장환형 (Large-circular) 안티센스를 이용한 난소암세포 성장 관련 유전자의 발굴

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Ovarian cancer related gene targeting with large circular antisense library

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Objective: The aim of this study is to find out the genes which are related to ovarian cancer cell growth using large circular antisense library.

Methods: Clones for antisense library were uni-directionally sub-cloned into pHS SK (-) vector. LC-antisense molecules were then purified from the culture supernatants of the bacterial competent cells superinfected with M13K07 helper bacteriophages. The LC-antisense library to 240 unigene clone was constructed and utilized in the identification of genes functionally involved in the growth of ovarian cancer cells.

Results: The 17 numbers out of the 240 numbers of the antisense library exerted a marked inhibitory effect on the growth of SK-OV 3.

Conclusion: The putative functional categorization of each gene was then conducted via public databases. These candidates may be used as target genes for drug development or adjuvant of conventional chemotherapeutic drugs.

Key Words: Ovarian neoplasms, Large circular antisense DNA, Gene therapy

Introduction

Ovarian cancer is the leading cause of death from gynecological cancer,1 and the second most common gynecological cancer in South Korea. Median overall survival ranges are 24~60 Month, and ~60% of patients die within 5 years of diagnosis. In spite of manipulation of existing antineoplastic drugs, there have been marginal gains. The most important factor of high mortality is the widespread metastasis at initial diagnosis.2 In addition, conventional antineoplastic drugs are typically non-selective cytotoxic molecules with significant undesirable systemic effects. Thus, in many cases effective therapy can not be offered to patients with ovarian cancer. Therefore, there are strong needs to research the mechanisms of carcinogenesis, invasion and metastasis of ovarian cancer for new therapeutic modality.

Various methods have been devised to study the ex-
pression of a large number of genes, generating vast amount of information. However, this information is limited to differential or sequential expression profiles of genes in different tissues or cells. Rapid accumulation of genomic sequence information and expression profiling has created a bottleneck in subsequent definitive gene functionalization and/or target validation. Most definitive functionalization of genes has been performed with various conventional gain-of-function or loss-of-function studies. Recently it is thought that cell-based array using transfection indeed paved the way for loss-of-function experiments. Effects of gene silencing in this platform can be monitored using downstream signaling events, apoptosis and other cellular processes. With the increase in genomic and proteomic databases, gene-silencing libraries may characterize genes involved in cancer development and progression. Loss-of-function has been performed either with gene knockdown using conventional antisense or its related technologies, or with gene knockout using homologous recombination. These approaches are limited in that they must be performed individually in a time consuming manner. Construction of an extensive antisense library may provide an answer to this information bottleneck for massive gene functionalization. Antisense (AS) – oligo libraries have been partially established and employed to obtain functional data of a large number of genes. Constructing such a library, however, can be costly and time consuming due to the process of target site search. Recently, high-throughput functional genomics using large circular (LC)–antisense molecules has been developed for the identification of genes associated with cancer cell growth. The LC–antisense DNA of recombinant bacteriophages may result in advantages for higher chances of binding to complementary target cDNA, owing both to its considerable length and high degree of sequence fidelity. LC–antisense DNA can also be easily generated in a high-throughput and large-scale mode in transformed E. coli cultures. In this study, we tried to find out genes which are related to ovarian cancer cell growth using large circular antisense library.

Materials and Methods

1. Cell culture RNA preparation

SK–OV 3, ovarian cancer cell line, was acquired from the Korean Cell Line Bank, and cultured in DMEM containing 10% fetal bovine serum (Welgene, Daegu, Korea). All cultures were maintained at 37\(^\circ\) in an atmosphere containing 5% CO\(_2\). Total RNA preparation was conducted with Welprep RNA isolation reagent (Welgene, Daegu, Korea), in accordance with the protocol recommended by the manufacturer. The quality of purified RNA was verified from the OD 260/280 ratios and via electrophoresis using agarose gels stained with ethidium bromide. RNA was used only in cases in which the OD 260/280 ratio was above 1.9.

2. Production of LC–antisense library

Either pSPORT1 or pT3T7–Pac phagemids, harboring human EST cDNA, were utilized in the production of single–stranded phage genomic DNA harboring the antisense cDNA sequences. The recombinant phagemids were transformed into competent E. coli cells, XL–1 Blue (Stratagene, La Jolla, CA, USA), which had been infected with the helper bacteriophage, M13K07 (New England Biolabs, Ipswich, MA, USA). The transformed cells were then plated on LB agar plates containing ampicillin (50 ug/mL) and kanamycin (70 ug/mL), and incubated overnight at 37\(^\circ\). A single colony was carefully isolated and seeded in 100 mL of LB media (bactotryptone 10 g, yeast extract 5 g, NaCl 10 g/1,000 mL, 50 ug/mL of ampicillin, and 70 ug/mL of