

Metastases in Immune-Mediated Dormancy: A New Opportunity for Targeting Cancer

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Abstract

The aim of any anticancer treatment is to avoid, control, or eliminate disseminated tumor cells. Clinical and experimental evidence has revealed that metastases can remain in a latency state, that is, metastasis dormancy. Three mechanisms are thought to be involved in cancer dormancy: cellular dormancy, angiogenic dormancy, and immune-mediated dormancy. Here, we review the mechanisms and cells involved in immune-mediated cancer dormancy and discuss current and future immunotherapeutic strategies. Recent results indicate that the immune system can restrain disseminated cancer cells, promoting their permanent dormancy. CD8⁺ T lymphocytes play a relevant role in maintaining immune equilibrium with metastatic dormant cells, and MHC class I surface expression on tumor cells may also be involved. Natural killer (NK) cells have an activator function that triggers a cytotoxic T lymphocyte (CTL) response. Furthermore, immune dormancy promotes cancer cell growth arrest and angiogenic control. Immunotherapeutic interventions in metastatic dormancy may help to control or eradicate cancer disease. Treatments that activate or increase the CTL immune response or reverse cancer cell-induced CTL immunosuppression might be useful to restrain or destroy metastatic cells. These objectives may be achieved by recovering or increasing MHC class I surface expression on cancer cells or even by activating NK cells. Immune-mediated metastasis dormancy provides an opportunity for targeting cancer in novel immune treatments. *Cancer Res*; 74(23); 6750–7. ©2014 AACR.

Introduction

In cancer progression, the migration and invasion of tumor cells to other organs is a fatal stage that may end with the onset of overt metastases and the death of patient. It is well established that the clinical detection or not of metastases determines the prognosis of the patient. However, many patients with no detectable metastases at the diagnosis develop overt metastases or primary tumor relapse some months, years, or even decades later. This latency period, during which cancer cells do not grow or progress and remain occult in a quiescent or equilibrium state, is known as "cancer dormancy". In these patients, disseminated tumor cells (DTC) can remain in a latent state as micrometastases, designated "dormant metastases". The dormancy of tumor and metastatic cells has been widely reported in human patients and in preclinical animal models (1). It usually occurs during metastatic progression, and its duration varies among different cancers or even

between different types of the same cancer. Thus, metastases are normally detected within 5 years of surgery in patients with breast cancer with HER2⁺ or triple-negative tumors but after 10 or even 20 years after surgery in those with ER⁺ breast cancer (2, 3). A latency period of 10 to 15 years is also frequently reported in patients with melanoma or renal cell carcinoma (4, 5), whereas the median postsurgical interval before metastasis detection exceeds 15 years in prostate cancer (6). The above clinical data suggest that dormant DTCs may cause metastatic relapse and that targeting them may be an effective anticancer treatment. Some mathematical models have shown dormancy to be a state of equilibrium that can persist for long time periods or even indefinitely, whereas others conclude that the state of equilibrium must end, with the escape or complete destruction of the cancer cells (7, 8). Both propositions have been supported by experimental data found in human and animal cancers. Thus, disseminated metastatic cells have also been found to remain in permanent dormancy throughout the life of animals, and autopsies of humans have detected small tumors or metastatic nodules that had remained in dormancy (9, 10).

Hence, dormancy is a well-documented stage in cancer progression, during which cancer cells are controlled and maintained in a latent state. Therapies designed to extend this period, destroy dormant cancer cells, or revert progressing cancer cells to a dormant state represent a promising approach against cancer. The design of such treatments requires knowledge of the molecular mechanisms involved in cancer dormancy, and some progress has been achieved in this respect over recent years.

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Mechanisms Involved in Cancer Dormancy

Considerable research has been devoted to the characterization of dormant DTCs, to the mechanisms involved in maintaining a nonexpanding state, and to the transition between dormancy and metastatic relapse. When a patient is diagnosed with cancer, solitary dormant tumor cells and dormant micrometastases might be present in multiple organs. The following mechanisms have been reported to underlie dormancy: cellular dormancy, through mitotic arrest or a balance between proliferation and apoptosis mediated by interaction between the microenvironment and residual cancer cells, angiogenic dormancy, and immune-mediated dormancy (1, 11, 12). The first two mechanisms have been extensively reviewed (13) and are only briefly discussed here. The present review focuses on immune-mediated metastatic dormancy, discussing the mechanisms and immune cells involved and identifying possible immunotherapy treatments to eliminate residual metastatic cells or maintain them in permanent immune dormancy.

Cellular dormancy and angiogenic dormancy

The interaction of solitary tumor cells with the different microenvironments and extracellular matrices encountered in secondary organs may determine their expansion or latency. Various authors have observed that the invasiveness of tumor cells is increased by the binding of urokinase plasminogen activator (uPA) to its receptor (uPAR) on their surface (14, 15). Conversely, a reduction in uPAR expression results in G₀-G₁ arrest and disrupts the uPAR complex, increasing p38 signaling (negative regulator of angiogenesis) and dormancy is promoted by a low ERK/p38 ratio. Thus, suppression of p38 activity in dormant tumor cells restored their proliferation (16). In addition, reduced PI3K-AKT signaling has been reported to play a role in DTC quiescence (17). The development of new blood vessels is required for this restoration of proliferation, and the shift from an anti- to pro-angiogenic microenvironment, the so-called "angiogenic switch", is considered a key factor in the transition to a clinically aggressive cancer (12, 18). In this regard, Achilles and colleagues reported that human cancers contain subpopulations of tumor cells that vary in their angiogenic ability (19). Thus, transfection of nonangiogenic human tumor cells with an activated *Ras* oncogene was found to produce a transition to the angiogenic phenotype in a mouse model of human osteosarcoma dormancy (18). Other experimental studies have demonstrated that DII4 and Notch3 play a role in angiogenic dormancy (20, 21). The two mechanisms mentioned above (quiescence and angiogenesis) have been demonstrated in local tumors in immunodeficient mice; however, it is not clear whether growth arrest and angiogenic dormancy take place during metastatic progression in humans, largely due to the difficulty of detecting and isolating dormant micrometastases in patients with cancer.

Immune-mediated dormancy

The direct involvement of the immune system in maintaining cancer cells in dormancy is supported by clinical and experimental evidence. There is a higher incidence of cancer

in patients undergoing immunosuppressive treatments (22), and there are reports of the nonintentional transplant of an occult tumor to an immunosuppressed host, suggesting that it had remained dormant for some years (23). DTCs in a dormant state have been observed in bone marrow from patients with breast cancer, which also showed a rise in several immune subpopulations, including natural killer (NK) cells, macrophages, and T lymphocytes, with increased expression markers of activation, proliferation, costimulation, and memory (24). All of these findings strongly suggest that the immune system recognizes these DTCs and that memory T lymphocytes migrate to the bone marrow in humans to control these dormant cancer cells. Similar data were observed in a mouse lymphoma model, in which a small number of tumor cells persisted in the bone marrow along with an increase in memory T cells (25, 26). In patients with B-cell lymphoma, therapy with monoclonal anti-idiotypic antibodies alone or in combination with cytokine or chemotherapy was found to promote tumor regression for long time periods, but persistent malignant cell clones in dormant stage were detected in these patients (27). These results are in line with experimental findings using a B-cell lymphoma 1 model (BCL-1) in BALB/c mice, in which 70% of mice immunized with anti-idiotypic vaccine before BCL-1 cell injection remained asymptomatic but with persistence of dormant BCL-1 cells (28). In further assays, CD8⁺ T cells and IFN γ were found to maintain these cells in dormancy (29). In addition, mice preimmunized with mitomycin C-treated or irradiated L5178Y lymphoma cells elicited a strong protective cytotoxic T lymphocyte (CTL)-mediated response that kept L5178Y tumor cells in a dormant state in the peritoneal cavity (30). A study by Eyles and colleagues in RET.AAD mice found that CD8⁺ T cells were involved in controlling the growth of early-disseminated melanoma cells (31). In a mouse model of chemically induced carcinogenesis, Koebel and colleagues found that adaptive immune components (e.g., CD4⁺ and CD8⁺ T lymphocytes, IFN γ , and IL12) are involved in controlling the state of equilibrium between primary tumor cells and the immune system (32). In this model, IL23 and IL12 directly participate in maintaining this equilibrium but in opposite directions, with the former promoting cancer persistence and the latter preventing cancer outgrowth (33). A related issue is whether direct immunosuppression by tumor cells might also promote escape from the state of equilibrium. A recent study examined the immune microenvironment profile in primary chemically induced murine sarcomas and reported that the equilibrium state was maintained by a balance between immunosuppressive cells, especially myeloid-derived suppressor cells (MDSC) and Treg cells and antitumor immunity cells (CTLs, NK cells, and $\gamma\delta$ T cells). The duration of this state of equilibrium largely depended on the CD8⁺/CD4⁺ and CD8⁺/Treg cell ratios among the immune cells that infiltrate the tumor microenvironment (34). This result is consistent with previous reports in human colon and ovarian cancer that CD8⁺ tumor-infiltrating lymphocytes and high CD8⁺/Treg cell ratios are associated with increased survival (35, 36). MDSCs can increase Treg cells, suppressing T-cell activation and cytotoxicity, and may even sustain tumor growth by protecting a fraction of proliferating

tumor cells from senescence (37). In a DA1-3b mouse model of tumor dormancy, the hosts were vaccinated with DA1-3b cells expressing CD40L or IL12 before the injection of parental DA1-3b cells, and a fraction of these mice did not develop leukemia but harbored dormant leukemic cells (38). It was observed that these cells escaped dormancy through an immunosuppressive mechanism in which B7-H1 and B7-1 receptors are overexpressed, indicating a direct involvement of CD8⁺ T lymphocytes in maintaining the dormant state (39). Furthermore, regulatory B cells might promote escape from dormancy by inhibiting antigen presentation by dendritic cells and macrophages. In this context, the presence of cancer-related autoantibodies in the serum or tumor microenvironment of patients with cancer is associated with a poor prognosis (40). Taken together, these data suggest that the balance between immune effector cells and suppressor immune cells may influence the fate of a tumor.

A novel preclinical model of permanent immune-mediated metastatic dormancy

Knowledge of the participation of the immune system in the evolution of the cancer to metastases is very limited. Most of the research data derive from immunodeficient mice models, in which the role of the immune system cannot be investigated, or from the study of experimental metastases in animal models, which do not resemble the metastatic process in humans. *In vivo* metastatic models need to recapitulate the distinct stages of the disease, the microenvironment, and the presence of an autologous immune response. It took our group more than two decades of research using a cancer murine model of spontaneous metastases to study the characteristics and mechanisms of the metastatic process. We recently reported a novel preclinical cancer model of permanent immune-mediated metastatic dormancy (10). The original cancer murine model comprises different clone cell lines derived from a methylcholanthrene-induced fibrosarcoma and cell lines derived from the spontaneous metastases obtained with each of these clones (41). We found an elevated spontaneous metastatic capacity in tumor clones that were highly positive for MHC-I and no metastatic capacity in MHC-I-negative clones and mice with the latter remained metastasis-free throughout their life (10, 42). We expected MHC-I-negative GR9-B11 tumor cell clone to lack migratory and invasive capacities, but when we repeated the same spontaneous metastasis assays in immunodeficient mice, a large number of overt pulmonary metastases appeared in all of the animals (10). Hence, the tumor cells were able to migrate and invade in the absence of a T lymphocyte-mediated immune response. The immune response was analyzed in tumor-bearing immunocompetent mice, detecting an increase in T lymphocytes, dendritic cells, and macrophages (10). We then investigated whether this immune response destroyed DTCs or merely kept them in a state of dormancy. Tumor-bearing mice were left for 4 months after primary tumor removal and then began to be depleted of T lymphocytes or asialo-GM1 cells. The mice were euthanized 3 months later, and complete necropsy revealed overt pulmonary metastases in all mice depleted of CD8⁺ T lymphocytes in 86% of those depleted of asialo-GM1⁺ cells, but

in only 25% of the mice depleted of CD4⁺ T lymphocytes (10). Tumor-bearing mice that were not immunodepleted remained metastasis free throughout their lives (10). According to these experimental animal data, spontaneous metastases can be restrained in permanent dormancy by CD8⁺ T lymphocytes and NK cells and, to a lesser extent, by CD4⁺ T lymphocytes (Fig. 1). Numerous metastases were observed in nu/nu BALB/c mice, despite their possession of large number of NK cells, indicating an absence of direct cytotoxic action by these cells (10). Furthermore, analysis of the immune response in tumor-bearing immunocompetent mice free of overt metastases found no increase in the number of NK cells, and only the depletion of CD8⁺ cells promoted the onset of overt metastases in all animals. It is therefore possible that NK cells may promote the development of a CTL immune response independent of CD4⁺ cells. Hence, MHC-I-negative GR9-B11 cells may activate NK cells, priming local dendritic cells and stimulating a strong protective response by CD8⁺ T cells. An increased population of dendritic cells was observed in GR9-B11-bearing mice (10). Given that the disseminated metastatic cells recovered MHC-I cell surface expression, they might be recognized and kept in dormancy by CD8⁺ T lymphocytes (Fig. 1). The study of another highly metastatic MHC-I-positive tumor clone in the same cancer model showed that immunotherapeutic treatment of the host avoided metastatic colonization (43); however, depletion of T lymphocytes after the immunotherapy promoted the awakening and growth of dormant metastases, which colonized the lungs and caused the death of these mice (unpublished observations).

Taken together, the above data demonstrate that CD8⁺ T lymphocytes participate in the control of dormant spontaneous metastases (Fig. 1). The findings of our group suggest that the interaction between MHC-I molecules on the cancer cell surface and T-cell receptors may also play an important role in dormancy. Schirmmayer and colleagues reported that a tumor-associated antigen (TAA) expressed by a small population of dormant cancer cells in the bone marrow favors the maintenance and high frequency of TAA-specific long-term memory CD8⁺ T cells (25, 26). Interestingly, MHC-I surface expression on these dormant tumor cells was upregulated with respect to the parental tumor cells. Another key issue is whether the dormant metastases in our model are in a quiescent state or in a state of equilibrium between cell proliferation and cell death. Cell lines derived from overt metastases obtained after immunodepletion of the hosts presented *in vitro* and *in vivo* proliferative capacity. These dormant metastatic cells therefore appear able to maintain a certain proliferative capacity. The possibility that the dormant metastatic cells were cancer stem cells can be ruled out, because they did not have the low expression of MHC-I molecules and high proliferative capacity characteristic of stem cells.

In most tumor models of dormancy in animals, the hosts are transgenic mice with a predisposition to develop cancer or are previously immunized with syngeneic tumor antigens/tumor cells or treated with anticancer therapies. We emphasize that the present dormant metastatic model is nontransgenic and that the hosts do not receive any anticancer therapy and are injected with nongenetically modified tumor cells. The model

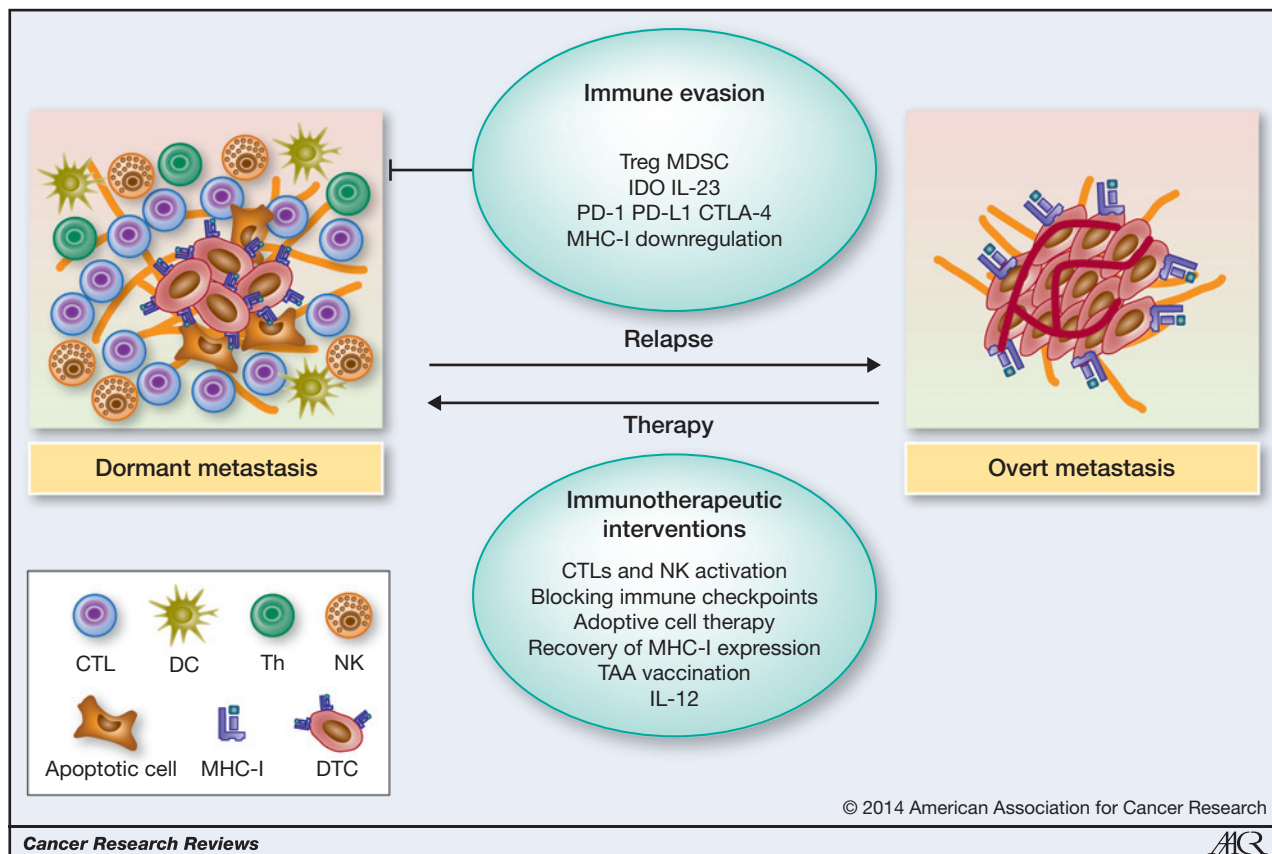


Figure 1. Immune-mediated metastatic dormancy stage: mechanisms involved and immunotherapeutic interventions. CTL activation by Th or dendritic cell-activated NK cells restrains metastases in dormancy. MHC class I surface expression on tumor cells may be involved in this process. This immune equilibrium can be disrupted by various immune evasion mechanisms, resulting in overt metastases. Different immunotherapeutic interventions may lead to the restraint or destruction of DTC.

also recapitulates all stages of human metastatic disease, making it ideal for studying the mechanisms and cells involved in immune-mediated metastatic dormancy.

Immune dormancy may promote cancer cell growth arrest and angiogenic control

Various authors have proposed that immune cell-mediated dormancy may require the induction of tumor cell growth arrest and angiogenic control. Thus, Koebel and colleagues reported that the state of equilibrium of the primary tumor mass in a mouse model was characterized by a combination of T-cell-mediated tumor cell rejection with increased apoptosis and decreased proliferation of the tumor cells (low Ki67 expression; ref. 32). In an RET.AA model, Eyles and colleagues found an association between the presence of DTCs in the lung and a reduction in Ki67 positivity with respect to the primary tumor (31). Muller-Hemerlink and colleagues reported that Tag-specific Th1 cells induced antiangiogenic chemokines and senescence in Tag-expressing tumor cells via IFN γ and TNFR1 signaling (44). The CD4⁺ T-cell-mediated antitumor effects observed were attributed to the release of the potent angiogenesis inhibitors CXCL9 and CXCL10 and to a reduced $\alpha\beta$ 3 integrin expression. They also found that secreted IFN γ and

TNF arrested cancer cells in G₀-G₁ by stabilizing the p16INK4a-Rb pathway without inducing their death. This cytokine-induced senescence required the combined action of STAT1 and TNFR1 signaling (45). Moreover, IFN- and TNF-induced *in vitro* growth arrest in other types of murine and human tumor cell lines (45). All of the above evidence suggests that the antitumor immune response may induce dormancy via nonimmune mechanisms.

Immunotherapeutic Interventions in Metastatic Dormancy

The aim of anticancer therapies is to completely eradicate the cancer clinically detected in a patient, avoiding tumor primary recurrence and dissemination and the growth of malignant cells at distant sites. As reported above, numerous studies have described a state of equilibrium between dormant metastatic cells and the anticancer immune response and have shown that these dormant cells can develop escape mechanisms and progress (Fig. 1). The development of animal models that closely mimic the immune-mediated dormant cancer state is crucial for evaluating novel immunotherapeutic strategies. Knowledge of the mechanisms involved in cancer dormancy and immune escape may help to restrain or eradicate

these dormant micrometastases. Dormant disseminated metastatic cells have a very low proliferation rate or are quiescent and are therefore not susceptible to conventional chemotherapy or radiotherapy. Furthermore, elevated chemotherapy and radiotherapy doses can impair the immune system, which may lead to the awakening of dormant cancer cells, whereas low doses can induce immunogenic death and the host immune response. Eradication of this small population of dormant metastatic cells is a challenging task due to the difficulty of their detection. In immune-mediated dormancy, immune cells may be a vehicle for finding and destroying dormant cells or for keeping them indefinitely in latency, as in our model. It may also be possible to revert progressing cancer cells to a stage of permanent dormancy (Fig. 1).

CTL activation

As noted above, CTLs participate in metastatic dormancy and may even restrain spontaneous metastases in permanent dormancy. There may therefore be a role for immunotherapies to promote CTL activation or reverse cancer cell-induced CTL immunosuppression, including treatments to block immune checkpoints such as the immunosuppressive receptors expressed on cancer cells or on CTLs (CD80, CD86, PD-L1, PD-1, CTLA-4; Fig. 1). In fact, various antibodies against PD-L1, PD-1, and CTLA-4 are currently undergoing clinical trials (nivolumab, MK-3475/MDPL3280A, MDX-1105), and the anti-CTLA-4 antibody ipilimumab is approved by FDA for clinical use and has achieved highly promising outcomes. Another possible approach would be to inhibit the signal pathways involved in the surface expression of these immunosuppressive receptors or in their signal transduction pathways. In this respect, researchers have reported the involvement of JAK/STAT, MEK, PTEN, and PI3K pathways (46, 47). The benefits to patients may be enhanced by combining these therapies. Recent clinical trials reported superior outcomes with a combination of T-cell-activating anti-PD-1 (nivolumab) and anti-CTLA-4 (ipilimumab) monoclonal antibody (mAb) than with each therapy alone (48). Other potentially valuable strategies include immunotherapies designed to activate the CTL response, such as the adoptive transference of lymphocytes, the activation, stimulation, or adoptive transference of dendritic cells, vaccination with tumor antigens or treatment with CpG oligonucleotides.

Upregulation of MHC-I surface expression

Given that MHC-I-positive surface expression on tumor cells may be involved in metastatic-immune dormancy, therapies designed to increase or recover MHC-I surface expression on metastatic cells may be effective to keep them in dormancy (Fig. 1). In cancer cells with reversible MHC-I defects (soft lesions), it may be possible to recover MHC-I surface expression by treatment with IFN type I and II and/or TNF α or by immunotherapies that promote the production of these cytokines (49). This approach offers the dual benefit of a possible increase in both MHC-I expression and CTL immune response. An increase in MHC-I can be achieved with chemotherapy (e.g., 5-aza-2'-deoxycytidine, anthracyclines, histone deacetylation inhibitors) and with radiotherapy (50, 51). Loss of *Fhit* tumor

suppressor gene expression was recently implicated in the coordinated transcriptional downregulation of APM components, MHC-I heavy chains, and β_2 -microglobulin genes, leading to partial or total losses of MHC-I surface expression on tumor cells (52). Hence, treatments to recover *Fhit* expression (e.g., demethylating agents or *Fhit* gene transfection) may also increase MHC-I surface expression in tumors with this defect. In contrast, when MHC-I losses are caused by structural defects (hard lesions) in MHC-I heavy chains, APM components, or β_2 -microglobulin genes, only transfection of the corresponding wild-type gene would increase MHC-I surface expression (49).

Balance between immune effector cells and immunosuppressive cells

The aim of therapeutic strategies in immune cancer dormancy should be to maintain a positive balance between immune effector cells and immunosuppressive cells. Thus, treatments against immunosuppressive mechanisms may include the destruction of Treg or MDSCs or the elimination of IDO or adenosine (Fig. 1; ref. 53). However, caution must be taken in administering immunosuppressive treatments to transplantation patients or those with autoimmune disease to avoid disrupting the dormant equilibrium state in favor of progression of the cancer cells (33). Furthermore, given the involvement of CTLs, IFN γ , and IL12 in cancer cell dormancy, immunosuppressive treatments that block them might trigger the awakening of dormant cancer.

Intervention in the IL12/IL23 axis

As already mentioned, it was recently reported that IL12 and IL23 may be responsible for controlling cancer outgrowth and thereby maintaining primary tumors in dormancy (Fig. 1; ref. 33). Stimulation of the production of IL12 through the administration of anti-CD40 agonist (stimulator of IL12 production from APCs) has a protective effect on dormant primary tumors, as also observed with the blockade of IL23 (33). A combination of anti-IL23p19 and anti-CD40 therapies may be a promising option for destroying dormant metastatic cells. Clinical trials of anti-IL23 mAbs are ongoing for different autoimmune diseases. In this regard, various studies found that IL12-activated NK/NKT cells may regulate dendritic cells via cell-cell contact and IFN γ secretion (54). This interplay between dendritic cells and NK cells may completely replace CD4⁺ T induction of CTLs, providing an alternative pathway. However, although the use of IL12 proved successful in numerous mouse models, clinical trials reported sporadic tumor regression and severe adverse effects in humans (55, 56). There is currently renewed interest in the therapeutic potential of IL12, and several new clinical trials are under way on its intratumoral administration as a means of avoiding toxic effects.

NK cell activation

The activation of NK cells is another possible approach. NK cells play a significant role in metastatic dormancy but through an activator function rather than a direct cytotoxic activity. There two options: maintaining NK cell activation to promote or to extend dormant state or stimulating NK cells to destroy

dormant metastatic cells. Treatment with protein-bound polysaccharide K increased the NK cell population, promoting the entry into dormancy of progressing metastases derived from a highly metastatic clone of GR9 (unpublished observations; ref. 43). Saudemont and colleagues reported that vaccination with *CXCL10*-transduced DA1-3b cells enhanced the NK response, eradicating the minimal residual disease produced by DA1-3b tumor cells, despite the expression of B7-H1 by these cells and their resistance to activated CTLs (38).

Modifying the cancer microenvironment

There is a constant interaction between cancer cells and host cells, and the tumor microenvironment plays important roles in the progression, restraint, or destruction of cancer cells. Anticancer therapies should target not only the cancer cells but also their microenvironment. One line of attack against DTCs is to identify organ-specific targets in their microenvironment. Thus, disruption of the binding of $\alpha 4$ integrins to VCAM-1 has been proposed in potential therapies against lung and bone metastases, and there are ongoing clinical trials on the effects of $\alpha 4$ integrin inhibitors (57). Another target in the tumor microenvironment is the microvasculature of potential metastatic niches. Zaho and colleagues recently reported that vaccination of HHD mice with multiple tumor-associated blood vessel antigen-derived peptides produced a CD8⁺ T-cell-dependent regression of colon carcinoma and melanoma and even provided long-term protection against disease relapse (58).

Combined therapies

The combination of vaccines with tumor cell antigens and antiangiogenic agents (e.g., TKI, VEGF/VEGFR antagonist) might improve the outcome of immunotherapy (59). Furthermore, combination therapies may be more effective, not only due to their summative effect in destroying cancer cells but also because a first treatment can sensitize tumor cells to a second treatment. Thus, Schreiber and colleagues found that the administration of chemotherapy and radiotherapy two days before adoptive T-cell immunotherapy achieved the eradication of established tumors expressing low levels of TAA by destroying their stroma. Antigen-loss tumor variants were also abolished by this destruction of cancer stroma (60). According to these results, changes induced in the tumor microenvironment might favor the destruction of dormant metastatic cells. Drugs such as cyclophosphamide, paclitaxel, 5-fluorouracil, 5-*axa*-2'-deoxycytidine, mitomycin, doxorubicin, and mitoxantrone have been found to activate antitumor activity in mice in combination with immunotherapy, increasing antigenic presentation (61, 62). Treatment with vinblastine produced the maturation of dendritic cells, inducing a CD8⁺ T-cell response (63), whereas 5-fluorouracil, CPT-11, and cisplatin increased the susceptibility of colon cancer cells to killing by CTLs (64). Further sensitization treatments that could be applied before immunotherapy include the administration of immune checkpoint inhibitors and the recovery of MHC-I surface expression on metastatic cells with low or negative expression. Therefore, future clinical research should focus not only on testing new

drugs or novel treatments but also on optimizing doses in combined therapies.

Implications and Future Directions

All of the results and evidence presented here indicate that the immune system may play a central role in metastatic dormancy (Fig. 1). An important function has been revealed for CTLs, which are even capable of restraining metastases in permanent dormancy. Hence, any anticancer treatment, especially immunotherapy, aimed at maintaining, promoting, or activating a CTL immune response or at reversing CTL immunosuppression may be effective to restrain or destroy metastases in dormancy or to revert metastases to a dormant state (Fig. 1). MHC-I surface expression on metastatic cells appears to play an important role in this dormant equilibrium state. Consequently, treatments designed to recover or increase MHC-I surface expression on DTCs may be useful to control these dormant metastatic cells. NK cells are also involved in metastatic dormancy through an activator function rather than a direct cytotoxic function. Treatments inducing NK cell proliferation and activation may be valuable to modulate the CTL response (Fig. 1). However, all these treatments and other immunotherapeutic treatments should be used not only after the onset of metastatic disease but also after the removal of primary tumors in cancers in which minimal residual disease is frequent (e.g., breast and prostate cancers, melanoma, lymphoma treated with anti-idiotypic antibodies, and chronic myelogenous leukemia treated with imatinib mesylate) and even when patients are in complete remission. Moreover, when the initial treatment of patients after primary tumor excision is chemotherapy or radiotherapy, this should be combined with or immediately followed by immunotherapy to enhance the control of the immune system over dormant metastatic cells. Thus, when we used a highly metastatic clone in our cancer model, the complete eradication of overt pulmonary spontaneous metastases was promoted by a 4-month immunotherapy regimen immediately after primary tumor removal (43). Hence, immunotherapy treatments can control metastases that would progress in an untreated immunocompetent host. Periodic immunotherapy treatments may be appropriate in some patients with the aim of transforming their cancer into a chronic nonfatal disease. However, caution must be taken in administering immunosuppressive treatments to transplanted patients or those with autoimmune diseases to avoid awakening dormant metastases. Immune-mediated metastatic dormancy may be an important phenomenon in cancer, and a greater understanding of the mechanisms and cells involved may help to design novel immune strategies for the control or destruction of cancer cells.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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