CANSA DETECTIVES

Gene Therapy for Cancer Treatment

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CANCER DETECTIVES

Booklet 4

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Targeting cancer cells

Elucidation of the structure of DNA in 1953 by James Watson and Francis Crick was a major development in the field of biology. This finding revealed important insights and provided the basis for understanding how genes are essential for life. Further analysis led to conceptualising the so-called 'central dogma' of biology, which refers to the sequential flow of information from DNA to protein (Figure 1). Although not accurate in all cases, this model has proved useful to normal biology and to understanding disease processes such as cancer. Essentially the principle is that information carried in DNA is initially transcribed to form RNA, which is then translated to form proteins. Proteins provide the myriad of functions that are required for cells to live and they also impart cellular structure. Developing these basic ideas led logically to the notion that mutations in DNA cause disruption to protein function to result in diseases such as cancer.

Detailed information on DNA and gene structure has recently enabled investigation of modification of genes as a means of treating disease. The term of 'Gene Therapy' was coined in the 1970s and refers to the use of procedures that are intended to treat or alleviate disease by genetically modifying the cells of a patient. To restore the health of cells, gene therapy may involve repairing damaged genes and silencing 'rogue' genetic elements that may be found in cancers. Gene therapy for cancer may involve different methods of countering a malignancy. These include eliminating cancer-causing agents such as a virus, the direct killing of cancer cells and augmenting cancer patients' immune response to malignant cells. To illustrate the power and versatility of gene therapy for cancer, the essentials of these three examples are described below.

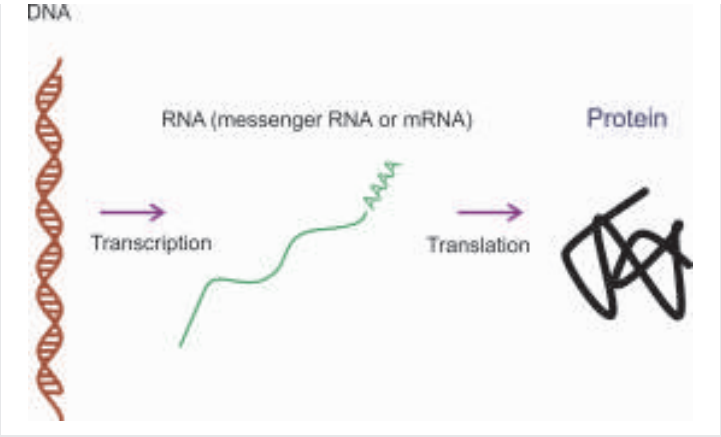

Figure 1: The 'Central Dogma' refers to the flow of information from DNA to RNA to proteins, which carry out essential functions within cells. When a mutation occurs in a gene (DNA), then the function of the encoded protein may be disrupted. If the protein is responsible for controlling cell division, then cancer may result.

Eliminating viral cancer-causing agents

An approach to treating cancer using gene therapy, which has been the main focus of our work in the Antiviral Gene Therapy Research Unit at the University of the Witwatersrand, is through elimination of risk factors for cancer. The strategy entails inactivation of viruses that are involved in cancer aetiology. A major aim of our work is to prevent emergence of liver cancer by countering hepatitis B virus (HBV) infection. Research has led to development of methods to silence genes by harnessing the RNA interference (RNAi) pathway (Figure 2) and also by engineering proteins that cause disabling mutations of HBV DNA sequences (Figure 3). As with development of most new therapies, advancing gene therapy for HBV infection involves a stepwise series of investigations.

The process requires preliminary analysis in simple models then progress to more complex disease simulations before embarking on trials in humans. Candidate drugs that show good efficacy in the simple models are often not effective in humans. It is only a small proportion (<1%) of candidate drugs that meet all requirements of safety and efficacy to proceed to clinical trial. Analysis may be started using computer simulations, but is typically initiated on cells in culture. From there, lead compounds are taken forward for testing in various in vivo disease models (e.g. in mice) and then in clinical trial.

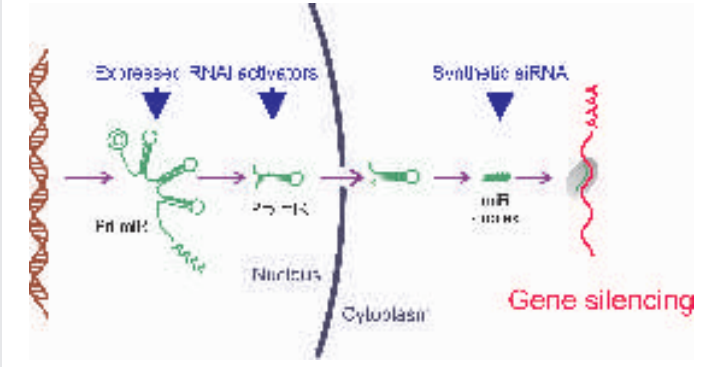

Figure 2: The RNAi pathway is initiated by production of pri-micro RNA (pri-miR), which is processed to form pre-miR and then mature miR short double stranded RNA. The sequence of one of the strands of the mature miR guides inhibition of gene expression through the formation of complementary pairing with a messenger RNA. The RNAi pathway may be reprogrammed by introducing artificial intermediates of the pathway into a cell (blue arrowheads). These mimics are processed by RNAi and cause silencing of a pathology-causing gene. Theoretically this approach may be used to silence any gene, including cancer causing genes.

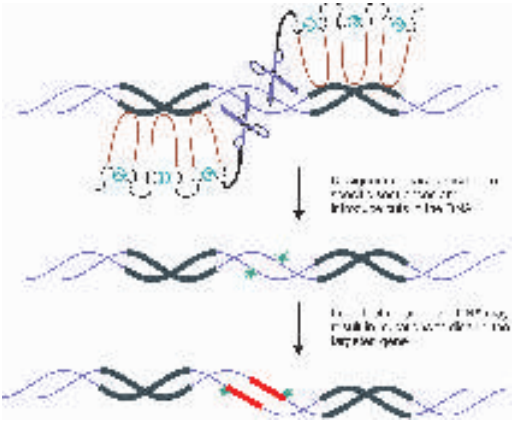

Figure 3: Designer nucleases are engineered to recognise specific DNA sequences and cut them. In this example, a pair of zinc finger nucleases cleaves DNA at a specific site, and resultant repair leads to introduction of disabling mutations. If the mutations are introduced into a cancer-causing gene, such as in HBV, then it is possible to arrest progression of the malignancy.

HBV Infection and Liver Cancer

Globally, liver cancer or hepatocellular carcinoma (HCC) is a major cause of mortality. The malignancy is characterised by a grave prognosis and high mortality. Liver cancer has a particularly high incidence in sub Saharan Africa, East and South East Asia where chronic HBV infection is common. There are many other causes of liver cancer and one that occurs commonly in developed countries is infection with hepatitis C virus (HCV). The general topic of liver cancer, and the role of HBV in causing this serious malignancy, was recently covered in some detail by Professor Michael Kew in another booklet of the CANSA detectives series.

Of the factors that have been identified to cause HCC, persistent HBV infection has the strongest association with the malignancy. The long term risk of HCC in HBV carriers has been reported to be in the range of 25-40%. Globally it is estimated that there are 387 million carriers of HBV. This enormous number makes HBV the major HCC-predisposing factor. Importantly, there is an effective vaccine against HBV, and this became compulsory for babies born in South Africa since 1995.

However, as the vaccine is ineffective against an established infection, people who are already infected remain at risk for developing liver cancer. An important current focus of preventing HCC is aimed at eliminating HBV infection. Available treatments for HBV infection rarely eradicate the infection. The virus may be suppressed, but after withdrawal of

treatment, the infection is capable of re-establishing itself. Developing more effective antiHBV agents therefore remains a global priority. With better understanding of the molecular pathogenesis of the cancer and significant advances in gene silencing technology, improved approaches are being devised to counter the malignancy. In particular, harnessing RNAi, as well as use of 'designer nucleases' to disable cancer-causing HBV genes is an exciting new approach to treating HCC. The technology is however at an early stage of development and there are several important hurdles that need to be overcome before it is widely applicable.

HBV Gene Silencing Using RNA interference

Discovery of RNAi was a landmark in the field of gene therapy. Elucidating this natural process provided the means for achieving powerful and specific silencing of pathology-causing genes. Research emanating from the Antiviral Gene Therapy Research Unit has demonstrated feasibility of harnessing RNAi to counter HBV. The methods that have been employed to silence HBV genes include use of chemically synthesised RNAi activators and also engineered artificial genes (expression cassettes) to reprogramme the pathway to act against HBV.

These different approaches to activating RNAi are illustrated in Figure 2. Chemically synthesised RNAi activators or short interfering RNAs (siRNAs) have the advantage of being amenable to convenient large scale preparation. This is important for a clinical setting since large amounts of siRNAs would be required for use as antiviral drug. Efficacy of siRNAs against HBV is however of short duration and repeat administrations are necessary to achieve a sustained therapeutic effect. Employing artificial genes to mimic intermediates of RNAi partially address this problem.

However these cassettes are difficult to deliver to the liver and sophisticated methods of engineering viruses to act as carriers of this class of HBV silencers are required. This is an expensive and time consuming process, which is also complicated by a risk of side effects caused by virus carriers. Although chemically synthesised antiHBV agents have a shorter duration of action against HBV, they have the advantage of smaller size and are therefore easier to deliver to HBV-infected liver cells. In addition, synthetic HBV gene silencers are not reliant on engineered viruses to reach the target tissue. Typically lipid (fat) molecules and various polymers may be coupled to the synthetic siRNAs to achieve delivery.

Several studies have been published by the Antiviral Gene Therapy Research Unit on this topic. Results have demonstrated that HBV can be effectively silenced in cell culture and in a stringent mouse model of the human condition of chronic HBV infection (Figure 4). Silencing of up to 95% was achieved in vivo, which should be sufficient for therapeutic application. Importantly, we have found that the engineered viruses (adenoviruses) that carry HBV-targeting expression cassettes have a strong activating effect on the immune system. This has two potentially significant consequences:

1. Toxicity may result from the immunostimulation, and
2. The duration of the silencing of HBV is attenuated as a result of the immune response to the carrier.

Consequently, the current focus of our work is on increasing duration of silencing and improving safety of antiHBV agents.

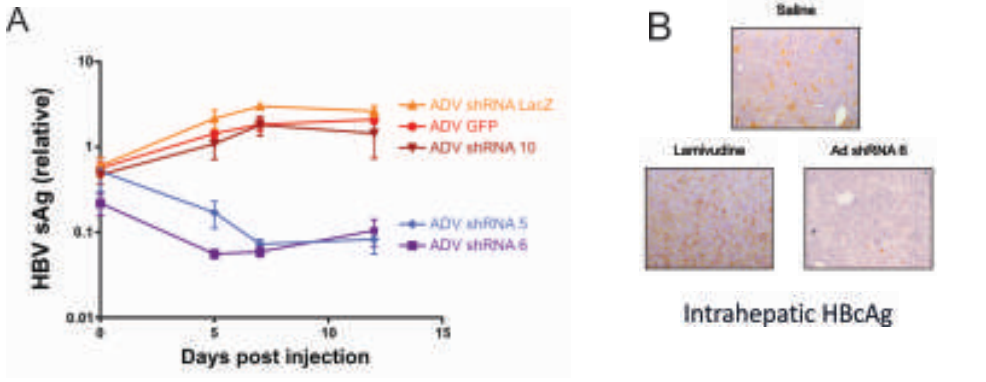

Figure 4: An example of RNAi-based gene therapy used to silence HBV in mice. In A, the viral surface antigen, a marker of HBV proliferation, was measured in the blood to assess the efficacy of engineered adenovirus vectors that produce artificial HBV-targeting gene silencers. The controls, orange, red and maroon lines, show that there is no effect against the virus. However, the antiHBV adenoviruses effectively suppress the virus marker of proliferation (blue and purple lines). Similarly (B), presence of another marker of the virus proliferation in the liver, the HBcAg (core antigen), was diminished. The HBcAg stains brown and it is clear that there is considerably more of this HBV marker in the control (saline and lamivudine-treated mice) than in the animals that received the vector that produces the HBV-silencing element.

Designing proteins that target specific genes

One of the major problems that is associated with HBV treatment is the fact that one of the viral intermediates, co-called cccDNA, is very stable and is resistant to effects of available drugs. Indeed, cccDNA may also be unaffected by RNAi-based approaches. To address this limitation, we have investigated the use of DNA disabling 'designer nucleases'. This class of potentially therapeutic genes has the very useful property of being capable of targeting and disabling specific DNA targets by introducing mutations at any intended site. Cutting the DNA with the nuclease provokes a repair processes to mutate the target (Figure 3). Currently there are two main groups of sequence-specific nucleases that have been developed for HBV treatment. They are transcription activator–like effecter (TALE) nucleases (TALENs) and polydactyl (many fingered) Zinc finger nucleases (ZFNs). ZFNs have been investigated for a long time and methodology for selection of engineered proteins with highly specific intended properties is now available. Moreover, use of ZFN-based therapies to eliminate the HIV-1 co-receptor CCR5 is being tested in clinical trial with promising results.

Although ZFNs have shown promise, the more recently described TALENs have been shown to have superior specificity, efficiency and safety when compared to ZFNs. Naturally, TALEs are produced by the Xanthomonas bacterial plant pathogen. They function by activating plant gene expression to favour Xanthomonas survival. Specificity is conferred by the tandemly repeat elements comprising 33-35 amino acids, each of which has specificity for a single DNA nucleotide. The convenient 'one repeat to one nucleotide' code is a useful property that has been exploited to generate designer TALEs. The technology also allows convenient addition of DNA-digesting domain to generate mutagenic TALENs (the 'designer nucleases').

Work in the Antiviral Gene Therapy Research Unit has also been focused on use of TALENs to disable HBV cccDNA. We propagated four different TALENs that recognise and cleave at specific sites within the virus DNA. The TALENs efficiently introduced mutations at the target sites, and as a result, the virus proliferation was severely attenuated in a mouse model of HBV infection(Figure 5). Impressive inhibition of HBV in cell culture and in mice augurs well for the use of this approach to treat HBV infection.

Work completed at AGTRU has established efficacy of gene silencing and use of designer nucleases to inhibit HBV infection in mice. Although these results are promising, the technology is still some way from approval for use in humans.

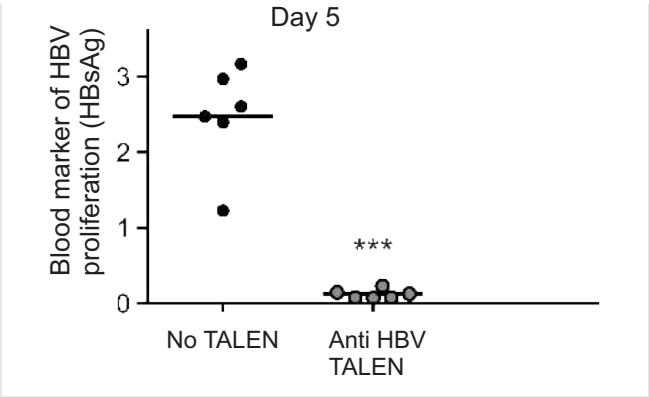

Figure 5: Silencing of HBV by engineered TALENs that introduce specific mutations in the virus genes. As in Figure 4, the surface antigen was used as a marker of virus proliferation in mice. The solid black and grey circles represent measurements from individual mice that received no TALEN or an HBV-targeting TALEN respectively.

Killing Cancerous cells using Gene Therapy

A rapidly advancing and exciting novel field of gene therapy is the selective killing of cancer cells with genes that cause toxicity to malignancies. Typically, cancer-killing genes are delivered with engineered viruses. Significant success has been achieved with so-called oncolytic viruses, which are capable of homing in on and destroying (lysing) cancer cells. These developments have largely taken place in the USA and little if any work on this topic is being carried out in South Africa. Oncloytic viruses originate from a wide range of natural viruses such as adenoviruses, measles virus and herpes simplex viruses. The natural toxic effects of these viruses are harnessed to kill cancerous cells. OncoVEX is an example of an oncolytic herpes simplex-derived virus that has shown exciting promise. Its use has reached an advanced stage of clinical testing for the treatment of malignant melanoma. US-based biotechnology company, BioVex, has been responsible for developing this oncolytic virus.

Immunostimulation to enhance killing of cancer cells

An exciting field that has made rapid recent advances is the engineering of immune cells to express receptors that are capable of recognising and activating the killing of cancerous cells. Since malignancies are by definition abnormal, it stands to reason that they produce a variety of proteins that are not found on normal cells. Engineering immune cells to enable specific identification and killing of malignant cells according to presence of certain cancer protein signatures has taken vast and rapid strides. It is now possible to take T-cells (lymphocytes) and introduce genes expressing receptors that are specific to cancer markers. Typically, T cell receptors or artificial chimaeric antigen receptors (CARs) have been used and both have shown impressive efficacy. The T cells increase their numbers when exposed to the cancerous antigen, and are then capable of causing highly effective cancer cell killing. Clinical trials are already in progress using this approach, and the technology is being developed for the treatment of ovarian and neural cancers as well as certain lymphomas and leukaemias.

The Way Forward

The enormous potential of gene and cell therapy has prompted enthusiasm for advancing novel and more effective cancer treatments. Developments have been exciting, and clinical trials are now in progress using gene and cell therapy for a wide variety of cancers. The first gene therapy drug has been licensed in Europe for the treatment of an inherited deficiency that causes hypercholesterolaemia. This is a significant milestone and if this treatment proves successful, which seems likely, it will pave the way for an upsurge of gene therapies for a wide variety of diseases that include cancer. Approaches, which include gene silencing, use of designer nucleases, oncolytic viruses and immunotherapy, all show promise. Nevertheless use of gene therapy for cancer still faces difficulties. These include identification of optimal targets, efficient and safe delivery of therapeutic genes and limitation of unintended off target effects. Good progress has been made with silencing of HBV using RNAi and designer nucleases. It is likely that treatment of these virus infections, as an HCC preventative measure, will be the first gene therapy-based approach to countering the malignancy. Currently, early diagnosis of HCC is critical for its effective treatment. Success of gene therapy for HCC is also expected to be dependent on identifying the malignancy in its early stages before tumour bulk becomes excessive. In the immediate future, it seems that gene therapy may well be used as an adjunct to other cancer treatments. Thus, the utility of gene therapy is also likely to be reliant on improvement of existing treatment and diagnostic modalities. Despite difficulties, intensive efforts from several quarters have given momentum to the field. It is difficult to anticipate technological advancements, but the discipline is likely to see considerable progress during the coming years. Hopefully there will be many breakthroughs that will soon make gene therapy an attractive approach to overcoming a wide range of cancers.

Scientists and postgraduate students that were involved in the project:

Dr Abdullah Ely, Dr Betty Mowa, Dr Carol Crowther, Dr Kristie Bloom, Dr Samantha Nicholson, Justin Hean, Musa Marimani & Dejana Ivacik.

Key recent papers from WITS

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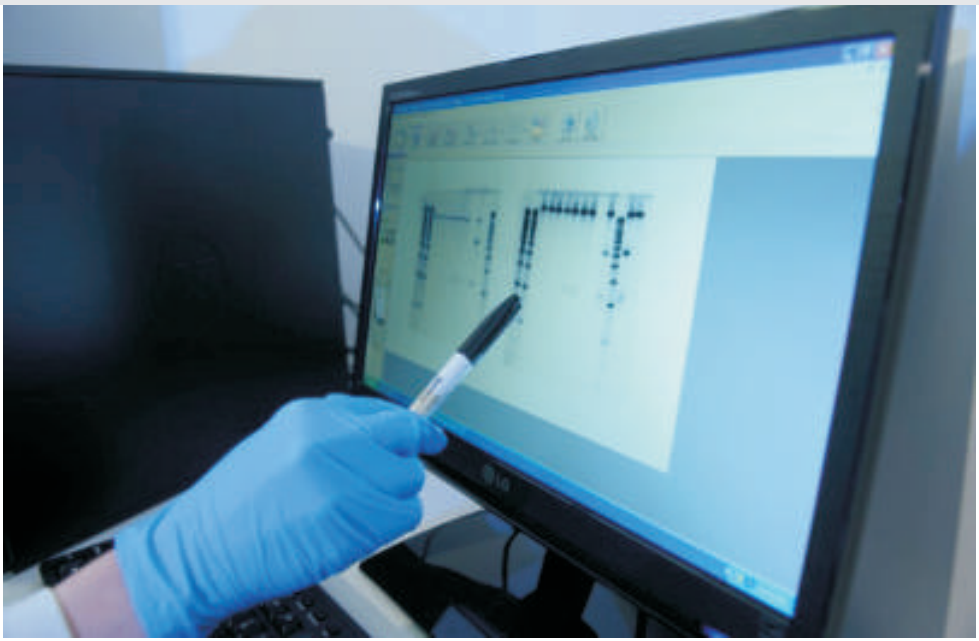
Back row left to right: Dejana Ivacik, Victoria Green, Musa Marimani, Marco Weinberg, Justin Hean



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