Online Submissions: wjg.wjgnet.com
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2007 November 28; 13(44): 5832-5844
World Journal of Gastroenterology ISSN 1007-9327

TOPIC HIGHLIGHT

Ignacio Gil-Bazo, MD, PhD, *Series Editor*

Is there a genetic signature for liver metastasis in colorectal cancer?

Cristina Nadal, Joan Maurel, Pere Gascon

Cristina Nadal, Joan Maurel, Pere Gascon, Servei Oncologia Mèdica, Institut Clínic de Malalties Hemato-Oncològiques, Hospital Clínic de Barcelona, c/Villarroel, 170, Barcelona 08036, Spain
Correspondence to: Cristina Nadal, Servei Oncologia Mèdica, Institut Clínic de Malalties Hemato-Oncològiques, Hospital Clínic de Barcelona, c/Villarroel, 170, Barcelona 08036, Spain. cnadal@clinic.ub.es
Telephone: +34-93-2275402 Fax: +34-93-4546520
Received: October 14, 2006 Revised: December 21, 2006

Abstract

Even though liver metastasis accounts for the vast majority of cancer deaths in patients with colorectal cancer (CRC), fundamental questions about the molecular and cellular mechanisms of liver metastasis still remain unanswered. Determination of gene expression profiles by microarray technology has improved our knowledge of CRC molecular pathways. However, defined gene signatures are highly variable among studies. Expression profiles and molecular markers have been specifically linked to liver metastases mechanistic paths in CRC. However, to date, none of the identified signatures or molecular markers has been successfully validated as a diagnostic or prognostic tool applicable to routine clinical practice. To obtain a genetic signature for liver metastasis in CRC, measures to improve reproducibility, to increase consistency, and to validate results need to be implemented. Alternatives to expression profiling with microarray technology are continuing to be used. In the recent past, many genes codifying for proteins that are directly or indirectly involved in adhesion, invasion, angiogenesis, survival and cell growth have been linked to mechanisms of liver metastases in CRC.

Key words: Colorectal cancer; Liver metastasis; Genetic signature; Expression profile; Arrays

Nadal C, Maurel J, Gascon P. Is there a genetic signature for liver metastasis in colorectal cancer? *World J Gastroenterol* 2007; 13(44):5832-5844

http://www.wjgnet.com/1007-9327/13/5832.asp

INTRODUCTION

Colorectal cancer (CRC) is the third most commonly diagnosed cancer, with a worldwide incidence of almost a million cases annually in both males and females[1]. Despite advances in screening, approximately 25% of patients have initially detectable liver metastases (synchronous metastases), and an additional 25% of patients will develop liver metastasis during the course of their disease (metachronous disease)[2]. Of all patients who die of advanced colorectal cancer (ACRC), 60% to 70% show liver metastasis[3]. Metastatic spread to the liver is the major contributor to mortality in patients with CRC.

CRC is a genetically heterogeneous and complex disease. Initially, two major pathways were described as being responsible for the CRC tumorigenic process: the chromosomal instability pathway and the microsatellite instability pathway. The chromosomal instability or classical pathway accounted for 85% of the tumorigenic processes and was mainly characterized by the sequential allelic losses on chromosomes 5q (APC gene), 17p (TP53) and 18q (DCC/Smad4). The microsatellite instability pathway (MNI), which is associated with the mutator phenotype, only accounted for 15% of the carcinogenic processes. Recently, it has been shown that colorectal carcinogenesis is much more complex, involving new pathways, such as the serrated, the TGFβ/Smad and epigenetic pathways, and also non-pure or mixed pathways[4-6].

The general mechanisms of tumorogenesis also include metastasis generation mechanisms. But, is the knowledge of CRC tumorigenic pathways extensible to metastasis generation? What do we really know about the molecular determinants of liver metastases formation in CRC?

MECHANISMS OF LIVER METASTASIS

Colorectal liver metastasis, or dissemination and colonization by colorectal tumor cells coming from the primary CRC to the liver, is a complex process and has many different steps. In order to metastasize, tumor cells detach from the primary tumor, invade and migrate through the stroma and intravasate into the lymphatic and/or venous vessels. With either as the vasculature entrance, cells will mainly end up travelling through the portal vein system. During transportation they manage to survive mechani-

Table 1 Summary of gene expression profile studies related to CRC liver metastasis

Source for transcription profile comparisons	Authors	Signature	Prediction
Primary tumors (Stage Ⅱ and Ⅲ)	Bertucci *et al*[11]	46 gene set	Lymph Node (+)
Primary tumors (Dukes C)	Arango *et al*[12]	Two different gene sets	Survival
Primary tumors (Stage Ⅱ and Ⅲ)	Barrier *et al*[13]	30 gene set	Lymph Node (+)
Primary tumors (Stage Ⅱ and Ⅲ)	Komuro *et al*[14]	Gene set	Stage Classification
Primary tumors (Stage Ⅱ and Ⅲ)	Kwon *et al*[15]	60 gene set	Lymph Node (+)
Primary tumors (Dukes B)	Wang *et al*[16]	23 gene set	Recurrence
Primary tumors (Stage Ⅱ to Ⅳ)	D´Arrico *et al*[17]	37 gene set	Distant Recurrence
Primary tumors and matched metastases	D´Arrico *et al*[17]	GnT-IV gene[1]	Liver Metastasis
Primary tumors and matched metastases	Koehler *et al*[18]	Not found	Liver Metastasis
Primary tumors and matched metastases	Agrawal *et al*[20]	11 gene set	Metastasis (including liver)
CRC cell lines[2]	Hegde *et al*[21]	11 gene set	Metastatic potential
CRC cell lines[2]	[11-14,16,17,22]	Individual genes[3]	Metastatic potential

[1]Mannosyl (alpha-1, 3-)-glycoprotein beta-1, 4-N-acetyl-glucosaminyl-transferase, which was found up-regulated in CRC liver metastases compared to primary CRC tumors; [2]Comparing SW480 to SW620; [3]Down-regulation of Cadherin 17 (CDH17)[11,22], Insulin-like growth factor 2 (IGF2)[14,17], Tyrosine 3-monooxygenase/tryptophan-5-monooxygenase activation protein (YWHAH)[12,16], DEK oncogene (DEK)[11,12] and GATA binding protein (GATA6)[11,14], up-regulation of Linker for activation of T cells (LAT)[14,16] and Protein Kinase, cAMP dependent, catalytic alpha (PRKACA)[12,14], and altered expression of IQ motif containing GTPase activating protein 1 (IQGAP1)[11,12], Tumor protein 53 (TP53)[11,12], Oligoadenylate synthetase 1 (OAS1)[11,12], Interferon regulatory factor (IRF2)[11,14], Retinoic acid receptor beta (RARB)[11,12] and Programmed cell death 10 (PDCD10)[12,13].

cal stresses and escape from the immune system. Some stresses keep acting once cells arrest in the liver capillaries. Some of the arrested cells manage to adhere to endothelial cells, contact the extracellular matrix and extravasate to the surrounding tissues. Kupffer cells, belonging to the monocyte-macrophage system, are a perfect barrier to unwanted hosts. Being in the liver parenchyma, tumor cells establish crosstalk with the stroma and create a microenvironment. Only if this microenvironment is favourable to tumor cells, signals of proliferation and neoangiogenesis will lead to macroscopic liver metastasis formation[7-9]. Even though liver metastasis accounts for the vast majority of all cancer deaths in patients with colorectal cancer, fundamental questions about the molecular and cellular mechanisms of liver metastasis still remain unanswered.

Genetic signatures: The breakthrough

The availability of DNA array technology, allowing genome-wide analyses of gene expression, has been providing new insights on the determination of gene expression or transcriptional profiles. Expression profiling studies in CRC have mainly focused on comparisons of normal mucosa, adenoma and primary carcinomas. Few studies have thrown light on differences between primary tumors and metastases. For this reason, in contrast to the many molecular alterations involved in the CRC adenoma to carcinoma step characterized to date, comparatively little information is available on the possible mechanisms of metastases, with even less for liver specific metastases[10].

There are two different aspects of metastasis to consider: metastatic ability and tropism or organ-specificity. Metastatic ability accounts for the potential to establish a distant secondary tumor. Organ-specificity or tropism means the capacity of this happening in a specific type of tissue. The ability to metastasize together with the specificity for it to happen in one organ and not in another can be genetically marked by what is called a metastatic signature. Studies looking at mRNA or protein levels take into account expression regulation, splicing mechanisms, epigenetic phenomena, and the complexity of post-translational changes or modifications. A metastatic signature, therefore, is not a gene list but is a translation of the functional status of gene expression. Metastatic signatures are gene expression patterns conditioned by both an intrinsic gene composition and phenomena regulating expression.

In order to determine metastatic signatures by microarray technology in CRC, three different strategies have been followed (Table 1). The first approach consists of comparing transcriptional profiles of primary CRC from metastasis-free patients to those affected by metastatic spread during a 5-year follow-up period. The main goal is finding gene expression profiles as prognostic markers of metastatic spread. Identification of a gene set capable of classifying CRC patients according to prognosis or 5-year survival rate was carried out by Bertucci *et al*[11]. A total of 219 genes and 25 genes were found to be respectively down- and up-regulated in metastatic samples when compared to non-metastatic patients. Moreover, a 46 gene set signature was isolated, discriminating between CRC with and without lymph node metastases. Arango *et al*[12] checked the expression profile of Dukes C CRC and reported two different signatures according to survival. Barrier *et al*[13] built an accurate 30-gene tumor-based prognosis predictor for stage Ⅱ and Ⅲ colon cancer patients, based on gene expression measures. The group of Komuro *et al*[14] analyzed gene expression profiles in a total of 89 CRC. After stratifying according to right and left locations, they were able to extract gene expression profiles characteristic of the presence versus absence of lymph node metastasis with an accuracy of more than 90%. Kwon *et al*[15] analyzed the gene-expression profiles of colorectal cancer cells from 12 tumors. Sixty genes possibly associated with lymph node metastasis in CRC were selected on the basis of clinicopathological data. Wang *et al*[16] analyzed RNA samples from 74 patients with Dukes' B CRC. Gene expression profiling identified a 23-gene signature that predicted recurrence. This signature was validated in 36 independent patients. The overall

performance accuracy was 78%. D´Arrico *et al*[17] compared the transcriptional profiles of 10 radically resected primary CRCs from patients who did not develop distant metastases within a 5-year follow-up period with those of 10 primary/metastatic tumor pairs from patients with synchronous liver metastases. The study was conducted on laser-microdissected bioptic tissues. Arrays of 7864 human cDNAs were utilized. Non-metastasizing primary tumors were clearly distinct from the primary/metastatic tumor pairs. Of 37 gene expression differences found between the 2 groups of primary tumors, 29 also distinguished nonmetastasizing tumors from metastases. The gene encoding for mannosyl (alpha-1, 3-)-glycoprotein beta-1, 4-N-acetyl-glucosaminyl-transferase (GnT-IV) became significantly upregulated in primary/metastatic tumor pairs ($P < 0.001$), supporting the existence of a specific transcriptional signature distinguishing primary CRCs with different metastatic potential[17].

The second approach consists of comparing gene expression in primary tumors with their matched metastases. Studies comparing gene expression between primary and corresponding metastases indicate that there is a high transcriptional resemblance. The above mentioned study found a striking transcriptional similarity between primary tumors and their distant metastases[17]. Another study by Koehler *et al*[18] determined expression profiles from 25 CRCs and 14 corresponding liver metastases using cDNA arrays containing 1176 cancer-related genes. Most primary tumors and matched liver metastases clustered together. A specific expression signature in matching metastases was not found, but a set of 23 classifier genes with statistically significant expression patterns in high- and low-stage tumors was identified. Gene expression studies in breast cancer also support the notion that primary tumors genetically resemble their matched metastases more than their primary counterparts[19]. Agrawal *et al*[20] found a signature of 11 markers for tumor progression when comparing gene expression among different stages, including liver metastases in a total of 60 samples.

Expression profiling using CRC cell lines with different metastatic potential is another approach[21,22]. Studies using cDNA microarrays have identified genes that are differentially expressed in primary *versus* metastatic CRC cell lines. Differential expression of 11 genes has been found in SW480 and SW620 CRC cell lines[21]. Unfortunately, metastatic signatures described in the above mentioned studies do not show much in common. Gene expression patterns do not overlap enough to show consistency. Only a few genes reported in at least two independent studies have been linked to metastatic ability (Table 1).

It is interesting that no expression profile has been specifically linked to liver metastases in CRC. Apart from gene expression profiling, other techniques, such as genomic profiling, have also been used to determine metastatic ability in CRC. Genomic analyses of primaries and their matched metastases[23] showed that CRC primary tumors resemble their corresponding metastases. Array-based comparative genomic hybridization (CGH) was used to detect genetic alterations in CRC that predicted survival after liver resection[24]. Genome wide copy number analysis revealed the involvement of Cycline D3 in liver metastases formation in CRC[25].

Genetic signatures: Handicaps and pitfalls

When determining metastatic expression profiles or signatures with array technology, several confounders have to be taken into account. Studies have employed important methodological differences, which are mainly due to the use of different array platforms (Affymetrix, cDNA nylon membranes) or experimental conditions. Tissue sampling is almost always an issue in this regard. Availability of frozen tissues is not the norm in many institutions. Formalin-fixed or paraffin-embedded tissues usually yield low quality RNA and/or DNA. The creation of frozen-tissue tumor banks is rapidly increasing. Also methodologies for RNA isolation can lead to different results. The number of samples used varies enormously in different studies. Relatively small cohorts of tumors have been analyzed in some studies, especially if they include the analysis of matched metastases. Selection of homogeneous samples among heterogeneous tumors can often be a problem. Anatomical localization (right *vs* left sided, colon *vs* rectum) and genetic instability status (MSI/classical) may justify the variability of CRC gene expression profiles characterized to date. Macrodissection techniques include tumor tissue with both tumor cells and tumor stroma and valid tissue samples should be at least 50% tumor cells. One of the major criticisms of "metastatic signature" -seeking studies is the fact that tumors are analyzed as a whole, mixing tumor cells with microenvironment and stroma components. Certainly, data coming from these experiments is a mixture representing gene expression of tumor cells, stroma cells as well as their interactions. Moreover, expression data can be highly conditioned by the host genetic background. Resulting data can be highly interesting in terms of defining prognosis, but not in understanding the mechanisms of metastasis generation. Microdissection techniques help to avoid this problem. Laser capture microdissection (LCM) allows isolation of only tumor cells and is considered the gold standard in microdissection procedures[26]. It is a time-consuming technique and it is not available at all institutions. Other strategies include subtracting non-tumor cell signatures from gene expression data[27]. It is still unclear whether the analysis of pure tumor cell populations will lead to an appropriate result in terms of prognostic value.

Description of metastatic signatures has been done on the basis of transcription analysis of tumors. Data from DNA microarray analysis is often overwhelming and mixed. Analysis of differentially expressed genes can be altered by the use of different criteria to define low-quality spots, different normalization procedures, different baseline references for ratio calculations, and arbitrary criteria for cut-off values applied to fold-change and significance level. Commonly, quantitative levels of expression are the basis for filtering the raw data. During filtering, information coming from qualitative data can be lost[10]. Moreover, the final data set has to be interpreted and integrated to make sense in biological terms. This step is highly subjective and probably often leads to false conclusions. Nearly

Table 2 Proteins related to liver metastasis formation and their function, and their differential expression when comparing primary tumors and liver metastasis by immunohistochemical technique

Proteins related to liver metastasis formation	Function	Liver expression compared to primary tumor (IHC)
E-Cadherin	Adhesion	Down-regulated[34]
Epithelial Cell Adhesion Molecule (EpCAM)	Adhesion	NA
P-Selectin and L-Selectin	Adhesion	NA
Carcinoembryonic Antigen (CEA)	Adhesion	NA
Integrin αvβ5	Adhesion, Survival	NA
sLex and sLea	Adhesion	Up-regulated[48,51]
Osteopontin (OPN)	Adhesion, Survival, Motility	Up-regulated[63]
Intracellular Adhesion Molecule (ICAM-1)	Adhesion	NA
Vascular Cell Adhesion Molecule (VCAM-1)	Adhesion	NA
CD44v6	Adhesion	NA
Cathepsine B	Invasion	NA
MMP-7	Invasion	Up-regulated[81]
MMP-2 and MMP-9	Invasion	Up-regulated[86]
Angiopoietin	Angiogenesis	Up-regulated[110]
Epidermal Growth Factor Receptor	Growth	Equal[125]
Urokinase Plasminogen Activator Receptor (uPAR)	Invasion, Motility, Dormancy	NA
Vascular endotelial Growth Factor (VEGF)	Angiogenesis	Equal[109]
Thrombospondin-1 (TSP-1)	Angiogenesis	NA
Angiostatin	Angiogenesis	NA
Endostatin	Angiogenesis	NA
Thimidine Phosphorylase (dThdPase or PDECGF)	Angiogenesis	NA
c-erb-2	Growth	NA
c-Src/β-Arrestin 1	Growth	NA
FAS Receptor (CD95)	Apoptosis	Down-regulated[134]
TRAIL Receptors (-R1, -R2, -R3 and -R4)	Apoptosis	NA
Nm23-H1 and Nm23-H2	Metastasis Suppresor Genes	NA
PRL-3	Motility, Extravasation	Up-regulated[157]

NA: Not available.

all studies lack internal and external validation tests for the generated lists of implicated genes. Different selection algorithms should be tested in order to improve the accuracy of the classifier sets[10].

In conclusion, to obtain a genetic signature for liver metastases in CRC, measures to improve reproducibility, increase consistency, and validate results need to be implemented.

Genes involved in liver metastasis formation in CRC

Alternatives to expression profiling by microarray technology have also been used in recent past years. Many genes codifying for proteins directly or indirectly involved in adhesion, invasion, angiogenesis, survival and cell growth have been linked to mechanisms of liver metastasis in CRC[28] (Table 2).

Adhesion: Different proteins involved in adhesion/deadhesion processes have been linked to liver metastasis development in CRC. Deadhesion is a necessary step for tumor cells to detach from a tumor and disseminate. Adhesion is needed for circulating cells to contact helping counterparts in the dissemination process. It is also needed to attach to the vascular endothelium, induce endothelial retraction, and subsequently bind to glycoproteins of the basement membrane to extravasate.

E-cadherin/α-catenin is a cell to cell adhesion complex that keeps tumor cells together. Cells detaching from the primary CRC undergo an epithelial to mesenchymal transition, during which E-cadherin downregulatates in favour of other cadherins, such as N-cadherin. This process is known as the "cadherin switch" and leads to acquisition of a mesenchymal phenotype that favours invasion and migration through the stroma and thus dissemination of tumor cells[29]. Downregulation of E-cadherin/α-catenin expression has been related to tumor aggressiveness[30,31] and metastatic potential[32,33] in gastrointestinal cancers. Low expression of α-catenin and E-cadherin in CRC patients has been associated with an increment of β-catenin[34-36], advanced stages[33,37,38] and acquisition of metastatic potential[39,40]. Immunohistochemical studies show that CRCs metastasizing to liver have a significant ($P = 0.014$) reduction or complete absence of E-cadherin expression when compared to non-liver metastases[34].

Epithelial cell adhesion marker (EpCAM) is a widely expressed adhesion molecule. It has been found to present a more diffuse pattern and higher expression in CRC compared to non-malignant tissues[41]. EpCAM plays a role in modulating cadherin mediated cell-cell interactions[42] and its expression has been linked to downregulation of cadherin levels[43], suggesting that this protein possibly plays a role in ETM processes, facilitating migration and dissemination of tumor cells. Supporting this notion, isolation of EpCAM positive cells in blood samples of advanced CRC patients[44] has recently been achieved. All these preliminary data suggest that possibly EpCAM plays a role in CRC cell dissemination. Whether there is liver specificity remains unknown.

Sialyl Lewis X (sLex or CD15s) and A (sLea) are oligosaccharides commonly found in surface glycoproteins

of metastatic tumor cells[45]. sLex and sLea are natural ligands for E-selectin, which is a receptor that has been found to be expressed by activated endothelial cells. Interaction between sLex and sLea induces endothelial adhesion of tumor cells and thus favours stasis, extravasation and metastases formation. sLex and sLea expression in primary CRC have been related to poor prognosis[46] and metastatic potential[46-48] in CRC patients. sLex and sLea stain significantly positive in vessel invasion CRC cells that develop metastases compared to those that do not (71.4% *vs* 31%)[49]. sLex and sLea have been found to be present on the surface of tumor cells[50] in CRC patients who develop liver metastases. Similarly, CRC liver metastases express sLex and sLea in a larger proportion of tumor cells than in primary tumors[48,51]. E-selectin is overexpressed by endothelial cells from tumor and non-tumor vessels in CRC patients who develop liver metastasis[52,53]. In general, as has been demonstrated in *in vivo* models, glycosylated and sialylated mucins are associated with liver metastasis formation[54]. Some proteins allow the adhesion of CRC cells with blood components, such as platelets and leukocytes. Among those proteins are P-Selectin and L-Selectin. This interaction facilitates tumor emboli formation, favouring protection of tumor cells from immune attack and also enhancing their ability to contact blood vessels by mechanical means. This interaction between tumor cells and blood cells also increases contact with the endothelial surface, facilitating stasis and thus enhancing the chances of extravasation[55].

Carcinoembryonic antigen (CEA) is a cell surface glycoprotein containing significant amounts of sLex and sLea. Expression of (CEA) has been clearly correlated to generation of liver metastases in experiments transfecting CEA to CRC cell lines or administering CEA in animal models previous to CRC cell injection[56]. Initially it was speculated that CEA would act as an adhesion molecule, facilitating tumor cell aggregation and interaction with the endothelial surface. However, studies with immunosuppressed mice show that administration of intravenous CEA results in an increase of hepatic colonization and retention of CRC cells, but not an increase of adhesion[57]. Kupffer cells that express a CEA receptor bind to and degrade it, activating a signaling cascade that ends up releasing IL-1, 6 and TNF-α which, in turn, facilitates CRC cell stasis and growth[58,59]. The ability to secrete CEA offers CRC cells a selective advantage in forming metastases in the liver.

Integrins are molecules that can bind to many ECM components, such as laminin, collagen, fibronectin and vitronectin. Cancer cells expressing integrins are more likely to adhere to ECM components surrounding microvasculature. High expression of $\alpha 6\beta 4$ and $\alpha 5\beta 3$ integrins has been related to a more aggressive CRC phenotype[60,61]. Intravital fluorescence-video microscopy has been used to investigate liver metastas formation by CRC cells in animal models[62] and results have shown that $\alpha v\beta 5$ integrin is useful as an adhesion molecule and its inhibition diminished liver metastas formation.

Osteopontin (OPN) is a secreted phosphoglycoprotein capable of binding and inducing integrin-mediated cell survival, motility and anti-apoptotic intracellular pathways. OPN has been isolated in gene expression profiling studies as a candidate marker for CRC progression[20]. CRC liver metastases express OPN at higher ratios than primary CRC or normal mucosa[63]. OPN up-regulation can occur due to TCF4/LEF transcription factor activation[64]. Mechanisms by which OPN promotes liver metastases formation in CRC are unknown, but could be related to up-regulation of Upa[65], c-Met receptor and integrins[66].

Other adhesion molecules, such as the intracellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1), have been measured in ACRC patients showing higher serum levels when compared to non-advanced CRC or healthy controls[67,68]. Nevertheless, neither clinical nor physiological relation has been established with specific development of liver metastases.

CD44 glycoprotein, more specifically v6 and v8-10 splicing variants, have been related to metastases and disease recurrence in CRC[69,70]. There is quite a bit of controversy regarding the real value of CD44 in liver metastases formation because plasma levels have not been linked to advanced stages of the disease[71] and immunohistochemical studies measuring CD44v6 staining have not found significant differences when comparing CRCs metastasizing to liver or not[34].

Invasion: Invasion processes are crucial for liver metastasis formation in CRC. Invasion occurs mainly due to basal membrane and extracellular matrix (ECM) degradation in both intravasation and extravasation steps. Some of the enzymes responsible for degradation are proteases. Among proteases, matrix metalloproteases (MMPs), cathepsins and plasminogen activators are the most relevant.

Matrylysin (MMP-7) is a proteolytic enzyme belonging to the MMPs family[72,73]. It is synthesized and secreted by tumor epithelial cells as a 28-kDa proenzyme, which can be activated through proteolytic removal of a 9-kDa prodomain from the N-terminus. The soluble activated form binds to the tumor epithelial cell surface. Both active forms, the soluble and the membrane-bound, have proteolytic activity. Its expression can be regulated by epidermal growth factor through transcription factors such as PEA3[74] or AP-1 and the β-catenin/ tcf4 complex. By degrading elastin, laminin, proteoglycans, osteopontin, fibronectin and type Ⅳ collagen, MMP-7 gains the capacity to invade. Matrilysin can also promote tumor invasion by activating other MMPs (MMP-2, MMP-9), through ectodomain shedding of E-cadherin[75] and receptor activator of nuclear factor-kappa B ligand (RANKL)[76] or through cleavage of adhesion molecules, such as integrin $\beta 4$[77].

Matrilysin has been found overexpressed in CRC[78]. MMP-7 overexpression in localized CRC disease has been correlated with invasion and liver metastasis formation[79,80]. Colorectal liver metastases show intense expression of MMP-7 compared to normal liver, and differences are more evident when comparing the MMP-7 activated form, measured by zymography, emphasizing the role of MMP-7 in CRC liver metastases formation[81]. While testing liver metastasis formation *in vivo*, it has been shown that treating colorectal cancer cells with MMP-7 specific antisense

oligonucleotides leads to a decrease in liver metastasis generation[82], while adding active MMP-7 results in an increase of liver metastasis generation[83].

MMP-9 and MMP-2 also seem to play a role in liver metastasis formation in CRC. High MMP-9 and MMP-2 levels have been detected by immunohistochemistry in the tumor-stroma interface in both primary CRC and liver metastases[84,85]. Moreover, MMP-2 and -9 activities seem to be higher in metastases than in the originating primary tumor[86]. A close correlation between high MMP-9 RNA levels and worse survival and higher risk of liver relapse after surgery has also been established[81].

Cathepsins have also been implicated in liver metastasis formation in CRC. They are a family of proteolytic enzymes with a wide variety of physiological functions. They act as serin-proteases, cystein-proteases or aspartate-proteases. They are stored as proforms in cell lysosomes and secreted to the ECM secondarily to inflammatory and oncogenic stimuli[87].

Cathepsins B, L and D are especially involved in ECM degradation in CRC. Their levels and activity[87-88] have been found to be elevated in the invasion edge of CRC. Still, Cathepsin B is the most valuable in determining invasion in CRC[89]. Cathepsin B degrades ECM directly or indirectly, by stimulating other proteases or blocking their inhibitors[87]. It can be detected in early stages of CRC but it is a good marker to determine metastatic disease[90,91]. High plasma and urine levels of Cathepsin B have been found in CRC patients[92]. *In vivo* experiments show that inhibition of Cathepsin B by selective compounds results in reduction of liver metastases formation up to 60% and reduction of liver metastases burden up to 80%[93]. A proteolytic profile, taking into account MMP and cathepsin expression, has been defined for CRC by some authors[94].

Urokinase plasminogen activator receptor (uPAR) is a factor involved in metastasis development in several cancers[95,96]. uPAR binding to urokinase plasminogen activator (uPA) enhances plasmin production which, in turn, degrades ECM and activates pro-MMPs. Inhibition of uPAR expression is associated with decreased motility and invasiveness in the human CRC cell line HCT116[97]. High uPAR expression in CRC has been related to low 5-year survival[98]. Use of antisense uPAR mRNA in a nude mice model inhibited CRC liver metastasis development[99].

During invasion, apart from basal membrane and ECM degradation processes, cancer cells have to migrate through the stroma. Clues for success are acquisition of a mesenchymal phenotype during ETM and ability to survive independently of the tumor cell population. To gain the ability to disseminate, tumor cells have to detach from the tumor population, overcome anoikis and transit from an epithelial to a mesenchymal phenotype. As a principle, cells need to be in contact with other cells in order to survive. If they lose contact or penetrate to the ECM they undergo anoikis. Overcoming anoikis, an apoptotic program related to tumor cell population detachment, is a necessary requirement to disseminate. Integrins are responsible for epithelial cancer cell cross-talk with the ECM in order to overcome anoikis, survive and migrate.

In vitro experiments have shown that activation of Src and Akt pathways are linked to decreased sensitivity of detached CRC cells to anoikis[100]. Down-regulation of $\alpha v\beta 3$ integrin has also been linked to resistance to anoikis in CRC cells[101,102]. Integrins can bind to many ECM components such as laminin, collagen, fibronectin and vitronectin. Cancer cells expressing these integrins are more likely to invade and migrate through the ECM[103,104]. High expression of $\alpha 6\beta 4$ and $\alpha 5\beta 3$ integrins has been related to more aggressive CRC phenotypes[60,61]. Intravital fluorescence-video microscopy has been used to investigate liver metastasis formation by CRC cells in animal models[62] showing that $\alpha v\beta$-integrin inhibition did not affect migration within the liver parenchyma. The role of integrins in the migration and invasion through the ECM in order to generate liver metastasis has not been extensively explored.

Angiogenesis: Different angiogenic factors have been related to metastasis formation because they can promote primary tumor growth and increase tumor cell chances to contact blood and thus disseminate. However, it is likely that angiogenesis plays a major role in metastasis generation regulating micrometastases outgrowth. Balance between angiogenic/antiangiogenic factors in the microenvironment of the metastatic tissue can promote metastasis formation by directly stimulating tumor cell growth or by increasing blood vessel formation and supply. Even in quiescent tumor cells, alteration of angiogenic balance can induce metastasis formation. This phenomenon is known as "angiogenic switch"[105] and causal factors are still under investigation.

Expression levels of vascular endothelial growth factor (VEGF) in primary CRC have been related to a poor prognosis[106]. VEGF isoform patterns have been defined using reverse transcription polymerase chain reaction (RT-PCR) analysis in 61 primary CRC. Patients developing liver metastases showed expression of VEGF121 + VEGF165 + VEGF189 at a significantly higher incidence (12 of 16, 75%) than those without liver metastasis (20 of 45, 44%) ($P = 0.036$)[107]. VEGF expression in primary CRC seems clearly associated with increased chances of dissemination. However, other studies support the contrary[108]. When VEGF mRNA levels were measured in 31 pairs of primary CRC and corresponding liver metastases, no significant differences were detected (median value 3.79 *vs* 3.97: $P = 0.989$). On an individual basis, there was a significant correlation in VEGF mRNA expression between primary CRCs and matched liver metastases ($r = 0.6627$, $P < 0.0001$). VEGF mRNA levels of patients having two or more liver metastatic tumors were significantly higher than those of patients who had solitary liver metastatic tumors in both primary cancer (5.02 *vs* 3.34: $P = 0.0483$) and liver metastases (4.38 *vs* 3.25: $P = 0.0358$)[109]. Together these results indicate that VEGF is probably not more active in metastases than in primary tumors. Despite that, increased blood supply and tumor vessel formation, as estimators of angiogenic activity, have been found to be higher in liver metastases that in primary CRC. Some molecular mediators have been thought to fulfill this role, such as angiopoietin-2 (Ang-2)[110].

Other distinctive molecules related to angiogenesis and

liver metastatic progression are platelet-derived endothelial cell growth factor or thymidine phosphorylase (PD ECGF or dThdPase). Inhibitors of angiogenesis, such as angiostatin, endostatin and thrombospondin-1 (TSP-1), either secreted by the primary or the metastatic CRC cells, can also regulate liver metastasis growth. Frequency of hepatic recurrence was significantly higher in patients with TSP-1-negative primary CRC[111]. Angiostatin transfected cells developed liver metastases in lower proportion than controls in animal models[112]. Removal of primary CRC resulted in an increase in metabolic activity in liver metastasis, while decreases in plasma levels of angiostatin and endostatin were observed. This finding indicates that primary tumors suppressed angiogenesis in distant metastases, and that removal of the primary lesion caused a flare-up in vessel neoformation and, thus, enhanced metabolic activity in liver metastases[113].

Other molecules mentioned above also contribute to liver metastasis formation through angiogenesis regulation. MMP-7 induces a direct proliferative effect on vascular endothelial cells[114] and produces angiogenesis inhibitors (angiostatin, endostatin, neostatin-7)[115] and activators (sVEGF)[116]. MMP-2 and MMP-9 stimulate degradation of ECM, increasing the availability of angiogenic activators. E-selectin acts by facilitating endothelial cell migration. α and β integrins play an important role by sending survival signals for endothelial cell maintenance[117].

Cell growth: Once established in the liver tissue microenvironment, micrometastases need growth factor stimuli in order to grow. Degradation of ECM results in an increased availability of growth and inhibitory factors. The resulting balance will then determine micrometastasic growth. Extrapolation to a non-physiological situation can be highly illustrative. Liver tissue thermal ablation was performed in mice models bearing CRC liver metastases. After ablation, increased expression of FGF-2 and VEGF was detected in the surrounding tissue. Subsequently, a greater amount of metastases occupied the regenerated thermal-ablated lobe compared with controls (55% ± 4% *vs* 29% ± 3%, $P < 0.04$)[118].

Tumor cells growth factor receptors also seem to determine success in metastatic liver growth. Her-2/neu has been detected by immunohistochemistry in 5% to 50% of primary CRC[119]. The mechanism of overexpression seems to be not linked to gene amplification. Her-2/neu positive CRCs were associated with higher postoperative non-liver specific recurrence rates (39.3% *vs* 14.6%, $P = 0.013$) and worse prognosis at 5 years (55.1% *vs* 78.3%)[120]. Other studies showed that primary CRC with high c-erbB-2 expression (27%), determined by immunohistochemical techniques, develop liver metastases more often than CRC with low c-erb-2 expression (3%)[49].

Epidermal growth factor receptors (EGFR) have been reported to be highly expressed and/or gene amplificated in 72% to 82% of metastatic CRC tissue samples[121-123]. Some studies have reported that expression of EGF receptors in CRC is associated with aggressiveness and metastatic ability. EGFR status has been shown to express similarly when measured in primary CRC and corresponding liver metastases[124]. However, some authors have seen that its status in the corresponding metastatic site is not always the same[125,126]. Conventional immunohistochemistry techniques have not been able to reveal any association between EGFR expression and outcome predicted by the biological role of EGFR in tumor behavior[127].

The C-Src gene, codifying for pp60 tyrosine kinase, has been reported to be mutated and thus is highly activated in CRC, implying an increase in proliferative potential. High activation is present especially in those CRC that metastasize to liver[128,129]. Prostaglandin E_2 (PGE_2)-induced transactivation of the EGF receptor (EGFR) in colorectal carcinoma cells has been recently found to be mediated by β-arrestin 1, which acts as an important mediator in G protein-coupled receptor-induced activation of c-Src. Interaction of beta-arrestin 1 and c-Src seems to be critical for the regulation of CRC metastatic spread of disease to the liver *in vivo*[130].

Cell survival: CRC cells need molecular factors, specifically growth factors, in order to survive in the liver parenchyma. However, there is also the need to survive immune system action (immunoescape) and to overcome anoikis.

Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), a member of the TNF family, is known to be expressed in human hepatic NK cells[131]. CRC cells expressing TRAIL-receptor would undergo apoptosis upon triggering the ligand. The same would happen in CRC cells expressing tumor necrosis factor receptor FAS (Apo-1; CD95) when contacting its corresponding ligand FASL (Apo-1L; CD95L) expressing cells, as activated lymphocytes.

During the CRC tumorigenic process, cells tend to down-regulate FAS receptor expression and up-regulate FASL[132]. Fas expression is significantly down-regulated in liver metastasis compared to corresponding primary colorectal carcinoma[133]. The link between functional Fas status and malignant phenotype was investigated using matched pairs of naturally occurring primary (Fas-sensitive) and metastatic (Fas-resistant) human colon carcinoma cell lines in both *in vitro* and *in vivo* (xenograft) settings. Results showed that loss of Fas function was linked to the acquisition of a detectable metastatic phenotype, however, only loss of Fas function was insufficient. Also, results showed that metastatic subpopulations pre-existed within the heterogeneous primary tumor and that anti-Fas interactions served as selective pressure for their outgrowth. Thus, Fas-based interactions may represent novel mechanisms for the biological or immunological selection of certain types of Fas-resistant neoplastic clones with enhanced metastatic ability[134]. Moreover, univariate and multivariate analyses revealed that Fas/CD95 expression in CRC resected liver metastases is a significant prognostic indicator of survival[135]. Increases in TRAIL sensitivity, due to changes in the balance between TRAIL receptors TRAIL-R1 and -R2 and "decoy" receptors TRAIL-R3 and -R4, have also been described during malignant progression in CRC. Still, studies measuring receptors by flow cytometry have not

been conclusive[136].

Experimental metastases studies with a CRC cell line allowed the characterization of metastatic derivatives, showing that they were less susceptible for killing by syngeneic NK cells, due to a decreased sensitivity towards TRAIL- and CD95L[137]. Data suggest that CRC cells forming metastases acquire the ability to surpass immune surveillance through desensitization to FAS/TRAIL killing. As discussed previously, integrins and Src activation may contribute to CRC progression and liver metastasis, in part, by activating survival pathways that decrease sensitivity of detached cells to anoikis[100].

Other molecules related to liver metastatic spreading: k-ras (12p) activation, present in 40% to 50% of sporadic CRC[4], has been related to a decrease in overall survival and disease free survival in CRC[6,138,139]. p53 (17p) abolition, occurring in 70% to 80% of CRC[4] and resulting in accumulation of abnormal protein detectable by immunohistochemistry, has been linked to a poor prognosis[6,140-142]. The deletion or mutation of the DCC (deleted in colorectal cancer) gene has also been related to poor prognosis tumors[143-146]. Even p53, Ras and/or DCC alterations have been linked to metastatic spreading in CRC, however, there is still no evidence specifically relating them to liver metastasis formation. The human nm23 genes, nm23-H1 and nm23-H2, are candidate metastasis suppressor genes. Their role in CRC is still confusing. Some authors claim that a reduced protein expression, secondary to gene alterations, is associated with metastasis development[147,148]. Genetic alterations were detected in four of eight CRCs associated with metastasis in lymph nodes, lung, or liver, while no alteration was observed in 12 additional CRC specimens without metastasis[149]. Others have found that gene overexpression is linked to higher recurrences, liver metastasis and decreased overall survival[150,151]. This contradiction could be explained if overexpression of nm23 was a reflection of a deletion in the nm23 gene, leading to accumulation of an altered protein product. However, more recent works have not been able to relate nm23 expression to prognosis[152-154]. The PRL-3 protein tyrosine phosphatase gene gained importance in 2001 when an article was published in Science showing that it was expressed at high levels in each of 18 cancer metastases studied but was expressed at lower levels in nonmetastatic tumors and in normal colorectal epithelium[155]. Subsequently, new data established an unexpected and unprecedented specificity in metastatic gene expression profiles: PRL-3 was apparently expressed in CRC metastasis to any organ but was not expressed in metastases of other cancers to the same organs or in nonmetastatic CRC[156]. At that time PRL-3 was determined to be a potential marker for liver metastasis of CRC with a negative impact in prognosis[157]. CRC specificity was objected to in further studies. Some authors claimed that PRL-3 acted by enhancing cell motility and thus facilitating extravasation into liver tissue[158]. The mechanism of action is still under investigation but it has already been related to integrin $\alpha 1$[159] and the Rho family of small GTPases[160].

CONCLUSION

A significant amount of experimental data points to tumor cells having a metastatic signature. This signature codifies not only for the ability to form metasteses but also for organ-specificity. DNA microarray technology has significantly improved efficiency in wide-range analysis of gene expression. Many authors have provided gene expression profiles that have been related to CRC liver metastases, however, in order to obtain a real genetic signature for liver metastases in CRC by transcription profiling, measures to improve reproducibility, increase consistency, and validate results need to be implemented. Seeking metastatic signatures through expression profiling is a tool to fight cancer, but its indiscriminate use can be misleading. Advances in molecular assays on isolated cells and in the study of cell-cell and cell-stroma interactions will likely enable the dissection of the metastatic cascade. Genes codifying for proteins directly or indirectly involved in adhesion, invasion, angiogenesis, survival and cell growth have already been linked to mechanisms of liver metastases in CRC. Improvement in knowledge of the molecular pathways involved in the development of colorectal liver metastasis will lead to a better approach to prevent and treat this disease.

REFERENCES

1 **Parkin DM**, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. *CA Cancer J Clin* 2005; **55**: 74-108

2 **Millikan KW**, Staren ED, Doolas A. Invasive therapy of metastatic colorectal cancer to the liver. *Surg Clin North Am* 1997; **77**: 27-48

3 **Weiss L**, Grundmann E, Torhorst J, Hartveit F, Moberg I, Eder M, Fenoglio-Preiser CM, Napier J, Horne CH, Lopez MJ. Haematogenous metastatic patterns in colonic carcinoma: an analysis of 1541 necropsies. *J Pathol* 1986; **150**: 195-203

4 **Vogelstein B**, Fearon ER, Hamilton SR, Kern SE, Preisinger AC, Leppert M, Nakamura Y, White R, Smits AM, Bos JL. Genetic alterations during colorectal-tumor development. *N Engl J Med* 1988; **319**: 525-532

5 **Fearon ER**, Vogelstein B. A genetic model for colorectal tumorigenesis. *Cell* 1990; **61**: 759-767

6 **Iniesta P**, de Juan C, Caldés T, Vega FJ, Massa MJ, Cerdán FJ, López JA, Fernández C, Sánchez A, Torres AJ, Balibrea JL, Benito M. Genetic abnormalities and microsatellite instability in colorectal cancer. *Cancer Detect Prev* 1998; **22**: 383-395

7 **Takayama T**, Miyanishi K, Hayashi T, Sato Y, Niitsu Y. Colorectal cancer: genetics of development and metastasis. *J Gastroenterol* 2006; **41**: 185-192

8 **Bird NC**, Mangnall D, Majeed AW. Biology of colorectal liver metastases: A review. *J Surg Oncol* 2006; **94**: 68-80

9 **Rudmik LR**, Magliocco AM. Molecular mechanisms of hepatic metastasis in colorectal cancer. *J Surg Oncol* 2005; **92**: 347-359

10 **Cardoso J**, Boer J, Morreau H, Fodde R. Expression and genomic profiling of colorectal cancer. *Biochim Biophys Acta* 2007; **1775**: 103-137

11 **Bertucci F**, Salas S, Eysteries S, Nasser V, Finetti P, Ginestier C, Charafe-Jauffret E, Loriod B, Bachelart L, Montfort J, Victorero G, Viret F, Ollendorff V, Fert V, Giovaninni M, Delpero JR, Nguyen C, Viens P, Monges G, Birnbaum D, Houlgatte R. Gene expression profiling of colon cancer by DNA microarrays and correlation with histoclinical parameters. *Oncogene* 2004; **23**: 1377-1391

12 **Arango D**, Laiho P, Kokko A, Alhopuro P, Sammalkorpi H, Salovaara R, Nicorici D, Hautaniemi S, Alazzouzi H, Mecklin JP, Järvinen H, Hemminki A, Astola J, Schwartz S, Aaltonen LA. Gene-expression profiling predicts recurrence in Dukes' C colorectal cancer. *Gastroenterology* 2005; **129**: 874-884

13 **Barrier A**, Lemoine A, Boelle PY, Tse C, Brault D, Chiappini F, Breittschneider J, Lacaine F, Houry S, Huguier M, Van der Laan MJ, Speed T, Debuire B, Flahault A, Dudoit S. Colon cancer prognosis prediction by gene expression profiling. *Oncogene* 2005; **24**: 6155-6164

14 **Komuro K**, Tada M, Tamoto E, Kawakami A, Matsunaga A, Teramoto K, Shindoh G, Takada M, Murakawa K, Kanai M, Kobayashi N, Fujiwara Y, Nishimura N, Hamada J, Ishizu A, Ikeda H, Kondo S, Katoh H, Moriuchi T, Yoshiki T. Right- and left-sided colorectal cancers display distinct expression profiles and the anatomical stratification allows a high accuracy prediction of lymph node metastasis. *J Surg Res* 2005; **124**: 216-224

15 **Kwon HC**, Kim SH, Roh MS, Kim JS, Lee HS, Choi HJ, Jeong JS, Kim HJ, Hwang TH. Gene expression profiling in lymph node-positive and lymph node-negative colorectal cancer. *Dis Colon Rectum* 2004; **47**: 141-152

16 **Wang Y**, Jatkoe T, Zhang Y, Mutch MG, Talantov D, Jiang J, McLeod HL, Atkins D. Gene expression profiles and molecular markers to predict recurrence of Dukes' B colon cancer. *J Clin Oncol* 2004; **22**: 1564-1571

17 **D'Arrigo A**, Belluco C, Ambrosi A, Digito M, Esposito G, Bertola A, Fabris M, Nofrate V, Mammano E, Leon A, Nitti D, Lise M. Metastatic transcriptional pattern revealed by gene expression profiling in primary colorectal carcinoma. *Int J Cancer* 2005; **115**: 256-262

18 **Koehler A**, Bataille F, Schmid C, Ruemmele P, Waldeck A, Blaszyk H, Hartmann A, Hofstaedter F, Dietmaier W. Gene expression profiling of colorectal cancer and metastases divides tumours according to their clinicopathological stage. *J Pathol* 2004; **204**: 65-74

19 **Weigelt B**, Glas AM, Wessels LF, Witteveen AT, Peterse JL, van't Veer LJ. Gene expression profiles of primary breast tumors maintained in distant metastases. *Proc Natl Acad Sci USA* 2003; **100**: 15901-15905

20 **Agrawal D**, Chen T, Irby R, Quackenbush J, Chambers AF, Szabo M, Cantor A, Coppola D, Yeatman TJ. Osteopontin identified as colon cancer tumor progression marker. *C R Biol* 2003; **326**: 1041-1043

21 **Hegde P**, Qi R, Gaspard R, Abernathy K, Dharap S, Earle-Hughes J, Gay C, Nwokekeh NU, Chen T, Saeed AI, Sharov V, Lee NH, Yeatman TJ, Quackenbush J. Identification of tumor markers in models of human colorectal cancer using a 19,200-element complementary DNA microarray. *Cancer Res* 2001; **61**: 7792-7797

22 **Dan S**, Tsunoda T, Kitahara O, Yanagawa R, Zembutsu H, Katagiri T, Yamazaki K, Nakamura Y, Yamori T. An integrated database of chemosensitivity to 55 anticancer drugs and gene expression profiles of 39 human cancer cell lines. *Cancer Res* 2002; **62**: 1139-1147

23 **Al-Mulla F**, Keith WN, Pickford IR, Going JJ, Birnie GD. Comparative genomic hybridization analysis of primary colorectal carcinomas and their synchronous metastases. *Genes Chromosomes Cancer* 1999; **24**: 306-314

24 **Mehta KR**, Nakao K, Zuraek MB, Ruan DT, Bergsland EK, Venook AP, Moore DH, Tokuyasu TA, Jain AN, Warren RS, Terdiman JP, Waldman FM. Fractional genomic alteration detected by array-based comparative genomic hybridization independently predicts survival after hepatic resection for metastatic colorectal cancer. *Clin Cancer Res* 2005; **11**: 1791-1797

25 **Tanami H**, Tsuda H, Okabe S, Iwai T, Sugihara K, Imoto I, Inazawa J. Involvement of cyclin D3 in liver metastasis of colorectal cancer, revealed by genome-wide copy-number analysis. *Lab Invest* 2005; **85**: 1118-1129

26 **Ma XJ**, Salunga R, Tuggle JT, Gaudet J, Enright E, McQuary P, Payette T, Pistone M, Stecker K, Zhang BM, Zhou YX, Varnholt H, Smith B, Gadd M, Chatfield E, Kessler J, Baer TM, Erlander MG, Sgroi DC. Gene expression profiles of human breast cancer progression. *Proc Natl Acad Sci USA* 2003; **100**: 5974-5979

27 **Stuart RO**, Wachsman W, Berry CC, Wang-Rodriguez J, Wasserman L, Klacansky I, Masys D, Arden K, Goodison S, McClelland M, Wang Y, Sawyers A, Kalcheva I, Tarin D, Mercola D. In silico dissection of cell-type-associated patterns of gene expression in prostate cancer. *Proc Natl Acad Sci USA* 2004; **101**: 615-620

28 **Neal CP**, Garcea G, Doucas H, Manson MM, Sutton CD, Dennison AR, Berry DP. Molecular prognostic markers in resectable colorectal liver metastases: a systematic review. *Eur J Cancer* 2006; **42**: 1728-1743

29 **Kang Y**, Massagué J. Epithelial-mesenchymal transitions: twist in development and metastasis. *Cell* 2004; **118**: 277-279

30 **Matsuura K**, Kawanishi J, Fujii S, Imamura M, Hirano S, Takeichi M, Niitsu Y. Altered expression of E-cadherin in gastric cancer tissues and carcinomatous fluid. *Br J Cancer* 1992; **66**: 1122-1130

31 **Karatzas G**, Karayiannakis AJ, Syrigos KN, Chatzigianni E, Papanikolaou S, Riza F, Papanikolaou D. E-cadherin expression correlates with tumor differentiation in colorectal cancer. *Hepatogastroenterology* 1999; **46**: 232-235

32 **Debruyne P**, Vermeulen S, Mareel M. The role of the E-cadherin/catenin complex in gastrointestinal cancer. *Acta Gastroenterol Belg* 1999; **62**: 393-402

33 **Ropponen KM**, Eskelinen MJ, Lipponen PK, Alhava EM, Kosma VM. Reduced expression of alpha catenin is associated with poor prognosis in colorectal carcinoma. *J Clin Pathol* 1999; **52**: 10-16

34 **Delektorskaia VV**, Perevoshchikov AG, Golovkov DA, Kushlinskiĭ NE. Immunohistochemical study of E-cadherin, beta-catenin and CD-44v6 expression in the cells of primary colon cancer and its metastases. *Arkh Patol* 2005; **67**: 34-38

35 **Bravou V**, Klironomos G, Papadaki E, Taraviras S, Varakis J. ILK over-expression in human colon cancer progression correlates with activation of beta-catenin, down-regulation of E-cadherin and activation of the Akt-FKHR pathway. *J Pathol* 2006; **208**: 91-99

36 **Hayashida Y**, Honda K, Idogawa M, Ino Y, Ono M, Tsuchida A, Aoki T, Hirohashi S, Yamada T. E-cadherin regulates the association between beta-catenin and actinin-4. *Cancer Res* 2005; **65**: 8836-8845

37 **Gofuku J**, Shiozaki H, Tsujinaka T, Inoue M, Tamura S, Doki Y, Matsui S, Tsukita S, Kikkawa N, Monden M. Expression of E-cadherin and alpha-catenin in patients with colorectal carcinoma. Correlation with cancer invasion and metastasis. *Am J Clin Pathol* 1999; **111**: 29-37

38 **Dorudi S**, Sheffield JP, Poulsom R, Northover JM, Hart IR. E-cadherin expression in colorectal cancer. An immunocytochemical and in situ hybridization study. *Am J Pathol* 1993; **142**: 981-986

39 **Roca F**, Mauro LV, Morandi A, Bonadeo F, Vaccaro C, Quintana GO, Specterman S, de Kier Joffé EB, Pallotta MG, Puricelli LI, Lastiri J. Prognostic value of E-cadherin, beta-catenin, MMPs (7 and 9), and TIMPs (1 and 2) in patients with colorectal carcinoma. *J Surg Oncol* 2006; **93**: 151-160

40 **Raftopoulos I**, Davaris P, Karatzas G, Karayannacos P, Kouraklis G. Level of alpha-catenin expression in colorectal cancer correlates with invasiveness, metastatic potential, and survival. *J Surg Oncol* 1998; **68**: 92-99

41 **Xie X**, Wang CY, Cao YX, Wang W, Zhuang R, Chen LH, Dang NN, Fang L, Jin BQ. Expression pattern of epithelial cell adhesion molecule on normal and malignant colon tissues. *World J Gastroenterol* 2005; **11**: 344-347

42 **Litvinov SV**, Balzar M, Winter MJ, Bakker HA, Briaire-de Bruijn IH, Prins F, Fleuren GJ, Warnaar SO. Epithelial cell adhesion molecule (Ep-CAM) modulates cell-cell interactions mediated by classic cadherins. *J Cell Biol* 1997; **139**: 1337-1348

43 **Winter MJ**, Nagelkerken B, Mertens AE, Rees-Bakker HA, Briaire-de Bruijn IH, Litvinov SV. Expression of Ep-CAM shifts the state of cadherin-mediated adhesions from strong to weak. *Exp Cell Res* 2003; **285**: 50-58

44 **Cohen SJ**, Alpaugh RK, Gross S, O'Hara SM, Smirnov DA, Terstappen LW, Allard WJ, Bilbee M, Cheng JD, Hoffman JP, Lewis NL, Pellegrino A, Rogatko A, Sigurdson E, Wang H, Watson JC, Weiner LM, Meropol NJ. Isolation and characterization of circulating tumor cells in patients with metastatic colorectal cancer. *Clin Colorectal Cancer* 2006; **6**:

125-132
45 **Berg EL**, Robinson MK, Mansson O, Butcher EC, Magnani JL. A carbohydrate domain common to both sialyl Le(a) and sialyl Le(X) is recognized by the endothelial cell leukocyte adhesion molecule ELAM-1. *J Biol Chem* 1991; **266**: 14869-14872
46 **Nakamori S**, Kameyama M, Imaoka S, Furukawa H, Ishikawa O, Sasaki Y, Izumi Y, Irimura T. Involvement of carbohydrate antigen sialyl Lewis(x) in colorectal cancer metastasis. *Dis Colon Rectum* 1997; **40**: 420-431
47 **Li XW**, Ding YQ, Cai JJ, Yang SQ, An LB, Qiao DF. Studies on mechanism of Sialy Lewis-X antigen in liver metastases of human colorectal carcinoma. *World J Gastroenterol* 2001; **7**: 425-430
48 **Yamada N**, Chung YS, Maeda K, Sawada T, Ikehara T, Nishino H, Okuno M, Sowa M. Increased expression of sialyl Lewis A and sialyl Lewis X in liver metastases of human colorectal carcinoma. *Invasion Metastasis* 1995; **15**: 95-102
49 **Yamaguchi S**, Ichikawa Y, Tanaka K, Ishikawa T, Masui H, Koganei K, Eguchi K, Ike H, Ohki S, Shimada H. Prognostic factors of colorectal cancer concerning metastases. *Gan To Kagaku Ryoho* 1996; **23**: 529-533
50 **Yasui N**, Sakamoto M, Ochiai A, Ino Y, Akimoto S, Orikasa A, Kitajima M, Hirohashi S. Tumor growth and metastasis of human colorectal cancer cell lines in SCID mice resemble clinical metastatic behaviors. *Invasion Metastasis* 1997; **17**: 259-269
51 **Hoff SD**, Matsushita Y, Ota DM, Cleary KR, Yamori T, Hakomori S, Irimura T. Increased expression of sialyl-dimeric LeX antigen in liver metastases of human colorectal carcinoma. *Cancer Res* 1989; **49**: 6883-6888
52 **Wittig BM**, Kaulen H, Thees R, Schmitt C, Knolle P, Stock J, Meyer zum Büschenfelde KH, Dippold W. Elevated serum E-selectin in patients with liver metastases of colorectal cancer. *Eur J Cancer* 1996; **32A**: 1215-1218
53 **Brodt P**, Fallavollita L, Bresalier RS, Meterissian S, Norton CR, Wolitzky BA. Liver endothelial E-selectin mediates carcinoma cell adhesion and promotes liver metastasis. *Int J Cancer* 1997; **71**: 612-619
54 **Bresalier RS**, Byrd JC, Brodt P, Ogata S, Itzkowitz SH, Yunker CK. Liver metastasis and adhesion to the sinusoidal endothelium by human colon cancer cells is related to mucin carbohydrate chain length. *Int J Cancer* 1998; **76**: 556-562
55 **Mannori G**, Crottet P, Cecconi O, Hanasaki K, Aruffo A, Nelson RM, Varki A, Bevilacqua MP. Differential colon cancer cell adhesion to E-, P-, and L-selectin: role of mucin-type glycoproteins. *Cancer Res* 1995; **55**: 4425-4431
56 **Thomas P**, Gangopadhyay A, Steele G, Andrews C, Nakazato H, Oikawa S, Jessup JM. The effect of transfection of the CEA gene on the metastatic behavior of the human colorectal cancer cell line MIP-101. *Cancer Lett* 1995; **92**: 59-66
57 **Jessup JM**, Petrick AT, Toth CA, Ford R, Meterissian S, O'Hara CJ, Steele G, Thomas P. Carcinoembryonic antigen: enhancement of liver colonisation through retention of human colorectal carcinoma cells. *Br J Cancer* 1993; **67**: 464-470
58 **Gangopadhyay A**, Lazure DA, Thomas P. Carcinoembryonic antigen induces signal transduction in Kupffer cells. *Cancer Lett* 1997; **118**: 1-6
59 **Gangopadhyay A**, Lazure DA, Thomas P. Adhesion of colorectal carcinoma cells to the endothelium is mediated by cytokines from CEA stimulated Kupffer cells. *Clin Exp Metastasis* 1998; **16**: 703-712
60 **Chao C**, Lotz MM, Clarke AC, Mercurio AM. A function for the integrin alpha6beta4 in the invasive properties of colorectal carcinoma cells. *Cancer Res* 1996; **56**: 4811-4819
61 **Agrez MV**, Bates RC, Mitchell D, Wilson N, Ferguson N, Anseline P, Sheppard D. Multiplicity of fibronectin-binding alpha V integrin receptors in colorectal cancer. *Br J Cancer* 1996; **73**: 887-892
62 **Enns A**, Korb T, Schlüter K, Gassmann P, Spiegel HU, Senninger N, Mitjans F, Haier J. Alphavbeta5-integrins mediate early steps of metastasis formation. *Eur J Cancer* 2005; **41**: 1065-1072
63 **Yeatman TJ**, Chambers AF. Osteopontin and colon cancer progression. *Clin Exp Metastasis* 2003; **20**: 85-90
64 **El-Tanani M**, Barraclough R, Wilkinson MC, Rudland PS. Metastasis-inducing dna regulates the expression of the osteopontin gene by binding the transcription factor Tcf-4. *Cancer Res* 2001; **61**: 5619-5629
65 **Tuck AB**, Arsenault DM, O'Malley FP, Hota C, Ling MC, Wilson SM, Chambers AF. Osteopontin induces increased invasiveness and plasminogen activator expression of human mammary epithelial cells. *Oncogene* 1999; **18**: 4237-4246
66 **Tuck AB**, Elliott BE, Hota C, Tremblay E, Chambers AF. Osteopontin-induced, integrin-dependent migration of human mammary epithelial cells involves activation of the hepatocyte growth factor receptor (Met). *J Cell Biochem* 2000; **78**: 465-475
67 **Sánchez-Rovira P**, Jimenez E, Carracedo J, Barneto IC, Ramirez R, Aranda E. Serum levels of intercellular adhesion molecule 1 (ICAM-1) in patients with colorectal cancer: inhibitory effect on cytotoxicity. *Eur J Cancer* 1998; **34**: 394-398
68 **Velikova G**, Banks RE, Gearing A, Hemingway I, Forbes MA, Preston SR, Hall NR, Jones M, Wyatt J, Miller K, Ward U, Al-Maskatti J, Singh SM, Finan PJ, Ambrose NS, Primrose JN, Selby PJ. Serum concentrations of soluble adhesion molecules in patients with colorectal cancer. *Br J Cancer* 1998; **77**: 1857-1863
69 **Wielenga VJ**, Heider KH, Offerhaus GJ, Adolf GR, van den Berg FM, Ponta H, Herrlich P, Pals ST. Expression of CD44 variant proteins in human colorectal cancer is related to tumor progression. *Cancer Res* 1993; **53**: 4754-4756
70 **Yamaguchi A**, Urano T, Goi T, Saito M, Takeuchi K, Hirose K, Nakagawara G, Shiku H, Furukawa K. Expression of a CD44 variant containing exons 8 to 10 is a useful independent factor for the prediction of prognosis in colorectal cancer patients. *J Clin Oncol* 1996; **14**: 1122-1127
71 **Masson D**, Denis MG, Denis M, Blanchard D, Loirat MJ, Cassagnau E, Lustenberger P. Soluble CD44: quantification and molecular repartition in plasma of patients with colorectal cancer. *Br J Cancer* 1999; **80**: 1995-2000
72 **Leeman MF**, Curran S, Murray GI. New insights into the roles of matrix metalloproteinases in colorectal cancer development and progression. *J Pathol* 2003; **201**: 528-534
73 **Ii M**, Yamamoto H, Adachi Y, Maruyama Y, Shinomura Y. Role of matrix metalloproteinase-7 (matrilysin) in human cancer invasion, apoptosis, growth, and angiogenesis. *Exp Biol Med* (Maywood) 2006; **231**: 20-27
74 **Lynch CC**, Crawford HC, Matrisian LM, McDonnell S. Epidermal growth factor upregulates matrix metalloproteinase-7 expression through activation of PEA3 transcription factors. *Int J Oncol* 2004; **24**: 1565-1572
75 **Noë V**, Fingleton B, Jacobs K, Crawford HC, Vermeulen S, Steelant W, Bruyneel E, Matrisian LM, Mareel M. Release of an invasion promoter E-cadherin fragment by matrilysin and stromelysin-1. *J Cell Sci* 2001; **114**: 111-118
76 **Lynch CC**, Hikosaka A, Acuff HB, Martin MD, Kawai N, Singh RK, Vargo-Gogola TC, Begtrup JL, Peterson TE, Fingleton B, Shirai T, Matrisian LM, Futakuchi M. MMP-7 promotes prostate cancer-induced osteolysis via the solubilization of RANKL. *Cancer Cell* 2005; **7**: 485-496
77 **von Bredow DC**, Nagle RB, Bowden GT, Cress AE. Cleavage of beta 4 integrin by matrilysin. *Exp Cell Res* 1997; **236**: 341-345
78 **Masaki T**, Matsuoka H, Sugiyama M, Abe N, Goto A, Sakamoto A, Atomi Y. Matrilysin (MMP-7) as a significant determinant of malignant potential of early invasive colorectal carcinomas. *Br J Cancer* 2001; **84**: 1317-1321
79 **Ishikawa T**, Ichikawa Y, Mitsuhashi M, Momiyama N, Chishima T, Tanaka K, Yamaoka H, Miyazakic K, Nagashima Y, Akitaya T, Shimada H. Matrilysin is associated with progression of colorectal tumor. *Cancer Lett* 1996; **107**: 5-10
80 **Adachi Y**, Yamamoto H, Itoh F, Hinoda Y, Okada Y, Imai K. Contribution of matrilysin (MMP-7) to the metastatic pathway of human colorectal cancers. *Gut* 1999; **45**: 252-258
81 **Zeng ZS**, Shu WP, Cohen AM, Guillem JG. Matrix metalloproteinase-7 expression in colorectal cancer liver metastases: evidence for involvement of MMP-7 activation in human cancer metastases. *Clin Cancer Res* 2002; **8**: 144-148

82 **Hasegawa S**, Koshikawa N, Momiyama N, Moriyama K, Ichikawa Y, Ishikawa T, Mitsuhashi M, Shimada H, Miyazaki K. Matrilysin-specific antisense oligonucleotide inhibits liver metastasis of human colon cancer cells in a nude mouse model. *Int J Cancer* 1998; **76**: 812-816

83 **Kioi M**, Yamamoto K, Higashi S, Koshikawa N, Fujita K, Miyazaki K. Matrilysin (MMP-7) induces homotypic adhesion of human colon cancer cells and enhances their metastatic potential in nude mouse model. *Oncogene* 2003; **22**: 8662-8670

84 **Parsons SL**, Watson SA, Brown PD, Collins HM, Steele RJ. Matrix metalloproteinases. *Br J Surg* 1997; **84**: 160-166

85 **Mc Donnell S**, Chaudhry V, Mansilla-Soto J, Zeng ZS, Shu WP, Guillem JG. Metastatic and non-metastatic colorectal cancer (CRC) cells induce host metalloproteinase production *in vivo*. *Clin Exp Metastasis* 1999; **17**: 341-349

86 **Karakiulakis G**, Papanikolaou C, Jankovic SM, Aletras A, Papakonstantinou E, Vretou E, Mirtsou-Fidani V. Increased type IV collagen-degrading activity in metastases originating from primary tumors of the human colon. *Invasion Metastasis* 1997; **17**: 158-168

87 **Schwartz MK**. Tissue cathepsins as tumor markers. *Clin Chim Acta* 1995; **237**: 67-78

88 **Mayer A**, Fritz E, Fortelny R, Kofler K, Ludwig H. Immunohistochemical evaluation of cathepsin D expression in colorectal cancer. *Cancer Invest* 1997; **15**: 106-110

89 **Adenis A**, Huet G, Zerimech F, Hecquet B, Balduyck M, Peyrat JP. Cathepsin B, L, and D activities in colorectal carcinomas: relationship with clinico-pathological parameters. *Cancer Lett* 1995; **96**: 267-275

90 **Khan A**, Krishna M, Baker SP, Banner BF. Cathepsin B and tumor-associated laminin expression in the progression of colorectal adenoma to carcinoma. *Mod Pathol* 1998; **11**: 704-708

91 **Hirai K**, Yokoyama M, Asano G, Tanaka S. Expression of cathepsin B and cystatin C in human colorectal cancer. *Hum Pathol* 1999; **30**: 680-686

92 **Hirano T**, Manabe T, Takeuchi S. Serum cathepsin B levels and urinary excretion of cathepsin B in the cancer patients with remote metastasis. *Cancer Lett* 1993; **70**: 41-44

93 **Van Noorden CJ**, Jonges TG, Van Marle J, Bissell ER, Griffini P, Jans M, Snel J, Smith RE. Heterogeneous suppression of experimentally induced colon cancer metastasis in rat liver lobes by inhibition of extracellular cathepsin B. *Clin Exp Metastasis* 1998; **16**: 159-167

94 **Murnane MJ**, Shuja S, Del Re E, Cai J, Iacobuzio-Donahue C, Klepeis V. Characterizing human colorectal carcinomas by proteolytic profile. *In Vivo* 1997; **11**: 209-216

95 **Crowley CW**, Cohen RL, Lucas BK, Liu G, Shuman MA, Levinson AD. Prevention of metastasis by inhibition of the urokinase receptor. *Proc Natl Acad Sci USA* 1993; **90**: 5021-5025

96 **Ossowski L**, Aguirre-Ghiso JA. Urokinase receptor and integrin partnership: coordination of signaling for cell adhesion, migration and growth. *Curr Opin Cell Biol* 2000; **12**: 613-620

97 **Ahmed N**, Oliva K, Wang Y, Quinn M, Rice G. Downregulation of urokinase plasminogen activator receptor expression inhibits Erk signalling with concomitant suppression of invasiveness due to loss of uPAR-beta1 integrin complex in colon cancer cells. *Br J Cancer* 2003; **89**: 374-384

98 **Ganesh S**, Sier CF, Heerding MM, Griffioen G, Lamers CB, Verspaget HW. Urokinase receptor and colorectal cancer survival. *Lancet* 1994; **344**: 401-402

99 **Ahmed N**, Oliva K, Wang Y, Quinn M, Rice G. Proteomic profiling of proteins associated with urokinase plasminogen activator receptor in a colon cancer cell line using an antisense approach. *Proteomics* 2003; **3**: 288-298

100 **Windham TC**, Parikh NU, Siwak DR, Summy JM, McConkey DJ, Kraker AJ, Gallick GE. Src activation regulates anoikis in human colon tumor cell lines. *Oncogene* 2002; **21**: 7797-7807

101 **Morozevich GE**, Kozlova NI, Chubukina AN, Berman AE. Role of integrin alphavbeta3 in substrate-dependent apoptosis of human intestinal carcinoma cells. *Biochemistry* (Mosc) 2003; **68**: 416-423

102 **Frisch SM**, Francis H. Disruption of epithelial cell-matrix interactions induces apoptosis. *J Cell Biol* 1994; **124**: 619-626

103 **Ohtaka K**, Watanabe S, Iwazaki R, Hirose M, Sato N. Role of extracellular matrix on colonic cancer cell migration and proliferation. *Biochem Biophys Res Commun* 1996; **220**: 346-352

104 **Ebert EC**. Mechanisms of colon cancer binding to substratum and cells. *Dig Dis Sci* 1996; **41**: 1551-1556

105 **Hanahan D**, Folkman J. Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis. *Cell* 1996; **86**: 353-364

106 **Takahashi Y**, Kitadai Y, Bucana CD, Cleary KR, Ellis LM. Expression of vascular endothelial growth factor and its receptor, KDR, correlates with vascularity, metastasis, and proliferation of human colon cancer. *Cancer Res* 1995; **55**: 3964-3968

107 **Tokunaga T**, Oshika Y, Abe Y, Ozeki Y, Sadahiro S, Kijima H, Tsuchida T, Yamazaki H, Ueyama Y, Tamaoki N, Nakamura M. Vascular endothelial growth factor (VEGF) mRNA isoform expression pattern is correlated with liver metastasis and poor prognosis in colon cancer. *Br J Cancer* 1998; **77**: 998-1002

108 **Saito S**, Tsuno N, Nagawa H, Sunami E, Zhengxi J, Osada T, Kitayama J, Shibata Y, Tsuruo T, Muto T. Expression of platelet-derived endothelial cell growth factor correlates with good prognosis in patients with colorectal carcinoma. *Cancer* 2000; **88**: 42-49

109 **Kuramochi H**, Hayashi K, Uchida K, Miyakura S, Shimizu D, Vallböhmer D, Park S, Danenberg KD, Takasaki K, Danenberg PV. Vascular endothelial growth factor messenger RNA expression level is preserved in liver metastases compared with corresponding primary colorectal cancer. *Clin Cancer Res* 2006; **12**: 29-33

110 **Ogawa M**, Yamamoto H, Nagano H, Miyake Y, Sugita Y, Hata T, Kim BN, Ngan CY, Damdinsuren B, Ikenaga M, Ikeda M, Ohue M, Nakamori S, Sekimoto M, Sakon M, Matsuura N, Monden M. Hepatic expression of ANG2 RNA in metastatic colorectal cancer. *Hepatology* 2004; **39**: 528-539

111 **Maeda K**, Nishiguchi Y, Kang SM, Yashiro M, Onoda N, Sawada T, Ishikawa T, Hirakawa K. Expression of thrombospondin-1 inversely correlated with tumor vascularity and hematogenous metastasis in colon cancer. *Oncol Rep* 2001; **8**: 763-766

112 **Mi J**, Sarraf-Yazdi S, Zhang X, Cao Y, Dewhirst MW, Kontos CD, Li CY, Clary BM. A comparison of antiangiogenic therapies for the prevention of liver metastases. *J Surg Res* 2006; **131**: 97-104

113 **Peeters CF**, de Geus LF, Westphal JR, de Waal RM, Ruiter DJ, Wobbes T, Oyen WJ, Ruers TJ. Decrease in circulating anti-angiogenic factors (angiostatin and endostatin) after surgical removal of primary colorectal carcinoma coincides with increased metabolic activity of liver metastases. *Surgery* 2005; **137**: 246-249

114 **Nishizuka I**, Ichikawa Y, Ishikawa T, Kamiyama M, Hasegawa S, Momiyama N, Miyazaki K, Shimada H. Matrilysin stimulates DNA synthesis of cultured vascular endothelial cells and induces angiogenesis *in vivo*. *Cancer Lett* 2001; **173**: 175-182

115 **Lin HC**, Chang JH, Jain S, Gabison EE, Kure T, Kato T, Fukai N, Azar DT. Matrilysin cleavage of corneal collagen type XVIII NC1 domain and generation of a 28-kDa fragment. *Invest Ophthalmol Vis Sci* 2001; **42**: 2517-2524

116 **Lee S**, Jilani SM, Nikolova GV, Carpizo D, Iruela-Arispe ML. Processing of VEGF-A by matrix metalloproteinases regulates bioavailability and vascular patterning in tumors. *J Cell Biol* 2005; **169**: 681-691

117 **Eliceiri BP**, Cheresh DA. Role of alpha v integrins during angiogenesis. *Cancer J* 2000; **6** Suppl 3: S245-S249

118 **Nikfarjam M**, Muralidharan V, Christophi C. Altered growth patterns of colorectal liver metastases after thermal ablation. *Surgery* 2006; **139**: 73-81

119 **Cunningham MP**, Essapen S, Thomas H, Green M, Lovell DP, Topham C, Marks C, Modjtahedi H. Coexpression of the IGF-IR, EGFR and HER-2 is common in colorectal cancer patients. *Int J Oncol* 2006; **28**: 329-335

120 **Park DI**, Kang MS, Oh SJ, Kim HJ, Cho YK, Sohn CI, Jeon WK,

Kim BI, Han WK, Kim H, Ryu SH, Sepulveda AR. HER-2/neu overexpression is an independent prognostic factor in colorectal cancer. *Int J Colorectal Dis* 2007; **22**: 491-497

121 **Saltz LB**, Meropol NJ, Loehrer PJ, Needle MN, Kopit J, Mayer RJ. Phase II trial of cetuximab in patients with refractory colorectal cancer that expresses the epidermal growth factor receptor. *J Clin Oncol* 2004; **22**: 1201-1208

122 **Cunningham MP**, Essapen S, Thomas H, Green M, Lovell DP, Topham C, Marks C, Modjtahedi H. Coexpression, prognostic significance and predictive value of EGFR, EGFRvIII and phosphorylated EGFR in colorectal cancer. *Int J Oncol* 2005; **27**: 317-325

123 **Delektorskaia VV**, Perevoshchikov AG, Kushlinskiĭ NE. Expression of nm23 and c-erbB-2 proteins in cells of primary colorectal cancer and its metastases. *Arkh Patol* 2003; **65**: 11-15

124 **Italiano A**, Saint-Paul MC, Caroli-Bosc FX, François E, Bourgeon A, Benchimol D, Gugenheim J, Michiels JF. Epidermal growth factor receptor (EGFR) status in primary colorectal tumors correlates with EGFR expression in related metastatic sites: biological and clinical implications. *Ann Oncol* 2005; **16**: 1503-1507

125 **Scartozzi M**, Bearzi I, Berardi R, Mandolesi A, Fabris G, Cascinu S. Epidermal growth factor receptor (EGFR) status in primary colorectal tumors does not correlate with EGFR expression in related metastatic sites: implications for treatment with EGFR-targeted monoclonal antibodies. *J Clin Oncol* 2004; **22**: 4772-4778

126 **Bralet MP**, Paule B, Adam R, Guettier C. Loss of epidermal growth factor receptor expression in lymph node and liver metastases of colon carcinoma. *J Clin Oncol* 2005; **23**: 5844; author reply 5844-5845

127 **Kountourakis P**, Pavlakis K, Psyrri A, Rontogianni D, Xiros N, Patsouris E, Pectasides D, Economopoulos T. Clinicopathologic significance of EGFR and Her-2/neu in colorectal adenocarcinomas. *Cancer J* 2006; **12**: 229-236

128 **Bolen JB**, Veillette A, Schwartz AM, Deseau V, Rosen N. Analysis of pp60c-src in human colon carcinoma and normal human colon mucosal cells. *Oncogene Res* 1987; **1**: 149-168

129 **Talamonti MS**, Roh MS, Curley SA, Gallick GE. Increase in activity and level of pp60c-src in progressive stages of human colorectal cancer. *J Clin Invest* 1993; **91**: 53-60

130 **Buchanan FG**, Gorden DL, Matta P, Shi Q, Matrisian LM, DuBois RN. Role of beta-arrestin 1 in the metastatic progression of colorectal cancer. *Proc Natl Acad Sci USA* 2006; **103**: 1492-1497

131 **Kashii Y**, Giorda R, Herberman RB, Whiteside TL, Vujanovic NL. Constitutive expression and role of the TNF family ligands in apoptotic killing of tumor cells by human NK cells. *J Immunol* 1999; **163**: 5358-5366

132 **Belluco C**, Esposito G, Bertorelle R, Alaggio R, Giacomelli L, Bianchi LC, Nitti D, Lise M. Fas ligand is up-regulated during the colorectal adenoma-carcinoma sequence. *Eur J Surg Oncol* 2002; **28**: 120-125

133 **Ogawa S**, Nagao M, Kanehiro H, Hisanaga M, Ko S, Ikeda N, Nakajima Y. The breakdown of apoptotic mechanism in the development and progression of colorectal carcinoma. *Anticancer Res* 2004; **24**: 1569-1579

134 **Liu K**, McDuffie E, Abrams SI. Exposure of human primary colon carcinoma cells to anti-Fas interactions influences the emergence of pre-existing Fas-resistant metastatic subpopulations. *J Immunol* 2003; **171**: 4164-4174

135 **Onodera H**, Mori A, Nagayama S, Fujimoto A, Tachibana T, Yonenaga Y, Tsuruyama T. Fas/CD95 signaling rather than angiogenesis or proliferative activity is a useful prognostic factor in patients with resected liver metastases from colorectal cancer. *Int J Colorectal Dis* 2005; **20**: 477-484

136 **Hague A**, Hicks DJ, Hasan F, Smartt H, Cohen GM, Paraskeva C, MacFarlane M. Increased sensitivity to TRAIL-induced apoptosis occurs during the adenoma to carcinoma transition of colorectal carcinogenesis. *Br J Cancer* 2005; **92**: 736-742

137 **Velthuis JH**, Stitzinger M, Aalbers RI, de Bont HJ, Mulder GJ, Kuppen PJ, Nagelkerke JF. Rat colon carcinoma cells that survived systemic immune surveillance are less sensitive to NK-cell mediated apoptosis. *Clin Exp Metastasis* 2003; **20**: 713-721

138 **Cerottini JP**, Caplin S, Saraga E, Givel JC, Benhattar J. The type of K-ras mutation determines prognosis in colorectal cancer. *Am J Surg* 1998; **175**: 198-202

139 **Tortola S**, Marcuello E, González I, Reyes G, Arribas R, Aiza G, Sancho FJ, Peinado MA, Capella G. p53 and K-ras gene mutations correlate with tumor aggressiveness but are not of routine prognostic value in colorectal cancer. *J Clin Oncol* 1999; **17**: 1375-1381

140 **Remvikos Y**, Tominaga O, Hammel P, Laurent-Puig P, Salmon RJ, Dutrillaux B, Thomas G. Increased p53 protein content of colorectal tumours correlates with poor survival. *Br J Cancer* 1992; **66**: 758-764

141 **Bosari S**, Viale G, Bossi P, Maggioni M, Coggi G, Murray JJ, Lee AK. Cytoplasmic accumulation of p53 protein: an independent prognostic indicator in colorectal adenocarcinomas. *J Natl Cancer Inst* 1994; **86**: 681-687

142 **Kressner U**, Bjørheim J, Westring S, Wahlberg SS, Påhlman L, Glimelius B, Lindmark G, Lindblom A, Børresen-Dale AL. Ki-ras mutations and prognosis in colorectal cancer. *Eur J Cancer* 1998; **34**: 518-521

143 **Hedrick L**, Cho KR, Fearon ER, Wu TC, Kinzler KW, Vogelstein B. The DCC gene product in cellular differentiation and colorectal tumorigenesis. *Genes Dev* 1994; **8**: 1174-1183

144 **Kataoka M**, Okabayashi T, Johira H, Nakatani S, Nakashima A, Takeda A, Nishizaki M, Orita K, Tanaka N. Aberration of p53 and DCC in gastric and colorectal cancer. *Oncol Rep* 2000; **7**: 99-103

145 **Sun XF**, Rütten S, Zhang H, Nordenskjöld B. Expression of the deleted in colorectal cancer gene is related to prognosis in DNA diploid and low proliferative colorectal adenocarcinoma. *J Clin Oncol* 1999; **17**: 1745-1750

146 **Saito M**, Yamaguchi A, Goi T, Tsuchiyama T, Nakagawara G, Urano T, Shiku H, Furukawa K. Expression of DCC protein in colorectal tumors and its relationship to tumor progression and metastasis. *Oncology* 1999; **56**: 134-141

147 **Yamaguchi A**, Urano T, Fushida S, Furukawa K, Nishimura G, Yonemura Y, Miyazaki I, Nakagawara G, Shiku H. Inverse association of nm23-H1 expression by colorectal cancer with liver metastasis. *Br J Cancer* 1993; **68**: 1020-1024

148 **Ayhan A**, Yasui W, Yokozaki H, Kitadai Y, Tahara E. Reduced expression of nm23 protein is associated with advanced tumor stage and distant metastases in human colorectal carcinomas. *Virchows Arch B Cell Pathol Incl Mol Pathol* 1993; **63**: 213-218

149 **Wang L**, Patel U, Ghosh L, Chen HC, Banerjee S. Mutation in the nm23 gene is associated with metastasis in colorectal cancer. *Cancer Res* 1993; **53**: 3652

150 **Indinnimeo M**, Giarnieri E, Stazi A, Cicchini C, Brozzetti S, Valli C, Carreca I, Vecchione A. Early stage human colorectal cancer: prognostic value of nm23-H1 protein overexpression. *Cancer Lett* 1997; **111**: 1-5

151 **Berney CR**, Yang JL, Fisher RJ, Russell PJ, Crowe PJ. Overexpression of nm23 protein assessed by color video image analysis in metastatic colorectal cancer: correlation with reduced patient survival. *World J Surg* 1998; **22**: 484-490

152 **Soliani P**, Ziegler S, Romani A, Corcione L, Campanini N, Dell'Abate P, Del Rio P, Sianesi M. Prognostic significance of nm23 gene product expression in patients with colorectal carcinoma treated with radical intent. *Oncol Rep* 2004; **11**: 1193-1200

153 **Lindmark G**. NM-23 H1 immunohistochemistry is not useful as predictor of metastatic potential of colorectal cancer. *Br J Cancer* 1996; **74**: 1413-1418

154 **Tabuchi Y**, Nakamura T, Kuniyasu T, Ohno M, Nakae S. Expression of nm23-H1 in colorectal cancer: no association with metastases, histological stage, or survival. *Surg Today* 1999; **29**: 116-120

155 **Saha S**, Bardelli A, Buckhaults P, Velculescu VE, Rago C, St Croix B, Romans KE, Choti MA, Lengauer C, Kinzler KW, Vogelstein B. A phosphatase associated with metastasis of colorectal cancer. *Science* 2001; **294**: 1343-1346

156 **Bardelli A**, Saha S, Sager JA, Romans KE, Xin B, Markowitz

SD, Lengauer C, Velculescu VE, Kinzler KW, Vogelstein B. PRL-3 expression in metastatic cancers. *Clin Cancer Res* 2003; **9**: 5607-5615

157 **Peng L**, Ning J, Meng L, Shou C. The association of the expression level of protein tyrosine phosphatase PRL-3 protein with liver metastasis and prognosis of patients with colorectal cancer. *J Cancer Res Clin Oncol* 2004; **130**: 521-526

158 **Kato H**, Semba S, Miskad UA, Seo Y, Kasuga M, Yokozaki H. High expression of PRL-3 promotes cancer cell motility and liver metastasis in human colorectal cancer: a predictive molecular marker of metachronous liver and lung metastases. *Clin Cancer Res* 2004; **10**: 7318-7328

159 **Peng L**, Jin G, Wang L, Guo J, Meng L, Shou C. Identification of integrin alpha1 as an interacting protein of protein tyrosine phosphatase PRL-3. *Biochem Biophys Res Commun* 2006; **342**: 179-183

160 **Fiordalisi JJ**, Keller PJ, Cox AD. PRL tyrosine phosphatases regulate rho family GTPases to promote invasion and motility. *Cancer Res* 2006; **66**: 3153-3161

S- Editor Wang GP **L- Editor** Lutze M **E- Editor** Ma WH