Bisphenol A disrupts seminoma cell proliferation following an inverted U-shaped non monotonic dose-response curve, due to its greater affinity for GPR30, the non classical membrane G Protein related Estrogen Receptor (GPER), than for ER beta.

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Background

Testicular germ cell tumours are the ***most frequent cancer of young men between 15 and 35 years of age.*** *While pathogenesis and reasons of an increasing incidence (about 2% each year) all over the world remain unknown, epidemiological and clinical data have suggested that* ***fetal exposure to environmental endocrine disruptors (EEDs) with estrogenic effects, could participate to testicular germ cell carcinogenesis.*** *However, EEDs (like bisphenol A) are often weak ligands for classical nuclear estrogen receptors.*

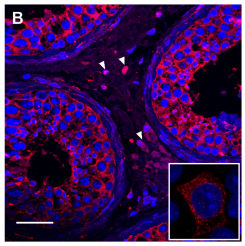
Using a human seminoma cell line (JKT-1), which expressed ER beta but not ER alpha, we previously reported that bisphenol A (BPA) could induce cell proliferation at a very low concentration (10^{-9}M) independently of the classical estrogen receptor ER beta *(Bouskine et al., Endocrinology, 2008).*

The non classical membrane G-protein coupled estrogen receptor (GPER or GPR30) has been recently shown to mediate the effects of several xeno-estrogens through rapid non genomic activation of signal transduction pathways in various human estrogen dependent cancer cells (breast, ovary, endometrium).

Methods

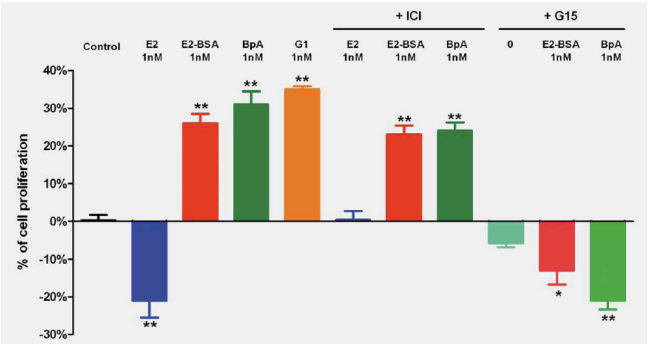
Quantification of *in vitro* seminoma cells (JKT-1) proliferation with various doses of E2 and BPA, with or without selective agonists and antagonists of ER beta (ZU, ICI) and GPER (G1, G15), in order to determine the affinity of BPA for ER beta and GPER.

Results

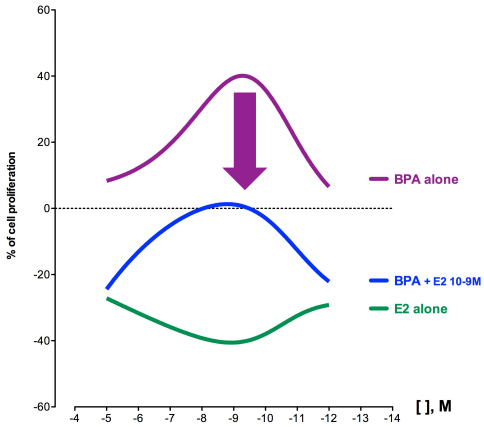


We previoulsy demonstrated that **GPER was expressed in testis,** both by somatic and germ cells and by seminoma cells (*Chevalier et al., PlosOne 2012; red fluoescence*). Using Western blotting and RT-PCR, we showed that **GPER was exclusively and significantly ($P < 0.05$) overexpressed in seminomas,** the most frequent testicular germ cell cancer, compared to non seminoma tumours.

When added at very low doses (nM;pM), E2-BSA (an impermeable E2-conjugate), G1 (a selective agonist of GPER) and **bisphenol A induced JKT-1 cells proliferation**. This effect was completely abolished by G15 (a GPER selective antagonist) and by siRNA invalidation, but not by ICI-182,780, a selective antagonist of ER.



We confirmed **in our model that E2 had a greater affinity for ER beta than for GPER,** with a maximal effect at the concentration of 10-9M. **At the opposite, BPA exhibited a greater affinity for GPER than for ER beta, with a maximal effect at the concentration of 10-9M.** The response of JKT-1 seminoma cells to E2 (from 10-5 to 10-12 M) followed a U-shaped nonmonotonic dose response (NMDR) curve. Interestingly, they responded to BPA in the opposite direction, **following an inverted U-shaped NMDR curve**, which could be totally shifted from top to bottom in case of coexposition to BPA and E2.



Conclusions

GPER is specifically overexpressed in seminoma tumors and able to trigger seminoma cell proliferation *in vitro*. It should therefore be considered rather than classical ERs when xeno-estrogens or other endocrine disruptors are assessed in testicular germ cell carcinogenesis.

In our model, BPA promotes seminoma cell proliferation at low doses (environmentally relevant) because it exhibits a greater affinity for GPER than for ER beta, explaining the inverted U-shaped NMDR curve observed.