Biology and genetics of colorectal cancer

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Colorectal cancer is an important disease with a large morbidity and mortality and increasing healthcare costs as multi-modality therapy becomes more widespread and new drugs appear. There are 678,000 colorectal cancer cases and 400,000 deaths from the disease worldwide. It is the second commonest cause of cancer death in the European Union, but unlike the commonest cause of cancer death, lung cancer, the basis of the initiation of this disease is currently not understood. Treatment is by surgery with additional adjuvant chemotherapy for local metastatic spread or palliative chemotherapy when there is evidence of spread outside of the operative field. Radiotherapy may be used for rectal carcinoma.

Molecular pathology should explain the cause of this disease, the reasons for progression, the different biological behaviours of each tumour and may predict how we treat each individual patient. We know more about the basis of colorectal cancer than any other human malignancy and with the sequencing of the human genome we are poised to make rapid advances. With the development of DNA chips, the simultaneous analysis of thousands of DNA sequences should be possible [1]. cDNA expression arrays will allow the rapid investigation of the pattern of gene expression of the estimated 30,000–40,000 genes in the genome [2] and tissue microarrays [3] will allow the simultaneous assessment of hundreds of tumours, we thus have unparalleled opportunities to understand this disease. In this paper, firstly, we will review the basic pathology of the process, secondly, we will discuss the molecular basis of the inherited and sporadic types and thirdly, we will demonstrate the use of this knowledge in the clinical situation.

Pathology

It is believed that colorectal adenocarcinomas develop through either the inheritance of a genetic defect or the induction of DNA damage by an, as yet, unknown mechanism in a stem cell in a colonic crypt. The lesion leads to an expansion of the population and the development of further genetic defects that give that population of cells a Darwinian advantage over their neighbours [4]. There are progressive defects in the key cellular pathways enhancing cellular proliferation, inhibiting apoptosis, preventing senescence and encouraging further evolutionary changes by a failure to recognise DNA damage when it occurs and the inactivation of DNA repair mechanisms. These problems lead to morphological changes that can be recognised macroscopically or under the microscope. They cause a normal colonic crypt to bud and become deformed into an aberrant crypt focus [5], to develop cellular changes of dysplasia and to grow above the surrounding mucosa and form a small adenoma. The adenoma grows and the cellular morphology becomes more abnormal. The epithelial cells develop the capacity to breakthrough the basement membrane and invade the surrounding tissues and subsequently involve lymphatics, nerves and blood vessels. Care must be exercised when comparing eastern and western papers detailing the events at the adenoma–carcinoma interface owing to differences in criteria used for the diagnosis of cancer [6]. Japanese pathologists tend to diagnose cancer prior to invasion if the cellular morphology is very atypical whereas western pathologists require invasion through the basement membrane or if in the colon the muscularis mucosae. After invasion, further molecular defects allow the cells to detach from the primary tumour, to survive in the bloodstream, lodge elsewhere in the body and to grow as new independent tumours in a number of preferred sites such as the liver or lung.

Inherited colorectal cancer

The two major forms of this disease are those where there is a known inherited component and those which tend to occur sporadically. Familial adenomatous polyposis is the most well known of the inherited diseases. It accounts for less than 1% of all

colorectal cancers. This is an autosomal dominantly inherited disease where patients begin to develop hundreds or thousands of adenomas in their teens and subsequently develop colorectal cancers in their 30's–50's. It is caused by an inherited germline defect in the adenomatous polyposis coli (*APC*) gene. The site of the mutation affects the phenotype [7]. Somatic inactivation of the second copy of *APC* by loss of heterozygosity, mutation or methylation leads to accumulation of beta-catenin in the cytoplasm and the subsequent abnormalities of the T cell factor (TCF)–lef pathway which interacts with c-myc, wnt's, and many other proteins [8]. APC also interacts with a wide range of other proteins such as axin, glycogen synthase kinase-3 beta (GSK3B), EB-1 [9]. Subsequent molecular defects occur which appear to be similar to those seen in sporadic colorectal cancer and the biological behaviour of the cancers seems to be identical. Genetic diagnosis is available to detect carriers of the disease.

The commonest form of inherited colorectal cancer is hereditary non-polyposis colorectal cancer (HNPCC) accounting for 1–3% of colorectal cancer [10,11]. The clinical suspicion is raised by the patient's history meeting the Amsterdam criteria. 40 to 50% of such patients are subsequently shown to have germ line mutations in the DNA repair genes *hMSH2* and *hMLH1* [12,13], with a small number having mutations in *hMSH6* [14,15], *PMS1* and *PMS2* [16]. These genes are involved in the recognition, excision and repair of expansions and reductions in microsatellite repeats (reviewed [17]). Failure of these excision repair mechanisms leads to the generation of increased or reduced numbers of the repeat sequence at that site and the appearance of new alleles when amplified by the polymerase chain reaction (PCR). These changes in the DNA can lead to frameshift mutations and production of abnormal non-functioning proteins. The inactivated proteins are different to those seen in FAP cancers in that transforming growth factor beta (TGF-beta) receptor type II [18], insulin-like growth factor II [19], BAX [20], TCF-4 [21], MBD4 [22], CHK-1 [23] etc. are involved. Ninety percent of the carrier's of germ line mutation cancers will have microsatellite instability. Some series report these cancers to be less aggressive [24,25] than many of their sporadic counterparts, but the biological reasons for this are not well understood. The tumours show an increase in lymphocytic infiltrates and may show a higher rate of apoptosis, as has been confirmed for mismatch repair sporadic tumours [26].

The diagnosis of these cancers involves a full family history, the testing of the tumours from more than one member of the family for immunohistochemical loss of hMSH2, hMLH1 or hMSH6 [27,28] with direct sequencing of any of the genes where the product is seen to be lost. In the absence of loss of these proteins, amplification of four markers for microsatellite instability will identify which abnormalities of mismatch repair exists and that subsequent sequencing of each of the genes is required. A recent paper confirmed that the dual microsatellite analysis and immunocytochemistry approach was the current gold standard [29].

Peutz-Jeghers syndrome is a rare autosomal dominant condition characterised by harmartomatous polyps throughout the gastrointestinal tract. There are also melanin spots on the lips and buccal mucosa. [30,31]. These patients have an increased risk of developing cancer; especially gastrointestinal, but also of the pancreas, breast, ovary and testis [32]. Recently, a defect in the *STK11/LKB1* gene has been identified as the cause for this syndrome [33]. This gene encodes a serine/threonine kinase [34] and may be involved in cell cycle arrest [35]. Mutations at the 19p13.3 [36] lead to inactivation of this gene. Less frequent causes are mutations at 19q and breakpoints at a pericentric inversion on chromosome 6 [37].

Juvenile polyposis is another rare syndrome with autosomal dominant inheritance. It is manifested as harmartomatous polyps, usually within the colon, but also arising in the stomach and small bowel. Unlike solitary juvenile polyps, juvenile polyposis patients have an increased risk of gastrointestinal malignancy [38]. Recently, it has been shown that a germ line mutation in *SMAD 4* (18q21.1 or *DDC4*) accounts for a significant proportion of these cases [39,40]. *SMAD 4* mutation leads to the downregulation of TGF-beta receptors. Normally TGF-beta has antiproliferative effects, so such downregulation leads to the loss of growth inhibition on colorectal tumour cells [41]. The increased neoplastic risk may be due to such mutations occurring in the stromal component, stimulating epithelial dysplasia and progression to invasive malignancy [40].

Polymorphisms can predispose to an increased risk of colorectal cancer. A polymorphism in the *APC* gene where there is elongation of a repeat sequence of A's causes a frameshift which leads to an abnormal APC protein. This is inherited in Askenazi Jews. [42] This so-called *I1307K* allele increases colorectal cancer risk by 1.5–1.7 by increasing the transition from polyps to carcinoma [43]. A large number of such polymorphisms are likely to be described in the future as advances in the detection of single nucleotide polymorphisms occurs with the widespread application of DNA chips.

Sporadic colorectal cancer

Sporadic colorectal cancers arise at a median age of 70–75 years. Seventy percent arise in the left side of the colon and there are differences in the age, sex and regional distribution of both adenomas [44] and carcinomas between both sides of the large bowel. They develop from adenomas; but there are at least three types of adenoma; polypoid, flat and serrated. The biology of the latter two is not well understood whereas that of polypoid adenomas is. The frequency of invasion increases in frequency with increasing size, dysplasia and the proportion of villousness within the adenoma. These abnormalities are caused by the development of a series of genetic abnormalities in tumour suppressor genes and oncogenes that give cells an evolutionary advantage over their neighbours.

The process appears to be initiated by abnormalities developing in the APC–beta catenin–lef pathway. Most frequently, this is inactivation of *APC*, but mutations that stabilise beta catenin can also occur and it is likely that other genes in this pathway can also be initiating events. Most frequently, the site of the first mutation in *APC* is in the mutation cluster region, with the second event caused by either loss of heterozygosity, mutation or methylation. The site of the first event appears to determine the type of second hit [45]. Following inactivation of the APC pathway, dysregulation of bcl-2 occurs. Bcl-2 is usually confined to a small cluster of cells at the base of the crypt, but with the onset of dysplasia bcl-2 expression is not repressed and cells can escape apoptosis. There is also the appearance of Cox-2 overexpression. In normal mucosa Cox-2 expression is not present, but this also appears with the onset of dysplasia. Cox 2 appears to interact with a number of important pathways such as bcl-2 [46], PPARdelta [47], nuclear factor (NF)–kappaB pathway [48] and, subsequently, angiogenesis [49]. In 40% of adenomas, mutations develop in codons 12,13,59 or 61 of Kirsten *ras* [50] and this appears to be associated with methylation of the O^6-methylguanine-DNA methyltransferase (MGMT) DNA repair enzyme [51,52]. *Ras* mutations appear to be more frequent in polypoid than flat adenomas [53]. Ras interacts with the epidermal growth factor receptor (EGFR)–Jun/Fos pathway, pro-caspase-9 and may interact with INK4a-ARF, cyclin D1 and c-myc [54]. Loss of the long arm of chromosome 18 leads to further growth of adenomas. The genes involved probably include *SMAD2* and *SMAD4*, *DCC* and others [55]. Mutations in *SMAD4* have been shown in 6% to 30% of cases [56]. Trisomies are also found, but their functional effects are not well characterised. Non-random gains of chromosome 7 have been consistently reported [57,58]. The onset of invasion is closely linked to two major changes of *TP53* mutation and the development of DNA aneuploidy, both of which will lead to further genetic abnormalities by the failure to identify genetic damage and the generation of new clones with different chromosomal numbers. *TP53* is a key gene with multiple functions and has been extensively reviewed [59,60]. As stated above, APC has a number of functions, but a particularly interesting new function appears to be concerned with the control of processes that prevent tetraploidy [61]. It has now been demonstrated that after APC inactivation, the frequency with which tetraploid cells are formed increases. This could be one of the underlying lesions that lead to chromosomal instability and the frequent finding of DNA aneuploidy. It remains to be explained how the early inactivation of APC might cause DNA aneuploidy since it is not frequently seen in adenomas until just prior to the onset of invasion [62–64]. An alternative suggestion is that the mutation of mitotic checkpoint genes such as *BUB1* [65] or abnormalities of the proteins concerned with separation of the chromosomes (reviewed [66,67]) leads to chromosomal instability. Following invasion, non-random losses occur on chromosomes 12, 14, 15, 22q and 8p with gains reported on 13q, 8q and 20q [68]. Many of these have also been reported in cytogenetic studies [69]. The mechanism of metastasis is not well understood, but may involve overexpression of the matrix metalloproteinase MMP-7. Accumulation of beta-catenin may also act as a transcriptional activator for this gene; therefore the *APC* gene may have a significant influence on later steps of tumour progression [70]. Overexpression of Cox-2 also seems to be important.

Some tumours do not develop DNA aneuploidy, but remain diploid or peridiploid with 42–46 chromosomes. These tumours show abnormal methylation of CpG islands. These are CpG rich sequences that are usually not methylated apart from genes on the inactivated X chromosome of females or imprinted genes on autosomal chromosomes [71]. They are frequently found in the 5′ regulatory regions of genes and if methylated can control gene expression. Methylation can occur in two situations. The first occurs with increasing age where certain genes become methylated [72]. This occurs in genes such as *oestrogen receptor alpha*, *N33*, *MyoD1*, *versican* and *IGF2* [73]. This is tissue-specific in that the changes in *oestrogen receptor alpha* are not seen in the breast, but are seen in the liver. This change was called

type A methylation for age-specific methylation [74]. Other genes appear to be only methylated in cancer the so-called type C methylation pattern. Examples of these genes are *hMLH1*, *MGMT*, *p16*, *E-Cadherin*, *Cox-2* and *THBS1* [74]. Tumours with high levels of methylation of the genes were called CpG island methylator phenotype tumours or CIMP for short. In a study by Costello et al. [75] 600 of 45,000 CpG islands were abnormally methylated with colorectal cancers showing one of the highest levels of methylation. An important feature of methylational silencing is the possibility of reversing it by treatment with AzaC. This has been successfully performed in vitro, but whether this is now an in vivo treatment option awaits data from clinical trials. Methylational silencing can be detected by molecular analysis of the CpG methylation status (for reviews see [76,77]), but it can also be assessed by immunocytochemistry where either total or partial loss of expression can be rapidly assessed. Methylation of the DNA mismatch repair gene *hMLH1* silences both alleles [78] leading to loss of protein expression and can easily be assessed by immunocytochemistry [77]. Loss of the protein leads to high levels of microsatellite instability due to mismatch repair and the generation of similar abnormalities to those described above for HNPCC. This accounts for 15% of sporadic colorectal cancers. These patients tend to have right-sided tumours which are more frequently polypoid, invade with a pushing border, poor or mucoid differentiation and have an increased lymphocytic reaction and an increased apoptotic rate [26]. The pattern of methylation of other genes is unknown. Recently, we have investigated the loss of expression of *hMLH1*, *MGMT* and *Cox-2* [79]. There was a significant association between the loss of expression of *hMLH1* and *MGMT*, but these did not overlap with loss of *Cox-2* expression. Both *hMLH1* and *MGMT* tended to be located in proximal cancers supporting the view that these occur more frequently on the right side of the colon [78]. Interestingly *hMLH1* methylation appears to occur at the adenoma–carcinoma interface and is lost throughout the tumour, whereas loss of *MGMT* expression can be more heterogeneous and is seen earlier in adenomas of both polypoid and flat types [80] and serrated adenomas [81]. Loss of *Cox-2* in our hands [79] does not associate with the CIMP phenotype, but may identify a group of tumours with a very good prognosis [82]. More work is clearly needed in this area to define the biological and clinical importance of this group of tumours subdividing them into those that have microsatellite instability and other types of CIMP abnormality. The cause of this aberrant methylation is currently unknown. A good review of chromosomally unstable and microsatellite unstable cancers is available in Ref. [83].

New data is emerging from large-scale studies of gene expression. In collaborative studies with Genentech on Affymetrix chips, the data showed that roughly 10% of 6000 genes were overexpressed at a much higher level and 10% underexpressed when comparing colorectal adenocarcinomas with their corresponding normal mucosa. Publications by Notterman et al. [1] and Kitahara et al. [84] used different cut-offs. Notterman using the Affymetrix 6500 chips showed 19 (0.48%) of the transcripts had at least 4–10.5 fold higher mRNA expression and 47 4–38 fold lower expression. They identified a large number of individual genes some of which could be hypothesised to be involved in colorectal cancer. Reverse transcriptase (RT)-PCR confirmation of some of these was obtained. Hierarchical clustering appeared to be able to separate the tumours, but this was after significant data removal with only 1096 genes and expression sequencing tags (EST's) included and stripping out of muscle- and connective tissue-associated genes. Hierarchical clustering usually requires a large amount of data manipulation and the robustness of these assays remains to be seen when tissue and tumour heterogeneity is not rigidly controlled. Kitahara et al. [84] used a printed cDNA array of 9216 genes and explored the cDNA expression patterns of laser capture microdissected tissue. They reported upregulation of 44 genes and downregulation of 191 genes for more than half of their 8 cancers analysed. RT-PCR data were consistent in 64 of 74 experiments revealing a concordance of 86.5%. They went on to test the other 12 collected tumours and confirmed the data. Several of the genes identified by both sets of workers were also abnormal in our data [85].

Clinical value of molecular pathology

Our increased understanding of the molecular biology of colorectal cancer can affect many clinical situations. Knowledge of the defects in hereditary colorectal cancer allows the diagnosis of such familial diseases and the detection of carriers. Molecular methods have been used in an attempt to screen patients for colorectal cancer looking for the presence of mutations in the stool or molecular abnormalities in DNA released from tumours into plasma and to identify patients with micrometastatic disease in lymph nodes. Many of these studies are 'proof of the principle' of the test, but do not inform the debate

about their robustness in routine use. We have known for many years that tumour cells can be found in the peripheral blood and that further testing of lymph nodes by techniques such as immunocytochemistry can identify involved nodes. What has not happened is the testing of these new techniques against the current gold standard to prove their benefit.

Common molecular targets are Ki-ras, *TP53* mutations and the presence of microsatellite instability. The drawback of such tests is the limited presence of the molecular abnormality under study e.g. 40% for Ki-ras, 70% for *TP53* and 15% for microsatellite instability. One test does not identify all cases. Promising studies include the following. Thebo et al. [86] looked at Ki-*ras* mutations in lymph nodes to upstage Dukes' B patients. None of 4 patients with mutation-free nodes developed recurrence, whereas 37.5% of those with positive lymph nodes did. Ki-*ras* mutations identified in plasma DNA have also been shown to be strongly associated with the presence of a colorectal neoplasm, bearing such mutations [87,88]. p53 antibodies have been demonstrated to be present in 14 of 54 colorectal cancer patients (26%) by an enzyme-linked immunoabsorbant assay (ELISA), with none being present in 24 patients with non-malignant digestive disease [89]. The identification of *TP53* [90] and Ki-*ras* mutations [91,92] from DNA shed from tumour into stool has proved possible. A small number of such samples, 22, have also been analysed using a panel of assays (including Ki-*ras*, *TP53*, *APC* and a microsatellite marker). This demonstrated sensitivity of 91% and specificity of 93% in identifying malignancy [93].

Testing the value of the newly emerging data will be facilitated by the use of tissue microarrays [94] which allow the simultaneous rapid assessment of hundreds of different tumours, hopefully from prospective randomised trials generating unchallengeable data of value to clinicians.

Molecular pathology and prognosis

Small scale clinical studies have generated conflicting data about the behaviour of the different types of cancer. In the late 80's, many studies of DNA aneuploidy showed a worse prognosis in these tumours compared to diploid cancers [95]. This has been recently confirmed by ourselves in large prospective study of 400 cancers within the United Kingdom Co-ordinating Committee on Cancer Research (UKCCCR) Axis study [96]. Kirsten *ras* mutations also confer a slightly worse prognosis in a meta-analysis [97]. Loss of Cox-2 expression has also been reported to confer an excellent prognosis in a small series of patients [82]. A number of studies have also reported a worse prognosis in patients with abnormal patterns of p53 expression [98–102]. Jen et al. [103] and Martinez-Lopez et al. [104] have reported a worse prognosis in patients with 18q deletions and Shibata et al. [105] using immunocytochemistry found a worse prognosis in patients who had lost staining for DCC. Those with a higher level of chromosomal deletions were also reported by Vogelstein [106] to have a worse outcome. Unluckily, many of these studies are on small numbers and do not take into account the possible confounding influences of molecular abnormalities affecting the response of the tumour to therapy. We have recently prospectively looked at allelic imbalance in 400 randomised patients in the UKCCCR Axis study and have seen no major clinically important effects on prognosis of allelic imbalance of *APC*, 17p, 18q or microsatellite instability [96]. We also investigated bcl-2 and hMLH1 by immunocytochemistry and found no significant effects. This is different to a recent publication from Perth, which suggested microsatellite instability is very important [107].

Response to therapy

The commonest therapy in colorectal cancer is 5-fluorouracil (5-FU) in combination with folinic acid. 5-FU is a pro-drug, which after intracellular conversion to active metabolites inhibits thymidylate synthase, impairing DNA synthesis largely in the S phase (replication). In addition, 5-FU metabolites can be falsely incorporated in to RNA and DNA, interfering with the normal protein production necessary for cell growth [108]. This has been shown to work in both the settings of adjuvant therapy [109] and in advanced disease. Assessment of a number of enzymes suggests that if there is a high level of thymidylate synthase, thymidine phosphorylase and dihydropyrimidine dehydrogenase (DPD) then the patients may not respond to 5-FU- based chemotherapy [110]. Loss of dUTPase expression has also been reported as indicating a better response to 5-FU [111]. Recently, a polymorphism in the thymidylate synthase promoter has been linked to a poor response to 5-FU [112]. The triple repeat was found in 40.2% of a Mediterranean population. The presence of the triple tandem repeat has been demonstrated in vitro to lead to an increase in TS expression [113]. However, all of these studies findings are based on relatively small numbers of cases usually treated outside of prospective randomised clinical trials and as

such do not provide enough evidence upon which to base clinical practice. Recent work in our laboratory on the UKCCCR Axis study has shown that loss of 18q appears to identify patients who will not respond to intraportal 5-FU [96].

The response of patients to the newer agents such as irinotecan may also be predicted by enzyme level. Irinotecan is a topoisomerase inhibitor (blocking cell division by inducing single-strand DNA breaks) and oxaliplatin (a third-generation platinum analogue) induces DNA cross-linkage and thus produces apoptotic death [108]. Therefore, treatment response may be predicted by the levels of enzymes such as topoisomerase 1 and by ERCCC1. Abnormalities of expression of other enzymes such as MGMT loss might identify small numbers of patients that might respond to other drugs such as dacarbazine, procarbazine, temozolomide, lomustine(CCNU), nimustine (ACNU) and carmustine (BCNU). Molecular pathological analysis will probably be essential for the inhibitors of other pathways such as EGFR and Vascular Endothelial Growth Factor (VEGF) in the same way as HER-2 analysis has been used for the planning of Herceptin treatment.

Immune therapies

Many cancers can be destroyed by a tumour-specific cell-mediated immune response, usually by cytotoxic (CD8) lymphocytes. However, colorectal tumours are poorly immunogenic and may evade immune destruction by various mechanisms involving so-called 'tumour tolerance'. To overcome this, several immunostimulatory approaches have been advocated to augment the innate immune response against tumours.

Firstly, some have used vaccination with autologous tumour cells. Utilising cells derived from the patient's own tumour to elicit a cell-mediated response. To increase efficacy Bacillus Calmette-Guerin (BCG) was co-administered (several trials have shown overall little benefit: [114,115]). Also investigated has been vaccination against tumour-associated antigens, such as carcinoembryonic antigen (CEA) which is expressed in 90% of colon cancer (several trials are ongoing). The potential of monoclonal antibody-directed therapy have been encouraged by the success of Herceptin in the treatment of breast cancer. The *HER-2/NEU* (human epidermal growth factor receptor) oncogene encodes a transmembrane tyrosine kinase receptor with extensive homology to the EGFR. Antibodies against this receptor (Herceptin) have shown good results. A 15% response as a single agent in metastatic breast cancer [116] and a phase III trial in combination with doxorubicin or paclitaxel produced markedly increased anticancer activity [117]. Antibodies against a highly expressed antigen (17-1A) have been utilised in the treatment of colorectal cancer.

17-1A is a murine monoclonal IgG2a antibody that recognises a 37–40 KDa cell surface glycoprotein, expressed on a range of malignant and normal tissue. It is reported to be an intercellular adhesion molecule [118]. Antibodies to this antigen are thought to destroy tumour cells by activating endogenous cytotoxic mechanisms including antibody-dependent cell-mediated cytotoxicity and possibly also antibody-dependent complement-mediated cytoxicity. It may also invoke anti-tumour activity indirectly by inducing an anti-idiotypic antibody response [119].

Therapy with the monoclonal antibody 17-1A (compared to observation alone) was shown to improve the survival of patients with Dukes' C colorectal carcinoma by the German group headed by Riethmuller [120]. This improvement was seen as a decrease in distant metastases rather than an improvement in local recurrence. Effects of the antibody on minimal residual disease may depend on the location of these dispersed cells being in the mesenchymal or reticuloendothelial compartments. Here, effector mechanisms like complement and killer cells abound while at the same time, the total volume of tumour cells to be destroyed is low [121]. Its failure to suppress local recurrence may be explained by the larger volume of disease satellites and by the surrounding environment, for example the poor blood supply in connective tissue [120]. This proposed mechanism of action would also mean that a prolonged follow-up period would be necessary, to see the full effects of the treatment. The drug could also have the potential to deal with micro-metastases that even 12% of Dukes' A cases have at presentation [121].

Although this trial was encouraging and involved follow-up over 5 years, only 166 in total (treated and untreated) were studied. Therefore, further trials have been undertaken in Europe and the United States.

Gene therapies

Gene therapy utilises genetic changes in colorectal cancer, so interfacing basic science with clinical medicine. Viral vectors are used to insert genes that encode cytokines, tumour suppressor genes and genes which sensitise cells to chemotherapy (e.g.

TP53). A phase I trial of such an approach [122] is utilising a synthesised adenoviral vector linking a CEA promoter to the structural gene for the bacterial enzyme cytosine deaminase. Cytosine deaminase metabolises the pro-drug 5-fluorocytosine to 5-FU, but only in CEA-expressing cells. At present, systemic gene replacement therapy of mutated tumour suppressor genes is not possible, but a regional form of such therapy is being developed for *TP53* directed at primary and metastatic neoplasms [123].

Conclusions

Molecular pathology has made great strides in recent times. This has afforded a rapid growth in our knowledge of colorectal cancer; which has just started to be translated into new diagnostic tests and treatments. These are the mere 'tip of the iceberg'. However, this potential should be tempered with the knowledge that these advances should only come into clinical practice if they are tested in the context of prospective randomised trials. This technology must prove its worth.

References

1 Notterman DA, Alon U, Sierk AJ, Levine AJ. Transcriptional gene expression profiles of colorectal adenoma, adenocarcinomas and normal tissue examined by oligonucleotide arrays. Cancer Res 2001, 61: 24–30.

2 Maughan NJ, Lewis F, Smith V. An introduction to arrays. J Pathol 2001, 193: 1–4.

3 Budendorf L, Nocito A, Moch H et al. Tissue microarray (TMA) technology: miniaturized pathology archives for high-throughput in situ studies. J Pathol 2001, 195: 72–79.

4 Breivik J, Gaudernack G. Carcinogenesis and natural selection: a new perspective to the genetics and epigenetics of colorectal cancer. Adv Cancer Res 1999, 76: 187–212.

5 Nascimbeni R, Villanacci V, Mariani PP, Di Betta E, Ghirardi M, Donato F, Salerni B. Aberrant crypt foci in the human colon. Am J Surg Pathol 1999, 23: 1256–1263.

6 Schlemper RJ, Borchard F, Dixon MF et al. International comparability of the pathological diagnosis for early cancer of the digestive tract. J Gastroenterol 2000 (35 Suppl): 12, 102–110.

7 Scott RJ, Meldrum C, Crooks R et al. Familial adenomatous polyposis: more evidence for disease diversity and genetic heterogeneity. Gut 2001, 48: 508–514.

8 Heppner Goss K, Groden J. Biology of the adenomatous polyposis coli tumour suppressor. J Clin Oncol 2000, 18: 1967–79.

9 Hinoi T, Yamamoto H, Kishida M et al. A complex formation of adenomatous polyposis coli gene product and axin facilitates glycogen synthase kinase-3 beta-dependent phosphorylation of beta-catenin and down-regulates beta-catenin. J Biol Chem 2000, 275: 34399–34406.

10 Evans DG, Walsh S, Jeacock J et al. Incidence of hereditary non-polyposis colorectal cancer in a population-based study of 1137 consecutive cases of colorectal cancer. Br J Surg 1997, 84: 1281–1285.

11 Aaltonen LA, Salovaara R, Kristo P et al. Incidence off hereditary nonpolyposis colorectal cancer and the feasibility of molecular screening for the disease. N Eng J Med 1998, 338: 1481–1487.

12 Wijnen J, de Leeuw W, Vasen H et al. Familial endometrial cancer in female carriers of MSH6 germline mutations. Nat Genet 1999, 23: 142–144.

13 Kolodner RD, Tytell JD, Scmeits JL et al. Germline mutations in colorectal cancer families. Cancer Res 1999, 59: 5068–5074.

14 Miyaki M, Konishi M, Tanaka K et al. Germline mutation of MSH6 as the cause of hereditary nonpolyposis colorectal cancer. Nat Genet 1997, 17: 271–272.

15 Akiyama Y, Sato H, Yamada T et al. Germline-mutation of the hHSH6/GTBP gene in an atypical hereditary nonpolyposis cancer kindred. Cancer Res 1997, 59: 5068–5074.

16 Nicolaides NC, Papadopoulos N, Liu B et al. Mutations of two PMS homologues in hereditary nonpolyposis colon cancer. Nature 1994, 371: 75–80.

17 Jiricny J, Nystrom-Lahti M. Mismatch repair defects in cancer. Curr Opin Genet Dev 2000, 10(2): 157–161.

18 Olschwang S, Hamelin R, Laurent-Puig P et al. Alternative genetic pathways in colorectal carcinogenesis. Proc Natl Acad Sci USA 1997, 94: 12122–12127.

19 Souza RF, Appel R, Yin J et al. Microsatellite instability in the insulin-like growth factor II receptor gene in gastrointestinal tumours. Nat Genet 1996, 14: 255–257.

20 Rampino N, Yamamoto H, Ionov Y et al. Somatic frameshift mutations in the BAX gene in colon cancers of the microsatellite mutator phenotype. Science 1997, 275(5302): 967–969.

21 Duval A, Gayet T, Zhou XP et al. Frequent frameshift mutations of the TCF-4 gene in colorectal cancers with microsatellite instability. Cancer Res 1999, 59: 4213–4215.

22 Riccio A, Aaltonen LA, Godwin AK et al. The DNA repair gene MBD4 (MED1) is mutated in human carcinomas with microsatellite instability. Nat Genet 1999, 23: 266–268.

23 Codegoni AM, Bertoni F, Colella G et al. Microsatellite instability and frame shift mutations in genes involved in cell cycle progression or apoptosis in ovarian cancer. Oncol Res 1999, 11: 297–301.

24 Thiodeau SN, French AJ, Cunningham JM et al. Microsatellite instability in colorectal cancer: different mutator phenotypes and the principle involvement of hMLH1. Cancer Res 1998, 58: 1713–1718.

25 Wright CM, Dent CF, Barker M et al. Prognostic significance of extensive microsatellite instability in sporadic clinicopathological stage C colorectal cancer. Br J Surg 2000, 87: 1197–1202.

26 Michael-Robinson JM, Biemer-Huttmann A, Purdig DM et al. Tumour infiltrating lymphocytes and apoptosis are independent features in colorectal cancer stratified according to microsatellite instability status. Gut 2001, 48: 360–366.

27 Cawkwell L, Gray S, Murgatroyd H, Sutherland F, Haine L, Longfellow M, O'loughlin S, Cross D, Kronborg O, Fenger C, Mapstone N, Dixon, Quirke P. Choice of management strategy for colorectal cancer based on a diagnostic immunohistochemical test for defective mismatch repair. Gut 1999, 45: 409–415.

28 Marcus VA, Madensky L, Gryfe R et al. Immunhistochemistry for hMLH1 and hMSH2: a practical test for DNA mismatch repair-deficient tumours. Am J Pathol 1999, 23: 1248–1255.

29 Terdiman JP, Gum JR, Conrad PG et al. Efficient detection of Hereditary Nonpolyposis Colorectal Cancer Gene Carriers by screening for tumor microsatellite instability before germline genetic testing. Gastroenterology 2001, 120: 21–30.
30 Peutz JLA. A very remarkable case of familial polyposis of mucous membrane of intestinal tract and accompanied by peculiar pigmentations of skin and mucous membranes. Ned Maandschr Geneeskunde 1921, 10: 134–146.
31 Jeghers H, Mckusick VA, Katz KH. Generalized intestinal polyposis and melanin spots of the oral mucosa, lips and digits. N Engl J Med 1949, 241: 1031–1036.
32 Giardielio FM, Welsh SB, Hamilton SR et al. Increased risk of cancer in peutz-jeghers syndrome. N Engl J Med 1987, 316: 1511–1514.
33 Hemminki A, Tomlinson I, Markie D et al. Localization of a susceptibility locus for peutz-jeghers syndrome. Nat Genet 1997, 15: 87–90.
34 Hemminki A, Markie D, Tomlison I et al. A serine/threonine kinase gene defect in peutz-jeghers syndrome. Nature 1998, 391: 184–187.
35 Tiainen M, Yukorkala A, Makela TP. Growth suppression by LKb1 is mediated by a G1 cell cycle arrest. Proc Natl Acad Sci USA 1999, 96: 9248–9251.
36 Mehenni H, Blouin JL, Radhakrishna U et al. Peutz-jeghers: confirmation of linkage to chromosome 19p13.3 and identification of a potential second locus; on 19p13.4. Am J Hum Genet 1997, 61: 1327–1334.
37 Entius MM, Keller JJ, Westerman AM. Molecular genetic alterations in hamartomatous polyps and carcinomas of patients with peutz-jeghers syndrome. J Clin Pathol 2001, 54: 126–131.
38 Howe JR, Mitros FA, Summers RW. The risk of gastrointestinal carcinoma in familial juvenile polyposis. Ann Surg Oncol 1998, 5: 751–756.
39 Howe JR, Ringold JC, Summer RW et al. A gene for familial juvenile polyposis maps to chromosome 18q21.1. Am J Hum Genet 1998, 62: 1129–1136.
40 Woodford-Richens K, Williamson J, Bevan S et al. Allelic loss at SMAD4 in polyps from juvenile polyposis patients and use of fluorescence in situ hybridization to demonstrate clonal origin of the epithelium. Cancer Res 2000, 60(9): 2477–2482.
41 Matsushita M, Matsuzaki K, Date M et al. Down-regulation of TGF-beta receptors in human colorectal cancer: implications for cancer development. Br J Cancer 1999, 80(1–2): 194–205.
42 Laken SJ, Petersen GM, Gruber SB, Oddoux C, Ostret H, Giardiello FM, Hamilton SR, Hampel H, Markowitz A, Klimstra D, Jhanwar S, Winaver S, Offit K, Luce MC, Kinzler KW, Vogelstein B. Familial colorectal cancer in Ashkenazi due to a hypermutable tract in APC. Nat Genet 1997, 17: 70–83.
43 Stern HS, Viertelhausen S, Hunter AGW, O'Rourke K, Cappelli M, Perras H, Serfas K, Blumenthall A, Dewar D, Baumann E, Lagarde AE. APC I1307K increases risk of transition from polyp to colorectal carcinoma in Ashenazi Jews. Gastroenterology 2001, 120: 392–400.
44 Vatn MN, Stalsberg H. The prevalence of polyps of the large intestine in Oslo: an autopsy study. Cancer 1982, 49: 819–25.
45 Lamlum H, Ilyas M, Rowan A et al. The type of somatic mutation at APC in familial adenomatous polyposis is determined by the site of the germline mutation: a new facet to Knudson's two hit hypothesis. Nat Med 1999, 5(9): 1071–1075.
46 Sheng H, Shao J, Morrow JD et al. Modulation of apoptosis and Bcl-2 expression by prostaglandin E2 in human colon cancer cells. Cancer Res 1998, 58: 362–366.
47 He TC, Chan TA, Vogelstein B et al. PPARdelta is an APC-regulated target of nonsteroidal anti-inflammatory drugs. Cell 1999, 99: 333–345.
48 Yamamoto Y, Yin MJ, Lin KM et al. Sulindac inhibits activation of the NF-kappaB pathway. J Biol Chem 1999, 274: 27307–14.
49 Tsujii M, Kawano S, Tsjui S et al. Cyclooxygenase regulates angiogenesis induced by colon cancer cells. Cell 1998, 93: 705–716.
50 Bos JL. Ras oncogenes in human cancer: a review. Cancer 1989, 49: 4682–4689.
51 Esteller M, Toyota M, Sanchez-Cespedes M et al. Inactivation of the DNA repair gene O6-methylguanine-DNA methyltransferase by promoter hypermethylation is associated with G to A mutations in K-ras in colorectal tumorigenesis. Cancer Res 2000, 60: 2368–71.
52 Whitehall VLJ, Walsh MD, Young JY et al. Methylation of O^6methylguanine DNA methyltransferase characterises a subset of colorectal cancer with low level DNA microsatellite instability. Cancer Res 2001, 61: 827–830.
53 Jackson PE, Hall CN, O'Conner PJ et al. Low O^6-akly guanine DNA-alkyltransfearse activity in normal colorectal tissue is associated with colorectal tumours containing GC → AT transition in the K-ras oncogene. Carcinogenesis 1997, 18: 1299–1302.
54 Arends JW. Molecular interactions in the Vogelstein model of colorectal carcinoma. J Pathol 2000, 190: 412–416.
55 Zhou S, Kinzler KW, Vogelstein B et al. Going MAD with SMADS. N Engl J Med 1999, 341: 1144–1146.
56 Riggens GJ, Thiagalingam S, Rozenbulm E et al. MAD related genes in human cancer. Nat Genet 1996, 13: 347–349.
57 Herbergs J, Arends JW, Bongers EM et al. Clonal origin of trisomy for chromosome 7 in the epithelial component of colon neoplasia. Genes, Chromosomes Cancer 1996, 16: 106–112.
58 Meijer GA, Hermsen MA, Baak JP et al. Progression from colorectal adenoma to carcinoma is associated with non-random chromosomal gains as detected by comparative genomic hybridisation. J Clin Pathol 1998, 51: 901–909.
59 Levine AJ. p53, the cellular gatekeeper for growth and division. Cell 1997, 88: 323–331.
60 Prives C, Hall PA. The p53 pathway. J Pathol 1999, 187: 112–126.
61 Fodde R, Kuipers J, Rosenberg C et al. Mutations in the APC tumour suppressor gene cause chromosomal instability. Nat Cell Biol 2001, 3: 433–438.
62 Quirke P, Durdey P, Dixon MF et al. The prognostic significance of DNA aneuploidy and cell proliferation in rectal adenocarcinomas. J Pathol 151, 285–292.
63 van den Inghe HF, Griffioen G, Cornelisse CJ. DNA aneuploidy in colorectal adenomas. Br J Cancer 1987, 55: 351.
64 Goh HS, Jass JR. DNA content and the adenoma-carcinoma sequence in the colorectum. J Clin Pathol 1986, 39: 387–392.
65 Cahill DP, Lengauer C, Yu J et al. Mutations of mitotic checkpoints genes in human cancer. Nature 1998, 392(6673): 300–303.
66 Nasmyth K, Peters J-M, Uhlmann. Splitting the chromo-

some: cutting the ties that bind sister chromatids. Science 2000, 288: 1379–84.
67 Jallepalli PV, Waizenegger IC, Bunz F et al. Securin is required for chromosomal stability in human cells. Cell 2001, 105: 445–447.
68 Ried T, Knutzen R, Steinbeck R et al. Comparative genomic hybrization reveals a specific pattern of chromosomal gains and losses during the genesis of colorectal tumours. Genes, Chromosomes Cancer 1996, 15: 234–245.
69 Muleris M, Salmon Rj, Dutrillaux B et al. Cytogenetics of colorectal adenocarcinomas. Cancer Genet Cytogenet 1990, 46: 143–156.
70 Brabletz T, Jung A, Dag S et al. Beta-catenin regulates the expression of the matrix metalloproteinase-7 in human colorectal cancer. Am J Pathol 1999, 155: 1033–1038.
71 Antequera F, Bird A. CpG islands [review]. EXS 1993, 64: 169–185.
72 Issa JP, Ottaviano YL, Celano P et al. Methylation of the oestrogen receptor CpG island links ageing and neoplasia in the human colon. Nat Genet 1994, 7: 536–540.
73 Issa JP. CpG-island methylation in ageing and cancer. Current Topics Microbiol Immunol 2000, 249: 101–118.
74 Toyota M, Ahuja N, Ohe-Toyota M, Herman JG, Baylin, Issa JP. CpG island methylator phenotype in colorectal cancer. Proc Natl Acad Sci USA 1999, 96: 8681–8686.
75 Costello JF, Fruhwald MC, Smiraglia et al. Abberant CpG-island methylation has non-random and tumour specific patterns. Nat Genet 2000, 24: 101–102.
76 Baylin SB, Herman JG. Promotor hypermethylation-can this change alone ever designate true tumour suppresser gene function. JNCI 2001, 93: 664–665.
77 Jubb A, Bell SM, Quirke P. An epi-mutator phenotype in colorectal cancer. J Pathol 2001, 195: 111–134.
78 Baylin SB, Herman JG, Graff JR et al. Alterations in DNA methylation: a fundamental aspect of neoplasia. Adv Cancer Res 1998, 72: 141–96.
79 Jubb AM, Bell SM, Quirke P. Cyclooxygenase-2 expression in sporadic colorectal cancer. Pathol Soc Great Britain 2001, July, abstract 15.
80 Sutherland F, Bell SM, Gray et al. Loss of MGMT expression occurs more frequently in flat adenomas than polypoid adenomas or hyperplastic polyps. Pathol Soc Great Britain 2001, July, abstract 118.
81 Whitehall VLJ, Walsh MD, Young JY, Leggett BA, Jass JR. Methylation of O^6methylguanine DNA methyltransferase characterises a subset of colorectal cancer with low level DNA microsatellite instability. Cancer Res 2001, 61: 827–830.
82 Sheehan KM, Shehan N, O'Donohue DP et al. The relationship between cyclooxygenase-2 expression and colorectal cancer. JAMA 1999, 282: 1254–1257.
83 Lindblom A. Different mechanisms in the tumorigenesis of proximal and distal colon cancers. Curr Opin Oncol 2001, 13: 63–9.
84 Kitahara O, Furukawa Y, Tanaka T et al. Alterations of gene expression during colorectal carcinogenesis revealed by cDNA microarrays after laser-capture microdissection of tumour tissues and normal epithelia. Cancer Res 2001, 61: 3544–9.
85 Maughan NJ, Heinemeyer E, Wieand D et al. Gene expression profiling and cluster analysis of colorectal cancer utilising DNA microarrays. Pathol Soc Great Britain 2001, July, abstract 26.
86 Thebo JS, Senagore AJ, Reinhold DS, Stapleton SR. Molecular staging of colorectal cancer: K-ras mutation analysis of lymph node upstages Dukes' B patients. Dis Colon Rectum 2000, 43: 155–162.
87 Kopreski MS, Benko FA, Borys DJ, Khan A, Mcgarrity JJ, Gocke CD. Somatic mutation screening: identification of individuals harbouring K-ras mutations with the use of plasma DNA. JNCI 2000, 92(11): 918–923.
88 De Kok JB, van Solinge WW, Ruers TJM et al. Detection of tumour DNA in serum of colorectal patients. Scand J Clin Lab Invest 1997, 57: 601–604.
89 Hammel P, Boissier B, Chaumette M-Tet et al. Detection and monitoring of serum p53 antibodies in patients with colorectal cancer. Gut 1997, 40: 356–361.
90 Euchi S, Kohara N, Komuta K, Kanematsu T. Mutations of the p53 gene in the stool of patients with resectable colorectal cancer. Cancer 1996, 77: 1707–1710.
91 Villa E, Dugani A, Rebecchi AM et al. Identification of subjects at risk for colorectal carcinoma through a test based on K-ras determination in stool. Gastroenterology 1996, 110: 1346–1353.
92 Puig P, Urgell E, Capella G et al. Improved detection of K-ras codon 12 mutations in fecal exfoliated cells. Lab Invest 1999, 79: 617–618.
93 Ahlquist DA, Skoletsky JE, Boynton KA et al. Colorectal cancer screening by detection of altered human DNA in stool: feasibility of a multitarget assay panel. Gastroenterology 2000, 119: 1219–1227.
94 Moch H, Kononen T, Kallioniemi OP et al. Tissue microarrays: what will they bring to molecular and anatomic pathology? Adv Anat Pathol 2001, 8: 14–20.
95 Jass JR, Mukawa K, Goh HS et al. Clinical importance of DNA content in rectal cancer measured by flow cytometry. J Clin Pathol 1989, 42: 254–259.
96 Barratt PL, Seymour MT, Stenning S, Birbeck KF, Quirke P, and the AXIS collaborators. Molecular markers and the prediction of prognosis and response to therapy in colon cancer. J Pathol 1999, 189 (Suppl): 1A-28A.
97 Andreyev HJ, Normal AR, Cunningham D et al. Kirtsen ras mutations in patients with colorectal cancer: the multicentre 'RASCAL' study. JNCI 1998, 90: 675–685.
98 Kahlenberg MS, Stoler DL, Rodriguez-Bigas MA et al. P53 tumour suppressor gene mutations predict decreased survival of patients with sporadic colorectal carcinoma. Cancer 2000, 88: 1814–1819.
99 Hamelin R, Laurent-Puig P, Olschwang S, Jego W, Asselain B, Remvikos, Girodet J, Salmon R, Thomas G. Association of p53 mutations with short survival in colorectal cancer. Gastroenterology 1994, 106: 42–48.
100 Rodrigues NR, Rowan A, Smith MEF et al. p53 mutations in colorectal cancer. Proc Natl Acad Sci USA 1990, 87: 7555–7559.
101 Remvikos Y, Tominaga O, Hammel P, Laurent-Puig P, Salmon RJ, Dutrillaux B. Increased p53 protein concentration of colorectal tumours correlates with poor survival. Br J Cancer 1992, 66: 758–764.
102 Bosari S, Viale G, Bossi P, Maggioni M, Coggi G, Murray JJ et al. Cytoplasmic accumulation of p53 protein: an independent prognostic indicator in colorectal adenocarcinomas. JNCI 1994, 86: 691–687.
103 Jen J, Kim H, Piantadosi S et al. Allelic loss of chromosome 18q and prognosis in colorectal cancer. N Engl J Med 1994, 331: 213–221.
104 Martinez-Lopez E, Abad A, Font A et al. Allelic loss on chromosome 18q as a prognostic marker in stage II colorectal cancer. Gastroenterology 1998, 114: 1180–1187.

105 Shibata D, Reale MA, Lavine P et al. Allelic loss on chromosome 18q as a prognostic marker in stage III colorectal cancer. Gastroenterology 1998, 114: 1180–1187.
106 Kern SE, Fearon ER, Tersmette. Clinical and pathological associations with allelic loss in colorecatl carcinoma. JAMA 1989, 261(21): 3099–3103.
107 Elsaleh H, Joseph D, Grieu F, Zeps N, Spry N, Iacopetta B. Association of tumour site and sex with survival benefit from adjuvant chemotherapy in colorectal cancer. Lancet 2000, 355: 1745–1750.
108 Nicum S, Midgley R, Kerr DJ. Chemotherapy for colorectal cancer. J R Soc Med 2000, 93: 416–419.
109 IMPACT (international multicentre pooled analysis of colon cancer trials) investigators. Efficacy of adjuvant fluorouracil and folinic acid in colon cancer. Lancet 1995, 345: 939–944.
110 Edler D, Hallstrom M, Johnston PG et al. Thymidylate synthase expression: an independent prognostic factor for local recurrence, a distant metastasis, disease-free and overall survival in rectal cancer. Clin Cancer Res 2000, 6: 1378–1384.
111 Lander RD, Lynch FJ, Groshen S, Xiong YP, Sherrod A, Caradonna SJ, Stoehlmacher J, Lenz HJ. DUTP nucleotidohydrolase isoform expression in normal and neoplastic tissue: association with survival and response to 5-Fluorouracil in colorectal cancer. Cancer Res 2000, 60: 3493–3503.
112 Villafranca E, Okruzhnov Y, Dominguez MA, Garcia-Foncillas J, Azinovic I, Martinez E, Illarramendi JJ, Arias F, Monge RF, Salgado E, Angeletti S, Brugarolas A. Polymorphisms of the repeated sequences in the enhancer region of the thymidylate synthase gene promoter may predict downstaging after preoperative chemoradiation in rectal cancer. J Clin Oncol 2001, 19: 1779–1786.
113 Horie N, Aiba H, Oguro K et al. Functional analysis and DNA polymorphism of the tandemly repeated sequences in the 5'-terminal regulatory region of the human gene for thymidylate synthase. Cell Struct Funct 1995, 20: 191–197.
114 Vermorken JB. Adjuvant immunotherapy in colorectal cancer. Semin Oncol 2000, 27 (5 Suppl 10): 66–71.
115 Hoover HC jr, Brandhorst JS, Peters LC. Adjuvant active specific immunotherapy for human colorectal cancer: 6.5-year median follow-up of a phase III prospectively randomized trial. J Clin Oncol 1993, 11: 390–399.
116 Cobleigh MA, Vogel CL, Tripathy NJ et al. Efficacy and safety of Herceptin as a single agent in 222 women with Her2 overexpression who relapsed following chemotherapy for metastatic breast cancer. Proc Am Soc Clin Oncol 1998, 17: 97a.
117 Slamon D, Leyland-Jones B, Shak et al. Addition of herceptin to first line chemotherapy for Her2 overexpressing metastatic breast cancer markedly increases anticancer activity: a randomised, multinational controlled phase III trial. Proc Soc Clin Oncol 1998, 17: 98a.
118 Utvinov SV, Velders MP, Bakker HAM, Fleuren GJ, Warnaar SO. Epcam: A human epithelial antigen is a homophilic cell-cell adhesion molecule. J Cell Biol 125, 2: 437–446.
119 Adkins JC, Spencer CM. Edrecolomab. Drugs 1998, 56: 919–626.
120 Riethmuller G, Schneider-Gadicke E, Schlimok G et al. Randomised trial of monoclonal antibody for adjuvant therapy of resected Dukes' C colorectal carcinoma cancer. German cancer Aid 17–1A study group. Lancet 1994, 343: 1177–1183.
121 Riethmuller G, Schlimok G, Lehhmann JM et al. Immunological analysis of micro-metastases and the metastatic phenotype of human tumours. In: Melcher SF et al. (Eds.), Progress in Immunology vol. VII. Springer-Verlag, Berlin, 1989, pp. 1079–1086.
122 Huber BE, Austin EA, Good SS et al. In vivo anti-tumour activity of 5-fluorouracil on human CRC cells genetically modified to express cytosine deaminase. Cancer Res 1993, 53: 4619–4626.
123 Bookstein R, Demers W, Gregory R et al. P53 gene therapy in vivo of hepatocellular and liver metastatic colorectal cancer. Semin Oncol 1996, 23: 66–77.