

NOVEL ANTI-MULLERIAN HORMONE RECEPTOR 2 BINDING PEPTIDE (AMHR2BP) AND ITS AFFINITY TO AMH RECEPTOR 2

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Abstract Body

BACKGROUND: Anti-Müllerian hormone (AMH) preserves ovarian follicle reserve by inhibiting hormone production, follicle development, and granulosa cells replication. Recently, we developed an AMH receptor 2 (AMH-R2) binding peptide (AMHR2BP) that mimics native AMH functions. AMHR2BP inhibited granulosa cells replication and function and preserved ovarian follicle number. Furthermore, AMHR2BP decreased follicle hormone production, replication and apoptosis, and modulated oocyte function. We sought to show the specific binding of AMHR2BP to the AMH receptor AMH-R2 by utilizing the AMH-R2-expressing, ovarian cancer cell line A2780, in vitro.

METHODS: We performed in vitro studies to detect and quantify biotin-labeled AMHR2BP in cells utilizing the avidin-biotin complex and immunofluorescence. This method involves biotinylated horseradish peroxidase preincubated with avidin to form large avidin-biotin complexes which bind to the biotinylated targets. To test whether AMHR2BP specifically binds to AMH-R2, total protein was extracted from the A2780 cells and incubated with the biotin-labeled AMHR2BP. Western Blot was performed hybridizing with both, the biotin-labeled AMHR2BP and AMH-R2 monoclonal antibodies. We utilized immunofluorescence to co-localize AMH-R2, AMHR2BP, and the complex AMH-R2+AMHR2BP, within the cells.

RESULTS: Western Blot studies showed that AMHR2BP binds specifically to AMH-R2 as indicated by the presence of a 62 kDa band corresponding to AMHR2BP and the same 62 kDa band corresponding to AMH-R2 antibody incubated at 1:500 concentration (Figure 1). Additionally, immunofluorescence showed the biotin-labeled AMHR2BP and AMH-R2 antibody co-localized on the cell membrane, as indicated by the merge between the biotin-labeled AMHR2BP (green) and AMH-R2 antibody (red) within the cells, which resulted in a yellow pigment (Figure 2).

CONCLUSIONS: We confirmed AMHR2BP's affinity with, and exclusive binding to, AMH-R2. We endorse using AMHR2BP in place of AMH in all applications where AMH's inhibitory effects on ovarian function and development are needed, which include, but are not limited to, fertility preservation during gonadotoxic therapy.