demonstration that $Cl-/HCO_3-$ exchange activity is present in intact hepatocytes and may be the principal mechanism responsible for recovery from intracellular alkaline challenge. These studies also provide a strong foundation for future investigation of related questions regarding the overall contribution of $Cl-/HCO_3-$ exchange to other cellular functions including biliary HCO_3- secretion and bile formation. Unlike $Na+/H+$ exchange and $Na+/HCO_3-$ cotransport, $Cl-/HCO_3-$ exchange is located in the canalicular membrane. These studies predict that intracellular alkalosis would be followed by rapid alkalinization of canalicular bile and that transport activity would be sensitive to $Cl-$ in the canalicular rather than the extracellular space. Regulation by factors other than $Cl-$ and HCO_3- concentration that alter the "set point" or rate of transport including $H+$ ions and Ca^{2+}- or cyclic AMP–dependent signaling will also be of great interest. Finally, it will be important to determine how regulation of $Cl-/HCO_3-$ exchange is integrated with other mechanisms of $H+$ and HCO_3- transport. It is attractive to speculate that a balance exists between HCO_3- influx through $Na+/HCO_3-$ cotransport and efflux through $Cl-/HCO_3-$ exchange. Because these transporters are located in separate membrane domains, coregulation might contribute to transcellular movement of HCO_3- from the sinusoidal to the canalicular space and the regulation of pHi.

GREG FITZ, M.D.
Department of Medicine
Duke University Medical Center
Durham, North Carolina 27710

REFERENCES

1. Kashiwagura T, Deutsch CJ, Taylor J, Erecinsha M, Wilson DF. Dependence of gluconeogenesis, urea synthesis, and energy metabolism of hepatocytes on intracellular pH. J Biol Chem 1984; 259:237-243.
2. Henderson RM, Graf J, Boyer JL. Na^+-H^+ regulated intracellular pH in isolated rat hepatocyte couplets. Am J Physiol 1987;252: G109-G113.
3. Renner EL, Lake JR, Scharschmidt BF. Na^+-H^+ exchange activity in hepatocytes: its role in regulation of intracellular pH. Am J Physiol 1988;256:G44-G52.
4. Gleeson O, Smith ND, Boyer JL. Bicarbonate dependent and independent pH regulatory mechanisms in rat hepatocytes. J Clin Invest 1989;84:312-321.
5. Fitz JG, Persico M, Scharschmidt BF. Electrophysiologic evidence for $Na+$-coupled HCO_3- transport in cultured rat hepatocytes. Am J Physiol 1989;256:G491-G500.
6. Fitz JG, Lidofsky SD, Weisiger RA, Xie M-H, Cochran M, Grotmol T, Scharschmidt BF. HCO_3--coupled $Na+$ influx is a major determinant of $Na+$ turnover and $Na+/K+$ pump activity in rat hepatocytes. J Membr Biol 1991;122:1-10.
7. Fitz JG, Lidofsky SD, Xie M-H, Cochran M, Scharschmidt BF. Plasma membrane $H+/HCO_3-$ transport in rat hepatocytes: a principal role for $Na+$-coupled HCO_3- transport [in press]. Am J Physiol 1991;24.
8. Benedetti A, Strazzabosco M, Corasanti J, Haddad P, Graf J, Boyer JL. Cl^--HCO^- exchanger in isolated rat hepatocytes: role in regulation of intracellular pH. Am J Physiol 1991;261:G512-G522.
9. Meier PJ, Knickelbein R, Moseley RH, Dobbins JW, Boyer JL. Evidence for a carrier-mediated chloride-bicarbonate exchange in canalicular rat liver plasma membrane vesicles. J Clin Invest 1985;83:1225-1235.
10. Fitz JG, Scharschmidt BF. Intracellular $Cl-$ activity in intact rat liver: relationship to membrane potential and bile flow. Am J Physiol 1987;252:G699-G706.

PROVOCATIVE GENE THERAPY STRATEGY FOR THE TREATMENT OF HEPATOCELLULAR CARCINOMA

Huber BE, Richards CA, Krenitsky TA. Retroviral-mediated gene therapy for the treatment of hepatocellular carcinoma: an innovative approach for cancer therapy. Proc Natl Acad Sci USA 1991;88:8039-8043.

ABSTRACT

An approach involving retroviral-mediated gene therapy for the treatment of neoplastic disease is described. This therapeutic approach is called "virus-directed enzyme/prodrug therapy" (VDEPT). The VDEPT approach exploits the transcriptional differences between normal and neoplastic cells to achieve selective killing of neoplastic cells. We now describe development of the VDEPT approach for the treatment of hepatocellular carcinoma. Replication-defective, amphotrophic retroviruses were constructed containing a chimeric varicella-zoster virus thymidine kinase (VZV TK) gene that is transcriptionally regulated by either the hepatoma-associated α-fetoprotein or liver-associated albumin transcriptional regulatory sequences. Subsequent to retroviral infection, expression of VZV TK was limited to either α-fetoprotein- or albumin-positive cells, respectively. VZV TK metabolically activated the nontoxic prodrug 6-methoxypurine arabinonucleoside (araM), ultimately leading to the formation of the cytotoxic anabolite adenine arabinonucleoside triphosphate (araATP). Cells that selectively expressed VZV TK became selectively sensitive to araM due to the VZV TK-dependent anabolism of araM to araATP. Hence, these retroviral-delivered chimeric genes generated tissue-specific expression of VZV TK, tissue-specific anabolism of araM to araATP, and tissue-specific cytotoxicity due to araM exposure. By utilizing such retroviral vectors, araM was anabolized to araATP in hepatoma cells, producing a selective cytotoxic effect.

COMMENTS

The rapid advance in gene transfer technology has fueled speculation with regard to future human applications. This article defines a gene therapy strategy for the treatment of HCC in patients and then thoroughly explores its feasibility *in vitro*. The strategy exploits two well-described phenomena, which are as follows: (a) the ability of tissue-specific regulatory elements to selectively drive gene expression in different types of cells and (b) the ability of the varicella-zoster virus thymidine kinase (TK) enzyme to metabolically activate the prodrug 6 methoxypurine arabinonucleoside (araM) to adenine arabinonucleoside triphosphate (araATP). The prodrug, araM, is not toxic, but araATP is toxic to the

cells in which it is synthesized. The TK gene was transferred into cultured hepatoma cells using a retroviral expression vector, and the gene was expressed when it was driven by the AFP promoter. When exposed to araM, the transfected cells evidently produced araATP and died.

This gene therapy strategy sets out to kill cancerous cells while allowing noncancerous cells to live. First, cancerous and noncancerous cells would be transduced with the TK gene driven by the α-fetoprotein (AFP) promoter. Next, the gene is ideally only turned on in the cancer cells. Tissue-specific promoters are used to selectively drive transgene expression in cancer cells as opposed to healthy ones. The strategy depends on transduced carcinomatous cells actively transcribing the TK gene when it is driven by the AFP regulatory unit, whereas nonmalignant hepatocytes would not turn on this gene. Finally, cells which turn on the TK gene activate the prodrug araM to araATP and die, whereas cells that do not express the TK enzyme should not activate the prodrug and therefore should survive.

For this gene therapy strategy to cure cancer *in vivo,* most or all tumor cells would be transduced with the TK retroviral expression vector driven by the AFP promoter. Tumor cells would activate transcription driven by the AFP regulatory unit, whereas healthy cells would not activate transcription. This assumes that all tumor cells possess the tissue specificity to activate transcription driven by the AFP regulatory unit. If a tumor contained cells with heterogeneous transcriptional promiscuity, clones of malignant cells that did not turn on genes driven by the AFP regulatory unit would be selected. Carcinomatous cells that did not activate transcription of the AFP promoter would not be ablated using this strategy.

Given the current efficiency of retroviral expression vectors, this technique is not presently a feasible approach to cure cancer *in vivo.* The investigators used retroviral expression vectors to transfer the TK gene into eukaryotic cells, a technique that is presently optimally effective at transfering genes into approximately 20% of hepatocytes in primary culture (1). The increased propensity of retroviruses to infect dividing cells (2) might favor the *in vivo* transduction of tumor cells over quiescent hepatocytes. *In vivo* gene transduction efficiency would probably still be low. Even under conditions of intense hepatocellular division after partial hepatectomy, 5% of hepatocytes were infected *in vivo* (3). Naturally, a miraculous propensity of retroviral vectors to transduce *in vivo* hepatocellular tumor cells cannot be formally excluded without attempting their infection.

The initially most promising liver-directed gene therapy strategies address diseases in which genetic supplementation of a small percent of cells could lead to a therapeutic effect. If only a small fraction of hepatocytes in a patient with HCC were transduced using this strategy, it would be unlikely to achieve a clinical effect. Initial gene therapy protocols addressing the treatment of malignancies have attempted to target tumors with immune modifiers so that transduction of a small percentage of the tumor cells could potentially have a clinical impact (4).

In summary, a thorough and provocative series of *in vitro* experiments were presented in this article. It is anticipated that more than a small fraction of tumor cells would have to be targeted with the gene therapy vector to ablate HCC *in vivo* using this strategy. Substantive advances in *in vivo* liver-directed gene transfer efficiency will be necessary for such an approach to cure HCC. Given the clinical course of unresectable HCC, it is compelling to develop treatments that could prolong life expectancy and improve the quality of life.

FREDERICK ASKARI, M.D., PH.D.
JAMES WILSON, M.D., PH.D.
Division of Molecular Medicine and Genetics
Department of Internal Medicine
University of Michigan Medical Center
Ann Arbor, Michigan 48105

REFERENCES

1. Chowdhury JR, Grossman M, Gupta S, Chowdhury NR, Baker JR Jr, Wilson JM. Long-term improvement of hypercholesterolemia after *ex vivo* gene therapy in LDLR-deficient rabbits. Science 1991;254:1802-1805.
2. Miller DG, Adam MA, Miller AD. Gene transfer by retrovirus vectors occurs only in cells that are actively replicating at the time of infection. Mol Cell Biol 1990;8:4239-4242.
3. Ferry N, Duplessis O, Houssin D, Danos O, Heard JM. Retroviral-mediated gene transfer into hepatocytes *in vivo.* PNAS USA 1991;88:8377-8381.
4. Rosenberg SA, Aebersold P, Cornetta K, Kasid A, Morgan RA, Moen R, Karson EM, et al. Gene transfer into humans: immunotherapy of patients with advanced melanoma, using tumor-infiltrating lymphocytes modified by retroviral gene transduction. N Engl J Med 1990;323:570-578.