Improved prospects for anti-cancer gene therapy

Although gene therapy holds great promise for treating cancer, delivering genes that kill tumour cells whilst leaving normal healthy cells intact remains a challenge. Recently, James McArthur and colleagues at Cell Genesys (Foster City, CA, USA) and GPC Biotech (Cambridge, MA, USA) revealed that a fusion of two tumour suppressor genes, p16 and p27, show selective anti-tumour activity *in vitro* and *in vivo*[1].

The cyclin-dependent kinase inhibitors, p16 and p27, have been shown to have anti-proliferative or apoptotic activity when overexpressed in malignant cells following adenoviral vector-mediated gene transfer[2,3]. Several fusion genes were tested for increased anti-tumour activity relative to their parent genes, and the p16–p27 fusion gene with the greatest activity was chosen for further investigation.

In vitro studies

In the first set of experiments, the ability of p27–p16 to cause selective apoptosis in human cancer cell lines was tested. These cell lines were derived from various sites, including the prostate, lung, colon, kidney, ovary and breast, and were transfected with ΔE1-adenovirus vectors carrying the fusion gene. Analyses performed 2–4 days after virus infection showed that, overall, p16–p27 expression induced apoptosis in 12 of the 14 tumour cell lines tested. Further study of lung, prostate and colon-derived cell lines revealed that the p16–p27 fusion gene elicited the same level of apoptosis at doses five-times lower than p16 alone and 50-times lower than p27 alone[1]. 'Interestingly, the apoptosis detected seemed to be independent of the cells' Rb or p53 gene status,' comments McArthur.

Rb and p53 are natural proteins that help to regulate cell proliferation. Most cells in an adult human do not proliferate frequently, if at all. These proteins are thought to have an important role in the control of cell division because they are mutated in a large proportion of tumours. In the absence of a functional p53 molecule, for example, the cell enters a state of uncontrolled division. 'We do not yet understand why p16–p27 acts independently of p53 or its precise mechanism of action. It appears to act at multiple points in the cell cycle and is present in the cytoplasm of the cell, as well as in the nucleus; ongoing studies are investigating what is going on at the molecular level,' says McArthur.

Michael Moeller (Department of Pathology, Odense University Hospital, Odense, Denmark) says that the fact that p16–p27 works independently of p53 could be particularly promising as 'the high-profile adenoviral cancer gene therapeutic ONYX-015 primarily, but maybe not entirely, depends on p53 inactivation.'

In vivo studies

In the first set of *in vivo* studies, McArthur and colleagues injected immunodeficient mice with untreated tumour cells or tumour cells pre-treated with the adenoviral vector carrying p16–p27, p16 alone or p27 alone. 'Pretreatment with the vector carrying the fusion gene completely prevented tumour formation in the mice,' reports McArthur. A dose titration of the tumour cells showed that p16–p27 resulted in at least 95% apoptotic cell death.

The ability of the fusion gene to act on established tumours in mice was then investigated using two mouse models. In the first, 3 million cells from a malignant cell line were injected into immunodeficient mice; about 3 weeks later, the adenovirus vector containing different genes was injected directly into the tumours that developed. The treatment was repeated every 2 days for 6 days. p16–p27-treated tumours showed significantly delayed growth compared with tumours treated with vector or with p16 only.

In the second model, established xenograft tumours were treated weekly on consecutive days with two doses of recombinant virus or control solution. Again, the p16–p27 fusion gene produced significantly higher anti-tumour activity. 'Injection of the tumours with p16–p27 induced tumour regression or arrested the growth of tumours in 50% of the treated animals,' says McArthur.

Future clinical application

A possible clinical application of p16–p27 could be in solid tumours of the brain that are life-threatening, such as gliomas, and primary liver tumours or liver metastases that are more dangerous than the original cancer. 'Having a specific "molecular knife" could make a big difference to patients with these types of tumours,' says McArthur but he warns that 'additional preclinical efficacy and safety studies must be done before we can move into clinical development.'

Moeller agrees that a more detailed analysis of the interactions of p16 and p27 are warranted. 'The results of this study also suggest that the anti-angiogenic function of p16 might contribute to the overall anti-tumour activity of p16–p27 and this will need to be investigated

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Box 1. ONYX-015 gives good Phase II clinical trial results

ONYX-015 is a modified adenovirus that replicates and lyses $p53^-$ but not $p53^+$ human tumour cells. Recently, a Phase II clinical trial reported that ONYX-015 treatment, combined with chemotherapy, was promisingly successful in 30 patients with head and neck cancers. Tumours disappeared completely in eight patients and another 19 experienced a dramatic reduction in tumour size[4]. French Anderson, who pioneered the first clinical trial of gene therapy for the genetic adenosine deaminase (ADA) deficiency in 1990, says that this latest trial of ONYX-015 'is particularly encouraging because the tumours disappeared in significant numbers of patients and have not recurred.'[5]

further.' Moeller also highlights that future studies should also address the problems of gene delivery. 'An adenoviral vector is only useful in solid tumours, and it has to be injected directly, making the treatment of small tumours or metastases very difficult.' Clinical trials might be some way off but he thinks they should investigate whether a combination therapy of p16–p27, chemotherapy and radiotherapy might improve the outcome, 'as it has in the recent and encouraging studies of ONYX-015' (Box 1).

Although unable to give full details at this stage, McArthur says that his team is testing second-generation molecules that are more potent than the p16–p27 fusion product. 'We are currently doing preclinical studies on these molecules and we expect that we can use them to overcome some of the delivery hurdles. We hope that the molecules we put into clinical trials will have broader activity against a wider variety of tumours and will be capable of dispersing throughout the body,' he says.

REFERENCES

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Key strategies in functional genomics for drug discovery

Speakers at the IIR conference entitled *Using Functional Genomics in Drug Discovery*, held on 27–28 September 2000 in London (UK) discussed a number of methods of using functional genomics, some of the problems and issues surrounding their use in drug discovery and many future concerns and future directions for the field.

Klaus Lindpaintner (Hoffman-La-Roche, Basel, Switzerland) highlighted that although many diseases are genetically defined, many are the result of a combination of genetics with other factors. For example, in Alzheimer's disease, the presence of the ApoE4 allele is associated with a twofold increase in incidence of the disease. However, although head trauma alone shows no effect on incidence, the presence of this allele together with trauma increases incidence of the disease tenfold. This highlights that diseases are more than just a genetic mutation but are a combination of genes and the environment: diseases such as haemophilia are mostly genetic, while diseases such as lung cancer are significantly induced through the environment (mostly smoking in this example).

John Morrison (AstraZeneca, Macclesfield, UK) presented data on asthma to highlight that combined genetics and genomics approaches are better than the individual approaches alone as it should enable the prioritization of candidate genes to reduce the number from tens of thousands with unknown function to approximately ten. This would be done through the use of data from several differential strategies and then identifying the genetic association with the disease using SNPs followed by searching databases such as Incyte's. There were concerns that the use of so many different technologies would lead to accumulative errors and an increase in the number of false-positives, but Morrison confirmed that these technologies were being run in parallel at AstraZeneca and that the experiments were designed specifically to avoid this problem.

Genomics for target validation

The information gleaned from the sequencing of the human genome should dramatically speed up the process of target validation. There are several methods with which to use this information, four different such approaches being presented at this conference.

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