연제 4

Construction and Expression of Tetracycline-inducible Vectors which contain Recombinant Chicken Interferon-gamma and Interleukin-15 gene

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Introduction

Tetracycline-regulated gene expression system has been successfully employed in mammalian cell cultures and transgenic mice. This inducible gene expression system has been developed using the regulatory elements of Tn10-encoded tetracycline resistance operon from *Escherichia coli*. This short regula tory sequence is bound tightly by the tetracycline repressor protein(TetR). TetR is converted into a transcriptional activator by fusion with VP16 trans-criptional activation domain. Subsequently, a reversed TetR-VP16 transactivator was developed which efficiently binds regulatory sequence only in the presence of tetracycline(1). In this study, we have evaluated the tetracycline-responsive regulatory system *in vitro*.

Materials and Methods

Tetracycline-inducible vector is derived from pcDNA3 (Invitrogen), which is replaced CMV promoter with the TRE-CMV promoter, reversed TetR-VP16 transactivator element and bovine growth hormone poly-A tail of plasmid pRetro-ON (Clontech co.). Chicken interferon-gamma or interleukin-15 gene was fused with this inducible vector. These vectors were transfected into chicken embryonic fibroblast (CEF), NIH3T3 and 293GP cell lines by liposome methods. After 48 hrs of transfection, transfected cells were screened by addition of 100 ng/㎖ or 200 ng/㎖ of anhydrotetracycline to the culture media. Total cellular RNAs were isolated by procedure of Chomczynski and the expression level of tetracycline-inducible vectors was analyzed by using RT-PCR.

Results and Discussion

Oligonucleotide primers covering open reading frame of recombinant chicken

INF-γ and IL-15 gene amplified a 517 bp or 446 bp full length DNA fragment from transfected and induced CEF, 293GP and NIH3T3 cell-line, respectively(2, 3). These results indicated that tetracycline-inducible vectors could be tightly regulated in vitro and manipulated for expression of foreign functional genes. Therefore, we established the inducible vector systems for expression of recombinant chicken INF-γ and IL-15 genes, while have a potential of anti-viral activity. Tetracycline-inducible vector system can not only be used to study gene function but may also be applied in the future to produce transgenic chickens.

(Key words; tetracycline, interferon-gamma, interleukin-15, RT-PCR)

References

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