Background

Sexual differentiation is critical for reproduction in nearly all metazoan. Defects in any of the genes involved in either testicular or ovarian development can result in differences of sex development (DSD). CBX2 is a chromatin modifier that plays an important role in sexual development and its disorders, and exists in two isoforms. The promoter of these variants is unknown, however there are hints of differential expression by the isoforms in different cell lines and tissues.

Objective

To characterize the differential regulation of CBX2 transcription in applicable cell lines.

Methodology

i) Cell culture & cloning:

To locate candidate CBX2 promoter regions, primer sequences were designed targeting transcription.

Amplified DNA fragments were cloned as reporter inserts into the pGL4.17 Vector which lacks a promoter, requires expression of SV40 T antigen, and encodes the luciferase reporter gene luc2.

ii) Dual luciferase system:

Custom promoter constructs were transfected in COS-1 cells (SV40 transformed cell type), and subsequently in HeLa & HEK293T cells to determine the regulation of CBX2 transactivation activity.

Results

Utilizing the dual-reporter assay system, we identified an optimal candidate CBX2 promoter construct that exhibited a 3.6 normalized fold change in activity when compared to a negative control (p=0.0074). (Fig.1)

Subsequently, the selected CBX2 promoter construct exhibited significant transactivation potential in the HeLa cell line (3.11 normalized fold change) (p=0.0038). (Fig.2)

The CBX2 promoter did not exhibit significant transactivation in the HEK293T cell line (1.5 normalized fold change) (p=0.0748). (Fig.3)

Conclusions

➢ Preliminary results indicate our promoter construct may be applied to characterize differential transactivation of CBX2 in cell models recapitulating ovaries, testis and adrenal cells, and thereby elucidate its functional role as transactivator, distinct from its known function as chromatin-modifier.

➢ Further study of the impact of CBX2 activation and suppression may shed light on potential pathological mechanisms involved in DSD, and ultimately its diagnosis and management.

References


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Figure 1. CBX2 promoter activity in COS-1 cells. Promoter activity of CBX2 constructs is expressed as relative to an empty vector (Luc). Error bars represent the mean ± SD of three independent experiments.

Figure 2. CBX2 promoter activity in HeLa cells. Promoter activity of CBX2 constructs is expressed as relative to an empty vector (Luc). Error bars represent the mean ± SD of three independent experiments.

Figure 3. CBX2 promoter activity in HEK293T cells. Promoter activity of CBX2 constructs is expressed as relative to an empty vector (Luc). Error bars represent the mean ± SD of three independent experiments.

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