

Editorial

Refined tools for the treatment of hepatocellular carcinoma

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See Articles, pages 728–735 and 736–743

With about one million new cases diagnosed annually world-wide and a three-fold increase in its incidence in the USA over the past decade, hepatocellular carcinoma (HCC) should be viewed as a major health problem for which there is a compelling need for better treatments [1].

Less than 1 in five patients with HCC have the fortune to be diagnosed at an early stage thus permitting a curative approach to be started. And for the remaining, doctors are not able to deliver much more than transient relief and hope. A significant proportion of patients is not offered any other therapy than the best supportive care. Moreover many others are given treatments that cannot be considered more than suboptimal since they barely improve survival [2]. On the other hand, we need effective therapies that could be provided in those areas of the world that bear the worst of the burden, especially largely underdeveloped countries in Africa and Southeast Asia. This means simple and, if at all possible, affordable therapies for patients with invasive or metastatic tumors.

In this issue of the Journal, two different approaches to the problem are presented involving new formulations of old drugs and technologically advanced procedures, respectively. But both approaches point towards the same goal, i.e. to get tumor cells exposed to high doses of antitumor agents, purportedly those that can be effective, while preserving patients from increased toxicity. Drug resistance via a number of mechanisms is a major challenge in the treatment of cancer. They include overexpression of drug export pumps such as multidrug resistant (MDR) protein but also decreased drug uptake, increased drug inactivation, overexpression of target enzymes, increased DNA repair ability and resistance to apoptosis [3]. Also, chemoresistance is fostered by impaired drug delivery to tumor cells due to physical barriers including an abundant

extracellular matrix and increased interstitial pressure within the tumor. But the actual contribution of these mechanisms to chemoresistance in individual cancer patients is poorly understood.

In the first approach, Barraud et al., make use of nanoparticles in an attempt to overcome the detrimental action of membrane transporters, essentially MDR, on the sensitivity of HCC cells to doxorubicin [4]. When compared to the free agent, doxorubicin-loaded acrylate nanoparticles had a higher activity against HCC cell lines and showed increased pro-apoptotic activity in a murine model that overexpresses MDR. In vitro, it was shown that resistance is overcome as a result of both the adsorption of nanoparticles to the cell surface and increased doxorubicin diffusion into the cell. So far, several attempts have been made to specifically target doxorubicin to tumor cells by drug encapsulation. Liposomal carriers tend to slowly accumulate preferentially in tumors as a result of enhanced permeability and retention and may increase drug concentration inside the tumor by more than 100-fold, thus overcoming the activity of multidrug transporter systems. Commercially available liposomal anthracyclines have shown a modest clinical value in the treatment of a number of malignancies but unfortunately not HCC, stressing the multifactorial nature of chemoresistance in humans. In two different phase II clinical trials, intravenous pegylated liposomal doxorubicin, although well tolerated, showed no ability to induce objective remissions [5,6]. On the other hand, these doxorubicin-loaded nanoparticles now tested by Barraud et al. have shown an acceptable toxic profile in an earlier phase I clinical trial [7] and a forthcoming trial to investigate its efficacy among HCC patients can be envisioned. Yet, a number of important side effects including myelosuppression, fatigue, vomiting, cardiomyopathy and hair loss render doxorubicin a rather unappealing agent. Historical reasons have made doxorubicin the gold standard for comparing systemic therapies

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against HCC although its activity is by no means better than that of a variety of other agents including many with a much better toxic profile. Consequently, nanoparticles or other liposomal carriers loading agents such as oxaliplatin, fluorouracil or gemcitabine deserve further attention.

The second approach is more sophisticated. Gene therapy is a novel platform, still as immature as promising, that attempts to treat human diseases by the transfer of genetic material with the aim of restoring a cell function that was lost or defective, introducing a new function or even interfering with an existing albeit deleterious function. In their paper, Stefani et al. [8] use gene transfer with two complementary goals. On one hand, to perform a sort of selective chemotherapy by producing high doses of a cytotoxic compound solely inside the tumor. On the other, to stimulate the immune response against the tumor by the also local production of cytokines in an environment that is rich in antigens arising from tumor cell debris. These two targets are aimed at simultaneously by using a retroviral vector that transfers the genes of interleukin 2 and of viral thymidine kinase. The latter is an enzyme that transforms the antiviral ganciclovir into a cytotoxic agent. This genetically mediated prodrug activation has proved effective in a wide variety of animal models of cancer but its clinical development has been limited by very mild activity [9]. The synergy with immune stimulation by intratumoral production of cytokines had also been established in animal models of HCC [10] and is appealing since genetic immunotherapy itself has shown a promising though weak antitumor activity against HCC [11].

The main hurdles that complicate clinical success concern the tools rather than the rationale for gene therapy, and delivery is probably the most prominent barrier. In vivo, available vectors have the ability to transduce only a small minority of cells within a tumor, and none is currently effective in targeting tumor cells following systemic administration. Although these limitations could be partially overcome by agents that induce strong bystander effects, including genetic prodrug activation therapy or genetic immunotherapy, the use of inefficient vectors may curtail the clinical development of such strategy. A viral vector is a defective virus able to penetrate the target cell and carry the transgene into the nucleus, but unable to replicate and produce disease in the treated patient. Retroviruses enter efficiently into dividing cells and integrate the genetic material into the host genome so that the progeny of the transduced cells also carry the transgene and accordingly they are most commonly used for permanent correction of genetic diseases using ex vivo gene transfer. The major drawback of retroviral vectors for cancer gene therapy, besides their potential for insertional mutagenesis, is that they infect and integrate only dividing cells. And dividing cells are only a small fraction of most solid tumors.

The growth fraction of a human tumor can be estimated from the proportion of tumor cells that are stained with a

monoclonal antibody such as Ki67 that reacts with a nuclear protein expressed in the G1, G2, S, and M phases of the cell cycle. Among patients with HCC, it has been shown that slow- and intermediate-growing tumors (doubling time > 100 and 50 days, respectively) only have 5–20% of Ki67-positive cells [12]. Consequently, only a minority of tumor cells would express the transgene in the most optimistic scenario. As a matter of fact, the results observed by Stefani et al. distinctly illustrate how tough this work of in vivo gene transfer can be. When gene transfer to tumor cells was carried out ex vivo to optimize transduction, most of the tumors regressed after animals were given the prodrug ganciclovir. But when gene transfer was attempted in vivo, a significant decrease in growth rate after ganciclovir administration was the best outcome reached.

These stumbling-stones should, however, be regarded as nothing but provocative and moreover should lead to continuous improvement in the design of gene transfer tools. The efficacy of cancer gene therapy depends on a variety of factors including the number and nature of cells to which the transgene is transferred by the vector, the intensity and duration of transgene expression, or the selection of the ideal therapeutic genes for each medical indication. In the end, gene therapy products should have benefits that outweigh the risks and should offer advantages over conventional and less expensive treatments before they are accepted in the general medical practice. A great effort is still needed, but sound basic research as that presented in this issue of Journal of Hepatology is the best way to take solid steps towards the final success in the task of developing better therapies for patients with HCC.

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