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Signal Transduction Inhibitors

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THE CONCEPT of employing elements of mitogenic signal transduction as targets for cancer chemotherapy originated from increasing knowledge of the mechanism of transformation by oncogenes. It has become clear that most oncogene products can be classified into three categories, representing (i) growth factors; (ii) growth factor receptors; or (iii) elements of growth factor signal transduction, including transcription factors. All these molecules are part of the information-transfer system regulating cellular proliferation. Malignant transformation can be explained as a dysfunction of signal transduction, resulting in autonomous growth where cells either generate their own growth-promoting stimuli or do not respond to growth-inhibitory signals. Agents which are able to counteract oncogene-induced growth stimulation may act potentially as tumour specific drugs. They could act by interference with autocrine cycles, blocking of constitutively active or overexpressed growth factor receptors, inhibition of constitutively active growth factor signal transduction elements or by restoring the function of deficient suppressor gene products.

Present activities along these lines include, in particular, development of growth factor antagonists, growth factor receptor blockers, and targets in growth factor signal transduction at the post-receptor level. Since overexpression of cellular *RAS* or expression of constitutively active *RAS* mutants are frequently correlated with human tumours, attempts to block ras functions will be discussed in some detail. We have recently found that expression of transforming *RAS* opens a plasma membrane Ca^{2+}-channel. This effect is ras-specific, essential for the mitogenic function of ras, and may be exploited to inhibit proliferation of *RAS*-transformed cells. *RAS* is also known to activate members of the protein kinase C (PKC) family in a variety of systems. This effect is also required for growth stimulation by *RAS*. Thus, PKC inhibitors are another potentially useful class of agents to block *RAS* transformed cells.

So far, clinical experience with drugs acting on elements of signal transduction is limited. Some phospholipid analogues introduced into the clinic several years ago are representatives of agents which act as signal transduction inhibitors. These compounds have been shown to inhibit PKC and phosphatidylinositol (PI) specific phospholipase C (PLC) at growth inhibitory concentrations [1, 2]. Hexadecylphosphocholine (HePC) is a prominent representative of this class of agents. With this compound as a lead substance, it was investigated whether these two effects (anti-PKC and anti-PLC) can be uncoupled with preservation of the anti-tumour effect. A further question concerned which effect is essential for the growth inhibitory activity, and which contributes to unwanted side-effects. Several new HePC-analogues were investigated including: octadecyl-[2-(N-methyl-piperidinio)-ethyl]-phosphate (D20133); octadecyl-(N,N-dimethyl-piperidinio-4-yl)-phosphate (D21266); octadecyl-[2-(trimethyl-arsonio)-ethyl]-phosphate (D21805); hexadecylphospho-L-serine (HePS) and hexadecylphosphono-L-serine (HePNS). Compounds D20133, D21266 and D21805 have been shown to combine enhanced anti-tumour activities with considerably lower general toxicities compared to HePC [3–5]. As HePC inhibits PKC competitively with regard to phosphatidylserine [1], HePS and HePNS (which more closely resemble phosphatidylserine) were expected to be stronger PKC-inhibitors. Studies revealed that all compounds inhibit PI specific PLC with IC_{50} values close the IC_{50} values for the anti-mitogenic effects. The inhibition of PLC leads to a reduced formation of thrombin-induced inositol-triphosphate (IP_3) and a subsequent depression of the agonist-mediated Ca^{2+}-signal. With the notable exception of HePS and HePNS, all compounds also act as PKC-inhibitors in cell free extracts and interfere with the TPA-induced activation of the Na^+/H^+-antiporter and *C-FOS* expression. Compounds lacking an anti-PKC effect, however, are equally potent inhibitors of the thrombin-induced progression of quiescent cells into S-phase. Thus, it is concluded that direct inhibition of PKC is not required for the antiproliferative activity of these agents. Compounds with lower general toxicities than HePC affect PLC and PKC with almost identical IC_{50} values. This indicates that the anti-PKC effect is not related to toxic side effects [10].

Signalling by PKC has also been shown to be implicated in the development of multidrug/resistance (MDR). Both expression of the *MDR-1* gene and the regulation of the activity of the *MDR-1* gene product, the p170 glycoprotein (Pgp), are in part regulated by PKC [6]. It appeared intriguing, therefore, to study the effect of a PKC inhibitor on *MDR-1* mediated drug resistance. The studies revealed that the PKC specific staurosporine derivative CGP41251 is indeed able to overcome resistance to doxorubicin and vinblastine in *MDR-1* overexpressing cells [7]. Depletion of phorbolester-responsive PKC isoforms by prolonged exposure to TPA, however, did not affect the modulatory activity of CGP41251. As CGP41251 predominantly affects the Ca^{2+}-sensitive PKC isoforms [8], which are depleted by TPA, the data strongly suggest that the modulation of drug resistance by the PKC inhibitor CGP41251 is not related to PKC inhibition. Bryostatin is another highly specific PKC modulator and currently in clinical trial as an antitumour agent. Bryostatin acts as an activator of PKC in short-term experiments, but causes a PKC depletion after chronic exposure [9]. In order to evaluate the contribution of PKC in

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drug resistant *MDR-1* transfected HeLa cells, cultures were PKC-depleted by prolonged exposure to bryostatin. This resulted in reduced activity of the Pgp as determined by the efflux of the Pgp substrate rhodamine 123. However, bryostatin was equally efficient in reducing the rhodamine efflux in experiments, where cells were exposed to the drug for 10 min, a condition which activates PKC and does not yet deplete the enzyme, or in cells where PKC was depleted by prior treatment with TPA. These findings suggest that bryostatin acts independently of PKC, probably by a direct interaction with the Pgp.

Thus, inhibitors of enzymes involved in signal transduction appear to be useful in tumour chemotherapy, but in some cases, the precise targets of the putative signal transduction modulators inside the cells still have to be elucidated.

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Retinoids in Oncology

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VITAMIN A (RETINOL) is an essential component of our diet and deficits can lead to visual and fertility problems, as well as loss of integrity of epithelia and mucus secreting cells [1]. Retinoids are vitamin A congeners and have changed the therapy of many skin diseases, such as acne and psoriasis [2, 3]. Their use in oncology stems back to the work of Rowe and Gorlin, Bollag and Lotan who showed their efficacy in preclinical models, developed thousands of derivatives and determined their anti-proliferative action [3, 4].

Retinoids are absorbed from the gastrointestinal mucosa in the form of chylomicrons (although some synthetic forms pass directly into portal circulation) and delivered from liver storage bound to retinol-binding proteins. Their growth modulation and differentiating action on cells which absorb them (disputed mechanism) is through modulation of gene expression, where nuclear retinoic acid receptors (RARs: alpha, beta, gamma) play a key role [5]. Resistance to the effects of retinoids seems to be a multi-step process. For all-*trans* retinoic acid, at least, this seems to be mainly the result of accelerated catabolism of the agent through cytochrome-P450 induction, increased oxidative cofactors and increased expression of cellular retinoic acid binding proteins (CRABP), all of which will ultimately lead to decreased effective concentrations of the retinoid.

All-*trans* retinoic acid and 13-*cis* retinoic acid are naturally occurring retinol derivatives, and many others (e.g. fenretinide (4-HPR), etretinate, acitretin) have been synthesised. These agents differ in their pharmacological properties in terms of bioavailability, half-life and spectrum of toxicity. Skin and mucous membrane toxicity is usually the limiting factor in the clinic; central nervous system toxicity can be manifested by headaches or even pseudotumour cerebri syndrome. Abnormal lipid metabolism occurs, and hepatic toxicity is common. Cardio-respiratory toxicity syndromes have been described in the treatment of promyelocytic leukaemia (PML) with all-*trans* retinoic acid [6].

PML is the first disease in oncology for which retinoids have been shown to be effective, although most responses are transitory. Acute promyelocytic leukaemia is an interesting model as there is a translocation which disrupts the gene encoding for RAR alpha, which fuses with the PML gene on chromosome 15. Sporadic reports on the use of retinoids in other haematological malignancies have appeared, and possibly juvenile chronic myelogenous leukaemia is a target for the use of 13-*cis* retinoic acid.

Initial attempts at treatment of solid tumours with retinoids have not been successful, but the combination of 13-*cis* retinoic

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