

Molecular Pathways: Emerging Pathways Mediating Growth, Invasion, and Metastasis of Tumors Progressing in an Irradiated Microenvironment

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Abstract

Radiotherapy is a well-established therapeutic modality in oncology. It provides survival benefits in several different cancer types. However, cancers relapsing after radiotherapy often develop into more aggressive conditions that are difficult to treat and are associated with poor prognosis. Cumulative experimental evidence indicates that the irradiated tumor bed contributes to such aggressive behavior. The involved mechanisms have for long remained elusive. Recent progress in the field revealed previously unrecognized cellular and molecular events promoting growth, invasion, and metastasis of tumors progressing in an irradiated microenvironment. Cellular mechanisms include inhibition of sprouting angiogenesis, formation of hypoxia, activation and differentiation of stromal cells, and recruitment of bone marrow-derived cells with vasculogenic and prometastatic activities. Identified pathways include TGF- β /ALK5, CXCL12/CXCR4, KITL/KIT, and CYR61/ α V β 5 integrin. The availability of pharmacologic inhibitors impinging on these pathways opens novel opportunities for translational and clinical studies. These experimental results and ongoing work highlight the importance of the irradiated microenvironment in modulating the tumor response to radiotherapy and open new opportunities for the development of novel therapeutic strategies for patients with cancer who relapse after radiotherapy. Here, we review and discuss recent advances in the field and their translational and therapeutic implications to human cancer treatment. *Clin Cancer Res*; 18(19); 5196–202. ©2012 AACR.

Background

Radiotherapy plays a prominent role in the treatment of various tumor types and provides significant survival benefits in many cancers, including the breast, prostate, rectum, brain, lung, and head and neck cancers (1). In breast cancer, radiotherapy, alone or in combination with chemotherapy, is widely used as adjuvant treatment after breast-saving surgery to reduce the incidence of locoregional and distant recurrences (2). The therapeutic effects of radiotherapy are traditionally considered because of the induction of double-strand DNA breaks in cancer cells causing cell-cycle arrest, senescence, or apoptosis (3). Consistent with this view, efforts aimed at understanding and improving the therapeutic efficacy of radiotherapy largely concentrated on the

study of mechanisms of DNA damage and repair (4). It is now becoming increasingly evident that ionizing radiation also induces modifications of the tumor microenvironment, which have a profound impact on tumor biology (5). This is particularly relevant to cancers relapsing after radiotherapy, which tend to develop into invasive and metastatic conditions with poor prognosis (6). Cumulative experimental evidence indicates that the irradiated tumor microenvironment actively contributes to such aggressive behavior. Here, we review and discuss recent advances in unraveling cellular events and molecular pathways of the tumor microenvironment modulated by ionizing radiation and affecting tumor growth, invasion, and metastasis.

The tumor bed effect

Experimental tumors implanted in a preirradiated bed grow with slower kinetics, an effect originally referred to as the tumor bed effect (TBE; refs. 7, 8). The TBE is dose dependent between 5- and 20-Gy single doses (9), occurs with fractionated therapy (e.g., daily doses of 2Gy; ref. 10) and within broad time intervals (0–90 days) between irradiation and tumor implantation (11), and is cell line dependent (10). A second element of the TBE is enhanced invasion and metastasis, which might seem paradoxical considering the reduced primary tumor growth (6, 12). Enhanced metastasis was observed regardless of whether the analysis was metachronic (i.e., equivalent tumor sizes at

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different time points; ref. 12) or synchronic (i.e., different tumor sizes at the same time point; refs. 13, 14). The TBE is a local effect, as it is only observed for tumors injected inside of the irradiated bed (14). These observations are of clinical relevance, as radiotherapy improves local tumor control, but tumor recurrences within a preirradiated field are often associated with an elevated risk of metastasis and poor prognosis (2, 15–17).

Inhibition of sprouting angiogenesis

Several microenvironmental events have been linked to the TBE, most notably the decrease in tumor vascularity (18–20). We know today that radiotherapy-induced modifications of tumor vasculature are due to direct effects of ionizing radiations on endothelial cells. Garcia-Barros and colleagues showed that high-dose ionizing radiation induces ceramide-mediated apoptosis of tumor-associated endothelial cells causing tumor vessel disruption and delayed tumor growth. Preventing ceramide-mediated endothelial cell apoptosis attenuated these effects indicating that endothelial cells are a therapeutically relevant target of radiotherapy (21). We showed that ionizing radiation suppresses *de novo* angiogenesis by inhibiting endothelial cell proliferation, migration, and sprouting, and by causing premature senescence, in part mediated by the TGF- β /ALK5 pathway (22). ALK5 inhibition rescued radiation-induced cell sprouting and migration defects *in vitro* and restored angiogenesis *in vivo* (22). Despite inhibited sprouting angiogenesis, vessels can still form, to a certain extent, in irradiated tumors. Hlushchuk and colleagues showed that intussusceptive angiogenesis replaces sprouting angiogenesis in irradiated tumors to support growth of surviving tumor cells (23). The resulting microvascular density is, however, reduced by 30% to 40% compared with nonirradiated tumors. The molecular mechanisms of intussusceptive angiogenesis remain largely unknown.

Hypoxia, vasculogenesis, invasion, and metastasis

Hypoxia, consequent to impaired angiogenesis, is considered as a major cause of the TBE (13, 14, 24). Indeed, tumor hypoxia is associated with a shorter disease-free survival in many human cancers (25). Many molecular mechanisms induced by hypoxia and promoting cancer progression have been unraveled (26) including in tumors growing in irradiated beds. Kioi and colleagues reported that hypoxia-mediated activation of hypoxia-inducible factor (HIF)-1 in glioblastoma cells after radiotherapy leads to increased expression of the chemokine CXCL12 (27). CXCL12 stimulates the recruitment of CXCR4⁺CD11b⁺ bone marrow-derived cells (BMDC) into irradiated tumors to promote matrix metalloproteinase (MMP)-9-dependent blood vessel formation by vasculogenesis, sufficient to support the growth of recurring tumors (27, 28). Using an orthotopic TBE model of breast cancer, we observed HIF-dependent induction of KIT in hypoxic tumors, causing the recruitment of bone marrow-derived prometastatic KIT⁺CD11b⁺ BMDCs to primary tumors and premetastatic lungs (29). Using a subcutaneous model of

TBE, we observed that recovered tumor cells expanded *in vitro* and reinjected in nonirradiated mice retained enhanced metastatic capacity (14). The cysteine-rich protein 61 (CYR61), a matricellular protein that regulates cell growth, differentiation, survival, and migration (30), and the adhesion receptor integrin α V β 5 emerged as 2 molecules cooperating to enhance metastasis. Importantly, CYR61 and α V β 5 promoted resistance to hypoxia, although they were not induced by hypoxia (14). These results support the notion that besides HIF-dependent adaptive reactions to hypoxia, selection of hypoxia-resistant cancer cells with superior metastatic capacities also contributes to the TBE (31).

Hypoxia-independent mechanisms

Ionizing radiation induces the generation of reactive oxygen/nitrogen species (ROS/RNS). When exceeding the cellular antioxidants defense, ROS/RNS induce damages to DNA, proteins and lipids resulting in cell-cycle arrest, apoptosis, cell activation, or differentiation (32). ROS/RNS also regulate cellular functions by acting as messenger molecules in signaling pathways and by direct effects on transcription (33). ROS/RNS also modify production and activation of TGF- β 1 (34, 35). Because ROS/RNS are rapidly induced, and persist over time by self-amplification, they are considered as main mediators of sustained radiation modifications of the microenvironment, in particular fibrosis (desmoplastic reaction; ref. 36). Ionizing radiation induces expression of growth and inflammatory factors and matrix proteins (5). For example, ionizing radiation induces endothelial cell activation resulting in the activation of the proinflammatory pathways NF- κ B (37), the expression of procoagulant proteins (e.g., thrombomodulin and von Willebrand factor), and leukocyte endothelial cell adhesion molecules (e.g., intercellular adhesion molecule-1 and vascular endothelial cell adhesion molecule-1; refs. 6 and 38). Irradiated fibroblasts differentiate into myofibroblasts (39) producing tissue-specific collagens, growth factors, and cytokines, such as platelet-derived growth factor (PDGF), interleukin (IL)-1 β , TNF, and TGF- β (5, 6, 40). Irradiated fibroblasts enhance the invasive capacity of cocultured pancreatic cancer cells (41). This effect was attributed to fibroblast-induced activation of MET, a receptor tyrosine kinase promoting cell growth and invasiveness through the Ras/mitogen-activated protein kinase (MAPK) pathway (42). Although further experiments are needed to confirm the role of MET in the TBE, recent evidence indicates that ionizing radiations induce MET expression in cancer cells via a ataxia telangiectasia mutated (ATM) and NF- κ B signaling pathway resulting in ligand-independent MET activation and enhanced invasiveness (43). Among all radiation-induced cytokines, TGF- β activation is of particular relevance, as it elicits strong and long lasting microenvironmental changes (e.g., suppressed angiogenesis, inhibited immune response, and fibrosis) and tumor reactions (e.g., invasiveness and epithelial-to-mesenchymal transition) concurrently to promote carcinogenesis and accelerate the development of highly malignant phenotypes (44, 45).

Induction of angiogenesis by low-dose radiotherapy

While high doses of ionizing radiation inhibit sprouting angiogenesis, low doses stimulate it. Sonveaux and colleagues originally reported that low doses of ionizing radiation stimulate endothelial cell migration and tubulogenesis *in vitro* and angiogenesis *in vivo* by activating the nitric oxide pathway (46). More recently, Sofia Vala and colleagues observed that doses lower or equal to 0.8 Gy cause ligand-independent activation of VEGF receptor (VEGFR)-2 resulting in enhanced endothelial cell migration, survival, and angiogenesis (47). Low-dose ionizing radiation promotes cancer cell dissemination and metastasis of leukemia and breast cancer cells, which are prevented by blocking VEGFR-2 activation (47). These observations might be relevant to human cancer therapy, especially in hyperfractionation protocols, as the tissue and the border of the irradiated field is exposed to lower doses compared with the bulk of the tumor (48).

Clinical-Translational Advances

The fact that tumor bed irradiation promotes metastasis in experimental models is well established. In contrast, whether this also occurs in patients treated with radiotherapy is still a matter of debate (6). Increased risk of developing distant metastases upon local recurrences after radiotherapy has been reported in some (15–17), but not all (49), studies. Because these analyses were retrospective and nonrandomized, it will be necessary to conduct prospective randomized studies to obtain conclusive results. Translational studies will be necessary to validate or invalidate whether cellular and molecular events observed in preclinical models also apply to human cancer. The fact that different and complementary mechanisms are at play in the TBE, that some of them may be tumor specific or dominant over others, and that tumors are highly heterogeneous tissues may complicate the interpretation of the results issued from these translational studies. Nevertheless, it is important to start considering which of the pathways emerging from preclinical studies may be a relevant target for future therapeutic approaches to blunt invasion and metastasis of cancers relapsing after radiotherapy (see Fig. 1 and Table 1).

HIF-1 pathway

Preclinical models of the TBE point to HIF-1 activation as an important event in promoting tumor invasion and metastasis, suggesting that HIF-1 inhibition might provide therapeutic benefits. Blocking HIF-1 transcriptional activity would prevent the release of CXCL12 and attraction of CXCR4⁺ myelomonocytic cells promoting the formation of novel vessels by vasculogenesis, the mobilization of prometastatic KIT⁺CD11b⁺ cells, and the expression of HIF1-dependent prometastatic genes, including the urokinase type of plasminogen activator receptor (uPAR), lysyl oxidase (LOX), plasminogen activator inhibitor (PAI) 1, MMP-2, Snail, or fibronectin (26). However, recent preclinical studies warrant caution in the use of HIF inhibitors in cancer, as different HIF isoforms (i.e., HIF-1, -2, -3) have

pleiotropic and sometimes opposing effects (50). For example, in contrast to its well-known tumor-promoting activity, HIF-1 has growth inhibitory and proapoptotic effects in some cancers (51, 52). At this point, it might be judicious to target prometastatic pathways downstream of HIF-1 rather than HIF-1 itself.

CXCL12/CXCR4 pathway

Inhibition of CXCR4 activation, using small-molecule (AMD3100) or neutralizing antibodies to CXCR4, prevented the recruitment of vasculogenic CD11b⁺ cells to glioblastoma relapsing after radiotherapy and inhibited tumor regrowth (27). These preclinical results are corroborated by the increased accumulation of CD11b⁺ cells observed in recurrent human glioblastoma when compared with untreated tumors. Thus, targeting the CXCL12/CXCR4 axis might add potential benefits to standard radiotherapy. This hypothesis could be rapidly tested in patients, as CXCR4 inhibitors are available for clinical use (53). It should be noted, however, that CXCR4 inhibition leads to a rapid mobilization of hematopoietic stem cells into the peripheral circulation, some of which may have protumoral activities.

KITL/KIT pathway

In our orthotopic breast cancer model of TBE, KITL silencing in tumor cells and systemic KIT inhibition with a blocking antibody or the tyrosine kinase inhibitor nilotinib (54) reduced the mobilization of KIT⁺CD11b⁺ cells and their recruitment to primary tumors and lungs and attenuated lung metastasis (29). Whether mobilized KIT⁺CD11b⁺ cells are present in the blood of patients with breast cancer treated with radiotherapy and their relationship to CD11b⁺ cells infiltrating the primary tumor and metastases is currently under investigation. Of interest, increased expression of KITL was reported in perinecrotic regions of glioblastoma and breast cancer tissues in association with poor prognosis (55). Several small-molecule tyrosine kinase inhibitors targeting KIT are already approved for clinical use (56), thereby facilitating the development prospective clinical studies aimed at inhibiting KIT in patients with post-radiotherapy recurrences.

CD11b⁺ cells

Zoledronic acid and liposomal clodronate were successfully used in postradiotherapy settings to target MMP-9-expressing tumor-recruited CD11b⁺ monocytes/macrophages and to deplete tumor-mobilized KIT⁺CD11b⁺ populations resulting in reduced tumor growth and metastasis, respectively (29). Whether biphosphonates may also be used in patients with cancer for the same purpose remains to be shown. Carrageenan, a sulfated polysaccharide extracted from red seaweeds, was also used to deplete monocytes/macrophages with similar inhibitory effects on tumor regrowth after radiotherapy (27). Inhibition of the CD11b/CD18 complex (β 2 integrin subfamily) using function-blocking antibodies effectively inhibited the recruitment of CD11b⁺ myeloid cells into irradiated tumors

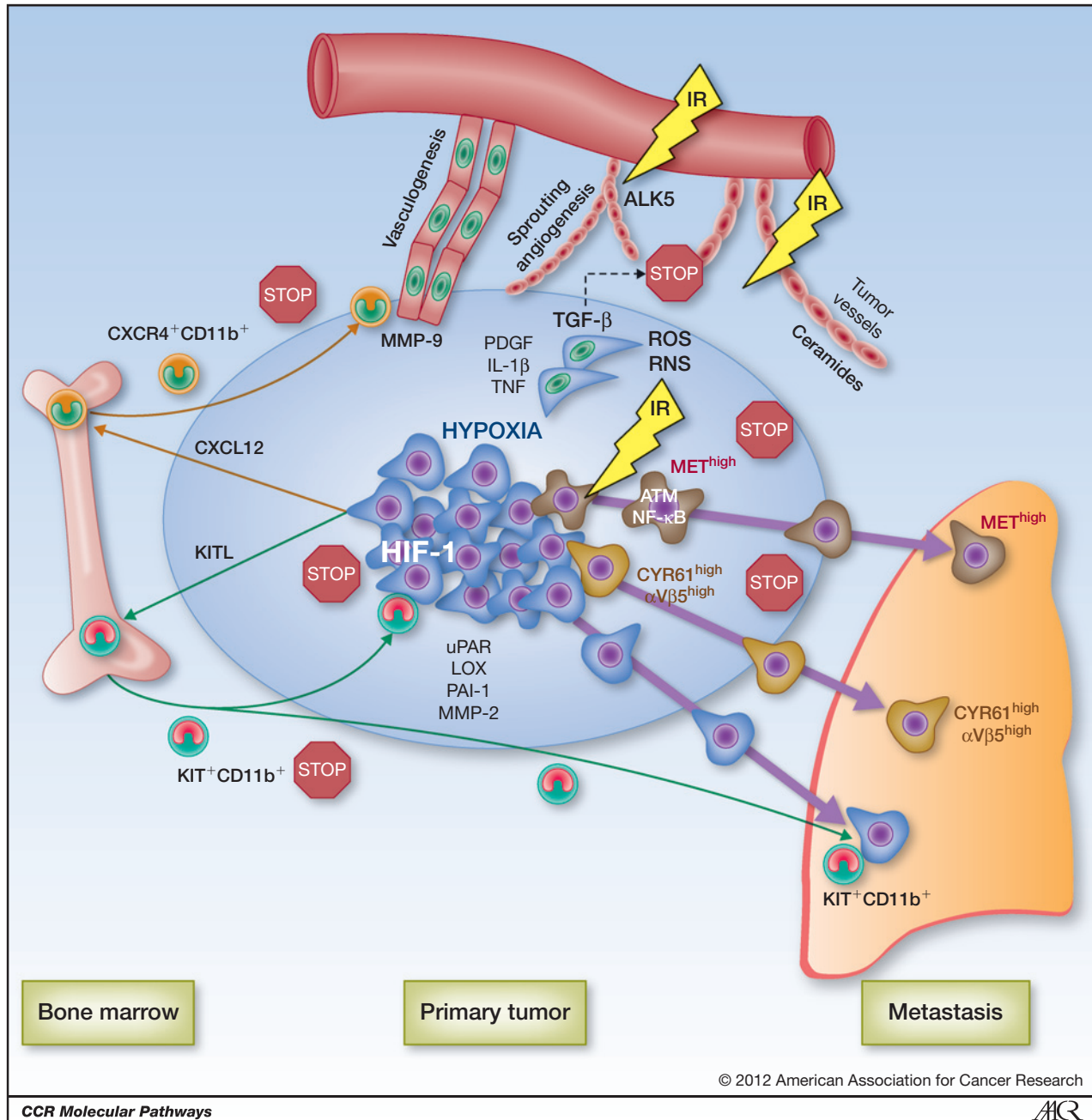


Figure 1. Emerging pathways mediating growth, invasion, and metastasis of tumors progressing after radiotherapy. High-dose ionizing radiation (IR) kills tumor-associated endothelial cells and inhibits sprouting angiogenesis. Tumor cells growing within this angiogenesis-suppressed microenvironment cause tissue hypoxia, which elicits HIF-dependent and HIF-independent responses. Hypoxia induces HIF-dependent expression of CXCL12 and KITL promoting the mobilization from the bone marrow and the recruitment to tumors or metastatic sites of cells promoting vasculogenesis (CXCR4⁺CD11b⁺ cells) or metastasis (KIT⁺CD11b⁺ cells). Hypoxia selects for invasive and metastatic tumor cells expressing high levels of αV integrins and CYR61. Hypoxia also stimulates the expression of additional prometastatic factors, such as uPAR, LOX, PAI-1, and MMP-2. Ionizing radiation also induces the expression of tumor-promoting cytokine and growth factor by stromal cells (TGF- β , PDGF, and IL-1 β) directly or indirectly (e.g., through ROS/NOS). Irradiated tumor cells increase expression of the proinvasive receptor MET, through the ATM/NF- κ B pathway. Together, these events concur to promote growth, invasion, and metastasis of tumors progressing in a preirradiated microenvironment. The "stop" signs indicate candidate target molecules that can be blocked with available inhibitors to suppress growth and metastasis of tumors growing in an irradiated bed. See Table 1 for a selection of inhibitory molecules. The proangiogenic effects of low-dose ionizing radiation are not depicted here.

and prevented their growth (57). Humanized antibodies to the CD11b/CD18 complex have been developed for human use (58) and could be tested in patients with postradiother-

apy recurrences. In contrast, humanized, CD11b-selective antibodies do not exist yet. Considering their essential role in mediating leukocyte recruitment to sites of infection,

Table 1. Synopsis of molecules promoting tumor growth and metastasis in a preirradiated bed and selected inhibitory drugs

Molecule	Target		Inhibitor	
	Class	Molecule	Class	Targeted event
α V β 3 integrin	Adhesion receptor	Cilengitide;17E6	Peptide (α V β 3/ α V β 5) Antibody (pan anti- α V)	Tumor cell adhesion, migration, invasion, and metastasis
β 2 integrins (CD11/18 complex)	Adhesion receptor	Rovelizumab	Antibody	Recruitment of CD11/18 ⁺ monocytes
KIT	Receptor tyrosine kinase	Imatinib and nilotinib	Tyrosine kinase inhibitors	Recruitment of KIT ⁺ CD11b ⁺ BMDC
MET	Receptor tyrosine kinase	PHA665752, JNJ-38877605, SU11274, PF-02341066, and XL880	Tyrosine kinase inhibitors	Tumor cell growth, survival, and invasion
CXCR4	Chemokine receptor	BKT140, ALX40-4C, and plerixafor	Small-molecule inhibitors	Recruitment of CD11b ⁺ BMDC
ALK5	TGF- β receptors	EW-7195, SB-431542, and PF-03446962	Kinase inhibitors	Inhibition of angiogenesis, invasion, and epithelial-to-mesenchymal transition
ALK1			Antibody	
HIF-1	Transcription factor	NSC-134754, PX-478, PX-12, YC-1, and topotecan	Small-molecule inhibitors; camptothecin analogue	Metabolism, migration, invasion, angiogenesis, and survival

NOTE: This table presents therapeutic targets and a selection of inhibitory molecules to consider in preclinical and clinical studies to impinge on prometastatic pathways activated by ionizing radiation.

broad and sustained inhibition of β 2 integrins bears the risk of increased infections.

HGF/MET pathway

A specific antagonist of hepatocyte growth factor (HGF), the MET ligand, suppressed growth, invasion, and metastasis of pancreatic cancer cells cocultured with irradiated fibroblasts (41). MET kinase inhibitors enhanced the efficacy of radiotherapy to halt tumor growth and prevent radiation-induced invasiveness (43). Considering the well-established role of the HGF/MET pathway in mediating invasive tumor growth (42) and the clinical development of novel MET inhibitors (59), MET should be considered as an attractive target to prevent or treat invasive growth of postradiotherapy tumor relapses.

TGF- β pathway

Because of its multiple tumor-promoting effects, the TGF- β pathway has been long considered as an appealing target in cancer (44). For example, genetic and pharmacologic targeting of the TGF- β receptor activin receptor-like kinase (ALK) 1 impaired tumor growth and angiogenesis in the Rip1Tag2 model (60), and TGF- β -neutralizing antibody enhanced efficacy of radiotherapy in a breast cancer model (61). Because TGF- β signaling is induced by ionizing radiation (62), and TGF- β receptor inhibitors are in clinical development, it appears reasonable to consider this path-

way as a valid therapeutic target to prevent invasion and metastasis in postradiotherapy recurrences. In view of the complexity, and sometimes opposing effects of TGF- β in cancer and its role in healthy tissue homeostasis, however, it may be prudent to focus on the inhibition of molecules up or downstream of TGF- β , such as AKT (63).

α V integrins

The function-blocking anti- α V monoclonal antibody 17E6 and the α V β 3/ α V β 5-specific peptidic antagonist cilengitide (EMD12197) prevented metastasis induced by tumor bed irradiation or CYR61 overexpression (14). Because cilengitide is currently in advanced clinical testing in patients with glioblastoma treated with radio- and chemotherapies (64), it seems reasonable to assess the effect of cilengitide in patients at high risk for postradiation relapses in other cancers. Of note, cilengitide, alone or in combination with radio- and chemotherapy, has shown high tolerability and low toxicity making it an ideal drug for combination therapies (65).

Conclusions

In recent time, we have gained insights in some of the cellular events and molecular pathways responsible for the TBE. These results represent conceptual advances to the understanding of the TBE and provide rationales to the development of new therapeutic approaches to prevent

invasion and metastasis of tumors locally relapsing after radiotherapy. However, they may only represent the tip of the iceberg of the radiation-induced microenvironmental modifications modulating cancer progression and many more mechanisms are likely to be discovered. Drugs targeting some of the uncovered pathways are already available for human use. We now need to move on and conduct innovative translational studies and combination trials to validate or invalidate in patients the molecular pathways uncovered in preclinical models. In addition, we need to determine predictive biomarkers, such as gene expression signatures in primary tumors, circulating molecules, or cell populations to identify patients at risk for relapse and progression after radiotherapy. Such patients would be the ones to benefit most from a concomitant therapy blunting tumor escape and evasive growth. The TBE may also serve as a model relevant to unravel pathways mediating evasive resistance to antiangiogenic therapies. Indeed, hypoxia and metabolic starvation caused by antiangiogenic treatments (66), much alike hypoxia caused by radiotherapy, may initiate microenvironmental modifications eliciting adaptive tumor responses or the selection of highly aggressive tumor cell populations. This would be an unexpected but welcome contribution to research in anti-

angiogenesis for an effect originally described nearly 100 years ago!

Disclosure of Potential Conflicts of Interest

C. Ruegg has ownership interest (including patents) and is cofounder and stockholder of Diagnoplex SA. No potential conflicts of interests were disclosed by the other authors.

Authors' Contributions

Conception and design: F. Kuonen, C. Ruegg

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): C. Secondini

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): C. Secondini, C. Ruegg

Writing, review, and/or revision of the manuscript: F. Kuonen, C. Ruegg

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