Protection against chemotherapy- and radiotherapy-induced cell damage

Drugs could be available within the next decade that could protect sensitive organs (such as the bone marrow) from the worst effects of anticancer treatments. This would enable the administration of higher doses of chemotherapy and radiotherapy and, hence, the possible achievement of higher cure rates with certain cancers. A single injection of such a compound, identified by researchers in the US, rescued mice completely from a radiation dose that normally kills 60% of the animals.

The compound, called pifithrin-alpha, works by inhibiting the protein p53: the name pifithrin is short for 'p-fifty three inhibitor'. Although inhibition of this molecule is normally associated with tumour formation, mice treated with it have so far survived for approximately nine months (about half the normal mouse life-span) without developing tumours[1].

Elena Komarova (Department of Molecular Genetics, University of Illinois, Chicago, IL, USA) said, 'We believe that our data are very promising. We hope that pifithrin can be used in the future as a drug to reduce the side effects of anticancer treatment, although obviously, before it can be used in the clinic, more long-term animal studies are needed to find out if it has dangerous side effects.' Komarova added that pifithrin could also be useful in other clinical conditions where p53 suppression might be desirable, such as ischaemia of the heart or brain, where cell death is also caused by p53 activation.

Mechanism of action of pifithrin

The idea that inhibiting p53 could help to improve cancer cure rates might initially appear counter-intuitive. In many cells, the gene that encodes p53 only stimulates protein manufacture when something goes wrong with the cell. The role of p53 is to force the cell into the apoptosis pathway, thus ensuring that damaged or potentially dangerous cells (such as malignant cells) are eliminated for the good of the organism. The observation that the gene for p53 is missing or mutated in most human tumours demonstrates the importance of this function.

In some tissues, however, high levels of p53 are normal. For example, in mice, high p53 levels are found in the bone marrow, in the epithelial lining of the intestines, and in the testis. These are the same tissues that are so sensitive to anticancer treatments in humans, leading to damage that limits the dose of cytotoxic drugs or radiation therapy. Studies have shown that cells in these tissues undergo apoptosis mediated by p53 shortly after exposure to gamma irradiation[2].

Andrei Gudkov (Associate Professor, Department of Molecular Genetics, University of Illinois) and his colleagues, in collaboration with Quark Biotech (Pleasanton, CA, USA), therefore decided to try to identify a compound that would reversibly inhibit p53. They wanted to know what effect a p53 inhibitor would have on normal tissues, if given at the same time as anticancer treatments. Giving a drug that suppresses p53 would not, they reasoned, help tumour cells to escape eradication, but would only help healthy cells to survive.

To identify a compound that could inhibit the action of p53, the researchers first produced cells that contained a reporter gene that makes cells a blue colour when p53 is activated. They then added individual compounds from a commercially available collection of 10,000 synthetic chemicals, along with a potent p53 inducer. Out of several compounds that blocked p53 production in this system, the benzothiazol-derivative pifithrin was chosen for detailed assessment.

Pifithrin is water soluble and stable, and has a molecular weight of 367. The effect of pifithrin on p53-mediated apoptosis was examined using mouse embryo fibroblasts transformed with *Ela* + *ras*, line C8, a cell line that normally undergoes p53-mediated apoptosis when exposed to a range of anticancer drugs, to UV light or to γ-radiation. This study showed that a ten micromolar concentration of pifithrin could inhibit this apoptotic response. Further studies have suggested that pifithrin acts downstream of p53, possibly by modulating the import and/or export of p53 to, or from, the nucleus. The effects of the drug are reversible and inhibition of p53 only occurred in the presence of the drug. Furthermore, when mice were given single intraperitoneal injections of pifithrin (2.2 mg kg^{-1}) followed by exposure to doses of γ-radiation that would normally kill 60% of the animals (6–14 Gy, depending on strain and age), all the mice survived. Some mice survived even higher doses of irradiation when given pifithrin. Moreover, the typical loss of weight associated with radiation was greatly reduced in mice treated with pifithrin compared with the few irradiated survivors that did not receive the drug.

Conclusion

Gudkov and Quark Biotech predict that pifithrin will probably enter Phase I clinical trials within a year. Komarova added that the team is planning a screening programme of natural

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compounds to try to identify additional p53 inhibitors, and will also examine the inhibitory properties of synthesized derivatives of pifithrin. David Patterson, President of the Eleanor Roosevelt Institute for Cancer Research (Denver, CO, USA), who was not involved in the study, said, 'This is a novel concept – to be able to suppress the damage of chemotherapy and X-rays with a drug – and it looks like it works extremely well.'

REFERENCES

1 Komarov, P.G. and Komarova, E.A. (1999) A chemical inhibitor of p53 that protects mice from the side effects of cancer therapy. *Science* 285, 1733–1737

2 Komarova, E.A. *et al.* (1997) Transgenic mice with p53-responsive lacZ: new insights into p53 function in normal development and in response to DNA damage *in vivo*. *EMBO J.* 16, 1391–1400

Sharon Kingman

Selective arginine receptors as antiviral compounds and molecular probes

It might soon be possible to design novel antiviral compounds and molecular probes using a new type of receptor molecule that selectively binds to the amino acid, arginine. Arginine-rich sequences are a feature of several pathogen proteins, including HIV proteins. Blocking the activity of RNA sequences that bind to these arginine-rich sequences might therefore be a novel way to inhibit viral and bacterial replication.

Thomas Bell and Alisher Khasanov (University of Nevada, Reno, NV, USA), together with Thomas James and Anton Filikov (University of California, San Francisco, CA, USA) and Mike Drew (University of Reading, Reading, UK) have synthesized a highly preorganized artificial receptor for guanidinium cations that they say can bind with high affinity and selectivity to arginine in polar solvents such as methanol and even water. The molecular device could then be used either as an antiviral agent or as a nuclear magnetic resonance (NMR) spectroscopic probe for studying arginine-containing protein structure and folding.

Crucial involvement of arginine

Arginine is crucial to the functioning of nucleotide-binding proteins that mediate several biochemical processes. The replication of the HIV-1 virus involves short, arginine-rich sequences in two important regulating proteins that invoke RNA binding, these being the transcriptional activator TAT and the REV protein. According to Bell and his colleagues[1], small molecules that are tailored to bind selectively to arginine could be used to inhibit these processes.

There have been various efforts to create an arginine receptor for this purpose but one of the problems encountered has been a lack of activity in water, which would then hinder antiviral activity. Such a receptor must also be highly specific for arginine alone and not bind to lysine side-chains. However, both arginine and lysine are found in human and viral RNA-binding proteins and so some sequence-specificity is likely to be necessary to discriminate between the two amino acids.

Thomas Schrader (Dusseldorf University, Dusseldorf, Germany) devised molecular 'tweezers' for binding arginine[2]. However, Bell highlighted that while the molecule resembles the natural arginine receptor, only modest binding was observed. This low activity might be explained by incomplete preorganization of the receptor molecule. In an attempt to avoid this problem, Bell and his colleagues have designed a recognition unit in which a relatively rigid array of hydrogen-bonding groups are fused together in a series of six-membered carbon rings. This design should create perfect 'host–guest' complementarity between the arginine residue and the receptor molecule. The incorporation of various polar groups could then be used to make the host molecule water-soluble.

Close examination

Bell's team examined the ability of their receptor to bind arginine using NMR spectroscopy. The signals for the receptor protons alone are shifted downfield when a guest arginine molecule binds to the receptor. By contrast, for lysine as the guest molecule, there is very weak binding and so a much smaller shift in the signals is observed. Indeed, a detailed analysis of the spectra revealed that their receptor binds to arginine approximately two hundred-fold more strongly than Schrader's molecular tweezers.

The primary mode of interaction between arginine and the receptor molecule is electrostatic bonding, rather than conventional covalent bonding. Hydrogen bonds form between complimentary charged oxygen atoms on the receptor and the hydrogens attached to the amino nitrogens on the arginine and between amino hydrogens and the nitrogens on the receptor pyridyl groups. By contrast, lysine lacks the

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