Institute of DNA Medicine
Department of Gene Therapy

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General Summary

Our purpose is to develop treatments for intractable diseases, including genetic diseases, cancer, and diabetes. We performed various studies and investigations this year. Below, we describe the progress in each of our projects.

Research Activities

Genetics Disease

1. Development of gene therapy for lysosomal storage diseases

We generated recombinant lentiviral vectors expressing missing enzyme of Krabbe disease and mucopolysaccharidosis (MPS) VII and administered these vectors to newborn model mice. For Krabbe disease, we detected increased body weight and delayed onset of clinical symptoms but no effect on severe progressive symptoms. For MPS VII, effects on body weight and life span were observed, and copies of lentiviral vector DNA were detected in several organs, including the brain, of the treated neonatal model mice, a finding that indicates efficient long-term expression. We have started to make a recombinant lentivirus for a new gene therapy for MPS II.

2. Pathophysiological analysis of Pompe disease

We analyzed the signaling pathway of endoplasmic reticulum stress-induced autophagy in fibroblasts from patients with Pompe disease. We found that treatment with SB203580, an inhibitor of p38 mitogen-activated protein kinase, significantly inhibits induction of autophagy, whereas no effect is observed following treatment with other mitogen-activated protein kinase inhibitors. Furthermore, we demonstrated phosphorylation of p38 by endoplasmic reticulum stress in patient fibroblasts.

3. Immune tolerance induction for enzyme replacement therapy

This year we worked to develop immune tolerance induction for enzyme replacement therapy for Pompe disease. Parenteral administration of an anti-CD3 antibody induced immune tolerance against infused enzyme in various mouse strains, including Pompe disease model mice. In addition, the anti-CD3 antibody prevented a lethal hypersensitivity reaction to the enzyme infusion. The effect described above persisted for up to 20 weeks, even if the enzyme was repeatedly infused. The anti-CD3 antibody prevented increases of enzyme-specific immunoglobulin (Ig) E antibodies and IgG antibodies. The anti-CD3 antibody is also effective for preexisting antibodies against the enzyme. The $CD4^+$ and $CD8^+$ effector cells and $CD4^+$ $CD25^+$ regulatory T cells decreased with the anti-CD3 antibody. The immune-tolerant effects of the anti-CD3 antibody were blocked if $CD4^+$ $CD25^+$ regulatory cells were depleted by an anti-CD25 antibody. This observation indicates that $CD4^+$ $CD25^+$ cells play a key role in the immunosuppressive effects of the

anti-CD3 antibody.

4. Antitumor effect and application to gene therapy of nafamostat mesilate for fatal gastrointestinal cancers

Recent studies have demonstrated that nuclear factor (NF)-κB plays important roles in the regulation of cell apoptosis, inflammation, and oncogenesis. Inhibition of NF-κB is a potential new strategy for the treatment of cancers. We have previously reported that nafamostat mesilate, a serine-protease inhibitor that is widely used to treat pancreatitis and disseminated intravascular coagulation and is used as an anticoagulant for hemodialysis in Japan, inhibits NF-κB activation and induces apoptosis of pancreatic cancer. Moreover, we showed that addition of nafamostat mesilate promotes gemcitabine- or paclitaxel-induced apoptosis through the inhibition of NF-κB activation of pancreatic cancer. The clinical usefulness of gemcitabine combined with nafamostat mesilate for patients with unresectable pancreatic cancer has been examined in a phase II study. Recently, we evaluated the antitumor effects of nafamostat mesilate on other gastrointestinal cancers.

Gene therapy combining gemcitabine with an adenoviral vector expressing tumor necrosis factor-alpha (TNF-α) is a new therapeutic approach for pancreatic cancer. However, such a combination therapy is limited owing to activation of NF-κB by TNF-α and gemcitabine. We hypothesize that the addition of nafamostat mesilate will enhance the antitumor effect of combination therapy with TNF-α gene delivery and gemcitabine for pancreatic cancer.

5. Gynecologic Oncology

1) Cyclin D1 is a prognostic indicator in advanced serous ovarian cancer

We have previously reported that high-resolution oligonucleotide copy number analysis shows that cyclin E1 gene (*CCNE1*) amplification is strongly associated with treatment resistance in serous epithelial ovarian cancer (EOC). We focused on 66 advanced cases of serous EOC and investigated the associations between the expression of G1-S-phase regulatory proteins and clinicopathological variables. Immunohistochemical analysis for cyclin D1, pRb, p16,p53, $p27^{Kip1}$, $p21^{Waf1/Cip1}$, and cyclin E was performed with formalin-fixed tissue sections of primary surgical specimens. Univariate analysis showed that overexpression of cyclin D1 was correlated with reduced progression-free survival ($p=0.00062$) and overall survival ($p=0.00037$). Multivariate analysis identified overexpression of cyclin D1 ($p=0.0019$), reduced expression of $p27^{Kip1}$ ($p=0.042$), and residual tumor volume ($p=0.0092$) as independent predictors of overall survival. Overexpression of cyclin D1 ($p=0.011$) and residual tumor volume ($p=0.006$) were significantly associated with first-line chemosensitivity. In advanced serous EOC, overexpression of cyclin D1 contributed largely to a poor prognosis due, perhaps in part, to chemoresistance. Cyclin D1 is a possible target for overcoming the refractory nature of advanced serous EOC.

2) Copy number analysis identifies novel interactions between genomic loci in ovarian cancer

We obtained genome-wide copy number alteration data from 4 different single nucleotide polymorphism array platforms, with a final data set of 398 ovarian tumors, mostly of the serous histological subtype. The large data set enabled refinement of minimal regions

and identification of rare amplicons, such as those at 1p34 and 20q11. We performed a novel co-occurrence analysis to assess cooperation and exclusivity of copy number alterations and analyzed their relationship to patient outcome.
Positive associations were identified between gains on 19q and 20q, gain of 20q and loss of X, and between several regions of loss, particularly 17q. We found weak correlations of genomic loci, such as 19q12, with clinical outcome.
3) IL- 6-STAT3-HIF signaling and therapeutic response to the angiogenesis inhibitor sunitinib in ovarian clear cell cancer
We analyzed the most comprehensive gene expression and copy number data sets to date to identify potential therapeutic targets of ovarian clear cell adenocarcinoma (OCCA). Analyses of gene expression and DNA copy number were performed with primary human OCCA tumor specimens, and the findings were confirmed with immunohistochemical studies of tissue microarrays. We found specific overexpression of the IL-6-STAT3-HIF (interleukin 6 signal transducer and activator of transcription 3 hypoxia-induced factor) pathway in OCCA tumors compared with that in high-grade serous cancers. Expression of parathyroid hormone-like hormone and high levels of circulating interleukin 6 in patients with OCCA may explain the frequent occurrence of hypercalcemia of malignancy and thromboembolic events in OCCA. We described amplification of several receptor tyrosine kinases, most notably MET, which are potential therapeutic targets. We reported sustained clinical and functional imaging responses in 2 patients with chemotherapy-resistant OCCA who were treated with sunitinib and found significant parallels with renal clear cell cancer. Our findings highlight important therapeutic targets in OCCA, suggest that more extensive clinical trials with sunitinib in OCCA are warranted, and provide significant impetus to the growing realization that OCCA is molecularly and clinically distinct from other forms of ovarian cancer.
6. Islet biology and molecular medicine in diabetes mellitus
Disassembly and degradation of islet cells are major obstacles in the analysis of isolated islets *in vitro*, possibly due to ablation of the innervating vessels and nerves. To address this problem, we have developed a novel method for long-term maintenance of basic structures and functional analysis of islets of Langerhans using Matrigel basement membrane matrix (BD Biosciences, San Jose, CA, USA) and subcutaneous transplantation. Islets isolated from mice were mixed with Matrigel and was subjected to subcutaneous allograft. Grafts excised 24 hours after transplantation showed almost normal morphology as islets. After 10 days, the transplanted islets showed lobulated structures, but neovessels were observed within the islets. These results suggest that the subcutaneous transplantation method with Matrigel to maintain isolated islets could lead to highly functional analysis of isolated islets.

Publications

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