

## Review Article

# Impact of Molecular Biology on Cancer Treatment: I Therapeutic Targets

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**ABSTRACT**

The study of cancer at the molecular level over the last two decades has led to the identification of major groups of genes which, when disrupted or mutated, can lead to the development of malignancy. Together

with other molecules, these genes, their RNA transcripts and their protein products are providing a wide range of targets for therapeutic intervention.

KEY WORDS: cancer, molecular targets, therapy

**INTRODUCTION**

The need for better cancer treatment is evident. In the developed world, approximately one in three persons contracts cancer and around one in four of these dies from the disease. The worldwide incidence of cancer is predicted to double from 10 to 20 million over the next two decades and the death rate will increase from 6 to 10 million. Advances in treatment with surgery, radiotherapy and chemotherapy have had a limited impact on mortality. Cures can be achieved in childhood cancers, testicular cancer and lymphoma, and improvements in survival rates have been made as a result of the adjuvant drug treatment of breast and colorectal cancer. However, the majority of human cancers are difficult to treat, especially in their advanced, metastatic forms. The need for new and effective forms of systemic therapy is pressing and the discovery of novel, mechanism-based agents directed against the molecular pathology of cancer is of enormous potential<sup>[1]</sup>.

It has been known for many years that cancer has a genetic component and it is clear that there is a multistage progression to malignancy. The application of modern molecular techniques to study cancer over the last 2 decades has led to the identification of 4 major groups of genes which are involved in tumourigenesis – oncogenes, tumour suppressor (TS), cell cycle control (CCC) and mismatch repair (MMR). Cellular oncogenes (proto-oncogenes) encode proteins, which are important in the control of cell proliferation, differentiation, cell cycle control and apoptosis. Mutations in these genes act dominantly and lead to a gain of function. In contrast, TS genes inhibit

cell proliferation by arresting progression through the cell cycle and block differentiation. CCC genes are involved in the positive and negative regulation of the cell cycle and they interact with oncogenes and TS genes, and in some cases may be considered to be such in their own right. To ensure that DNA replication is complete and that any damaged DNAs repaired, cells must pass through specific checkpoints and MMR genes ensure that damaged DNA is repaired. There is compelling evidence for the importance of these genes in the etiology of many human tumours.

**RECEPTORS AS TARGETS****Receptor tyrosine kinases – ERBB**

Receptor tyrosine kinases (RTKs) are important regulators of intercellular communication controlling cell growth, proliferation, differentiation, survival and metabolism. Deregulation of protein tyrosine kinase activity usually results in RTKs with constitutive or greatly enhanced signalling capacity leading to malignant transformation<sup>[2]</sup>. Protein tyrosine kinases (PTKs) are potential targets because in several cancers their activity is up-regulated by gain-of-function mutations or over-expression. PTK activity can be up-regulated by several mechanisms: genomic re-arrangements e.g. *BCR-ABL\** in chronic myelogenous leukaemia (CML); point mutations e.g. *Flt-3* in acute myelogenous leukaemia (AML) and *c-kit* (the receptor for stem cell factor) in gastrointestinal stromal tumours; over-expression e.g. epidermal growth factor receptor (EGFR) in various cancers; and ectopic or inappropriate expression of growth factors such as vascular endothelial growth factor (VEGF) and its receptors

(\*The convention used in this review is italics for a gene and normal case for its protein product e.g., *MYC* and MYC)

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on endothelial cells which are involved in angiogenesis<sup>[3]</sup>. ERBB receptors belong to the epidermal growth factor (EGF) family of structurally related RTKs and four ERBB members have been identified so far: ERB1 (EGFR, HER1); ERBB2 (HER2, Neu); ERBB3 (HER3); and ERBB4 (HER4)<sup>[4]</sup>.

Prevention or inhibition of RTK signalling includes selective targeting of the extracellular ligand-binding domain, the intracellular tyrosine kinase or the substrate-binding region. Pharmacological agents such as monoclonal antibodies (Mabs), antibody conjugates, antisense oligonucleotides and small chemical compounds have been developed for these purposes, for example Imatinib (Gleevec/Glivec) which is being used for the treatment of CML and gastrointestinal tumours<sup>[2-4]</sup>.

Small molecule tyrosine kinase inhibitors that have been developed for the treatment of ERBB1 and ERBB2 expressing tumours include ZD1839 and OSI-774 (ERBB1) and tryphostins, 4,5-dianilinophthalamide and emodin (ERBB2). These agents have shown considerable promise *in vitro* and in preclinical animal models. Both ZD1839 and OSI-774 have shown activity in Phase I and II clinical trials and further clinical trials in a variety of tumour types are currently underway. Second generation inhibitors are under development by a number of pharmaceutical companies<sup>[4]</sup>.

Additional strategies for the inhibition of RTKs include the use of immunotoxins. One promising immunotoxin is the EGF fusion protein DAB389EGF, which contains the enzymatically active and membrane translocation domains of diphtheria toxin and sequences for human EGF. A variety of EGFR-expressing tumours, such as breast cancer and non-small cell lung cancer, have been shown to be sensitive to DAB389EGF in preclinical studies and this recombinant toxin is now under evaluation in Phase II clinical trials<sup>[2]</sup>.

### **BRC-ABL**

The tyrosine kinase activity of the BCR-ABL oncoprotein results in reduced apoptosis and thus prolongs survival of CML cells. The tyrosine kinase inhibitor Imatinib selectively suppresses the proliferation of BCR-ABL-positive cells and is an example of a rationally designed, molecular-targeted drug for the treatment of a specific cancer (CML)<sup>[5,6]</sup>. Three large multinational studies in patients with late chronic-phase CML, in whom previous interferon treatment had failed, have shown that achievement of a haematological and cytogenetic response increased the earlier the treatment was started with Imatinib in the course of the disease and that these responses were associated with improved survival and

progression-free survival<sup>[6]</sup>. Preclinical studies have shown that the combination of Imatinib with various anticancer agents might have synergistic effects and several phase I/II studies are evaluating the feasibility of combining Imatinib with interferon, polyethylene glycol (PEG)ylated interferon, cytarabine and other single-agent or combination chemotherapy regimens, in patients with either chronic-phase or advanced CML<sup>[6]</sup>. Combinations of Imatinib and -irradiation or alkylating agents such as busulfan or treosulfan are being evaluated for their synergistic activity in BCR-ABL-positive CML cell lines. Such data will provide the basis to further develop Imatinib-containing conditioning therapies for stem cell transplantation in CML<sup>[5]</sup>.

### **Estrogen receptor (ER)**

Estrogen (estradiol) is a steroid hormone that affects growth, differentiation and function of the female reproductive organs, including the breast, uterus and ovaries and also plays several other important physiological roles e.g. in maintaining bone density and protecting against osteoporosis. Estrogen also promotes cancer cell growth in the breast and the uterus. All of these effects are mediated by estrogen binding to ERs and the ER regulates gene transcription both directly, by binding to an estrogen-responsive element in gene promoters, and indirectly, by binding through other transcription factors<sup>[7]</sup>.

Estrogen has been a major target in the treatment of breast cancer since the end of the 19th century and tamoxifen was the first selective estrogen receptor modulator (SERM) to be developed. It has estrogen-like actions in maintaining bone density and in lowering circulating cholesterol, but antiestrogenic actions in the breast. It has proved to be valuable in the treatment of ER-positive breast cancer. The finding that tamoxifen could inhibit the growth of breast cancer, but at the same time stimulate the growth of endometrial cancer in the nude mouse model, indicated that its mode of action is specific to a target tissue. The overall conclusion from clinical trial data is that there is a 2-3 fold increase in the risk of endometrial cancer in tamoxifen-treated postmenopausal patients. Another SERM, raloxifene, binds to ERs to competitively block-estrogen-induced DNA transcription in both the breast and the endometrium. However, its poor bioavailability and its short biological half-life mean it is not as effective an anti-tumour agent as tamoxifen<sup>[8]</sup>.

The role of tamoxifen in chemoprevention (i.e. breast cancer prevention) in high-risk pre- and post-menopausal women is more controversial with conflicting results being reported from studies that have addressed this question<sup>[8]</sup>. Use of

raloxifene in postmenopausal women with osteoporosis decreased the risk of vertebral fractures, increased bone mineral density in the spine and reduced the risk of invasive breast cancer by 72% and the risk of ER-positive breast cancer by 84%<sup>[9]</sup>. A Phase III, double-blind trial of tamoxifen and raloxifene in which post-menopausal women are randomized to tamoxifen or raloxifene orally for 5 years, will compare the relative merits of raloxifene and tamoxifen for the prevention of invasive breast cancer, as well as their effects on the cardiovascular system and bones<sup>[8]</sup>.

The molecular determinants for the tissue specificity of SERMs are under investigation and it is known that tissue-specific co-regulator expression levels determine tamoxifen's different effects on breast and endometrial tissue. This improved understanding of the mechanism of action of SERMs should lead to better SERMs without carcinogenic side effects<sup>[10]</sup>.

### Retinoic acid receptor (RAR) and retinoid X receptor (RXR)

Retinoids are natural derivatives of vitamin A or retinol. The retinoid signal is mediated through RARs and RXRs on target cells, each of which comprise three isotypes –  $\alpha$ ,  $\beta$ ,  $\gamma$  – as well as several isoforms. RARs and RXRs are transcription factors that act predominantly as RAR-RXR heterodimers, positively or negatively modulating gene transcription. Natural and synthetic retinoids are effective inhibitors of tumour cell growth *in vitro* and *in vivo* but the natural derivatives have limited therapeutic use due to their toxicity. Synthetic compounds selective for the different retinoid receptor isotypes are currently undergoing clinical evaluation. In addition, the combination of retinoids with other chemotherapeutic agents may also be of value in cancer therapy<sup>[11]</sup>.

### Peroxisome proliferator-activated receptor (PPAR)

PPAR is a nuclear receptor and transcription factor that regulates the expression of many genes relevant to carcinogenesis. Deficient expression of PPAR can be a significant risk factor for the development of cancer but, paradoxically, in some cases overexpression can enhance carcinogenesis. In experimental models ligands for PPAR have been shown to suppress breast carcinogenesis and to induce differentiation of human liposarcoma cells. By analogy to the SERM concept, it has been suggested that PPAR modulators (SPARMS), designed to have desired effects on specific genes and target tissue without undesirable effects on others, will be clinically important in the future for chemoprevention and chemotherapy of cancer<sup>[12]</sup>.

## OTHER TARGETS

### Proteasomes

Protein degradation is fundamental to cell viability and the primary component of the protein degradation pathway in the cell is the 26S proteasome which is a large multiprotein complex present in the cytoplasm and the nucleus of all eukaryotic cells. The central role of the proteasome in controlling the expression of regulators of cell proliferation and survival has led to interest in developing proteasome inhibitors as anti-cancer agents. Studies *in vitro* and *in vivo* have shown that proteasome inhibitors have activity against a variety of tumours and one of these agents, PS-341 (bortezomid, VELCADE<sup>TM</sup>), has been tested in clinical trials. These phase I trials showed that the treatment was well tolerated as a single agent and preliminary evidence of biological activity was seen in some patients, thereby providing the rationale for Phase II and III trials in multiple myeloma. Phase II trials in several haematological malignancies and solid tumour types are also in progress and additional trials of bortezomid, in combination with other cytotoxic regimens, will focus on its activity in solid tumours<sup>[13]</sup>. Drugs that affect protein degradation by the proteasome are a potentially promising class of agents that are just beginning to be explored.

### p53

Mutations in this TS gene occur in half of all human cancers and regulation of the protein is defective in a variety of others. Strategies directed at treating tumours that have p53 mutations include gene therapy, viruses that only replicate in p53 deficient cells, and the search for small molecules that reactivate mutant p53. Potentiating the function of p53 in a non-genotoxic way in tumours that express wild type protein can be achieved by inhibiting the expression and function of MDM2 (a negative regulator of p53)<sup>[14]</sup>.

Over 6,000 papers have described p53 alterations in human tumours -15,121 somatic and 196 germline mutations in p53 are catalogued in the International Association of Cancer Registries (IARC) database<sup>[15]</sup>. Over 1,700 different mutations in p53 have been reported. The mutations are found throughout the open reading frame (ORF) as well as at splice junctions, and although the most common site for mutations is in exons 5-8, which encode the DNA binding domain of the protein, over 13% of mutations lie outside this region<sup>[14]</sup>. Mutation of p53 is often associated with a poor prognosis.

In the past decade the genetic and biochemical analysis of the p53 pathway that leads from cellular stress (through p53 activation) to growth arrest and

apoptosis, has identified many targets for therapeutic development. It has also led to the realization that the toxicity and efficacy of many of the current treatments are also affected by the activity of the *p53* pathway. Most cytotoxic drugs induce the *p53* response in normal tissues, hence contributing to their toxicity, whereas tumours that retain the normal *p53* gene function are in many cases more responsive to treatment<sup>[14,16,17]</sup>.

The various therapeutic approaches based around the *p53* pathway can be summarized as follows: 1) treatments for tumours in which the *p53* gene is mutant - including gene therapy with wild type *p53*, exploiting the absence of *p53* to enable selective drive of therapeutic gene expression, exploiting the absence of *p53* to enable selective viral replication, exploiting small-molecule inhibitors of the *p53* response, mimicking the function of downstream genes, reactivating mutant *p53*; 2) treatments for tumours in which the *p53* gene is wild type (activating the function of the endogenous *p53* gene in the tumour) - including inhibiting *MDM2*, blocking the *p53*-*MDM2* interaction, inhibiting nuclear export and mimicking *p14<sup>ARF</sup>* which is a small protein-activator of the *p53* response<sup>[14]</sup>.

Lack of functional *p53* in tumours, either through mutation or by other mechanisms, such as overexpression of *MDM2*, can affect the efficacy of standard radiation and chemotherapy. The relationship between *p53* status and sensitivity to chemotherapy has been extensively studied in breast and ovarian cancers. The majority of findings from these studies show that mutation or alteration in *p53* can lead to decreased sensitivity and resistance to cytotoxic drugs. Numerous *in vitro* and *in vivo* studies have also shown that loss of *p53* function increases post-irradiation clonogenic cell survival. This correlates with an abrogated G1 checkpoint control and changes in apoptosis<sup>[17]</sup>. Collectively, the evidence indicates an association between lack of functional *p53* and inability of tumour cells to undergo apoptosis in response to chemotherapy and/or radiotherapy. Restoration of normal *p53* function in tumours might restore the apoptotic pathway and therefore lead to an increased response to conventional therapeutics<sup>[17]</sup>.

A low molecular weight compound (PRIMA-1) has been found to be capable of inducing apoptosis in human tumour cells through restoration of the transcriptional transactivation function to mutant *p53*. This molecule restored sequence-specific DNA binding and the active conformation to mutant *p53* proteins *in vitro*, and *in vivo* in mice it showed an anti-tumour effect without apparent toxicity. This molecule may serve as a lead compound for the development of anti-cancer drugs targeting mutant

*p53*<sup>[18]</sup>. Numerous small molecular weight agents have been identified that are capable of reactivating both wild type and mutant *p53 in vivo*, and these hold great promise for treatment in the future<sup>[19]</sup>.

Death receptors - members of the tumour necrosis factor receptor (TNFR) superfamily - signal apoptosis independently of *p53*. Decoy receptors, in contrast, are a non-signalling subset of the TNFR superfamily that attenuate death receptor function. Agents that are designed to activate death receptors (or block decoy receptors) might therefore be used to kill tumour cells that are resistant to conventional cancer therapy. Concomitant with the evaluation of the safety and efficacy of such agents in preclinical models is the identification of suitable candidates for clinical investigation. The identification of more TNF and TNFR superfamily members through the Human Genome project has yielded novel apoptosis based approaches that have the potential to expand cancer therapy in a new direction<sup>[20]</sup>.

### Raf kinases

Raf kinases are proto-oncogenes that work at the entry point of the mitogen-activated protein kinase/extracellular-signal-regulated kinase (MAPK/ERK) pathway, a signalling module that connects cell surface receptors and RAS proteins to nuclear transcription factors. The pathway is hyperactivated in 30% of human tumours and impinges on all the functional hallmarks of cancer - immortalization, growth factor-independent proliferation, insensitivity to growth-inhibiting signals, ability to invade and metastasize, ability to attract blood vessels, and evasion of apoptosis. Raf is an attractive target for therapy as a single inhibitor could block several cancer-promoting elements at once<sup>[21]</sup>.

Although Raf activation is still incompletely understood, three approaches are currently under investigation to inhibit the Raf-MEK (MAPK/ERK kinase) pathway. The first is the use of antisense RNA to downregulate Raf-1 protein levels. The second is the use of chemical Raf inhibitors such as BAY 43-9006, which has entered Phase I trials after encouraging preclinical results. The third approach is inactivation of MEK by Raf and PD184322 is a drug that does this effectively in preclinical studies with colon cancer xenografts in nude mice and which is now proceeding to clinical trials<sup>[21]</sup>.

### Cyclin-dependent kinases (CDKs)

With the recent understanding of the role of CDKs in cell cycle regulation and the discovery that approximately 90% of all neoplasia is the result of CDK hyperactivation, leading to the abrogation of the Rb pathway, novel CDK modulators are being

developed. Most CDK inhibitors have anti-proliferative properties associated with apoptosis-inducing activity and display anti-tumour activity. However, their cellular targets remain to be identified<sup>[22]</sup>. The first two CDK modulators tested in clinical trials, flavopiridol and UCN-01, demonstrated significant preclinical activity in haematopoietic models. Both compounds have also demonstrated activity in some patients with non-Hodgkin's lymphoma. The best schedule to be administered, combination with standard chemotherapeutic agents and demonstration of CDK modulation in tumour samples from patients in these trials are important issues that need to be addressed in order to ensure the best possible use of these agents<sup>[23]</sup>.

### Angiogenesis

Angiogenesis and lymphangiogenesis are thought to be essential for tumour progression and metastasis<sup>[24,25]</sup>. The initial encouraging results obtained with anti-angiogenic agents meant that there was a rush to take this research from the bench to the clinic. However, this has been tempered by the realization that anti-angiogenic therapy is not the panacea for cancer. There are many possible reasons for this, including endothelial and tumour cell heterogeneity, the presence of survival factors within the tumour micro-environment, the problem of defining the best dose and schedule and angiogenesis-independent regrowth of tumours<sup>[26]</sup>. More than 300 angiogenesis inhibitors have been discovered to date and there are currently over 80 anti-angiogenic agents in clinical trials involving over 10,000 patients<sup>[24,27]</sup> but so far no therapy based on angiogenic modulation has shown sufficient clinical benefit to be approved for such an indication<sup>[24]</sup>. It is clear that not enough was known about the molecular mechanisms of tumour angiogenesis when trials of anti-angiogenic compounds began in the 1990s, and the manner in which these drugs are administered must be changed to achieve maximum clinical efficacy<sup>[28]</sup>.

It has been argued that the traditional strategies that are used for assessing efficacy of anti-cancer therapies in clinical trials are not appropriate for agents that modulate angiogenesis since most angiogenic modulators are cytostatic, slowing or stopping tumour growth, without producing an objective remission. It has been suggested also that imaging studies, for example MRI, could have a key role in assessing the efficacy of treatments<sup>[24]</sup>.

Cancer cells begin to promote angiogenesis early in tumourigenesis and this 'angiogenic switch' is characterized by oncogene-driven tumour expression of pro-angiogenic proteins, such as vascular endothelial growth factor (VEGF), basic

fibroblast growth factor, interleukin-8, placenta-like growth factor (PLGF), transforming growth factor- $\beta$ , platelet-derived endothelial growth factor, pleiotrophin and others<sup>[29]</sup>.

Paradoxically, tumour progression is associated with both increased microvascular density and intratumoural hypoxia. This paradox arises because the tumour vasculature is structurally and functionally abnormal, resulting in perfusion that is characterized by spatial and temporal heterogeneity<sup>[30]</sup>. In addition, decreased aerobic (hypoxic) conditions in tumours induce the release of cytokines that promote vascularization and thereby enhance tumour growth and metastasis<sup>[31]</sup>. Hypoxia-inducible factor 1 (HIF-1) controls oxygen delivery (via angiogenesis) and metabolic adaptation to hypoxia (via glycolysis). In xenograft models tumour growth and angiogenesis are correlated with HIF-1 expression. HIF-1 consists of a constitutively expressed HIF-1 $\alpha$  subunit and an oxygen and growth factor-regulated HIF-1 $\beta$  subunit. Three members of the HIF-1 family have been cloned to date: HIF-1 $\alpha$ , HIF-2 $\alpha$ , HIF-3 $\alpha$ . HIF-1 $\alpha$  has been the most extensively characterized and in human cancers it is over-expressed as a result of intratumoural hypoxia and genetic alterations affecting key oncogenes and TS genes. HIF-1 $\alpha$  over-expression in biopsies of brain, breast, cervical, esophageal, oropharyngeal and ovarian cancers is correlated with treatment failure and mortality. Genes that are involved in many processes are transcriptionally activated by HIF-1 including those that are involved in important aspects of cancer biology such as angiogenesis, cell survival, glucose metabolism and invasion. Since increased HIF-1 activity promotes tumour progression, inhibition of HIF-1 could represent a novel approach to cancer therapy and two potential candidates for HIF-1 targeted therapy are renal cell carcinoma and glioblastoma multiforme<sup>[32,33]</sup>.

Five mammalian VEGF family members have been identified to date: VEGF, VEGF-B, VEGF-C, VEGF-D and PLGF. Almost all types of cancer cells express VEGF, which uses VEGF receptor 1 (VEGFR-1) and VEGFR-2 for signalling. Associations have been observed between VEGF expression, the vascular density in human tumours and patient prognosis<sup>[25]</sup>. Several studies have shown that over-expression of VEGF-C or VEGF-D induces lymphangiogenesis and promotes tumour metastasis in mouse tumour models. Using such a model it has been demonstrated that VEGFR-3 signalling can be inhibited by recombinant adenoviruses expressing the VEGFR-3-Ig fusion protein (which binds VEGF-C) resulting in suppression of tumour lymphangiogenesis and metastasis to regional lymph nodes, but not lung metastasis<sup>[25]</sup>.

Although anti-angiogenic therapy is a promising approach, concerns have been raised that it will select for highly aggressive, hypoxia-adapted tumour cells. Tumour cells deficient in p53 display a diminished rate of apoptosis under hypoxic conditions, which might reduce their reliance on vascular supply, and hence their responsiveness to anti-angiogenic therapy. Although anti-angiogenic therapy targets genetically stable endothelial cells in the tumour vasculature, genetic alterations that decrease the vascular dependence of tumour cells can influence the therapeutic response of tumours to this therapy<sup>[34]</sup>. In addition, the assumption that selection for endothelial cells that are resistant to the therapy is unlikely to occur has been called into question by the identification of mutations affecting proteins in apoptotic pathways in endothelial cells of patients with primary hypertension. The combination of anti-angiogenic agent and an inhibitor of HIF-1 might be particularly effective, as the angiogenesis inhibitor would cut off the tumour's blood supply and the HIF-1 inhibitor would prevent the ability of the tumour to adapt to the ensuing hypoxia. Under these conditions of severe intratumoural hypoxia, a therapeutic window for inhibition of HIF-1 activity is most likely to exist. The dramatic effects of total HIF-1 deficiency on vascular development in mice also suggest that inhibition of HIF-1 could potentiate the effect of angiogenesis inhibitors and reduce the potential for the development of drug resistance<sup>[30]</sup>.

The blood vessels of individual tissues are biochemically distinct, and pathological lesions put their own 'signature' on the vasculature. The development of targeted pharmaceuticals necessitates the identification of specific ligand-receptor pairs and knowledge of their cellular distribution and accessibility. Using new methods, such as *in vivo* screening of 'phage libraries', which permits the identification of organ-specific and disease-specific proteins expressed on the endothelial surface, it is now possible to decipher the molecular signature of blood vessels in normal and diseased tissue<sup>[35]</sup>. Since in tumours both blood and lymphatic vessels differ from normal vessels, peptides and antibodies that recognize these vascular signatures and can be used in targeted delivery therapeutic approaches are being developed<sup>[35-38]</sup>. Pigment-epithelium derived factor is an example of a naturally occurring angiogenesis inhibitor which has an important role in vascularisation in the eye, targets only new vessel growth and has shown good potency in *in vitro* and *in vivo* models<sup>[39]</sup>. However, an important challenge for the successful translation of angiogenesis inhibitors into clinical application is the lack of markers to determine efficacy in most cases<sup>[29]</sup>.

Polymorphisms in the angiogenic genes/factors may in part explain the variation in tumour angiogenesis, which has been observed between individuals. The establishment of a DNA repository containing samples from over 1,800 breast cancer patients to identify gene polymorphisms in angiogenesis-related genes that play an important role in tumour growth and progression illustrates the intensive efforts that are underway in this area<sup>[40]</sup>. It is clear that in order to optimize anti-angiogenic therapy a much greater understanding of the fundamentals of angiogenesis will be required which should lead to new approaches of attacking tumour vasculature<sup>[41]</sup>.

### Epigenetic silencing

Epigenetic inactivation of genes that are crucial for the control of normal cell growth is a characteristic of cancer cells<sup>[42,43]</sup>. These epigenetic mechanisms include crosstalk between DNA methylation, histone modification and other components of chromatin higher-order structure, and lead to the regulation of gene transcription. Unlike mutagenic events, epigenetic events can be reversed to restore the function of key control pathways in malignant and pre-malignant cells and re-expression of genes epigenetically inactivated can result in the suppression of tumour growth or sensitization to other anti-cancer therapies<sup>[43]</sup>. Small molecules that reverse epigenetic inactivation are now undergoing clinical trials. This, together with epigenomic analysis of chromatin alterations such as DNAmethylation and histone acetylation, opens up the potential to define epigenetic patterns of gene inactivation in tumours and to use drugs that target epigenetic silencing<sup>[42]</sup>.

Two key changes in chromatin are associated with epigenetic transcriptional repression - DNA methylation and histone modifications. DNA methylation is the only commonly occurring modification of human DNA and results from the activity of a family of DNA methyltransferase enzymes (DNMT). DNA methylation leads to the binding of a family of proteins known as methyl-binding domain (MBD) proteins. Several of the members of this family have been shown to be associated with large protein complexes containing histone deacetylase (HDAC). To date several trials using agents that target DNMTs and HDACs have been completed or are underway<sup>[42]</sup>.

### Mitochondria

Genetic and/or metabolic alterations in this organelle are causative or contributing factors in a variety of human diseases including cancer. Point mutations, deletions or duplications of mitochondrial DNA are found in many cancers and the

accumulation of mutations in mitochondrial DNA has been found to be tenfold greater than that in nuclear DNA. The many distinct differences in mitochondrial structure and function between normal cells and cancer cells provide molecular sites against which novel and selective chemotherapeutic agents might be targeted<sup>[44]</sup>.

A new class of anti-cancer agents {lipophilic cations (DLCs)} has been developed that exploits the higher mitochondrial membrane potential seen in some carcinoma cells versus control epithelial cells. Although the use of DLCs as anti-cancer agents has shown promise, there is at present no real understanding of the biochemical basis for the increased mitochondrial membrane potential in carcinoma cells. Knowledge of the specific biochemical alterations leading to the increased membrane potential should lead to a more rational approach to the choice of highly selective DLCs for clinical use in the future<sup>[44,45]</sup>.

### Carbohydrates

Experimental evidence directly implicates complex carbohydrates in recognition processes, including adhesion between cells, adhesion of cells to the extracellular matrix, and specific recognition of cells by one another. In addition, carbohydrates are recognized as differentiation markers and as antigenic determinants. Modified carbohydrates and oligosaccharides have the ability to interfere with carbohydrate-protein interactions and therefore, inhibit the cell-cell recognition and adhesion processes, which play an important role in cancer growth and progression. Galectins are a family of proteins that share an affinity for -galactoside moieties and significant sequence similarity in their carbohydrate-binding sites. Many epithelial tumours, such as colon, thyroid and breast express both galectin-1 and -3. Increased expression of galectin-1 by tumour cells is positively correlated with a metastatic phenotype and a poorly differentiated morphology. Selectins are a group of cell adhesion molecules that bind to carbohydrate ligands and play a critical role in host defence and in tumour progression and metastasis.

Interfering with normal cell recognition using a large or a small sugar molecule has been reported to block the progression of tumours by interfering with angiogenesis, cell-cell, cell-matrix interactions, tumour invasion, and metastasis and a modified natural polysaccharide modified citrus pectin (MCP) has been shown to have anti-tumour effects *in vitro* and in animal models. In Phase II clinical trials on colorectal cancer patients, MCP showed clinical activity, with five out of 23 patients showing tumour stabilization and one patient showing tumour shrinkage<sup>[46]</sup>.

### Cyclooxygenase 2 (COX-2)

COX-2 is an inducible prostaglandin G/H synthase, which is over-expressed in several human cancers. Oncogenes, growth factors, cytokines, chemotherapy and tumour promoters stimulate COX-2 transcription via protein kinase C and RAS-mediated signalling. For example, the level of COX-2 is elevated in breast cancers that over-express HER-2/neu as a result of increased signalling. The use of non-steroidal anti-inflammatory drugs (NSAIDs), which are prototypic COX-2 inhibitors, is associated with a reduced risk of several malignancies, including colorectal cancer<sup>[47]</sup>. Treatment with celecoxib, a selective COX-2 inhibitor, has been shown to reduce the number of colorectal polyps in patients with familial adenomatous polyposis (FAP)<sup>[48]</sup>. Selective COX-2 inhibitors are being evaluated in conjunction with chemotherapy and radiotherapy in patients with cancers of the colon, lung, esophagus, pancreas, liver, breast and cervix. These studies should provide information on whether selective COX-2 inhibitors are effective in either preventing or treating cancer<sup>[47,49]</sup> and the results of these clinical trials are awaited.

### Antisense RNA or oligonucleotides

Following the initial discoveries of natural antisense RNAs in prokaryotes, numerous applications of antisense RNA-mediated regulation have been demonstrated in a variety of experimental systems<sup>[50,51]</sup>. These non-translated mRNAs directly repress gene expression by hybridizing to a target RNA, rendering it functionally inactive. Specificity of antisense RNA for a particular transcript is conferred by extensive sequence complementarity with the 'sense' or target RNA. Translation of a target mRNA is inhibited following formation of a sense-antisense RNA hybrid. In addition, the duplex molecule may become sensitive to double-strand-specific cellular nucleases. Other effects of antisense RNA may include transcriptional attenuation of the mRNA and also disruption of post-transcriptional processing events<sup>[51]</sup>.

Oncogene DNA and RNA differ in nucleotide sequences from normal proto-oncogene DNA and RNA, and it is therefore theoretically possible to design specific antisense molecules to block translation of oncogene mRNA. There have been many attempts to reverse the transformed phenotype by expressing large amounts of mRNA from the DNA strands complementary to the one coding an aberrant oncogene protein. In the nuclei of the cells the two complementary mRNA strands hybridize to form a double-stranded structure that effectively prevents translation of the mRNA. It is now possible to design antisense oligonucleotides (ODNs), or catalytic antisense RNAs (ribozymes),

which can pair with and functionally inhibit the expression of any single stranded nucleic acid. These compounds interact with mRNA by Watson-Crick base-pairing and are therefore, highly specific for the target protein. This high degree of specificity has made them attractive candidates as therapeutic agents<sup>[52]</sup>. To give just one example out of many, ODNs directed at HER2 are in preclinical evaluation for the treatment of breast cancer<sup>[2]</sup>. With the implementation of gene therapy in early clinical trials, oligonucleotide mediated suppression of gene expression has emerged as an important complementary strategy to gene therapy. Evaluation of the antisense blocking of specific genes involved in cancer, AIDS and a variety of other diseases has resulted in questions arising about how these genes really work<sup>[53,54]</sup>. Even though the phosphorothioates are generally believed to represent the first generation of antisense nucleotides, they suffer from certain drawbacks and non-specific side effects<sup>[55]</sup>. *In vivo* data is mainly limited to methylphosphonates and in particular phosphorothioates, which have entered clinical trials as the first generation of antisense compounds.

However, as stressed in a review of the antisense treatment of viral infection<sup>[56]</sup>, many simple but critical questions remain unanswered and this is also true of its application in cancer. A review in the mid 1990s<sup>[57]</sup> focused on those aspects of chemistry and mechanism that were thought to be important and relevant for the therapeutic use of deoxynucleotide agents. Most of these, as well as the promise and the shortcomings<sup>[58,59]</sup> in the field of antisense are still relevant today.

In haematological disorders antisense ODNs are being employed as *ex vivo* bone marrow purging agents and as potential drugs for direct *in vivo* administration to patients with leukaemia<sup>[60,61]</sup>. *In vitro* data from cell culture experiments showed that an antisense ODN (G3139) designed to hybridize with the mRNA of *BCL2* can sensitize lymphoma cells to the apoptotic effects of chemotherapeutic agents. A Phase I study in 21 patients with *BCL2*-positive relapsed non-Hodgkin's lymphoma patients who received an 18-mer phosphorothioate ODN complementary to the first six codons of the *BCL2* open reading frame (G3139) showed that no systemic toxicity was seen at daily doses up to 110.4 mg/m<sup>2</sup> and that *BCL2* protein was reduced in seven of 16 assessable patients<sup>[62]</sup>. Phase I and II studies are also being undertaken to test G3139 in combination with docetaxel in patients with advanced breast cancer, hormone-refractory prostate cancer and other solid tumours<sup>[63]</sup>.

ISIS 5132 is an antisense oligonucleotide which has been shown to reduce Raf-1 mRNA levels in the

blood cells from treated patients in Phase 1 clinical trials. The results of Phase II trials are awaited.

Another target of antisense ODNs is protein kinase C- $\alpha$  (PKC- $\alpha$ ), which belongs to a class of serine-threonine kinases. An antisense ODN directed against PKC- $\alpha$  has been evaluated in Phase I and II studies in patients with low-grade lymphomas, and in combination with carboplatin and paclitaxel in patients with stage IIB or IV non-small cell lung cancer. Antisense ODNs against *RAF-1*, *HRAS*, *MYB*, protein kinase A and DNA methyltransferase are also undergoing preliminary clinical investigation in patients with a variety of cancers including haematological, colorectal, breast and ovary<sup>[63]</sup>.

It has become clear that antisense therapeutics is considerably more problematic than was naively assumed initially and the approach has yet to have a substantial impact on clinical practice. However, there is considerable evidence that antisense ODNs are effective *in vitro*. Critical analysis of the molecular and cellular behaviour of antisense ODNs indicate that the clinical strategies that have been utilized so far are sub-optimal for a number of reasons including unfavorable antisense chemistries, the wrong target or failure to achieve intracellular access. Considerable further basic research is required and an optimal antisense strategy is therefore some years away<sup>[61]</sup>.

### RNA interference/inhibition (RNAi)

RNAi is an innate cellular process, which is activated when a double stranded RNA (dsRNA) molecule of greater than 19 duplex nucleotides enters the cell, causing the degradation of not only the invading dsRNA molecule, but also single stranded RNAs (ssRNAs) of identical sequence, including endogenous mRNAs. RNA interference methods, like antisense strategies, are based on nucleic acid technology. However, unlike the antisense approach, double stranded RNA activates a normal cellular process leading to a highly specific RNA degradation and to cell-to-cell spreading of this gene silencing effect in several RNAi models. This systemic property potentially provides great promise for therapy because the delivery problems that have plagued other nucleic acid based therapies could be at least partly alleviated in RNAi-based gene silencing applications<sup>[64-66]</sup>.

The demonstration that a single base difference in synthetic small inhibiting RNAs (siRNAs) can discriminate between mutated and wild type (WT) p53 in cells expressing both forms, and can result in the restoration of WT protein<sup>[67]</sup>, indicates the potential of this approach. A better description of the systemic nature of the response in whole

animals together with the ongoing improvements in *in vivo* nucleic acid delivery technologies could enable RNAi to be used therapeutically, as a single agent or in combination, sooner than is predicted at present<sup>[64,66,67]</sup>.

The second part of this review will deal with gene therapy, immunotherapy and future prospects.

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## REFERENCES

1. Workman P, Kaye SB. Translating basic cancer research into new cancer therapeutics. *Trends Mol Med* 2002; 8:1-9.
2. Zwick E, Bange J, Ullrich A. Receptor tyrosine kinases as targets for anticancer drugs. *Trends Mol Med* 2002; 8:17-23.
3. Fabbro D, Parkinson D, Matter A. Protein tyrosine kinase inhibitors: new treatment modalities? *Curr Opin Pharmacol* 2002; 2:374-381.
4. de Bono JS, Rowinsky EK. The ErbB receptor family: a therapeutic target for cancer. *Trends Mol Med* 2002; 8:19-26.
5. Topaly J, Freuhauf S, Ho AD, *et al.* Rationale for combination therapy of chronic myelogenous leukaemia with imatinib and irradiation or alkylating agents: implications for pretransplant conditioning. *Br J Cancer* 2002; 86:1487-1493.
6. Capdeville R, Buchdunger E, Zimmermann J, *et al.* Glivec (STI571, imatinib), a rationally developed, targeted anticancer drug. *Nature Rev Drug Discov* 2002; 1:493-502.
7. Osborne CK. Steroid hormone receptors in breast cancer management. *Breast Cancer Res Treat* 1998; 51:227-238.
8. Park WC, Jordan VC. Selective estrogen receptor modulators (SERMs) and their roles in breast cancer prevention. *Trends Mol Med* 2002; 8:82-88.
9. Cauley JA, Norton L, Lippman ME, *et al.* Continued breast cancer risk reduction in postmenopausal women treated with raloxifene: 4-year results from the MORE trial. Multiple outcomes of raloxifene evaluation. *Breast Cancer Res Treat* 2001; 65:125-134.
10. Shang Y, Brown M. Molecular determinants for the tissue specificity of SERMs. *Science* 2002; 295:2465-2468.
11. Zusi FC, Lorenzi MV, Vivat-Hannah V. Selective retinoids and rexinoids in cancer therapy and chemoprevention. *Drug Deliv Today* 2002; 7:1165-1174.
12. Sporn MB, Suh N, Mangelsdorf DJ. Prospects for prevention and treatment of cancer with selective PPARgamma modulators (SPARMs). *Trends Mol Med* 2001; 7:395-400.
13. Adams J. Potential for proteasome inhibition in the treatment of cancer. *Drug Discov Today* 2003; 8:307-315.
14. Lane P, Lain S. Therapeutic exploitation of the p53 pathway. *Trends Mol Med* 2002; 8:38-42.
15. www.iacr.fr/p53
16. Hupp TR, Lane DP, Ball KL. Strategies for manipulating the p53 pathway in the treatment of human cancer. *Biochem J* 2000; 352:1-17.
17. Chang EH, Pirollo KF, Bouker KB. Tp53 gene therapy: a key to modulating resistance to anticancer therapies? *Mol Med Today* 2000; 6:358-364.
18. Bykov VJN, Issaeva N, Shilov A, *et al.* Restoration of the tumour suppressor function to mutant p53 by a low-molecular-weight compound. *Nature Med* 2002; 8:282-288.
19. Lane DP, Hupp TR. Drug discovery and p53. *Drug Discov Today* 2003; 8:347-355.
20. Ashkenazi A. Targeting death and decoy receptors of the tumour-necrosis factor superfamily. *Nature Rev Cancer* 2002; 2:420-430.
21. Kolch W, Kotwaliwale A, Vass K, *et al.* The role of Raf kinases in malignant transformation. *Expert Rev Mol Med* 2002; 25 April, <http://www.expertreviews.org/02004386h.htm>
22. Knockaert M, Greengard P, Meijer L. Pharmacological inhibitors of cyclin-dependent kinases. *Trends Pharmacol Sci* 2002; 23:417-425.
23. Senderowicz AM. Development of cyclin-dependent kinase modulators as novel therapeutic approaches for Haematological malignancies. *Leukemia* 2001; 15:1-9.
24. Cristofanilli M, Charnsangavej C, Hortobagyi GN. Angiogenesis modulation in cancer research: novel clinical approaches. *Nature Rev Drug Discov* 2002; 1:415-426.
25. He Y, Kozaki K, Karpanen T, *et al.* Suppression of tumour lymphangiogenesis and lymph node metastasis by blocking vascular endothelial growth factor receptor 3 signalling. *J Natl Cancer Inst* 2002; 94:819-825.
26. Sweeney CJ, Mille, KD, Sledge GW. Resistance in the anti-angiogenic era: nay-saying or a word of caution. *Trends Mol Med* 2003; 9:24-28.
27. Madhusudan S, Harris AL. Drug inhibition of angiogenesis. *Curr. Opin. Pharmacol* 2002; 2:403-414.
28. McCarty MF, Liu W, Fan F, *et al.* Promises and pitfalls of anti-angiogenic therapy in clinical trials. *Trends Mol Med* 2003; 9:53-58.
29. Kerbel R, Folkman J. Clinical translation of angiogenesis inhibitors. *Nature Rev Cancer* 2002; 2:727-739.
30. Semenza GL. HIF-1 and tumour progression: pathophysiology and therapeutics. *Trends Mol Med* 2002; 8:62-67.
31. Brahimi-Horn C, Berra E, Pouyssegur J. Hypoxia: the tumour's gateway to progression along the angiogenic pathway. *Trends Cell Biol* 2001; 11:32-36.
32. Pili R, Donehower RC. Is HIF-1a a valid therapeutic target? *J Natl Cancer Inst* 2003; 95:498-499.
33. Semenza, G.L. Targeting HIF-1 for cancer therapy. *Nature Rev Cancer* 2003; 3:721-732.
34. Yu JL, Rak JW, Coomber BL, *et al.* Effect of p53 status on tumour response to antiangiogenic therapy. *Science* 2002; 295:1526-1528.
35. Pasqualini R, Arap W, McDonald DM. Probing the structural and molecular diversity of tumour vasculature. *Trends Mol Med* 2002; 8:563-571.
36. Ruoslahti E. Drug targeting to specific vascular sites. *Drug Discov Today* 2002; 7:1138-1143.
37. Laakkonen P, Porkka K, Hoffman JA, *et al.* A tumour-homing peptide with a targeting specificity related to lymphatic vessels. *Nature Med* 2002; 8:751-755.
38. Bikfalvi A, Bicknell R. Recent advances in angiogenesis, anti-angiogenesis and vascular targeting. *Trends Pharmacol Sci* 2002; 23:576-582.
39. Tombran-Tink, J, Barnstable, CJ. Therapeutic prospects for PEDF: more than a promising angiogenesis inhibitor. *Trends Mol Med* 2003; 9:244-250.
40. Balasubramaniam SP, Brown NJ, Reed MWR. Role of genetic polymorphisms in tumour angiogenesis. *Br J Cancer* 2002; 87:1057-1065.

41. Munn LL. Aberrant vascular architecture in tumours and its importance in drug-based therapies. *Drug Discov Today* 2003; 8:396-403.
42. Brown R, Strathdee G. Epigenomics and epigenetic therapy of cancer. *Trends Mol Med* 2002; 8:43-48.
43. Jones PA, Baylin SB. The fundamental role of epigenetic events in cancer. *Nature Rev Genet* 2002; 3:415-428.
44. Modica-Napolitano, J.S. and Singh, K.K. (2002) *Expert Rev. Mol. Med.*, 11 April, <http://www-ermm.cbuc.cam.ac.uk/12004453h.htm>
45. Weissig V, Torchilin VP, editors. Drug and DNA delivery to mitochondria. *Adv Drug Deliv Rev* 2001; 49: Nos. 1-2 (entire issue).
46. Nangia-Makker P, Conklin J, Hogan V, *et al.* Carbohydrate-binding proteins in cancer, and their ligands as therapeutic agents. *Trends Mol Med* 2002; 8:187-192.
47. Subbaramaiah K, Dannenberg AJ. Cyclooxygenase 2: a molecular target for cancer prevention and treatment. *Trends Pharmacol Sci* 2003; 24:96-102.
48. Steinbach G, Lynch PM, Phillips KS, *et al.* The effect of celecoxib, a cyclooxygenase-2 inhibitor, in familial adenomatous polyposis. *New Engl J Med* 2000; 342:1946-1952.
49. Iñiguez MA, Rodríguez A, Volpert OV, *et al.* Cyclooxygenase-2: a therapeutic target in angiogenesis. *Trends Mol Med* 2003; 9:73-78.
50. Green PJ, Pines O, Inouye M. The role of antisense RNA in gene regulation. *Ann. Rev Biochem* 1986; 55:569-597.
51. Takayama KM, Inouye M. Antisense RNA. *Crit Rev Biochem Mol Biol* 1990; 25:155-184.
52. Rossi JJ. Therapeutic antisense and ribozymes. *Br Med Bull* 1995; 51:217-225.
53. Gura T. Antisense has growing pains. *Science* 1995; 270:575-577.
54. Rojanasakul Y. Antisense oligonucleotide therapeutics: Drug delivery and targeting. *Adv Drug Deliv Rev* 1996; 18:115-131.
55. Stein CA, Cheng YC. Antisense oligonucleotides as therapeutic agents - is the bullet really magical? *Science* 1993; 261:1004-1012.
56. Whitton JL. Antisense treatment of viral infection. *Adv Virus Res* 1994; 44:267-303.
57. Heidenreich O, Kang S-H, Xu X, *et al.* Application of antisense technology to therapeutics. *Mol Med Today* 1995; 1:128-133.
58. Wagoner R. The state of the art in antisense research. *Nature Med* 1995; 1:1116-1118.
59. Stein CA. Does antisense exist? *Nature Med* 1995; 1:1119-1121.
60. Agarwal N, Gewirtz AM. Oligonucleotide therapeutics for hematologic disorders. *Biochim Biophys Acta* 1999; 1489:85-96.
61. Clark RE. Antisense therapeutics in chronic myeloid leukaemia: the promise, the progress and the problems. *Leukemia* 2000; 14:347-355.
62. Waters JS, Webb A, Cunningham D, *et al.* Phase I clinical and pharmacokinetic study of bcl-2 antisense oligonucleotide therapy in patients with non-Hodgkin's lymphoma. *J Clin Oncol* 2000; 18:1812-1823.
63. Tamm I, Dorken B, Hartmann G. Antisense therapy in oncology: new hope for an old idea? *Lancet* 2001; 358:489-497.
64. Shuey DJ, McCallus DE, Giordano T. RNAi; gene-silencing in therapeutic intervention. *Drug Deliv Today* 2002; 7:1040-1046.
65. Agami R. RNAi and related mechanisms and their potential use for therapy. *Curr Opin Chem Biol* 2002; 6:829-834.
66. Shi Y. Mammalian RNAi for the masses. *Trends Genet* 2003; 19: 9-12.
67. Martinez LA, Naguibneva I, Lehrmann H, *et al.* Synthetic small inhibiting RNAs: efficient tools to inactivate oncogenic mutations and restore p53 pathways. *Proc Natl Acad Sci U.S.A.* 2002; 99:14849-14854.