INVITED COMMENTARY

Progress in Gene Therapy: Seeing Is Believing

The past few decades have seen enormous progress in molecular genetics. Techniques such as Southern blotting, Northern blotting, polymerase chain reaction, nucleotide sequencing, chromosome walking, and genetic transfer allow the specific isolation, characterization, and modification of genetic information. Optimists have characterized these developments as “mankind about to determine its destiny,” whereas others are afraid that scientists are about to “start playing God.”

Developments in molecular genetics have given us insights, at the molecular level, into vital processes in living organisms, such as embryonic development, growth regulation, differentiation, pathogenesis, and carcinogenesis. Insights into the mechanism of pathologic processes, such as developmental disorders and carcinogenesis, have stimulated efforts to develop therapeutic approaches to prevent or correct these processes. Techniques to directly change the genetic information of a cell have raised high expectations of the therapeutic potential of genetic manipulation. In vitro, genes have been introduced successfully into cells, thus changing the genotype and the phenotype of cells. These developments have raised hopes that diseases appearing to be incurable can soon be cured.

Although the basic concept of gene therapy is simple and straightforward, it turned out to be much more complicated in actual practice. In their efforts to transfer genes into animals, investigators quickly realized that numerous technologic problems had to be overcome before gene therapy could be added to the armamentarium of therapeutic approaches of modern medicine. The main obstacles of effective gene therapy are the limited efficiency of the transfer, the limited specificity of the transfer, and the limited duration of the expression of the newly introduced gene.

The design of an effective vehicle to transport the gene into the target cell, the so-called vector, appeared to be of crucial importance. For gene transfer, viral vectors are used. However, most viruses do not allow the incorporation of large genes into their genome. Retroviruses seemed to be attractive candidates because of their ability to stably incorporate their genetic information in the genome of the target cell. Unfortunately, the efficiency of their transfer was low and restricted to dividing cells. Adenoviruses, although efficient vehicles for gene transfer, are easily recognized and neutralized by the immune system. Systematic research during the past decade has revealed new viral constructs for in vivo gene transfer based on lentiviruses, adenovirus-associated viruses, and replication-selective lytic viruses (1).

As indicated, the concept of gene therapy has raised high expectations in the medical community and in the general public. However, progress is slower than expected, and in the late 1990s, hope turned into skepticism. The death of 18-yr-old Jesse Gelsinger, caused by an unexpectedly vigorous immune response toward an injected adenoviral vector in a gene therapy trial, marked the first crisis in the field (2). However, since this sad incident, a few encouraging successes have been reported. A group at the Pasteur Institute in Paris published the first unequivocal results showing that gene therapy can treat patients with the rare immune disease severe combined immunodeficiency-X1 (3). Investigators at the Children’s Hospital of Philadelphia and at Stanford University proved that hemophilia in patients could be alleviated by intramuscular injection of an adenovirus-associated viral vector containing the factor VIII gene (4). A group at the University of Pittsburgh used gene therapy to repair a defect in mice with a type of muscular dystrophy mimicking Duchenne’s syndrome (5). With a replication-selective lytic viral construct, termed ONYX-015, tumor-selective tissue destruction has been documented in patients with recurrent refractory squamous cell carcinoma of the head and neck (6,7).

For further development of gene therapy, it is of the utmost importance that investigators have tools to determine the success of the site-directed gene transfer. In virtually all gene therapy studies, determining whether the transferred gene is expressed in the target cell is important. In that context, investigators need to determine the efficiency, the specificity, and the durability of expression of the therapeutic gene. Reporter gene imaging is a technique that can potentially measure gene expression noninvasively. Imaging reporter gene expression can be a valuable tool to optimize in vivo gene transfer. The study that Yahoubi et al. (8) describe in this issue of The Journal of Nuclear Medicine marks another step in the development of gene therapy. The radiopharmaceutical in reporter gene imaging is a substrate of the transferred gene, the marker gene. When the marker gene is expressed in the target cell, the gene product (an enzyme) converts the radiopharmaceutical into a metabolite that is selectively trapped within the transfected cell. Alternatively, the transferred gene can induce the expression of a receptor on the cell membrane (i.e., the NaI symporter gene or the dopamine recep-
tor gene). In the latter form of reporter gene imaging, the radiopharmaceutical is a radiolabeled ligand that specifically binds this receptor (i.e., radioiodide or radiolabeled dopamine, respectively).

At the Memorial Sloan-Kettering Cancer Center, Tjuvajev et al. (9) were the first to develop an elegant approach to visualize the expression of the viral enzyme, human simple virus type-1 thymidine kinase (HSV1-tk). They synthesized radiolabeled analogs of penciclovir, 9-[4-(18F)-fluoro-3-(hydroxymethyl)butyl]guanine (18F)FHBG—originally developed by Alauddin et al. (15)—was selected for further development (14). Yaghoubi et al. (8) report on the first use of 18F-labeled analog in 10 healthy volunteers. This 18F-labeled substrate of the HSV1-tk gene product can be synthesized within a reasonable time (95–100 min), at a high specific activity (>37,000 GBq/mmol), and with good radiochemical purity (99%).

The data reported by Yaghoubi et al. (8) show that [18F]FHBG was excreted mainly through the kidneys but that a considerable portion was cleared through the hepatobiliary route. Dosemetric analysis of the images indicated that the highest radiation dose was absorbed by the bladder wall (0.1 mGy/MBq) and that the effective dose was acceptable (<0.016 mGy/MBq).

The sensitivity of [18F]FHBG in visualizing cells expressing the HSV1-tk gene has not yet been determined in patients. In mice, cells transfected with the HSV1-tk gene (by intratumoral injection with various viral vectors) could be visualized even in the liver (16). Yaghoubi et al. (8) indicate that the compound rapidly cleared from virtually all HSV1-tk–negative tissues in the human volunteers. However, whether [18F]FHBG will show HSV1-tk–transfected tissues largely depends on the extent to which [18F]FHBG has accumulated in these tissues. The hepatobiliary excretion of the compound and its excretion in the gut will hamper visualization of HSV1-tk expression in the gut region. The hepatobiliary clearance of a portion of the [18F]FHBG results from the lipophilic character of the compound. Because of its lipophilicity, the compound is able to cross the membrane of the target cell and interact with the HSV1-tk. A marker gene that is expressed on the cell surface, preferably encoding a receptor, will allow the use of a more hydrophilic radiopharmaceutical that is excreted exclusively through the kidneys.

In summary, sensitive imaging of reporter gene expression can provide a valuable method to optimize gene therapy. By developing such a technique, nuclear medicine can contribute to the progress in this exciting field. Therefore, we look forward to the first reports on the use of [18F]FHBG in patients whose tumors were transfected with an HSV1-tk–containing vector.

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