Prospects for the treatment of endocrine-responsive tumours

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Abstract

Much has been achieved over the last 30 years to improve the treatment of hormone-dependent cancer of the breast, ovary and prostate. The development of the antioestrogen tamoxifen (Nolvadex) spearheaded a range of drugs that counter the growth-promoting action of the female and male sex steroid hormones. An important additional benefit of endocrine therapies has been their low toxicity compared with conventional cancer chemotherapy thereby providing effective treatment with few serious side-effects and a sustained quality of life. Although some currently available therapies improve patient disease-free survival and overall survival, particularly when given in an adjuvant setting, they are not cures. There is, therefore, a continued need to develop newer therapies that extend the effectiveness of those currently available. This is particularly important when tumours either fail to respond or develop resistance to endocrine therapy. In this review, we examine how our improved understanding of the factors that influence the progression of endocrine-related tumours is leading to the development of novel therapies to treat both hormone-dependent and -independent tumours.

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The success of endocrine therapies

Antioestrogens

Although the adverse effect of oestrogens on breast cancer progression was recognised at the end of the last century, it was only 30 years ago that a low molecular weight oestrogen antagonist was identified and subsequently developed successfully for the treatment of breast cancer (Harper & Walpole 1966). The availability of pharmaceutical agents which were later shown to achieve the same clinical benefit as oophorectomy was a revolution in the management of hormone-dependent breast cancer. More recently, the identification of the breast cancer susceptibility genes BRCA1 and BRCA2 has increased our awareness of high-risk groups and led to a greater prevalence in the use of diagnostic tests to predict the risk of developing breast cancer. This has understandably produced greater pressure from women's health groups to extend the use of pharmaceutical agents into preventative medicine in order to avoid traumatic preventative surgery such as a double mastectomy.

Much has been learnt since the early 1960s about the nature of the oestrogen receptor (ER) and the molecular mechanism by which it regulates gene expression but mysteries still remain. For example, it is still unclear

which oestrogen-regulated factors are critical in promoting the growth of breast cancer epithelial cells. Tamoxifen was developed as a non-steroidal, orally active antioestrogen, initially as a contraceptive agent, but was recognised as having potential benefit to treat hormone-responsive breast cancer patients (Harper & Walpole 1966). Antioestrogens act by competing with 17β -oestradiol for binding to a site within the ER ligand-binding domain (Fig. 1). Whereas the binding of oestradiol alters the conformation of the ligand-binding domain to promote the interaction with other transcription factors, the binding of antioestrogen prevents the formation of the active conformation (Brzozowski *et al.* 1997, White & Parker 1998). It has become fashionable to describe partial agonists, such as tamoxifen and raloxifene, as SERMs (selective estrogen receptor modulators) since it is felt that this better describes their antagonist effect in some target tissues, such as the breast, and agonist effect in others, such as bone (Grese & Dodge 1998). One possible explanation as to why tamoxifen is a partial agonist is that the drug inactivates only one of the two regions within the ER responsible for the activation of gene transcription (Green & Chambon 1988, McInerney *et al.* 1998). Furthermore, non-classical oestrogen response elements have been identified, termed raloxifene response ele-

Figure 1 Endocrine therapy. Oestrogens and androgens are important mitogens promoting the growth of hormone-dependent breast and prostate cancer respectively. Total oestrogen blockade is achieved by the combination of an LHRH agonist to suppress the release of LH, an aromatase inhibitor to suppress the production of oestrogens in post-menopausal patients and an antioestrogen to antagonise the action of oestrogens on the receptor. Total androgen blockade is achieved by the combination of an LHRH agonist and an antiandrogen. AR, androgen receptor.

ments, in the promoters of genes that are the targets of the agonist effect of SERMs in bone (Yang *et al.* 1996). The picture is even further complicated by the discovery of a second oestrogen receptor (ERβ) and the finding that tamoxifen, raloxifene and the pure anti-oestrogen ICI 164,384 are pure antagonists of ERβ when measured using classical oestrogen response elements (Barkhem *et al.* 1998, McInerney *et al.* 1998). Despite the complexity of selectively modulating ER activity, a number of tamoxifen-derived SERMs, such as arzoxifene (LY 353,381), droloxifene, toremifene and idoxifene are being developed. Some of these have been used to treat osteoporosis in post-menopausal women, but increasingly are being considered as alternatives to tamoxifen for the treatment of breast cancer. The hope is that such alternatives will be as effective as tamoxifen in treating

breast cancer but with a reduced risk of developing endometrial cancer. Clinical trials, such as STAR (Study of Tamoxifen and Raloxifene), are being planned that are specifically designed to establish the efficacy and safety of alternative SERMs to treat patients with breast cancer.

Extensive clinical use has demonstrated tamoxifen to be a well-tolerated and effective agent producing an objective response in approximately a third of unselected treated patients with advanced breast cancer, and disease stabilisation in a further 20% (Baum 1997). In the adjuvant setting, tamoxifen is currently the endocrine therapy of choice, irrespective of age or menopausal status, with increasing benefit observed as the length of treatment is increased to at least 5 years (Early Breast Cancer Trialists' Collaborative Group 1998). Because the action of antioestrogens is mediated by the ER, it is not surprising

that ER-positive tumours are most responsive to therapy (Bezwoda *et al.* 1991), although a small rate of response is seen in apparently ER-negative tumours. As the risks and benefits of treatment with tamoxifen have become established, the use of the drug has now been extended beyond the adjuvant setting to include prophylactic use in healthy women who are considered to be at risk of developing breast cancer. The results of these prevention studies have been controversial, with the National Surgical Adjuvant Breast and Bowel Project trial in the USA being stopped early because of clear evidence of a benefit with tamoxifen treatment (Fisher *et al.* 1998) whilst other smaller studies have failed so far to demonstrate a significant benefit (Powles *et al.* 1998, Veronesi *et al.* 1998). It is likely that differences in the selection of patients and definition of 'high' risk contribute to this apparent discrepancy. Further studies are required to establish whether patients who are genetically predisposed to developing breast cancer will also benefit from the early use of tamoxifen.

Eventually, many patients with primary breast cancer treated with tamoxifen will relapse (Rutqvist 1998). The reasons why tumours develop resistance to tamoxifen are unknown but may be related to the partial agonist action of tamoxifen-stimulating growth. Alternatively, it is possible that genetic or epigenetic changes within the tumour activate hormone-independent mitogenic pathways; for example it has been observed that c-erbB2 is expressed in a greater proportion of patients who relapsed early (Houston *et al.* 1999). Support for the first proposal, that with time tamoxifen can begin to stimulate the growth of tumours, comes from both pre-clinical and clinical studies using pure antioestrogens such as ICI 182,780 (Faslodex) and EM-800 (England & Jordan 1997). In a small phase II study ($n=19$) with ICI 182,780, approximately two thirds of the patients who relapsed on tamoxifen therapy responded to treatment with the pure antioestrogen with a median duration of response of 26 months (Howell *et al.* 1996). Interestingly, it has been demonstrated in model systems that additional mitogenic signal transduction pathways are able to cross-talk with the oestrogen receptor. In one example, activation of the epidermal growth factor receptor (EGFR) stimulates the MAP kinase signalling pathway that leads to ER phosphorylation and activation even in the absence of oestrogen (Kato *et al.* 1995, Bunone *et al.* 1996). Importantly, the same authors demonstrate that pure antioestrogens such as ICI 182,780, but not tamoxifen, block growth factor-induced ER activity.

A second more recent example is the activation of ER by cyclin D1 in the absence of oestrogen (Neuman *et al.* 1997, Zwijsen *et al.* 1997). Cyclin D1 is overexpressed in approximately half of breast cancer cases (see cell cycle inhibition below), suggesting that the elevation of this G1

phase cyclin may stimulate breast cancer growth through the ER, in addition to its more classical role as an activator of cyclin-dependent kinases (CDK). Notably, the expression of cyclin D1 potentiates the effect of oestrogens (Zwijsen *et al.* 1997). Studies employing oestrogen-responsive reporter genes suggest that tamoxifen and pure anti-oestrogens can completely (Neuman *et al.* 1997) or partially (Zwijsen *et al.* 1997) block the ligand-independent action of cyclin D1. Furthermore, studies in cells engineered to overexpress cyclin D1 indicate that antioestrogens can inhibit the growth of these cells (Pacilio *et al.* 1998). These studies therefore suggest that the increased expression of cyclin D1 is unlikely to account for the development of resistance to tamoxifen although it is conceivable that high cyclin D1 levels could reduce the effect of tamoxifen.

Other recent studies have shown that aspartate 351 within the ER ligand-binding domain makes a critical contact with the side chain of the antioestrogen raloxifene (Brzozowski *et al.* 1997). Intriguingly, the mutation of residue 351 to tyrosine breaks the key interaction with raloxifene and converts this antagonist into an agonist (Levenson & Jordan 1998). One interpretation of these studies is that antioestrogens, such as raloxifene and tamoxifen, use the interaction with aspartate 351 to prevent the transcriptional activation region (AF2) within the ER ligand-binding domain from adopting a conformation where it can bind to additional co-activator transcription factors. Therefore, it is possible that ER mutations that affect aspartate 351 directly, or influence the conformation of AF2, could be selected for in the tumours of relapsed patients, although no such mutations have been identified to date.

An alternative epigenetic mechanism to explain the conversion of tamoxifen-sensitive tumours to resistance is that there is an increase in the concentration of ER co-activators that stabilise the ER in the active conformation (Levenson & Jordan 1998). Importantly, the activity of the pure antioestrogen ICI 182,780 appears to be independent of aspartate 351 (Levenson & Jordan 1998), possibly because the bulky alkyl side chain of ICI 182,780 prevents the ER adopting the active conformation or because this drug can prevent ER dimerisation and/or promote ER degradation (White & Parker 1998). This distinct mechanism of action may explain why ICI 182,780 is effective in producing a response in patients who have relapsed on tamoxifen and could provide an advantage over other antioestrogens (such as raloxifene) that are dependent upon maintaining the ER ligand-binding domain in the inactive conformation.

Antiprogestins

One of the actions of oestrogens is to increase the level of the progesterone receptors (PR) and the presence of

receptors for both steroid hormones in a breast tumour predicts a high response rate to endocrine therapy (McGuire 1978). Although the co-expression of both receptors is thought simply to indicate that the ER is functional, antiprogestins, such as mifepristone (RU-486), inhibit the growth of breast cancer cell lines *in vitro* (Bardon *et al.* 1985) suggesting a direct role for the PR in promoting the growth of breast cancer. *In vivo* studies, utilising oestrogen-primed MCF-7 xenografts in nude mice, have demonstrated that monotherapy with tamoxifen, mifepristone or an alternative antiprogestin onapristone, reduces tumour growth (El Etreby & Liang 1998). Furthermore, the combination of tamoxifen with mifepristone prevented tumour growth and delayed or prevented tumour escape. These studies suggest that such agents may be beneficial in treating breast cancer patients.

Mifepristone and onapristone have been evaluated in the clinic as first line therapy for primary breast cancer (Perrault *et al.* 1996, Robertson *et al.* 1999). Partial responses were observed in 10.7% of PR-positive breast cancer patients who had received no prior therapy when treated with 200 mg mifepristone daily (Perrault *et al.* 1996). Better responses have been observed with onapristone where 56% of 18 breast cancer patients showed a partial response, and 11% showed stable disease after receiving 100 mg daily (Robertson *et al.* 1999). A complication in the use of these agents to treat breast cancer is that they also possess antiglucocorticoid activity (Van Look & Hertzen 1995) and are associated with liver function abnormalities (Robertson *et al.* 1999). Newer agents are being developed that have reduced antiglucocorticoid activity (Wagner *et al.* 1999) and it remains to be seen whether more selective antiprogestins will be beneficial in the treatment of breast cancer.

Aromatase inhibitors

In post-menopausal women, a major amount of oestrogen is generated by the peripheral conversion of androstenedione to oestrone by the p450 enzyme aromatase. The enzyme is found in a variety of tissues, such as fat, stromal tissue and in breast tumours themselves (Santen *et al.* 1997), and contributes significantly to levels of circulating oestrogens in these patients. A second source of oestrogens in post-menopausal women is the local production of oestrone from oestrone sulphate, which is present at higher levels within the tumour compared with normal tissue or plasma (Pasqualini *et al.* 1997). The enzyme responsible for this conversion, oestrone sulphatase, is present in a greater proportion of breast tumours than aromatase and it is suggested that it is responsible for a greater production of oestrogens (Santen *et al.* 1986). In both cases, oestrone is converted to 17 β -oestradiol within the tumour by 17 β -hydroxysteroid dehydrogenase (17 β -HSD). It has therefore been proposed that inhibitors that

block aromatase, oestrone sulphatase or 17 β -HSD would be effective in controlling the local production of oestrogens in postmenopausal patients.

Traditional second line therapies for the treatment of hormone-dependent tumours that have become refractory to tamoxifen have included high dose progestins, such as megestrol acetate, and non-selective aromatase inhibitors such as aminoglutethimide. Potent and selective new generation aromatase inhibitors such as anastrozole (Arimidex), letrozole, exemestane and vorozole that prevent the peripheral synthesis of oestrogens (Fig. 1) have recently been developed and have been shown to provide clinical benefit as second line therapy in post-menopausal patients (Dowsett 1997).

In clinical trials with post-menopausal patients who have relapsed on tamoxifen therapy, second generation aromatase inhibitors have demonstrated similar response rates to megestrol acetate but with improved duration and patient survival and decreased side effects, in particular less weight gain (Buzdar *et al.* 1998, Dombernowsky *et al.* 1998). Anastrozole, however, has shown a significant survival benefit compared with megestrol acetate (Buzdar *et al.* 1998). Other trials have shown letrozole to be superior to aminoglutethimide (Gershanovich *et al.* 1998). These agents are, therefore, of proven value as second-line therapy in treating post-menopausal patients with hormone-dependent cancer who have relapsed after treatment with tamoxifen. An assessment of the relative merits of anastrozole and letrozole will require a direct head-to-head comparison of the two drugs. Continuing clinical trials also aim to assess the value of different aromatase inhibitors versus antioestrogens as first-line therapy for post-menopausal patients (Blamey 1997, Harvey 1998) and as adjuvant therapy in the treatment of early disease (Lønning 1998).

A variety of inhibitors of both oestrone sulphatase (Li *et al.* 1998) and 17 β -HSD (Tremblay & Poirier 1998) have been identified and indeed many progestins inhibit both enzymes (Pasqualini *et al.* 1998). However, whether such agents will find a place in the clinic is perhaps questionable, given that the plasma level of oestrone and oestrone sulphate is effectively controlled in patients treated with aromatase inhibitors (Lønning *et al.* 1997, Geisler *et al.* 1998).

Gonadotrophin-releasing hormone agonists

Luteinising hormone-releasing hormone (LHRH) agonist analogues were developed to block follicular activity by desensitising the pituitary gland to LHRH. The resulting reduction in LH in pre- and peri-menopausal women leads to a reduction in the production of oestrogens from the ovaries, and in men to a decrease in the production of testosterone by the testis (Fig. 1; Furr 1989). Drugs such as goserelin (Zoladex), buserelin and leuprolide are

modified peptides, which can be administered as slow-release, long-term, 1 or 3 monthly depots, with the advantage over surgical castration in that treatment can be reversed. Clinical trials with these agents have demonstrated them to be effective in 30-60% of pre-menopausal patients with advanced breast cancer (Blamey *et al.* 1992, Dowsett *et al.* 1992). Furthermore, consistent with the mechanism of action, responses are much better in pre- rather than post-menopausal patients (Dowsett *et al.* 1992). Treatment with LHRH agonists lowers plasma oestradiol to post-menopausal levels and treatment with goserelin monthly depot has been shown to be as effective as oophorectomy in improving both disease-free and overall survival in pre-menopausal women with advanced breast cancer (Taylor *et al.* 1998). Recently, a meta-analysis of four randomised trials, including 506 patients, tested the hypothesis that 'complete oestrogen blockade' by combined LHRH agonist plus tamoxifen treatment is more effective than medical ovarian suppression alone (Klijn *et al.* 1998). The combination treatment was superior to LHRH agonist alone with respect to objective response and both progression-free and overall survival, indicating that the combined treatment modality should be proposed as the new standard treatment in pre-menopausal women with advanced breast cancer. Currently, trials are also being performed to evaluate the effect of increasing oestrogen blockade further by the addition of an aromatase inhibitor, an LHRH agonist and tamoxifen in pre-menopausal women (Blamey 1997). Finally, the LHRH analogue, goserelin, is currently being investigated in an extensive adjuvant clinical trial programme, particularly in comparison with cytotoxic therapy (CMF: cyclophosphamide, methotrexate and 5-fluorouracil) (Kaufmann 1998) since the effect of CMF in pre-menopausal patients may be mediated by ovarian suppression (Boccardo *et al.* 1996, Jonat 1998).

LHRH antagonists have been developed more slowly because early compounds lacked sufficient potency and induced histamine release and local inflammation that could be severe (Schmidt *et al.* 1984, Weinbauer & Nieschlag 1992, Reissmann *et al.* 1995, Schally & Comaru-Schally 1997). More recently, potent LHRH antagonists such as aborelix, have been developed which have little local irritancy potential and can be delivered in depot preparations, although durations of more than one month may be difficult to achieve (Menon *et al.* 1998). Such compounds have the advantage that they will produce an immediate fall in serum LH and sex steroid hormones in men and women and so will avoid any potential for an initial stimulatory hormone response that can occur, particularly in men, with LHRH agonists. However, since they are competitive antagonists, adequate blood levels must be present continuously to prevent access of LHRH to the receptor. This places severe

demands on delivery systems. In contrast, LHRH agonists induce LHRH receptor down-regulation, which recovers more slowly on withdrawal, and so specifications for delivery are likely to be less demanding: this might lead to fewer treatment failures or breakthroughs with LHRH agonists. The results of current phase III trials and direct comparisons with LHRH agonist depots will determine the future place of LHRH antagonists in the therapy of breast and prostate cancer.

Androgen blockade to treat prostate cancer

The demonstration in the early 1940s that prostatic cancer is dependent upon androgens (Huggins & Hodges 1941) has led to the development of increasingly sophisticated regimes to treat patients with advanced prostate cancer. Surgical castration is one means to control prostate cancer by reducing the level of circulating androgens. LHRH agonists suppress LHRH receptor content of the pituitary gland and consequently LH secretion and can, therefore, be used in men to achieve chemical castration to treat advanced metastatic prostate cancer (Furr 1989). Goserelin monotherapy is as effective as surgical castration in the treatment of prostate cancer (Kaisary *et al.* 1991, Vogelzang *et al.* 1995) with response rates of around 85% but with the advantage of any adverse effects being reversible.

By analogy with the treatment of breast cancer, non-steroidal pure antiandrogens such as flutamide, nilutamide and bicalutamide (Casodex) have been developed that antagonise the action of androgens at the level of the androgen receptor (AR) (Fig. 1; Furr 1996). One use of antiandrogens has been to suppress the effect of any initial stimulatory response in patients treated with LHRH analogues, since these agents produce a transient stimulation in LH levels with consequent increase in the production of testosterone (Waxman *et al.* 1985). The use of bicalutamide (150 mg daily) alone has been demonstrated to provide a similar survival outcome to surgical castration in non-metastatic advanced prostate cancer patients but with the significant advantage of improved sexual interest and physical capacity (Iversen *et al.* 1998). Continuing clinical trials with bicalutamide as monotherapy will determine the full advantages of the drug both in locally advanced and metastatic disease as well as in early prostate cancer.

A major use of antiandrogens has been in combination with LHRH analogues to achieve complete androgen blockade. This is because androgens, such as androstenedione and dehydroepiandrosterone, are produced by the adrenals and possess about a fifth of the biological activity of testosterone. Moreover, to a small extent these androgens are converted to testosterone in peripheral tissue. LHRH analogues are unable to reduce the levels of these androgens and, therefore, the combination of an

antiandrogen with an LHRH analogue may provide a more effective therapy (Labrie *et al.* 1993, Schellhammer *et al.* 1997a, Sarosdy *et al.* 1998).

More recently a major comparative study of surgical castration versus surgical castration combined with flutamide has failed to show any advantage for the combination (Eisenberger *et al.* 1998), which has caused considerable controversy. Interestingly, the actual time to treatment failure and survival are similar for the combination therapy of LHRH with flutamide or surgical castration with flutamide. However, there is a major difference in time to treatment failure and survival between LHRH agonist treatment and surgical castration in these studies, which was not found in a direct comparison of medical and surgical castration alone (Kaisary *et al.* 1991, Vogelzang *et al.* 1995). Such a difference in response to equivalent standard therapy might indicate different patient selection and this could explain the observed differences between this and other maximal androgen blockade trials. A meta-analysis (Prostate Cancer Trialists' Collaborative Group 1995) of combination of LHRH agonist and antiandrogen showed a non-significant trend in favour of combination therapy. However, this analysis has been criticised (Klotz & Newman 1996). There is a lack of consensus about the merits of this analysis since it includes many small studies and a range of antiandrogens, including cyproterone acetate which may have stimulatory activity in some tumours. Moreover, other meta-analyses have shown a significant benefit for maximal androgen blockade (in respect of objective response, risk of progression and overall survival) (Bertagna *et al.* 1994, Caubet *et al.* 1997).

Intermittent androgen withdrawal therapy has also been proposed in the treatment of prostate cancer with the objective of preventing the development of tumour resistance to endocrine therapy (Bruchovsky *et al.* 1997). Cycles of therapy are given to achieve maximal response with therapy reinitiated when tumour escape occurs. Such therapy has the advantage of periods of drug withdrawal and restoration of normal sexual function. This creative concept has been used to good effect in open trials (Bruchovsky *et al.* 1997), but requires large randomised trials against continuous comparable therapy to determine whether survival advantages can be demonstrated.

There has been considerable controversy over the relative potency of some of the pure antiandrogens, with Luo *et al.* (1996) championing the superiority of flutamide. Furr (1997), in reviewing the data, has concluded that the majority of preclinical and clinical studies confirm the increased potency and tolerability of bicalutamide.

Clinical studies have demonstrated that bicalutamide is at least as effective as flutamide when used in

combination with an LHRH agonist (Schellhammer *et al.* 1997a) and that the combination of leuprolide with flutamide is significantly less effective than the combination of goserelin or leuprolide with bicalutamide (Sarosdy *et al.* 1998). When compared with flutamide, bicalutamide also has a superior tolerance profile (Schellhammer *et al.* 1997a).

The major side-effects of LHRH agonists are loss of sexual activity and hot flushes and for antiandrogens, gynaecomastia is the most frequent side-effect. It will be interesting to see whether sildenafil has any role to play in restoring libido in surgically or medically castrated patients and whether the use of aromatase inhibitors will prevent gynaecomastia. It is also very important to know if the addition of aromatase inhibitors to pure antiandrogens will cause a further significant increase in circulating androgens, that could compromise anti-tumour efficacy, before any recommendations can be made about its value in the prostate cancer setting. Such trials are in progress.

Inhibitors of the enzyme 5 α -reductase, such as finasteride, have also been developed that prevent the conversion of testosterone into the 5-10 times more potent androgen 5 α -dihydrotestosterone (DHT). Finasteride is used to treat benign prostate hyperplasia (Gormley *et al.* 1992). However, inhibiting 5 α -reductase does not reduce the concentrations of testosterone and, due to the lack of feedback inhibition, could lead to an increase in testosterone concentrations (Lamb *et al.* 1992, Presti *et al.* 1992). Recent studies have, therefore, suggested that finasteride monotherapy is inadequate for the treatment of advanced prostate cancer (Ornstein *et al.* 1996). It is possible that the use of a 5 α -reductase inhibitor would decrease the production of DHT from adrenal androgens in patients treated with an LHRH agonist. Trials to combine a 5 α -reductase inhibitor with an antiandrogen in order to maintain sexual function have had some success (Fleshner & Trachtenberg 1993). Nevertheless, increases in serum testosterone were observed and those with the highest increase developed gynaecomastia. It remains to be established whether the addition of a 5 α -reductase inhibitor to the combination of LHRH agonist and antiandrogen therapy will provide any additional benefit.

Antiandrogen withdrawal can lead to further tumour responses. It is likely that some of these responses are due to mutation in the androgen receptor because both steroidal and non-steroidal antiandrogens have been shown to act in a stimulatory manner as agonists at a number of mutant receptors (Veldscholte *et al.* 1992, Sharief *et al.* 1995, Bubley & Balk 1996). Antiandrogen withdrawal responses have also been seen with bicalutamide, although less frequently than with flutamide (Dupont *et al.* 1993, Scher & Kelly 1993, Sartor *et al.* 1994, Small & Carroll 1994, Figg *et al.* 1995, Liebertz *et al.* 1995, Nieh 1995, Small & Srinivas 1995,

Schellhammer *et al.* 1997b). However, bicalutamide, unlike flutamide, still inhibits the growth of cell lines expressing mutant receptors and so mutation of these receptors is unlikely to be the complete explanation for androgen withdrawal responses (Veldescholte *et al.* 1992, Culig *et al.* 1994, Peterziel *et al.* 1995, Bubley & Balk 1996).

Prospects for treating hormone non-responsive endocrine tumours

Some ER-positive tumours fail to respond to endocrine therapy or eventually develop resistance. Furthermore, approximately a third of breast cancer patients are ER negative and are, therefore, far less likely to respond to endocrine therapy. The treatment of hormone-independent tumours has not, thus far, been as successful as the treatment of those that remain responsive to hormones. The current options available include radiation and combination chemotherapy with, for example, anthracyclines, anti-tumour antibiotics, vinca alkaloids and more recently platins and taxanes. Our increasing knowledge of the genetic changes that occur as tumours develop and progress has uncovered a web of oncogenes and tumour suppressor genes that together conspire to transform a normal hormone-regulated epithelial cell into one that can grow and spread independently from the host regulatory signals. Some of the changes identified to date may offer alternative approaches to control the growth of hormone-independent tumours but with the advantage of a toxicity profile closer to that of the endocrine therapies than the current poorly selective chemotherapy approaches.

Retinoids

Retinoids have been known for some time to play a crucial role in regulating the differentiation and proliferation of epithelial cells, and are also potent inducers of apoptosis. Furthermore, there are many cellular and animal studies to indicate that retinoids are effective in preventing or suppressing tumour growth (Gottardis *et al.* 1996a). The effects of retinoids appear to be mediated by nuclear retinoid receptors that regulate specific gene expression due to their interaction with specific DNA response elements. Broadly two types of retinoid receptor have been identified; retinoic acid receptors (RAR) and retinoid X receptors (RXR). The retinoid, 9-cis retinoic acid, binds to both types of receptor whereas all-trans retinoic acid is selective for RAR. As a further level of complexity, there are three subtypes of RAR and RXR termed alpha, beta and gamma; each exists in a variety of alternatively spliced forms with different patterns of tissue expression. Finally, the functional retinoid receptor consists of a heterodimer between an RAR and an RXR and the active

form of other nuclear hormone receptors consists of a heterodimer with RXR (Mangelsdorf *et al.* 1993).

Evidence is beginning to emerge that retinoids may have utility in the treatment of cancer in the clinic. Tretinoin (all-trans retinoic acid) has been approved in the USA for acute promyelocytic leukaemia, a condition that involves the t(15;17) chromosomal translocation between the PML gene and that for RAR α (Gillis & Goa 1995). The retinoid N-(4-hydroxyphenyl) retinamide (4 HPR; fenretinide) is currently being evaluated for the prevention of breast and prostate cancer. The results thus far (in prostate cancer) have not, however, been encouraging. In a small study involving 22 patients, 8 patients with negative biopsies at the start of the trial were found to be positive by the end of 12 months resulting in the closure of the study (Pienta *et al.* 1997).

9-Cis-retinoic acid (Panretin; LGD 1057) is a more potent inhibitor of mammary carcinogenesis induced by N-nitroso-N-methylurea (NMU) than all-trans retinoic acid in rats (Anzano *et al.* 1994). Furthermore, its combination with low levels of tamoxifen was particularly effective, with a doubling in the number of animals that were tumour free at autopsy and significantly diminished tumour numbers and tumour burden. In phase I clinical trials, the dose-related toxicities associated with 9-cis retinoic acid were headache (3/41) and skin toxicity (11/41), manifested as mild and limited to skin dryness and erythema, but no tumour responses were observed (Rizvi *et al.* 1998a). Topical use of the compound has been demonstrated to be effective in approximately a third of patients with Kaposi's sarcoma. Phase II trials should be in progress to evaluate 9-cis retinoic acid in breast and prostate cancer and the National Cancer Institute (NCI) are sponsoring trials to examine the combination of tamoxifen with 9-cis retinoic acid.

More selective retinoid receptor ligands have been developed. For example, LGD 1550 is a potent (1-4 nM) alpha, beta and gamma RAR-selective agonist (Shalinsky *et al.* 1997). In tumour xenograft models, the compound was very effective in inhibiting growth of a squamous cell carcinoma, but no objective tumour responses were observed in a phase I/II dose-ranging trial in 25 advanced cancer patients (Rizvi *et al.* 1998b). LGD 1069 (Targretin) is a highly selective RXR agonist with low affinity for RARs. Like 9-cis retinoic acid, LGD 1069 prevents the formation of tumours in the NMU-initiated rat model (Gottardis *et al.* 1996b) and the combination of LDG 1069 and tamoxifen was more effective than either agent alone (Bischoff *et al.* 1998). In phase I studies with LGD 1069, liver enzyme changes were the most common dose-limiting adverse effects (Miller *et al.* 1997). Less prominent reactions included hypertriglyceridaemia and hypercalcaemia and it would be interesting to learn whether these effects are mediated by the interaction of

Figure 2 Defective signalling pathways in breast and prostate cancer. Cellular proliferation in tumours is induced by deregulated signalling pathways that increase the activity of cyclin-dependent kinases. Some of the pathways increase the level of cyclin D, an activator of CDK4 and CDK6, whereas others modulate the activity of CDK2 by decreasing the level of CDK inhibitors such as p15^{INK4B} and p27^{Kip1}. The net effect is accelerated entry into the cell cycle and a lower dependency on exogenous growth factors. A variety of receptor antagonists and kinase inhibitors that block these aberrant signalling pathways are being evaluated in the clinic.

RI/RII, receptor I and II; PDGFR, platelet derived growth factor receptor.

RXR with other nuclear hormone receptors. Notably, the characteristic retinoid toxicities, such as cheilitis, headache, and myalgias/arthralgias, were mild or absent and responses were observed in patients with cutaneous T-cell lymphoma. Clearly, further clinical studies are required to evaluate these interesting new compounds. Time will tell whether retinoids will be effective agents against advanced solid tumours and whether they will be sufficiently well tolerated to consider their use for tumour prevention, as is suggested by a large number of pre-clinical studies.

Aberrant growth factor signalling

A variety of aberrant signal transduction pathways have been implicated in the growth of human tumours and a variety of drugs are being developed to block these pathways (Fig. 2). The epidermal growth factor (EGF) receptor family comprises a small number (erb-B1 to erb-B4) of trans-membrane receptor tyrosine kinases that appear to play an important role in growth of endocrine-responsive tumours. EGF and transforming growth factor α (TGF) are mitogens for a variety of epithelial cells that bind and activate the EGF receptor (EGFR or erb-B1). In some cases, the tumour itself is capable of expressing EGFR ligands thereby providing an autocrine growth

signal (Sherwood & Lee 1995). The EGF receptor is overexpressed in a wide range of human epithelial tumours including those of the breast (Harris *et al.* 1992) and prostate (Sherwood & Lee 1995). With respect to breast cancer, it is noteworthy that the expression of EGFR is inversely correlated with ER expression (Harris *et al.* 1992) and associated with a poorer prognosis. Additional members of the EGFR family have also been implicated in tumorigenesis; the erb-B2 gene (the neu oncogene) is amplified in a small proportion of breast and ovarian cancers and is similarly associated with a poor prognosis (Slamon *et al.* 1989) and erb-B3, a receptor for heregulin, is overexpressed in a variety of solid tumours (Gullick 1996). Ligand binding promotes receptor homo-dimerisation and activation of the intracellular tyrosine kinase (Honegger *et al.* 1989). In addition, hetero-dimerisation can occur so that, for example, ligand bound EGFR can activate the intracellular domain of erb-B2 (Gulliford *et al.* 1997). Once activated, the tyrosine kinase domain of a receptor phosphorylates specific sites on the intracellular domain of the partner receptor creating phospho-tyrosine motifs that are specifically recognised by Src homology domain 2 (SH2) domains within additional signal transduction proteins (Moran *et al.* 1990). As a result of the interaction, these proteins are recruited to the cell membrane initiating a cascade of signals that ultimately can lead to cell division.

Developing agents that antagonise EGFR action may, therefore, be particularly effective against hormone-independent breast and prostate cancer and several such compounds are now being evaluated in clinical trials. One approach has been to use monoclonal antibodies that bind to the extracellular domain of the EGFR (Modjtahedi *et al.* 1996) or erb-B2 (Baselga *et al.* 1998). An antibody to EGFR (C225) is in phase I/II trials in breast cancer in combination with paclitaxel and with doxorubicin for advanced prostate cancer. The efficacy of a recombinant humanised monoclonal antibody against erb-B2 (trastuzumab; Herceptin) has been assessed in clinical trials with breast cancer patients with metastatic disease and has recently been approved by the Food and Drug Administration (FDA). Objective responses were seen in 5 of 43 assessable patients, and included one complete remission and four partial remissions (Baselga *et al.* 1996). Pre-clinical models demonstrate that a combination of paclitaxel and the erb-B2 antibody resulted in superior tumour growth inhibition when compared with either paclitaxel or antibody alone (Baselga *et al.* 1998). Clinical trials combining Herceptin with cisplatin, paclitaxel or anthracycline plus cyclophosphamide indicate that the addition of antibody doubles the response to chemotherapy in a group of erb-B2-positive patients (Slamon *et al.* 1998).

An alternative approach has been to identify low molecular weight inhibitors that bind to the ATP binding site of the EGFR tyrosine kinase (Klohs *et al.* 1997). These agents are potent and selective inhibitors of the kinase *in vitro* and are able to block receptor autophosphorylation in cells stimulated with EGF (Moyer *et al.* 1997, Lydon *et al.* 1998). Pre-clinical models have demonstrated EGFR tyrosine kinase (TK) inhibitors to be well tolerated and effective agents against a variety of EGF-dependent tumour xenografts grown in athymic nude mice including those of vulval, prostate and ovarian origin (Woodburn *et al.* 1997). Interestingly, EGFR TK inhibitors not only produce the anticipated cytostatic response in tumour models but are also capable of producing tumour regression (Woodburn *et al.* 1997, Lydon *et al.* 1998) suggesting that these agents alter the balance between mitogenesis and apoptosis (Moyer *et al.* 1997). Furthermore, studies in cell culture suggest that the combination of an EGFR antibody with chemotherapy (Hoffmann *et al.* 1997) or radiation (Balaban *et al.* 1996) may act synergistically to induce tumour regression, suggesting that EGFR TK inhibitors could enhance the effect of conventional agents. Since EGFR and erb-B2 heterodimerise in cells and act synergistically in transgenic models to enhance tumour formation (Muller *et al.* 1996), then EGFR TK inhibitors may also be effective against tumours that overexpress erb-B2. A number of EGFR TK inhibitors (ZD 1839, CP 358774, CGP 59326) are in phase I/II clinical trials.

Breast and prostate tumours also express platelet-derived growth factor (PDGF). The prognostic significance of the expression of PDGF is suggested by studies demonstrating that breast cancer patients with tumours staining positively for PDGF have a highly significant shorter survival than patients with no immunostaining (Seymour & Bezwoda 1994). Furthermore, patients with PDGF-positive tumours treated with combination chemotherapy have a significantly lower response rate than those that have PDGF-negative tumours. Other studies have suggested that PDGF may be an important mediator of benign prostate hyperplasia in response to inflammation (Vlahos *et al.* 1993). The immunosuppressive drug and dihydroorotate dehydrogenase inhibitor, leflunomide (SU101) may also interfere with PDGF signalling pathways since it has, in addition, been demonstrated to be a tyrosine kinase inhibitor (Xu *et al.* 1996), including that of the PDGF receptor. The compound is currently in phase II studies in patients with hormone-refractory prostate cancer.

Cell cycle inhibition

The observation that a marker of cell proliferation such as Ki-67 is associated with reduced patient survival and disease-free interval in breast (Archer *et al.* 1995) and

prostate (Stapleton *et al.* 1998) cancer suggests that enhanced cellular proliferation contributes to tumour progression. Moreover, there is evidence for an increase in cellular proliferation for recurrent prostate tumours after radiotherapy or surgery (Grossfeld *et al.* 1998). There are many pathways which could be deregulated in tumours in order to increase cell proliferation, including the overexpression of growth factors and their receptors (e.g. TGF- α /EGFR), the loss of growth inhibitory pathways (e.g. TGF- β), the loss of tumour suppressor genes (e.g. the retinoblastoma protein pRb) and the stimulation of endocrine-responsive tumours by steroid hormones.

Although we still do not understand at the molecular level precisely how steroids regulate entry into the cell cycle, much has been learnt recently about some of the principal factors involved. In general, steroid hormones regulate the activity of CDKs through the accumulation of D cyclins with little change in the level of cyclin E, CDK2 or the CDK inhibitor proteins such as p21^{Cip1} and p27^{Kip1} (Planas-Silva & Weinberg 1997). The increase in cyclin D1 activates CDK4 and CDK6 which phosphorylate pRb. This promotes the release of pRb from the E2F family of transcription factors allowing them to activate the transcription of genes involved in S phase transition (Sherr 1996). Model cell systems have provided evidence that treating breast cancer cells with oestrogens (Watts *et al.* 1995) or progestins (Musgrove *et al.* 1993) stimulates the accumulation of cyclin D1 and that the artificial elevation of cyclin D1 can mimic the mitogenic effect of steroids (Musgrove *et al.* 1996). Furthermore, the treatment of ER-positive cells with antioestrogens reduces the level of cyclin D1 and the cells accumulate in the G1 phase of the cell cycle (Watts *et al.* 1995). These studies have been supported by experiments in mice that demonstrate that oestrogens stimulate the expression of cyclin D1 in mammary epithelial cells and that progestins further enhance the level of cyclin D1 mRNA (Said *et al.* 1997). It appears unlikely that the ER directly regulates transcription of the cyclin D1 gene (Altucci *et al.* 1996), suggesting that it is probably acting further upstream in the signalling pathway. In addition to changes in cyclin D1-CDK4 activity, oestrogens stimulate cyclin E-CDK2 activity by promoting the displacement of the CDK inhibitor p21^{Cip1} (Planas-Silva & Weinberg 1997).

Approximately half of primary breast cancers overexpress cyclin D1 (Bartova *et al.* 1994, Marsh & Varley 1998) and the gene encoding this protein is amplified in about a third of these cases, suggesting that increases in the level of cyclin D1 could promote breast tumour growth. In support of this, transgenic mice overexpressing cyclin D1 driven by the mouse mammary tumour virus promoter, demonstrate abnormal mammary cell proliferation and develop mammary adenocarcinoma after a latency period of greater than a year (Wang *et al.*

1994). Interestingly, female mice that are null for the cyclin D1 gene show a defect in mammary epithelial proliferation during pregnancy indicating that cyclin D1 is a critical mediator of steroid hormone-induced cell proliferation in the mammary gland (Sicinski *et al.* 1995). A recent twist in this story has been the demonstration that cyclin D1 can bind to and activate the ER directly (Zwijnsen *et al.* 1997) and it could, therefore, be speculated that the ER may mediate the oncogenic effects of cyclin D1 in breast tumours. Interestingly, tumours that are ER positive also have a tendency to express higher amounts of cyclin D1 (Gillett *et al.* 1996), but this could either indicate that oestrogens stimulate the level of cyclin D1 or that cyclin D1 promotes the growth of breast tumours by binding and activating ER. No matter how cyclin D1 is working, it is important to note that antioestrogens can inhibit this ligand-independent ER activation in cell lines forced to overexpress cyclin D1 (Pacilio *et al.* 1998). One of the natural protein inhibitors of CDK4 is p16^{INK4A}. Inactivation of the p16 gene by deletion, mutation, or silencing of gene transcription by methylation, have all been observed in a variety of solid tumours including breast cancer (Geradts & Wilson 1996, Marsh & Varley 1998), but only rarely in prostate cancer (Jarrard *et al.* 1997). Animal models suggest that p16 is a tumour suppressor gene and argue that the loss of p16 may release the inhibition of D1/CDK4 activity thus promoting cell cycle entry. More recently, however, the picture has become more complicated by the finding that a second protein, encoded in an alternative reading frame (ARF) of the INK4A gene locus, is also a tumour suppressor protein that functions by regulating p53 levels (Chin *et al.* 1998). It would appear that both the p16 and ARF protein are important in preventing unregulated cell proliferation and, therefore, contribute to tumour progression.

Other changes in cell cycle regulatory proteins have been observed in endocrine-responsive tumours besides changes in cyclin D1, p16 and ARF. Of particular interest has been the observation that low levels of p27^{Kip1}, and to a lesser extent elevated levels of cyclin E, are associated with poor prognosis in a variety of solid tumours including breast (Porter *et al.* 1997) and prostate (Tsihlias *et al.* 1998). As cyclin E is an activator of CDK2, and p27^{Kip1} an inhibitor, these results suggest that an increase in the activity of CDK2 is important in tumour progression, although it is unclear at the present time as to whether these changes are cause or effect. Similarly, amplification and overexpression of the cyclin E gene has been observed in ovarian cancer; however, there is little evidence for amplification of the cyclin E gene in breast tumours (Courjal *et al.* 1996). Only a few examples of mutation or deletion of the p27^{Kip1} gene have been reported, rather it has been proposed that other proteins that regulate the level of p27^{Kip1} may be aberrant in tumours, resulting in

Figure 3 Increased survival signalling in cancer. A number of defective apoptosis pathways have been identified in breast and prostate tumours. The loss of functional p53 leads to reduced apoptosis in response to DNA damage and elevated levels of Bcl-2 and survivin help to protect tumour cells from apoptosis. Insulin-like growth factors act as survival factors and part of their activity appears to be mediated by the activation of PI3 kinase. Bcl-2 antisense may increase the effectiveness of radio- and chemo-therapeutic agents by reducing the ability of the tumour to prevent apoptosis.

IGFR, insulin-like growth factor receptor; Apaf-1, apoptotic protease-activating factor 1; DR, death receptor.

the enhanced destruction of the CDK inhibitor (Loda *et al.* 1997). The levels of p27^{Kip1} can be regulated by a variety of negative growth factor and oncogene signalling pathways. For example, TGF- β induces increases in the level of the CDK inhibitor p15^{INK4B}, resulting in the displacement of p27^{Kip1} from CDK4 to CDK2 and the inhibition of this kinase (Reynisdottir *et al.* 1995). Furthermore, mutations within the transcription factor SMAD4/DPC4, a mediator of TGF- β signalling, occur at low frequency within a variety of tumours, including those of the breast (Schutte *et al.* 1996) and prostate (MacGrogan *et al.* 1997). The levels of p27^{Kip1} are also influenced by the activation of the c-myc oncoprotein. Increased c-myc induces cyclin E/CDK2 activity. This, in turn, phosphorylates p27^{Kip1} promoting its dissociation from CDK2 complexes and its subsequent degradation (Muller *et al.* 1997). The amplification and over-expression of the myc oncogene is observed in approximately a quarter of primary breast cancers and is

associated with a poor prognosis (Varley *et al.* 1987, Roux-Dosseto *et al.* 1992). Therefore, mutations in SMAD4 or the overexpression of c-myc are likely to make tumour cells more sensitive to factors that elevate D cyclins. Indeed, the regulation of D cyclins by ras may help explain the cooperation of ras and myc to transform primary rat embryo fibroblasts (Leone *et al.* 1997).

The large body of evidence implicating aberrant cell cycle factors with the progression of endocrine-responsive tumours suggests that small molecule inhibitors of CDKs could be effective agents in controlling this disease. For example, a broad spectrum CDK inhibitor, flavopiridol (NSC 649,890), derived from a natural alkaloid obtained from the Indian plant *Dysoxylum benectariferum*, has been developed (Sedlacek *et al.* 1996) and is currently in phase II clinical trials. Flavopiridol inhibits CDK1, CDK2, CDK4 and CDK7 with similar potencies (100 to 400 nM). It induces cytotoxicity in cells at 20-200 nM with some evidence for apoptosis and is effective in animal tumour

models and, in particular, against prostate cancer xenografts (Sedlacek *et al.* 1996). Many pharmaceutical companies are aiming to develop more selective CDK inhibitors that may prove effective in treating tumours that have developed hormone independence.

Apoptosis

It has become clear over the last few years that tumour cells, in addition to having deregulated mitogen signalling pathways, have aberrant cell death pathways providing them with a greater chance for survival (Fig. 3). Apoptotic mechanisms also play an important role in mediating the response to endocrine therapy since an increase is observed in the number of apoptotic bodies within tumours in patients treated with antioestrogens (Ellis *et al.* 1997) and antiandrogens (Montironi *et al.* 1998). A number of oncogenes and tumour suppressor genes such as Bcl-2 and p53 have roles in regulating apoptosis. Bcl-2 appears to protect cells from apoptosis by preventing the release from mitochondria of factors such as cytochrome c that are required to activate destructive caspase proteases (Reed 1997, Kroemer *et al.* 1998). Bcl-2 is overexpressed in a proportion of breast and prostate tumours and may, therefore, be expected to provide a survival advantage. However, an examination of breast cancer patients has demonstrated that the expression of Bcl-2 is tightly associated with ER expression and that those patients with elevated levels of Bcl-2 appeared to benefit most from endocrine therapy (Gee *et al.* 1994, Berardo *et al.* 1998). Studies in cell culture may provide an explanation for this link since oestrogens stimulate the expression of Bcl-2, possibly as a means to reduce cell death whilst stimulating mitogenesis (Teixeira *et al.* 1995). Moreover, the same authors demonstrate that, in the presence of oestrogen, the MCF-7 breast cancer cell line is more resistant to apoptosis induced by adriamycin than when the ER is antagonised with a pure antioestrogen (ICI 164,384). That this effect is mediated by Bcl-2 is suggested by the observation that the effect of oestrogen can be mimicked by the exogenous expression of Bcl-2. If such *in vitro* studies translate to the clinic, then they would suggest that cytotoxic drugs administered in combination with tamoxifen to lower Bcl-2 levels could benefit patients. Some studies in prostate cancer have suggested a correlation between the expression of Bcl-2 and poor prognosis (Bauer *et al.* 1996, Matsushima *et al.* 1997), whereas other studies have suggested no correlation (Grossfeld *et al.* 1998) and show that a high level of Bcl-2 is associated with early prostate cancer (Statton *et al.* 1996). Interestingly, androgen-independent prostate tumours express higher levels of Bcl-2 (McDonnell *et al.* 1992) and their treatment with hormone ablation therapy increases the level of Bcl-2 (Statton *et al.* 1996). These observations suggest that Bcl-2 could promote a survival

advantage against apoptosis induced by hormone withdrawal. If Bcl-2 promotes cell survival in response to radiotherapy and chemotherapy, then it would be expected that the cancers of patients expressing high levels of Bcl-2 would have a poorer response to treatment. There is some evidence that this is true in prostate cancer (Huang *et al.* 1998), where a significantly greater number of tumours from patients who failed radiation therapy expressed Bcl-2. Because the regulation of apoptosis by Bcl-2 is complex, involving the interaction of a number of related proteins which either promote or prevent apoptosis, it is likely that additional proteins are deregulated in endocrine-responsive tumours to reduce the apoptosis threshold. For example, Bax is one family member that antagonises the action of Bcl-2, whose expression is lower in a subset of metastatic breast cancer patients that respond poorly to chemotherapy (Krajewski *et al.* 1995). Antisense therapies directed towards the Bcl-2 oncogene are now being evaluated in the clinic in patients with non-Hodgkin lymphoma, a tumour which often contains a t(14;18) translocation involving the Bcl-2 gene and increased amounts of the protein (Cotter 1997). If successful, it will be interesting to determine whether reducing the level of Bcl-2 in androgen-independent prostate cancer will promote tumour regression, either as stand-alone therapy, or in combination with radiation or chemotherapy.

The level of the tumour suppressor protein p53 is stabilised in response to DNA damage (Lane & Hall 1997). Since p53 is a transcription factor, its stabilisation enhances the expression of p53-regulated genes. One of the genes regulated by p53 encodes the CDK inhibitor p21 to promote cell cycle arrest (Deng *et al.* 1995). Other genes stimulated by p53 include Bax (Miyashita *et al.* 1994) and Fas (Owen-Schaub *et al.* 1995) which promote apoptosis. Furthermore, p53 suppresses the expression of Bcl-2 (Miyashita *et al.* 1994) thereby further sensitising cells to apoptosis. The p53 gene is frequently mutated in a wide variety of solid tumours, including those of the breast and prostate, and is linked with poor prognosis (Gasparini *et al.* 1998). The loss of p53 or the expression of a dominant negative mutant can prevent apoptosis in response to radiation in a transgenic mouse model (Lowe *et al.* 1993) and the loss of functional p53 increases the radioresistance of tumour cell lines (McIlwrath *et al.* 1994). Using immunohistochemistry, it is not clear whether the level of p53 (an indication of aberrant p53 function) predicts clinical response to therapy in primary breast cancer but interesting associations have been made between the presence of particular p53 mutations and the poor response of the tumour to treatment with tamoxifen, radiotherapy or chemotherapy (Bergh *et al.* 1995, Aas *et al.* 1996). Studies in patients suggest that the level of p53 is elevated in prostate tumours recurring after radiotherapy

(Grossfeld *et al.* 1998, Huang *et al.* 1998), perhaps indicating that there is selection of tumour cells with mutant p53, because of their reduced propensity to undergo apoptosis or cell cycle arrest.

The role of additional factors in regulating apoptosis to promote endocrine-responsive tumour progression or response to therapy remains to be established. For example, insulin-like growth factors (IGF) are mitogens for breast and prostate cells that appear to function as survival factors reducing the level of apoptosis. Recent studies have suggested that high plasma levels of IGF-I are associated with an increased risk of developing prostate cancer (Chan *et al.* 1998) and IGF family members may be indicators of prognosis in breast cancer but, because of the number of factors involved, the picture remains complicated (Lee *et al.* 1998). One of the second messenger pathways that appears to be involved in suppressing apoptosis is the phosphoinositide pathway. A number of growth factors including IGFs activate phosphatidylinositol-3 kinase (PI3K) resulting in the elevation of 3-phosphorylated inositol lipids. These bind to a variety of proteins that contain a pleckstrin homology domain, thereby recruiting them to the plasma membrane and inducing enzyme activity (Shepherd *et al.* 1998). One such protein is the oncoprotein Akt, also known as protein kinase B, and the expression of a constitutively active form of Akt can suppress apoptosis in a variety of models, possibly by indirectly modulating Bcl-2 activity (Datta *et al.* 1997). More recently, it has been demonstrated that Akt can phosphorylate and inhibit the activity of caspase 9 (Cardone *et al.* 1998), providing a further potential mechanism to explain the ability of Akt to suppress apoptosis. Interestingly, the PTEN tumour suppressor gene encodes a protein with phosphoinositol-3 phosphatase activity that counters the activity of PI3K (Maehama & Dixon 1998). Disruption of the gene is associated with Cowden's disease and predisposes these families to breast cancer (FitzGerald *et al.* 1998). Furthermore, the gene is mutated, deleted or repressed in approximately 10-20% of spontaneous cases of prostate cancer (Cairns *et al.* 1997, Whang *et al.* 1998). The loss of PTEN may therefore lead to elevated levels of phosphatidylinositol-3 lipids, increased activity of proteins such as Akt and, therefore, reduced apoptosis.

Additional factors that may influence apoptosis in tumours include fas, and other members of the tumour necrosis factor (TNF) receptor family. Recent studies have suggested that cell lines derived from primary prostate tumours are sensitive to fas-mediated apoptosis, whereas those derived from metastases are more resistant (Hedlund *et al.* 1998). It will be of interest to determine whether these differences are due to changes in the level of proteins such as Bcl-2. In addition, novel apoptosis genes associated with endocrine-responsive tumours continue to

be identified. One such gene is survivin, which is related to an insect virus protein IAP (inhibitor of apoptosis). Survivin is undetectable in terminally-differentiated adult tissues but is prominently expressed in lung, colon, pancreas, prostate and breast tumours (Ambrosini *et al.* 1997). Other studies have identified a novel TNF-related apoptosis-inducing ligand (TRAIL) that induces apoptosis of many transformed cell lines, but not of normal tissues, due to the latter expressing decoy receptors that bind TRAIL but lack the intracellular effector death domain (Pan *et al.* 1997).

Evidence is beginning to emerge that as endocrine-responsive tumours develop they become more resistant to apoptosis through either the increased expression of proteins that prevent apoptosis or the loss of proteins that promote apoptosis. In addition, the treatment of endocrine-responsive tumours with endocrine agents, radiation or chemotherapy is likely to select for cells with increased resistance to apoptosis. Identifying pharmacological agents that can either suppress these antiapoptotic pathways or override them by activating apoptosis pathways directly should, therefore, provide new approaches to treat tumours directly or sensitise them to conventional agents in order to improve efficacy.

Angiogenesis

A proliferating tumour mass, whether the primary tumour or a small clump of cells lodged at a distant metastatic site, can only grow beyond a couple of millimetres in size before physical constraints prevent the essential supply of nutrients. It is, therefore, essential for tumours to establish their own vasculature in order to be able to survive and grow (Bicknell & Harris 1996, Eckhardt & Pluda 1997). Exploiting this angiogenic process has become an attractive ploy to target a wide variety of solid tumours with the added bonus that resistance to therapy is less likely to develop, because the target is an endothelial cell rather than a genetically unstable tumour cell (Boehm *et al.* 1997).

There is now good evidence that endocrine-responsive tumours produce a variety of positive and negative angiogenic factors that influence the vascularisation process. Some of the positive factors that encourage the formation of new blood vessels include members of the fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF) families, pleiotrophin, placental growth factor, midkine and thymidine phosphorylase (platelet-derived endothelial cell growth factor) (Relf *et al.* 1997, Choudhuri *et al.* 1997, Ferrer *et al.* 1997), whilst the angiogenic inhibitors include angiostatin (O'Reilly *et al.* 1994) and endostatin (Boehm *et al.* 1997). Because of continuous tumour cell proliferation, the centre of the tumour becomes hypoxic. The lack of oxygen rapidly stimulates the tumour cells to express VEGF which then

Figure 4 Inhibiting angiogenesis. Tumour cells produce a variety of angiogenic and antiangiogenic factors that influence the growth of the primary and secondary metastatic tumours. Biopharmaceuticals are being developed that mimic the activity of angiostatin to suppress angiogenesis. Other approaches to selectively prevent angiogenesis include inhibition of the receptor for VEGF (VEGFR) using receptor antagonists and kinase inhibitors.

induces the growth of new vasculature in order to nourish the tumour (Shweiki *et al.* 1992). The importance of factors such as VEGF to neo-vascularisation and tumour growth has been demonstrated in animal models. The treatment of nude mice with a neutralising anti-VEGF antibody prevented the growth of the human prostate tumour cell line DU 145 (Borgstrom *et al.* 1998). Furthermore, a dominant-negative Flk-1 (a receptor for VEGF), contained within a retrovirus to permit its delivery to the endothelial cells of a nude mouse *in vivo*, is able to suppress the growth of a variety of solid tumour types including those of the breast and ovary (Millauer *et al.* 1996). As far as angiogenesis inhibitors are concerned, it is interesting that, in an animal model, a primary tumour is able to inhibit the growth of remote metastases and that the removal of the primary tumour provides a stimulus for the metastases to neovascularise and grow (O'Reilly *et al.* 1994). A fragment of plasminogen, termed angiostatin, is produced by primary tumours and the systemic

administration of angiostatin is capable of inhibiting the growth of solid tumours in mice (O'Reilly *et al.* 1994, 1996). It has also been demonstrated that prostate tumour cell lines express enzymes that can convert plasminogen or plasmin into angiostatin (Gately *et al.* 1996). Together, these results indicate that tumour growth is achieved through a balance of both positive and negative angiogenic factors.

Measuring the extent of vascularisation in tumours by determining the microvessel count (MVC) has provided a link between the apparent level of angiogenesis and poor prognosis in a variety of endocrine-responsive tumour types (Weidner 1998). An examination of prostate cancer patients demonstrated that the mean MVC was higher in patients with metastases than those without (Weidner *et al.* 1993). In a study of both node-positive and node-negative breast cancer patients, MVC was determined to be the most accurate prognostic factor for both disease-free and overall survival (Gasparini *et al.* 1998, Jacquemier *et al.*

1998). Although essential in the developing embryo, angiogenesis in the adult is limited to menstruation and wound healing. Therefore, a targeted inhibitor of angiogenesis is anticipated to arrest the growth of the primary tumour, and consequently any metastases, with minimal side effects and with far less chance for resistance to develop. There is considerable excitement, together with a lot of hype, about the development of angiogenesis inhibitors (Augustin 1998, Harris 1998, Marshall 1998, Phillips 1998). It has been suggested that because of the panoply of angiogenic factors that a non-specific inhibitor of angiogenesis, such as a suramin-like compound, will be required (Relf *et al.* 1997) and thalidomide is being re-examined for use in patients, now that it has been recognised as an inhibitor of angiogenesis (D'Amato *et al.* 1994). Other approaches target the tumour endothelial cell. For example, CM101 is an analogue of a bacterial polysaccharide that selectively binds to tumour endothelium and stimulates the death of the cells by inducing inflammation (Wamil *et al.* 1997), and TNP-470 is an analogue of the antibiotic fumagillin that inhibits the growth of endothelial cells (Castronovo & Belotti 1996). More targeted agents include angiostatin and endostatin as well as antibodies to VEGF and inhibitors (SU5416, ZD4190) of the VEGF receptor tyrosine kinase (Strawn *et al.* 1996, Ogilvie *et al.* 1999) (Fig. 4). Many of these agents are now in clinical trials. Despite the many claims surrounding angiostatin and endostatin they still need to clear a number of hurdles before they will be available to test in the clinic. Drugs that target the endothelial cell should be effective against a wide variety of solid tumours and, as an added bonus when treating patients with endocrine cancer, should be equally effective against both hormone-dependent, as well as hormone non-responsive tumours. Despite the excellent activity of antiangiogenic drugs in animal models, the importance of angiogenesis and vasculature remodelling for tumour growth in humans, compared with animal models, remains to be established. The results of clinical trials with these novel agents are, therefore, awaited eagerly.

Hope for the future

Our understanding of the mechanisms that regulate endocrine-responsive tumour development have changed radically since the association between steroid sex hormones and tumour growth was first recognised at the turn of the century. We are now poised to exploit this increased level of understanding with the development of valuable new drugs with which to treat these tumours. The pioneering basic research work during the 1980s has been translated into new cancer therapeutics that are progressing through clinical trials in the 1990s. It is hoped that

some will provide significant benefit to patients at the start of the new millennium.

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