

LH receptor structure-function and role in ovarian cancer

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The LH receptor (LHR), one of the three glycoprotein hormone receptors, binds two gonadotropins, LH and CG. Structurally and functionally the receptor can be divided into three portions: the ectodomain (ECD) that is comprised of leucine-rich repeats capped by Cys-rich regions and responsible for high affinity ligand binding, the transmembrane region that contains the seven membrane-spanning helices and extracellular and intracellular loops, and the cytoplasmic carboxy-terminal tail. Expression and function of this G protein-coupled receptor is necessary for early male sex differentiation, gonadal steroidogenesis in men and women, and ovulation. In addition to these roles in reproductive physiology, LHR has also been implicated in ovarian cancer. The goals of this study are to elucidate the structure-function relationships of LHR and examine its putative effects on human ovarian cancer cells. Structure-function studies, based on human CG (hCG) binding and hCG-mediated signaling (cAMP), were performed using transfected HEK293 cells expressing wild type and mutant LHR. These methods were complemented with molecular modeling of the LHR ECD and mass spectrometry to probe hCG-LHR contacts. The experiments relating to ovarian cancer were conducted with stable transformants of SKOV3 cells expressing LHR. In addition to determining the binding and signaling properties of the LHR-expressing SKOV3 cells, Taqman expression array analysis, Western blots, and various inhibitors to Ser/Thr and Tyr protein kinases were used to characterize LHR activation and the effects on cell migration, invasion, and proliferation. Using several templates for homology modeling, a working model of the LHR ECD structure was developed. A combination of experimental and computational studies has shown the importance of the DRY motif and the intracellular loops in receptor activation and G protein coupling. A major unresolved component of ligand-mediated receptor activation is the mode of communication of the ligand-bound ECD with the transmembrane portion of LHR. In LHR-expressing SKOV3 cells, an increase in ErbB2 (Her2) gene expression and protein levels, including P-ErbB2, were found in response to LH and hCG. These effects could also be mediated by addition of 8-Br-cAMP and forskolin to the cells and blocked by the addition of an inhibitor of PKA, showing the importance of signaling via cAMP and PKA activation in up-regulation of the ErbB2 gene. The array analysis indicated a phenotype of increased expression of adhesion genes and decreased expression of metalloprotease genes in response to LH. Interestingly, the gonadotropin-mediated up-regulation of ErbB2 is insufficient to increase cell proliferation, invasion, and migration. In summary, this study has provided: (a) an overview of the LHR structure and a possible mode of activation, and (b) a delineation in SKOV3 LHR-expressing cells of the mechanism by which LHR activation leads to an up-regulation of ErbB2, a member of the EGF receptor tyrosine kinase family. Supported by NIH DK33973 and DK69711.