Review

Tumor Markers in Cancer Patients. An Update of their Prognostic Significance. Part II

N.J. AGNANTIS, A.C. GOUSSIA, A. BATISTATOU and D. STEFANOU

Department of Pathology, Medical School, University of Ioannina, 45110 Ioannina, Greece

Abstract. *The primary determinant of outcome in patients with cancer is the development of distant metastasis. Metastasis is a multistep process involving disruption of cell-matrix adhesion, dissolution of the extracellular matrix, angiogenesis, invasion in the blood vessel wall, extravasation and establishment of a secondary growth. Nowadays, a large number of biochemical and cell biological studies have indicated the important role of extacellular matrix adhesion molecules, proteinases and angiogenic factors in the dissemination of cancer. Cell adhesion molecules, such as integrins, E-cadherin, catenins and CD44 appear to have some prognostic significance, especially in gastric, colorectal and lung cancer patients. Since matrix degrading proteinases are involved in cancer spread, they should be good candidates as prognostic factors. The proteinase which has been investigated in greatest detail is uPA in breast cancer. As a marker of cancer, its main value is to aid in selecting the subgroups of node-negative breast cancer patients that are unlikely to benefit from adjuvant chemotherapy. Cathepsin D and metalloproteinases (MMPs) look promising prognostic markers but further work is needed to establish their utility. Intratumoral angiogenesis is a putative prognostic indicator for some types of cancer. High expression of the angiogenic factor VEGF is associated with angiogenesis and an unfavourable survival.*

The most characteristic phenomenon of malignant tumors is their metastatic ability. Despite advances in surgery and patient management, most deaths are caused by metastasis. Recent developments in molecular biology have demonstrated that the metastatic process is related to the interactions of tumor cells and micro-environmental factors such as adhesion molecules, proteolytic enzymes and angiogenic factors.

Adhesion molecules

Cell adhesion events are thought to play an important role in tumor metastasis. Knowledge of molecular mechanisms in cell-cell and cell-matrix adhesion has increased dramatically during the past decade. It is now evident that interactions between tumor cells and neighbouring elements, such as matrix and endothelium, determine invasion and metastasis (1).

Various molecules on the tumor cell surface mediate these interactions, either directly, like integrins or other adhesion molecules, or as receptors for various growth factors. Integrin-mediated interactions are among the most important determinants for organ-specific metastasis.

In colorectal cancer, significant correlations between integrin expression and tumor progression as well as clinical stage have been reported (2). The transformation from benign to malignant neoplasms was characterized by diminished expression of α_6-, β_1- and β_4-integrin subunits (2). Reduced α_2- integrin expression was statistically associated with advanced colorectal cancer stages. A strong correlation was also observed between the expression of the α_6-laminin and the degree of tumor differentiation, the invasive properties and the metastatic ability (3).

Serum E-selectin levels have been found elevated in patients with metastasis compared to those without (4). However, studies on the prognostic role 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. The expression of E-cadherin and catenin is down-regulated in poorly-differentiated colon cancer cells. Immunohistochemical loss or heterogeneous expression of E-cadherin in colorectal

Correspondence to: Niki J. Agnantis, MD, PhD, FRCPath, Professor of Pathology, Director and Chairman, Department of Pathology, Medical School, University of Ioannina, University Campus, 45110 Ioannina, Greece. Tel: ++ 30 26510 97792, Fax: ++ 30 26510 97858, e-mail: nagnanti@cc.uoi.gr

Key Words: Tumor markers, breast cancer, prognosis.

cancer cases has been closely related to advanced clinical stage, widespread lymph node and distant metastases, as well as to venous invasion (5). It was also significantly associated with an increased incidence of recurrence and reduced overall and disease-free survival. Significant down-regulation of α-catenin expression has been associated with higher metastatic potential and unfavourable prognosis.

Up-regulation of CD44 has been observed as a very early event in the progression of colon carcinomas (6). Several reports have mentioned that CD44 expression is related to a higher metastatic potential of the tumors. Correlations between CD44 expression or its variants and survival have recently been demonstrated, but in other studies these markers did not emerge as prognostic factors (7,8).

In gastric carcinomas, CD44 expression and especially the CD44-9v isoform has been correlated with poor tumor differentiation, lymph node metastasis and hepatic metastasis (9-11). A significant survival advantage in patients with low expression of CD44mRNA compared to those with high expression has been reported.

Joensu *et al.* were the first investigators researching immunohistochemically the correlation between CD44 expression and breast cancer patients outcome (12). In their study of 198 adenocarcinomas, they found CD44 positivity more often in estrogen receptor- negative cases and in poorly-differentiated tumors. In addition, patients with node-positive breast cancers and high CD44 expression had an adverse outcome. However, there are conflicting results in literature with other studies showing no correlation between CD44 and prognosis (13).

In lung cancer, reduced E-cadherin expression has been associated with tumor dedifferentiation, lymph node metastasis, advanced disease stage and poor prognosis (14-16). Reduced expression of both E-cadherin and β-catenin was a significantly unfavourable prognostic marker (14). Moreover, a significantly lower survival rate for patients with reduced β-catenin expression has been noticed.

In pancreatic carcinoma, the expression of E-cadherin was inversely correlated with tumor progression and the development of metastasis (17). Increased expression of CD44 and especially its variants 6 and 2 has been associated with decreased overall survival (18). However, Gansauge *et al.* found that the low levels of soluble CD44 variant 6 predict poor patient prognosis (19).

Low expression of E-cadherin has been correlated with early recurrence in cases of hepatocellular carcinoma (20). Patients with E-cadherin, α-catenin and γ-catenin underexpression as well as with β-catenin overexpression had poor survival. (20). CD44 overexpression has been correlated with poor histological grade (21).

An inverse correlation between E-cadherin expression and grade as well stage of prostate cancers has been reported (22). Studies analysing both E-cadherin and β-

catenin expression correlate their expression with improved survival (23). Loss of CD44 expression in radical prostatectomy specimens was an independent predictor for recurrence (24). Down-regulation of CD44 and CD44v6 assessed on archival needle biopsies was predictive of poor outcome (25).

In some cases of renal carcinomas, CD44 expression was up-regulated during tumor progression (26). No significant associations of CD44 with any prognostic markers has been reported in endometrial carcinomas (27).

Proteolytic enzymes

The biological functions of proteinases in cancer progression include activation of latent growth factors or growth factors anchored to the extracellular matrix (ECM) and dissolution of the ECM and basement membranes (BM), thereby governing the migration of cancer cells and cell to cell or cell to matrix attachment. In addition, fragments formed following proteolytic digestion of the ECM and BM may act as chemokines or angiogenesis-modulated factors (28,29).

Several different families of proteinases have been identified *e.g.,* the matrix metalloproteinases, the serine (plasminogen activator, cathepsin G, elastase), the aspartyl (pepsin A, retropepsin, cathepsins D and E) and the cysteine proteases (cathepsin B, H, L and S).

As proteases are causally involved in cancer spread, their activities or concentrations in primary carcinomas might be expected to correlate with metastatic potential and thus with patient prognosis. Over the past years numerous studies have tested this issue and their results have shown that certain proteases are among the most powerful biological prognostic factors for cancer described to date. In addition, selective inhibition of tumor-associated proteases should have a significant impact on treating cancer.

Among the proteases, the matrix metalloproteinases (MMPs) and the plasminogen activation system (uPA) are of particular interest, not only due to their increased expression correlated with the invasiveness of several tumor types, but also because their down-regulation by inhibitors (TIMPs, uPAI) has been shown to reduce the invasiveness in manipulated cell lines (30).

Most of the work relating MMPs to prognosis has been carried out with gastrointestinal cancers. Thus, high levels of MMP-2 and MMP-9 have been shown to predict adverse outcome in patients with gastric cancers, while high concentrations of both MMP-1 and MMP-9 were related to poor prognosis in colorectal cancer (31). In breast carcinomas, MMPs are expressed at higher levels than in precancerous or normal breast tissues (32). Increasing levels of MMP-2 are found as lesions progress from dysplasia through carcinoma *in situ*. MMP-9 is expressed more

strongly in larger tumors but it does not appear from published reports to be a likely candidate as a tumor marker of metastasis or early relapse. Paradoxically, high levels of TIP-1 and TIMP-2 have also been linked to metastatic spread and poor prognosis, especially in breast cancer patients (33,34). Although this seems to contradict the hypothesis that MMPs activity promotes tumor invasion, it may reflect the need for some regulation of the increased metalloproteinase activity.

MMP-2, MMP-9 and their inhibitors have been found to be related to recurrence and survival after hepatocellular carcinoma resection (35). Moreover, in pancreatic carcinomas, high expression of MMP-2 and MMP-9 has been shown to be correlated with invasion and metastasis (36).

The urokinase plasminogen activator (uPA), its receptor (uPAR) or its plasminogen activator inhibitor-1 (PAI-1) play a key role in cancer invasion and metastasis. The PAI-1 protein is a multifaceted proteolytic factor. It not only functions as an inhibitor of the uPA, but also plays an important role in signal transduction, cell adherence and cell migration. Thus, an apparent paradox exists considering its name – although it inhibits uPA during blood coagulation, it actually promotes invasion and metastasis. Many studies have consistently associated high breast tumor levels of either or both factors with poor prognosis, including the subgroup with node-negative breast cancer patients (37-39). In clinical practice, the main application of these two markers is likely to be selecting the indolent node-negative breast cancer patients who do not need adjuvant chemotherapy. High levels of PAI-2, another inhibitor of uPA, have been shown to be associated with improved outcome. A dissemination risk index that takes into account the proteolytic activity of uPA and the two inhibitors has been proposed (40).

Although not extensively investigated in breast cancer, several studies have reported a correlation between high levels of uPA and poor prognosis in patients with colorectal cancer (41). High levels of uPA were also found to correlate with aggressive disease in patients with both gastric and esophageal cancers (41). Elevated uPA and PAI-1 levels were shown to be associated with poor prognosis, predicting shorter survival in gastric cancer patients (41). In preliminary studies, uPA has been shown to be a prognostic indicator in a variety of other cancers such as ovarian, renal, hepatocellular, pancreatic and lung cancer (41).

Although not as widely investigated as uPA, high levels of cathepsin B have been found to correlate with aggressive disease in multiple types of tumors, including breast, colorectal and lung carcinomas (42-45). Most of the studies are retrospective and, with one exception, contain relatively low numbers of patients. In only one large study of 1500 patients, cathepsin B was shown to be an independent prognostic marker for both relapse-free and overall survival

in patients with breast cancer (44). However, cathepsin B does not predict outcome as strongly as uPA (45).

Almost all the published data relating to the prognostic value for cathepsin D have focused on breast cancer. In most studies high levels of cathepsin D have been related to poor prognosis (46,47). Recently, the prognostic value of cathepsin D was confirmed in a meta-analysis of 11 published studies containing approximately 2700 patients (48). In this work, high levels of cathepsin D, detected by immunoradiometric assay, predicted a worse outcome in patients free of nodal metastasis. In contrast, the immunohistochemical detection of cathepsin D has led to conflicting results regarding the prognostic value of cathepsin D (46,49). In some studies, its immuno-histochemical overexpression has been associated with high histological grade, lymph node positivity, increased risk of recurrence and reduced disease-free survival. However, other studies did not find any correlation between cathepsin D and prognosis.

Angiogenesis and angiogenesis-related markers

The role of angiogenesis in the growth, progression and metastatic spread of solid tumors is well established. Formation of new blood vessels within the tumor stroma is an essential requirement for the growth of neoplasms. Several studies have shown that neoangiogenesis is also crucial for tumor progression and metastatic spread; in other studies, however, the opposite conclusion has been drawn. The most commonly used measure of angiogenesis is the immunohistochemical detection of intratumoral blood vessels stained by anti-CD31, anti-CD34 and anti-Factor VIII antibodies. The conflicting conclusions may be due to the varying methods used to measure intratumor microvessel density (MVD) and the significant interobserver variation that exists in interpretation of the number of positive vessels and the optimal way in which fields are selected (hot spot *versus* general counting of vessels).

In recent years, many angiogenesis-related markers such as vascular endothelial growth factor (VEGF), basic fibroblast growth factor (b-FGF), platelet-derived endothelial cell growth factor (PD-ECGF), thrombospondin (TSP), angiogenin and endostatin (ES) levels have been evaluated and in some studies they have been found to be related to prognosis.

In breast cancer patients most studies have reported a relationship between high vessel counts and a worse clinical outcome, although no association with prognosis was found in others (50-52). Weidner *et al.* counted microvessels in the most densely vascularized areas of 49 breast cancer cases and found a correlation between the frequency of metastasis and the number and density of vessels (50). Subsequent studies by Horak *et al.* (51) and Fox *et al.* (53) confirmed the above

finding. Weidner *et al.,* in his series of 165 breast cancer patients with a median follow-up period of 4 years, found significant associations between increased MVD and relapse-free as well as overall survival (50). An association with nodal and distant metastases was also observed. By multivariate analysis, neoangiogenesis had been found to be an independent prognostic marker in both relapse-free and overall survival (50). Increased VEGF immunohistochemical expression or VEGF mRNA up-regulation has been correlated with worse survival, however most data are related to small retrospective studies (54-56).

Macchiarini *et al.* reported the first data concerning the role of angiogenesis in early stages of non-small cell lung cancers (NSCLC) (57). High MVD has been correlated with a high rate of metastasis, while in multivariate analysis high MVD was an independent marker of poor prognosis (57). In subsequent studies a significant association between MVD and the rate of relapse and poor outcome was demonstrated (58,59). Fontanini *et al.* reported an association between high MVD and lymph node metastasis, distant metastasis and survival (58). In the study of Lucchi *et al.* on 227 patients with surgically treated stage I NSCLC, the investigators found that MVD was associated with overall and disease-free survival (60). In addition a correlation with histological type was reported, since a high MVD was observed more frequently in adenocarcinomas than in squamous cell carcinomas. In the recent studies of Giatromanolaki *et al.*, high MVD was related to a high metastatic ability and a poor outcome (61,62). Moreover, in the same study the prognostic significance of MVD was independent of p53 and Ki-67 expression.

High VEGF expression has been correlated with a shorter overall and recurrence-free survival in some studies, but not in others (63,64). In addition, VEGF was correlated with poor prognosis only in patients with squamous cell carcinomas (65), but Ohta *et al.* found a similar association in both squamous cell carcinomas and adenocarcinomas (66). Moreover, VEGF has been correlated with high MVD in some studies (16,67) as well as with mutant p53 expression (67).

In a large study of 223 NSCLC cases, PD-ECGF was significantly co-expressed with VEGF and both factors were associated with high angiogenesis and poor survival (68). A similar correlation between PD-ECGF and VEGF has been reported by Volm *et al.* (69) but not by others (70).

bFGF and bFGF-receptor-1 expression has been associated with poor patient prognosis in several studies (71,72). Other investigations found a significant correlation with patient outcome only for bFGF-receptor-1 but not for bFGF (72). In contrast, high serum levels of bFGF were found to be related to a better outcome (73).

The studies on the prognostic role of MVD in colorectal carcinoma have produced inconclusive and conflicting results (74-77). Frank *et al.* found a significant association between tumor angiogenesis and survival (74). In addition, a significant association between MVD and hematogeneous metastasis was observed. The significant association of high MVD with survival found in node-negative patients suggested that angiogenesis is an early step in colorectal tumor progression. In contrast, Lindmark *et al.* showed an adverse prognostic value of angiogenesis since higher MVD was associated with longer survival (75), while in a recent study no prognostic significance was reported (78).

In gastric cancer, a correlation between increased vascularity and poor prognosis as well as with hematogeneous metastases and the rate of recurrence has been reported (79,80) In addition, high VEGF expression has been correlated with hematogeneous metastasis and poor patient prognosis (79). Moreover, PD-ECGF has been associated with proliferation activity and poor clinical outcome. There are no data about the prognostic significance of b-FGF in gastric cancer patients, however the expression of b-FGF and its receptors has been correlated with angiogenesis in several studies.

In bladder cancer, an association between MVD and tumor stage, the presence of vascular invasion and survival has been demonstrated in cases of various stages of the disease (81,82). Particularly, in invasive tumors an increase of mortality and an increased rate of lymph node metastasis has been reported. Similarly, in a current study, an association between high MVD and recurrence as well as shorter survival has been demonstrated (83). VEGF mRNA has been found to be related to high rate of recurrence, while other investigators did not demonstrate any such correlation (84,85). Interestingly, higher VEGF mRNA levels were found in superficial rather than in invasive tumors, suggesting a probable role of VEGF in the early stages of the disease.

Elevated PD-ECGF mRNA has been found in invasive rather than in superficial tumors (86,87). Moreover, in superficial cases increased PD-ECGF expression has been related to early recurrence and short survival (87). Interestingly, data in the urine of patients with bladder cancer have shown that a high concentration of b-FGF is correlated with tumor stage (88).

In patients with endometrial cancer, high MVD was associated with tumor grade, depth of myometrial invasion, lymph node involvement, disease stage and poor prognosis (89-91). However, other studies have not reported the same results (92). In addition, VEGF has been associated with high MVD and poor outcome (91). Interestingly, it has been shown that the ratio VEGF/flk-1 (KDR) was a more important marker of poor survival than VEGF alone (93). Sivridis *et al.* failed to show any association between PD-ECGF reactivity in cancerous endometrial lesions and prognostic factors (94). In contrast, stromal PD-ECGF reactivity was related to invasion and advanced stage.

Similarly, Seki *et al.* did not find any relationship between PD-ECGF and prognosis (90).

In hepatocellular carcinoma, high MVD has been found to be closely associated with tumor size, disease recurrence and disease-free survival (95,96). In serum samples, high VEGF was correlated with vessel invasion, advanced stage and metastasis (97). Moreover, PD-ECGF expression was related to vessel invasion (98).

MVD was recently reported to be highly predictive of survival in low stage renal tumors (99). Studies in pancreatic cancer did not show any prognostic value for MVD (100). In contrast, the expression of PD-ECGF was associated with reduced survival. No prognostic significance for VEGF expression was reported in most investigations and only one demonstrated a relationship to shorter survival (100,101).

Conclusion

The establishment of prognostic markers for cancer is useful to determine prognosis as well as suitable adjuvant therapies. Thus, the selection of patients based on their prognosis may lead to more appropriate management.

Numerous cell surface molecules have been identified that are functionally involved in malignant transformation, tumor progression and the development of metastases. Adhesion molecules such as integrins, E-cadherin and its intracellular partner proteins α- and β-catenin and CD44 appear to have some prognostic significance, especially in gastric, colorectal and lung cancer patients. The value of these markers needs further investigation and future studies must focus on multimarker comparisons and clinical outcome correlation.

Proteases, such as uPA and its receptor, MMPs and cathepsins have all been associated with cancer patient prognosis. Interestingly, high levels of their inhibitors are also associated with shorter survival. uPA and its inhibitor PAI-1 are the strongest prognostic factors for breast cancer patients next to lymph node status, associated with both disease-free and overall survival. Nowdays, there is a strong evidence to recommend routine uPA/PAI-1 testing in breast cancer, particularly in node-negative patients, in order to guide clinicians towards appropriate treatment strategies. Currently, researchers are exploring whether drugs targeting uPA and /or PAI-1 can also help in the treatment of cancer patients.

Finally, assessment of angiogenesis and angiogenic factors such as VEGF and PD-ECGF further contribute to the identification of high-risk cancer patients.

References

1   Bruun JA, Bergijk EC, Kootstra CJ and de Heer E: Fatal attraction: adhesion molecules and disease. J Pathol *182*: 371-372, 1997.

2   Haier J, Nasralla M and Nicolson GL: Cell surface molecules and their prognostic values in assessing colorectal carcinomas. Ann Surg *231*: 11-24, 1999.

3   Streit M, Schmidt R, Hilgenfeld RU, Thiel E and Kreuser ED: Adhesion receptors in malignant transformation and dissemination of gastrointestinal tumors. Recent Results Cancer *142*: 19-50, 1996.

4   Alexiou D, Karayiannakis AJ, Syrigos KN, Zbar A, Kremmyda A, Bramis I and Tsigris C: Serum levels of E-selectin, ICAM-1 and VCAM-1 in colorectal cancer patients: correlations with clinicopathological features, patient survival and tumour surgery. Eur J Cancer *37*: 2392-2397, 2001.

5   Kaihara T, Kusaka T, Nishi M, Kawamata H, Imura J, Kitajima K, Itoh-Minami R, Aoyama N, Kasuga M, Oda Y, Hattozi M and Fujimozi T: Dedifferentiation and decreased expression of adhesion molecules E-cadherin and ZO-1 in colorectal cancer are closely related to liver metastasis. J Exp Clin Cancer Res *22*: 117-123, 2003.

6   Kim H, Yang X-L, Rosada C, Hamilton SR and August JT: CD44 expression in colorectal adenomas in an early event occurring prior to K-ras and p53 gene mutation. Archives Biochem Biophys *310*: 504-507, 1994.

7   Mulder J-WR, Kruyt PM, Sewnath M, Oosting J, Seldenrijk CA, Weidema WF, Offerhaus GJ and Pals ST: Colorectal cancer prognosis and expression of exon-v6-containing CD44 proteins. Lancet *344*: 1470-1472, 1994.

8   Koretz K, Moller P, Lehnert T, Hinz U, Otto HF and Herfarth C: Effect of CD44v6 on survival in colorectal carcinoma. Lancet *345*: 327-328, 1995.

9   Ichikawa W: Positive relationship between expression of CD44 and hepatic metastases in colorectal cancer. Pathobiology *62*: 172-179, 1994.

10 Washington K, Gottfried MR and Telen MJ: Expression of the adhesion molecule CD44 in gastric adenocarcinomas. Hum Pathol *25*: 1043-1049, 1994.

11 Goussia A, Ioachim E, Agnantis NJ, Zagorianakou P, Vougiouklakis Th, Tsianos E and Kappas A: Expression of cell adhesion molecule CD44 in human gastric cancer. A correlation with p53 and Rb proteins, the HLA-DR antigen, and the proliferative activity. J Tumor Marker Oncol *15*: 129-135, 2000.

12 Joensuu H, Klemi PJ, Toikkanen S and Jalkanen S: Glycoprotein CD44 expression and its association with survival in breast cancer. Am J Pathol *143*: 867-874, 1993.

13 Zoller M and Kaufman M: CD44 and metastasis. Onkologie *17*: 114-122, 1994.

14 Kase S, Sugio K, Yamazaki K, Okamoto T, Yano T and Sugimachi K: Expression of E-cadherin and β-catenin in human non-small cell lung cancer and the clinical significance. Clin Cancer Res *6*: 4789-4796, 2000.

15 Bremmes RM, Veve R, Gabrielson E, Hirsch FR, Baron A, Bemis L, Gemmill RM, Drabkin HA and Franklin WA: High-throughput tissue microarray analysis used to evaluate biology and prognostic significance of the E-cadherin pathway in non-small cell lung cancer. J Clin Oncol *15*: 2417-2428, 2002.

16 Stefanou S, Goussia A, Arkoumani E and Agnantis NJ: Expression of vascular endothelial growth factor and E-cadherin in non-small cell lung cancer. Anticancer Res *23*: 4715-4720, 2003.

17 Kuniyasu H, Ellis LM, Evans DB, AbbruzzeseJL, Fenoglio CJ, Bucana CD, Cleary KR, Tahara E and Fidler IJ: Relative expression of E-cadherin and type IV collagenase genes predicts disease outcome in patients with resectable pancreatic carcinoma. Clin Cancer Res *5*: 25-33, 1999.

18 Gotoda T, Matsumura Y, Kondo H, Saitoh D, Shimada Y, Kosuge T, Kanai Y and Kakizoe T: Expression of CD44 variants and its association with survival in pancreatic cancer. Jpn J Cancer Res *89*: 1033-1040, 1998.

19 Gansauge F, Gansauge S, Rau B, Scheiblich A, Poch B, Schoenberg MH and Beger HG: Low serum levels of soluble CD44 variant 6 are significantly associated with poor prognosis in patients with pancreatic carcinoma. Cancer *80*: 1733-1739, 1997.

20 Endo K, Ueda T, Ueyama J, Ohta T and Terada T: Immunoreactive E-cadherin, alpha-catenin, beta-catenin, and gamma-catenin proteins in hepatocellular carcinoma: relationships with tumor grade, clinicopathologic parameters, and patients survival. Hum Pathol *31*: 558-565, 2000.

21 Endo K and Terada T: Protein expression of CD44 (standard and variant isoforms) in hepatocellular carcinoma: relationships with tumor grade, clinicopathological parameters, p53 expressio, and patient survival. J Hepatol *32*: 78-84, 2000.

22 Verhagen PCMS, Tilanus MGJ, de Weger RA, van Moorselaar RJA, van den Tweel JG and Boon TA: Prognostic factors in localised prostate cancer with emphasis on the application of molecular techniques. Eur Oncol *41*: 363-371, 2002.

23 Vis AN, Noordzij MA, Fitoz K, Wildhagen MF, Schroder FH and van der Kwast TH: Prognostic value of cell cycle proteins p27kip1 and MIB-1, and the adhesion protein CD44s in surgically treated patients with prostate cancer. J Urol *164*: 2156-2161, 2000.

24 Richmond PJ, Karayiannakis AJ, Nagafuchi A, Kaisary AV and Pignatelli M: Aberrant E-cadherin and alpha-catenin expression in prostate cancer: correlation with patient survival. Cancer Res *57*: 3189-3193, 1997.

25 Aaltomaa S, Lipponen P, Ala-Opas M and Kosma VM: Expression and prognostic value of CD44 standard and variant v3 and v6 isoforms in prostate cancer. Eur Urol *39*: 138-144, 2001.

26 Gunthert U, Stauder R, Mayer B, Terpe H-J, Finke L and Friedrichs K: Are CD44 isoforms involved in human tumor progression? Cancer Surveys *24*: 19-42, 1995.

27 Saegusa M, Hashimura M and Okayasu I: CD44 expression in normal, hyperplastic, and malignant endometrium. J Pathol *184*: 297-306, 1998.

28 Cottam DW and Rees RC: Regulation of matrix metalloproteinases: their role in tumor invasion and metastasis. Int J Oncol *2*: 861-872, 1993.

29 Slater M: Dynamic interactions of the extracellular matrix. Histol Histopathol *11*: 175-180, 1996.

30 Cox G and O'Byrne KJ: Metalloproteinases and cancer. Anticancer Res *21*: 4207-4220, 2001.

31 Duffy MJ and McCarthy K: Matrix metalloproteinases in cancer: prognostic markers and targets for therapy. Int J Oncol *12*: 1343-1348, 1998.

32 Garbett EA, Reed MWR and Brown NJ: Proteolysis in human breast and colorectal cancer. Br J Cancer *81*: 287-293, 1999.

33 McCarthy K, Maguire T, McGrael G, McDermott E, O'Higgins N and Duffy MJ: High levels of tissue inhibitor of metalloproteinase-1 predicts poor outcome in patients with breast cancer. Int J Cancer *84*: 44-48, 1999.

34 Remackle A, McCarthy K, Noel A, Maguire T, McDermott E, O'Higgins N, Foidart JM and Duffy MJ: High levels of TIMP-2 correlate with adverse prognosis in breast cancer. Int J Cancer *89*: 118-121, 2000.

35 Sakamoto Y, Mafune K, Mori M, Shiraishi T, Imamura H, Mori M, Takayama T and Makuuchi M: Overexpression of MMP-9 correlates with growth of small hepatocellular carcinoma. Int J Oncol *17*: 237-243, 2000.

36 Koshiba T, Hostani R, Wada M, Miyamoto Y, Fujimoto K, Lee JU, Doi R, Arii S and Imamura M: Involvement of MMP2 activity in invasion and metastases of pancreatic cancer. Cancer *82*: 642-650, 1998.

37 Duffy MJ: Proteases as prognostic markers in cancer. Clin Cancer Res *2*: 613-618, 1996.

38 Look MP and Foekens JA: Clinical relevance of the urokinase plasminogen activator system in breast cancer. ARMIS *107*: 150-159, 1999.

39 Janicke F, Prechtl A, Thomssen C, Harbeck N, Meisner C, Untch M, Sweep CG, Selbmann HK, Graeff H and Scmitt M: German N0 Study Group. Randomized adjuvant chemotherapy trial in high-risk, lymph-node negative breast cancer patients identified by urokinase-type plasminogen activator and plasminogen activator inhibitor type 1. J Natl Cancer Inst *93*: 913-920, 2001.

40 Ferrandina G, Scambia G, Bardelli F, Benedett Panici P, Mancuso S and Messori A: Relationship between cathpsin-D content and disease-free survival in node-negative breast cancer patients: a metaanalysis. Br J Cancer *76*: 661-666, 1997.

41 Duffy MJ, Maguire T, McDermott EW and O'Higgins N: Urokinase plasminogen activator: a prognostic marker in multiple types of cancer. J Surg Oncol *71*: 130-135, 1999.

42 Campo E, Munoz J, Miquel R, Palacin A, Cardesa A, Sloane BF and Emmert-Buck MR: Cathepsin D expression in colorectal carcinomas correlate with tumor progression and shortened survival. Am J Pathol *145*: 301-309, 1994.

43 Sukoh N, Abe S, Ogura S, Isobe H, Takekawa H, Inoue K and Kawakami Y: Immunohistochemical study of cathepsin B, prognostic significance in human lung cancer. Cancer *74*: 46-51, 1994.

44 Foekens JA, Kos J, Peters HA, Krasovec M, Look MP, Cimerman N, Meijer-van Gerder ME, Henzen-Logmans SC, van Putten WL and Klijn JG: Prognostic significance of cathepsin B and L in primary human breast cancer. J Clin Oncol *16*: 1013-1021, 1998.

45 Maguire TM, Shering SG, Duggan C, McDermott E, O'Higgins NJ and Duffy MJ: High levels of cathepsin B predict poor outcome in patients with breast cancer. Int J Biol Markers *13*: 139-144, 1998.

46 Westley B and May FEB: Cathepsin D and breast cancer. Eur J Cancer *32A*: 15-24, 1996.

47 Foekens JA, Look M, Bolt-de Vries Meijer-van Gelder ME, van Putten WLJ and Klijn JGM: Cathepsin-D in primary breast cancer: prognostic evaluation involving 2810 patients. Br J Cancer *79*: 300-307, 1999.

48 Ferrandina G, Scambia G, Bardell F, Benedetti P, Mancuso S and Messori A: Relationship between cathepsin-D content and disease-free survival in node-negative breast cancer patients: a metaanalysis. Br J Cancer *76*: 661-666, 1997.

49 Rochefort H: Cathepsin D in breast cancer: a tissue marker associated with metastasis. Eur J Cancer *28A*: 1780-1783, 1992.

50   Weidner N, Folkman J, Popzza F, Bevilacqua P, Allred EN, Moore DH, Meli S and Gasparini G: Tumor angiogenesis: a new significant and independent prognostic indicator in early-stage breast carcinoma. J Natl Cancer Inst *84*: 1875-1887, 1992.

51   Horak ER, Leek R, Klenk N, LeJeune S, Smith K and Stuart N, Greenall M, Stepniewska K and Harris AL: Angiogenesis, assessed by platelet/endothelial cell adhesion molecule antibodies, as indicator of node metastases and survival in breast cancer. Lancet *340*: 1120-1124, 1992.

52   Leek RD: The prognostic role of angiogenesis in breast cancer. Anticancer Res *21*: 4325-4332, 2001.

53   Fox SB, Leek RD, Smith K, Hollyer J, Greenall M and Harris AL: Tumor angiogenesis in node- negative breast carcinomas-relationship with epidermal growth factor receptor, estrogen receptor, and survival. Breast Cancer Res Treat *29*: 109-116, 1994.

54   Anan K, Morisaki T, Katano M, Itubo A, Kitsuki H and Uchiyama A: Vascular endothelial growth-factor and platelet-derived growth-factor are potential angiogenic and metastatic factors in human breast-cancer. Surgery *119*: 333-339, 1996.

55   Gasparini G, Toi M, Gion M, Verderio P, Dittadi R and Hanatani M: Prognostic significance of vascular-endothelial growth-factor protein in node-negative breast carcinoma. J Natl Cancer Inst *89*: 139-147, 1997.

56   Leek RD, Hunt NC, Landers RJ, Lewis CE, Royds JA and Harris AL: Macrophage infiltration is associated with VEGF and EGFR expression in breast cancer. J Pathol *190*: 430-436, 2000.

57   Macchiarini P, Fontanini G, Hardin MJ, Squartini F and Angeletti CA: Relation of neovascularization to metastasis of non-small cell lung cancer. Lancet *340*: 145-146, 1992.

58   Fontanini G, Bigini D, Nignati S, Basolo F, Mussi A, Lucchi M, Chine S, Angeletti CA, Harris AL and Bevilacqua G: Microvessel count predicts metastatic disease and survival in non-small cell lung cancer. J Pathol *177*: 57-63, 1995.

59   Yuan A, Yang PC, Yu CJ, Lee YC, Yao YT, Chen CL, Lee LN, Kuo SH and Luh KT: Tumor angiogenesis correlates with histologic type and metastasis in non-small cell lung cancer. Am J Resp Crit Care Med *152*: 2157-2162, 1995.

60   Lucchi M, Fontanini G, Mussi A, Vignati S, Ribechini A, menconi GF, Bevilacqua G and Angeletti CA: Tumor angiogenesis and biologic markers in resected stage I NSCLC. Eur J Cardiothorac Surg *12*: 535-541, 1997.

61   Giatromanolaki A, Koukourakis MI, Theodossiou D, Barbatis K, O'Byrne K, Harris AL and Gatter KC: Prognostic value of angiogenesis in operable non-small cell lung cancer. J Pathol *179*: 80-88, 1996.

62   Giatromanolaki A: Prognostic role of angiogenesis in non-small cell lung cancer. Anticancer Res *21*: 4373-4382, 2001

63   Takahama M, Tsutsumi M, Tsujiuchi T, Kido A, Okajima E, Nezu K, Tojo T, Kushibe K, Kitamura S and Konishi Y: Frequent expression of the vascular endothelial growth factor in human non-small cell lung cancers. Jpn J Clin Oncol *28*: 176-181, 1998.

64   Ohta Y, Tomita Y, Oda M, Watanabe S, Murakami S and Watanabe Y: Tumor angiogenesis and recurrence in stage I non-small cell lung cancer. Ann Thorac Surg *68*: 1034-1038, 1999.

65   Volm M, Koomagi R and Mattern J: Prognostic value of vascular endothelial growth factor and its receptor Flt-1 in squamous cell lung cancer. Int J Cancer *74*: 64-68, 1997.

66   Ohta Y, Endo Y, Tanaka M, Shimizu J, Oda M, Hayashi Y, Watanabe Y and Sasaki T: Significance of vascular endothelial growth factor messenger RNA expression in primary lung cancer. Clin Cancer Res *2*: 1411-1416, 1996.

67   Giatromanolaki A, Koukourakis MI, Kakolyris S, Turley H, O'Byrne K, Scott PA, Pezzella F, Georgoulias V, Harris AL and Gatter KC: Vascular endothelial growth factor, wild-type p53, and angiogenesis in early operable non-small cell lung cancer. Clin Cancer Res *4*: 3017-3024, 1998.

68   O'Byrne KJ, Koukourakis MI, Giatromanolaki A, Cox G, Turley H, Steward WP, Gatter K and Harris AL: Vascular endothelial growth factor, platelet-derived endothelial cell growth factor and angiogenesis in non-small cell lung cancer. Br J Cancer *82*: 1427-1432, 2000.

69   Volm M, Mattern J and Koomagi R: Expression of platelet-derived endothelial cell growth factor in non-small cell lung carcinomas: relationship to various biological factors. Int J Oncol *13*: 5975-5979.

70   Aikawa H, Takahashi H, Fujimura S, Sato M, Endo C, Sakurada A, Kondo T, Tanita T, Matsumura Y, Ono S, Saito Y and Sagawa M: Immunohistochemical study on tumor angiogenic factors in non-small cell lung cancer. Anticancer Res *19*: 4305-4309, 1999.

71   Takanami I, Tanaka F, Hashizume T, Kikuchi K, Yamamoto Y, Yamamoto T and Kodaira S: The basic fibroblast growth factor and its receptor in pulmonary adenocarcinomas: an investigation of their expression as prognostic markers. Eur J Cancer *32A*: 1504-1509, 1996.

72   Volm M, Koomagi R, Mattern J and Stammler G: Prognostic value of basic fibroblast growth factor and its receptor (FGFR-1) in patients with non-small cell lung carcinomas. Eur J Cancer *33*: 691-693, 1997.

73   Brattstrom D, Bergqvist M, Larrsson A, Holmertz J, Hesselius P, Rosenberg L, Brodin O and Wagenius G: Basic fibroblast growth factor and vascular endothelial growth factor in sera from non-small cell lung cancer patients. Anticancer Res *18:* 1123-1127, 1998.

74   Frank R, Saclarides TJ, Leurgans S, Speziale NJ, Drab EA and Rubin DB: Tumor angiogenesis as a predictor of recurrence and survival in patients with node-negative colon cancer. Ann Surg *222*: 695-699, 1995.

75   Lindmark G, Gerdin B, Sundberg C, Pahlman L, Bergstrom R and Glimelius B: Prognostic significance of the microvascular count in colorectal cancer. J Clin Oncol *14*: 461-466, 1996.

76   Tanigawa N, Amaya H, Matsumura M, Lu C, Kitaoka A, Matsuyama K and Muraoka R: Tumor angiogenesis and mode of metastasis in patients with colorectal cancer. Cancer Res *57*: 1043-1046, 1997.

77   Sokmen S, Sarioglu S, Fuzun M, Terzi C, Kupelioglu A and Aslan B: Prognostic significance of angiogenesis in rectal cancer: a morphometric investigation. Anticancer Res *21*: 4341-4348, 2001.

78   Yoshimura H, Chikamoto A, Honda T, Tashiro K, Nakamoto T, Takano M, Takagi K, Nagasue N and Soma G: Relationship between microvessel quantification and inducibility of endogenous tumor necrosis factor in colorectal adenocarcinoma. Anticancer Res *20*: 629-633, 2000.

79   Saito H, Tsujitani S, Nondo A, Ikeguchi M, Maeta M and Kaibara N: The expression of vascular endothelial growth factor correlates with hematogenous recurrence in gastric carcinoma. Surgery *125*: 195-201, 1999.

80 Saito H and Tsujitani S: Angiogenesis, angiogenic factor expression and prognosis of gastric carcinoma. Anticancer Res *21*: 4365-4372, 2001.

81 Philip EA, Stephenson TJ and Reed MW: Prognostic significance of angiogenesis in transitional cell carcinoma of the human urinary bladder. Br J Urol *77*: 352-357, 1996.

82 Streeter EH and Crew JP: Angiogenesis, angiogenic factor expression and prognosis of bladder cancer. Anticancer Res *21*: 4355-4364, 2001.

83 Chaudhary R, Bromley M, Clarke NW, Betts CD, Barnard RJ, Ryder WD and Kumar S: Prognostic relevance of micro-vessel density in cancer of the urinary bladder. Anticancer Res *19*: 3479-3484, 1999.

84 Crew JP, O'Brien T, Bradburn M, Fuggle S, Bicknell R, Cranston D and Harris AL: Vascular endothelial growth factor is a predictor of relapse and stage progression in superficial bladder cancer. Cancer Res *57*: 5281-5285, 1997.

85 Chow NH, Liu HS, Chan SH, Cheng HL and Tzai TS: Expression of vascular endothelial growth factor in primary superficial bladder cancer. Anticancer Res *19*: 4593-4597, 1999.

86 O'Brien TS, Fox SB, Dickinson AJ, Turley H, Westwood M, Moghaddam A, Gatter KC, Biocknell R and Harris AL: Expression of the angiogenic factor thymidine phosphorylase/platelet-derived endothelial cell growth factor in primary bladder cancers. Cancer Res *56*: 4799-4804, 1996.

87 Arima J, Imazono Y, Takebayashi Y, Nishiyama K, Shirahama T, Akiba S, Furukawa T, Akiyama S and Ohi Y: Expression of thymidine phosphorylase as an indicator of poor prognosis for patients with transitional cell carcinoma of the bladder. Cancer *88*: 1131-1138, 2000.

88 Nguyen M, Watanabe H, Budson AE, Richie JP and Folkman J: Elevated levels of the angiogenic peptide basic fibroblast growth factor in urine of bladder cancer patients. J Natl Cancer Inst *85*: 241-242, 1993.

89 Wagatsuma S, Konno R, Sato S and Yajima A: Tumor angiogenesis, hepatocyte growth factor, and c-Met expression in endometrial carcinoma. Cancer *82*: 520-530, 1998.

90 Seki N, Kodama J, Hongo A, Miyagi Y, Yoshinouchi M and Kudo T: Angiogenesis and platelet-derived endothelial cell growth factor/thymidine phosphorylase expression in endometrial cancer. Int J Oncol *15*: 781-786, 1999.

91 Sivridis E: Angiogenesis and endometrial cancer. Anticancer Res *21*: 4383-4388, 2001

92 Morgan KG, Wilkinson N and Buckley CH: Angiogenesis in endometrial carcinoma. Int J Cancer *6*: 385-388, 1996.

93 Giatromanolaki A, Sivridis E, Brekken R, Thorpe PE, Anastasiadis P, Gatter KC, Harris AL, Koukourakis MI and Tumour and Angiogenesis Research Group: The angiogenic pathway "VEGF/flk-1(KDR)" in endometrial carcinomas: prognostic and therapeutic implications. Cancer *92*: 2569-2577, 2002.

94 Sivridis E, Gaitromanolaki A, Koukourakis MI, Bicknell R, Harris AL and Gatter KC: Thymidine phosphorylase expression in endometrial carcinomas. Cl Exp Metast *17*: 445-450, 1999.

95 Qin L-X and Tang Z-Y: The prognostic molecular markers in hepatocellular carcinoma. World J Gastroenterol *8*: 385-392, 2002.

96 El-Assal ON, Yamanoi A, Soda Y, Yamaguchi M, Igarashi M, Yamamoto A, Nabika T and Nagasue N: Clinical significance of microvessel density and vascular endothelial growth factor expression in the angiogenesis of cirrhotic liver. Hepatology *27*: 1554-1562, 1998.

97 Poon RT, Ng IO, Lau C, Zhu LX,Yu WC, Lo CM, Fan ST and Wong J: Serum vascular endothelial growth factor predicts venous invasion in hepatocellular carcinoma: a prospective study. Ann Surg *233*: 227-235, 2001.

98 Zhou J, Tang ZY, Fan J, Wu ZQ, Li XM, Liu YK, Liu F, Sun HC and Ye SL: Expression of platelet-derived endothelial cell growth factor and vascular endothelial growth factor in hepatocellular carcinoma and portal vein tumor thrombus. J Cancer Res Clin Oncol *126*: 57-61, 2000.

99 Yoshimo S, Kato M and Okada K: Prognostic significance of microvessel count in low stage renal cell carcinoma. Int J Urol *2*: 156-160, 1995.

100 Ellis LM, Takahashi Y, Fenoglio C, Cleary K, Bucana C and Evans DB: Vessel counts and vascular endothelial growth factor expression in pancreatic adenocarcinoma. Eur J Cancer *34*: 337-340.

101 Ikeda N, Adachi M, Taki T, Huang C, Hashida H, Takabayashi A, Sho M, Nakajima Y, Kanahiro H, Hisanaga M, Nakano H and Miyake M: Prognostic significance of angiogenesis in pancreatic cancer. Br J Cancer *79*: 1553-1563, 1999.