CHARACTERIZATION OF THE ETS DOMAIN OF HUMAN ESX GENE: CONSTRUCTION, CLONING AND EXPRESSION

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The Ets family of eukaryotic transcription factors presents a conserved sequence, the Ets domain, which comprises the DNA-binding domain of these proteins. They act as positive or negative regulators of gene expression involved in several biological processes. ESX is an Ets factor expressed specifically in epithelial cells and induced during terminal differentiation, being involved in human mammary gland development and cancer malignancy. It is transcriptionally stimulated in early stages of breast cancer, suggesting a role in tumorigenesis of mammary secretory epithelium. The aims of this work are the synthesis of the ESX region that encodes the Ets domain, expression in Escherichia coli, and purification for further crystallization and structural analysis. Expression assays of the recombinant protein using different E. coli strains were carried out and the expression of the protein was detected in the insoluble fraction. Optimization of the protein expression of the human ESX domain will be necessary to implement highyield purification protocols. Recombinant homogeneous protein will be then used in crystallization trials, three-dimensional structure determination and DNA binding studies. The present work paves the way for our understanding of the molecular mechanisms of DNA recognition by members of the Ets domain family of eukaryotic transcription factors.

Key Words: Breast cancer. ESX. Ets.