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# Role of GPR30 in testicular germ cell tumors

## A potential new anticancer target

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Estrogens play several key roles in humans, especially in women. These hormones not only contribute to the development and function of the female mammary and reproductive systems, but also have biological effects in the cardiovascular, musculoskeletal, immune and central nervous systems. The effects of estrogens are mediated by two nuclear estrogen receptors (ERs), ERα and ERβ, both belonging to the nuclear receptor family of transcription factors.[1,2]

G-protein-coupled receptor 30 (GPR30) is a seven-transmembrane-spanning receptor. It has been recently found to bind 17β-estradiol with high affinity and to mediate estrogenic signals.[3-5] GPR30 was proposed to be an estrogen receptor responsible for both rapid non-genomic events and genomic transcriptional events of estrogens,[6-8] hence its official new acronym is GPER1 (G protein-coupled estrogen receptor 1). At the cellular level (**Fig. 1**), GPR30 was reported to influence growth factor signaling pathways including trans-activation of the EGF receptor, intracellular $Ca^{2+}$ mobilization, phosphoinositide-3-kinase translocation, Src activation, ERK activation and cAMP production and to modulate downstream transcription factor networks.[5-10] Interestingly, GPR30 triggers the release of the membrane-tethered Heparin-Binding EGF (HB-EGF), which, in turn, binds to unoccupied EGF receptors resulting in their activation.[11] This mechanism of action may possibly explain the EGF-like effects of estrogen measured in female reproductive tissues in mice, and provides the means for ER-negative breast cancer cells to remain estrogen responsive.[11] Considering that ERs play important roles in the induction and progression of estrogen-related cancers, such as breast, endometrial, ovarian, adrenocortical, prostate, colorectal, liver and lung cancer, it is likely that a relationship exists between the novel receptor GPR30 and cancer.[7,12]

GPR30 expression has been detected in several human tumor specimens or cell lines, including breast cancer, endometrial cancer, ovarian cancer, choriocarcinoma, thyroid cancer, lung cancer and lymphomas.[4,12-14] Experimental evidence supports a strong association between GPR30 and cancer proliferation, migration, invasion, metastasis and tumor cell differentiation.[12,15-21] GPR30 was shown to mediate the proliferative effects of 17β-estradiol in breast cancer cells lacking ERα and ERβ.[20] He et al.[21] demonstrated that estrogen promotes cell proliferation in two endometrial cancer cell lines, KLE ($ER^-$) and RL95-2 ($ER^+$), through activation of GPR30 and the MAPK signaling pathway. In the ovarian cancer cell line BG-1, GPR30 mediates the growth response to estrogen through the EGF-receptor-MAPK signaling pathway.[15]

GPR30 seems to be also a tumor prognostic factor and a predictive marker of drug resistance in a few estrogen-related tumors.[11,14-24] Filardo et al.[18] have carried out immunohistochemistry for ER and GPR30 in 361 breast carcinomas. Overexpression of GPR30 was positively associated with tumor size (>2 cm), the presence of distant metastases and HER-2/neu overexpression. These data

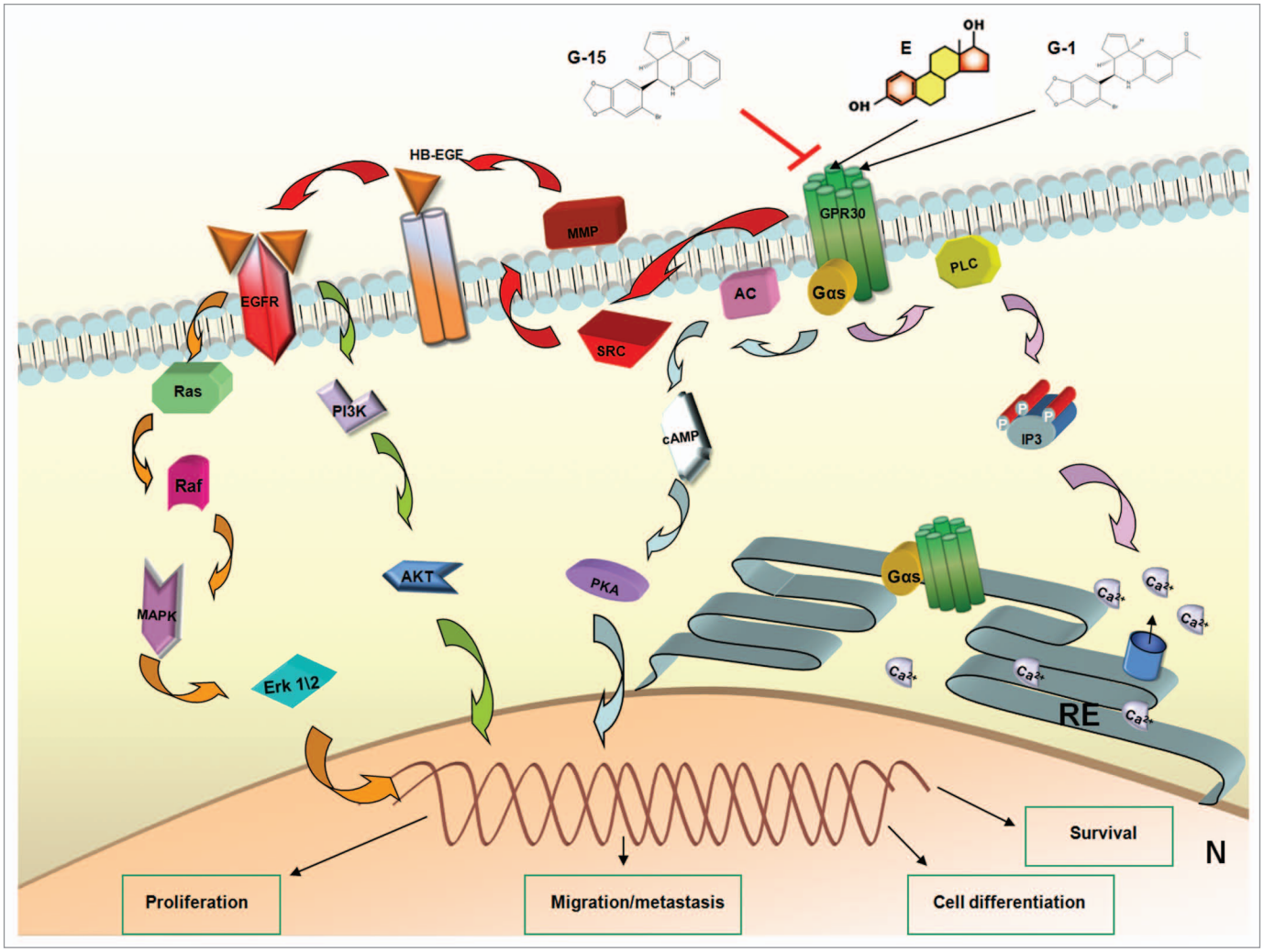


**Figure 1.** Signaling pathways activated by GPR30: GPR30 influences growth factor signaling pathways including trans-activation of the EGF receptor, intracellular $Ca^{2+}$ mobilization, phosphoinositide-3-kinase translocation, Src activation, ERK activation, and cAMP production and modulates downstream transcription factor networks that lead to cancer proliferation, migration, invasion, metastasis and tumor cell differentiation. AC, adenylate cyclase; GPR30, G-protein-coupled receptor; PLC, Phospholipase C; IP3, Inositol trisphosphate; cAMP, 3'-5'-cyclic adenosine monophosphate; $Ca^{2+}$, calcium ion; SRC, Src tyrosine kinase; ERK12, extracellular signal-regulated kinase; Gαs, G proteins alpha; MMP, matrix metalloproteinase; HB-EGF, membrane-tethered Heparin-Binding EGF; PI3K, Phosphoinositide-3-kinase; AKT, Serine/threonine protein kinase; EGF\R, epidermal growth factor receptor; Ras, RAt sarcoma protein; Raf, serine/threonine-selective protein kinase; MAPK, mitogen-activated protein kinase; G-15, selective GPR30 antagonist; G-1, GPR30 agonist; E, estrogen; RE, endoplasmic reticulum; N, nucleus; Arrow, direct stimulation; Red line, direct inhibition.

represent the first assessment of GPR30 in malignant human breast carcinoma and indicate that GPR30 overexpression may be a predictor of biologically aggressive disease in breast cancer. Interestingly, in models of drug resistance to selective estrogen receptor modulators (SERMs) and aromatase inhibitors, GPR30 is overexpressed, suggesting that GPR30 upregulation may compensate for a deficiency in estrogen-mediated proliferative signaling.[22] GPR30 overexpression occurred more frequently in endometrial carcinomas exhibiting deep myometrial invasion, advanced stage, high-grade and biologically aggressive histological subtypes.[23] In addition, in patients with endometrial carcinoma showing high GPR30 expression, the overall survival rate was significantly worse than that of patients with low GPR30 expression (65.2 and 100%, respectively).[23] A similar correlation has been reported in ovarian cancer.[24]

Testicular germ cell tumors (TGCTs) are the most common cancer in young men between the ages of 15 and 34 years.[25,26] ERβ is expressed in normal testicular cells and it is downregulated in seminomas and embryonal cell carcinomas.[27,28] However, the estrogen response system seems to have a role in the carcinogenesis of TGCT. The estrogen excess theory is the major hypothesis for the pathogenesis of this tumor.[29] According to this theory, the initiating events that lead to tumor development occur early in utero during the prenatal period. Exposure to high levels of maternal estrogens, secondary to the endogenous hormonal imbalances, to the exogenous administration of hormones or to the contact with environmental estrogens is associated with a high risk of TGCT.[29]

In a recent issue of *Cancer Biology & Therapy*, Franco et al.[30] evaluated GPR30

expression in post-puberal TGCTs (30 seminomas, 5 teratomas, 12 embryonal carcinomas and 20 intratubular germ cell tumors) by immunohistochemical analysis. High GPR30 protein expression was seen in all intratubular germ cell tumors, seminomas and embryonal carcinomas, whereas in teratomas the immunoreactivity was low. Western blot analysis, performed on the same samples, showed that the GPR30 levels correlated with the immunohistochemical data. GPR30 protein expression was also evaluated in GC1 and TCam-2 cell lines, derived from an immortalized type B murine spermatogonia and a human seminoma, respectively. The GPR30 protein was detected in the cytoplasm and along the plasma membrane of GC1 cells, with a diffuse expression, whereas in the seminoma cell line, TCam-2, the protein was identified in specific compartments, probably into the Golgi.

An agonist, G-1, with selectivity for GPR30 over ERα and ERβ, was reported by Bologa et al.[31], and is now being used extensively to study this receptor. G-1 induced proliferation in ovarian carcinoma cell lines,[32] while G-1 reduced mouse brain microvascular endothelial cell proliferation by inhibition of DNA synthesis and by accumulation of cells in S and $G_2$ phase.[33] On the other hand, a selective GPR30 antagonist, G15, has recently become available. This compound binds to GPR30 with high affinity and acts as an antagonist of estrogen signaling through GPR30.[34] Future studies utilizing GPR30-selective agonists and antagonists (**Fig. 1**) will further define the in vitro and in vivo effects of GPR30 modulation in TGCTs, opening a new scenario in the treatment of these tumors with a new targeted therapy.

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