Abstract. Established staging models for colorectal cancer (CRC) have mostly been based on morphological and clinicopathological criteria, which, while predictive of outcomes at extreme stages (e.g., Dukes’ A and D), are less informative at the intermediate stages (e.g., Dukes’ B and C). Although this traditional staging has improved survival by adjuvant therapy, a significant percentage of patients develop locoregional recurrences or metastases to the liver and other organs after curative tumor resection. Therefore, it is important to explore the use of new molecular and biological markers, particularly those that provide clues to tumor aggressiveness, in order to target therapeutic outcomes. The clinical usefulness of markers may vary with ethnicity and the anatomical location of the tumor; thus, combinations of markers are needed to develop a "predictive gene expression index" that will identify the underlying potential of the tumor for successful spread to a distant site.

Colorectal cancer (CRC) is the second most common malignancy in industrialized countries (1). CRC kills patients by metastatic destruction of the liver and/or obstruction of the GI tract, mainly via spreading through lymph nodes (LN). Hence, nodal involvement at the time the primary tumor is resected (pN) has been the "gold standard" for determining the prognosis of CRC patients (2). The pathways by which CRC leads to death are depicted in Figure 1. Genetic or epigenetic changes during CRC carcinogenesis lead to overexpression, suppression or gene mutation, which collectively may affect tumor cells and their interaction with the harboring host. Altered gene expression frequently results from mutations at different stages of tumor development, or from changes in the regulation of mutated genes (1).

Until now, established staging models for CRC have been based on morphological, histopathological and clinical criteria (e.g., pTNM and tumor localization), which do not adequately characterize the tumor’s metastatic potential. For example, while traditional staging systems are highly predictive of outcomes at extreme stages (e.g., Dukes’ A and D), they are less informative for the intermediate stages (e.g., Dukes’ B and C). Although traditional clinicopathological staging has improved survival by adjuvant therapy, a significant percentage of patients develop locoregional recurrence or metastases to the liver, lung or other organs after curative tumor resection and a combination of chemotherapy and locoregional therapy (3). Therefore, it is important to explore the use of new biological marker entities—particularly those that provide clues to aggressiveness—in order to augment current clinical staging and develop molecular markers with targeted therapeutic modalities. The hope is to find a marker (or markers) capable of differentiating patients with aggressive from those with indolent disease. The former group could then receive adjuvant therapy, while those patients effectively cured by surgery would avoid the cost, inconvenience and side-effects of the treatment (1, 4, 5).

Whereas the molecular phenotypes of early hereditary CRC lesions have been described as part of either the...
suppressor gene pathway of familial adenomatous polyposis (FAP) coli, or a mutator phenotype leading to microsatellite instability, only a few phenotypes have been ascribed to sporadic CRC, particularly those molecular changes resulting in aggressive lesions. The complication arises because, invariably, the molecular phenotypes that predict an outcome vary with the ethnic group of the patient and the anatomic site of the tumor. This is not unexpected since diet influences cancer and dietary habits vary with ethnicity. Anatomical location is an important variable because of differing embryological origins, varying vascular and lymphatic patterns, and phenotypic variations of epithelial mucosal functions and biomarkers (1, 2). CRC enhances our understanding of tumor progression and metastasis primarily because there is almost a step-wise advancement of the disease, which is marked by measurable genetic and associated phenotypic alterations. Metastatic death appears to be the end product of the development of clones capable of independent growth, invasion, adhesion, avoidance of apoptosis and angiogenesis. Although significant progress has been made in understanding the sequential genetic steps leading to the development of cancer, the precise genes and associated molecular pathways underlying the development of metastatic potentials are still poorly understood. These events are simply illustrated in Figure 2.

Biological and Molecular Markers (MMs)

MMs, as used in this article, refer to the evaluation of specific molecules in the colorectum as markers of either disease or induced changes in normal or diseased tissue. Since much of the research employing MMs translates the results of laboratory research to clinical use, it is designated as "translational research" (5). During the 1950s and 60s, pathologists relied on morphology, histochemistry, biochemical and enzymatic assays for disease characterization. By the 1970s, immunohistochemical (IHC) and ligand assays were employed, while by the 80s the advent of PCR was prognostically used to monitor the effectiveness of adjuvant therapy (e.g., detection of micrometastases and circulating tumor cells using specific markers) (1). Biological markers have also been used for the selection and evaluation of specific therapies (e.g., immunotherapy and gene therapy) (6, 7). Although markers can also be used for diagnosis and early detection of CRC (8), this article focuses mainly on prognosis.

Carcinoembryonic antigen (CEA). CEA has been proposed to be involved in several biological processes such as cell adhesion, immunity, protecting tumor cells from apoptosis and the experimental enhancement of metastatic potential (9). A number of randomized controlled trials showed that screening for CRC can reduce mortality (1). Therefore, a serum marker employing CEA has been developed as a convenient parameter for CRC screening. However, because of inadequate sensitivity and specificity, CEA has been considered an unsuitable marker as a screen for the early detection of CRC (10). Nevertheless, recent expert groups have concluded that preoperative measurement of serum CEA is desirable, since it provides independent prognostic information helping surgical management, and also provides a baseline level for subsequent determinations (10, 11). Moreover, CRC patients with stage 2 or 3 disease, who may be candidates for liver resection, were recommended for CEA serum test every 2-3 months for at least 3 years after the initial diagnosis, with testing carried out less frequently (e.g., every 6 months) after year three. Additionally, the CEA serum concentration may be of use in stratifying patients for therapy after removal of liver metastases. A clinically significant increase in CEA was arbitrarily defined as an increase in concentration of at least 30% over the previous value; however, this definition is not exclusive since a smaller percentage increase (i.e., 15 to 20%), maintained over at least 3 successive determinations, could prompt further investigations (10). A major limitation in monitoring with serum CEA is that 20-30% of CRC patients fail to produce elevated serum levels, despite the presence of advanced disease. For such patients, other serum markers such as CA 19-9, CA 242, CA 72-4, CA 50, TPA (tissue polypeptide antigen), TPS (tissue polypeptide-specific antigen) and tissue inhibition of metalloproteinase 1 (TIMP-1) have been suggested (10).

RT-PCR has also been used to evaluate the prognostic value of CEA as a marker for micrometastasis in high-risk CRC patients (1). The results have been mixed, with some studies showing this method to be more sensitive than IHC techniques, while others did not support that assertion.
Thus, currently, no conclusion can be arrived at without a well-designed prospective study to evaluate the sensitivity and specificity of conventional RT-PCR methodology for detecting CEA micrometastasis (1).

A more recent study employing quantitative real-time RT-PCR, which provides a more dynamic range of quantitation than conventional PCR, to detect micrometastases of cancer cells in lymph nodes (LN), ascites or the circulatory system of CRC patients, showed that postoperative increase of CEA mRNA is a predictive marker for micrometastases (12). In a study conducted in 2004, in which expression of CEA in LNs of CRC patients was determined by quantitative real-time RT-PCR, CEA was found to lack detection specificity, since it was also detected in most LNs of control subjects (13). In another real-time RT-PCR study, the presence of different CEA splice variants in WBCs of CRC patients and normal controls — as detected by different melting temperatures—affected the detection specificity of CEA in whole blood samples containing both WBCs and shed carcinoma cells, suggesting that CEA splice variants represent a physiological product distinct from the CEA expressed in malignancy (14). When a more specific marker (e.g., guanylyl cyclase C) was used in the 2004 study mentioned above, it exhibited higher signaling specificity than either CEA or CK 20, although its expression was detected in at least one LN in 8117 CRC patients undergoing surgery for non-cancer-related conditions (13).

It should be noted that RNA-based assays do not target tumor-specific markers; rather, they assay only tissue-specific ones. Thus, unless specific CRC genes expressed only in CRC patients and only in colonic tissues are used (1), it is doubtful that these PCR assays will achieve a high degree of specificity (13). Moreover, the clinical significance of the prognostic MM must be unambiguously demonstrated, e.g., they should exhibit a gradual increase in tissues as the colonic tissues go through the various stages of progression (e.g., from adenoma to carcinoma) (1).

Kirsten (K) ras oncogene. The ras protooncogene is a GTPase that encodes a 21 kDa membrane-associated protein (p21), producing different signal transduction pathways from transmembrane growth factor receptor to cytoplasmic kinases and, ultimately, to the nucleus regulating, for example, cell proliferation. The ras oncogene is activated in carcinogenesis either by overexpression of p21, or by expression of a point-mutated form of p21 leading to a constitutive activity of ras and possibly to a malignant transformation of the cell (15). Up to 70% of CRCS exhibit prognostically unfavorable mutations of the
K-ras; these mutations occur early in the progression of colorectal adenoma to carcinoma (Figure 1). Mutant k-ras 2 has been detected in serum (16).

An international collaborative study, comprising more than 22 research groups in 13 countries, responded to questionnaires related to Ki-ras mutation data on 2721 CRC patients. The data were coded and entered into a database called "RASCAL" and statistically analyzed by multivariate analysis. Mutations of the transition type were observed particularly in codon 12 and were G to T, resulting in a change from amino acid glycine to valine, and not A to C-type transition. These mutations increased the risk of recurrence and death. However, no correlations have been found between mutation and sex, tumor site or Dukes' stage (17).

Adenomatous polyposis coli (APC) suppressor gene. APC is a tumor suppressor gene located on chromosome 5q21. APC, together with glycogen synthase kinase 3β, provides a signal that targets the degradation of cytoplasmic β-catenin. In the presence of either mutant APC or β-catenin, this process is disrupted. Excess β-catenin is translocated to the nucleus, where it binds with T-cell factor 4 to up-regulate target genes including c-myc, cyclin D1, PPARG, and c-jun. β-catenin binds to APC in a mutually exclusive manner with E-cadherin at the plasma membrane (4). Studies have shown that mutations in this gene occur in most sporadic colorectal cancers and in up to 30% of familial adenomatous polyposis and initiated colorectal neoplasia, whereas other mutations, such as those in p53, are present only in the later stages of colorectal neoplasia, or may be present in non-neoplastic hyperplloietive cells such as those in c-Ki-ras. However, it not easy to detect these mutations in the APC gene because mutations can occur virtually anywhere within the first 1600 codons of the gene. Moreover, the type of mutation (base substitutions or insertions, or deletions of diverse length) varies widely among tumors. These mutations are even more difficult to detect in stool DNA, where they may be present in less than 1 in 100 APC genes in the sample (1).

Expression studies using cDNA microarrays, after introduction of the wild-type APC gene into SW48 colon cancer cells, identified a novel human gene, APCDD1, whose expression was elevated in 18 out of 27 primary colon cancer tissues as compared with corresponding non-cancers. Exogenous APCDD1 promoted the growth of colon cancer cells both in vitro and in vivo, whereas transfection with antisense S-oligoodeoxynucleotides decreased tumor growth suggesting that APCDD1 is directly regulated by the β-catenin/transduction pathway (Tcf) complex, and that its elevated expression is likely to contribute to colorectal tumorigenesis (18). Studies in colon cancer cells treated with alkylating agents showed that the DNA damage-induced level of APC mRNA requires p53 (19). Although most studies on APC were concerned with its screening potential, this marker also has potential prognostic significance because of its involvement in many pathways of CRC carcinogenesis (1).

β-catenin adhesion molecule. Abnormal levels of β-catenin are believed to contribute to neoplastic transformation by causing accumulation of cyclin D1 and were frequently reduced (~80%) in primary colorectal carcinomas (20). β-catenin forms complexes with APC tumor suppressor protein, and β-catenin expression levels were affected by exogenously-induced APC protein. The expression levels of APC protein in tumor tissues were more than three times greater than those in corresponding normal mucosa (21). It has been hypothesized that β-catenin forms a complex with E-cadherin in vivo. The down-regulation of β-catenin expression is associated with malignant transformation, and colorectal cancer cells may have impaired E-cadherin-mediated cell adhesiveness because of the down-regulation of catenin expression (1). IHC studies of formalin-fixed, paraffin-embedded polyps of CRC patients showed that cytoplasmic β-catenin accumulation as a result of APC mutations seems to drive p53 overexpression, whereas nuclear β-catenin translocation appeared to be related to a pattern of invasion of neoplastic cells (22).

p53 (TP53) suppressor gene. p53, which resides at chromosomal location Ch17p13.1, is the most commonly altered gene in solid human neoplasia. Approximately half of CRCs show p53 (TP53) gene mutations, with higher
frequencies in distal colon and rectal tumors, and lower frequencies in proximal tumors and those with microsatellite instability or methylation phenotypes. Accumulation of mutant p53 has frequently been identified by IHC and, in some cases, leads to accumulation in the extra-cellular environment, with potential use as biomarkers in blood (1). Alterations of p53 appear to have little or no prognostic value for CRC patients treated by surgery alone, but are associated with worse survival for patients treated with chemotherapy (23). Studies showed nuclear p53 (p53\textsuperscript{nuc}) may have prognostic value, mainly in proximal tumors of Caucasian patients (2). Other studies showed bad survival outcome in patients with mutations in both K-ras and TP53 (24).

**Bcl-2.** Bcl-2 was so named so because it was discovered as part of the most common translocation in human B cell lymphoma. In human, the first regulator for programmed cell death emerged when the bcl-2 gene, activated by chromosomal translocation in follicular lymphomas, permitted the survival of cytokine-dependent hematopoietic cells in quiescent state in the absence of cytokines. This discovery established that cell survival and proliferation were under separate control and that disturbances in both were likely to contribute to neoplasia (8). Apoptosis eliminates cells with damaged DNA or aberrant cell cycle (i.e., those most likely to create a neoplastic clone) (25).

The phenotypic expression of bcl-2 could be a useful prognostic marker in CRC (8). In univariate Kaplan-Meier analysis, expression of bcl-2 was associated with better overall survival of both African-American and Caucasian patients with distal, but not proximal, CRC. Moreover, these studies suggested that bcl-2 expression is useful in determining prognosis before clinicopathological staging, and in selection of therapeutic modality in patients with distal CRC (2, 26). Although a trend toward an inverse correlation between bcl-2 and p53 expression was found, the prognostic value of bcl-2 expression was independent of p53 status (27).

Work on syngenic CRC cell lines, using an RNA interference technique, indicated a new cell death pathway in which bcl-2 constitutively suppresses p53-dependent apoptosis. Silencing of bcl-2 induced massive p53-dependent apoptosis. The bcl-2/p53 axis required bax and caspase 2 as apoptotic mediators. Treating the cells with chemotherapeutic agents, such as 5-FU, in order to activate p53, can also induce apoptosis. This new pathway can regulate apoptosis by targeting bcl-2 in CRC cells in response to genotoxic stress (28).

**Survivin.** The inhibitors of apoptosis (IAPs) proteins are a widely-expressed gene family of apoptotic inhibitors, whose apoptotic suppressor ability exceeds other families of apoptotic inhibitors, including the bcl-2 family (29). The central mechanism of apoptotic suppression appears to be through direct caspase and pro-caspase inhibition (primarily caspase 3 and 7) and modulation of transcription factors v-Rel and NF-\textsuperscript{1}B, as the IAPs have been shown to be induced by these transcriptional factors in multiple cell lines (30).

Survivin is an IAP present in most transformed cell lines and cancers tested to date, but not in differentiated adult tissue, though abundantly expressed in transformed cell types and a variety of human cancers in vivo. Survivin has been shown to inhibit caspase directly and apoptosis in general. Moreover, survivin protein levels correlate inversely with 5-year survival rates in colorectal cancer (29). A study employing a real-time RT-PCR has shown survivin \(\beta\) to be highly expressed in human colonocytes in the stool of colon adenocarcinoma patients (31).

An IHC study of 171 CRC patients, who underwent curative tumor resection, but received neither chemotherapy nor radiation therapy before surgery, with data analyzed by Cox multivariate proportional hazard model, showed that apoptosis inhibition by survivin alone, or in cooperation with bcl-2, was an important predictive/diagnostic parameter of poor outcome (32). Another IHC study, that looked at 43 hyperplastic polyps, 171 adenomas with low dysplasia, 42 adenomas with high dysplasia and 60 carcinomas, in which tissue expression of survivin, p53 and bcl-2 was examined, found that survivin and p53, but not bcl-2, increased the transition from adenoma with low dysplasia to high dysplasia/carcinoma. This transition was also associated with a significant decrease in apoptotic index and a significant increase in Ki-67 labelling index and microvessel density. Bcl-2 showed an aberrant expression throughout the course of their sequence. Survivin, p53 and bcl-2 all showed significantly lower suppression in preneoplastic polyps than in adenoma or carcinoma, being similar to that in normal colon mucosa. The lack of expression of all genes is noteworthy considering that hyperplastic polyps, unlike adenomas, have a tendency to become malignant (33). Further work is needed to elucidate the role of survivin in CRC progression.

**Mucin 1 (MUC1) and mucin 2 (MUC2).** There are 8 mucin gene family members (MUC1-MUC8), which are produced by epithelial cells. Differential expressions of MUC1 and MUC2 have been reported in colorectal adenomas and CRCs. Early studies on CRC, as well as other human malignancies, have suggested that increased expression of the core peptide of MUC1 was associated with poor prognosis (1). The role of MUC2 in predicting clinical outcome was, however, controversial, as some studies on CRC neoplasia showed the increased expression of MUC2 to be associated with poor patient survival, while studies on other neoplasia (e.g., pancreatic adenocarcinoma, biliary carcinoma and gastric carcinoma) showed association with better patient prognosis (34).
In a more recent IHC study, evaluating the expression of MUC1 and MUC2 in 166 archival CRC specimens from African-Americans and 108 Caucasian patients, who had been analyzed previously for p53nuc accumulation, univariate Kaplan-Meier and multivariate Cox proportional hazard analysis showed that MUC1 expression was more frequent in advanced stages of CRCs, whereas MUC2 expression was higher in the mucinous type of CRCs. Moreover, MUC1 expression was found to be an indicator of high-risk of death from CRC in Caucasians, but not in African-Americans. Additionally, Caucasians with CRCs showing concomitant expression of MUC1 and p53nuc demonstrated the lowest probability of overall survival; a finding that may be useful in developing new therapeutic regimens. No prognostic value was found for MUC2 alone, or in combination with p53nuc, in either group (35). No variation of the prognostic value of MUC1 was reported based on the anatomical location of the tumor (2). The combination of MUC1, bcl-2 and p53nuc in prognosticating Caucasian patients showed enhanced survival in patients lacking p53nuc and MUC1, but expressing bcl-2 (Figure 3) (2).

Insulin-like growth factors (IGFs). IGF-1, -2 and the IGF-1 membrane receptor (IGF-1R) are mitogens that play a pivotal role in regulating cell proliferation, differentiation, apoptosis, transformation and carcinogenesis in several tumors, among them CRCs. IGF-1 and -2 bind to and activate the IGF-1R, inducing cellular proliferation and growth (1). Both IGF-1 and -2 are produced in many tissues in response to growth hormone stimulation, and can be found in serum, either free (active form), or more often bound to binding proteins (BPs) in inactive form; the effect of BPs are regulated in part by proteases (36). IGF-1 and -2 are single-chain polypeptides having 62% homology in their amino acid sequences; their structures resemble that of proinsulin and both share additional structure similarities. The IGF-1 and -2 genes are located on chromosomes 12 and 11, respectively. The IGF-1R is a glycopeptide located on the cell membrane. It is a tetramer of two individual α-subunits and two individual β-subunits. Structurally, the IGF-1R resembles the insulin receptor and there is 60% homology between them (36). Binding of the IGFs to the IGF-1R activates the receptor’s tyrosine kinase activity, triggering a cascade of reactions among a number of molecules included in the signal transduction pathways. In addition to mediating the mitogenic and antiapoptotic reactions of IGFs, IGF-1R is also involved in cell transformation (36). The prognostic significance of these factors has been studied.

A study aimed at analyzing the changes in expression levels of the IGF-1R in 40 paired samples of carcinomatous colon tissue by quantitative RT-PCR, IHC and ligand binding, showed that the overall mean IGF-1R mRNA level was 5-fold higher in tumor versus adjacent normal mucosa. These results were also confirmed at the protein level by IHC and receptor-binding studies. Moreover, IGF-2 mRNA levels were significantly elevated in CRC and the IGF-2 mRNA log ratio in tumors versus normal tissue was elevated more than 2-fold in 28 out of 40 paired samples. Additionally, a positive correlation was observed between overexpression of IGF-2 and IGF-1R in tumors (37). IGF-1 and IGF-2 transcripts showed 73- and 42-fold increases, respectively, in serial analysis of gene expression (SAGE) by CRC database (38). Studies showed an increase in CRC risk among subjects with elevated serum IGF-2 levels (39). One study suggested the increased expression of IGF-2 to be a strong predictor of liver metastases (40); however, this study lacked adequate statistical analysis and employed a group that already showed liver metastases. A recent carefully conducted IHC study in 713 CRC patients that correlated molecular findings with clinicopathological data and with clinical follow-up findings, and evaluated survival by Kaplan-Meier plots and Cox regression analysis, concluded that the prognostic effects of IGF-1 and -2 are limited; however, the identification of IGF-positive CRC was thought to be beneficial for predicting new therapies targeting the IGF system (41).

Tumor growth factor beta (TGF-β). TGF-β, a member of a family of dimeric polypeptide growth factors, is a multifunctional protein that modulates cell growth, differentiation, extracellular matrix production and
immunosuppression. There are three isoforms of TGF-β (1, 2 and 3); each isoform is encoded by a distinct gene and is expressed in both a tissue-specific and a developmentally-regulated fashion. The TGF-β1 isoform is the most abundant member of the family. TGF-β has been shown to inhibit the growth of normal jejunal crypt cells of the small intestine and most well-differentiated and some moderately-differentiated human colon cancer epithelial cell lines. In contrast, many cell lines, including those established from colorectal cancers, are resistant to TGF-β-induced growth inhibition. Messenger RNA from TGF-α and TGF-β were found in various tumor lines, especially colon cancer cell lines, suggesting that these endogenous factors could be involved in the paracrine stimulation of stromal cells. (1). The TGF-β transcript showed a 24-fold increase in expression in the CRC SAGE database (38).

Colonic tumors of human origin were reported to produce abundant TGF-β, which alluded to that molecule’s critical role in tumor growth, and studies showed that TGF-β plays an important role in early and late stages of colon carcinogenesis (42). In a DNA microarray study, investigating potential effectors of TGF-β-mediated suppression of colon cancer in a non-transformed TGF-β-sensitive cell line (VAC0330) derived from a human adenomatous colon polyp, a gene, PMEPA1, highly regulated by TGF-β treatment, was identified as early as 2 h after TGF-β treatment. Moreover, this gene was not inhibited by pretreatment of cells with cycloheximide, suggesting that PMEPA1 is a direct target of TGF-β signaling. PMEPA1 expression was maintained in a TGF-β-independent manner after malignant transformation and metastases, suggesting that late colon cancer retains a strong capacity to execute many steps of the normal colonic differentiation program (43).

Because cell cycle checkpoints are often deregulated in oncogenesis, a better understanding of how TGF-β mediates the cell cycle progression of cancer cells may ultimately have therapeutic implications. Therefore, the molecular mechanism of cell cycle inhibition by TGF-β was investigated in human colon carcinoma FET cells. TGF-β1 was found to inhibit DNA synthesis and to dramatically increase p21WAF1 protein levels after release from growth arrest, leading to an increased association with cdk2, but had no effect on the cyclin E-cdk2 bound p27 levels, which remained relatively unchanged. TGF-β1 stimulation of p21WAF1 protein was regulated at the posttranscriptional and transcriptional levels through a p53-independent pathway. Posttranscriptional control is dependent on treatment with TGF-β1 in early G1, whereas TGF-β1-mediated transcriptional control does not appear to be a function of the time of TGF-β1 treatment during cell cycle progression. This is a new mechanism of TGF-β1 inhibition (44). There is only circumstantial evidence of the biphasic role of TGF-β in human cancer (i.e., suppression of cellular proliferation in early stages and progression of cancer in later stages as cells acquire resistance to growth inhibition and secrete large amounts of the cytokine), but this role has not been validated in animal models (45). TGF-β is an important marker for diagnosis and prognosis in CRC (46).

Cdns (p21WAF1 and p27KIP1). Proteins such as p21WAF1, p27KIP1 and p57KIP2 form a family of cdk inhibitors, the CDKIs. The p27KIP1 inhibits G1-associated-cdn complexes as well as cyclin-bcl-2 complexes. These inhibitors contain a conserved cdk/cyclin inhibitory domain plus additional domains specific for each member. In contrast to the INK4, inhibitors of the p21WAF1-type inhibitors bind and inhibit multiple cdk/cyclin complexes, including those containing cdk2 (pt, p16, p18, p19). A strong positive correlation between cyclin D1 and Rb expression and the level of expression of the CDKI protein p27KIP1 was found. This correlation suggests the existence of a homeostatic feedback loop between positive and negative acting components of the cell machinery (47). The homeobox genes cdx1 and cdx2 (48, 49) up-regulate when the myc gene suppresses the transcription of the p21WAF1 gene (50).

Using a cDNA microarray to study gene expression in non-dysplastic colonic mucosa of the mucinous and nonmucinous types showed that the p21WAF1 gene was overexpressed only in cells derived from the mucinous colon carcinoma (51), suggesting that these phenotypes may develop along different genetic pathways. A possible explanation for the overexpression may be that in 70% of mucinous carcinoma, the p53 gene is not mutated, thus being able to induce p21WAF1 expression (51).

The expression of the cyclin-dependent kinase inhibitor p27KIP1, which inhibits the G1- to S- transition of the cell cycle, is located on chromosome 12p. It is a putative tumor suppressor and no mutations were reported for it in a variety of tumors, suggesting that this protein may play a positive role in the growth of tumors. An IHC assessment of 98 CRC patients revealed a significant correlation between high p27KIP1 protein expression and high cyclin D1 expression in the adenoma polyps and in the subset of carcinoma that had only nuclear p27KIP1 expression. There was a significant correlation between p27KIP1 expression and tumor grade (i.e., well- and moderately-differentiated carcinoma had high p27KIP1 expression, whereas poorly-differentiated carcinoma had lower expression). Comparison of Northern and Western blots did not show a correlation between the level of p27KIP1 mRNA and a corresponding protein, which is consistent with the evidence that this protein is regulated mainly via a posttranscriptional mechanism. These findings allude to the existence of a homeostatic feedback mechanism that may have been lost in high-grade carcinoma that expresses low levels of p27KIP1 (52).
Another IHC study in 178 primary CRC cancer, 34 LN metastases and 48 normal mucosa, showed that loss of p27KIP1 was found in 51% of primary tumors, 68% of metastases and 56% of normal samples. In patients with Duke's B or with proximal tumors, the loss of p27KIP1 predicted poorer prognosis. However, there were no significant differences in patients with other individual Duke's stage or in distal tumors, suggesting that p27KIP1 might be a useful prognostic marker to identify the more progressive tumors (53). Moreover, in a study in 41 CRC patients with right-sided colon cancer, including 18 cases with regional LN metastases and 23 cases with negative LN, immunostaining for p27 found decreasing expression of this protein associated with large tumor size. Multivariate analysis revealed that low p27 expression in primary cancer was correlated with LN metastases. However, it did not correlate with any other histological parameter (54). Another study, that looked at 171 CRC carcinoma specimens using anti-Ki67 and anti-p27 antibodies and assessed mutated p53 expression, found no association among p27 expression, mutated p53 accumulation, the Ki67 labelling index and the apoptotic index, but a multivariate analysis found p27 expression to be an independent and a significant predictor of the overall survival. Patients with low p27 expression and low apoptotic index had the poorest prognosis (55).

A study in which proteasome-mediated degradation of p27 activity was compared with its protein level in a subset of tumor samples, the carcinoma with low or no p27 protein displayed enhanced proteolytic activity specific for p27, suggesting that low p27 expression can result from increased proteasome-mediated degradation rather than altered gene expression. Multivariate analysis showed that p27 was an independent prognostic marker associated with poor prognosis, particularly in stage II tumors, and thus this marker may help in the selection of patients who may benefit from adjuvant therapy (56). In a HIC study that analyzed p27KIP1 in 80 CRC, the protein was localized heterogeneously in the nuclei of cancer cells. The mean apoptotic index was significantly higher in p27-positive than in -negative patients, and PCNA labelling was not correlated with p27 expression. The data, when analyzed by univariate Kaplan-Meier and Cox's multivariate proportional hazard statistics, showed that overall survival was significantly lower for patients who were p27-positive compared to those who were negative, and in p27KIP1-positive patients, no significant differences between prognosis and AI or PCNA-LI were found by univariate analysis. Multivariate analysis revealed the p27 expression to be an independent prognostic marker of outcome in CRC patients, as no correlation was found between p27 status and clinicopathological parameters, probably through the role of p27 in promoting apoptosis (57).

An IHC study assessed the tumor location and prognostic significance of p27 expression as it relates to tumor size, stage and patient ethnicity in 85 African-Americans and 121 Caucasians, and data analyzed with Kaplan-Meier and Cox statistics found no prognostic significance for p27 expression in stages I, II or IV CRC, or association with either ethnicity or tumor location. The results also suggested that decreased expression of p27 was an indicator of poor prognosis and aids in identifying a subset of patients with an aggressive form of stage III CRC (58).

The prognostic significance of p21, p27, p53 and COX2 expression was examined by RT-PCR in 62 patients (from 45 to 97 years old) from whom fresh tissue was obtained, and CP characteristics from 1354 patients (between 40 to 80 years old) who underwent resection. The data were analyzed by $\chi^2$ test for CP, Kaplan-Meier for cumulative survival rate, Wilcoxon test for significance and Welch $t$-test for mRNA expression of genes (59). The results showed that CRC developed more frequently at the right side of colon in the elderly compared to the young. The incidence of CRC was higher in men than in women, but with advancing age the incidence in women tended to increase. Moreover, a higher incidence of right colon was observed in women than men. CRC in patients > 80 years old showed deeper invasion, was larger in diameter, more advanced, had a higher positive ratio of LN metastases and showed significantly lower levels of p27 mRNA, suggesting that decreased survival was due to lower p27 expression. Low expression of p53, and no changes in p21 or COX2 mRNA levels with advanced age, suggested a weak relationship among these markers and CRC progression in the elderly (59).

**Src protooncogene.** Src kinase family members, a group of non-receptor tyrosine kinases, simultaneously activate multiple signaling pathways, including those mediating proliferation, cell cycle progression, migration, cytoskeleton organization and cell survival. Dysregulation of Src via post-translational modifications of genetic alterations leads to constitutive kinase activity and cellular transformation. Src is a 60 kDa protein kinase. A point mutation at tyrosine residue 527 renders a constitutively active c-Src protein with transforming capacity (60). Src activation has been implicated as an early event in colorectal carcinogenesis, and over 80% of colon cancers have elevated Src activity. Src expression and activity is decreased in colon carcinoma cell lines, and corresponding decreases in tumorigenicity in nude mice were observed (61).

The myriad of cellular processes affected at the level of gene expression have been assessed in 9 rodent fibroblast lines (3Y1) transfected with c-Src, v-Src or Src 531 (a human Src mutant containing a truncated mutation leading to activation, increased transformation and tumorigenesis). The orthologous human genes present on the Affymetrix Hu95A GeneChip.
(12K named genes) were extracted and expression profiles were compared between the Src-induced rodent cell lines and staged colon tumors where Src is known to be activated. Approximately 600 co-regulated genes were identified, that correlated with high tyrosine kinase activity and soft agar colonization. This transformation "fingerprint" was composed of five gene clusters containing between 21-306 genes/cluster. Cell cycle control genes (e.g., cyclin D1) were elevated 2 to 3-fold in all five stages of colon cancer. Cytoskeletal proteins, associated with actin-based motility or intracellular transport, were also overexpressed in tumors (62).

Proteolytic enzymes, that partially mediate invasion, such as MMP-9, cathepsin-L or uPA, are induced by v-Src and the expression of activated Src in colon carcinoma cell lines leads directly to increased tumor eruption and expression of the urokinase-type plasminogen (u-PAR) gene at the transcriptional level (63), resulting in increased ability to proteolytically cleave laminin, a component of the basement membrane that is degraded during metastases. These results strengthen the role of Src in the development and progression (i.e., invasion and metastasis) of human colon cancer.

**Urokinase activation system (uPA).** A protease that has been implicated as a marker of invasion and metastasis is the urokinase-type plasminogen activator (uPA), a component of the uPA system. It is a 55 kDa serine protease that, by activating its substrate plasminogen to active plasmin, cleaves components of the extracellular matrix (ECM) structures (e.g., fibrin, fibronectin, proteoglycans) and basal membranes (e.g., laminin, collagen IV), thereby facilitating invasion and metastasis. Dissolution of ECM also liberates sequestered growth factors that enhance cell proliferation, migration and angiogenesis. Moreover, some of the processed ECM components, by themselves, may promote the metastatic process (63). Other components of the system include two receptors [a glycolipid-anchored receptor (uPAR) and plasminogen receptor] and three inhibitors (plasminogen activator inhibitors PAI-1, PAI-2 and protease nexin 1) (64). The urokinase receptor (uPAR) gene, which is located on chromosome 19q13, spans 7 exons. Transcription of the gene yields a 1.4 kb mRNA, or an alternatively spliced variant lacking the membrane attachment peptide sequence. Like classical housekeeping genes, the sequence for the u-PAR gene lacks TATA and CAAT boxes and contains a GC-rich proximal sequence with multiple SP1 consensus elements. Three main transcriptional start sites are apparent, revealing similarity to the consensus initiator sequence of the dihydrofolate reductase (DHFR) gene (63,64). The uPA system has been implicated in remodeling of the extracellular matrix, enhancing both cell proliferation and migration, and modulating cell adhesion. Moreover, data from model systems suggests that the uPA system may also play a role in the early phase, as well as in late phases of tumor progression (64). Figure 4 depicts the steps by which the uPA system induces progression.

Studies showed an overexpression of the uPAR gene in diverse tumors (e.g., colon, esophageal, gastric, breast and lung), as compared to normal surrounding tissue and/or stromal cells, suggesting that the u-PAR molecule is a critical component of the uPA system for invasion and metastasis, thus showing promise of being a molecular marker.

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### Table I. Genes with prognostic importance in CRC carcinogenesis.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Full Name</th>
<th>GenBank Accession #</th>
</tr>
</thead>
<tbody>
<tr>
<td>CEA</td>
<td>Carcinoembryonic antigen</td>
<td>AK026884</td>
</tr>
<tr>
<td>Ki-ras</td>
<td>Kirsten ras protooncogene</td>
<td>P01118</td>
</tr>
<tr>
<td>APC</td>
<td>Adenomatous polyposis coli tumor suppressor gene</td>
<td>NM_0000038</td>
</tr>
<tr>
<td>β-catenin</td>
<td>Adhesion molecule β-catenin</td>
<td>X89578</td>
</tr>
<tr>
<td>p53</td>
<td>Tumor suppressor gene p53 (TP53)</td>
<td>K03119</td>
</tr>
<tr>
<td>Bcl-2</td>
<td>Antiapoptotic oncogene bel-2</td>
<td>Q9Y3E5</td>
</tr>
<tr>
<td>p27kip1</td>
<td>Cdk inhibitor p72 suppressor gene</td>
<td>BC014544</td>
</tr>
<tr>
<td>IGF-1 &amp; -2</td>
<td>Insulin-like growth factor II receptor, forms 1 and 2</td>
<td>X07868/AF069333</td>
</tr>
<tr>
<td>TGFβ1</td>
<td>Transforming growth factor beta 1</td>
<td>NM_000660</td>
</tr>
<tr>
<td>MMP</td>
<td>Matrix metalloproteinase</td>
<td>L23808</td>
</tr>
<tr>
<td>MUC-1 &amp; -2</td>
<td>Mucin 1 &amp; 2 family members</td>
<td>NM_002456/NM_002457</td>
</tr>
<tr>
<td>Survivin</td>
<td>Survivin β</td>
<td>AB028869</td>
</tr>
<tr>
<td>Src</td>
<td>Protooncogene Src tyrosine kinase</td>
<td>X04000</td>
</tr>
<tr>
<td>uPAR</td>
<td>Urokinase-type plasminogen activator receptor</td>
<td>K03226</td>
</tr>
<tr>
<td>CD44</td>
<td>Cell adhesion molecule CD44</td>
<td>AF096644</td>
</tr>
<tr>
<td>OPN</td>
<td>Osteopontin (secreted phosphoprotein 1)</td>
<td>BC022844</td>
</tr>
</tbody>
</table>

Ahmed: Molecular Staging Models of Colorectal Cancer (Review)
target for therapy (65). The uPA system promotes the processing of diverse proteins in the ECM, which is necessarily a first step in cancer invasion and metastases (66). Moreover, it activates the precursor form of certain matrix metalloproteinases (MMPs) (67), the formation of which allows further remodeling of the ECM. It also catalyzes the conversion of pro-uPA to active uPA, leading to further plasmin formation, and plasmin in turn activates or releases growth factors such as FGF2 and TGFβ (68).

In colon cancer, the amount of uPAR expression is regulated mainly at the transcriptional level by cis-elements and trans-acting factors that mediate multiple means of induction or suppression by different regulators involving signaling cascades, oncogenes or tumor suppressor genes. For example, studies on colon cancer showed that both the constitutive and phorbol 12-myristate 13-acetate (PMA)-inducible expression of uPAR gene required a footprinted region located at base pairs (bp) -190/-171 of the promoter containing an AP-1 consensus motif bound with jun-D, c-fos and fra-1 oncogenes. This motif also mediates the induction of the uPAR gene expression by factors such as epidermal growth factor (EGF), vascular endothelial factor (VEGF), transforming growth factor beta, type 1 (TGF-β1), interferons alpha and gamma (INF-α and INF-γ), protein kinases A and C (PKA and PKC), the MAPK and the JNK-signaling pathways in diverse cell culture models (69).

In colon cancer, activated k-ras oncogene was shown to regulate uPAR-mediated proteolysis, in part, via region-190/-171 of the promoter through an AP-1 transcription factor-dependent mechanism, whereas inhibition at the transcriptional level suppressed uPAR-mediated proteolysis (70). The binding of the SP1 transcription factor to region-152/-153 of the uPAR promoter was shown to induce both PMA-uPAR promoter activity and u-PAR gene expression by the constitutively active c-src oncogene in colon cancer (63). Furthermore, it has been demonstrated that c-src overexpression confers invasiveness to rat colonic cells (71). In colon cancer HCT116 cells, the binding of the transcription factor NFκB to promoter region -51/-30 led to constitutive expression of the uPAR cell, whereas co-transformation with a dominant negative IκB-kinase-2 expression vector reduced uPAR promoter activity by 75%, thereby implicating this factor in the regulation of uPAR (72). Transcriptional suppression of the u-PAR gene was shown to be mediated by a PEA3/ets-silencing motif element at -248 bp as a mediator of β3A-integrin-induced suppression of uPAR pro-rated activity in CHO cells (73). The tumor suppressor gene pdcdd4 has been hypothesized to down-regulate u-PAR gene expression in part by inhibiting u-PAR gene transcription and a region containing a putative binding site for Sp-1, GATA-2 and NF-1 (-4021/-350 bp). The PEA3/ets motif at -248 bp has also been suggested to mediate this suppression (74). The information presented above on regulation of uPAR suggests that future therapeutic strategies might be directed towards the signal transduction level and transcription mechanisms, in addition to those therapies currently directed at the protein levels (e.g., antibodies) (64, 65).

Matrix metalloproteinases (MMPs). MMPs comprise a large family (> 20) of metal-dependent endopeptidases with proteolytic activities central to the degradation of basement membrane components (e.g., collagen Type IV, laminin and heparin sulfate proteoglycans), which is believed to play an important role in tumor invasion and metastasis and they are important in creating and maintaining an environment supporting the initiation and maintenance of growth of primary and metastatic tumor. This occurs via the activation of matrix metalloproteinase 2 (MMP2). A subset of MMPs, known as membrane-type (MT) MMPs, also contain a transmembrane domain but, unlike other members of the MMP family, MT-MMPs are not excreted, but instead remain attached to cell surfaces. Although not all MT-MMPs are fully characterized, there is good evidence that one of their functions is to localize and activate secreted MMPs, particularly gelatinase A (MMP2) and collagenase 3 (75). MT1-MMP expression has been correlated with tumor invasiveness in a variety of tumor types, including breast, liver, lung, gastric, head and neck and cervical cancers (1). A correlation of increased MT1-MMP mRNA with advanced tumor stage has been demonstrated in colon cancer from patients with adenocarcinoma, using the Northern blotting technique (76). The presence of MMP1 in colorectal cancer was found to be associated with poor prognosis and bad prognostic value independent of Dukes’ stage (77). MT1-MMP was found to be overexpressed in invasive colon cancer, but not in colonic polyps (78). Activation of an inactive precursor of MMP2 (pro-MMP2) by MT1-MMP was proposed as a key step in tumor invasion or metastasis (79).

MMP7 (matrilysin) mRNA, with elastolytic activity, was reported to be specifically expressed in colorectal cancers and adenomas; its message is believed to be located in the tumor cells and is believed to be of prognostic value in CRC (1). MMP7 mRNA was overexpressed in human colorectal carcinomas, its expression in tumor tissues increased with increasing Dukes’ stage (80) and this enzyme can be a reliable marker of occult lymph node metastasis in CRC patients (81). In a study of 100 patients with esophageal carcinoma, those individuals with tumors that demonstrated no matrilysin expression had a better disease-free and overall survival (82).

Human macrophages produce a unique metalloproteinase that possesses the capacity to degrade elastin (1). Studies employing nucleotide arrays showed that HME mRNA transcripts were 5.1-fold more expressed in adenocarcinomas
than in normal colon tissue (83). MMP2 and MMP9 expression in primary tumors was associated with liver metastases in pancreatic and colon carcinoma, and the balance of activity between these two markers and TIMP2 may be relevant to carcinoma invasion and metastases to liver in pancreatic and CRC cancers (84).

MMP7 and mMP1 are activated by β-catenin in tumor cells. MMP7 is an important invasion and metastatic factor, being expressed in 75% of colon carcinomas (85). Matrilysin has been shown to correlate with nodal or distal metastasis in CRC. In an IHC study aimed at clarifying the association of matrilysin expression with CP parameters in early invasive CRCs, 38 early invasive colorectal carcinomas treated by local excision or radical surgery were evaluated. Tumor budding was estimated by the number of differentiation units along the entire invasive margin. Univariate and multivariate analysis showed that matrilysin expression alone was significantly associated with distant metastases, and that both tumor budding and matrilysin expression were significantly associated with adverse outcome, whereas histological differentiation, vessel invasion or depth of invasion were not associated with either outcome, whereas histological differentiation, vessel invasion or depth of invasion were not associated with either distant metastases or adverse outcome. Tumor budding at the invasive margin and matrilysin expression were more useful in identifying high-risk groups for adverse outcome in patients with early invasive CRC (86).

The relationship between matrilysin expression and Dukes’s type was investigated in an IHC study using 83 surgically resected CRCs, including 5 with liver metastases. Furthermore, the effect of matrilysin on the in vivo invasive and metastatic potential of CRC cells transfected with matrilysin cDNA was examined after subcutaneous injection into SCID mice. The results showed the expression of matrilysin to correlate significantly with the presence of nodal or distal metastases, and matrilysin transfectants formed invasive tumors and multiple liver metastases in SCID mice which correlated with the number of metastatic lesions, without producing any significant difference in the subcutaneous tumor growth from mock transfectants, indicating that matrilysin plays a critical role in the metastatic pathway of CRC (87).

Studies analyzing serum-free MMP2, the MMP2/TIMP2 complex and total amounts of MMP9, TIMP1 and TIMP2, were of limited value for tumor staging and prognosis of CRC (88).

These observations link MMPs with aggressive progression and suggest that the tumor-related expression of MMPs may provide important prognostic information that could help in developing therapeutic modalities, including targeting inhibition of MMPs.

**Variants of the cell adhesion molecule CD44.** The CD44 molecule is a transmembrane glycoprotein, having various functions such as a lymphocyte homing receptor on circulating lymphocytes, binding to collagen, fibronectin and hyaluronate to confer cell-matrix contacts and mediation of cell-cell adhesion. CD44 molecules have heterogeneous isoforms, which are attributed both to variable exon usage with alternative splicing and to differential glycosylation within the extracellular domain. Molecular cloning of CD44 disclosed the genomic region coding for exons 6-15, which are components of the extracellular domain of CD44 and can be incorporated alternatively into CD44-mRNA. Studies suggested that there are various isoforms of CD44 generated by this alternative splicing of exons 6-15 (variant exons 1-10) (1).

CD44 splice variants are frequently, but not always, expressed in advanced stages of tumor progression. In colorectal carcinogenesis, expression of exon v5 is an early tumor marker, because it can be detected on small dysplastic polyps but not on normal colon epithelium (89). In an IHC study, the CD44 cleavage products –produced in primary human tumors by membrane-associated MMPs– could be detected in 90% (9 out of 10) colon carcinomas, suggesting that these products play an important role in the pathogenesis of human tumors (90).

Stable transfectants of standard and variant isoforms of CD44 were examined in a human colon carcinoma cell line, that does not express CD44S (W620 cells), by an in vitro cell motility system. Results showed that, contrary to common models, expression of standard CD44 increases cell motility, whereas CD44 isoforms containing an exon sequence associated with metastatic dissemination have a slowing effect, probably due to alteration in their cell adhesion properties, and cell proliferation was also depressed in that variant (91).

Production of variant exon 3 of CD44 (CD44v3), a receptor for heparin sulfate, and expression of heparanase (an enzyme that degrades extracellular glycoprotein to release heparin sulfate molecules), were detected by IHC and in situ hybridization, respectively, in 145 cases of CRC. Coexpression of heparanase and CD44v3 was observed in 12%, 32% and 57% of Dukes’ B, C and D, respectively, and survival analysis found significantly poorer prognosis in patients showing concurrent expression of both markers than in patients not showing both (92).

The loss of expression of the CD44-v6 isoform seems to be associated with a poor prognosis in colorectal cancer because of the development of tumor metastases (1). Normal mucosa shows weak subnuclear localization of CD44-v6 after immunostaining. Overall correlations were not found with either tumor type or stage or patient survival in one study (93). Another study reported a significant correlation between expression of CD44-v6, Dukes’ stage, metastasis and patient survival (94). Expression of CD44H, CD44-v9 and CD44-v6 in normal colon tissue was decreased compared with corresponding primary colorectal tumors, and increasing CD44-v6 expression correlated with
progressive tumor stage and differentiation (95). Using a multivariate analysis, the expression of another CD44 exon, CD44-v8-10, emerged as an independent prognostic indicator for lymph node and hematogenous metastasis and overall survival (96). Moreover, one of the CD44 receptors, hyaluronate, was also correlated with colorectal cancer survival and recurrence. The intensity of hyaluronate immunostaining in tumor epithelium indirectly predicted survival and recurrence-free survival (97).

The expression of CD44-mRNA examined in 90 specimens from 44 patients with colorectal carcinoma or adenoma polyps, and in peripheral blood lymphocytes from 7 healthy volunteers, suggested that various forms of CD44-mRNA might be expressed in an early stage of colorectal carcinogenesis (98). CD44v2-v10 are widely expressed in normal colonic crypt epithelium, predominantly in the crypt base. CD44-v6, the epitope most commonly associated with tumor progression and metastasis, was not only expressed by many benign colonic tumors, but was expressed as frequently in normal basal crypt epithelium as in malignant colonic tumor cells and, surprisingly, was even absent from some metastatic colorectal tumors. Expression of none of the CD44 variant epitopes was found to be positively correlated with tumor progression or with colorectal tumor metastasis to the liver; results which are inconsistent with a role for CD44 variants as indicators of colon cancer progression (99).

Osteopontin (OPN). OPN is a member of a family of secreted proteins called SIBLINGS; all of its genes are clustered on chromosome 4, and are also expressed in the skeleton (100). OPN is a secreted integrin-binding protein that has been expressed in multiple malignant tissues and has been employed as a marker of tumor progression in breast, lung and prostate cancer (101). Experimental evidence suggests that OPN may play multiple roles in promoting tumor progression, including inhibiting macrophage function and enhancing growth of metastases, altering adhesion, increasing cell migration and invasiveness, inducing uPA expression (102) and stimulating proliferation through integrin-mediated intracellular signaling (103).

In serum, OPN is bound to complex factor H. This complex must be disrupted to generate free protein that can be measured. In a study designed to measure the serum level of OPN in several cancers, including colon, serum from 20 patients and 77 normal subjects was collected and samples were analyzed by competitive ELISA. The sensitivity and specificity of the determination were measured by receiver operating characteristic curves. A value of 449 ± 22 ng/ml and sensitivity/specificity was high (104). OPN has been found in transformed cells in culture (105, 106) and was expressed in CRC tumor specimens, as measured by DNA microarrays in individually analyzed colon cancer (83).

A study was carried on CRC patients in an attempt to identify molecular markers that correlate with CP staging for progression, hence of clinical relevance. It utilized Affymetrix oligonucleotide arrays to measure gene expression. Tissue samples were pooled from 5-10 subjects per stage at various stages of tumor progression, in order to reduce the risk of any one sample contributing bias to the pool, making analysis faster, convenient and less costly. A modified algorithm was constructed to analyze the data. The data were confirmed by Northern blot analysis and IHC staining. OPN induction showed progressive increase from normal mucosa to resected liver metastases, where fold increases ranged from 10- to 20-fold over adenomas and normal samples (107). More work is needed to determine the suitability of this marker for molecular staging in CRC.

Conclusion

Since the metastatic potential of a tumor appears to be encoded in the bulk of a primary tumor (108), then the detection procedure—whether IHC method, molecular measurement of DNA mutations, or determination of gene expression for micrometastases—should be tailored to provide information about the metastatic potential of the cells they intend to detect (15). Moreover, it is often difficult to distinguish between a local recurrence, which reflects an inadequate surgical procedure, and distant treatment, which is a result of the biological outcome of the tumor-host interaction. Thus, attention should be directed not only to detection per se, but also to identifying the tumor’s underlying potential for successful spread and growth at a distant site (1, 13).

The clinical usefulness of most markers may vary with ethnicity and the anatomic location of CRC; thus, combinations of molecular markers have been suggested to be equivalent to clinicopathological staging in predicting an outcome (1-5). Moreover, MMs can augment clinicopathological staging to provide a stronger indication of clinical outcome, thus facilitating therapeutic decisions, especially intermediate stages (II and III) of CRC. It is unlikely that one or two markers will be sufficient to develop an approach to molecular staging, but a combination of carefully selected multiple markers may be necessary. The fact that some genes show increased, whereas other show decreased expression, and no single gene can predict progression at all times, necessitates the development of a "predictive gene expression index (PGEI)". This PGEI, which can be derived using mathematical models, is defined as a product of two or more genes divided by the expression of one or more genes. It was shown to be a more reliable indication of tumorigenic expression than a single gene (109). Development of this PGEI for CRC metastasis will facilitate the molecular prognosis of this preventable disease. Table I depicts the tumor markers reviewed herein for their aggressive potential.
References


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