Oestrogen receptor α and foxp2: investigating a possible interaction and itsregulation

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Introduction: Forkhead box P2 (FOXP2) regulates the expression of various genes and is associated with language and speech, neural development and outgrowth and cancer. It impedes tumour progression through the TGFβ/SMAD signalling pathway in breast cancer. As transcription factors rarely function in isolation, this study aims to investigate whether FOXP2 directly associates with oestrogen receptor α (ER1), a nuclear receptor that blocks the TGFβ/SMAD signalling pathway through interactions with various proteins.

Methodology: The FOXP2's DNA-binding forkhead domain (FHD) and nuclear receptor box- containing region (NRB), and ER1's ligand-binding domain (LBD) were overexpressed in *E. coli* cells and purified using immobilised-metal affinity chromatography and size-exclusion chromatography. The proteins were structurally characterised using far-UV circular dichroism and intrinsic tryptophan fluorescence. Thereafter, fluorescence anisotropy experiments were performed to evaluate the interaction between the proteins and the regulatory effect of oestrogen on the interaction.

Results: Following their successful overexpression and purification, both proteins were confirmed to be primarily alpha-helical and structurally intact. Fluorescence anisotropy revealed that ER1 LBD interacts with the FOXP2 FHD but not FOXP2 NRB. Inclusion of oestrogen has no effect on the protein-protein interactions.
Discussion and Conclusion: This study provides evidence of an association between FOXP2 and ER1, the effect of which requires further investigation to elucidate what implications, if any, the interaction may have on the functional activities of either protein and ultimately, breast cancer, neurodevelopmental disorders, and the current understanding of the link between gender and speech.
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References: Blane, A. and Fanucchi, S., 2015. Effect of pH on the Structure and DNA Binding of the FOXP2 Forkhead Domain. *Biochemistry*, *54*(25), pp.4001-4007. Bowers, J.M., Perez-Pouchoulen, M., Roby, C.R., Ryan, T.E. and McCarthy, M.M., 2014. Androgen modulation of Foxp1 and Foxp2 in the developing rat brain: impact on sex specific vocalization. *Endocrinology*, 155(12), pp.4881-4894.
Chen, M.T., Sun, H.F., Li, L.D., Zhao, Y., Yang, L.P., Gao, S.P. and Jin, W., 2018. Downregulation of FOXP2 promotes breast cancer migration and invasion through TGFβ/SMAD signaling pathway. *Oncology letters*, 15(6), pp.8582-8588.
Ito, I., Hanyu, A., Wayama, M., Goto, N., Katsuno, Y., Kawasaki, S., Nakajima, Y., Kajiro, M., Komatsu, Y., Fujimura, A. and Hirota, R., 2010. Estrogen inhibits transforming growthfactor β signaling By promoting Smad2/3 degradation. *Journal of biologicalchemistry*, *285*(19), pp.14747-14755.

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