Research Center for Medical Sciences
Core Research Facilities for Basic Science
(Division of Molecular Genetics)

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General Summary

We can now analyze a person's whole genome. These technological developments have started a new era of medicine. The etiology and therapy of disease will be studied on the basis of genetics. As physicians of today, our research fields are the epigenetic control of cancers and neurodegenerative disorders. Gene therapy has become an attractive procedure to cure diseases. We contribute to gene therapy through the development of adenovirus vectors.
Our division plays a role in supporting various research studies. We served more than 8,000 sequence analysis. The management of the cell sorter and the next-generation sequencer were satisfactory.

Research Activities

Molecular pharmacology of anticancer agents
Ample evidences indicate that epigenetic dysfunctions play an important role in leukemogenesis. We previously demonstrated that bromodomain and extra terminal domain inhibitor 151 (I-BET151) is a promising agent for some myeloid leukemias. The acquisition of resistance to a drug by leukemic cells is a critical hurdle for achieving clinical success. We established I-BET151–resistant U937 cells (U937R) and compared the characterization of these cell lines. Gene expression profiles by means of microarray analysis disclosed no involvement of the ATP-binding cassette genes for the resistance. Because the expression of immune-system–related genes is modulated by I-BET151, we studied the effect of the nuclear factor kappa B pathway on the growth of parental and resistant cells. Both types of cell were treated with I kappa B kinase inhibitor VII (IKKi). The U937R cells treated with low concentrations of IKKi (1 to 4 μM) were more sensitive than parental cells. Interestingly, 1 μM of IKKi did not alter the expression of either *MYC* or *BCL2* in U937R cells. These results suggest that the survival of U937R cells is dependent on the nuclear factor kappa B pathway.

Cancer Molecular Biology
To reveal the appearance mechanism of a malignant tumor complicated by a congenital disorder, we used a next-generation sequence to analyze the exhaustive cancer-related gene expression. We reported on a 7-year-old girl with Maffucci syndrome in whom acute myeloid leukemia with a cup-like nuclear invagination had developed. The rate of malignant transformation in Maffucci syndrome is high; however, hematopoietic malig-

nancies rarely arise in this syndrome. In patients with Maffuccie syndrome, somatic mutations of *isocitrate dehydrogenase (IDH)* have been reported. In this patient we found the insertion frameshift at the nucleophosmin gene (*NPM1*) in leukemic cells and hemangioma. These results suggested that the multiple somatic mutations of the *IDH1* and *NPM1* gene in hemangioblasts were related to the development of cup-like acute myeloid leukemia associated with Maffucci syndrome. We also analyzed the medulloblastoma that was complicated with Gorlin syndrome and the Wilm's tumor that was complicated with phacomatosis pigmentokeratotica.

Development of the adenovirus vector systems

Because the adenovirus vector (AdV) is an attractive tool for gene expression and for the regulation of gene expression, it is applied to many areas of research. We have already established the cell-specific gene expression system using the combination of AdV and site-specific recombinase Cre or FLP. Because AdVs offer a transient expression system, they can also be applied to the differentiation instructions from stem cells to purpose cells. We constructed 16 AdVs for the induction of neuron cells from induced pluripotent stem cells. With this method, the efficacy of motor neuron induction was 7 times greater than with a standard method. Furthermore, because many immature cells remained when induction was being differentiated, we generated the AdVs carrying the drug-resistant gene driven by a neuron-specific promoter. These AdV systems may contribute to the analysis of the cause of the neurologic disease.

We also developed a protocol to cure hepatitis B virus (HBV) infection with an AdV. Current therapeutic treatments for HBV infection most often use nucleotide analogs, such as lamivudine and interferon α. However, these treatments cannot effectively eliminate the covalently closed circular DNA, which present in infected hepatocytes and continue to serve as the template for the pregenomic RNA. Consequently, complete clearance of HBV is difficult. We succeeded in constructing AdVs expressing 3 genomic RNAs targeting the HBV genome. The expressed genomic RNAs together with CRISPR-associated protein 9 using AdVs efficiently direct cleavage and cleavage-mediated mutation occurred in covalently closed circular DNA. These results suggest that an AdV expressing multiplex genomic RNA targeting the HBV genome might be used as an effective tool to treat chronic HBV infection.

Publications

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