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Biology of metastasis and its clinical implications: renal-cell cancer

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Summary. The ability of malignant cells to metastasize from a primary tumor and from secondary lesions is the most life-threatening aspect of cancer. Reported factors enabling this metastatic cascade to occur include reduced levels or an absence of cell-adhesion molecules, proteolytic enzymes, and angiogenic factors. The metastatic cell must also escape immune destruction. Defects in lymphocytes from renal-cell carcinoma patients with abnormalities in their proliferation, receptor structure, and signal transduction are present. The pathologic stage has been the most consistent single prognostic factor to influence survival. Other factors include the performance status, age, and histology grade and may include serum interleukin 6 (IL-6) levels and ploidy. Current and future therapeutic approaches that interfere with this metastatic cascade include applications of cytokines, antiadhesion-molecule strategies, and antisense nucleotides. An improvement in our understanding of the biology of metastases is essential before a significant increase in the cure rate can be realized.

Background

Renal-cell carcinoma (RCC) accounts for about 3% of adult malignancies, is the third most common type of urologic tumor after cancers of the prostate and bladder, and accounts for approximately 85% of all primary kidney neoplasms. Its peak incidence occurs in the sixth and seventh decades of life, with the mean age at presentation being 57 years. RCC is rare before the age of 20 years (< 1%) and has no racial predilection. Men are more commonly affected than women (ratio, 2 : 1). It is more prevalent among urban inhabitants than among rural dwellers [21]. In 1993 there were approximately 27,200 new cases of renal cancer and more than 10,900 deaths in the United States [12]. Although previously known as the "internists' tumor", in 1995 RCC should properly be called the "radiologists' tumor" because of the increasing number of tumors incidentally diagnosed on ultrasound or computerized tomography (CT) scans performed for nonrenally related problems [57]. Most patients with localized tumors can be cured by surgical resection, and the numbers of incidentally discovered low-stage tumors that are amenable to partial or total nephrectomy appear to be increasing [24]. Nonetheless, more than half of the new cases occurring each year involve regionally advanced or metastatic disease at presentation, and these patients face a collective median survival of 7 months and a 1–2% chance of surviving for 5 years or more [13, 24]. These patients with advanced disease represent a special clinical challenge that requires an improvement in our understanding of the biology of metastases before a significant increase in the cure rate can be realized.

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Metastatic cascade

The ability of malignant cells to metastasize from a primary tumor and form secondary lesions is the most life-threatening aspect of cancer. Understanding how malignant tumor cells are capable of spreading from the primary tumor to form secondary tumors at other sites in the body is a major goal of cancer research. The analyses and experimental animal data relative to tumor-cell populations with different metastatic properties led to the "cascade" theory of metastasis, suggesting that metastasis occurs via a complex series of sequential steps (Fig. 1) whereby malignant cells detach from the primary tumor mass and invade adjacent host-tissue barriers [39, 40]. Tumor cells involved in metastasis are not merely extensions of the original cancer but have their own specific characteristics, which as a whole differ from those of the primary tumor; indeed the propensity to metastasize is found only in a minority of the cells in any given tumor [40]. To metastasize, tumor cells must (1) lose homotypic adhesion to adjacent tumor cells, (2) invade into a vascular or lymphatic vessel, (3) evade host vascular defense mechanisms, (4) adhere to the endothelium or subendothelial basement membrane at a secondary site, (5) intravasate at a secondary site, and (6) finally develop a vascular network to allow for nutrition and secondary growth. What factors enable tumors to evolve from a solitary lesion to this disseminated malignant state?

The initial event is the tumor cell's ability to migrate from the tumor mass. This results from chemotactic factors, loss of adhesive interactions of cells, mechanical pressure exerted by the tumor mass itself, and direct adhesive interactions between tumor cells and vascular or

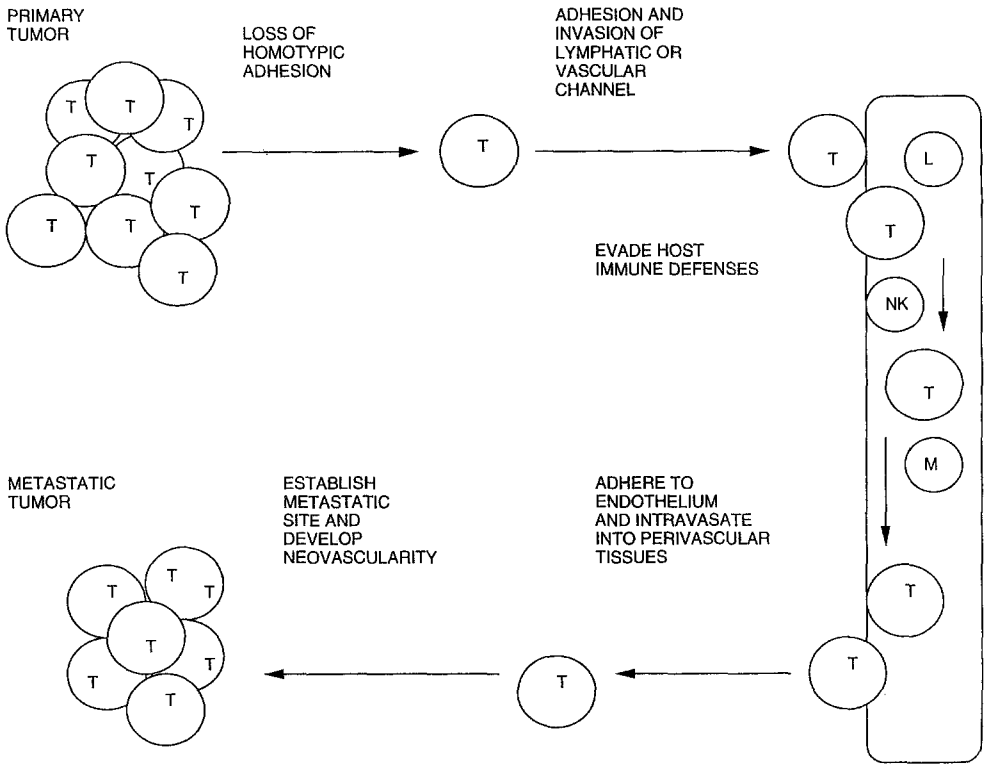

Fig. 1. Metastatic cascade. *NK* Natural killer cell, *L* lymphocyte, *M* macrophage *T* tumor cell

lymphatic endothelium. Reduced levels or an absence of cell-adhesion molecules such as E-cadherin have been reported in cancer cell lines and fresh tumor samples, most notably prostate and bladder cancers. Umbas et al. [54] reported on 92 patients' samples of prostate cancer stained immunohistochemically for E-cadherin. Approximately 50% of the tumors examined showed reduced or absent E-cadherin protein staining. A strong association between a high-grade tumor and aberrant E-cadherin staining of the tumor was noted [54]. A subsequent study has demonstrated that aberrant staining for E-cadherin is a powerful predictor of poor outcome in terms of both disease progression and overall survival [55]. Similar findings have been reported in transitional-cell carcinoma of the bladder ([8]; also see Schmitz-Dräger et al., this issue). Data on the expression of E-cadherin in RCC are much more limited. In one study, E-cadherin was expressed by 12 of 34 RCCs evaluated and showed a clear correlation with grade, as all 12 of the positive RCCs were of grade 2 or less and all grade 3 tumors lacked expression of E-cadherin. These observations suggest that loss of E-cadherin expression may be responsible for loss of homotypic adhesion in high-grade RCC [50].

The next step in the metastatic cascade is the dissolution of the extracellular matrix. This occurs by the actions of a variety of proteolytic enzymes, including type IV collagenase. This protein is a major structural component of the basement membrane [27]. Plasminogen activator, which activates many other proteolytic enzymes, has also been proposed to play a major role in the plasminogen activator-plasmin-collagenase cascade. Inhibition of the enzymatic degradation of the extracelluar matrix may be a place in the metastatic cascade where therapy can be initiated. One study using SN12M cells, a human RCC cell line, has suggested that an aminopeptidase inhibitor may halt the conversion of type IV procollagenase to its active form or the secretion of the collagenases from tumor cells [58].

The mechanisms of tumor cell-endothelial cell interactions are believed to be similar to the extravasation of lymphocytes into perivascular tissue, which involves the binding of specific cell-surface receptors on the lymphocytes to their appropriate counterreceptors on endothelial cells [37, 38]. Tumor-cell-surface receptors attach to specific components in the matrix, such as fibronectin and laminin. Laminin is a large glycoprotein found predominantly in the basement membrane, where one end of the molecule binds to type IV collagen. High concentrations of these receptors have been demonstrated in several neoplastic cell lines, and interference with these receptors' ability to bind matrix has been associated with diminished metastasis in a normal melanoma model system [4, 28]. Recent studies have demonstrated that several other cytokine-inducible endothelial cell-adhesion molecules (CAMs), including endothelial leukocyte-adhesion molecule-1 (ELAM-1), intercellular adhesion molecule-1 (ICAM-1), and vascular cell-adhesion molecule-1 (VCAM-1), play an important role in leukocyte-endothelial interactions [10, 11]. When expressed on activated endothelial cells, these CAMs have been shown to mediate the adherence of melanoma, leukemia, and colon carcinoma to vascular endothelium [42, 48].

Recently, Steinbach et al. [47] showed that stimulation of human umbilical-vein endothelial cells (HUVEC) with interleukin 1β (IL-1β), tumor necrosis factor α (TNFα), or phorbol myristate acetate (PMA) resulted in up to a 3-

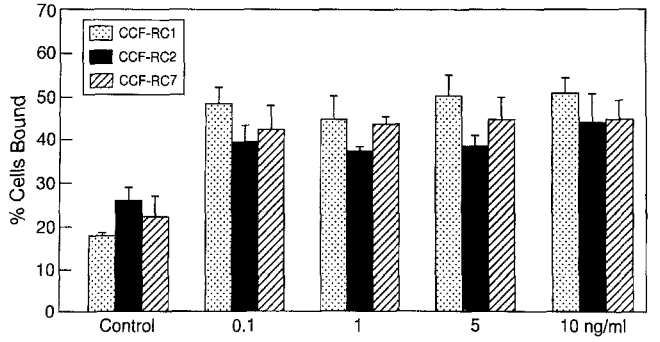

Fig. 2. Effect of different doses of rIL-1β on the adhesion of RCC cell lines to endothelium. Observations at all dose levels were significantly different from control values ($P < 0.001$)

fold increase in RCC adhesion to HUVEC (Fig. 2). Immunocytometry demonstrated the presence of the ligands sialyl Lewis X and VLA-4 on the RCC cell surface, and the interactions of these tumor-cell-surface ligands with VCAM-1 and ELAM-1 in the presence of the above-mentioned cytokines induced this increased tumor-endothelial cell adherence. Time-course experiments were also performed and demonstrated that this increase in adherence persisted for up to 24 h. This suggests that these molecules may play an important role in the ability of RCC both to extravasate from the primary tumor and to intravasate into the vasculature [47].

If the tumor successfully penetrates the vascular endothelium, the malignant cell gains access to the systemic circulation. However, this does not guarantee metastatic deposit. The tumor cell must be capable of evading host defenses such as lmyphocytes, macrophages, and natural killer cells and of surviving the mechanical trauma of the blood flow. Mechanical arrest and adhesion of the malignant cells to the capillary endothelium is then necessary to prevent spontaneous detachment, recirculation, and eventual cell death [1]. Similar tumor cell-endothelial cell interactions may again play an important role in the tumor cell's ability to migrate into the perivascular tissues. Once a metastatic focus has been established, additional factors concerning the long-term viability of these seeded tumor cells then come into play.

Initially, nutrients for metastatic tumor cells established at a distant site arrive by simple diffusion. However, solid tumors are incapable of growing beyond 1–2 mm in diameter without organized vascularization [16]. Numerous potent angiogenic factors, including basic fibroblast growth factor (bFGF), transforming growth factor-α (TGFα), tumor angiogenesis factor (TAF, angiogenin), and vascular endothelial growth factor (VEGF), have been identified [17]. For example, implantation of human RCC in the rabbit cornea has resulted in new vascular growth from the limbus toward the tumor implant. Bard et al. [3, 35] have suggested that an angiogenic substance is secreted from the RCC tumor cells, resulting in neovascularity. This factor is present in clear-cell and granular-cell carcinomas and, less so, in papillary RCC and may be the reason why vascular tumors have a more aggressive biological nature [3, 35]. This could potentially be an excellent model for the study of the effects of these angiogenic agents and how each relates to individual tumor angiogenesis. The question as to whether RCC tumor cells with metastatic ability retain this capability to stimulate angiogenesis has not yet been studied, but the answer is likely positive. In another study, high levels of VEGF mRNA were found by in situ hybridization to be expressed in tumor cells in 11 of 12 RCC patients, the only exception being a case of an avascular papillary variant. Both tumor cells and endothelial cells in small vessels adjacent to the tumor stained strongly for VEGF protein by immunohistochemistry [9]. These studies strongly suggest that angiogenic factors play a role in allowing RCC to establish metastatic foci.

Role of the immune system

The metastatic process consists of a series of linked steps, all of which must be successfully completed by tumor cells if metastasis is to develop. The process is probably not random and favors the survival and growth of a few populations of cells from the parent neoplasm [1]. Metastasis is a highly selective process that is influenced both by the intrinsic properties of tumor cells and by host factors. Theoretically, certain conditions need to occur before viable metastases can develop. The original tumor cell must escape immune destruction. Possibly, the tumor itself may be capable of altering its own microenvironment either by producing endogenous factors or by exhibiting altered expression of certain cell-surface molecules, thus preventing the development of a normal host immune response. These alterations would allow the tumor to grow and accumulate additional genetic mutations, which eventually permit expression of metastatic ability.

Growing experimental evidence suggests that RCC induces a selective immune unresponsiveness that may prevent tumor rejection at an early stage and permits unabated growth. This evidence includes:

1. Proliferative unresponsiveness of T-TILs (T-cell tumor-infiltrating lymphocytes) as compared with T-PBLs (T-cells from peripheral blood lymphocytes) derived from the same patient. This unresponsiveness is evident despite normal levels of CD3 and IL2Rα/β (interleukin 2-receptor alpha- and beta-chains) in T-TILs [2].

2. Deficiencies in expression of a protein, granzyme B, known to play a role in the cytotoxic effector function of T-cells. Studies reveal that mRNA levels of this enzyme after appropriate stimulation measured via RT-PCR (reverse transcriptase-polymerase chain reaction) in T-TILs were depressed or absent in five of nine patients with RCC despite expression by T-PBLs (Kudoh et al., manuscript in preparation).

3. Abnormalities in the structure of the TCR (T-cell receptor) in T-TILs versus T-PBLs. Finke et al. [14] demonstrated in Western-blotting studies a marked decrease in expression of the TCRζ chain in the T-TIL specimen as compared with the T-PBL sample from the same patient. A decrease in TCRζ-chain but not CD3ε expression was also detected in frozen sections of RCC tumors by immunohistology.

4. A marked decrease in the expression of two tyrosine kinases linked to TCR/CD3 signaling pathway, p561ck and p59fyn, has been detected in T-TILs versus T-PBLs. Western-blotting studies showed a marked decrease in the expression of these kinases in 10 of 11 patients in T-TILs, whereas T-PBLs expressed detectable but lower than normal levels of both kinases [14]. It has been hypothesized that a soluble product poduced by the tumor cells or resulting from the immune response might be responsible for inducing the changes detected both in the TCR and in the pattern of signal transduction. Further studies have shown that the described defects in T-TILs are reversible in vitro when T-cells are removed from the tumor environment [56].

5. Normal proximal tubular cells express low levels of CD54 (ICAM-1), whereas primary clear-cell RCCs express high levels of CD54. CD54 appears to be an important mediator of autologous TIL interactions in RCC, including tumor lysis and TIL proliferation [46]. Since ICAM-1 expression stimulates natural killer cell (NK)-mediated tumor lysis, overexpression by RCC suggests a defect in the lytic arm of the immune response or an alteration in the feedback regulation affecting the immune cells. Together, these observations suggest that immune defects in patients with RCC may be an important initial step in permitting tumor cells to proliferate and accumulate the genetic alterations associated with the metastatic phenotype.

Clinical implications and prognostic factors for metastases

With recent radiographic improvements, increasing numbers of RCCs are now being discovered at an earlier stage. However, a large percentage of patients continue to present with metastatic disease at the time of initial diagnosis. Even though spontaneous regressions of RCC with metastatic disease have been reported, these occur infrequently. Various clinical prognostic factors have been evaluated in an effort to determine which factors have relevance in influencing prognosis, predicting survival, and determining the optimal form of therapy. The tumor stage continues to be the best overall predictor currently known. Many other factors, including the patient's age and sex, the size and side of the primary tumor, the performance status, the number of metastatic sites, the specific organ involved with metastatic disease, and the interval between the diagnosis of the primary tumor and the diagnosis of the metastatic disease, may also serve as independent predictors and are under investigation.

Stage

Regardless of the tumor-staging system used, the pathologic stage has been the most consistent single prognostic factor to influence survival. Survival is inversely correlated with increasing tumor stage. Patients with distant metastatic disease have an approximate 5-year survival rate of 5–10% and a 10-year survival rate of 0–7% [43, 44]. Extension through Gerota's fascia and regional lymph-node or contiguous organ involvement have also been associated with decreased survival [6].

Solitary metastasis

Because of the dismal results of radiotherapy, chemotherapy, and hormonal therapy, surgery has been advocated for patients with a solitary metastasis. Solitary metastasis occurs in less than 5% of patients with metastatic disease, and these patients have a better prognosis than do patients with multiple metastases [20]. Nephrectomy in metastatic patients is usually performed only in individuals who are symptomatic from their primary tumor or have a solitary metastasis that can also be resected at the time of nephrectomy or in patients undergoing experimental therapies in which a nephrectomy is an entrance requirement for the trial. In one study of patients treated with excision of their solitary metastasis, a 33% 5-year survival rate was noted [44]. The prognosis is even better for those who have an isolated pulmonary focus. A survival advantage also appears to exist for those patients who have a lengthened interval between the time of initial presentation and the time at which metastatic disease is discovered [40].

Performance status

Performance status, as judged by standardized scales, has proved to be an important independent prognostic factor for patients with metastatic RCC in several studies. Patients with a good performance status have survived more than 3 times as long as those with a poor performance status and appear to respond best to therapy with biologic response modifiers [31].

Interleukin 6

Serum levels of IL-6 may also serve as a prognostic factor in patients with RCC. Blay et al. [5] evaluated 138 individuals with metastatic RCC and found detectable levels of IL-6 (above 76 pg/ml) in 48% of patients as compared with 11% of 70 normal controls. A detectable pretreatment level of IL-6 was found to be an adverse prognostic factor on the basis of the likelihood of a partial or complete response to therapy with systemic IL-2 (6% of patients with detectable levels achieved a partial or complete response as compared with 26% of those with undetectable levels), the time from diagnosis to onset of metastases (7 versus 22 months), and the median survival (8 versus 16 months). Furthermore, no patient with serum IL-6 levels above 300 pg/ml achieved a partial or complete response, and this group had the shortest median survival (5 months). Two other smaller studies have demonstrated similar findings [19, 45].

Several observations also suggest that the acute-phase reaction properties of IL-6 may mediate some RCC-related paraneoplastic syndromes. These occur in about 10% of patients and are characterized by fever, hepatosplenomegaly, and reversible hepatic dysfunction (Stauffer's syndrome). Prolonged erythrocyte sedimentation rates (ESRs) and elevated levels of C-reactive protein (CPR) have been noted in patients whose tumors express IL-6, with the elevations in CRP being proportional to the serum levels of IL-6 in patients with both localized and metastatic disease [5, 49, 53]. These studies suggest that

serum levels of IL-6 may be a clinically useful marker for patient stratification in the design of new immunotherapeuty trials [23].

Sex

The role of sex as a prognostic factor remains somewhat controversial. Whereas some studies have found no adverse impact conferred by male gender [43], others have reported a significantly worse prognosis for men than for women [52]. However, in the latter study, the men tended to present with more advanced stages of RCC, and when a multivariate analysis was performed to account for this difference in stage, the survival difference between men and women disappeared. Thus, these data indicate that gender cannot be used as an independent prognostic factor for RCC [52].

Age

Age appears to have no significant influence on the prognosis for patients with disseminated RCC. Naito et al. [36] evaluated 57 patients with stage IV RCC and concluded that the patient's age and sex and the side of the primary tumor were not important factors in prognosis. Various researchers have studied the prognostic significance of age at presentation to address the question of a more aggressive form of RCC in younger patients. Two large studies conducted at the Mayo clinic in different eras could find no statistically significant difference in survival according to age at diagnosis [26, 41]. No adverse prognostic impact by race or tumor side has been reported [43].

Histology

RCCs can be broadly grouped into four histologic types: clear cell, granular cell, tubulopapillary, and sarcomatoid [34]. However, mixed histologic forms of these tumors are not uncommon. The clear-cell type is most common, with the cytoplasm containing cholesterol, triglycerides, and glycogen. In decreasing order of prevalence, granular cells exhibit a homogeneous eosinophilic cytoplasm, large nuclei, and abundant mitochondria. A tubulopapillary tumor is small, confined to the cortex, nearly completely encapsulated, and displays little anaplasia, if any. The sarcomatoid form is composed primarily of spindle -shaped cells.

Two other less-well-known forms of RCC, the chromophobe and collecting-duct types, have both been described as having morphologic and immunohistochemical features of collecting-duct epithelia. The chromophobe type was first described in 1974 in rats and was described in more detail in 1988 by Thoenes et al. [51]. The tumor mass is a well-circumscribed solitary mass, with transparent to slightly eosinophilic cells surrounding vascular channels. Metastases in this cell type are rare. The collecting-duct cell has also been reported to be the cell of origin for RCC in a very limited number of cases. This variety of tumor is usually located in the medulla and is also known as Bellini-duct carcinoma. Monosomies and a strong family history have been associated with this tumor.

Clinical experience suggests that tumors with spindle cells are significantly more malignant than other cell types. Preliminary evidence suggests that the granular-cell and chromophobe-cell forms of RCC may have better survival, whereas the clear-cell and collecting-duct-cell varieties may carry a worse prognosis. There remains a definite lack of correlation of tumor-cell type to tumor stage and/or grade, and further studies need to be performed to determine if the tumor-cell type can be used as a potential clinical predictor of outcome in RCC patients.

Patterns of tumor growth include papillary, trabecular, tubular, cystic, and solid types, and all these types are unrelated to clinical behavior except for the papillary pattern, which constitutes 5–15% of all RCCs. A 5-year survival of 85% has been reported as compared with 52% for non-papillary carcinomas [32].

Ploidy

The DNA content (ploidy) of the tumor has also been considered a potential independent biologic prognostic factor of significance. Preliminary results indicate that patients with diploid tumors may have a survival advantage as compared with patients with aneuploid tumors [29, 30]. If these modalities are ever to be instituted clinically on a regular basis, trials controlling for tumor stage and grade will need to be performed.

Grade

Of all the pathology studies done to evaluate RCC, the nuclear grade of the tumor may have the greatest prognostic significance. Both Skinner et al. [44] and Fuhrman et al. [18] have developed a similar grading system based on nuclear characteristics. Grade 1 tumors have small nuclei and absent nucleoli. Grade 2 tumors possess slightly enlarged nuclei that are often pyknotic and have nucleoli. Grade 3 tumors have enlarged irregular nuclei and prominent nucleoli. Grade 4 tumors contain bizarre giant nuclei and are often multilobulated. Skinner et al. [44] found a strong correlation between tumor grade and survival, even among tumors of the same stage. Fuhrman et al. [18] could separate patients into three categories on the basis of survival: (1) a good prognosis, grade 1; (2) an intermediate prognosis, grades 2 and 3; and (3) a poor prognosis, grade 4. Studies conducted by Selli et al. [43] and Medeiros et al. [33] showed that grade 1 and grade 2 tumors correlated with a significant increase in disease-free survival over the higher grades of tumors.

Potential therapeutic approaches

Future clinical trials must acknowledge the role of the prognostic factors and biologic properties outlined herein and stratify patients accordingly to help define the subset with the best likelihood of therapeutic response. Treatment with biologic response modifiers has emerged as the therapy of choice for established metastatic disease. Response rates of 15–40% have been reported for treatment with a variety of systemically delivered cytokines with or

without concurrent infusion of immune-active lymphokine-activated killer (LAK) or TIL cells. Patients with a good performance status and a minimal metastatic burden appear to respond best to this approach. Although complete response appear to be durable, only a modest improvement in overall cure rates has been obtained with cytokines as compared with chemotherapy.

The optimal approach to prevent growth of micrometastasis in this population has yet to be defined. Current cytokine or combined cytokine-chemotherapeutic regimens are aimed at overcoming immune defects that permit tumor progression with or without concomitant cytoreduction. These approaches are currently limited by the toxicity of systemic cytokine administration and undefined response rates. Vector-mediated gene therapy exploiting autologous or HLA-modified allogeneic RCC cells modified to secrete high doses of cytokines represent another approach to overcome host immune tolerance. Four phase I clinical trials using this paracrine approach are currently in progress in humans with advanced RCC.

Several animal studies have demonstrated that the high local doses of cytokines achievable with this technology can induce systemic immunity to tumor rechallenge as well as eradicate a minimal metastatic disease burden. Furthermore, this form of active-specific immunotherapy holds promise for the establishment of long-lasting tumor-specific immunity that may protect against recurrence for the lifetime of the patient. However, the complexity of the tumor-host immune interaction makes it likely that a combined therapeutic approach will be necessary to improve the cure rate.

The biological mechanisms underlying metastases are fertile source for the development of other therapeutic strategies to be used alone or in conjunction with immune-based therapies. For example, agents that induce expression of E-cadherin or similar cell-surface adhesion molecules might prevent the development of new metastatic foci by inhibiting cells from breaking their initial attachment to the primary tumor. Bracke et al. [7] showed that tamoxifen was capable of increasing the function of E-cadherin and inhibiting invasion in vitro in human breast-cancer cells. Although no study conducted to date has confirmed this finding in RCC, the therapeutic concept remains promising. Further down the cascade, if an effective anticollagenase could be developed that would reproducibly inhibit degradation of the extracellular matrix, treatment could prevent access to the systemic circulation. Antiadhesion-molecule strategies include monoclonal antibody therapy against the adhesion molecules, preventing tumor-endothelial interactions required for extravasation and intravasation. Another possibility for therapeutic intervention could be the use of antisense oligonucleotides to offer the potential to block the expression of specific genes within cells. The recently described von Hippel-Lindau (VHL) tumor-suppressor gene, which is often mutated in sporadic RCC, is a potential target for this approach.

Once a metastatic site has been established, the focus of therapy needs to address either limitation of its continued growth and development or eradication of the site itself. The therapeutic institution of antiangiogenic factors could prevent continued growth of the metastasis and limit its site to a size that simple diffusion could support. Although this would not eliminate the disease from the host, it might allow a prolongation of survival or permit a more effective immune response by limiting the tumor burden. Lastly, palliation of the symptoms to improve the quality of life of the disseminated RCC-afflicted patient must not be ignored. It will be interesting to learn whether anti-IL-6 antibodies will reduce or eliminate the symptoms of RCC-related paraneoplastic syndromes.

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