Estrogen receptors in bladder cancer, a preliminary study about their effects and relevance for diagnostics

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Abstract

Bladder cancer (BlaCa) is more frequent in men than in women still, women are more likely to be diagnosed with invasive disease and have poor survival. Recent studies suggest that estrogen may have a role in urogenital schistosomiasis (UGS) and in schistosoma-related BlaCa. Epidemiological and molecular evidences support a possible role of estrogens in bladder carcinogenesis, however their contribution remains elusive. In this work the expression of estrogen receptors (ER) α and β in human BlaCa of urothelial cell origin (IPO-P series, n = 81), tumors from patients infected with S. haematobium (Angola series, n = 37), mainly SCCs, and in the bladder of patients with UGS without cancer (n = 35) was characterized and correlated with clinicopathological features. Human BlaCa cell lines HT1374, T24 and 5637 were characterized in terms of estrogen receptors expression and response to clinically used ER antagonists Tamoxifen (Tam) and Fulvestrant (ICI 182 780).

Results

ERα is expressed in almost 50% of IPO-P tumors and in 44% of the cases from Angola, mainly in the basal layer of the urothelium and tumor cells with low nuclear atypia (Fig. 1).

- expression more intense in the normal appearing urothelium than tumor
- subepithelial cells of NMIBC express more ERα than in MIBCs
- expression in benign urothelium associated with recurrence and poor survival
- higher expression in cancer cells and adjacent urothelium of specimens with schistosoma eggs
- UGS without tumor had higher ERα expression among older patients.

Expression of ERβ was analyzed in the Angola series and is ongoing in the IPO-series. ERβ is expressed in all layers of the urothelium and in both high and low grade tumor cells (Fig. 2). Both nuclear and cytoplasmic staining. Staining intensity is higher in samples with UGS-related BlaCa when compared to UGS alone. Expression seems more frequent in females than males.

ER expression in human bladder cancer cell lines:
5637 and T24 cells BlaCa cells express ERα mRNA, albeit in much lower levels than T-47D (Fig. 3). HT1376 cell line does not express ERα mRNA at detectable levels. ERβ mRNA was not detected in any of the cell lines

Effect of E2 and ER antagonist on BlaCa cells expressing ERα:
5637 cells where stimulated with E2 at 1 nM and had no significant effect at lower or higher concentrations (Fig. 4). T24 cells did not respond to E2. Tam induced significant inhibition of viability in both BlaCa cell lines and T-47D cells at conc. of 500-1000 nM, with greater potency in 5637 cells. Growth inhibitory effects were observed with ICI starting at 10 nM for 5637 and T-47D cells with 500 nM being the lowest concentration producing maximum effect. T24 cells were also inhibited by ICI, but only at high doses (1000 nM) (Fig. 5).

Conclusions

ERβ is the predominant ER in BlaCa, but ERα is also expressed in a significant proportion of the cases. The expression of ER was not associated with gender, but could be an indicator of aggressiveness and poor prognosis. Overall, ERs expression was more intense in cases of UGS when compared BlaCa without UGS, this could be related with the presence of the parasite. Schistosomes produce estradiol-related metabolites seen in the urine and sera of patients with UGS. This analysis provides insights into the role of ERs in bladder cancer development and progression however, for in vitro studies, it would be useful to use human BlaCa cell lines expressing ERβ to better represent the pattern observed in human tumors. Endogenous expression of ERβ is frequently absent in cell in culture, but can be induced through transfection.
Understanding the role ERs in BlaCa can offer new diagnostic and therapeutic options using ER antagonists currently used in the clinics for breast cancer.

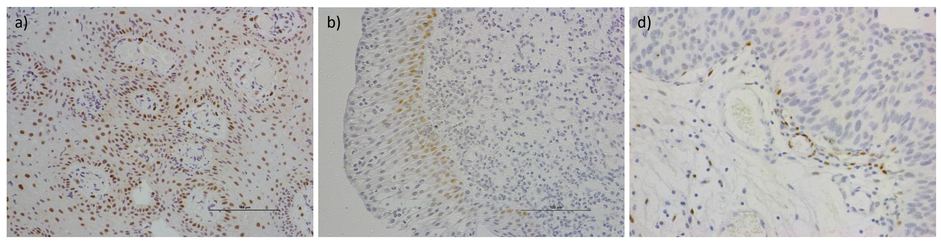

Fig. 1 Representative images of ERα expression in tumor cells (a) and adjacent urothelium (b) and subepithelial stroma (c).

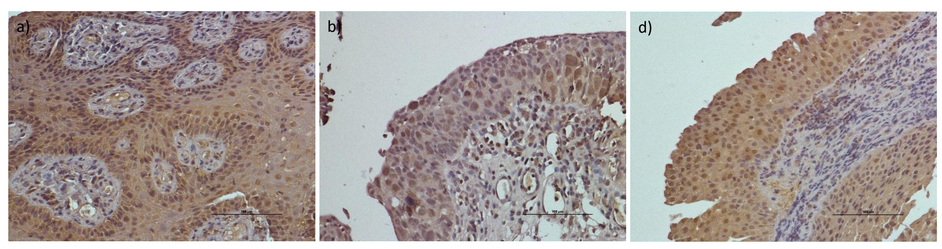

Fig. 2 Representative images of ERβ expression pattern in tumor cells (a), adjacent urothelium (b) and UGS without tumor (C).

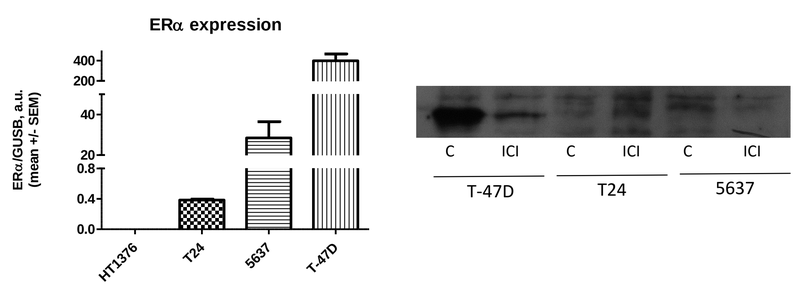

Fig.3 Expression levels of ERα in BlaCa cell lines evaluated by qRT-PCR and western blotting. For WB cells were treated for 24h with ICI or control vehicle. ERα loss after ICI treatment confirms the specificity of the band.

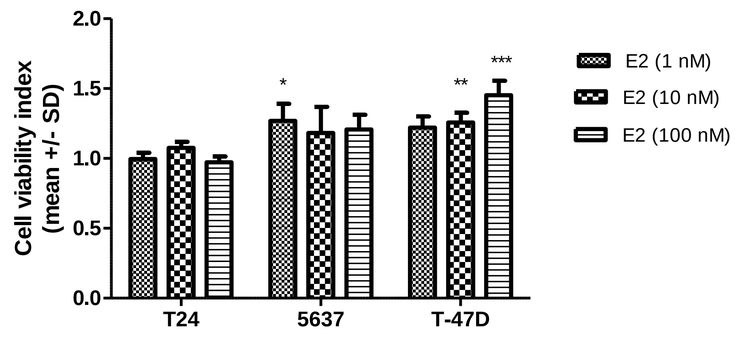

Fig. 4 Relative effect of 17β-estradiol (E2) on BlaCa cell lines and T-47D (used as reference) after72 h of treatment, data normalized to vehicle control. Cell viability evaluated using Presto Blue and confirmed by direct cell counting. Statistical significance is relative to vehicle controls. (*P<0.05, **P<0.01, ***P<0.001)

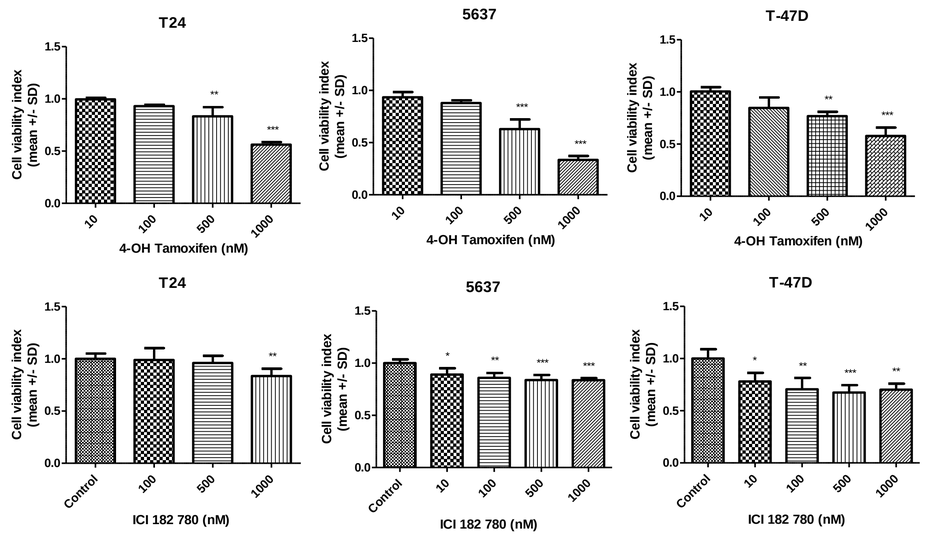

Fig. 8 Effect of ER antagonists on BlaCa cells viability after72 h treatment, data normalized to vehicle control. Statistical significance is relative to vehicle controls. (*P<0.05, **P<0.01, ***P<0.001)

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