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Protein Kinase Inhibitors for the Treatment of Disease: The Promise and the Problems

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1 The Promise

The reversible phosphorylation of proteins, catalysed by protein kinases and phosphatases, was first identified as a regulatory device in the 1950s, and it has been established for many years that this control mechanism regulates most aspects of cell life. However, it was only in the 1990s that interest in developing inhibitors of protein kinases and phosphatases started to enter centre stage (see Cohen 2002a,b for historical reviews). The first two drugs shown to target these classes of enzyme were cyclosporin, an inhibitor of protein phosphatase 2B (PP2B, also called calcineurin) (Liu et al. 1991) and rapamycin, an inhibitor of the protein kinase mTOR (mammalian target of rapamycin) (Heitman et al. 1991), which are the immunosuppressants that have permitted the widespread use of organ transplantation. However, these drugs were developed and approved for clinical use before their mechanism of action was identified. Fasudil, an isoquinoline sulphonamide that inhibits several protein kinases with relatively low potency, such as the Rho-dependent protein kinases (ROCK) (Davies et al. 2000), was developed by Hiroyoshi Hidaka in the 1980s and approved in Japan in 1995 for the treatment of cerebral vasospasm. ROCK can constrict blood vessels by inhibiting smooth muscle myosin phosphatase, but whether the clinical efficacy of fasudil results from its inhibition of ROCK, another protein kinase(s) or a completely different target, is unclear. Current information about this drug is discussed by Hidaka et al. (in Part 4).

Glivec (also called imatinib and STI-571), developed by Nick Lydon and his colleagues at Novartis, was the first drug to be developed by targeting a specific protein kinase and was approved for clinical use in the USA in 2001. It targets the protein tyrosine kinase c-Abl, which is mutated to the constitu-

tively active BCR-Abl fusion protein in nearly all cases of chronic myelogenous leukaemia (CML). The spectacular efficacy and minimal side effects of Glivec, first highlighted by Brian Druker, resulted in the most rapid approval of a drug in FDA history and was a landmark event in this area. The development of Glivec and its implications for the future of drug discovery in this area are discussed by Fabbro et al. (in Part 4). Interestingly, Abl is not the only protein tyrosine kinase targeted by Glivec. It also inhibits the c-Kit receptor tyrosine kinase and the platelet-derived growth factor (PDGF) receptor. The c-Kit receptor is mutated to an abnormally active form in many gastrointestinal stromal tumours (GISTs) and the efficacy of Glivec for the treatment GISTs is equally impressive, resulting in its approval for this therapeutic use in 2002. The potential of Glivec to treat several types of cancer is discussed by Druker (in Part 4).

Following on from the successful launch of Glivec, Iressa a potent inhibitor of the epidermal growth factor (EGF) receptor tyrosine kinase was approved in Japan in 2002 and in the USA in 2003 for the treatment of some types of lung cancer. Developed by AstraZeneca, this drug is discussed by Wakeling (in Part 4). Drugs that inhibit the vascular endothelial-growth factor (VEGF) or fibroblast growth factor (FGF) receptor tyrosine kinases are undergoing phase III clinical trials and may be among the next protein kinase inhibitors to be approved for clinical use. VEGF and FGF play key roles in angiogenesis, and inhibitors of their receptors destroy the tumour's vascular supply. For this reason these compounds may be useful for the treatment of several types of cancer.

Compounds that inhibit protein serine/threonine kinases are also undergoing human clinical trials in a number of therapeutic areas. For example, at least four companies have inhibitors of p38 mitogen-activated protein (MAP) kinase in the clinic. These compounds suppress the production of tumour necrosis factor (TNF) and some other proinflammatory cytokines and show efficacy for the treatment of rheumatoid arthritis and other chronic inflammatory diseases. These programmes are discussed by Kumar and Blake (in Part 2). In the same section, Meijer (in Part 2) discusses inhibitors of cyclin-dependent protein kinases (CDKs), which are undergoing clinical trials as anti-cancer agents, and inhibitors of GSK3 which, although at the preclinical stage, have shown potential for the treatment of several diseases including type II diabetes (Cline et al. 2002; Ring et al. 2003) and stroke (Cross et al. 2001). Inhibitors of MAP kinase kinase 1 (MKK1, also called MEK) and RAF (product of the proto-oncogene *Raf*) are undergoing clinical trials as anti-cancer agents, and inhibitors of mixed lineage kinase 3 (MLK3) to prevent neurodegeneration (reviewed in Cohen 2002b). However, this is only the 'tip of the iceberg'. Over the past few years protein kinases have become the second most studied group of drug targets after G protein-coupled receptors, accounting for a quarter or more of drug discovery programmes

worldwide. The number of protein kinase inhibitors undergoing human clinical trials at the present time almost certainly exceeds 100.

The discovery that PP2B, a serine/threonine-specific protein phosphatase, was inhibited specifically by cyclosporin highlighted the potential of protein phosphatases as drug targets, and programmes to develop specific inhibitors of several of these enzymes are underway. Protein tyrosine phosphatase IB (PTP1B) appears to be one of the enzymes that dephosphorylates and inactivates the insulin receptor, because mice that do not express it are hypersensitive to insulin and maintain normal blood glucose levels at half the normal circulating of insulin (Elchebly et al. 1999). In addition, these mice do not become obese when fed a high-fat, high-carbohydrate diet. For these reasons, PTPIB is potentially an attractive target for the development of a drug to treat diabetes and/or obesity, as discussed by Cheng et al. (in Part 3). However, although interesting compounds have been developed that are relatively specific inhibitors of PTP1B, as discussed by Møller (in Part 3), no inhibitors of this enzyme appear to have entered clinical trials. CD45 is another protein tyrosine phosphatase that is potentially an attractive drug target, because it is only expressed in cells of the immune system and is essential for T cell activation. Inhibitors of CD45 therefore have the potential to be effective immunosuppressants, but may lack the side effects associated with cyclosporin and rapamycin whose targets (PP2B and TOR) are expressed in nearly all cells and tissues. This topic is discussed by Alexander (in Part 3).

A number of toxins and tumour promoters are potent inhibitors of several members of one of the major classes of protein serine/threonine phosphatases, termed the PPP subfamily. They include the marine toxins responsible for diarrhetic seafood poisoning (okadaic acid and related compounds) and the algal toxins that are a threat to water supplies (microcystins) (reviewed in MacKintosh and MacKintosh 1994). Indeed, microcystins are the most potent liver carcinogens known to man. One might therefore predict that compounds which inhibit the catalytic subunits of these protein phosphatases would frequently be oncogenic and of little use as therapeutic agents. However, as discussed by Honkanen (Part 3), both fostriecin and cantharidin, which inhibit the same protein phosphatases, are cytotoxic for tumour cells and have been tested in phase I human clinical trials as anti-cancer agents. Not surprisingly, there are a number of side effects associated with the use of these compounds, and it seems more likely that drugs will eventually be developed that disrupt the functions of protein serine/threonine phosphatases in more subtle and specific ways. For example, the ability of the serine/threonine-specific protein phosphatase 1 (PP1) to dephosphorylate many proteins is controlled by its interaction with a great variety of 'targeting' subunits that direct it to specific subcellular locations and confer unique regulatory properties upon it. The form of PP1 associated with liver glycogen, which dephosphorylates and activates glycogen synthase, com-

prises the catalytic subunit of PP1 complexed to a glycogen-targeting subunit G_L. The ability of the PP1–G_L complex to dephosphorylate glycogen synthase is prevented when the active form of glycogen phosphorylase (termed phosphorylase a) binds to the extreme C-terminus of G_L, providing a mechanism for inhibiting glycogen synthesis when glycogenolysis is activated and vice versa (Armstrong et al. 1998). A drug that prevented the interaction of phosphorylase a with G_L would have the potential to lower the concentration of glucose in the blood by activating glycogen synthase and so stimulating the conversion of glucose into liver glycogen.

1.1 The Problems

There are over 500 protein kinases encoded by the human genome, most of which are members of the same superfamily. This has created a plethora of potential targets that can be studied in a unified way, but has highlighted the difficulty in developing compounds that are capable of inhibiting one of these enzymes specifically. The development of Glivec has shown that inhibition of more than one protein kinase can sometimes be beneficial, allowing the same drug to have more than one therapeutic use. However, more frequently one would expect such a lack of specificity to give rise to unwanted or unacceptable side effects. The recent availability of large panels of protein kinases (e.g. Davies et al. 2000; Bain et al. 2003) has been of considerable help in assessing the specificities of protein kinase inhibitors, and it is to be expected that such panels will continue to expand and eventually include the entire repertoire of protein kinases.

Lack of specificity may also mean that the therapeutic effect of a drug is actually mediated by inhibition of another protein kinase and not by inhibition of the kinase for which it was originally developed. For example, inhibitors of the cell cycle regulator CDK2 have been developed that suppress the proliferation of tumour cells, but these compounds may actually exert their therapeutic effects by inhibiting other protein kinases, such as CDK7 and/or CDK9, which are regulators of RNA polymerase II. It is therefore unclear whether the effects of these compounds are really mediated via CDK2. In order establish that the therapeutic effect of a drug is mediated by inhibition of a particular protein kinase one needs to show that the effects of the drug disappear in cells that express a drug-resistant mutant of the protein kinase (Eyers et al. 1999). It is possible to convert protein kinases to drug-resistant forms by single amino acid replacements (Brown et al. 1995; Eyers et al. 1998) so that, as for other types of drug, the development of drug resistance is a potential hazard. Mutations in Abl that make it resistant to Glivec are the cause of relapse in patients with chronic myelogenous leukaemia (Gorre et al. 2001). However, resistance to Glivec is mainly seen in patients

with the most advanced stage of this disease, where extensive genomic instability has already taken place.

Most of the protein kinase inhibitors developed thus far target the ATP-binding site and must therefore be of sufficient potency to compete with the millimolar concentrations of ATP that are present in the intracellular milieu. Clearly, it is possible to develop compounds with the requisite in vivo potency, as shown by the number of compounds undergoing human clinical trials. However, this remains a challenging problem, especially for protein kinases that bind ATP particularly tightly. Some of the most interesting protein kinase inhibitors developed thus far, including Glivec (Schindler et al. 2000) and the p38 MAP kinase inhibitor BIRB 796 (Pargellis et al. 2002), not only target the ATP-binding site, but also trigger structural changes that induce the inactive conformations of these protein kinases. Two other compounds, PD 98059 and U0126, do not target the ATP-binding site at all, but bind to the inactive conformation of MKK1, preventing it from being activated by the protein kinase Raf (Alessi et al. 1995; Davies et al. 2000). The development of more compounds that prevent one protein kinase from activating another may be a promising strategy for novel drug development in this area, since many of these enzymes are components of protein kinase 'cascades'. Another way of generating compounds that are not ATP-competitive would be to target the binding sites for protein substrates, a topic discussed by Lawrence (in Part 1).

There are about 150 protein phosphatase catalytic subunits encoded by the human genome, and they fall into three main superfamilies. The generation of compounds that discriminate between different protein phosphatases is therefore also a challenging one. However, in contrast to protein kinases, the option of targeting an ATP binding pocket does not exist. Moreover, the protein substrate-binding cleft can be very polar, as in the case of PTP1B (Kellie 2003). This has made it difficult to develop compounds that combine high potency with cell permeability. The only protein phosphatase inhibitor that has advanced to human clinical trials, cyclosporin, inhibits PP2B in an unusual way; it binds to the protein cyclophilin, and the cyclosporin–cyclophilin complex then inhibits the protein phosphatase (Liu et al. 1991). As discussed earlier, it seems more likely that the future of drug discovery in this area may lie in targeting the regulatory subunits of serine/threonine-specific protein phosphatases.

Finally, it is important to mention that inhibitors of protein kinases are not only becoming important for the treatment of disease, but also as reagents for the study of cell signalling. The huge number of citations garnered by the publications that have introduced these compounds to the scientific community are a reflection of the widespread need for these compounds by the scientific community. For example, I was surprised to learn from the Institute for Scientific Information that the paper we published in 1995 with David Dudley and Alan Saltiel at Parke Davis on the mechanism

of action of PD 98059 (Alessi et al. 1995) was the UK's most frequently cited original research paper over the past 10 years in the fields of biology and biochemistry, while our publication with Peter Young and John Lee at SmithKline Beecham on the specificity of SB 203580 (Cuenda et al. 1995), a prototypic p38 MAP kinase inhibitor, was the UK's sixth most cited original research paper over this period. Although many compounds are advertised for sale as 'specific protein kinase inhibitors', in practice many have turned out to inhibit so many protein kinases that conclusions drawn from their use are likely to be erroneous (Davies et al. 2000; Bain et al. 2003). The number of really useful protein kinase inhibitors that are available commercially is still rather limited, but the number will increase considerably over the next few years. I believe that pharmaceutical companies have much to gain from the discoveries that will be made by exploiting these compounds, and it is to be hoped that many more will be released for general use in the future.

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