Effects on preclinical and clinical studies

With the advent of pharmacogenomics, we should be able to move from empirical prescription using 'mass markers' to rational 'individualized' prescriptions, avoiding trial-and-error prescribing as well as reducing the impact of the side effects from inappropriate drugs. In the future, we should therefore be able to profile molecular targets and apply this knowledge to patients.

Lindpaintner predicted that although pharmacogenomics will enable more testing to be done prior to animal studies, there will always be a need to go into animals at some stage before going into humans. However, there should be an overall reduction in animal testing as animals should only need to be exposed to pharmacogenomically tested drugs. Meanwhile, in clinical trials, Jon Morrison suggested that as there will be a need for stratification of patients into homogenous groups for clinical trials, diseases will need to be reclassified at the molecular level and patients will be classified on the variability of expression of drug targets.

Diagnostic use

Lindpaintner predicted that in the future there will be a need for an increased use of *in vitro* diagnostics, for more differential molecular diagnosis and for an increase in the integration of diagnostics and therapeutics. This increased use of diagnostics should shorten discovery and development cycles, maybe not so much on individualized projects but to a more significant degree overall, due to fewer failures because of targeting *bona fide* disease mechanisms. In the short-term, Lindpaintner suggested that pharmacogenetics would include adverse effect profile pharmacogenetics and efficacy profile pharmacodynamics; in the mid-term, it would cover genotyping for target identification and expression profiling; in the long-term, there would be a move towards causative and predictive pharmacogenetics as well as new target discovery using, for example, expression profiling.

Public perceptions

Lindpaintner also expressed concern over the way that the bioethical problems are being handled. He suggested that, at the moment, there is a widespread fear by the public of abuse of genetic information and that a focus on confidentiality will limit the utility and use of information for the patients' benefit. He therefore felt that there is a need for a societal consensus that protects the individual while enabling the beneficial use of the information. A prerequisite to this, therefore, is that genetic scientists discuss these issues openly with the public. He also felt that as the process of pharmacogenomic testing proves to be effective, people will be more willing to have predisposition testing.

Antibody drugs

Carl Webster (Cambridge Antibody Technology, CAT, Royston, UK) discussed the use of phage antibody libraries and proposed that they are a key part of future target validation and drug discovery strategies. He suggested that the advantages of using these libraries are clonal diversity and that the antibodies have a high level of affinity. These antibodies can be used for high-throughput validation of genomics targets, as research reagents and for proof-of-principle studies. Webster also emphasized that monoclonal anti-bodies against these genomics targets are expected to become a substantial drug class of the future. There are several human monoclonal antibodies already in clinical trials, with S2E7 (BASF, Ludwigshafen, Germany), the potential rheumatoid arthritis agent, being the first (and only one so far) to enter Phase III trials.

The future

Lindpaintner concluded by predicting that genetics should change medicine in evolutionary and incremental terms because of more sophisticated patient-specific information. However, he said that conceptually, genetics would make no difference as it is just another step along in the history of medicine, as really genetic information is just biological information but on a different level.

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Rebecca N. Lawrence

Pseudocomplementary strategy strengthens PNA therapeutic potential

Peptide nucleic acids (PNAs), synthetic oligomers that mimic naturally occurring DNA and RNA, have the potential to act as antisense and antigene drugs, but several limitations have so far hindered their development as therapeutic agents. Recently, Vadim Demidov, Maxim Frank-Kamenetskii (Center for Advanced Biotechnology, Boston University, MA, USA) and colleagues published a new study on the properties of pseudocomplementary PNAs

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(pcPNAs) that could circumvent some of these limitations[1].

'We show for the first time that pcPNAs can protect selected DNA sites from the action of restriction enzymes and DNA methyltransferases. This ability should provide greater opportunities to use pcPNAs to develop powerful PNA-based antigene drugs that can regulate genes and correct harmful mutations,' explains Demidov. This has implications for both gene therapy and the treatment of cancer. pcPNAs could also prove useful for the diagnosis of latent viral infections caused by a provirus integration into the host genome.

PNA origins

PNAs arose at the interface between chemistry and biology almost ten years ago[2]. A PNA molecule is a synthetically modified nucleic acid (Fig. 1) in which the entire sugar–phosphate backbone is replaced by a structurally homomorphous and biologically more stable pseudo-peptide backbone linked periodically to normal and/or modified nucleobases. Although structurally similar, PNA molecules have very different physicochemical and biochemical characteristics to natural amino acids.

As David Corey (University of Texas, TX, USA), a recognized expert in the PNA field, explains: 'PNAs are a DNA/RNA mimic containing an uncharged amide backbone, and have shown a remarkable ability to invade duplex DNA (Fig. 2).

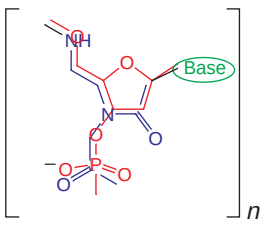

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Figure 1. *Comparison of peptide nucleic acid (blue) with DNA (red) backbones.*

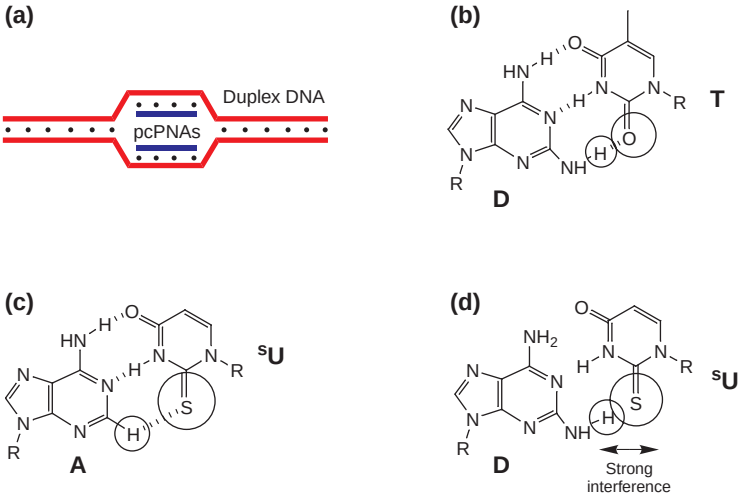

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Figure 2. *(a) The double-duplex invasion complex formed by a pair of pcPNA oligomers inside the duplex DNA site with a mixed composition of all four nucleobases. (b,c) Base pairs form between diaminopurine (D) and thymine (T) or adenine (A) and thiouracil (^{s}U). (d) The steric clash between bulky substitutions in D- and ^{s}U-modified nucleobases prevents them from formation of the D-^{s}U base pair[11]. Pseudocomplementary modifications of guanosine and cytosine with similar properties have also been designed[12].*

This ability is relevant to medicine because one can imagine that PNAs could be developed into artificial gene repressors or artificial gene activators. One would need only to know the sequence of the target promoter region to generate a drug.'

Problems with PNAs

Less encouragingly, PNAs are large molecules with relatively poor solubility and poor bioavailability. However, PNA solubility enhancers can be incorporated into the PNA structure and several approaches have been developed to improve the efficiency of intracellular PNA delivery[3]. There are also problems in getting PNA molecules to displace strands of duplex DNA – a process that is central to the development of PNA-based antigene drugs. 'First, the process requires low, non-physiological concentrations of salt and, second, requires homopurine–homopyrimidine target sequences,' points out Demidov.

The first limitation can be overcome by incorporating positive charges into the PNA molecule using intracellular processes such as DNA supercoiling and transcription that facilitate PNA invasion of the DNA duplex. The second has, until recently, been impossible to solve but Demidov suggests that applying the pseudocomplementarity principle to PNA represents the rational way forward (Box 1).

pcPNAs and the therapeutic challenge

'The principal challenge, of course, is to get PNAs to bind to the target sequence of all four nucleobases, and that is addressed by this study,' comments Corey, who peer-reviewed the paper[1] before publication. 'This discovery greatly extends the range of target sequences that should be accessible to PNAs, and potential target sites will exist in almost any promoter region,' he says. Challenges remain, such as determining the factors that facilitate access to duplex DNA sequences within cells and optimizing specificity. 'The work of Demidov and his collaborators now

Box 1. What are pseudocomplementary PNAs?

Last year, Peter Nielsen (Copenhagen University, Copenhagen, Denmark) and colleagues showed that pairs of PNA molecules carrying ordinary guanine (G) and cytosine (C) but containing 2,6-diaminopurine and 2-thiouracil instead of adenine (A) and thymine (T), recognize their natural A–T or G–C counterpart, but do not recognize each other[10]. Pseudocomplementary PNAs (pcPNAs) therefore do not bind to each other, but they can bind to any chosen sequence of DNA or RNA.

'A previous study has already shown that pcPNAs bind to duplex DNA by selectively targeting any designated site with all four nucleobases (A, G, C and T), but it gave only an initial description of novel PNA–DNA complexes,' stresses Demidov. The next study of pcPNAs, performed in collaboration with Nielsen, provides significant characterization of new PNA generation and demonstrates its impressive potential for sequence-specific blocking of DNA methylation and restriction enzymes.

'Whereas binding of common PNAs to duplex DNA is limited by sites consisting of only purines (A and G), pcPNAs target duplex DNA in a virtually sequence-unrestricted manner via a structurally unusual double-duplex mode of strand invasion,' reports Demidov. As a result, he adds, pcPNAs can be added to the repertoire of artificial reagents that make it possible to target and manipulate duplex DNA without sequence limitations.

makes it possible to design and execute key experiments aimed at controlling intracellular gene expression,' adds Corey.

The future

Although the strategy of pseudocomplementarity extends the potential of PNAs, translating such fundamental laboratory research into clinical applications typically takes about two decades. 'Considering that PNA has only been around for ten years, it is unreasonable to expect that PNA-based drugs will be marketed in the near future,' says Demidov. 'Too many problems still have to be resolved,' he warns. Nevertheless, the prospect for developing such drugs looks so promising that several pharmaceutical companies worldwide are involved in PNA development. For example, ISIS Pharmaceuticals (Carlsbad, CA, USA), a leader in the antisense drug technology, is aiming to generate novel classes of PNA-derived antisense/antigene therapeutics.

In addition, the Danish start-up company Pantheco (Copenhagen, Denmark) hopes to develop a new generation of PNA-based drugs for the treatment of infectious diseases caused by antibiotic-resistant microorganisms. This project follows on from the recent description of PNAs target-selective antimicrobial effect[4,5]. Recent studies have shown that telomerase could be a selective target for PNA (Refs 3,6,7) and that PNAs could potentially be employed for anticancer therapy[8,9].

The onus for the foreseeable future will be on basic work to elucidate the properties of pcPNAs. Currently, the Boston team is studying the mechanism of pcPNA double-duplex invasion. 'This might have implications for pcPNA affinity and specificity, and could help with the design of bis-pcPNAs,' says Demidov. In the next 1–2 years, he continues, we will extend our research on interactions of pcPNA–duplex DNA complexes with the key DNA processing enzymes. 'Understanding more about pcPNA will at least enable us to move on to the next stage of drug development,' he adds.

A full up-to-date review of PNAs and their potential future as gene therapeutic drugs will be coming up shortly in *Drug Discovery Today*.

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