

## GPR30: A new potential therapeutic target in human testicular germ cell tumors

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**SUMMARY** The G protein-coupled estrogen receptor (GPR30) is suggested to exert a role in non-nuclear estrogen signalling and is over-expressed in a variety of hormone dependent tumors. It is well known that estrogens and xenoestrogens are involved in testicular germ cell tumorigenesis. Different studies show that down regulation of estrogen receptor  $\beta$  (ER $\beta$ ) associates with GPR30 over-expression both in human testicular carcinoma *in situ* (CIS) and seminomas and that the mitogenic role exerted by 17 $\beta$ -oestradiol induces the activation of extracellular signal-regulated kinase 1/2 (ERK1/2) through GPR30. In conclusion, the exposure to oestrogens or oestrogen-mimics, in some as of yet undefined manner, diminishes the ER $\beta$ -mediated growth restraint in CIS and in human testicular seminoma, indicating that GPR30 could be considered a potential therapeutic target to design specific inhibitors.

**Keywords** GPR30, testicular cancer, estrogen receptor, seminomas

The estrogen receptor  $\beta$  (ER $\beta$ ) subtype is the principal mediator of oestrogen action in promoting germ cell survival and development. After activation, these receptors, in association with various coactivators as RING-finger protein 4 (RNF4) and repressors as POZ-AT HOOK Zinc Finger -Containing Protein 1 (PATZ1), act as nuclear transcription factors for targeted genes. It has been well documented in literature that ER $\beta$  is instead down regulated in seminomas and embryonal cell carcinomas (1-7).

In the last few years, G protein-coupled receptor 30 (GPR30) was demonstrated to be capable of mediating estrogen actions in a wide variety of cell types including germ cells and Sertoli cells; in fact, it has been shown that GPR30 may mediate actions important for Sertoli cell function and maintenance of normal testis development and homeostasis. GPR30 has been recently found to bind 17 $\beta$ -estradiol (E2) with high affinity and to mediate estrogenic signals controlling the proliferative effects of E2 in ER-negative SKBr3 breast cancer cell lines since GPR30 depletion, by using antisense oligonucleotides or RNA interference (RNAi) strategies, abrogated E2-stimulated growth in these cells (8,9). GPR30 activates numerous cell signaling pathways including calcium mobilization, adenylyl cyclase, MAP kinase and phosphatidylinositol 3-kinase, in large part *via* the transactivation of epidermal growth factor receptors (EGFRs) (10).

These observations led to the hypothesis that GPR30 activation may represent an alternative pathway for estrogen-mediated activity in high grade and advanced stage in various epithelial tumors that are more often ER negative.

Recent published studies correlate the GPR30, and ER $\beta$  expression in testicular human carcinoma *in situ* (CIS) and seminomas (8) (Table 1). First, the down-regulation of ER $\beta$ , observed in seminomas, was in accordance with our previously published data, and from animal models and human cell culture studies suggesting that ER $\beta$  may control cell proliferation during germ cells cancer progression (8,9). These considerations induce to hypothesize that exposure to estrogens, in some as of yet undefined manner, diminishes the ER $\beta$ -mediated growth restraint in spermatogonia, which favors unscheduled cell proliferation. The affected spermatogonia or their descendants may then be able to escape normal cell cycle regulation and be at a higher risk of undergoing malignant transformation (9).

Recently, we have shown that ER $\beta$  interacts with High Mobility Group A1 (HMGA1) and PATZ1 in normal germ cells, while down regulation of ER $\beta$  is concomitant with transcriptional coregulators HMGA1 and PATZ1 over-expression and cytoplasmic localization both in human testicular seminomas and in TCam-2 seminoma cell line (5,6). We also observed

**Table 1. Immunohistochemical markers in TGCTs subtypes**

Items	HMGA1	HMGA2	PATZ1	GPR30	ER $\beta$	RNF4
Seminoma	+ $\odot$	-	+ $\odot$	+	-	-
Embryonal carcinoma	+ $\odot$	+	+ $\odot$	+	-	-
Teratoma	-	-	+ $\odot$	+/-	-	-
Yolk sac	-	+	+ $\odot$	+	-	-

Notes: +, expressed; + $\odot$  cytoplasmic localization; -, not expressed; +/- variable expression.

that 17 $\beta$ -oestradiol induces an HMGA1 increased cytoplasmic expression correlates with an ER $\beta$  down-regulation in TCam2 cell line (5). In addition, our group has published that GPR30 is over-expressed in human testicular seminomas, that are more often ER $\alpha/\beta$  negative (9) (Table 1).

The relationship between estrogen signaling and its multiple regulatory interactions with growth factor and other kinase signaling pathways involves complex patterns of genomic and non genomic cross-talk. Estrogen, as well as many of the classic ER antagonists and estrogen receptor modulators (SERMs, including fulvestrant and tamoxifen) activate signaling pathways *via* GPR30 (9,10). In addition, in our recent published study we have shown by using the TCam2 seminoma cell line that 17 $\beta$ -estradiol induces Extracellular signal-Regulated Kinase 1 and 2 (ERK1/2) activation and c-fos increased expression in absence of ER $\beta$  and in presence of GPR30 (9). Studies that evaluate GPR30 expression in relation to the classical steroid receptors (ER $\alpha/\beta$ ) and response to chemotherapy are needed to elucidate the value of GPR30 as a prognostic indicator (8,9). Since many G protein-coupled receptors, including GPR30, induce EGFR phosphorylation, the inter-receptor cross-talk demonstrated by this paradigm represents a novel opportunity for therapeutic intervention (8,9). Therefore, the expression or function of GPR30 with selective agonists and/or antagonists could be an effective treatment strategy, in conjunction with standard chemotherapy. In fact, we have demonstrated that G15, a new selective GPR30 antagonist, inhibits estrogen-induced proliferation in TCam2 seminoma cell line (3,9,10).

In conclusion, the design of specific GPR30 inhibitors could represent a useful molecular target to block neoplastic germ cells with a high proliferative rate suggesting its potential therapeutic role for the treatment of CIS and seminomas.

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