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Cell Surface Molecules and Their Prognostic Values in Assessing Colorectal Carcinomas

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Objective

Carcinomas of the colon and rectum are the third leading cause of cancer-related deaths. Although advances in the surgical treatment of primary colorectal cancers have lead to improvements in patient survival at early tumor stages, treatment of more progressive cancers has not resulted in dramatic improvements in patient survival. However, the selection of patient subgroups based on their prognosis and other characteristics could result in improved outcomes from adjuvant therapies in patients with Dukes B and C carcinomas.

Methods

The authors reviewed the available data on the value of cell surface molecules in assessing the prognosis of colorectal carcinomas, paying specific attention to the evaluation of statistical analysis and multivariate procedures.

Results

Cell surface molecules have been identified on colorectal carcinoma cells whose expression appears to be related to malignant transformation, tumor progression, or patient prognosis. Among these cell surface molecules, various cell adhesion molecules, growth factor receptors, proteinases, and their receptors and inhibitors have been identified as potentially useful prognostic markers.

Conclusions

Although data exist on the prognostic values of certain cell surface markers, the use of multivariate analysis for the identification of valuable prognostic factors remains uncommon. Using reproducible and standardized multivariate analysis procedures, new tumor markers should be carefully examined for their biologic and prognostic relevance before being considered as potentially useful in the management of colorectal cancers.

Carcinomas of the colon and rectum will affect approximately 6% of the population (1 of 17) in the United States during their lifetime. Approximately one third of the estimated 130,000 new patients per year will die within 5 years of cancer-related problems, mostly resulting from metastatic lesions. Thus, colorectal carcinomas are the third leading cause of cancer-related deaths among women and men,[1] and they are the most important malignancies of the gut.

Colorectal carcinomas are one of the best models for the investigation of genetic alterations that lead to malignant transformation and tumor progression. Various chromosomal mutations and deletions are known to be necessary in the adenoma–carcinoma progression sequence that includes different stages of hyperplasia and malignant transformation to an invasive carcinoma.[2] Little is known, however, about the genetic alterations and cellular mechanisms responsible for the final steps in the progression sequence that lead to invasion and metastasis.

Tumor invasiveness and the development of metastases are the most important factors, besides the quality of primary surgery, in determining the prognosis of patients with colorectal carcinomas.[3,4] Patients with advanced local carcinomas or lymph node metastases can benefit from adjuvant therapy,[5] but metastatic involvement of lymph nodes or metastasis into distant organs reduces the median patient survival dramatically.[6,7] Because of the enormous differences between early and advanced stages of local tumors, the search for valuable prognostic markers remains an important subject of clinical research. Despite numerous investigations of these factors, only some of the data can be used for determining an individual patient's risk after surgery for tumor recurrence or formation of distant metastases. For example, elevated preoperative levels of carcinoembryonic antigen (CEA) may be relevant for a patient's prognosis, but there seems to be controversy on the usefulness of CEA determinations in individual patients. More appropriate evaluation can be achieved by following the postoperative kinetics of CEA, which more closely follows

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Table 1. SURFACE MOLECULES WITH POTENTIAL PROGNOSTIC PROPERTIES IN COLORECTAL CARCINOMAS

Group	Name	Expression	Function
Integrins	VLAs LFAs gp	Colonocytes, EC, fibroblasts, leukocytes, platelets	ECM–cell adhesion, leukocyte–EC adhesion, platelet–EC adhesion, gut homing receptor
Immunoglobin gene superfamily	ICAM VCAM PECAM (CD31)	EC, fibroblasts, leukocytes	Antigen recognition, leukocyte adhesion and trafficking
	CEA (CD66)	Colonocytes	Homotypic cell adhesion
	Cadherins	Colonocytes	Homotypic cell adhesion
Lectins	sLe^x sLe^a (CA 19-9) sLe^x Galectin-3	Colonocytes, EC	ECM–cell adhesion, heterotypic EC adhesion
Selectins	E-, P-, L-selectin	EC, leukocytes, platelets	Leukocyte–EC adhesion, platelet–EC adhesion
Growth factor receptors	EGF-R TGF-R IGF-R VEGF-R PD-ECGF c-Met bFGF	All cells	DNA synthesis, growth, motility, protein secretion
Proteoglycan receptors	CD44	Colonocytes, EC	Hyaluronate adhesion
Protease receptors	MT-MMP uPA-R	Stromal cells, EC, colonocytes	Protease activation
Sex hormone receptors	Androgen receptor Progesterone receptor Estrogen receptor Prolactin receptor	Stromal cells, EC, colonocytes	Growth regulation
Apoptosis receptor	APO-1 (CD95)	All cells	Apoptosis regulation
Cytokine receptors	TNF-R IL-2R IL-6R	Colonocytes, EC, fibroblasts	Cell activation

VLA, very late antigens; LFA, leukocyte function-associated antigens; gp, platelet glycoproteins; ICAM, intercellular adhesion molecules; VCAM, vascular cell adhesion molecule; MAdCAM, mucosal addressin cell adhesion molecule; PECAM, platelet endothelial cell adhesion molecule; CEA, carcinoembryonic antigen; EGF-R, epidermal growth factor receptor; TGF-R, transforming growth factor receptor; VEGF-R, vascular endothelial growth factor receptor; IGF-R, insulin-like growth factor receptor; PD-ECGF, platelet-derived endothelial cell growth factor; c-Met, hepatocyte growth factor/scatter factor receptor; bFGF, basic fibroblast growth factor; MT-MMP, membrane-bound matrix metalloproteases; uPA-R, urokinase-type plasminogen activator receptor; TNF-R, tumor necrosis factor receptor; IL, interleukins; ECM, extracellular matrix; EC, endothelial cells.

the presence of residual tumor.[8–10] Other strategies for the evaluation of patient prognosis have been developed during the last decade, such as genetic markers of progression or cellular proteins made by the tumor. This review summarizes data on surface proteins of colorectal carcinoma cells and their prognostic relevance (Table 1).

INVASIVE AND METASTATIC PROPERTIES

The prevention of death in colorectal cancer patients is dependent on understanding the mechanism of carcinoma cell spread from the primary tumor to distant organ sites. Although the pathogenesis of metastasis is the subject of numerous studies in basic and clinical research, the complex mechanisms that make a colorectal tumor cell metastatic are not well established. Colorectal cancer cells with different metastatic properties have been isolated from the same parent tumor, supporting a polyclonal character of these carcinomas.[11,12] Also, metastatic tumor cells show phenotypic instability, which leads to gradual shifts in their behavior and diversification of cells to form heterogeneous subpopulations that differ in their metastatic properties.[13] Colorectal carcinoma cells are known to undergo diversification as a result of quantitative changes in gene expression. As the tumor cells diversify, particular cell clones begin to dominate the cell population because of growth advantages and host selection. This leads to waves of clonal cell proliferation.[14]

Clonal selection and diversification leading to tumor heterogeneity are major problems for the establishment of prognostic parameters for colorectal tumors. All the com-

mon techniques currently available for the characterization of carcinomas and the evaluation of their potential metastatic behavior are based on statistical analyses and the use of average values. In contrast, the development of metastases is a nonrandom process, in which the survival and distant growth of a single cell or a small number of cells can lead to death.[15] Therefore, the search continues for key events, genes, and molecules that are characteristic for the metastatic behaviors of tumor cells and that could be useful for predicting patient outcome.

The sequential model for the development of metastases involves tumor growth, neovascularization and invasion at the primary sites, followed by penetration into lymphatics and blood vessels or through the peritoneum. At the end of this process, circulating tumor cells must adhere to vessel walls of distant host organs, invade surrounding tissues, and survive and grow.[16] During these steps, interactions between tumor cells and the extracellular matrix (ECM) or surrounding cells are required.[17] Various molecules on the tumor cell surface mediate these interactions, either by direct contact, such as integrins or other adhesion molecules, or as receptors for soluble peptides or hormones, such as receptors for various growth factors. Usually the same molecules are also found on normal colonic epithelium, where they function in the maintenance of tissue structure and normal cellular regeneration.

Cell adhesion events are thought to play an important role in tumor metastasis. The detachment of tumor cells from the primary site, the homotypic interactions between tumor cells and with host cells during transport in the circulation, and the cellular interactions with the endothelium and ECM in distant organs are important for the formation of secondary tumors. The multiple surface molecules that mediate these adhesive interactions include lectins, glycosyl transferases, integrin and nonintegrin adhesion molecules, and glycolipids. The detachment of tumor cells from the primary carcinoma is characterized by the loss of cell–cell adhesion, and the cadherin–catenin systems appear to play an essential role in this process. In poorly differentiated carcinomas, loss of epithelial cell contacts is frequently observed, allowing the cells to break away from the primary tumor, invade surrounding tissue, and be released into the lymphatics and blood circulation. Adhesion of circulating tumor cells to organ endothelial cells and subendothelial ECM is mediated by several adhesion systems that may involve the same endothelial cell surface receptors used by leukocytes. For example, contacts between carcinoma cells and the microvascular endothelium seem to be related to expression of selectins, sialyl-Lewis^x (sLe^x) and other carbohydrates, intercellular adhesion molecules (ICAM), and possibly annexins.[18] However, integrin-mediated interactions with the subendothelial ECM are among the most important determinants for organ-specific metastasis.

Penetration from the primary site into the circulation and extravasation into the host organ requires the release or activation of degradative enzymes, some classes of which appear to be metalloproteases, cathepsins, plasminogen activators, and endoglycosidases such as heparanase. Once malignant cells have arrived at and invaded secondary organ sites, they must stimulate local blood vessel growth. Neovascularization is induced and regulated by various angiogenetic molecules, which bind to specific cell surface receptors (e.g., vascular endothelial growth factor receptor, platelet-derived growth factor receptor [PDGF-R]). Finally, the neovascularized tumor must grow, and paracrine and autocrine growth factors appear to be essential in this process. Autocrine or paracrine growth factor receptors (e.g., epidermal growth factor receptor, fibroblast growth factor receptor, insulin-like growth factor) important in this process interact with intracellular proteins involved in signal transduction and can eventually modify cellular behavior.

CADHERIN–CATENIN SYSTEM

Cadherins belong structurally to the immunoglobin gene superfamily, a family of transmembrane glycoproteins responsible for calcium-dependent intercellular adhesion. They are divided into >10 subclasses, which are distinct in their immunologic characteristics and tissue distributions. Cadherins are involved in subclass-specific cell–cell interactions that play a role in selective cell adhesion at various developmental tissue stages.[19] Cadherins mediate homotypic adhesion in developing tissues, and they are connected with catenins at their cytosolic domains.[20] Inactivation of cadherins causes the disruption of cell–cell adhesion, and overexpression of cadherins leads to tighter cell–cell contacts. Continued expression and functional activity of E-cadherin are required for epithelial cells to remain integrated within the epithelium.[21] Catenins belong to the group of cytoplasmic plaque proteins that connect cell surface molecules with the actin cytoskeleton. α-Catenin is required for cadherin-mediated adhesion, and it indirectly links cadherins to actin by means of a specific binding site. β-Catenin is a necessary intermediate in the linkage of α-catenin to the cytoskeleton.[21]

Like other carcinomas, such as those of the breast, bladder, and lung, the expression of E-cadherin and α-catenin is downregulated in poorly differentiated colon cancer cells.[22,23] Using an immunohistochemical technique, Cowley and Smith[24] compared E-cadherin expression and tumor morphology in carcinomas that had invaded vascular spaces with extravascular carcinomas, both in primary tumors and in their lymph node metastases. In 40% of cases at the primary site, in lymph node metastases, or at both sites, E-cadherin levels were higher in the often tiny intravascular tumor compartment than in the adjacent but much larger extravascular tumor compartment. E-cadherin was poorly expressed within the extravascular compartment. In analyzing the expression of E-cadherin in colorectal carcinomas, it has been shown that this receptor may serve as an independent prognostic marker in Dukes stage B colon cancers to identify patients with a poor prognosis and designate them

for adjuvant therapy after curative surgical treatment.[25] Mohri[26] wrote that the loss or heterogeneous expression of E-cadherin in colorectal cancers correlated closely with advanced clinical stage, advanced tumor penetration, undifferentiated tumor histology, widespread lymph node involvement, liver metastasis, and penetration into the lymphatic and venous channels. The loss or heterogeneous expression of E-cadherin in tumor tissues was also significantly associated with an increased incidence of tumor recurrence after apparently curative resection, reduced overall survival rates, and reduced disease-free survival rates. A multivariate analysis disclosed that the expression of E-cadherin in tumor tissue was a significant prognostic variable independent of other clinicopathologic features. However, in another study, this marker did not predict outcome in the important group of moderately differentiated Dukes B colon cancers.[27] Using in situ hybridization, low levels of expression of E-cadherin were found to be independent prognostic factors on multivariate analysis and were significantly associated with metastasis or recurrent disease in N_0 colon carcinomas.[28] This has been confirmed by another group.[29] Collectively, there appears to be a statistically significant correlation between reduced expression of E-cadherin and loss of tumor differentiation. Thus, the frequency of reduced E-cadherin expression is generally greater in tumors with aggressive histopathologic characteristics, lymph node involvement, and distant metastases.

E-cadherin expression is considered to play a major role in the homotypic adhesion of cancer cells. Therefore, suppression of E-cadherin expression or its function might enhance the release of cancer cells from the primary lesion. Functionally, E-cadherin is thought to be regulated by its associated cytoplasmic proteins, including α-catenin. Normal epithelium expresses α-catenin strongly without exception. However, α- and β-catenin expression was frequently reduced (approximately 80%) in primary colorectal carcinomas.[30,31] Normal mucosa, as well as colon adenomas, showed strong membranous α-catenin expression. In the normal colon, catenin expression was observed in the crypt and surface epithelium; the cells showed reactivity both at the membrane and in the cytosol.[32] Significant downregulation of α-catenin expression in colorectal cancer has been associated with poor differentiation, higher metastatic potential, and unfavorable prognosis.[33,34] It has also been found that β-catenin forms complexes with adenomatous polyposis coli (APC) tumor suppressor protein, and β-catenin expression levels are 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.[35,36] In coexpression experiments, approximately 80% of the colon carcinomas showed similar expression of E-cadherin and β-catenin, whereas the other tumors showed strong positive staining for E-cadherin and reduced expression of β-catenin.[37] These findings support the hypothesis that β-catenin forms a complex with E-cadherin in vivo. The downregulation of β-catenin expression is associated with malignant transformation, and colorectal cancer cells may have impaired E-cadherin–mediated cell adhesiveness because of the downregulation of catenin expression.[38]

INTEGRIN SYSTEM

Integrins are heterodimeric transmembrane adhesion molecules that mediate interactions between cells and the ECM. More than 20 different integrin heterodimers are known, but β_1- and β_3-integrins appear to be the most important integrins expressed on tumor cells.[39] Each integrin subfamily is determined by the β-subunit. For the specificity of interactions with heterogeneous ligands, both subunits are necessary, but integrins are either monospecific or can bind to several different ECM components. Integrins are expressed on endothelial cells, epithelial cells, platelets, and leukocytes, among others, and tumor cells.[40,41] Most tissues express only a restricted number of integrins. These include those thought to function as collagen/laminin receptors. Changes in integrin receptors for fibronectin or vitronectin might have importance for the metastatic phenotypes of colorectal carcinomas.

Integrins mediate adhesion through various ECM binding sites, depending on the matrix components involved. Once cell adhesion occurs, integrin receptors generate regulatory signals in cells that allow them control over cell migration and invasion into host organs. These cell–ECM interactions are mediated through various transmembrane receptors, including integrins, which are intracellularly linked to cytoskeleton components[42] and signal transduction molecules.[43,44] Recent studies have shown that cross-talk between integrins and other cell surface receptors is involved in these signal transduction processes.[45] An example of this is the apparent cross-talk between integrin receptors and growth factor receptors. Thus, the presence of transforming growth factor-β can modulate integrin-mediated adhesive properties and differentiation states of colon carcinomas.[46]

Previous studies have demonstrated a correlation between the organ preference of metastasis and the in vitro adhesion rates of malignant cells to various endothelial cells[47,48] or subendothelial ECM.[49] Using colon carcinoma cell lines with different metastatic properties, we have shown that metastatic behavior correlates with different integrin-mediated adhesive properties.[50,51] However, differences in integrin expression could not explain these differences. Therefore, integrin-mediated specific tumor cell adhesion includes complex intracellular interactions with signaling cascades and cytoskeleton components.[52,53] In addition, distinct organ site-specific basement membrane composition has been found.[17] Tumors, such as colon carcinomas, produce their own ECM components that can change peritumoral stroma composition.[54,55] This includes the release of unusual ECM components, such as oncofetal fibronectin,

which is correlated with a poor prognosis in colorectal carcinomas.[56]

A broad spectrum of integrin expression in certain patterns was found in normal tissues, primary tumors and metastases.[46] In normal colonocytes, α_2-integrin staining was strongest in crypt cells, whereas α_1, α_3, and α_v, and β_1, β_3, and β_4 predominated in superficial enterocytes. In adenomas, monolayered glands showed integrin patterns that differed slightly from both crypt and superficial enterocytes. Complex glands in villous adenomas showed decreased integrin staining and basal polarization.[57] β_1-Integrins were usually found to be expressed in adenomas, but the $\alpha_2\beta_1$-integrin appeared to be lost in focal areas of cell contacts.[58] Colon carcinomas tend to have weaker integrin staining than adenomas or normal cells[59]; however, they also show considerable heterogeneity of $\alpha_2\beta_1$-integrin expression.[58] Also, α_5 was frequently found to be expressed in invasive colon carcinomas, whereas the expression of this integrin subunit is usually poor or absent in normal epithelium.[60,61] In another study, however, α_3 showed variable expression, with a diffuse distribution at the cell surface in peripheral areas of colorectal carcinomas, correlating with the histologic stage of malignancy, whereas the expression of α_5-subunits was almost absent.[62] Various combinations of α- and β-subunits are found only in transformed cells. The α_6-integrin subunit is normally paired with β_1-subunits, but in colon carcinoma cells coexpression of α_6- and β_4-subunits was frequently found.[63] There is also evidence that the α_v-subunit is involved in the regulation of apoptosis, or programmed cell death.[64] Also, colon carcinomas might induce or modify integrin expression on tumor-associated endothelial cells. The $\alpha_v\beta_3$-integrin was found to be overexpressed on tumor vasculature.[65] In contrast to the variability of integrin expression on colon carcinoma cells, analysis of adhesion molecules of the integrin family on lymphocytes immigrating into tumor tissue indicated no specific expression of individual β-integrins.[66]

Correlations have been reported between integrin expression and tumor prognosis and clinical stage. The transformation from benign to malignant neoplasia was found to be associated with infiltrative growth and characterized by diminished or lost expression of α_6-, β_1-, and β_4-integrin subunits.[67] When compared with their primary tumors, colorectal carcinoma liver metastases showed roughly similar patterns of integrin expression. In various studies, the expression of integrins in normal tissue was determined and compared with different stages of colorectal carcinomas.[62,68] Generally, a high variability of integrin expression seemed to be related to the degree of differentiation of the original tumor.[69] However, reduced α_2-integrin expression was statistically associated with advanced cancer stages. A strong correlation was also observed between the expression of the α_6-laminin receptor and the degree of colorectal carcinoma differentiation, invasive properties, and metastatic abilities.[25]

SELECTIN SYSTEM

Selectins are adhesion molecules that use carbohydrates as receptor ligands. They are important in the interactions of cells with leukocytes/lymphocytes (L-selectin), platelets (P-selectin), and endothelial cells (E-selectin). The action or increased expression of selectins depends on cell activation of endothelial cells or leukocytes/lymphocytes by interleukins, tumor necrosis factor (TNF), or toxins and of platelets by thrombin, histamine, O_2 radicals, and other procoagulatory substances. All selectins have a similar structure with a N-terminal lectin domain, epidermal growth factor-like domains, different numbers of complement binding domains, a transmembrane domain, and a short intracellular portion. Selectins mediate the recognition of carbohydrates, such as sLe^a, sLe^x, and the MECA-70 antigen. Although the members of the selectin family are structurally related, they have disparate functions. Selectins play a central role in targeting of circulating tumor cells to the endothelial cells of the host organ. In this manner, they help determine the organ preference of metastasis.

Various selectins are expressed in different tissues and on different cell types. E-selectin is expressed on endothelium but not on colonocytes.[70] Approximately 30% of the intraepithelial lymphocytes in the colonic epithelium express L-selectin.[71] E-selectin was shown to be present on the endothelial cells of small vessels adjacent to cancer cell nests both in primary and in metastatic lesions. In these tissues, E-selectin was observed on the endothelial cells lining the lumen of small vessels. The degree of expression of E-selectin was inversely correlated with the distance of the blood vessels from the cancer cell nests: endothelial cells adjacent to the metastatic lesion expressed E-selectin more extensively than those adjacent to the primary tumor foci.[72] Serum E-selectin levels were also significantly elevated in the patients with metastasis versus those without.[73] There were also weak but significant correlations between serum E-selectin levels and CEA or CA 19-9 levels.[73] However, in one study E-selectin immunostaining did not correlate with cell infiltration.[74] Studies on the prognostic value of selectins for estimating patient survival have not been published, so it is difficult to assess the potential value of using selectin expression for patient prognosis.

LECTINS AND GLYCOCONJUGATES

Normal colonic epithelial cells undergo maturation as they traverse the crypt to the luminal surface, and changes occur during this process in the expression of specific cell surface oligosaccharides. The binding of lectins to goblet cell mucins and other glycoconjugates changes as the cells migrate from the crypt and undergo differentiation. These sialylated carbohydrate structures on mucins play a role in colorectal cell adhesive interactions involving both basement membrane ECM and endothelial cell-associated ligands.

The affinities and antigenic structures of the sialylated carbohydrates are regulated by the activities of glycosyltransferases and other membrane-bound enzymes, some of which are upregulated in colon carcinomas.[75] Additional stepwise modifications in glycoconjugate expression occur in premalignant and malignant neoplasms.[76] Various glycoconjugates differ in their affinities for different cellular components, local distribution within crypts, and regional distribution between right (ascending colon) and left (rectum) segments of the large bowel.[77] CA 19-9 and sLe^x are tumor-associated antigens that have been found expressed in the whole colorectum, whereas other sialylated carbohydrates, such as sLe^b and sLe^y, were found only in the distal colon.[78]

Colorectal carcinomas with increased metastatic potential and with a poor prognosis are characterized by a high content of certain carbohydrate antigens. The levels of these carbohydrate antigens apparently increase during colorectal carcinoma progression from nonmetastatic to metastatic tumors.[79] For example, the levels of tumor-associated sLe^x antigens were inversely correlated with the postsurgical survival of patients with colon carcinoma, as revealed by retrospective studies.[80] Disease-free survival rates of patients with sLe^x-positive tumors were significantly poorer than those with sLe^x-negative tumors. A multivariate analysis revealed that the sLe^x status was an independent predictive factor for colorectal disease recurrence, depth of invasion, and histologic type, whereas sLe^a status, age, gender, tumor location, nodal status, and vessel invasion were not.[81] Increased sialylation of mucin-associated carbohydrates, such as sLe^x, is generally characteristic of colon cancer cells that are likely to metastasize. Metastases have been found to express decreases in mucin core structures, reciprocal increases in sialylated mucins, and increases in peripheral sLe^x compared with the primary tumors from which they arose.[82] The levels of this carbohydrate antigen apparently increase during colorectal carcinoma progression from nonmetastatic to metastatic tumors, and they inversely correlate with postoperative survival.[83]

Previously, certain antigens related to blood serum antigens were correlated with tumor progression and prognosis. For example, the CA 19-9 antigen was studied for years before it was identified as a monosialosyl Le^a blood group antigen. Levels in adenoma and carcinoma specimens were significantly higher than in the normal mucosa.[84,85] Higher tumor stages correlated with higher tissue marker values of CA 19-9.[86] Other lectins, such as lactose-binding lectins or galectin-3, also showed significant correlations to the Dukes stages and appear to be related to neoplastic transformation and metastatic progression.[87,88] These results were related to other known prognostic factors such as CEA.[89]

IMMUNOGLOBIN SUPERGENE FAMILY

Cell adhesion molecules with an immunoglobin-like (Ig-like) structure in their extracellular portions are thought to have wide-ranging functions and to participate in a variety of homophylic and heterophylic interactions.[90] Members of this family, such as ICAM-1, ICAM-2, and vascular cell adhesion molecule (VCAM-1), are known to participate in heterotypic cell–cell adhesion. Receptors for certain growth factors (e.g., PDGF, colony-stimulating factor-1), T-cell receptors (CD4, CD8), tumor cell antigens (CEA), and a group of molecules that mediate cell–cell interactions between platelets and endothelial cells (CD31, ICAM-1, VCAM-1) belong to various Ig-like subgroups. This latter group takes part in cell–cell interactions by binding to other adhesion molecules, such as integrins or selectins, that are important in tumor cell interactions and metastasis.

Immunohistochemical localization and in situ hybridization have revealed a lack or low expression of ICAM-1 on normal colonic epithelium.[70] In colonic tissues, ICAM-1 immunostaining was restricted to the ECM and vascular endothelium. The vast majority of normal tissue samples revealed only faint ICAM-1 immunoreactivity; however, moderate to strong immunostaining was found in $>80\%$ of cancerous tissues. ICAM-1 was more intensely expressed in well-differentiated carcinomas as well as in the adenomatous parts and transition zones of cancers. In normal tissues, VCAM was seen only in isolated lymphoid aggregates.[70] Similar to ICAM-1, colon cancers exhibited markedly enhanced VCAM-1 immunostaining in the endothelial cells of small blood vessels. The intense vascular immunostaining of ICAM-1 and VCAM-1 was associated with the presence of CD3-positive T lymphocytes.[74]

CEA is a highly glycosylated cell surface protein and a member of the Ig-like superfamily. It is produced in large amounts in essentially all colon and several other adenocarcinomas; therefore, it has been widely used as a clinical tumor marker. Endothelial cells express CEA on their cell surfaces. Therefore, CEA-expressing adenocarcinomas may adhere to endothelial cells, in part by CEA–CEA interactions. Thus, CEA interactions may facilitate tumor cell extravasation and hematogenous metastasis formation.[91] CEA is expressed intracellularly as well as extracellularly. The intracellular expression of CEA appears to be associated with the degree of atypia in histologic sections.[92] The concentrations of CEA in tumor specimens showed a high degree of correlation with the risk of relapse.[93,94] Conversely, there was no correlation between tissue CEA content and tumor differentiation.[95] Immunohistochemical expression confirmed the predictive value of CEA contents in colorectal tumor specimens.[96] Serum CEA levels and the CEA tissue contents determined by immunohistochemical staining correlated with patient survival, and they appear to have similar prognostic values.[95]

Ig-like receptors have also been useful in assessing angiogenesis, a crucial step in tumor growth and progression. Its quantitation by microvessel counting has prognostic value in several types of malignancies. The expression of endothelial cell-specific CD31 has been used to evaluate the onset of angiogenesis in colorectal tumors, and microvessel

quantitation has been used to assess its prognostic significance. The density of microvessels in the tumor can be determined using endothelial cell-specific antigens and is a reliable marker for the angiogenesis that is an early, critical step in colorectal tumorigenesis. High expression of CD31 was not associated with metastasis formation, disease stage, or patient survival[97]; however, using von Willebrand factor for endothelium-specific immunostaining, high microvascular counts were a prognostic predictor for a longer survival time independent of Dukes stage.[98] The presence of p53 protein overexpression was also found to be associated with a high microvascular density.[99]

CD44, ITS ISOFORMS AND RECEPTORS

CD44 is a cell surface adhesion molecule family with different splice variants that is expressed on endothelial cells and various tumor cells.[100] Multiple functions have been attributed to the CD44 family of molecules. CD44 plays a role in the production and catabolism of hyaluronate, which is primarily located in the liver and lymph nodes. It mediates cell–cell contacts with glycosaminoglycans, such as hyaluronate, on fibroblasts, on endothelial and hematopoietic cells, and in the ECM. Hyaluronate and CD44 have been proposed to be important in tumor invasiveness, cell migration, and angiogenesis.[100] CD44 splice variants are frequently but not always expressed in advanced states 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.[101] 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.[102] For example, CD44-v6 immunoreactivity was detected in 100% of adenomas and in >90% of colorectal carcinomas, but expression was mostly weak in only approximately one third of liver metastases. Normal mucosa shows weak subnuclear localization of CD44-v6 after immunostaining. Overall correlations were not found with tumor type, stage, or patient survival by Coppola et al.[103] Another study reported a significant correlation between expression of CD44-v6, Dukes stage, metastasis, and patient survival.[104] Expression of CD44H, CD44-v9, and CD44-v6 was decreased compared with corresponding primary colorectal tumors,[105] and this group also showed that increasing CD44-v6 expression correlated with progressive tumor stage and differentiation.[106] These correlations were confirmed at the mRNA level using reverse transcriptase–polymerase chain reaction.[107] Using a multivariate analysis, the expression of another CD44 exon, CD44v8-10, has emerged as an independent prognostic indicator for lymph node and hematogenous metastasis and overall survival.[108] In addition to CD44, one of its commonly found receptors, hyaluronate, has also been correlated with colorectal cancer survival and recurrence. The intensity of hyaluronate immunostaining in tumor epithelium independently predicted survival and recurrence-free survival.[109]

GROWTH FACTOR RECEPTORS

Growth factor receptors mediate a wide diversity of signals from the cell surface into the cell. Specific receptor occupation with growth factors can induce or inhibit cell growth, motility, and protein expression or secretion. Their autocrine or paracrine activity seems to be coregulated by other cell signaling systems, such as integrins (discussed above). Growth factor receptors are found on all cells, but their pattern of expression is highly heterogeneous and dynamic. Growth factor receptors are transmembrane molecules that often have enzymatic activity, mostly kinase activities, at their cytosolic domains. Their signaling pathways to the nucleus are often associated with oncogene products, such as the central protooncogene Ras, which is found in all eukaryotic cells.

The most important growth factors for determination of the growth properties of epithelial malignancies are epidermal growth factor (EGF) and transforming growth factor (TGF). No consensus about the involvement of the EGF receptor (EGF-R) in colorectal carcinomas has been attained, although it is assumed to play a role in the invasion and metastasis to lymph nodes and in recurrence at regional and distant sites. EGF-R has been detected both in adenomas and carcinomas.[110] Significantly increased levels of EGF and EGF-R were found in some neoplastic samples compared with surrounding mucosa,[111] but increased expression of EGF-R seems to be uncommon in colonic adenocarcinomas.[112] Some studies found significant correlations between EGF-R protein expression (or its mRNA) with Dukes classification, differentiation, and survival, whereas others could not confirm these results.[113–116] The expression of other growth factor receptors, such as TGF-R and amphiregulin (AR), have been correlated with Dukes stage and differentiation.[113] Often EGF-R and TGFs and their receptors are coexpressed in colorectal tissues. EGF-R expression and its mRNA levels appear to be related to TGFα staining in normal and adenomatous tissue.[117] TGFβ type I and II receptors were found to be overexpressed in tumors compared with normal samples, and there appeared to be a relation between the abundance of type II receptors and the degree of differentiation of the colorectal tumors, but not the Dukes staging or the locations of the carcinomas.[118]

HER-2/neu oncogene encodes a transmembrane tyrosine kinase receptor with homology to EGF-R and is often amplified or overexpressed in adenocarcinomas. Normal mucosa does not usually express HER-2/neu protein, but a significant number of benign lesions and adenocarcinomas were found to overexpress this protein. Carcinomas were significantly more positive than benign lesions. A significant correlation was found with differentiation, Dukes classification, and relapse-free and postoperative survival.[119]

AR, a protein structurally related to EGF and TGFα, is also functionally related to this family of growth regulatory molecules and can bind and activate EGF-R. Immunostaining and in situ hybridization detected AR protein and its mRNA in primary and metastatic colorectal tumors in liver but not in normal colon or uninvolved liver.[120]

Vascular endothelial growth factor (VEGF) is a well-known tumor and normal cell growth factor that induces angiogenesis. Expression of VEGF was found to be significantly reduced in metastatic colorectal liver tumors compared with primary lesions. However, the levels of VEGF in primary colorectal tumors did not predict risk of liver metastasis or survival duration in one study.[121] In another study, tumors with high VEGF expression and detection of the high-affinity VEGF receptor (KDR) on tumor endothelium were associated with metastasis formation.[122] Further studies indicated that patients with high VEGF expression in their primary colorectal cancers had a high likelihood of recurrence.[123] Various isoforms of VEGF have been identified in colorectal cancers. The detection of mRNA isoforms correlated with metastasis and a poor prognosis.[124]

The insulin-like growth factor (IGF) is known to induce or modify growth properties in various tissues. Although its receptor is expressed on colorectal carcinoma cells, none of the clinicopathologic parameters showed any association with IGF-1R status. Differences were not observed in the overall survival period between patients with IGF-1R–positive tumors and those with IGF-1R–negative tumors.[125] Other growth factor receptors, such as those for platelet-derived endothelial cell growth factor, c-Met (receptor for hepatocyte growth factor/scatter factor), or fibroblast growth factor, were investigated in animal models, where they demonstrated various correlations with colorectal tumorigenesis, invasion, or tumor vessel count.[126] However, data on human colon cancer specimens did not show significant correlations between the expression of these receptors and patient prognosis.[127]

PROTEASE ACTIVATORS AND PROTEASES

Although proteases are mostly nonintegral membrane or secreted molecules, they can be found in membrane receptor-bound forms or closely related to specific receptors that are involved in the release and activation of degradative enzymes. Therefore, proteases and their receptors should be considered as cell surface or surface-related molecules. Various kinds of degradative enzymes are involved in the dissolution of tumor-surrounding ECM as a prerequisite for tumor invasion at the primary site. Degradation of the ECM is also required for extravasation of tumor cells into the distant host organs. Various classes of degradative enzymes can be released by malignant cells and surrounding stromal cells, including the plasminogen activators, cathepsins, metalloproteinases, and endoglycosidases. Cells can also produce inhibitors of degradative enzymes. Therefore, maintenance or disturbance of the degradative enzyme/inhibitor balance plays an essential role in invasion and metastasis formation.

Receptor-bound urokinase-type plasminogen activator (uPA) and its receptor (uPA-R) and its inhibitor (PAI-1) seem to play an important role in the dissolution of the surrounding ECM and the formation of tumor stroma. Secreted uPA binds with high affinity to its specific receptor uPA-R on the cell surface. These processes appear to be prerequisites for invasion and metastasis. The binding of uPA to uPA-R has at least two important consequences: it enhances the rate of plasminogen activation on the cell surface, and it focuses the uPA proteolytic activity at the leading front of migrating cells.[128] Several recent findings suggest that surface-bound uPA is essential for the invasive ability of tumor cells, although the emerging data suggest concerted action of uPA and uPA-R with other secreted and cell-bound proteases, such as metalloproteinases and cathepsin B. Increased uPA, uPA-R, or PAI-1/2 correlated with tumor progression and shortened disease-free or overall survival.[129] Interestingly, the numbers of uPA-R–positive cells along the invasive margins of tumors were significantly less in patients with liver metastases than in patients without liver metastasis, and the uPA-R–positive cells were also less in cases with an infiltrating margin than in cases with an expanding margin.[130] This suggests that simple relations between one degradative enzyme and its membrane receptor, such as uPA–uPA-R, with tumor progression may be an oversimplification. Disturbances in plasmin formation take place in distinct stromal compartments, but not on epithelial cells. These imbalances appear to be maximized in invasive neoplasias.[131] Thus, it may be that changes in uPA–uPA-R may be more important than the absolute levels of these markers. Low tissue plasminogen activator (tPA), high levels of uPA-related antigen, and a high uPA:tPA antigen ratio as well as PAI-2 antigen were associated with poor overall survival.[132,133]

Expression of cathepsin D has been suggested to affect the invasiveness of carcinoma cells. Secretion of cathepsins appears to be mostly by stromal cells, such as fibroblasts. In colorectal carcinomas, cathepsin D was also found to be expressed by malignant cells. Because colorectal carcinomas showed a high variance of immunostaining for cathepsin D, the prognostic value of its expression remains uncertain. Although overexpression of cathepsin D was found in some studies,[134–136] its independent prognostic value was described for patient survival and Dukes stage in only one report.[135] Colorectal carcinomas express higher levels of cathepsin L than normal colonic tissues.[137] However, studies on the prognostic value of cathepsin L are not available.

Matrix metalloproteases (MMP) are a family of metal-dependent endopeptidases with proteolytic activities for various components of the ECM. Their activity is regulated by specific tissue inhibitors of metalloproteinases (TIMP) and by activation through membrane-bound MMP (MT-MMP).[138] Depending on their substrate specificity, MMPs

are grouped into collagenases (or gelatinases), stromelysins, matrilysin, and MT-MMP. The most important collagenases are MMP-2 and -9, which can hydrolyze ECM collagens. Using quantitative zymography for detection of proteolytic activity, higher amounts of MMP-2 and -9 were found in carcinomas, correlating with Dukes stage but not differentiation or survival.[138–140] Further, in specimens from metastases originating from primary tumors of the colon, significantly enhanced type IV collagen degrading enzyme activity was observed relative to the primary tumor.[141] Immunologic staining has also been seen in tumor-infiltrating neutrophils and macrophages located adjacent to invasive tumor glands where cancer cells were not stained. In normal colon tissue, staining for MMPs was seen only in scattered neutrophils in vessels and in macrophages in Peyer's patches.[142] The degree of tissue expression of MMP-9 by host cells in colorectal cancers appeared to be inversely associated with liver metastasis and an infiltrating growth pattern.[130] Both increased levels of proenzyme and active enzyme forms of gelatinase A (MMP-2) and increased cathepsin B activity were localized in regions of tumor invasion compared with the levels found in a matched number of normal epithelial cells. In this study, the levels of progelatinase B (MMP-9) were also increased in the tumors.[143]

Other MMPs have been found to be expressed by colorectal cancer cells or stromal cells. The presence of MMP-1 (fibroblast-type collagenase) in colorectal cancer was found to be associated with a poor prognosis and had prognostic value independent of Dukes stage.[144] MMP-11 (stromelysin-3) expression was characteristic for tumors of epithelial origin and was overexpressed in colon carcinomas, including in situ lesions.[145] These results were confirmed by detection of mRNA for all stromelysins, which were expressed in the majority of colon carcinomas examined.[146] The overexpression of MMP-11 was localized in stromal fibroblasts and correlated with tumor invasion and progression.[147] Matrilysin (MMP-7) mRNA was also detected in cancerous tissue but not in adjacent normal colon tissue.[148] This upregulation of secretion and the activation of matrilysin apparently occur during malignant conversion of colonic epithelium.[149] In an interesting study, the expression of this MMP-7 mRNA was used to detect occult lymph node metastases with a high sensitivity.[150]

Inhibitors of MMPs have also been examined for their expression in colorectal carcinomas and normal epithelium. For example, TIMP-1 and TIMP-2 were immunolocalized in scattered stromal cells, whereas epithelial cells of normal mucosa and hyperplastic polyps were weakly stained. Immunolocalization of TIMPs demonstrated gradual increases from tubular adenomas to villous adenomas, and in situ carcinomas showed a definite positive immunolocalization.[151] The distribution of TIMP-1 mRNA and protein showed similar increases in expression during malignant transformation.[152]

The expression of several proteinases and their inhibitors in a given tumor may provide information independent of clinical stage and may identify crucial variations in tumor behavior. On occasion these data have been combined, and it was shown in one study that cathepsins and MMPs could be combined into proteinase profiles.[153] The combination of MMP-9 and cathepsin B and L showed significant correlation with tumor stage. Moreover, a combined role for MMP-9, uPA, and uPA-R expression has been assumed in colon cancer tissue as important cancer progression/promoting factors, but they might also be related to host defense mechanisms when they are expressed by infiltrating host cells.

OTHER SURFACE MARKERS

Various other cell surface markers have been examined for their usefulness as prognostic factors. Histocompatibility antigen-A, -B, -C, and -DR expression in colorectal carcinoma seems to be irrelevant in vivo, and they are not related to the survival and growth of residual tumor cells after putatively curative colorectal tumor resection.[154,155] The expression of the motility-related protein-1 or CD9 showed a significant correlation with higher frequency of venous vessel invasion and liver metastasis.[156]

Although overexpression has been described for sex hormone receptors, such as androgen, progesterone, prolactin, and estrogen receptors, correlations with histologic findings, clinical stage, or prognosis have not been established.[157–159]

Some cell surface receptors regulate cell survival, and these are of interest in malignant tumors with high growth fractions or low death rates. APO-1 is a cell membrane protein identical to the Fas antigen (now designated CD95). It is a member of the NGF/TNF receptor superfamily, which is strongly involved in the regulation of apoptosis. Using immunohistochemistry, APO-1 was found to be expressed routinely at the basolateral membrane surface of normal colon epithelia. In a minor fraction of colon adenomas and in approximately 40% of the carcinomas, APO-1 expression was diminished. APO-1 expression was completely abrogated in approximately 50% of carcinomas, predominantly in the nonmucinous type, and the level of APO-1 expression in carcinomas was correlated with the mucinous type.[160]

Various cytokines and interleukins (IL) can take part in the activation and regulation of cellular functions, such as endothelial cell activation and leukocyte trafficking. Therefore, the expression of cytokines and their receptors is thought to be an important determinant for tumor cell behavior. When colorectal tumor cells were examined for IL-6, a large subset of colon cancer specimens were strongly immunostained. The expression of IL-6 was less conspicuous and less frequent in the epithelial cells of normal colonic mucosa compared with colon carcinomas. IL-6 receptor mRNA was also detected at twice the levels in colonic carcinomas than in normal colon tissues.[161] One study investigated soluble IL receptors in the peripheral blood from patients with colon carcinoma and found significantly

higher levels of sIL-2-R that correlated with clinical stage.[162] However, the relation of IL levels in the blood might be related to immune response in these patients.[163] Data on the prognostic value of cytokines and their receptors are not available.

CONCLUSIONS

The establishment of useful prognostic markers for colorectal cancer appears to have utility in determining prognosis as well as adjuvant therapies that might be clinically applied. Thus, selecting patients based on their prognosis may lead to more appropriate subgrouping of candidates for particular adjuvant therapies. Numerous cell surface or surface-related molecules have been identified that are functionally involved in neoplastic transformation, tumor progression, and the development of metastases. Therefore, it is not surprising that many data exist on the prognostic values of these markers. However, few studies have used multivariate analyses to identify valuable prognostic factors. Future examinations of potentially useful markers will have to take this into consideration. Also, the more immediate search for prognostic indicators and metastatic site preferences in tumors expressing particular molecular profiles has been partly hampered by the small numbers of patients studied by most investigators. Thus, marker trends in tumor subsets barely achieve significance in most studies.

Currently, adhesion molecules, such as β_1-, β_4-, and α_6-integrins, E-cadherin and its intracellular partner proteins α-, β-catenin, CD44-v6 and other splice variants, sLe^x, CEA, as well as the angiogenesis-related molecules von Willebrand factor, VEGF, MMP-9, uPA and its receptor (uPA-R) and inhibitor (PAI), appear to be the most significant prognostic markers for patients with colorectal cancer. Some studies suggest that EGF-R and cathepsin D also have prognostic significance. Taken together, several surface molecules were found to be useful for the evaluation of prognosis, but in some studies contradicting results, possibly because of small numbers of patients, different sources (genetics) of patients, or use of univariate analyses or other methodologic considerations, will require further investigation on the usefulness of these markers in assessing colorectal cancer outcome.

Perhaps the next phase should be to evaluate promising markers in defined combinations in larger studies to establish the relative importance of these markers in tumor prognosis, survival, and response to treatment. The usefulness of surface markers in routinely processed archival material from human tumor specimens must also be carefully examined in multivariate analyses. Future developments in therapy, such as definition of subgroups for particular adjuvant therapies, may rely on the knowledge that may emerge from such work.[164] New markers for evaluation of an individual tumor's progression and prognosis should also be undertaken. This can be achieved only with a better understanding of the cell surface biochemistry and molecular biology of colorectal carcinomas. All potential prognostic factors will have to demonstrate clear biologic relevance using reproducible and standardized procedures to be useful in the management of colorectal cancers.

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