTCTTCT-3’ forward and QHSPR2 5’-GCTGTATTACCCGGT- CATAAACA-3’ reverse. The transcript levels of Sprouty-2 mRNA were normalized to 18S rRNA.

**Immunohistochemistry studies in GLC culture**

Fixed GLC adherent to plastic Labtek slides were removed from storage in –70°C in 95% ethanol, air dried for 20 min and fixed for 10 min in ice-cold acetone. Immunohistochemistry was performed according to the Histostain Plus Kit (Zymed Lab-SA System, Zymed Laboratories, San Francisco, CA, USA) with some modifications. Briefly, slides were air dried and then fixed for 10 min in ice-cold acetone. Slides were then washed in phosphate-buffered saline (PBS), treated with 0.3% Triton X-100 in PBS for 4 min followed by another wash with PBS. Endogenous peroxidase was quenched with H₂O₂ for 10 min in ice-cold acetone. Slides were then washed in phosphate-buffered saline (PBS), treated with 0.3% Triton X-100 in PBS for 4 min followed by another wash with PBS. Endogenous peroxidase was quenched with H₂O₂ for 10 min. Slides were pre-incubated with blocking CAS-Block (cat.on. 00-b120) buffer (Zymed Laboratories). Rabbit antibodies against Sprouty-2 were kindly provided by Dr. G. Christofori. The specificity of the antibodies against Sprouty-2 was previously confirmed by peptide competition experiments, as well as by excluding cross-reactivity with Sprouty-4 (Impagnatiello et al., 2001). The primary antibodies (diluted 1:500) were incubated for 1.5 h at room temperature. The secondary antibody used was the Envision™ Systems horse-radish peroxidase (HRP) anti-rabbit (DakoCytomation, Glostrup, Denmark). Mouse anti-human inhibin alpha (Serotec, Oxon, UK) (diluted 1:40) was used as marker of steroidogenic GLC (Speroff et al., 1999) along with the Envision™ System, HRP anti-mouse as the secondary antibody. Antibody detection was performed according to the Histostain Plus Kit, developed with Single Solution AEC (aminooxy carbazole) and counterstained with hematoxylin (Biogenex, San-Ramon, CA, USA) before mounting with glycerol, polyvinyl alcohol and Tris buffer containing 0.05% sodium azide (GVA) (Zymed Laboratories).

**Immunohistochemistry for Sprouty-2 in human and rat ovary sections**

Immunohistochemistry for Sprouty-2 was performed on 4 µm sections of formalin fixed paraffin embedded ovarian specimens (5–8 sections from each group). The sections were deparaffinized and rehydrated. Tissue was then denatured for 3 min in a microwave oven in citrate buffer (0.01 M, pH 6.0). The slides were washed, blocked and incubated at room temperature with the primary antibody using the Envision, peroxidase-conjugated anti-rabbit antibodies (Dako, Carpinteria, CA, USA). Sections were incubated with the above mentioned rabbit antibodies against Sprouty-2 that cross react between human and mouse antigen, or with Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% horse serum as control, followed by incubation (30 min) with Envision, peroxidase-conjugated anti-rabbit antibodies. Colour was developed using substrate-chromagen solution of AEC (Sigma, St. Louis, MO, USA), followed by counterstain with Mayer’s hematoxylin.

**Statistical analysis**

Data were analysed by Student’s t-test and are presented as the mean ± SEM.

**Results**

**Expression of Sprouty-2 mRNA in human GLC**

Sprouty-2 mRNA expression was investigated in pooled GLC from several follicles and in GLC from single follicles by RT–PCR. Sprouty-2 mRNA was identified in pooled GLC. To exclude the possibility that Sprouty-2 mRNA was originated from leukocytes, anti-CD45 and anti-CD66b immunobeads were used to eliminate these cells from the follicular fluid. Similar mRNA expression of Sprouty-2 was found with or without the removal of WBC from the follicular fluid, suggesting that GLC indeed express mRNA of Sprouty-2 (Figure 1). Nonetheless, GLC from some (but not all) individual follicles expressed Sprouty-2 mRNA, suggesting that Sprouty-2 is differentially expressed in single follicles (Figure 1, inset).

**Sprouty-2 expression in granulosa-lutein cells**

![Sprouty-2 expression in granulosa-lutein cells](image)

**Regulation of Sprouty-2 mRNA expression in GLC by FGF and EGF**

According to previous publications Sprouty-2 expression was regulated by FGF and EGF (Casci et al., 1999; Kramer et al., 1999; Cabrita and Christofori, 2003; Rubin et al., 2005). We examined the effect of these growth factors on Sprouty-2 mRNA expression in GLC by using real-time quantitative PCR. For this purpose, GLCs were incubated with the following growth factors: basic FGF, FGF4, FGF10 and EGF for 1 and 2 h. Similar effect was noted after 1 h and 2 h. The expression of Sprouty-2 mRNA increased by 1.85-fold following incubation with 10 ng/ml bFGF and by 2.2-fold following incubation with 20 ng/ml EGF (P value = 0.05 and 0.004, respectively) (Figure 2). In contrast, 25–50 ng/ml FGF4 and 500–1000 ng/ml FGF10 did not enhance Sprouty-2 mRNA expression.

**Detection of Sprouty-2 protein in human GLC**

To examine whether Sprouty-2 protein is produced by GLC, we immunostained granulosa cell culture with specific antibodies for Sprouty-2 and inhibit subunit alpha which serves as a marker for GLCs. Sprouty-2 protein was present in GLCs of inhibin positive culture, supporting Sprouty-2 production by GLCs (Figure 3).

**Detection of Sprouty-2 protein in the human CL in vivo**

To corroborate our in vitro findings with in vivo evidence, paraffin embedded sections of early and mature (Figure 4) corpora lutea derived from women that underwent removal of the ovaries because of non-malignant pathology were immunostained. Sprouty-2 was detected in the cytoplasm of GLC of both early and mature human CL, whereas theca lutein cells did not express Sprouty-2 (Figure 4).

**Localization of Sprouty-2 in rodent ovarian folliculogenesis**

The rat model was investigated to confirm our findings in the human ovary with another species. Also, a well controlled ovarian stimulation in the rat enabled more precise cycle timing and a study of other
Figure 2. Regulation of Sprouty-2 mRNA in granulosa-lutein cells (GLC) by bas f basic fibroblast growth factors (bFGF) and epidermal growth factor (EGF). The effect of bFGF, FGF4, FGF10 and EGF on Sprouty-2 mRNA expression was studied in cultured GLC using real-time quantitative PCR. GLC were incubated with each of the growth factors for 1 and 2 h. The expression of Sprouty-2 mRNA increased by 1.85-fold following incubation with bFGF and by 2.24-fold following incubation with EGF (P value = 0.05 and 0.004, respectively). In contrast, we did not observe an increase in Sprouty-2 mRNA expression following incubation with FGF4 and FGF10 (P value = 0.67 and 0.19, respectively). The dose of each growth factor that yielded the largest effect is shown in the figure. The data are presented as mean ± SEM.

Figure 3. Expression of Sprouty-2 protein in human granulosa-lutein cells (GLC). Using immunocytochemistry with anti-Sprouty-2 antibody, Sprouty-2 protein was immunostained (A) in the cytoplasm of cultured GLC that also express inhibin A (B). (Inset) Negative control where only rabbit serum was used devoid of primary anti-Sprouty-2 antibody. (Bar = 100 µm, magnification x100).

Figure 4. Expression of Sprouty-2 protein in early and mature human corpus luteum (CL). Paraffin embedded sections of early (upper panel) and mature (lower panel) human corpora lutea derived from women that underwent removal of the ovaries because of a non-malignant pathology were immunostained for Sprouty-2. The granulosa-lutein cells (GLC) comprising of the early CL wall (A and B) as well as the lobulated mature CL (A' and B') were immunoreactive with Sprouty-2 antibody, whereas the theca lutein cells (TLC) and the ovarian stroma (OvS) were nonreactive with the Sprouty-2 antibody. (C and C') Negative control of a consecutive section using rabbit serum without primary antibody. (Bar = 100 µm; A, C, A' and C', magnification x100; B and B', magnification x400.)

Figure 5. Localization of Sprouty-2 in rodent ovarian folliculogenesis. Immunohistochemistry using anti-Sprouty-2 were performed on paraffin embedded sections of rat ovary. Ovaries were examined before and 24 and 48 h after pregnant mare serum gonadotrophins (PMSG) administration and 12, 24 and 48 h after HCG injection. In serially sectioned ovaries from unstimulated rats no expression of Sprouty-2 was noted in the ovary (A). In PMSG-treated rats as well as 12 h after HCG exposure, expression of Sprouty-2 was noted exclusively in the ovarian secondary interstitial tissue (B–D). Twenty-four hours following an ovulatory dose of HCG, Sprouty-2 was expressed in granulosa-lutein cells (GLC) of the forming corpora lutea, as well as in the interstitial tissue (E, inset). Granulosa cells of unruptured follicles did not express the protein (F, arrowheads) in contrast to GLC of the corpus luteum (CL) that strongly expressed Sprouty-2 (F). (Bar = 100 µm; magnification (A), x25; (B–F), x100; insets, x200.

Sprouty-2 was preferentially localized to GLC forming the CL. Of note is that granulosa cells of unruptured follicles did not produce the protein in contrast to GLC of the CL that were consistently immunoreactive for Sprouty-2 (Figure 5F).
Discussion

Here we report, for the first time the presence of Sprouty-2 mRNA and protein in human granulosa cells from preovulatory follicles following HCG exposure. However not every follicle was found to contain Sprouty-2 expressing granulosa cells. Similarly in the rat model only granulosa cells of ruptured follicles were immunoreactive for Sprouty-2, whereas those of unruptured follicles were not. In the cow, using suppressive subtractive hybridization analysis, Sprouty-2 mRNA was identified only in granulosa cells of follicles whose oocytes developed to blastocysts but not in granulosa cells of follicles whose oocytes failed to further develop (Robert et al., 2001). Taken together, the differential expression of Sprouty-2 in preovulatory follicles may be associated with their capacity to ovulate.

The localization of Sprouty-2 in cell subpopulations throughout the ovarian cycle was partially studied in humans and confirmed in the rodent ovulatory model. In the rat during folliculogenesis Sprouty-2 was detected exclusively in interstitial theca cells, whereas following ovulation and CL development Sprouty-2 was expressed by both theca and GCL. In the human ovary throughout the luteal phase, Sprouty-2 was detected solely in GCL. This suggests a species divergence of Sprouty-2 expression, where rodent theca cells express Sprouty-2, whereas human theca cells do not. Nonetheless, the expression of Sprouty-2 in GCL is shared by both species.

CL development depends on dramatic tissue remodelling and well-orchestrated regulation of vascular outgrowth and regression. This process includes rapid proliferation of lutein cells along with a wave of vascular sprouting that eventually regress during luteolysis (Fraser and Wulff, 2001). The extensive follicular tissue growth and vascularization is regulated by various differentiation and angiogenesis promoting factors, such as EGF (Serta and Seibel, 1993; Khan-Dawood et al., 1995) and bFGF (Stirling et al., 1991; Redmer and Reynolds, 1996; Yamamoto et al., 1997).

The abundance of bFGF-positive small capillaries in the CL, in a proximity to Sprouty-2 producing lutein cells, observed in our study, suggests a regulatory dialogue between Sprouty-2 originated from lutein cells and bFGF-regulated endothelial cell proliferation. Lutein cell proliferation in this stage may be attributed in part to bFGF and possibly other growth factors, such as EGF (Budnik and Mukhopadhyay, 1998). EGF was absent from preovulatory granulosa cells whose oocytes failed to further develop (Robert et al., 2001). Taken together, the differential expression of Sprouty-2 in preovulatory follicles may be associated with their capacity to ovulate.

Indeed, our novel findings show that Sprouty adds another layer of complexity to the regulation of RTK-mediated signal transduction in GCL of the CL.

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References


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