The molecules of cancer

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The past decade has brought remarkable advances in our ability to dissect the cancer cell into its molecular components and compare it with its normal counterpart. In doing so, numerous genes have been discovered whose encoded products are vital to normal growth regulation. Their disruption can lead to the development of malignancy. The first set to be identified—the oncogenes—were found as hijacked pieces of cellular genetic information lurking in the relatively simple genomes of the RNA tumour viruses. These organisms, of little relevance to human cancer, cause a wide range of tumours in cats, rodents, monkeys and birds. The isolation and cloning of specific sequences from the virus genome, which could transform cells in culture, eventually led to the realisation that these genes were derived from cellular homologues in vertebrates.

The past 5 years has seen considerable progress in the identification of the products of these genes. In each case the encoded protein has some function in the complex growth control cascade that transmits signals from the outside of a cell to its nucleus. Some proteins have been well defined structurally, and their upstream and downstream interactions clearly elucidated. There are still many gaps in our knowledge of this intricate pathway, although new technology has accelerated the analysis of these molecular cogs.

The discovery that defects in the structure or function of genes whose normal function is to slow down growth can also lead to malignancy has stimulated considerable interest (Table 1). These tumour suppressor genes or anti-oncogenes were first identified by classical genetic studies in rare tumours such as retinoblastoma and familial Wilms’ tumour. They almost certainly represent a class of genes whose products are widely involved in the suppression of cancer. Defects in their products have now been associated with various common cancers.

The discovery of new growth control systems has been a major breakthrough in our understanding of the molecular biology of cancer with many exciting clinical implications. Amplification or increased expression of certain oncogenes has been related to prognosis. Specific oncogene mutations are associated with certain cancer types. This information could well be of use in making treatment decisions. Perhaps of greater significance is the potential for developing new drugs to inhibit growth by interacting with these new molecular targets. In this way novel, systemic, selective agents are likely to become available for clinical trial in the near future.

Table 1. Cancer genes

| Dominant oncogenes |
|-------------------|
| Indirectly related genes |
| Xeroderma pigmentosum |
| Ataxia telangiectasia |
| Bloom’s syndrome |
| Fanconi’s anaemia |
| Tumour suppressor genes |

The discovery of oncogenes

The evidence that specific genes have a role in carcinogenesis first came from the RNA tumour viruses. These small organisms possess only three genes; two encode structural proteins and the third is for reverse transcriptase, the enzyme that produces a DNA copy of the viral RNA, thus allowing incorporation of the viral genome into the host DNA. The addition of a fourth gene confers on these viruses the ability to induce tumours rapidly (Fig. 1). These transforming genes were termed viral oncogenes (v-oncs). They were cloned and sequenced and found to have homologues present in normal cellular genomes (c-oncs) [1]. This surprising observation led to the understanding that the viral oncogenes were in fact cellular in origin and represented the incorporation of growth control genes in an activated state in the viruses. The confusing nomenclature of oncogenes has arisen because the viral genes were named after the tumours in which they were first described. Thus v-myc represents the oncogene responsible for myelocytomatosis, a chicken leukaemia, and v-src that for chicken sarcoma [2] (Table 2).

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New technology

Several techniques have been used to explore the molecular biology of these genes and their proteins. Techniques such as Southern blotting, polymerase chain reaction, DNA sequencing, dot blot hybridisation, Western blotting and immunohistology have allowed the analysis of DNA, RNA and protein in clinical samples from a variety of neoplasms. A major problem has been that many of the reagents for such exploration are only just becoming available. Monoclonal antibodies constructed against a variety of oncogene products are now commercially available [3]. It is likely that, over the next few years, such reagents will make possible the dissection of the full catalogue of the fifty or more oncogene products. There are now hints that the information provided from such analyses can give us a molecular blueprint for a cell and can help to predict the likely course of disease in an individual patient. The temporal and spatial expression of these genes produces the molecular framework from which the destiny of the tumour and hence the patient derives (Fig. 2). They also provide novel targets for pharmacological and immunological attack.

Oncogene function

Growth factors

Growth factors are extracellular proteins that act as growth modulators. Some growth factors will transform normal cells *in vitro*. Conversely, in some systems the induction of cell transformation can result in an increased production of growth factor [4]. The observation that growth factors can both initiate transformation and be a product of it suggests the possibility of self-perpetuating positive feedback loops with unregulated cell division as a consequence. This phenomenon, termed autocrine secretion, has been implicated in situations involving rapid growth such as wound repair and embryogenesis as well as malignant transformation. Both transformation and growth factor stimulation result in similar biochemical changes such as increased tyrosine phosphorylation and altered cellular lipid metabolism [5]. One human oncogene, c-sis, codes for part of platelet derived growth factor (PDGF) [6]. Certain small-cell lung cancer lines grown in the laboratory actively secrete bombesin, a self-stimulating growth factor without which they are unable to reproduce. Tumour growth factor alpha (TGF alpha) binds to the epidermal growth factor receptor and acts in a complex way, stimulating the growth of different kinds of cells. A related product, TGF beta, can be inhibitory in certain circumstances [7]. The complex inter-relationship of extracellular factors with a plethora of surface recep-

Table 2. Some examples of viral oncogenes and their cellular counterparts

| Oncogene | Species of retrovirus origin | Tumour | Human gene | Function of product |
|----------|-------------------------------|--------|------------|--------------------|
| v-src    | Chicken                       | Sarcoma| c-src      | Tyrosine kinase    |
| v-ras    | Rat                           | Sarcoma| c-ras      | GTP binding protein|
| v-myc    | Chicken                       | Leukaemia| c-myc   | Nuclear binding protein|
| v-fes    | Cat                           | Sarcoma| c-fes      | Tyrosine kinase    |
| v-sis    | Monkey                        | Sarcoma| c-sis      | Growth factor      |
| v-erbB   | Chicken                       | Erythroblastosis| v-erbB | EGF receptor      |
| v-myb    | Chicken                       | Myeloblastosis| c-myb   | Nuclear binding protein|
| v-fms    | Cat                           | Sarcoma| c-fms      | Growth factor receptor|
| v-abl    | Mouse                         | Leukaemia| c-abl   | Tyrosine kinase    |
| v-fos    | Mouse                         | Osteosarcoma| c-fos   | Nuclear binding protein|

The three-letter code is derived from the type of tumour or the species affected by the virus.
tors will eventually determine the growth status of a cell and thus its malignant potential.

**Growth factor receptors**

Several oncogene encoded proteins form part of a cell surface receptor. The structure of these receptors is similar. There is an external or ligand-binding domain, a transmembrane portion and an internal component that contains tyrosine kinase activity [8]. The receptor is switched on when tyrosine kinase activity is enhanced, so resulting in the phosphorylation of intracellular proteins. Activation is caused by external ligand binding and a subsequent change in the shape of the external domain. This in turn leads to aggregation of receptors with the drawing together of the tyrosine kinase internal domains, so stimulating activity. There is also some evidence that internal regulatory regions exist to allow responses to intracellular events. The receptor can be seen as a controller susceptible to several influences—an integrator of several disparate stimuli.

Small alterations in the receptor can produce defects in the regulation of tyrosine kinase activity. An example of this is c-erbB1 which codes for the cellular receptor of epidermal growth factor (EGF) [9]. The equivalent viral gene v-erbB, which has transforming activity, encodes a receptor with a truncated external domain. In addition there can be alterations in the regulatory region internally. Transformation can occur by the viral gene product assuming a locked-on configuration tricking the cell into rapid growth. The second mechanism is alteration in tyrosine kinase activity. An example of this is the c-fms oncogene which codes the receptor for the colony stimulating factor (CSF) in differentiating macrophages. The transforming viral gene v-fms possesses enhanced kinase activity compared with its cellular counterpart [10].

**Intracellular messengers**

Transduction of signal from surface to nucleus is clearly an essential component of intracellular communication. Candidates for these intracellular messengers have been identified although their exact integration is not clear. The best example of oncogene involvement is the ras family [11]. These gene products have structural similarities to the G and M proteins that control adenylate cyclase activity. Linked through their C terminus to the cell membrane, ras-encoded products bind GTP and hydrolyse it to GDP. Another protein, GTPase activating protein (GAP), enhances this reaction and may well act as the downstream effector molecule. Various mutants of ras found in tumours have lower GTPase activity and lower potential for activation by GAP. Such proteins are more likely to be kept in the GDP-bound inactive state, leading to growth stimulation. Proteins like ras are part of a second messenger system providing links between the different control components in the cell.

**Nuclear acting oncogenes**

Several oncogenes such as myc, myb and fos encode nuclear associated proteins. Their precise localisation and function are unknown but they are almost certainly intimately involved in the control of transcription. Indeed, recent laboratory studies have shown a close relationship between transcription factors and oncoproteins [12]. When serum is added to serum-deprived quiescent fibroblasts, a sudden burst of mitotic activity is seen within a few hours. Time is required for the early signals of cell division to take effect. This model system demonstrates that the induction of two oncogenes, c-fos and c-jun, is amongst the first nuclear events to occur. The encoded proteins form a DNA binding complex which activates the transcription of specific genes [13]. Abnormalities in the structure or function of such complexes can lead to transformation.

**Tumour suppressor genes (Anti-oncogenes)**

The oncogenes discovered through the RNA viruses are dominantly active. The presence of excess or mutated product leads to neoplasia. But the defective expression of some normal gene products can have the same effect. The retinoblastoma gene (RB) located on the long arm of chromosome 13 is a good example. This rare childhood tumour occurs either as an inherited form or, more rarely, in a sporadic manner (Fig. 3). In familial cases, a karyotypic defect on chromosome 13 has been observed, not just in the tumour but in all cells of the affected individual [14]. In the tumour the relevant gene on the chromosome derived...
from the other parent—the other allele—is also defective. The incidence is much higher in affected families. With one chromosome already damaged, only a single further tumourigenic event is needed. Presumably this event is only carcinogenic in the developing and not the mature retina, so explaining the age distribution of the patients.

The discovery of this genetic mechanism has led to tremendous activity to identify the RB gene and its product [15]. It turns out to be a 110 kd protein which binds avidly to the transforming proteins E1A and large T of the tumour virus SV40 [16]. This physical link opens new avenues in which to explore RB protein function. Clearly it interacts with nuclear structures to slow down cell division (Fig. 4). Being mopped up by other proteins or its defective production releases a cell from some inhibitory influence. Intriguing data on reduced RB protein content in various common cancers such as those of breast, bladder and lung have recently been obtained [17]. The defective expression of a product is much more difficult to assess in clinical samples by either immunochemistry or immunohistology than the presence of high levels or a mutated protein. For this reason these results need further verification to determine their real significance.

There are several other human cancers in which specific loss mutations have been detected. These include multiple endocrine neoplasia, familial Wilms' tumour, acoustic neuroma, colon cancer, small cell lung cancer and certain melanomas [18]. The gene for familial adenomatous polyposis fits the model well. It is located on chromosome 5 and the heterozygote develops benign multiple polyps. Further mutation to homozygosity results in progression to adenocarcinoma [19]. Mutations at the same site are present in at least 30% of sporadic colorectal carcinomas, suggesting that the mechanism may be important in the development of the type of tumour [20]. More recently another tumour suppressor gene has been identified which is deleted in 70% of colon carcinomas [21].

Located on chromosome 18, it appears to code for surface glycoproteins involved in cell adhesion. This gene, called DCC (deleted in colorectal carcinomas), is large with a transcript size of 11 kb and is highly expressed in normal brain. The study of serial genetic changes in colorectal cancer and its precursor lesions may well result in much clearer understanding of the molecular nature of this disease.

Evidence is now accumulating that the paternal and maternal alleles of tumour suppressor genes may have differential susceptibilities to mutation. This phenomenon, termed genomic imprinting, was first shown in retinoblastoma, where germ line mutations were mainly found in the paternally derived chromosome 13 [22]. Even in the sporadic forms there is some evidence pointing to the paternal chromosome being the first to acquire the defect. These observations may have relevance to genetic counselling as well as suggesting the presence of intriguing molecular mechanisms of chromosome tagging.

Although little is known about the products of tumour suppressor genes, they are likely to inhibit cell turnover in the relevant tissues. There are several possible mechanisms for this: they may control the secretion of inhibitory growth factors such as TGF beta, tumour necrosis factor and various other cytokines. Other candidates are the processes controlling the integrity of contact inhibition, the proteins that inhibit oncogene effects and the inhibitors of gene transcription (Fig. 5).

The evidence for the existence of tumour suppressor genes is now strong (Table 3). As well as clinical data, there is good evidence from animal model systems in which tumours follow a recessive inheritance pattern (Table 4). Neuroblastosomas in Drosophila and melanomas in Xiphophorus (the swordfish) are two
good examples [23,24]. Furthermore, their existence explains the results of rather puzzling cell fusion experiments first reported almost three decades ago [25]. Fusing cancer and normal cells can often result in the hybrids having a normal growth control mechanism. The normal partner has provided an effective suppressor component. The mechanism of suppression can now be studied experimentally by investigating the artificial transfer of single cloned genes. Recently the transfection of RB into the malignant cell lines has been shown to convey properties of the benign phenotype [26]. Such studies may be of therapeutic relevance if selective vectors suitable for in vivo use can be developed.

**Diagnostic and prognostic potential**

**DNA**

Karyotypic abnormalities were noted in various cancers several decades ago. The refinement of modern molecular biology has allowed the examination of specific segments of chromosomes for amplification and mutation. Table 5 lists genes found to be amplified in certain tumours. For some tumours there is a correlation between amplification and clinical outcome. The tightest example is neuroblastoma where disease-free and absolute survival is strongly correlated with amplification of the N-myc gene [27]. A weaker, but equally interesting, correlation has been observed with the erbB1 and c-myc genes in breast cancer [28,29]. Related to c-erbB1, which encodes the receptor for epidermal growth factor, is another gene, c-erbB2. It has structural homology to c-erbB1 but codes for a different receptor whose ligand has yet to be identified [30]. Amplification and expression of this gene may correlate with prognosis in breast cancer [31]. Studies are in progress to evaluate the long-term prognostic significance of these abnormalities. Why gene amplification should result in a change in the physiology of the cell remains unclear. A third member, c-erbB3, has just been cloned and sequenced [32]. The main problem is that the function of the proteins encoded by these genes is not known.

Mutations at certain sites within oncogenes can also lead to growth abnormalities; a good example is the c-ras family. Here mutations create proteins that are less able to hydrolyse GTP [33]. Although GTP binding is one of the functions of ras, its other functions remain a mystery. Furthermore, it is clear that normal p21 ras interacts with GTPase activating protein (GAP) which speeds up the hydrolysis of GTP a hundred-fold. Mutant ras proteins have been shown not to interact as efficiently with GAP. This could explain the continued transduction of activated signal through the ras pathway. Although ras amplification with subsequent over-expression has been detected in cervical, ovarian and bladder cancer, it was in a relatively small proportion of tumours [34]. In around a quarter of tumours examined, ras mutations at amino-acid positions 12, 13 and 61 can be detected [35]. The several methods used have different sensitivities, so explaining the wide variation in results. The induction of foci of cells lacking contact inhibition after transfection was the first method to be used. More elaborate methods include the polymerase chain reaction, probe shift detection and RNAase A mismatch cleavage analysis [36].

From such studies it is now clear that certain tumours are associated with a high frequency of mutations at specific sites in a specific member of the ras

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**Table 3. Evidence for tumour suppressor genes**

| Hybridisation studies | Recessive cancer syndromes | Tumour inhibitory factors | Differentiation induction | Negative oncogene expression | Inhibition by transfection |
|-----------------------|---------------------------|--------------------------|--------------------------|-----------------------------|---------------------------|

**Table 4. Tumour suppressor genes**

| Deletion or alteration of specific genes recognised karyotypically or by molecular analysis and associated with tumour development |
|---------------------------------------------------------------|
| Retinoblastoma 13q 14 |
| Wilms' tumour 11p 13 |
| SCLC 3p 14-23 |
| FAP 5q 21-22 |
| Colorectal Ca 5q 21-22 |
| Renal cell Ca 3p 13-14 |
| Meningioma 22p |
| Acoustic neuroma 22q 12-13 |

| Recessive models |
|------------------|
| Drosophila Neuroblastoma |
| Xiphophorus Melanoma |

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Table 5. Amplification of oncogenes in DNA from human tumours, and correlation with clinical outcome

| Gene       | Tumour          | Amplification | Rearrangement | Correlation |
|------------|-----------------|---------------|---------------|-------------|
| c-myc      | Breast          | +             | -             | ++          |
|            | Burkitt’s lymphoma | -             | +             | -           |
|            | Stomach         | +             | -             | +           |
| N-myc      | Neuroblastoma   | +             | -             | +++         |
|            | Retinoblastoma  | +             | -             | +           |
|            | APML            | +             | -             | +           |
|            | Breast          | +             | -             | +           |
| L-myc      | Lung            | +             | -             | +           |
|            | Breast          | +             | -             | -           |
| c-abl      | CML             | -             | +             | -           |
| c-myb      | AML             | +             | -             | -           |
|            | Colon           | +             | -             | -           |
| c-erbB1    | Breast/lung     | +             | -             | ++          |
|            | Glioma/bladder  | -             | -             | -           |
| c-erbB2    | Breast          | +             | -             | ++          |
|            | Stomach         | +             | -             | +           |
|            | Salivary gland  | +             | -             | -           |
|            | Kidney          | +             | -             | -           |
| Ki-ras     | Colon           | +             | -             | -           |
|            | Ovary           | +             | -             | -           |
| N-ras      | Breast          | +             | -             | -           |
| gli        | Brain           | +             | -             | -           |

+ in the final column indicates good correlation of amplified gene with poor prognosis.

family (Table 6). Perhaps the most startling observation is the almost consistent mutation at position 12 in Ki-ras in pancreatic cancer [37] and the association of position 12 N-ras mutants with various haematological malignancies [38]. Clearly the elucidation of the molecular mechanisms involved could provide vital clues as to the origin and perhaps optimal treatment of these neoplasias.

Specific chromosomal translocation is a common feature of certain tumours. An example is Burkitt’s lymphoma which almost invariably carries a translocation involving chromosome 8:14. The break-point in chromosome 8 is at the site of the c-myc oncogene [39]. In the more common follicular lymphoma, translocations between chromosome 14 and 18 have been noted. The break-point region has been cloned and designated bcl-2 [40]. This region may well be an oncogene. The Philadelphia chromosome in chronic myeloid leukaemia is a chromosome 9:22 translocation. Here the c-abl oncogene, which is related to the v-onc of the murine Abelson leukaemia virus, is moved from chromosome 9 into a fragmented segment of chromosome 22. Examination of the break-point shows that it always occurs within a 5.8 kb segment designated bcr (break-point cluster region) [41]. An mRNA transcript has been detected in CML cells which is a fusion product of the c-abl and bcr regions. Furthermore, the protein product of this message has a molecular ratio of 210,000 and appears to have deregulated tyrosine kinase activity. By constructing antibodies against this protein, new diagnostic and, possibly, therapeutic agents may become available. The detection of translocations by extremely sensitive molecular methods such as PCR can be used to detect as few as one cell in 10,000 bearing the abnormality in blood or bone marrow. This level of sensitivity provides a powerful tool to determine endpoints to drug or biological therapy for haematological malignancies [42].

Table 6. Activated ras oncogenes in human tumours

| Tumour      | Number | N-ras(%) | Ki-ras(%) | Ha-ras(%) |
|-------------|--------|----------|-----------|-----------|
| Pancreas    | 54     | 0        | 94        | 0         |
| Colon       | 234    | 0.3      | 36        | 0         |
| Lung        | 133    | 0        | 14        | 0.3       |
| Thyroid     | 42     | 12       | 14        | 24        |
| Melanoma    | 95     | 11       | 12        | 6         |
| Urothelial  | 84     | 0        | 14        | 10        |
| MDS         | 110    | 17       | 0.8       | 3         |
| AML         | 154    | 32       | 3         | 0.5       |
| ALL         | 64     | 22       | 0         | 0         |
RNA

Over the past 4 years an intensive investigation of oncogene transcription in clinical samples has been carried out. Many tumours have been collected, RNA isolated, and the number of copies present per cell estimated, using hybridisation. The simplest and most commonly used method has been dot blotting [43]. Problems abound, however. First, RNA degrades very rapidly, even from the time of surgical clamping. Furthermore, RNA is difficult to process because of its instability. Dot blotting techniques can be tremendously variable and critically depend on the quality and length of the specific probe used. For these reasons, much of the work in the literature is difficult to reproduce and the results must be viewed with some scepticism. Correlations between outcome have been claimed for c-myc and c-ras, in colorectal and breast cancer [44]. However, careful study of DNA, RNA and protein in the same tumour samples is badly needed to clarify the problems.

Protein

In order to examine the relevance of oncoproteins, antibodies have been constructed to synthetic peptides. Such peptides are chosen from hydrophilic plots of the predicted amino acid sequence of the oncogene product. The hydrophilic sequences are likely to be on the outside of the molecule and therefore to constitute antigenic determinants. Polyclonal and monoclonal antibodies are now available to many oncoproteins. These reagents can be used in immunohistochemistry Western blotting. Correlations between prognosis and gene expression have been made for c-myc, N-myc, c-ras, c-erbB1 and c-erbB2 in several tumours [45,46].

Although histology is good for giving geographical information about the distribution of oncoproteins in normal and malignant tissues, it is poor for quantitation. Published papers on experiments using human tumour material do not always report in detail their methods of collection or storage of the specimens. In order to assess the stability of oncogene encoded proteins following collection or storage of human tumour biopsies, we have examined the rate of decay of the c-myc, c-erbB2 and c-erbB1 proteins in several tumours [47] in xenografts. Solid tumours, containing amplified copies of each oncogene, were established in nude mice, and the stability of the oncogene protein in portions of each tumour, left in phosphate buffered saline at room temperature for varying time intervals, was examined by immunoblotting. Intact c-erbB1 and c-erbB2 oncproteins were present even after 24 hours under these conditions while the c-myc protein was apparently rapidly degraded after 20 minutes (Fig. 6). These data demonstrate that oncogene products decay at different rates after tumour resection, and that collection of human biopsies should take this into account in order to provide the basis for consistent measurements of protein expression.

Sensitive flow cytometric assays have also been developed precisely to quantitate oncoproteins in nuclei isolated from wax-embedded tumours [48]. Correlations are apparent between differentiation state and clinical outcome with the concentrations of p62 c-myc and other proteins in lung, colonic, testicular and cervical cancer.

Therapeutic potential

The development of effective systemic therapy has revolutionised the treatment of certain cancers such as Hodgkin’s disease, leukaemia and testicular tumours. But the vast majority of patients with solid tumours are essentially incurable once the disease metastasises [49]. The success of combination chemotherapy now seems to have reached a plateau. Furthermore, few novel drugs are reaching clinical trial—and most are analogues of existing structures which may be useful because of reduced side effects. The discovery of oncogenes and tumour suppressor genes provides new molecular targets for the development of selective anticancer agents (Table 7).

Monoclonal antibodies (MCAs) against growth factors and their receptors have been demonstrated to inhibit tumour growth in various model systems. Thus MCAs to bombesin can drastically reduce the growth of xenografts of human small cell lung cancer lines which require this factor [50]. Antibodies against the external domain of c-erbB1 and c-erbB2 have also been shown to inhibit xenograft growth [51]. Antibodies against cytoplasmic and even nuclear oncoproteins may be effective at destroying partially viable cells which allow entry of antibody. Radiolabelled MCAs against the c-myc oncoprotein can localise small cell lung cancer by immunoscintigraphy [52]. The technology of MCA targeting using antibodies against surface molecules is well developed but badly needs new
Table 7. Molecular targets for new drug development

| Growth factors | Antagonists                  |
|---------------|-----------------------------|
|               | Toxic fusion proteins       |
|               | Antibodies                  |
|               | Downregulation              |
| Receptors     | Signal interference         |
|               | Downregulation              |
| Protein kinases| ATP analogues               |
|               | Substrate analogues         |
|               | Suicide peptides            |
| ras proteins  | GTP analogues               |
|               | GAP binding peptides        |
| Nuclear oncoproteins | Analogues       |
|               | Partition shifters          |
|               | Antisense oligos            |

targets to develop increased specificity of binding with high affinity. In this way drugs and radiation can be selectively deposited on the tumour.

Tyrosine kinase growth factor receptors provide an area of considerable interest. Molecules which mimic the ligand can displace the physiological factor and need not be stimulatory. Toxics fusion proteins can be constructed between potent toxins and TGF alpha which are then selectively taken up by cells expressing high levels of EGF receptor. LHRH analogues have been clinically useful in the management of prostate cancer. These analogues operate by downregulating the expression of the gonadotrophin receptor in the anterior pituitary, reducing LH and FSH secretion and inducing a medical orchidectomy [53]. Recently, gonadotrophin receptors have been demonstrated on prostate cancer cells, suggesting another important site for their action [54]. The mechanism of downregulation is not clear but provides an incentive to investigate similar strategies using tyrosine kinase receptor systems.

A particularly intriguing observation has been the constitutively activated c-erbB2 gene mutants which result from single amino acid changes within the transmembrane domain. Conceptually it was difficult to imagine how this section of the molecule could be relevant to its activity. Molecular modelling using computer graphics elegantly demonstrated that the transforming mutants allow hydrogen bonding between the transmembrane sections of different c-erbB2 encoded proteins [55]. It seems that the aggregation of this receptor is the physiological response to ligand binding. In this way the external signal results in increased tyrosine kinase activity of the internal domain. This model suggests that amphipathic peptides, which bind to the intramolecular aggregation sites, could be inhibitory. Experiments to verify this prediction are now in progress. Similarly, a range of agents with potential tyrosine kinase inhibitory effects is being examined (Table 8).

Within the cell, targets such as cytoplasmic protein kinases, mutant ras proteins and nuclear oncoproteins could all result in novel agents for clinical trial. Mutant ras proteins are an obvious target for the development of selectively acting inhibitors. Position 12 Ki-ras mutants are frequent in pancreatic, colon and lung adenocarcinomas. These diseases alone affect some 30,000 people annually in the UK, resulting in 25,000 deaths. The acylation of the C terminus cysteines allows the insertion of ras into the cell membrane and is one possible site for interference. Inhibitors of mevalonic acid such as compactin can block acylation and reduce cell turnover. The guanine nucleotide binding domain provides another possible target. Analogues of GTP may reduce the downstream activity of mutant ras to normal levels. The ras–GAP interaction is another area under intense investigation. Now that ras proteins have been crystallised and high resolution structural information is becoming available, rational drug design is feasible [56]. Furthermore, elegant in vitro models are available in which to test putative drugs. Cells transfected with mutant ras together with a revertant partner can be tested in parallel for growth inhibition and anchorage dependence—the latter by decreased colony formation in soft agar and the loss of focus formation in monolayer culture. Such systems permit the random screening of many compounds in an empirical manner to search for potential mutant ras blockers. Selectivity against cancer cells may require painstaking clinical studies to determine optimal scheduling in the same way that effective combinations of cytotoxic drugs were devised. Looking further into the future, it should become possible to control gene expression selectively.

Anti-sense oligonucleotides which inhibit the expression of oncoproteins would have considerable potential if methods could be found for delivering them to tumours in vivo. Already a pentadecadeoxyribo-nucleotide complementary to the initiation codon and four downstream codons of human c-myc has been shown to inhibit the in vitro growth of a human leukaemia cell line [57] and the mitogen-induced

Table 8. Growth factor receptors as targets for new drug development

| Ligand antagonists       | LHRH, bombesin, subs.P            |
| Synthentic peptides      | EGF, TGF                          |
| SDM of GF genes          | TGF-exotoxin                      |
| Toxic fusion proteins    | Anti-GF                           |
| Monoclonal antibodies    | TGF, bombesin, PDGF               |
| Anti-GF                  | EGF, TGF                          |
| Extracellular domain binding | Monoclonals, peptides              |
| Dimerisation inhibitors  | c-erbB2                           |
| External domain hindrance| FSBA, genistein,amiloride          |
| Transmembrane domain blockers | ST 638, staurosporine             |
| Tyrosine kinase inhibitors| erbstatin                         |

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stimulation of T lymphocytes [58]. Specific molecular targets for such attack may be present in certain tumours. The chromosome 8:14 translocation found in most Burkitt lymphomas results in the c-myc gene being inserted in the heavy chain immunoglobulin locus. In many cases the breakpoint on chromosome 8 lies downstream of the normal promoter. Transcription is started by a normally cryptic promoter in the first intron. A 21 base sequence complementary to this region can selectively block both c-myc expression and growth of tumour but not normal cells [59]. At the present time it is difficult to see how such observations can be translated into clinical gain. But it may be possible to stabilise oligonucleotides by methylphosphonate backbones, so preventing their degradation by nucleases, or to develop effective packaging systems for their delivery. In future, complicated gene therapy using novel vectors may be able to replace missing or defective tumour suppressor genes and return aberrant growth control to normal.

The speed of acquisition of knowledge in this area is so remarkable that time scales are difficult to predict. Table 9 indicates some of the likely changes in cancer therapy over the coming two decades. But it is almost certain that as we enter the next millennium there will be a vital role for the molecules of cancer in both the diagnosis and treatment of this common disease.

Table 9. Likely developments in cancer patient care over the next two decades

|                      | In 1990                      | By 2000                      | By 2010                      |
|----------------------|-----------------------------|-----------------------------|-----------------------------|
| Diagnosis            | Tumour markers              | PET scanning                | Gene prediction             |
|                      | CFMIR                       | Molecular histology         | Prognostic profiling        |
|                      | Immunohistology             |                             |                             |
| Radiation            | Computerisation after loading| Conformational therapy      | Computer optimisation        |
|                      |                             | MCA targeting               | Individualisation of dose   |
| Drugs                | Cytotoxics                  | Anti-growth factors         | Transcription control       |
|                      |                             | Receptor blockade           | Gene transfer               |
|                      |                             | one blockers                |                             |
| Hormones             | Hormones                    | Receptor regulation         | Gene control                |
|                      | Anti-hormones               |                             |                             |
| Biologics            | Interferons                 | LAK/TIL expansion           | Immune tuning               |
|                      | Interleukins                | Network control             |                             |
|                      | MCA therapy                 |                             |                             |
|                      | BM laundering               |                             |                             |

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