Practice of Gene Analysis in Human Cancer

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The continuity of life has been mediated by the transmission of genetic information from generation to generation in every organism. Since the identification of the structure of DNA by Watson and Crick in 1953, huge information about human genome was supplied by the scientists. After the completion of human genome sequencing by the human genome project, we learned that much more effort would be necessary to clarify the biological significance of any gene and their abnormalities linked with diseases.

Cancer is one of the diseases, which occurs with genetic abnormalities acquired by external and internal factors. Two main groups of genes namely, Oncogenes and Tumor Suppressor Genes, regulate cell growth and survival. While oncogenes force the cell to proliferate and growth, tumor suppressor genes balance the oncogenes and suppress the proliferation of the cell. Therefore any abnormalities occurring in one of these genes will make susceptible the cell to carcinogenic stimuli and finally lead to cancer development. Activation of oncogenes (gain of function) and inactivation of tumor suppressor genes (loss of function) will favor the cell in the trend of proliferation.

Recent developments in molecular biology and genetics provided us to analyse the roles of these genes in human cancer. These molecular methods such as polymerase chain reaction (PCR), loss of heterozygosity, comparative genomic hybridization, mutation analysis, single stranded conformational polymorphism, sequencing and cloning identified the function and abnormalities of many genes in various human diseases including cancer.

Since the public opening of human genome project in 2000, almost all regions of the human chromosomes were sequenced. This data showed a less number of genes about 25.000 as opposed to the previous assumptions up to the 100.000 genes. However, due to the frequent occurrence of splicing variants and complexity of post-translational modifications such as phosphorylation, acetylation, ubiquitination and sumolation, retrieval of the information from the human genome is just at the beginning step. Moreover, recent researches with the discoveries of siRNAs and microRNAs demonstrated that these splicing mechanism and RNA machinery are even more complex than expected. Furthermore, 5‘ and 3‘ untranslational regions of the genes and uncoding introns are getting more attentions with their newly identified roles in cellular processes and diseases.

For the long time, we have been dealt with the roles of tumor suppressor genes especially ING family genes in head and neck carcinomas. Two major groups of tumor-associated genes, oncogenes and tumor suppressor genes (TSGs), have been implicated in the carcinogenic process. Tumor suppressor genes are defined as genetic elements whose loss or mutational inactivation allows cell to display one or more phenotypes of neoplastic growth[1]. The five members of the ING family were recently identified and all contain a highly conserved plant homeodomain (PHD) finger motif in the carboxy (C)-terminal end of the proteins. We previously identified the tumor suppressive roles of ING1 and ING3, for the first time in a human cancer [2-3]. We recently showed the tumor suppressive role of a novel member of ING family, ING4 [4].

To identify the tumor suppressive function of a gene, the basic method is loss of heterozygosity (LOH) analysis of the targeted region, followed by mutation analysis of the candidate gene. These methods were based on the well-known Knudson‘s 2 hits hypothesis. According to this hypothesis, a tumor suppressor gene is inactivated through deletion of the one of its allele and the mutation of the rest allele. However, recent researches demonstrated that only a few percentages of TSGs are mutated, though LOH is frequently seen. This result led to a new conception, at which a class 2 TSG was defined. Class 2 TSG shows frequent deletion in one allele but no or few mutation in the rest allele, which resulted in the haploinsufficiency of the left allele. This haploinsufficient allele resulted in the decreased mRNA expression of a targeted gene and the patients were prone to cancer development when they were exposed to carcinogens such as smoking, UV, x-ray and others. The second method for detecting the function of a candidate TSG is clarification of its mutation status. This is done by several methods such as single stranded conformational polymorphism (SSCP) followed by direct sequencing or direct or cloned sequencing.

By using microsatellite analysis for LOH and SSCP and/or sequencing, we identified the frequent deletion of several chromosomal regions in human genome such as 13q34, 7q31, 12p12, 19p13 and found out some genes as a TSG such as ING1, ING3, ING4, BRG1 in head and neck cancer[2-5]. In this symposium, an outline of oncogenes and tumor suppressor genes in cancer and their identification methods exemplifying our researches and data will be given.

References

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