Gender medicine indicates that diseases can manifest differently in men and women and thus diagnosis, therapy and medication have to be gender-specific. Namely, thyroid diseases are common afflictions, with a much higher prevalence in females than in males. Both benign and malignant thyroid tumors are more prevalent in women than men, so female sex hormones may have an etiological role in these conditions. Immunohistochemistry and binding assays have identified estrogen receptor (ERs) isoforms (ERα and ERβ) in thyroid tissue and overexpression of ERs has been associated with neoplastic thyroid tissues. Furthermore, it has been shown that 17beta-estradiol (E2) enhances cell growth in FRTL-5 cells (rat thyrocytes) where it inhibits the sodium/iodide symporter (SLC5A5, NIS) gene expression. However, our knowledge on how E2 affects thyroid function and namely the thyroid-specific gene expression is very scarce.

The tissue-specific expression of the thyroid differentiation markers has been extensively studied and their transcriptional regulation results mediated by a set of transcription factors including TTF-1/Nkx2-1, Pax8 and TTF-2/FoxE1. During development and in the adult life, these transcription factors are present in other cell types, but their concomitant expression is restricted to thyrocytes only. Their expression is required for the early stages of thyroid morphogenesis and is crucial for the normal thyroid function and homeostasis.

In this study we have analysed the role of E2 in regulation of thyroid-specific gene expression. In order to verify the interaction between ER and TTF-2, we have performed the two-hybrid assay in mammalian cells. We show that Gal4-TTF-2 has modest trans-activation activity on Gal4-luc vector. Similarly, the estrogen receptors, expressed alone, have weak activity on the GAL4-dependent promoter. However, when Gal4-TTF-2 and ERα or Gal4-TTF-2 and ERβ are co-expressed, Gal4-TTF-2 is able to recruit the ERs on the promoter, resulting in a robust stimulation of the reporter gene transcription. These experiments strongly suggest that TTF-2 is able to form a complex with either ERα or ERβ. It is worth to point out that these experiments have been performed in HeLa cells that do not express endogenous ERs. To determine whether estrogen could affect TTF-2 activity in thyrocytes, we used p4xZ-Luc vector, a TTF-2-specific artificial promoter driving the luciferase gene expression. We have performed, in FRTL-5 cells, transfection experiments of p4xZ-Luc in presence or in absence of E2. The experiments show that E2 down-regulates the promoter activity and so strongly suggest that E2 inhibits TTF-2 activity. To confirm this result in the context of a thyroid-specific promoter we have used the pNIS-luc vector. We have co-transfected the pLKO-i77 vector, expressing the shRNA able to silence TTF-2 expression. In absence of estrogen, the silencing of TTF-2 down-regulates the luciferase gene expression. This result confirms that TTF-2 regulates NIS gene transcription. Interestingly, in presence of E2, NIS promoter activity is very similar in presence and in absence of TTF-2. Hence, in presence of E2, TTF-2 is not able to trans-activate the promoter. This result suggest that E2 inhibits TTF-2 activity on NIS promoter as well.

Thus, we have provided some experimental evidence that the estrogen receptors interact with TTF-2 and E2 inhibits its transcriptional activity. Taken together, these data suggest that estrogen receptors may acts as a TTF-2 corepressor.

References:
Keywords: thyroid, estrogen, FoxE1/TTF-2, thyroid-specific gene expression
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