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European Journal of Cancer 36 (2000) 1706–1712

European Journal of Cancer

www.ejconline.com

Therapeutic opportunities from tumour biology in metastatic colon cancer

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Received 5 May 2000; accepted 21 May 2000

Abstract

Tumour metastasis is the major cause of morbidity and mortality from colorectal cancer. While improvements in quality of life and patient survival have been made over the past 10 years, the majority of patients with metastatic colorectal cancer will die from their disease. As knowledge of the biology of colon cancer and its invasion/metastasis programme evolve, this presents new therapeutic opportunities for pharmacological and genetic intervention. This review discusses the current approaches to metastatic colorectal cancer therapy, details genomic and biological variance between primary and metastatic tumours, and highlights approaches for harnessing these differences to improve therapy. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Colorectal cancer; Metastasis; Developmental therapeutics; Gene therapy

1. The clinical problem

Colorectal cancer is a common disease in all the developed nations of the world [1]. It ranks second in incidence to lung cancer in men and breast cancer in women. Since it is a disease of the elderly it is predicted to become even more prevalent in the ageing population. Colon cancer is equally common in men and women, but men predominate in rectal cancer incidence, for reasons that are presently unclear.

Unfortunately, the early symptoms of colorectal cancer may be unspecific or non-existent. Thus, it is not uncommon to encounter patients with vague alteration in bowel function as their only complaint. This has two direct and serious consequences. The first is that a high proportion of patients (up to 30% in most literature series) will present as emergencies. Such presentations with obstruction and perforation are associated with a poorer prognosis than 'cold' cases. The second is that most patients present when the tumour is at a relatively advanced stage. Since prognosis is directly related to stage at presentation in this disease, this inevitably results in poorer outcome than if the disease presented at an earlier stage. It is this precept which underpins the current prospective screening studies for colorectal cancer.

The pathological stage at diagnosis is determined by the extent of local invasion and the presence of disease in lymph nodes [1]. Dukes' stage A and B are localised to the bowel wall, while Dukes' stage C has evidence of lymph node invasion and Dukes' stage D has distant metastases. Approximately 10% of patients will present with Dukes A, 20–30% with Dukes B and 30–40% with Dukes C. The remaining patients will present with metastatic disease at diagnosis. The most common site of metastasis is the liver, likely as a consequence of tumour seeding through the portal drainage of blood from the intestine. Overall 60% of patients will develop metastatic disease at some stage in their illness and almost all of these will succumb to the disease. At present radical surgical resection of primary and secondary disease provides the only realistic chance for long-term survival [2].

In advanced colorectal cancer (defined as beyond the scope of surgical resection) chemotherapy has a definite palliative role [3]. Modest survival benefits are possible with 5-fluorouracil (5-FU)/folinic acid (FA) regimens. However, there is no consensus as to the optimal regimen in this situation. Nor is there consensus as to the route of drug administration, with continued interest in the regional hepatic delivery of chemotherapy [4,5].

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PII: S0959-8049(00)00150-7

In the last five years, a group of new agents have been introduced in the therapy of colorectal cancer [6]. These consist of orally administered fluoropyrimidines, dihydropyrimidine dehydrogenase (DPD) inhibitors, irinotecan and oxaliplatin. The integration of these new agents into our therapeutic strategy is at an early stage. However, real benefits to patients from the use of these new drugs is already apparent [6]. The major challenge now in this area is to convert patients from an incurable situation into a curable one by the judicious application of multi-modality therapy (Table 1).

In the last 10 years developments in anaesthetic and surgical techniques have allowed the evaluation of resection of metastatic disease from the liver and lungs. Results from a number of studies performed in highly selected patients indicate 5-year survival can be as high as 40% [7,8]. Even re-resection of recurrent disease is becoming more acceptable [9]. The logical extension to the development in surgery and chemotherapy is to combine both approaches to try to cure patients with originally 'non-resectable' liver metastases. This aggressive treatment strategy can result in 50% 5-year survival rates [10]. Further investigations of combination chemotherapy and surgery are now ongoing to extend these findings with the goal of 'curative therapy' for metastatic colorectal cancer.

2. Metastases are not biologically or genetically identical to primary tumours

While therapy used in the treatment of metastatic colon cancer is targeted towards secondary lesions, often little is known of the biological make-up of metastatic deposits. In general, markers of prognosis or response are assessed in the context of the primary tumour, with the assumption that this reflects the situation in the secondary disease. This is often due to a lack of availability of tissue from metastatic lesions, as such biopsies are technically difficult to obtain and are associated with a degree of patient morbidity. However, several lines of evidence suggest that secondary tumours are genetically distinct from their progenitor tumour cells, and indeed metastatic tumours at different sites within the same patient may display heterogeneity.

Global assessment of genomic alterations in DNA from tumour tissue by comparative genomic hybridisation (CGH) identified distinct differences in chromosomal alterations between primary colorectal tumours and metastases (Table 2) [11,12]. A study of unpaired primary colorectal and secondary liver tumours found that liver lesions contained a higher number of chromosomal aberrations (mean 12.6) than primary colon tumours (mean 9.6) [11]. Alterations in liver metastases included those seen in the primary tumours. In the one case where the primary and secondary tumours were available from the same patient, a number of common alterations were shared, with the liver metastases found to contain additional novel aberrations [11]. Evidence for expansion of genomic change during metastasis was also observed by Al-Mulla and colleagues [12] using CGH in paired primary colorectal cancer and secondary deposits from either lymph nodes or liver. In the two cases where secondary tumours from both the lymph node and liver were available to compare with the primary colorectal tumour, differences between the secondary tumours were observed in one patient, whereas the second case demonstrated similarities between the secondary metastatic tumours [12]. These results indicate not only genetic heterogeneity between primary tumours and metastasis, but also that genomic differences exist in secondary lesions from discrete sites.

Such differences between primary and secondary colorectal tumours are likely to affect the response of secondary lesions to chemotherapy. The mainstay of

Table 1
Treatment options for metastatic colorectal cancer

Currently in clinical evaluation
Systemic chemotherapy[a]
Intrahepatic chemotherapy[a]
Combined systemic/intrahepatic chemotherapy[a]
Immunotherapy/tumour vaccines
Under investigation in preclinical models or early clinical studies
Intrapulmonary chemotherapy for lung metastasis[a]
Tissue-specific gene therapy

[a] Are being used in both palliative and neoadjuvant settings.

Table 2
Frequencies of genomic alterations in primary colorectal cancer and metastatic tumours[a]

Chromosome	Gain/loss	Primary colorectal tumour ($n=22$) n (%)	Secondary liver/ lymph node metastases ($n=22$) n (%)
4	loss	11 (50)	14 (64)
6p	gain	7 (32)	5 (23)
7p	gain	7 (32)	9 (41)
8p	loss	10 (45)	13 (59)
8q	gain	9 (41)	11 (50)
9q	loss	0 (0)	3 (14)
11q	loss	3 (14)	7 (32)
13q	gain	11 (50)	14 (64)
15	loss	6 (27)	10 (45)
16q	gain	5 (23)	7 (32)
17p	loss	6 (27)	13 (59)
17q	gain	4 (18)	5 (23)
17q	loss	0 (0)	5 (23)
18	loss	17 (77)	21 (95)
20q	gain	14 (64)	14 (64)
22q	loss	3 (14)	15 (68)

[a] Data from Refs. [11] and [12].

advanced colorectal cancer treatment is 5-FU, a pyrimidine analogue which targets the action of thymidylate synthase (TS). 5-FU acts by inhibiting TS, which is essential for both DNA and RNA synthesis. Several studies have shown TS levels in secondary colorectal tumours can be used to predict response to 5-FU-based treatment regimens [13,14]. However, measuring TS levels in primary tumours did not predict response in patients with metastatic disease [15], suggesting that enzyme levels in the primary tumour do not equate with levels in the secondary metastases against which the therapy is targeted. In addition, Gorlick and associates [16] demonstrated that both TS mRNA and TS protein are significantly higher in lung metastases compared with liver metastases, which may explain the lack of response often seen with pulmonary metastases. Similarly, a higher proportion of abdominal recurrences had elevated levels of TS compared with liver metastases [17].

5-FU is degraded and inactivated by DPD. A low ratio of DPD in paired tumour and normal tissue was correlated with response to 5-FU-based therapy in head/neck cancer [18]. Therefore, knowledge of DPD levels within metastatic colorectal tumours might predict the chances of achieving a clinical response. In the single study comparing DPD mRNA and DPD activity in unpaired primary colorectal tumours and secondary liver metastases, higher DPD levels were demonstrated in liver lesions [19], indicating that measuring DPD levels in primary colorectal tumours may be of little benefit.

The most comprehensive comparison of primary and metastatic colon cancer evaluated six components of the G1/S cell cycle checkpoint in 42 primary colon tumours and lymph node metastasis [20]. The patterns of expression of p53, p27 and Rb were not significantly different between primary and secondary tumours, supporting the use of the primary tumour for predictive purposes (Table 3; Fig. 1). In contrast, the expression patterns of p21, cyclin D1 and proliferating cell nuclear antigen (PCNA) were not concordant between colorectal tumours and their lymph node metastases, limiting the predictive ability of these markers from primary tumour analysis. Indeed, cyclin D1 was overexpressed at a higher frequency in lymph node metastases compared with colorectal tumours, suggesting a role in the metastatic process, possibly by propelling the cell cycle forward and affording a growth benefit. Conversely, p21 was demonstrated in a higher proportion of primary tumours compared with lymph node metastases. It may be that loss of this cell cycle inhibitor contributes to uncontrolled proliferation and therefore invasion.

Table 3
Comparison of G1/S phase cell cycle protein expression in primary and secondary colorectal cancer

Protein	Tissue examined[a]				Outcome
	Colorectal (CRC)		Lymph node (LNM)		
	Positive	Negative	Positive	Negative	
p53	67	33	76	24	CRC = LNM
Rb	93	7	93	7	CRC = LNM
p27	93	7	95	5	CRC = LNM
cyclin D1	50	50	74	26	CRC < LNM
p21	52	48	38	62	CRC > LNM

Adapted from Ref. [20].
[a] Numbers are percentages.

Taken together, these studies highlight biological differences which exist between primary colorectal tumours and their metastases, and indeed disparities between metastases at distinct sites within the same patient. Such variation may reflect the clone in the primary tumour from which the metastases were derived, the inherent nature of the secondary disease, or the microenvironment of the metastatic deposit. Regardless of the mechanism behind such differences, caution must be exercised when assessing potential response markers in the context of primary colorectal cancer.

3. Opportunities for therapeutic intervention

3.1. Enlightened biology to generate enlightened therapy

It is time for genomic and biological approaches which have the specific aim of identifying new targets for treating metastatic disease. The data from the two CGH studies in metastatic colon cancer identify loss of chromosome 22q as a high frequency event (Table 2), when compared with primary tumour [11,12]. Using data from both papers, loss of this chromosome arm was observed in 10/17 liver metastases and 3/22 primary tumours. With complete sequence analysis of chromosome 22 recently completed [21], this opens up an opportunity to use *in silico* approaches to identify putative therapeutic targets for metastatic colorectal cancer. Advances in gene expression arrays and proteomic technologies are also revealing new strategies for prognostic and therapeutic developments.

3.2. Tumour selectivity for genetic medicine

Poor response rates to current treatment regimens for patients with advanced colorectal cancer have stimulated interest in developing novel, more efficient therapies for this disease. Approximately 60% of colorectal cancer patients develop liver metastases and liver resection is currently the sole treatment for improved long-term survival in these patients. Various gene therapy strategies are currently under evaluation for the treatment of advanced colorectal cancer, with most attempting to

exploit differences between liver metastases and normal hepatic tissue for targeted treatment [22].

The potential use of 'suicide' genes to selectively activate pro-drugs in target tissues, such as colorectal liver metastases, has undergone extensive evaluation in animal models and cell lines. Initial approaches used adenoviral vectors to introduce into cells the transgene under the control of a strong promoter such as the herpes simplex virus (HSV) or cytomegalovirus promoters. One widely studied system utilises recombinant adenoviral vectors containing the cytosine deaminase (*CD*) gene, an enzyme that converts the pro-drug 5-flucytosine (5-FC) to 5-FU, currently the most effective agent in the treatment of advanced colorectal cancer. *CD*/5FC suicide gene therapy has recently entered phase I and II clinical trials. The *CD*/5-FC system has shown promising results for the treatment of colon carcinomas in animal models [23]. However, a major limitation of this approach is severe toxicity caused by activation of the 5-FC pro-drug in normal tissues, as until recently the *CD* gene has been expressed under the control of non-specific promoters. Several approaches to improve the specificity of gene therapy are currently being evaluated.

To take advantage of the biological differences between tumour and normal tissue, a number of *in vitro* studies have investigated the use of the carcinoembryonic antigen promoter (*CEA*) to drive specific expression of 'suicide' genes [24–27]. *CEA* is overexpressed in colorectal tumours and thereby allows selective expression of the foreign gene and subsequent enhanced activation of the pro-drug in tumour tissues. Animal studies demonstrate that *CEA* promoter systems are an effective tool for selective expression of

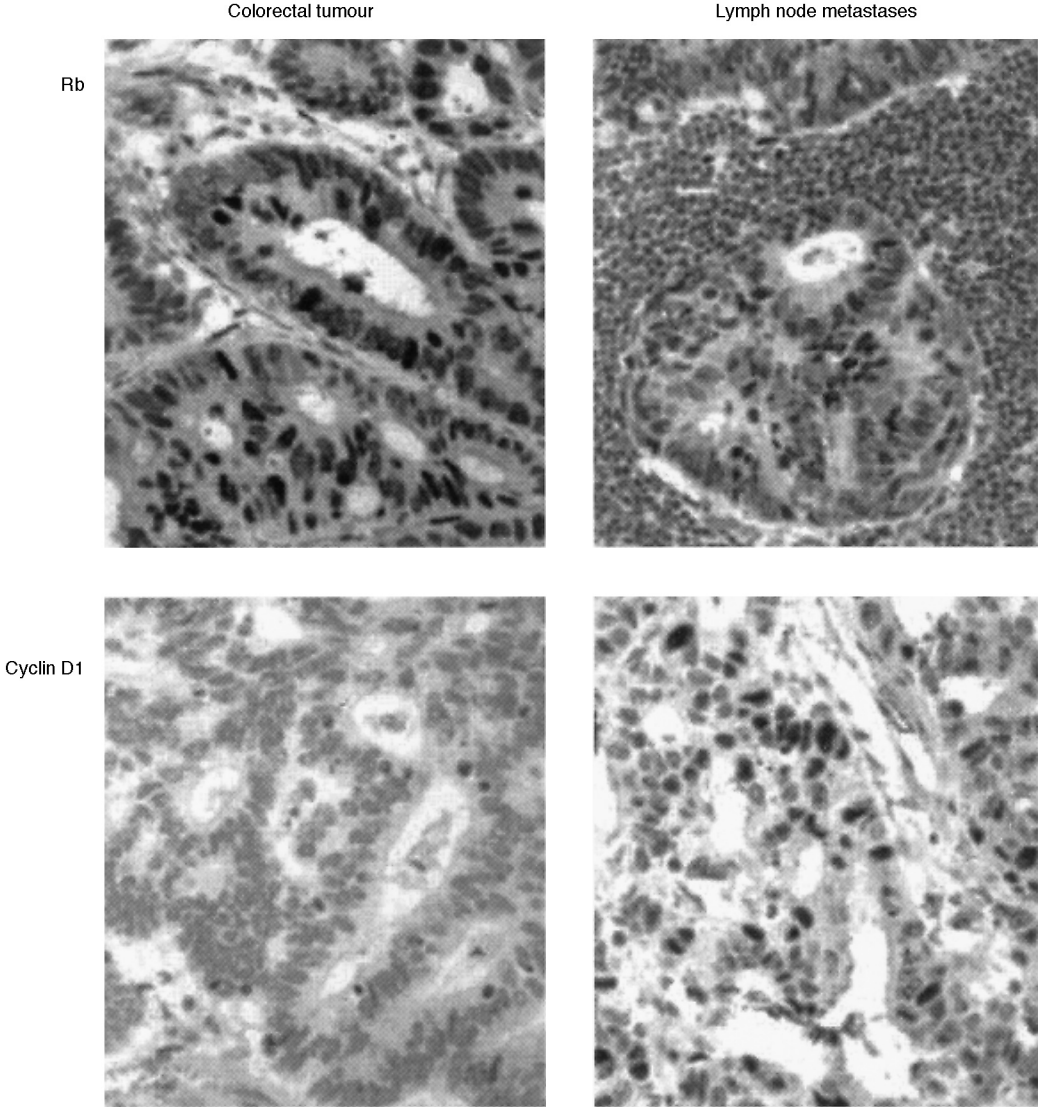

Fig. 1. Differential protein expression during metastasis. Immunohistochemical analysis of paired colorectal tumours and lymph node metastases. Similar staining patterns in both tumour types are observed for Rb immunostaining, whereas only the lymph node metastasis shows cyclin D1 immunoreactivity in the nuclei of this particular tumour. See [20] for additional details.

transgenes in colorectal carcinoma cells and liver metastasis and result in prolonged survival and reduced systemic toxicities associated with activation of the pro-drug 5-FC [24,26,27]. However, few *CEA* systems demonstrate significant antitumour activity *in vivo*, as it is a relatively weak promoter, compared with viral promoters such as HSV.

A novel dual gene therapy system for colorectal cancer, utilising the *CEA* promoter, has recently been developed *in vitro* to allow enhanced 'suicide' gene expression while maintaining tumour specificity. Human cancer cell lines were infected simultaneously with an adenoviral vector containing the *Cre* recombinase gene under the control of the *CEA* promoter and an adenoviral vector containing the HSV-thymidine kinase (*TK*) gene construct, with *TK* separated from its promoter by a neomycin (*neo*) gene [28]. Excision of the *neo* gene by *Cre* is required to allow expression of TK. This system increased the sensitivity of human cancer cell lines to the pro-drug gangciclovir (GCV), which is activated by TK, compared with cells treated with an adenoviral vector containing the *TK* gene directly under the control of the *CEA* promoter. This probably reflects stronger expression of the transgene from the viral promoter while maintaining tumour selectivity through the requirement for *CEA*. The use of similar systems to selectively and efficiently activate other pro-drugs and the efficacy of such gene therapy approaches *in vivo* require further investigation.

Another promising strategy for tumour-specific gene therapy takes advantage of colorectal tumour biology by using replication-defective adenoviral vectors. A *TK*-deleted recombinant vaccinia virus was used to deliver the 'suicide' gene in *CD*/5-FC gene therapy of a murine disseminated colon liver metastases model [29–31]. This vector will only replicate in the presence of TK. The system provided tumour-specific gene expression, due to the high levels of TK in tumour tissues, with improved survival and complete tumour response in some cases [30]. A second replication-defective vector approach utilises a recombinant HSV vector, hrR3 which lacks ribonuclease reductase activity and therefore is unable to replicate in the absence of host ribonucleotide reductase. hrR3 selectively replicates in actively dividing liver metastases that have high ribonucleotide reductase levels but only minimally in quiescent normal hepatocytes *in vivo* [32]. *In vitro* analysis demonstrated that hrR3 effectively kills cultured colon carcinoma cells. The use of the HSV-*TK*/GCV suicide gene therapy system did not increase the oncolytic activity of hrR3 [33].

A number of approaches utilise differences in p53 function in tumour and normal cells for tumour-specific gene therapy schedules. *TP53* is a tumour suppressor gene mutated in approximately 50% of human cancers [1]. In some studies, loss of p53 function is associated with resistance to chemotherapy and decreased survival in colon cancer patients [1]. p53 deficiency has been exploited both in the development of tumour-selective vectors and in gene replacement therapy. A modified adenoviral vector, ONYX-015 lacks the E1B 55 kDa protein, which is required for inactivation of host p53 and subsequent efficient viral replication in host cells. *In vitro* studies in human colon cancer cells with differential p53 function demonstrate that ONYX-015 replicates much more efficiently and is cytotoxic to p53-deficient cancer cells compared with the parent cells with wild-type (wt)-p53 [34]. In addition, ONYX-015 has significant *in vivo* antitumour activity against p53-deficient human carcinoma xenografts and acts synergistically with the chemotherapy agents, cisplatin and 5-FU [34]. ONYX-015 had no cytotoxic activity against a human glioblastoma xenograft with normal p53 function [34].

In addition to the use of endogenous p53 status to mediate tumour-specific adenoviral replication, *TP53* has been used as a therapeutic transgene to restore p53 function in cancer cells. Preclinical studies *in vitro* and *in vivo* demonstrate that restoration of p53 function induces apoptosis in cancer cells [35]. *TP53* gene replacement therapy is safe and demonstrated antitumour activity in Phase I clinical trials of non-small cell lung cancer (NSCLC) and head/neck cancer patients [35]. *In vitro* studies demonstrate that infection of NSCLC cells with the wt-*TP53* gene in a recombinant adenoviral vector has anti-angiogenic effects on infected and adjacent cells [36].

Preclinical studies *in vitro* and *in vivo* demonstrate that delivery of *TP53* as a transgene in a replication-defective vector inhibits tumour growth [37]. This strategy may provide enhanced tumour specificity for gene replacement therapy although whether this provides any clinical benefit has not been investigated. Treatment of cancer cells with cisplatin prior to infection with adenovirus/p53 resulted in higher therapeutic gene expression and time to disease progression appeared to be improved [38].

The effectiveness of gene replacement therapy in treating metastases is limited by the low levels of transgene expression. Combined treatment with adenovirus/p53 and 2-methoxyoestradiol, which induces and stabilises wt-p53 protein in cancer cells, inhibits the growth of human metastatic lung cells *in vivo* and *in vitro* in a synergistic fashion [39,40]. *TP53* gene replacement therapy demonstrates promising antitumour activity in the treatment of metastatic disease.

Vehicles for targeted delivery of systemically administered drugs or surgical procedures that allow regional delivery, can be used to improve tumour selective pro-drug activation. Targeting of pro-drugs, in addition to suicide gene targeting, may improve the specificity and efficacy of gene therapy schedules involving activation of a pro-drug by an expressed foreign gene, for the

treatment of colorectal liver metastases. Regional delivery of pro-drugs using intrahepatic perfusion (IHP), has been combined with gene therapy to improve hepatic conversion of pro-drugs [41]. In addition, liposomal encapsulation of GCV enhanced efficacy and decreased toxicity in gene therapy (HSV-*TK*) of liver metastases in rats [42].

There are many tumour-specific factors which appear worthy of exploitation as novel gene therapy approaches. For example, the cytochrome P450, CYP1B1, which metabolises xenobiotics, is expressed at a high frequency in a wide range of human cancers, including cancers of the breast, colon, lung, oesophagus, skin, lymph node, brain and testis but not in the corresponding normal tissues [43]. Normal liver or small intestine also do not express CYP1B1 [43]. This tumour-specific activity may be utilised in a similar fashion to the *CD* gene, as a 'built in' gene therapy approach using CYP1B1-activated pro-drugs.

4. Concluding comments

New targets are clearly needed for curative therapies to be developed for metastatic colorectal cancer. Taking advantage of current biological differences in secondary metastasis and expanding this knowledge to a more comprehensive level, offers the best hope of achieving this goal. Using biological findings to develop therapy is not a novel concept. In the 1950s, 5-FU was developed from the observation that tumours have greater uracil utilisation than normal tissues [44]. Similar rationale have guided the development of topoisomerase I inhibitors for colorectal cancer, based on differences in topoisomerase I protein expression [45]. As these simple observations have resulted in the most active current therapy for colorectal cancer, understanding the biological pathways which regulate the molecular signature of metastatic disease offers great promise for therapeutic outcome for patients.

Acknowledgements

Research in the Author's laboratory is supported by Scottish Hospitals Endowments Research Trust, The Dr James A. Mearns Trust, and the Aberdeen Colorectal Initiative.

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