

Comparison of the promoter sequence of cryptdin4 from defensin14 knock out mice with wild type mice

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Introduction

Vertebrate animals' epithelial cells secret several kind of antimicrobial peptides, which are effective molecules against invading pathogens and this ability belongs to innate immune system. Among all the antimicrobial peptide, beta defensin family is most well known. It has antimicrobial ability against Gram-negative bacteria but not Gram positive ones.

Mouse beta defensin14 (mBD14) is an ortholog of human beta defensin3 because they share structural and functional similarities. Like defensin14, most of these antimicrobial molecules are similar in mice and human, including cryptdin family which resemble human alpha defensins. Cryptdin4 belongs to the family and is expressed by paneth cells of the small intestine. Cryptdin4 gene has two exons, transcriptional start site, intron, and 3' flanking sequences, which is very similar to other paneth cell alpha defensin genes and only has a repeated 130 bp unique region of itself.

Recent study by W.Muller's group (unpublished data) suggested that the expression of gene cryptidin4 has a tendency to increase in the beta defensin14 knock out mouse compared to wild type mice.

To identify any regulative relationship between the two genes, I compared of the cryptdin4 promoter sequence of defensin14 knock out mice with wild type mice and found the find possible mutation site in defensin14 knock out mice, which may occurred on transcriptional binding site on the promoter of cryptdin4. The mBD14 knock out mouse is generated from 129 background mouse and back crossed several generations with C57BL6 mouse, which is used as the wild type mouse in this study. Here I sequenced the promoter region of mice gene cryptdin4 of both wild type and defensin14 knock out mouse and compared them with published sequence data to examine the possible mutation.

Method

Mouse DNA extraction

DNA is extracted from mouse ear using Extract-N-Amp^{tm} Tissue PCR Kit.

Primer design

Two sets of primers(set one: forward primer 5'-GCAGCCTAGCATACGACTCA-3' and reverse primer 5'-GGCAGAGAGGAGGACAAGTG-3', set two: forward primer 5'- GGTCCACACTAGAGAAGGC-3' and reverse primer 5'- TGTGTGTGTGTGTGTGTAGT-3') for Cryptdin4 DNA amplification are designed using primer3 website (http://primer3.ut.ee/)

Cryptdin4 DNA amplification and selection

Using mouse ear punch DNA extraction and amplify cryptin4 gene region by PCR, select desired DNA by electrophoresis and extract from gel.

Ligation to the vector

Ligate cryptidin4 DNA product to PgemT-vector which including an A-tailing procedure.

Transformation

Introduce vector to alpha select chemically competent cells (bacteria), and identify the success of transformation by Blue-white selection using X-gal and IPTG.

Colony PCR

Pick the positive colony from blue-white selection using T7 (5'-TAATACGACTCACTATAGGG-3') and SP6 (5'-TAATTAGGTGACACTATAG-3') primers. Examine positive colonies by electrophoresis.

Mini prep and sequencing

Mini prep the positive colony using QIAprep Spin Miniprep Kit and send to sequencing.

Sequence analysis

Compare the obtained WT and defensin14 KO cryptdin4 sequence with database using blast website (http://blast.ncbi.nlm.nih.gov/Blast.cgi?CMD=Web&PAGE_TYPE=BlastHome)

Search transcriptional factor binding site inside the sequence that include possible mutation using website TFSEARCH(http://www.cbrc.jp/research/db/TFSEARCH.html).

Result and discussion

Through the comparison of defensin14 KO and WT DNA sequences, transcriptional factors which bind to transcriptional binding sites that contain possible mutations are CdxA, Skn-1, and HSF. Direct relationship between these factors and Cryptdin4 gene is still not clear and further discussions should be made.