Institute of DNA Medicine
Department of Gene Therapy

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Research Activities

Genetic disease

1. The main achievements of our laboratory this year are as follows.

1) Development of gene therapy for lysosomal storage disease: We generated recombinant lentivirus vectors expressing the enzymes missing in Pompe disease, Krabbe disease, and mucopolysaccharidosis type VII and administered the vectors to newborn model mice. In the case of Pompe disease, enzyme expression and a reduction in glycogen levels were observed for at least 4 months in the heart, which is main affected organ in this disease. Moreover, there was no immunological response against enzymes or viral vectors and no elevation of alanine aminotransferase.

2) Screening for Pompe disease among patients with muscular dystrophy: We screened for Pompe disease among patients in whom muscular dystrophy was diagnosed with dried blood spots. So far, Pompe disease has not been found in any of the 130 patients examined. We will continue this study in the future.

3) Establishment of induced pluripotent stem cells from patients with lysosomal storage disease: To understand the pathological mechanisms and the development of therapies for lysosomal storage diseases (LSDs), we successfully established various induced pluripotent stem (iPS) cells from a mouse model of an LSD.

4) Establishment of vascular endothelial cells from patients with Fabry disease: To understand the pathophysiological mechanism of Fabry disease, which affects the vascular system, we established vascular endothelial cells from patients with Fabry disease.

2. Diabetes mellitus: *In vivo* gene transfer of the cell cycle regulator gene in diabetic mice

Increased expression of $p16^{INK4a}$ is a major cause for diabetes-associated inactivation of the cyclin D/cyclin-dependent kinase (CDK) complex which eventually reduces the islet beta cell mass in diabetes. We aimed to reactivate CDK4 to recover beta cell mass and glucose tolerance by transferring the $CDK4^{R24C}$ gene, a variant that promotes the G1/S transition, to adult mice with diabetes. In R24C-treated mice the number of terminally differentiated beta cells was 250% greater than in sham-treated mice, and blood glucose levels were significantly lower. Restoration of aberrant islet structure by treatment with cell-cycle regulator genes is useful for regenerative medicine in diabetes.

3. Hepatocellular carcinoma

In our previous studies, we developed methods of gene therapy for hepatocellular carcinoma (HCC) and metastasis to the liver, which is an important prognostic factor for gastrointestinal cancers. In this study, we have explored a gene-therapeutic approach to stimulate antitumor immunity by adenovirus-mediated transfer of CD40

ligand to treat HCC and cancers metastatic to the liver. We demonstrated antitumor effects in both preventative and therapeutic models of HCC and liver metastasis. These studies suggest that cellular and humoral immunity contribute to this antitumor effect. Next, we will study orthotropic models of HCC and liver metastasis and then study the available clinical approaches via the hepatic artery.

Gynecologic oncology

1. Genomic identification of significant targets in cancer analysis of ovarian carcinomas

We analyzed somatic DNA copy number variation in 78 specimens of various histological types of ovarian carcinomas using genomic identification of significant targets in cancer analysis. Regions with a p-value <0.01 after corrections for multiple hypothesis testing (indicating nonrandom distribution across subtypes) in order of significance included 8p23.3, 6q24.3, 11p15.5, 8p21.2, 16q22.1, 22q13.31, 4q22.1, 5q22.2, 7p22.3, and 14q24.2; these regions were more common in serous tumors. Although no regions of copy number variation were significantly associated with any histological subtype other than serous tumors, an overrepresentation of amplification of 20q13.2 (ZNF217), 17q12 (CCL4), and 8q13.2-q21.11 (NCOA2) was observed in clear-cell tumors, and amplification of 1q21.3 (CTMP) and 1q42.13 (RAB4A) was observed in endometrioid tumors.

2. Serum expression of indoleamine 2,3-dioxygenase was positively associated with impaired survival in patients with serous type ovarian cancer.

We have previously reported that the indoleamine 2,3-dioxygenase (IDO) screened with the GeneChip is positively associated with paclitaxel resistance and with impaired survival in patients with serous-type ovarian cancer. We established an enzyme-linked immunosorbent assay with an anti-IDO antibody for serum and measured serum IDO titers in 26 specimens of ovarian cancer. We compared the expression pattern in surgical specimens and the corresponding serum IDO titer and found an association in the serous type. These results suggest that the serum IDO titer may be a biomarker for the serous type of ovarian cancer.

Fetomaternal medicine

1. Establishment of an immortalized human extravillous trophoblast cell line by retroviral infection with E6/E7/human telomerase reverse transcriptase

Investigation into the function of human trophoblasts has been limited by a lack of suitable cell models. We aimed to obtain human normal trophoblast cell lines with a long lifespan and consequently establish an ideal *in vitro* cell model. Primary human trophoblast cells were derived from a placenta obtained at elective abortion in the 7th week of gestation. The cells were immortalized by infection with retroviral expression vectors containing type 16 human papillomavirus E6 and E7 in combination with human telomerase reverse transcriptase. The cell line was characterized. Immunocytochemical studies revealed an extravillous trophoblastic phenotype with positive staining for human chorionic gonadotrophin chain β, cytokeratin 7, HLA-G, and CD9. Transwell insert invasion assay showed the invasiveness of this cell line, and gelatin zymography detected the secretion of matrix metalloproteinases 2 and 9. Karyotype analysis showed an almost normal chromosomal number with small deviations ranging

from 46 to 48, and nude mouse assay showed no tumorigenecity. This newly immortalized cell line, HChEpC1b, is a useful model for the study of extravillous trophoblast function.

Publications

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