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Review

Prognostic factors in gastric cancer

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Background Despite gastric cancer being common, its prognosis has not been improved significantly in recent years. Now, greater insight has been gained into the biological properties of tumour cells, how they become malignant and what mechanisms they may use to invade and metastasize. This involves tumour-associated protease systems, loss or mutation of adhesion molecules and changes in genetics. The view of gastric cancer is changing: it is not only a solid tumour but also exhibits a minimal residual disease component even in the early stages of disease. Such biological tumour characteristics may provide new prognostic factors and also potential new therapeutic options.

Methods This is an update of prognostic factors in gastric cancer, emphasizing new biological features, some of which have been investigated by this group over the past few years. Current results are discussed in the light of 212 references obtained from the Medline database from 1979 to 1997.

Results There is high probability that some of the factors reviewed, such as c-*erb*B-2, individual course and phenotyping of disseminated tumour cells will become significant new prognostic variables. This is true also, to a lesser extent, of cathepsin D, matrix metalloproteinase 2 combined with activators or tissue inhibitor of metalloproteinases 2, CD44, E-cadherin, p53 and *cripto*. Plasminogen activator inhibitor 1 (PAI-1), a member of the urokinase-type plasminogen activator (uPA) system, can already be defined as an established new prognostic factor in gastric cancer.

Conclusion PAI-1 should be considered prognostically in addition to established tumour classifications. Moreover, the uPA system is a target for future therapeutic concepts. Further analysis of factors describing tumour biology should lead to new, functionally orientated, tumour classifications in gastric cancer.

The overall outcome for patients with gastric carcinoma has not significantly improved over the past decade. Excluding early gastric cancer (with 5-year survival rates of about 80–90 per cent[1]) and gastric cancer in Japan (with about 70 per cent 5-year survival most probably owing to higher proportions of early-stage cancer and of curative resections[2]), the present chance of 5-year survival is estimated at 20 per cent[3] to 45 per cent[1,4]. With an incidence of between 22 and 30 per 100 000 European inhabitants[1,5], however, gastric cancer still holds its position as one of the most common cancer types in the world. In such a setting investigations into factors determining individual prognosis and the development of therapeutic concepts are of major importance.

Prognostic factors in cancer have many aspects[2]. At present, most serve to predict clinical outcome for individual patients, aiming especially at the identification of subgroups at high risk of relapse. In cancers with proven effective adjuvant treatment modalities, prognostic variables help to classify patients into therapeutic groups which statistically should benefit from adjuvant treatment protocols. Ideally, prognostic factors should also suggest options for therapeutic intervention. Unfortunately, in the case of gastric cancer the established prognostic factors have not reached this level of sophistication up to now. For instance, in contrast to breast cancer, adjuvant therapeutic strategies either have not been proved to be of significant benefit, or selection criteria for those patients who might benefit from adjuvant protocols have not yet been identified.

During the past decade efforts have been made to identify new prognostic variables. In contrast to almost all of the established factors, these variables were orientated at tumour biology, in a period when knowledge of tumour cell behaviour was increasing dramatically[6] (*Table 1*). To permit separation from the primary tumour and invasion of surrounding structures, tumour cells have been shown to downregulate the expression of adhesion molecules[7–9] and to overexpress complex tumour-associated protease and protease inhibitor systems. This effects the malignant criteria of invasiveness and metastasis[6,10–15]. Systemic spread of tumour cells may be demonstrated in the very early stages of solid cancers[16–25] and recently the authors have provided strong evidence for the biological autonomy of an early systemic disease component in gastric cancer[26]. Finally, outgrowth and establishment as distant metastasis are aided by proteolytic enzymes, but angiogenetic factors, growth factors and their receptors[27], and proliferative capacity are decisive in this context. Increasing numbers of investigations are being carried out on certain oncogenes, tumour suppressor genes and other molecular mechanisms[27–29].

This review is an update of prognostic factors in gastric cancer, with emphasis on new biological variables. These variables are more able than established factors to yield useful information about the biology of individual tumours. Potentially they promise not only more detailed and functionally orientated tumour classifications but also the possibility of individual therapeutic targets for the future.

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Conventional prognostic variables

The first section of the review briefly summarizes results associated with variables that are defined by the condition of the patient, the treatment and the morphology of the tumour. These classical variables have been studied in large multivariate analyses in the past.

Patient-related factors

Gastric cancer preferentially affects patients aged 50 years and over[1]. It also tends to affect more men than women[1,30]. It has therefore been proposed that age and sex might be prognostic risk factors. In this regard the results of studies have been contradictory. Some multivariate analyses with more than 500 patients[4,31–34] have reported independent prognostic influence, others have not[35–39]. Female sex was reported to be associated with better prognosis in one study on R_0 (curatively resected) patients[40], and for certain subgroups in two other reports[35,41]. Other possible patient-associated factors, such as pattern of symptoms, weight loss, co-morbidity and immune status, have not yet been demonstrated as independent significant factors in multivariate analysis. In summary, there is no definite consensus on the significance of patient-related variables as independent factors determining survival.

Table 1 Overview of steps of tumour invasion and metastasis and biological features correlated with them

Metastatic status	Potential parameters relevant
Primary tumour	Stroma induction/angiogenesis: protease inhibitors (PAI, TIMP) Growth and proliferation: tyrosine kinase receptors (c-*erb*B-2, c-*met*), growth factors and their receptors (EGF receptor, *cripto*), signal transduction (c-*ras*), cell-cycle regulators (*pic1*, G1 and G2 cyclins, CDKs, *MTS*1), tumour suppressor mutations (*p53*, *nm23*), block of apoptosis (*bcl-2*), genetic instability
Segregation from the primary tumour	Loss of adhesion molecules, tumour-associated proteases/inhibitors
Invasion of surrounding tissue	Tumour-associated proteases (uPA system, MMPs, cathepsins and others)
Invasion of blood vessels	Tumour-associated proteases (uPA system, MMP-2, MMP-9 and others)
Systemic dissemination	Disseminated tumour cells (CK18 positive)
Adhesion and extravasation	Adhesion molecules, tumour-associated proteases
Establishment as metastasis	Interaction with microenvironment: tumour-associated proteases and inhibitors, adhesion molecules, loss of MHCI as immunological escape[200] Stroma induction/angiogenesis: protease inhibitors (PAI, TIMP) Growth and proliferation: tyrosine kinase receptors (c-*erb*B-2, c-*met*), growth factors and their receptors (EGF receptor, *cripto*), signal transduction (c-*ras*), cell-cycle regulators (*pic1*, G1 and G2 cyclins, CDKs, *MTS*1), tumour suppressor mutations (*p53*, *nm23*), block of apoptosis (*bcl-2*), genetic instability

PAI, plasminogen activator inhibitor; TIMP, tissue inhibitor of metalloproteinases; EGF, epidermal growth factor; CDK, cyclin-dependent kinase; UPA, urokinase-type plasminogen activator; MMP, matrix metalloproteinase; CK, cytokeratin; MHC, major histocompatibility complex

Treatment-related factors

There is no doubt that survival is decisively determined by the ability to perform macroscopically and microscopically complete surgical resection of tumour. Median survival time after non-curative resection (R_1 or R_2) has been reviewed[42] and ranges from 7 to 11 months; after explorative laparotomy or palliative gastroenterostomy it is 3–5 months. In contrast, median survival time after curative resection ranges from 35 to 75 months[2]. A curative tumour resection must still be recognized as the most powerful prognostic variable.

In addition, there is consensus that the quality of the resection is relevant to survival. Several studies have demonstrated especially that extended lymph node resection, including compartments I and II, performed without increased risk of perioperative morbidity, is associated with better long-term survival[33,34,43–46]. Siewert *et al.*[46] have demonstrated that radical lymph node dissection, defined as removal of 26 or more nodes, is an independent prognostic factor in patients with stage II and IIIA disease. There is also evidence that the hospital is an independent prognostic factor[31,32], with a higher probability of survival for patients operated in centres with a broad experience in oncological surgery.

Perioperative allogeneic blood transfusion has an adverse effect on the immune response, inducing a higher risk of postoperative infection[47] and, potentially, supporting the establishment of minimal residual tumour disease[48]; an association with poorer survival in gastric cancer has been reported[48,49], but the independent influence of allogeneic blood on survival still remains to be shown.

No advantage could be shown for adjuvant chemotherapy after curative tumour resection in terms of longer disease-free or overall survival time[1,50]. This can also be stated for a combination of chemotherapy (5-fluorouracil (5-FU) fluorouracil adriamycin metiotrexate (FAM)) and radiation therapy, adjuvant radiation therapy or intraoperative radiation therapy[1,51,52]. Randomized trials evaluating the prognostic value of defining patient subgroups profiting from neoadjuvant chemotherapy have to be performed[53]. Neoadjuvant chemotherapy may potentially increase survival mainly by increasing the chance of curative resection in advanced stages of gastric cancer. Indeed, there have been studies[1,54,55] in which resection rates between 62 and 93 per cent, including 71–100 per cent R_0 resections, could be achieved. However, the results of such studies are difficult to interpret as they were non-randomized and the criteria for non-resectability of treated cancers were not standardized.

There is still controversy regarding chemotherapy for non-resectable advanced gastric cancer. However, in recent years new combinations of chemotherapeutic agents with acceptable monoactivity in gastric cancer (which means objective remission rates of about 20 per cent) have been developed, such as methotrexate, 5-FU and adriamycin (FAMTX); adriamycin, cisplatin and etoposide; leucovorin, 5-FU and etoposide; leucovorin; and 5-FU[56–58]. Such therapy may reach 40–50 per cent objective remissions, including 10 per cent complete remissions. A randomized study comparing FAM and FAMTX revealed significantly higher remission rates and

significantly longer median survival in patients treated with FAMTX[59]. Modern chemotherapy of non-resectable advanced gastric cancer must therefore be assumed as influencing prognosis.

Established tumour-related factors

There is general consensus that the anatomical extent of gastric cancer is an independent prognostic variable. This has been shown for local infiltration depth (pT), nodal involvement (pN) and distant metastasis (M)[60–63]. In addition, the number of affected lymph nodes is associated with survival[2,61,64]. There is also a correlation between tumour site and survival, with proximal carcinomas (gastro-oesophageal junction, cardia, upper third of the stomach) associated with poorer survival[65,66]. Some studies report an independent prognostic impact for this variable[31,36,65,67].

For none of the existing histological classifications (World Health Organization[68], Laurén[69], Ming[70]) has an independent influence on prognosis been demonstrated up to now[2,63,71,72]. For tumour cell differentiation (G), results are controversial. Multivariate prognostic relevance of tumour cell dissociation at invasion fronts was reported by Gabbert *et al.*[73], and Nakane *et al.*[67] demonstrated a significant prognostic impact of grading. Other studies did not find an association with prognosis[4,35,36,63,65,74].

Lymphangiosis carcinomatosa has been noted as an independent prognostic factor in some larger studies[73,75]. In the authors' own study of 203 patients, a significant impact was revealed especially for disease-free survival[63,74]. Other investigators have not observed this[36,40,76]. Invasion of blood vessels by tumour cells may also correlate independently with poorer survival[40,75], but not all agree on this[36,63,76]. Contradictory results have also been obtained for Borrmann's macroscopic gastric cancer classification[36,38,63,67,76] and so it cannot be considered as an established prognostic variable.

Tumour markers

Measurement of the serum tumour markers, carcinoembryonic antigen (CEA), CA 19-9 and CA 72-4, has become standard as an indicator of relapse during follow-up after gastric cancer resection. Correlation with prognosis has been reported for perioperative levels of these serum markers, and also for CA 125, Sialyl Tn antigen, ST-439 and α_1-fetoprotein[77]. Independent prognostic influence has been shown for CEA[67,68] and CA 19-9[79]. However, the strongest impact is now ascribed to the combination of CEA and CA 72-4[80].

New functional biological variables

In recent years more insight has been gained into the mechanisms that underlie the ability of tumour cells to behave in a malignant fashion (*Table 1*). Some of the biological factors involved in these mechanisms have been investigated for their potential role as prognostic factors. The following is an overview of these functional factors.

Tumour-associated proteases and protease inhibitors

Tumour cell invasion and metastasis are biologically dependent on the proteolytic destruction of surrounding matrix components, including the basement membrane of vessels to enable the systemic circulation to be reached. Evidence has accumulated that this is achieved by a series of tumour-associated serine, aspartic, cysteine and threonine proteases, and metalloproteinases[6,10–15,81–83]. The proteolytic factors and specifically corresponding inhibitors employed by tumour cells, either by secretion or stromal induction, are different from physiological factors that play central roles in several remodelling processes: embryogenesis, fibrinolysis, inflammation, angiogenesis, wound healing[10,12,82]. However, investigations indicate that some of these protease systems are overexpressed in tumours and that tumours with a high level of proteolytic activity are more invasive than others[10,83–89]. This has been shown especially for the urokinase-type plasminogen activator (uPA) system, which includes the 55-kDa serine protease uPA, its specific cell membrane-bound receptor (uPA-R) and plasminogen activator inhibitor (PAI) 1 and 2 as specific inhibitors. uPA is able to induce destruction of a pattern of surrounding matrix elements by activation of plasminogen to active plasmin, and potentially directly to activate collagenase IV (matrix metalloproteinase (MMP) 2 and 9, see below) for basement membrane degradation[10–12,82]. In this way the uPA-R has a pivotal role as a concentrator of the activity of bound uPA, focusing proteolysis at tumour invasion fronts and so potentiating the effectivity of uPA[85,89,90]. The inhibitors, especially PAI-1, are essential for transcellular recirculation of uPA-R, guaranteeing a dynamic and flexible proteolytic system at the tumour cell surface[10,88]. Moreover, it is speculated that PAIs protect the inner part of a tumour or growing metastasis from self-destruction by overwhelming proteolysis, and are essential for the neoangiogenesis of outgrowing metastases[10,63,82,91].

In view of this association with tumour cell aggression, a potential role of the uPA system as a prognostic marker is suggested. Indeed, the prognostic relevance of this system has been studied and confirmed in many cancer types[92–98], revealing especially uPA and PAI-1 as independent prognostic variables. For gastric cancer the authors' group has demonstrated immunohistochemically with semiquantitative scoring that there is an overexpression of the uPA system[63,74]. Univariately, uPA, uPA-R and PAI-1 (but not PAI-2) were associated significantly with disease-free and overall survival (*Fig. 1*). In overall multivariate analysis, PAI-1 was revealed as a strong and independent prognostic variable[63]. These results corresponded exactly with a study by Nekarda *et al.*[99] performed on 76 patients with gastric cancer using an enzyme-linked immunosorbent assay. In another study on 160 gastric cancers[100], the significant association of the uPA system with prognosis was further confirmed. In a continuing analysis an especially strong and independent prognostic value of the uPA system was found in diffuse and poorly differentiated intestinal/gastric cancer[74], indicating the potentially similar biological behaviour of these types of tumour. Summarizing these results and considering similar observations for other solid carcinomas[92–98], the prognostic importance of the uPA system can be seen as a general principle in oncology, and PAI-1 can be considered as an established independent prognostic factor in gastric cancer.

The situation is different for the MMPs. The MMPs comprise a large family of zinc-dependent proteases which are capable of degrading different elements of stromal matrix (e.g. collagens, proteoglycans, laminin, fibronectin)[81] and are known to date to be inhibited by three different forms of tissue inhibitor of metalloproteinases

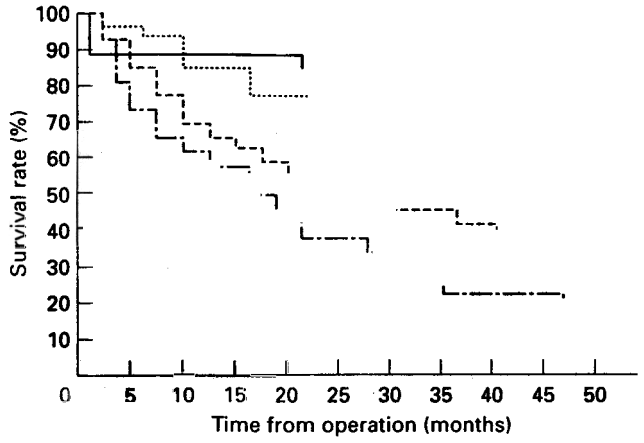

Fig. 1 Overall survival of 189 patients with gastric cancer according to immunohistochemical detection of plasminogen activator inhibitor 1 in tumour cells (semiquantitative score 0–3)[63]. Score 0 (———): eight patients, two events, mean survival time (MST) 44·5 months. Score 1 (.....): 46 patients, 14 events, MST 42·7 months. Score 2 (– – –): 74 patients, 38 events, MST 31·5 months. Score 3 (—·—·—): 61 patients, 39 events, MST 26·0 months. $P = 0{\cdot}0019$ (Mantel–Cox test)

(TIMPs), TIMP-1, TIMP-2 and TIMP-3[83,101]. Large prognostic studies on MMPs and TIMPs in gastric cancer have not yet been performed, with the exception of MMP-2, the 72-kDa form of collagenase IV. MMP-2 is capable of degrading the basement membrane component, collagen IV (besides collagen V, laminin and fibronectin)[83] and may be activated by uPA[11,82]. In contrast to the 92-kDa form of collagenase IV, MMP-9, it was postulated as a characteristic for the malignant phenotype in gastric tissue by Schwartz *et al.*[86], has been preferentially detected in advanced gastric cancers and correlates with vascular invasion[102]. Thus, an association with prognosis has been mooted. The authors have studied MMP-2 in a series of 203 patients, but only tendential univariate correlation with survival was detected and it failed as an independent parameter of prognosis (unpublished results). An association of MMP-2 with prognosis was noted in the subgroup of patients with high expression of uPA receptor. This probably indicates that MMP-2 is only of prognostic relevance if its activating proteases are overexpressed. A study by Grigioni *et al.*[103], moreover, indicates that the balance between MMP-2 and its inhibitor TIMP-2 could be of relevance. In summary, further prognostic studies on MMP-2, also considering co-expression of activating enzymes (e.g. membrane-type MMP) and inhibitors (TIMP-2) are necessary.

Another class of proteolytic enzymes located preferentially in lysosomes is the cathepsins. In particular, the oestrogen-inducible cathepsin D[104] has been investigated in breast cancer and there is consensus that raised tissue cathepsin D is a significant prognostic indicator of disease recurrence[105]. Cathepsin D is a lysosomal scavenger which plays an important role in intracellular protein metabolism, and in conversion of proenzymes and prohormones. The secreted enzyme is activated into a 34-kDa and 14-kDa dimer[105], and can degrade extracellular matrix components such as proteoglycans[106]. It further activates cathepsin B, which in turn activates uPA[82,107]. Moreover, a mitogenic activity has been mooted for cathepsin D[108]. In gastric cancer, strong immunohistochemical cathepsin D staining at advancing tumour margins has been reported[109], and it may have a role as a poor prognostic marker. However, an immunohistochemical study from Greece on 62 patients with gastric cancer (polyclonal antibody, chemotherapy in advanced stages) indicated an association with favourable prognosis[110]. The authors' group performed a prognostic study in a series of 203 patients using immunohistochemistry and a monoclonal antibody against cathepsin D. A significant association of cathepsin D in tumour cells was found with poorer disease-free and overall survival of patients who had curative resection (unpublished results). For disease-free survival, cathepsin D was an independent risk factor on multivariate analysis. As for MMP-2, there was a special prognostic relevance of cathepsin D in patients with parallel high expression of uPA-R, indicating that the aggressive potential of malignant tumours may derive from the concerted action of interacting proteolytic systems. With respect to other cathepsins (B, E, G), cathepsin B is also hypothesized to correlate with poor prognosis in gastrointestinal cancers[86,111]. However, no definitive large prognostic study on this factor in gastric cancer is available.

Observations that the prognostic relevance of MMP-2 and cathepsin D is improved when parallel high expression of the uPA system is taken into account prompt one to speculate on other protease systems that interact with this system. The uPA system is subject to several interactions (*Fig. 2*), of which activation of plasminogen and MMP-2 by the uPA system and activation of uPA by cathepsins have already been discussed. Furthermore, α_2-antiplasmin and α_2-macroglobulin are inhibitors of active plasmin[82]. Besides its inhibitory function, α_2-macroglobulin may enhance transcellular circulation of uPA-R, mediated by a specific low density lipoprotein receptor-related protein/α_2-macroglobulin receptor[112–115]. This receptor is potentially necessary for internalization of uPA-R/uPA/PAI complexes and, in combination with PAI-1, for uPA-R recirculation[10,112,116].

Trypsin is a proteolytic activator of pro-uPA[82] and is inhibited by α_1-antitrypsin[117]. In contrast, chymotrypsin inactivates uPA-R by cleavage of one of the three uPA-R protein domains[88]. Thus, the chymotrypsin inhibitor α_1-antichymotrypsin is potentially protective to the uPA-R. Thrombin may proteolytically cleave uPA to an inactive enzyme form[82]. It is antagonized by antithrombin 3. However, antithrombin 3 is also able to inactivate uPA[118]. The tissue-type plasminogen activator (tPA) in analogy to uPA can activate plasminogen[82]. In contrast to uPA, overexpression or prognostic impact has not yet been shown for tPA[119–121].

Consideration of these interactive protease systems[122] has revealed an additional univariate prognostic impact of α_1-antitrypsin, α_1-antichymotrypsin and α_2-macroglobulin in the authors' series of 203 patients with gastric cancer and identified the combination of high expression of uPA-R, PAI-1, antichymotrypsin and α_2-macroglobulin as a high-risk protease pattern (unpublished results). As indicated above, all of the last three variables are essential for transcellular recycling of the uPA-R. From this it may be concluded that uPA-R recycling is of decisive importance for tumour cell aggression and prognosis in individual gastric carcinomas.

In summary, there is no doubt that tumour-associated proteases and inhibitors, and especially the uPA system, should be considered when predicting the prognosis of patients with gastric cancer. For the uPA system,

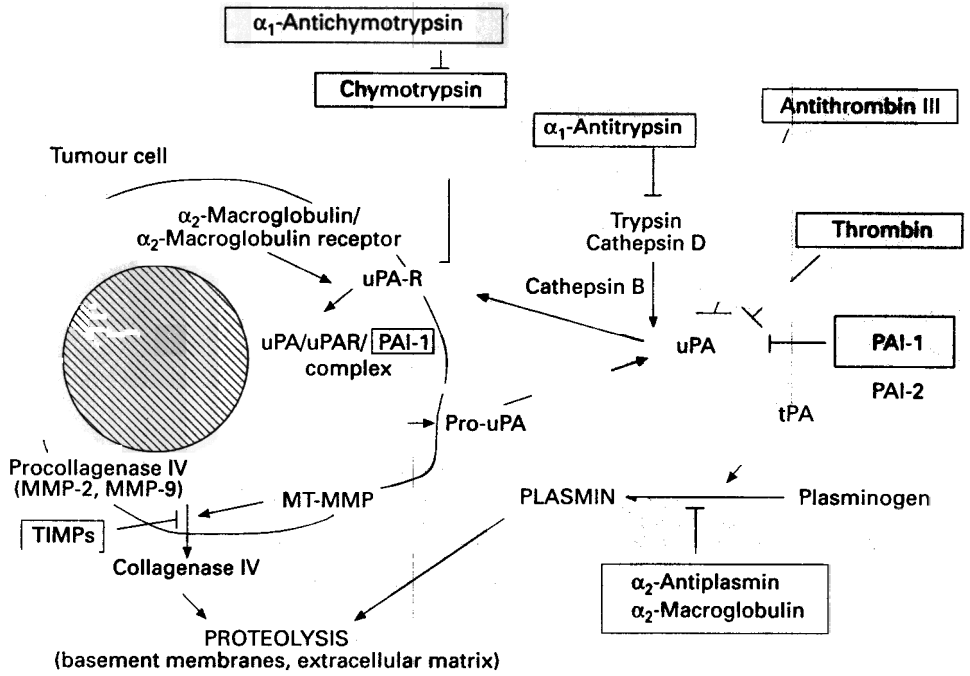

Fig. 2 Schematic overview of some of the multiple interactions of the urokinase-type plasminogen activator (uPA) system with other proteases/inhibitors. Activation is shown by arrows and inhibition by bars (——|) with inhibitors in boxes. uPA-R, urokinase-type plasminogen activator receptor; PAI, plasminogen activator inhibitor; tPA, tissue-type plasminogen activator; MMP, matrix metalloproteinase; MT-MMP, membrane-type matrix metalloproteinase; TIMP, tissue inhibitor of metalloproteinases

emphasizing PAI-1, the authors believe that this factor characterizing the biology of tumour cells should be integrated into existing established tumour classifications.

Adhesion molecules

Recent studies on the mechanism of tumour metastasis indicate not only that an increase in proteolytic activity contributes to this process which characterizes malignancy but also that dysregulation of adhesion mechanisms increases the ability of tumour cells to detach from the primary site[7,123,124]. Downregulation of adhesion molecules is involved; these are classified into the cadherins, integrins, selectins, the hyaluronate receptor family including CD44, the sialomucin family, and the immunoglobulin superfamily including intercellular adhesion molecule (ICAM) and vascular adhesion molecule[7].

Perhaps the most conclusive experimental studies showing an association of downregulation with increase of metastasis have been carried out on E-cadherin[7]. E-cadherin is a 120-kDa, calcium-dependent surface glycoprotein which is expressed exclusively by epithelial cells[7]. It has been shown that invasive tumour cell lines have often lost E-cadherin[8] and that invasiveness of malignant cell lines can be reduced by transfection with E-cadherin complementary DNA[8]. On the other hand, antibodies against E-cadherin can induce the ability to invade collagen gels[124]. *In vivo*, downregulation of E-cadherin has been reported for various human cancers[125–129]. Nevertheless, adequate prognostic clinical studies on the expression of E-cadherin in gastric cancer are still missing. One immunohistochemical study[9] on 60 patients (31 who had curative resection) showed reduced expression of E-cadherin in 92 per cent of cases, significant correlation with dedifferentiation (G_{3-4}, Laurén diffuse type) and significant univariate association of low E-cadherin expression with disease-free and overall survival. An independent impact on prognosis, however, could not be shown.

A larger study of 125 patients with gastric cancer was performed by Yonemura *et al.*[130], also using immunohistochemistry. This investigation is interesting since E-cadherin staining is set in relation to staining for uPA in the same tumour series, and it has been hypothesized by Frixen and Nagamine[131] that loss of E-cadherin function potentially leads to upregulation of uPA. Correspondingly, Yonemura *et al.*[130] found an independent prognostic impact for uPA/E-cadherin status in their gastric cancer series, the worst prognosis being confined to uPA-positive and E-cadherin-negative tumours. However, as discussed previously, the prognostic relevance of the uPA system alone is very strong, and the multivariate results given for the combination of the parameters mentioned could result from a strong influence of uPA. Two further studies on E-cadherin in gastric cancer did not show multivariate impact[132,133]. In conclusion, prognostic impact of E-cadherin still has to be revealed in gastric cancer.

Another adhesion molecule which has been studied at some length in the context of cancer is CD44, a member of the hyaluronate receptor family[7]. It specifically binds to hyaluronic acid, is expressed by a wide range of cell types, and exists in a pattern of different isoforms generated by alternative splicing of messenger RNA and differential glycosylation[7]. These isoforms have in part lost the ability to bind hyaluronic acid as an extracellular matrix component[7]. Thus, abnormal transcripts and isoforms of CD44 have been suggested as playing a role in tumour invasion[134–138].

CD44 splice variants have also been detected in gastric cancer[134,139–141] and, from observation of different expression patterns of CD44 isoforms in Laurén diffuse and intestinal types, it has been speculated that these gastric cancer types may have different genetic pathways[134]. An immunohistochemical study[142] has indicated that CD44 is an independent prognostic factor in gastric cancer. However, this preliminary study showing univariate and multivariate prognostic significance of CD44 and its isoform CD44 9v was performed on 31 patients who had curative resection, with a mean follow-up time of 17 months. Investigations on the isoform CD44 9v were restricted to 22 of those patients and so further studies involving larger patient numbers are needed.

Involvement in tumour progression has been reported for adhesion molecules of the immunoglobulin superfamily (with architecture similar to that of immunoglobulins)[143]. A circulating form of ICAM-1 has been found at increased levels in those with cancer[144]. For the integrin family, very late antigen (VLA) 4, a molecule which is involved also in normal leucocyte traffic, is hypothesized to be used by tumour cells for haematogenous dissemination[7]. Of the selectin family, E-selectin has been shown to mediate carcinoma cell binding to endothelium[145] and thus could play a role in metastasis. However, there is no evidence so far that any of these factors may have an impact as prognostic variables in gastric cancer.

In conclusion, of the complex array of adhesion molecules no factor has yet been established as a definite new prognostic variable in gastric carcinoma.

Molecular variables

The rapid progress in molecular biology has given increasing evidence that transformation from a normal epithelial cell to a malignant cell is a multistep process and results from accumulation of multiple gene abnormalities[27–29,146]. Potentially, the patterns of overexpressed or down-regulated factors and enzymes described in the last two sections must be seen as the 'symptoms' of these genetic alterations. For gastric cancer, multiple gene alterations have been described, including oncogenes coding for tyrosine kinase receptors (including c-*met* and c-*erb*B-2), genes encoding growth factors and growth factor receptors (epidermal growth factor (EGF) receptor, *cripto*), genes involved in intracellular signal transduction (the *ras* family), regulators of the cell cycle, tumour suppressor genes (including *p53*, APC), genes preventing apoptosis (*bcl-2*) and genetic instability[28]. Alterations within these groups seem to occur in different patterns in well and poorly differentiated gastric carcinomas, so different genetic pathways are postulated for these differently differentiated stomach cancers[29].

Of the proto-oncogenes encoding tyrosine kinase receptors, the c-*met* gene encoding hepatocyte growth factor receptor has been shown to be frequently amplified in advanced gastric cancers[28,29,147,148]. Kuniyasu *et al.*[148] demonstrated significant correlation of expression of an aberrant transcript of c-*met* with tumour stage, lymph node metastasis and depth of tumour invasion. A prognostic value, however, has not yet been determined for this proto-oncogene.

In contrast, there is evidence that c-*erb*B-2 may be of significant prognostic value in gastric cancer; c-*erb*B-2 encodes a 185-kDa transmembrane glycoprotein with tyrosine kinase activity (p185), suggesting that this product is a potential growth factor receptor for an unidentified ligand[149–153]. One study even suggests that c-*erb*B-2 mediates tumour progression and metastasis by inhibiting expression of E-cadherin[28]. Amplification of c-*erb*B-2 has been observed preferentially in well differentiated gastric cancers[154–156]. Some clinical studies provide evidence of a role for this marker in survival analysis. An immunohistochemical study using a polyclonal antibody on 260 gastric cancers[157] found correlation with histological type, pN and serosal tumour invasion, and revealed significant univariate association of p185-positive tumours with poorer survival. This was also confirmed in a study by Uchino *et al.*[158] involving 108 patients which also used a polyclonal antibody and immunohistochemistry. Ten year survival of patients with p185-positive lesions of 37 per cent was significantly lower than the 91 per cent survival of those with p185-negative tumours. Yonemura *et al.*[159] and Jaehne *et al.*[160] found independent prognostic relevance of c-*erb*B-2 in series of 189 and 58 patients respectively. In the present authors' series of 203 patients, high expression of p185 protein was of independent prognostic impact (unpublished data). On the other hand, another immunohistochemical study[161] did not show p185 as a prognostic variable. In spite of this, there is overall strong evidence that c-*erb*B-2 might serve as a prognostic indicator in gastric cancer.

Of the growth factor and growth factor receptor gene family, it has been shown that overexpression of EGF receptors and EGF/transforming growth factor α receptors correlates with biological malignancy[162–164]. For gastric cancer, an autocrine stimulating pathway promoting tumour growth, cell division and even uPA/uPA-R expression has been suggested for EGF[165,166]. In particular, expression of *cripto*, a recently identified member of the EGF family, has been described as associated with gastric cancer[167,168]. Regarding prognostic relevance, there have been a few studies indicating that synchronous expression of EGF and EGF receptor in gastric cancer correlates with poor prognosis[169–171]; for expression of *cripto* an association with tumour stage and prognosis has been reported[168]. However, larger studies assessing the independent prognostic impact of these factors in multivariate analysis still remain to be done.

In intracellular signal transduction, the *ras* genes (N-*ras*, K-*ras*, H-*ras*) are assumed to play central roles, thereby also influencing cell proliferation and cell differentiation[27]. They encode proteins located to the inner layer of cell membranes, preferentially mediating signals of tyrosine kinase receptors by controlled guanosine 5′-triphosphatase (GTPase) activity[27]. Mutations of c-*ras* sites can eliminate the GTPase potential, leading to a permanent state of activation of the encoded RAS protein[27]. In gastric cancer, *ras* mutations have been detected[2,172,173], and a potential role in prognosis has been implicated[172]. However, this remains to be shown in large prognostic studies.

Of the group of cell-cycle regulators, *pic1* (p53-regulated inhibitor of cyclin-dependent kinases (CDKs)) and *MTS1* (encoding p16 protein) have been shown to be directly involved in cancer genesis[28]; *pic1* can be induced by the wild type of the tumour suppressor gene *p53*, but not by its mutants[174], and leads to direct inhibition of DNA replication, thereby preventing cells from entering the S phase[175]. Moreover, from experiments on gastric cancer cell lines *pic1* is hypothesized to suppress cdk2 and G1 cyclins[28]. Thus, mutation of *p53* or *pic1* can lead to

overexpression of cdk2 and G1 cyclins, and to upregulated growth of gastric cancer cells. Correspondingly, overexpression of cyclin E has been reported in gastric cancers[28]. However, to the authors' knowledge there has been no prognostic study on *pic-1*, cdk2 or cyclin E in gastric cancer. The same can be said for mutations of *MTS1* which inhibits CDK4/cyclin D activity[176].

For the group of tumour suppressor genes, most prognostic studies in gastric cancer have been performed on *p53*. The wild-type form of p53 not only induces *pic1* as stated above, arresting the cell cycle in G1 and preventing cell-cycle progression into the S phase[175], but also induces apoptosis[175] after DNA damage[27]. Recent studies indicate that wild-type p53 regulates apoptosis in a manner that protects against malignant transformation[175]. In view of this, it becomes clear how loss of function of p53 by mutation can lead to transformation and/or tumour growth. Indeed in 60 per cent of gastric cancers, regardless of the histological type, allele loss and mutations on *p53* can be detected[28]. However, concerning the prognostic value of *p53* mutations in gastric cancer (mainly investigated immunohistochemically) there is no definite consensus. Mutant p53 proteins have been shown to accumulate within cell nuclei and, in contrast to wild type p53, have a prolonged half-life[177]. Thus, they can be detected immunohistochemically[178] at much higher probability than the wild type[177]. Nevertheless, the main difficulty in immunohistochemical studies is that most of the available antibodies to p53 proteins are not specific for mutant p53, but also bind to wild-type p53[177]. Of the available immunohistochemical studies, two demonstrated a univariate and multivariate significant impact of accumulation of p53 on survival in 125 and 206 patients respectively[179,180]. One study[181] confirmed shorter overall and disease-free survival in 55 patients. A recent report from Oiwa *et al.*[182] postulates an impact of p53 on prognosis in early gastric cancer because of a significant association with the penetrating A type[183]. Two other studies did not find a prognostic relevance of p53 accumulation[184,185]. A recent report from Gabbert *et al.*[186] of 418 patients also failed to describe an association of p53 expression with survival. These authors used a monoclonal antibody, however, which again recognized both mutant and wild-type p53. In conclusion, p53 cannot so far be regarded as an established prognostic factor in gastric cancer.

The expression of *nm23*, a suppressor gene for tumour metastasis and potentially tumour development[27,28,187] encoding nucleotide diphosphate kinase and PuF, a *c-myc* transcription factor[28], has been shown to be reduced in metastatic gastric carcinoma[188,189]. To the authors' knowledge, prognostic studies on *nm23* have not yet been introduced. Mis-sense mutations of another tumour suppressor gene involved in cell–cell adhesion, *APC*, are frequently detected in gastric cancers[28]. However, investigations on prognostic impact of *APC* mutations remain to be carried out.

The *bcl-2* gene encodes for a membrane-associated protein which blocks apoptotic cell death[27,190]. Ayhan *et al.*[191] demonstrated that loss of heterozygosity at the *bcl-2* gene locus could be detected in well differentiated gastric cancers in contrast to poorly differentiated ones. A prognostic role of *bcl-2* in gastric cancer has not been established up to now.

Finally, the concept is increasingly accepted that multiple genetic alterations affecting certain DNA sequences are involved in the development of malignancy. These regions are simple repeated sequences that are genetically unstable and prone to replication errors[192,193]. This genetic instability was detected at microsatellite loci particularly in poorly differentiated gastric cancers[194], in contrast to well differentiated tumours, suggesting a role for genetic instability in the genesis of poorly differentiated gastric cancer.

In summary, most evidence so far indicates a prognostic impact of c-*erb*B-2 and *p53* mutations in gastric cancer. However, the role of the latter in particular has still to be established definitely.

Minimal residual tumour disease

As a final new biologically defined prognostic variable in gastric cancer minimal residual tumour disease is proposed; this does not exist only in haematological malignancies[195,196], it is also suspected in solid cancers. Even in early stages of diverse carcinoma types, single disseminated tumour cells can be detected in the bone marrow compartment as cytokeratin (CK) 18-positive cells using immunocytochemistry[16–25,197–201]. This early systemic disease is mooted as the cause of the fatal clinical course of cancer, inducing recurrence months and years after and in spite of curative resection[26]. Disseminated tumour cells at the time of operation are significantly associated with clinical prognosis in diverse carcinoma types, including breast and colon cancer[16,20,21,24,202]. Disseminated tumour cells were detected before surgery in the bone marrow in 109 of 217 patients (57 per cent) resected for gastric cancer[17]. This has been corroborated by Juhl *et al.*[203], who found disseminated tumour cells in bone marrow and peritoneal washings in 25 of 48 patients. Nevertheless, the simple presence of these cells at the time of operation was not an independent prognostic parameter[17]. However, when the quantity of tumour cells was considered an independent impact on prognosis could be demonstrated.

To find further evidence of the biological autonomy of the systemic disease component in gastric cancer, sequential bone marrow aspirations were performed after curative surgery during follow-up of 78 patients[26]. The individual postoperative tumour cell course correlated significantly with later clinical outcome; 90 per cent of patients who later relapsed showed an increase in or constantly high numbers of tumour cells in bone marrow, whereas patients without recurrence had a reduction in or elimination of tumour cell numbers. An increase of five or more tumour cells in 10^6 6 months after curative tumour resection was a strong independent prognostic parameter[26].

As individual tumour cell courses in bone marrow appeared to be very heterogeneous, it was speculated that these cells were of varying biological and metastatic capacity[204,205]. In view of the association of the uPA system with aggressive tumour potential, an attempt was made to identify metastatic phenotypes by immunocytochemical double-staining for uPA-R[206]. A significant correlation was identified of uPA-R expression of disseminated tumour cells at the time of operation with later increasing tumour cell counts and survival[26,207].

In conclusion, it is suggested that minimal residual disease exists in gastric cancer with a high probability, that aggressive phenotypes of the disseminated cells can probably be identified by uPA-R analysis and that perioperative evidence of uPA-R on such cells might be a new biological prognostic variable. However, this hypothesis has to be verified in a larger series of patients.

Conclusion

Current results strongly suggest that tumour classification in gastric cancer will be extended by variables describing tumour biology. From the persuasive studies on the uPA system, performed not only in gastric cancer but also on several other cancer types[92–98], which all yield similar results, it can be stated that this tumour-associated protease system (and especially PAI-1) should be considered as an established new and independent prognostic factor in gastric cancer. It should therefore be considered in addition to established tumour classifications (defined by R category, pTNM, tumour localization, quality of tumour resection and hospital experience). Moreover, the authors believe that intensifying attempts to characterize protease patterns may lead to a more individualized characterization of the invasive potential of a tumour.

Of the genetic parameters, there is a high probability that c-*erb*B-2 will become a further new prognostic factor in gastric cancer. There is also evidence that the growth factor receptor *cripto* and the tumour suppressor p53 may be shown as new prognostic factors in future studies. Of the tumour-associated proteases besides the uPA system, evidence has accumulated for cathepsin D as a probable

Table 2 Status of current variables as prognostic factors in gastric cancer

	Established risk factor	High (or good) probability of becoming relevant	Potential relevance, yet to be tested	No relevance or low probability of becoming relevant
Established prognostic variables				
Patient-related			Age Sex	Pattern/duration of symptoms, weight loss, co-morbidity, immune status
Treatment-related	Surgical curability Quality of curative resection Hospital level		Allogeneic or autologous blood transfusion Adjuvant chemotherapy (FAMTX, EAP) Neoadjuvant chemotherapy Chemotherapy in non-resectable cancers (FAMTX, EAP, ELF)	Adjuvant chemotherapy (5-FU, FAM) Adjuvant radiation therapy Intraoperative radiation therapy
Tumour-related	pTNM Tumour localization		Grading (G) Lymphangiosis carcinomatosa Blood vessel infiltration	Laurén, Ming WHO classification Borrmann
Tumour markers in serum		Combination of CA 72-4 and CEA	CEA, CA 19-9, CA 72-4 CA 125, Sialyl Tn antigen ST-439, AFP	
New functional biological variables				
Tumour-associated proteases/protease inhibitors	uPA system (PAI-1)	(Cathepsin D) (MMP-2 in combination with activating factors or in balance with TIMP-2)	MMPs, MT-MMP TIMPs Cathepsins B, E, G α_2-Macroglobulin α_2-Macroglobulin receptor α_1-Antichymotrypsin α_1-Antitrypsin, protease patterns Other proteases like interleukin-1 converting enzyme)[81] (not discussed in this review)	α_2-Antiplasmin Plasminogen Antithrombin III tPA
Adhesion molecules		(CD44/CD44 splice variants) (E-cadherin)	ICAM, VLA-4, E-selectin	
Molecular parameters		c-*erb*B-2/p185 (p53) (*cripto*)	c-*met* (HGF receptor) c-*ras* EGF and EGF/TGF-α receptor *pic1*, *MTS1* (p16) G1 and G2 cyclins Cyclin-dependent kinases *nm23*, *APC* *bcl-2* Genetic instability	
Minimal residual disease		Follow-up course of disseminated tumour cells in bone marrow (CK18 positive), uPA-R expression of disseminated tumour cells	Further phenotypic characteristics of disseminated tumour cells Perioperative evidence of disseminated tumour cells in bone marrow	

5-FU, 5-fluorouracil; FAM, fluorouracil adriamycin methotrexate; FAMTX, methotrexate, 5-FU and adriamycin; EAP, adriamycin, cisplatin and etoposide; ELF, etoposide, leucovorin and 5-FU; TNM, tumour node metastasis; WHO, World Health Organization; CEA, carcinoembryonic antigen; AFP, α-fetoprotein; uPA, urokinase-type plasminogen activator; PAI, plasminogen activator inhibitor; MMP, matrix metalloproteinase; MT-MMP, membrane-type matrix metalloproteinase; TIMP, tissue inhibitor of metalloproteinases; tPA, tissue-type plasminogen activator; ICAM, intercellular adhesion molecule; VLA, very late antigen; HGF, hepatocyte growth factor; EGF, epidermal growth factor; TGF, transforming growth factor; CK, cytokeratin; uPA-R, urokinase-type plasminogen activator receptor

prognostic factor. To a lesser extent MMP-2, in combination with activating factors or in balance with TIMP-2, may be shown as a prognostic factor in the future. Of the adhesion molecules, there is the possibility that E-cadherin and CD44 splice variants will become new prognostic factors. In addition, individual follow-up and phenotyping of disseminated tumour cells in bone marrow is a promising new approach to prognosis and tumour biology. An overview on the current status of prognostic factors in gastric cancer is shown in *Table 2*.

Benefits for the patient with gastric cancer can at present be obtained in establishing more detailed risk profiles, orientated at the specific functional properties of the tumour. By biological grading, new patient groups can be defined which should enable more precise planning of adjuvant treatment. However, increasingly attempts are being made to use these biological factors as new targets for anti-invasive and antimetastatic cancer therapy[208–212]. This has already resulted in the first clinical attempts to affect the uPA system (especially uPA-R) and the MMPs by synthetic inhibitors. Potentially, these and further ongoing approaches will lead to therapeutic inhibition of the proteolytic and thus the invasive potential of human cancer.

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