

## GPER (G protein-coupled estrogen receptor 1) is involved in brood pouch cell proliferation of seahorse

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The brood pouch of seahorse is functionally equivalent to the amniote uterus. In the embryo-carrying stage, the inner epithelium and loose connective tissue layer is highly distended and rich in blood vessels. G protein-coupled estrogen receptor 1 (GPER) is widely distributed in tissues and high expressed in placenta. GPER combined with estrogen (E2) to transactivate the EGF receptor (EGFR), and also induced the secretion of connective tissue growth factor (CTGF), and was involved in cell migration and proliferation, as well as vascularization. The seahorse GPER consisted of 1026 bp bases, encoding 341 amino acids, including a seven-transmembrane domain. The branch-site model test showed that seahorse GPER gene is under strong positive selection. One paralleled replacement from Serine to Cysteine at position 142 was identified in placental mammals and seahorse. Transcriptomic analysis revealed GPER was highly abundant in brood pouch compared to the other estrogen receptors, implying that GPER were the main receptor in responsible for estrogen effects. The expression of relevant downstream genes including CTGF and EGFR were also high, implying that GPER were probably relevant to cell growth and vascular proliferation in brood pouch. An intraperitoneal E2 injection test was finished to test estrogen effects mediated by GPER in brood pouch. The qPCR analysis showed that relative expression of GPER increased with serum E2 level, accompanied by an increase in CTGF and EGFR expression.

In conclusion, our results suggest that GPER play an important role in promoting the brood pouch cell proliferation and vascularization.

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