Supplemental Data

FIGURE LEGEND

Localization of FOXL2 protein in the mouse ovary with an N-terminal anti-FOXL2 antibody.

A) Western blot analysis of cell lysates from each of the respective cell lines resulted in 2 bands of 45 and 90 kilodaltons. Serving as a negative control, antisera was preabsorbed with an excess of FOXL2 N-terminal peptide that blocked both bands from detection (data not shown). B) Anti-FOXL2 (N-terminal antibody) was incubated with murine ovarian sections representing different stages of follicular development. FOXL2 is highly expressed in the granulosa cell layer from the primary follicle stage through the large antral stage. C) Antisera preabsorbed with an excess of FOXL2 N-terminal blocking peptide. Scale bars represent 30 µm, 60-fold magnification.

Materials and Methods

N-Terminal FOXL2 Antibody

A rabbit anti-peptide N-terminal FOXL2 antibody that was obtained through Affinity Bio-Reagents (ABR) (Golden, CO) as follows. Nucleotide sequences of murine FOXL2 were used to generate a synthetic peptide representing the 14 N-terminal amino acids (MMASYPEPEDTAGT) for immunization. An antiserum was collected, tested for titer and used in western analysis and immunohistochemistry.
**Western Analysis**

Cell lysates were collected from the αT3-1 pituitary gonadotrope-derived (mouse) cell line, the CHO and KK1 ovarian (hamster and mouse, respectively) cell lines and the BeWo human coriocarcinoma cell line. Lysates were homogenized in RIPA buffer (20 mM Tris (pH 8.0), 137 mM NaCl, 10% glycerol, 1% NP40, 0.1% SDS, 0.5% deoxycholate, 2 mM EDTA, 5 mM sodium vanadate, 5 mM benzamidine, 1 mM phenylmethylsulfonyl fluoride (PMSF)) and assayed for total protein (Pierce BSA). Five µg of total protein /cell line was combined with 2X SDS, denatured and subjected to SDS polyacrylamide gel electrophoresis (acrylamide: bis-acrylamide ratio of 29:1). This was followed by electro-blotting to nitrocellulose (Bio-Rad) membrane. Membranes were then blocked in 5% non-fat dried milk in Tris buffered saline (TBS-T; 140 mM NaCl, 10 mM Tris, 0.1% Tween 20, pH 7.4). Anti-FOXL2 antibody (1:5000 dilution in 1% milk) was applied for an 8 hour incubation at 4° C on an orbital shaker. The blot was washed for 30 minutes (3 washes X 10 minutes) with TBS-T and then incubated with a goat anti-rabbit HRP conjugated secondary (1:5000) for 2 hours. The blot was washed for 60 minutes (6 X 10 minutes) with TBS-T following the secondary antibody incubation and then visualized by chemiluminescence using Pierce Super Signal reagents.