

Metastases and their microenvironments: linking pathogenesis and therapy

Angels Sierra*

*Centre d' Oncologia Molecular, Institut de Recerca Oncològica-IDIBELL, Hospital Duran i Reynals (C.S.U.B.),
Gran Via, km 2.7, E-08907 L' Hospitalet de Llobregat, Barcelona, Spain*

Received 23 May 2005; received in revised form 4 July 2005; accepted 7 July 2005

Abstract

The pathogenesis of metastasis depends on multiple favorable interactions of tumor cells with host homeostatic mechanisms. Interruption of one or more of these interactions can lead to the inhibition or eradication of cancer metastases. For many years, all efforts to treat cancer concentrated on the inhibition of growth or the destruction of tumor cells. A strategy of both eradication of tumor cells (e.g. by chemotherapy and immunotherapy) and modulation of the host microenvironment (e.g. tumor vasculature and hypoxia) is an additional, relatively novel approach to cancer treatment. Recent advances in our understanding of the biological basis of cancer metastasis open up unprecedented opportunities for translating basic research to clinical treatment of cancer. This research includes the unraveling of the genetic make-up of tumors and genome-wide expression analyses, thereby identifying many potential targets for therapy. Drugs acting on tumor cells which have a metastasis-prone mutational or expression status (by classical or targeted chemotherapy) as well as drugs affecting host-mediated survival pathways must be combined in order to create therapeutic synergy. Therapeutic maneuvers may target receptor tyrosine kinases (EGFR, VEGFR, FGFR), chemokines or G-protein-coupled receptors (CXCR4, CXCR2, EphB2), hypoxia-inducible factor (HIF), and signaling pathways (c-Src, PI3K, Akt, chaperon complexes) in tumor cells. Moreover, stromal and immunological cells and their cytokines coordinate critical pathways that exert important roles in the ability of tumors to invade and metastasize, thus suppressive cytokines (IL-6 and IL-10) and neutralizing specific antibodies might subvert conditions for metastasis.

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Keywords: Apoptosis; Drug Resistance; Metastases; Microenvironment; Therapy

1. Introduction

Great strides have been made in early detection and treatment of solid tumors, which are the most common forms of cancer and responsible for the majority of cancer-related deaths in Western industrialized countries. Early diagnosis and treatment give a high probability of permanent remission or cure (Parkin et al., 2001; Miyoshi et al., 2003). Because of this progress, tumor mortality is linked increasingly to early metastasis, which is often occult at the time of primary diagnosis. When solid secondary tumors are established, the chances of long-term survival fall from over 90% to around 5% (Greenberg et al., 1996). Thus, following surgery or radiotherapy on primary solid tumors, the priority is to prevent

the development of metastatic lesions from cancer cells, particularly to lymphatic, bone, liver, lung and central nervous system tissues (Chambers et al., 2002; Pantel et al., 1999). At the metastatic stage of the disease, there are few good treatment choices left and the patient's chance of survival is low. Therefore, to increase treatment success, it is important to integrate findings arising from the use of novel technical approaches such as genome-wide expression profiling of primary tumors and micrometastases with our knowledge of the biology of the metastatic process.

This paper discusses findings and ideas about the biology of the metastatic process which determines the success of cells in tissue invasion, survival in the circulation, extravasation and arrest within the secondary organ parenchyma. The outcome of the metastatic process depends on multiple interactions of metastasizing tumor cells with host homeostatic mechanisms. Thus, anti-metastasis therapy, which targets not

* Tel.: +34 93 260 74 29; fax: +34 93 260 74 26.

E-mail address: asierra@iro.es.

only the tumor cells but also the homeostatic factors that promote organ-specific tumor cell growth and survival, has a better chance of success.

2. Pathogenesis of metastasis

The development of cancer cells with the capacity to invade tissues and metastasize includes steps which allow them to escape the primary tumor, travel to distant sites and colonize organs in which, at least initially, nutrients and space are not limiting. This process includes several rounds of mutation and selection (Vogelstein and Kinzler, 2004) resulting in high genetic instability which further drives tumor progression. In particular, mutations in oncogenes and in tumor-suppressor genes provide cancer cells with a selective growth advantage and lead to the clonal outgrowth of a tumor (Cahill et al., 1999).

Some mutated genes are positively selected as relevant to the acquisition of the metastatic phenotype, such as Bax, MSH6, or IGFRII, against other neutral genes irrelevant to it (Perucho, 2003). Permanent or transient genomic instability might be ascribed to deficiencies in numerous cellular processes, including mitotic-checkpoint regulation, DNA-damage signaling and repair, telomere maintenance and centrosome function (Zhitovovskiy and Kroemer, 2004). Alterations in these molecular processes provide increased chances for the accumulation of mutations that further their own survival and proliferation. Cells are selected that produce their own mitogenic signals, suppress contact inhibition, evade apoptosis or support neovascularisation (Bernards and Weinberg, 2002; Leroi et al., 2003; Hanahan and Weinberg, 2000; Breivik, 2001). Consistent changes in gene expression occur in cancer cells acquiring traits that form the metastatic phenotype. Though the molecular mechanisms remain essentially unknown, their independent activities, with deleterious effects on normal cells, may coincide in tumor cells to promote metastasis (McCawley and Matrisian, 2000; Wells, 2000).

Recently, a comprehensive gene expression profile of each cell type in normal breast tissue *in situ* and in invasive breast carcinoma was determined (Allinen et al., 2004). The results showed extensive gene expression changes to occur in stromal, myoepithelial and malignant cells during cancer progression, and a significant fraction of the altered genes to encode secreted proteins and receptors.

The propensity to metastasize might be hardwired early during tumor development, even when clinical metastasis appears much later. However, whether this propensity is hardwired in all neoplastic cells, or whether such changes continue to occur as metastasis progresses, still remains largely unknown. New studies using mRNA expression profiling provide the opportunity to have a closer look at the molecular networks in cells contributing to metastasis (Webb, 2003). It has been found that solid tumors may carry a specific gene expression signature most associated with metastasis

and poor clinical outcome, suggesting that the metastatic potential of human tumors is encoded in the bulk of a primary tumor and that tumors likely to metastasize are fundamentally different (van't Veer et al., 2002; Weigelt et al., 2003; Ramaswamy et al., 2003; Bernards, 2003; Golub, 2004; Liotta and Kohn, 2001). To mention one example, patients presenting with a good prognostic fingerprint had a 95% chance of surviving the next decade, whereas those with a bad fingerprint had only a 55% chance of surviving (van't Veer et al., 2002). The genes making up the "poor prognosis" signature or "metastatic predisposition" signatures could represent genes expressed by the tumor cells themselves or by stromal cells including vasculature, connective tissue, or immune cells (Hynes, 2003).

A comprehensive genomic analysis of primary breast tumors and matched single cytokeratin-positive epithelial cells from bone marrow showed that genomic aberrations were greater in patients who had developed metastases. This suggested an independent evolution model of metastatic cells after early separation from the primary tumor (Schmidt-Kittler et al., 2003). The evidence reinforces the view that tumor cells disseminate very early and evolve to metastatic disease independently of the primary tumor. In fact, some metastases bear almost no genomic resemblance to the primary tumor from the same patient (Bissig et al., 1999). Even in experimental situations *in vivo*, metastatic cells from lung, bone and lymph node belonging to the same tumor showed different genomic DNA fingerprinting (Gu et al., 2004), suggesting that the driving force for metastasis development is selection of cells with the best conditions for survival in each microenvironment.

There is ample evidence that the movement of cancer cells through the body is not random and that different types of cancer cells have different destinations. A specific set of genes that mediate bone metastasis has been described. Cells over-expressing IL-11, MMP-1, connective tissue growth factor (CTGF), CXCR-4 and osteopontin (OPN), were highly metastatic to the bone *in vivo*. In contrast, cells without this phenotype had low potential for bone metastasis, but high potential for metastasis to the adrenal glands (Kang et al., 2003).

In an experimental metastasis model of human small-cell lung cancer in mice, differential expression profiles for metastases to lung, liver, kidney and bone were reported. However, the model did not distinguish how much of the difference arises from tumor responses in different environments (Kakiuchi et al., 2003). Recently, Montel et al. (2005) demonstrated, in a xenogenic breast cancer model, selective enrichment of cells that metastasize to lymph nodes and lungs, with a similar expression of genes, and different from those in parental cells. These differences are thought to reflect epigenetic changes in tumor cells in response to their *in vivo* microenvironment. Thus, the metastatic behaviour of tumor cells is derived from intrinsic properties of the primary tumor, secondary to carcinogenic inducers, towards an organ-specific phenotype (Fig. 1). The host immune response

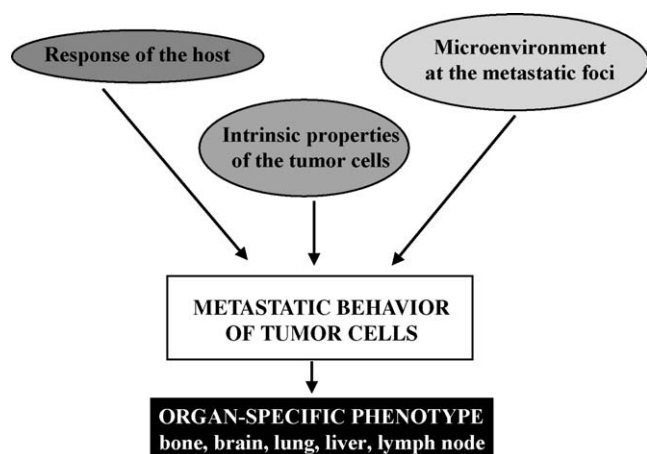


Fig. 1. The organ-specific growth of metastatic cells in secondary organs is a consequence of the presence of stromal elements in normal organs that facilitate organ-specific homing, leading to a cascade of tumor-stromal interactions that constitute the organ-specific phenotype of cells. Tumor cells, when they acquire the ability to metastasize, to survive and to disseminate by clonal evolution or by parallel evolution from the primary tumor, have to evade the host's immunological response and take advantage of the microenvironmental factors. The cross-talk between death and survival pathways at the metastatic foci determines the organ-specific phenotype of tumor cells.

is critical in the tolerance of altered phenotypes and contributes, along with the microenvironment at the metastatic foci, to determining whether the tumor cells will remain dormant or micrometastases develop into clinically observable disease.

3. “Cross-talk” between metastatic tumor cells and microenvironment

Clinical observations of cancer patients and experimental studies in mice have conclusively demonstrated that certain tumor types metastasize to specific organs independent of vascular anatomy, tumor size or the number of tumor cells delivered to each organ (Clarke and Dickson, 1997). To explain this specificity, the soil and seed theory holds that different organs provide growth conditions that are optimal for specific cancers (Fidler, 2002).

3.1. Capacity of tumor cells to metastasize

In breast cancer experimental models, cells disseminated early after implantation in the mammary gland do not require extensive tumor growth to enter the vascular and lymphatic systems (Rubio et al., 2001). This suggests that in clinical situations systemic disease may be prevalent earlier than usually suspected. Indeed, many of the tumor cells may travel as single cells and attach to the lung endothelium after arrival. Then, surviving tumor cells might proliferate intravascularly and extravasation of the tumor cells occurs when intravascular micrometastatic foci outgrow the vessels they are in (Wong et al., 2002).

However, whereas large numbers of cells from a primary tumor may gain access to the circulation, few of them will give rise to metastases. Growth of metastases is differentially affected by the physiological environment of the target organs as well as by the capacity of the tumor cells to overcome death signals (Goodison et al., 2003). Time-lapse video-microscopic observation of melanoma cells injected into the circulation has documented the failure of solitary cells to initiate growth in metastatic sites as well as the need for a permissive environment to sustain their growth (Luzzi et al., 1998). In fact, an early event in metastasis inefficiency is cell death. Apoptotic cells are found in the peripheral blood of cancer patients with a frequency that exceeds the number of intact circulating tumor cells (Mehes et al., 2001).

3.2. The role of the micro-environment in metastasis

The locally activated host microenvironment (both cellular and extracellular elements) in turn modifies the proliferative and invasive behaviour of metastatic cells (Hanahan and Weinberg, 2000; Park et al., 2000) and recruits new vasculature and stromal cells through production and secretion of stimulatory growth factors and cytokines. Recent investigations have revealed dual roles for cytokines in suppressing and promoting cancer formation. The complexity of cancer cell/stromal and immune-cell cross-talk determines the outcome of the host response. Moreover, tumor cells generate many of their own growth signals, thereby reducing their dependence on stimulation or inhibition from their normal tissue microenvironment (Ethier, 1995; Dranoff, 2004). Indeed, stromal cells and their cytokines coordinate pathways that are critical in tumor invasion and metastasis (Cheng and Weiner, 2003).

The extracellular matrix (ECM) provides a structural basis for multicellularity, whereas growth factors provide the information required for the formation of complex tumor tissues. ECM proteins induce signals through their cell receptors, such as integrins; the information can be transferred from the ECM to the cell interior directly, via mechanical forces, or can be mediated by ECM-associated growth factors (Taipale and Keski-Oja, 1997; Rizki and Bissell, 2004).

The metastatic process is the result of integration of many localized cellular adhesion and migration processes, actomyosin polymerization and assembly to filaments, and is regulated by integrins, matrix-degradation enzymes, cell-cell adhesion molecules and cell-cell communication (Sheetz et al., 1998; Friedl and Wolf, 2003; Cukierman et al., 2001). Depending on the cell type and ECM substrate, focal contact assembly and migration are regulated by different integrins, which bind fibronectin ($\alpha_5\beta_1$), laminin ($\alpha_6\beta_1$ or $\alpha_6\beta_4$), vitronectin ($\alpha_5\beta_3$) and collagen ($\alpha_2\beta_1$), and other non-integrin receptors, such as CD44, discoidin receptors, CD26, immunoglobulin superfamily receptors and surface proteoglycans. The engagement of integrins and other adhesion receptors leads to the recruitment of surface proteases towards attachment sites, which in turn

degrades ECM components that are in close proximity to the cell surface: seprase binds to the $\alpha_3\beta_1$ -integrin, MMP1 binds to the $\alpha_2\beta_1$ -integrin and MMP2 binds to the $\alpha_v\beta_3$ -integrin. ECM degradation causes cell expansion and migration (Jia et al., 2004). Remodeling of the ECM, which is restricted to the immediate environment of the cell, seems to be a necessary step in local invasion (Noel et al., 1998).

3.3. Tumor cell interactions with the microenvironment during metastasis

Progression to metastasis involves aberrations in the interactions of multiple cell types with each other and with other components of the microenvironment. One example is the case of bone metastasis, which affects common tumors such as breast, lung and prostate carcinomas. In osteolytic metastasis, there is a “vicious circle” in the bone microenvironment, whereby interactions between tumor cells and osteoclasts lead to both osteolysis and tumor growth (Mundy, 2002; Chang et al., 2004). Molecular mechanisms include the tumor cell production of parathyroid-hormone-related peptide (PTHrP) and bone-derived growth factors that occur as a consequence of increased bone resorption (Sloan and Anderson, 2002; Montell, 2003).

Genetic screening of *Drosophila* has shown that cooperation between oncogenic RAS expression and inactivation of any one of a number of genes affecting cell polarity leads to metastatic behaviour, including basement membrane degradation, loss of E-cadherin expression, migration and invasion (Pagliarini and Xu, 2003). This suggests that the genetic alterations sufficient to cause noninvasive tumors to growth, can indeed make additional contributions to the development of metastatic behaviour in combination with other tumor-initiating alterations.

Recent studies described the relationship between the EphA2 receptor tyrosine kinase and metastasis (Hu et al., 2004). This receptor is stimulated by ligands that are anchored to the membrane of adjacent cells. When malignant cells lose cell–cell contacts, the EphA2 ligand binding is decreased, which promotes oncogenesis (Walker-Daniels et al., 2003; Ogawa et al., 2000). EphA2 selectively inhibits cell–cell adhesion by increasing cell attachment to and up-regulating the ECM protein fibronectin, which enhances malignancy. This can be reversed by specific antibodies targeting EphA2, which in turn decreases fibronectin expression and induces apoptosis.

The transcription factor Twist contributes to the conversion of early-stage tumors into invasive malignancies, by increasing the ability of cancer cells to enter circulation and seed metastasis through favoring epithelial–mesenchymal transition (EMT). This includes loss of E-cadherin expression and activation of mesenchymal markers (Kang and Massague, 2004; Yang et al., 2004).

Another example of cooperation between genes favoring metastasis is the link between HER2, a member of the

epidermal growth factor receptors involved in breast cancer progression and metastasis, and CXCR4, a chemokine receptor that regulates the directional trafficking and invasion of metastasis sites by breast cancer cells (Staller et al., 2003; Smith et al., 2004; Balkwill, 2004). Both proteins may integrate cell migration, adhesion and invasion (Benovic and Marchese, 2004). HER2 signaling increases the expression of the chemokine receptor whose ligand, stromal derived factor 1 (SDF-1) is highly expressed in lung, liver and bone marrow, in which metastases of breast cancer commonly develop (Li et al., 2004). Moreover, blockade of the CXCR4/SDF1 signaling pathway with an anti-CXCR4 antibody also decreased transendothelial breast cancer cell migration and vascular permeability (Lee et al., 2004).

It has been recently described how TrkB, a neuronal receptor that binds the brain-derived neurotrophic factor (BDNF), enables cells to survive and grow independently of their anchorage to a matrix protein. Tumor cells can survive by means of an autostimulatory (autocrine) signaling loop mediated by both molecules that also can be produced in response to tumor hypoxia (Douma et al., 2004).

Hypoxia is involved in metastasis, angiogenesis and selection of cells with a more malignant phenotype (Wouters et al., 2004). The onset of neovascularization in a primary tumor is triggered by immune/inflammatory responses, genetic mutations and metabolic stress induced by fluctuations in oxygen tension though activation of hypoxia-inducible factor-1 (Michiels, 2004). Tumor hypoxia stimulates the formation of new blood vessels by increasing secretion of vascular endothelial cell growth factor (VEGF) (Semenza, 2001) and stimulates tumor invasion by activating hepatocyte growth factor (HGF) (Pennacchietti et al., 2003). In addition, lack of oxygen causes activation of CXCR4, a homing molecule that enables migrating cells to target specific organs (Bernards, 2003).

4. Modulation of tumor cell death by the microenvironment

It is now recognized that resistance to cell death – particularly apoptotic cell death – is an important aspect of tumorigenesis, metastatic progression and resistance to anti-cancer drugs (Malaguarrera, 2004; Shoemaker, 2000). It is currently believed that the metastatic cascade involves a series of interrelated events, in some of which tumor cells withstand severe pro-apoptotic pressures from host-cell cytokines and growth factors (Evan and Vousden, 2001; Wells, 2000; Yousefi et al., 2003). Indeed, defects in cell-death pathways are hallmarks of metastasis.

The most common and well-defined form of programmed cell death is apoptosis, which is one of the mechanisms by which chemotherapy destroys cancer cells. The apoptosis theory of cancer cell death following therapy interprets the levels of pro- or anti-apoptotic proteins, which may predict treatment response, at least in hematological malignancies (Sun

et al., 2004; Taylor et al., 2000; Brown and Attardi, 2005). Despite the wealth of data, no clear patterns have emerged for most solid tumors, probably because tumor cells can still be induced to die by non-apoptotic mechanisms, such as necrosis, senescence, autophagy and mitotic catastrophe (Okada and Mak, 2004; Edinger and Thompson, 2004).

Apoptosis is triggered by an initiation phase that is highly dependent on cell type and apoptotic stimuli. In the subsequent effector phase, the cells change biochemically, which leads to the systematic activation of catabolic hydrolases that participate in the degradation phase of apoptosis through the cleavage of proteins and DNA (Nicholson and Thornberry, 2003; Danial and Korsmeyer, 2004). The mitochondrial pathway is thought to be the principal target of survival signaling pathways, which act by stabilizing mitochondrial function and integrity and by suppressing release of cytochrome *c* (Debatin et al., 2002). Anti-apoptotic oncoproteins from the Bcl-2 family, which exert their principal effects through stabilization of the mitochondrion, could enhance cancer cell survival and adaptation to a new microenvironment, leading to resistance to chemotherapy (Coultas and Strasser, 2003; Ladeda et al., 2001; Letai et al., 2004; Martin et al., 2004; Weintraub et al., 2004).

Anti-apoptotic proteins from the Bcl-2 family (Bcl-2 and Bcl-x_L) have a role in the pathogenesis of metastasis (Fig. 2). They enhance metastasis of cancer cells by inducing extracellular matrix independence, which prolongs survival in the absence of cell attachment to extracellular matrix proteins

rescuing cells from anoikis (Rodeck et al., 1997; Fernandez et al., 2000). The selection of metastatic cells for anoikis resistance resulted in an increase in metastatic potential in parallel with multiple alterations in their phenotypic properties (Zhu et al., 2001). These alterations, which do not accelerate primary tumor development (Martin et al., 2004), enhance mutagenicity by altering the activity of enzymes that are involved in DNA mismatch repair (La Thangue, 2005), inducing chemotherapy resistance (Real et al., 2004; Pinkas et al., 2004).

Factors in the microenvironment can regulate molecules involved in control of apoptotic cell death following exposure to anti-cancer drugs (Real et al., 2004; Taylor et al., 2000). Some fibroblast growth factors induce drug resistance in metastatic cells, which elude cytotoxic insult (Song et al., 2000; Coleman, 2003). The soluble factors released from stromal cells in secondary organs might influence growth and survival of metastatic cells under chemotherapy, by modulating expression of anti-apoptotic proteins (Ladeda et al., 2001). In fact, microenvironmental factors at the metastatic foci seem to be more important in increasing drug resistance than the expression of Bcl-2 or Bcl-x_L in metastatic cells (Gu et al., 2004).

Activation of the latter “intrinsic” apoptotic pathway is the goal of many of the new cancer drugs (Table 1). Some of the cytotoxic compounds acting on mitochondria specifically target proteins such as Bcl-2, overcoming the cytoprotective effect on Bcl-2-like proteins (Costantini et al.,

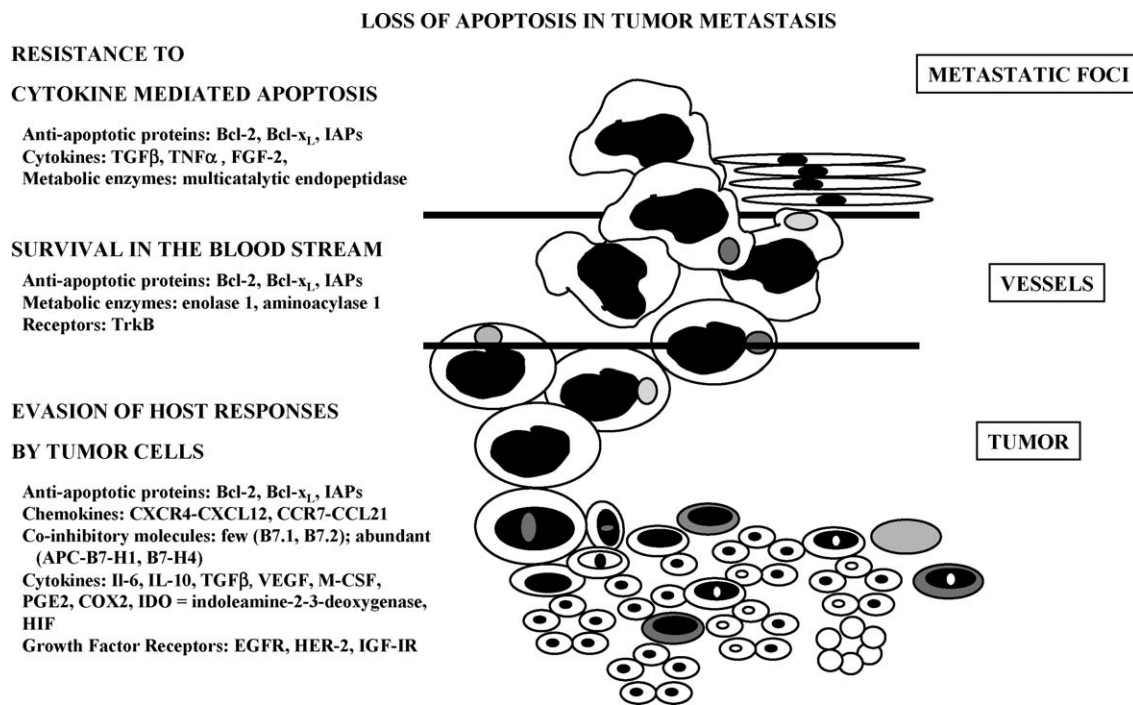


Fig. 2. Defects in cell death pathways are hallmarks of metastasis. Tumor cells which lose the ability to initiate or complete a programmed cell death program can evade immunological host responses, contributing to the anergy of immune cells against tumors. This characteristic improves chances of survival and evades immunological responses during cell mobilization through blood and lymph nodes. The overexpression of anti-apoptotic proteins also inhibits anoikis, the death of cells that achieve extracellular matrix independence. At the end, the success of metastatic growth at metastatic foci depends on the ability of cells to survive cytokine- or cytotoxicity-mediated apoptosis.

Table 1
Examples of drug discovery strategies, targeting core components of cell death pathways in the tumor microenvironment

Action	Target	Strategy	Agent	References
Oncogene	ERBB2 EGFR	Receptor tyrosine kinase antibodies	Cetuximab, Herceptin	Huang and Olliff (2001), Beslija et al. (2003), Malaguarnera (2004), Verweij (2004)
Anti-apoptotic proteins	Bcl-2, Bcl-x _L , XIAPs	Anti-sense therapy, antagonists, Smac	GENTA, IDUN, GEMIN-X, Agera	Debatin et al. (2002), Simoes-Wüst et al. (2002), Reed (2003), O'Neill et al. (2004), Schimmer et al. (2004), Garber (2005)
Angiogenesis	VEGF	Flk-1 kinase inhibitors, blocking VEGFR signaling, VEGF neutralization	SU5416	Bange et al. (2001), Steeg (2004), Wouters et al. (2004)
Hypoxia	HIF	Reduces angiogenesis and invasion	YC-1	Vogelstein and Kinzler (2004), Wouters et al. (2004)
Host-tumor interactions	Stromal antigen FAP	Tumor stromal fibroblast antibody	Sibrotuzumab	Cheng and Weiner (2003)
Chemokine receptors	CXCR4, CXCL12	Chemokine receptors antibodies, antagonists	Anti-CXCR4, AMD3100 Anti-CXCL12	Balkwill (2004), Smith et al. (2004), Zou (2005)
Cytokine therapies and immuno-suppressive networks	Suppressive cytokines IL-6 and IL-10, and T cells	Enhances: tumor antigen presentation, T-cell function, T-cell recruitment, blocking the common pathway STAT3 or SOCS1	GM-CSF, IFN- γ , IL-2, IL-3	Dranoff (2004), Zou (2005)

2000). This kind of agent enforces death in cells in which upstream signals that normally lead to apoptosis have been disabled (Zhivotovsky and Orrenius, 2003; Huang and Olliff, 2001). Metastatic cells might become resistant to treatment by modifying their expression levels or the function of proteins involved in apoptosis signaling pathways that enables cells to take advantage of microenvironmental factors (Hellman, 1997; Taylor et al., 2000; Zhang et al., 2000; Morin, 2003). For instance, breast cancer cells have an autocrine production of cytokines that determine the susceptibility or resistance of tumors to drug treatment (Conze et al., 2001). Apoptosis-based therapy could be used to specifically lower the threshold of apoptosis in cancer cells, overcoming resistance to conventional treatment (O'Neill et al., 2004). Oligonucleotide anti-Bcl-2 and Bcl-x bispecific antisense treatment sensitize breast carcinoma cells (Simoes-Wüst et al., 2002) and prostate cancer cells to chemotherapy (Miayake et al., 2000). Design of small-molecule inhibitors of Bcl2 and Bcl-x_L is an exciting area for current anti-cancer drug development (Real et al., 2004; Wang et al., 2003).

The XIAP (X-linked inhibitor of apoptosis), a key component of the intrinsic pathway and its cellular antagonistic protein (Smac/DIABLO) led to the development of Smac mimetic molecules, which effectively bind to several members of the inhibitor of apoptosis family proteins including XIAP, cIAP1, and cIAP2, which are in pre-clinical stages of development (Garber, 2005).

The “extrinsic” cell death pathway is also an important target. Particularly, recombinant soluble TRAIL induced apoptosis in a broad spectrum of cancer cell lines and also in in vivo xenograft models of human cancers. In addition, several gene therapy approaches have been developed to specifically target tumor cells (Fulda and Debatin, 2004; Danial and Korsmeyer, 2004).

5. Therapeutic targets for metastasis, aimed at selective chemoresistance in the microenvironment

The selective nature of the metastatic process and the rapid evolution and phenotypic diversification of clonal tumor growth result from the inherent genetic and phenotypic instability of many clonal populations of tumor cells (Fidler, 2003). Consequently, different metastases from the same tumor may contain different genetic changes, added to the genetic changes from the dynamic and stochastic evolutionary force that varies with differing somatic environments (Liotta and Kohn, 2001).

Chemotherapy uses powerful drugs designed to induce cancer cells to commit suicide. So why do not all tumors succumb to these drugs? The cell sensitivity to chemotherapeutic drugs is dependent on host cellular and tissue response and not solely on the genetic alterations of tumor cells. There is now much evidence that the microenvironment regulates tissue specificity and contributes significantly to metastasis and

chemoresistance. If clinical metastases are a product of their microenvironment, we can identify the molecular signals that participate in metastatic cell-host cross-talk and use them in therapy (Bissell and LaBarge, 2005).

Cancer cells in secondary organs may coexist in several states. Solitary cells can die, remain dormant or begin to proliferate to form preangiogenic micrometastases. These micrometastases may also die, become functionally dormant (in which apoptosis and proliferation are balanced, leading to no net growth) or become vascularized and grow progressively. Cells in each of these states may be differentially responsive to various therapies (Chambers, 2004). Cellular proliferation at the metastatic site must be considered a potential rate-limiting step of metastasis. Persistence of solitary cells at a secondary site contributes to tumor dormancy at first diagnosis and to tumor recurrence, because they may not be susceptible to current therapeutic strategies targeting proliferating cells (Naumov et al., 2002). Because many metastasis suppressor genes such as NM23, MKK4 or KiSS1 act at the final stage of tumor cell colonization at the metastatic site, re-expression of metastasis suppressors by micrometastatic tumor cells may have therapeutic effects on cancer progression (Steeg, 2004). Indeed, an important aspect of cancer treatment is tumor “dormancy”, which describes a prolonged quiescent state during which metastasis progression is not clinically detected (Yefenof et al., 1993). Metastatic cells may be present after surgery but remain dormant for several reasons, including the inability to induce angiogenesis or to change the balance between other growth-inducing/inhibiting factors in the tumor microenvironment. These factors may also determine the length of the period between dissemination and appearance of clinically observable metastases (Karrison et al., 1999; Hart, 1999). In addition, it was suggested (Holmgren et al., 1995), that dormancy could be the consequence of opposing tumor cell proliferative tendencies by anti-angiogenic factors that indirectly promote apoptosis. Progression may depend on the balance between the in situ tumor’s total angiogenic defence, in which case the future of inhibitors of blood-vessel growth is to prevent disease in those individuals whose genetics favor progression (Folkman and Kalluri, 2004).

Hypoxia stimulates tumor invasion by activating hepatocyte growth factor. Thus, the anti-angiogenic therapy effect, which acts by depriving tumors of oxygen, may depend on the tumor cell response to hypoxia (Pennacchiotti et al., 2003). Certain anti-angiogenic factors can also transiently “normalize” the abnormal structure and function of tumor vasculature to make it more efficient for oxygen and drug delivery (Eichhorn et al., 2004; Jain, 2005). Moreover, several new therapeutic strategies target the hypoxia-inducible factor (HIF) pathway, critical for the induction of hypoxia tolerance, apoptosis resistance and cancer progression (Erler et al., 2004; Wouters et al., 2004). In addition, increased glycolysis, due in part to mitochondrial respiration injury and hypoxia, decreases sensitivity to common anti-cancer agents. Depletion of ATP by glycolytic inhibition also potently induces

apoptosis in multi-drug resistance cells (Gatenby and Gillies, 2004; Xu et al., 2005).

In summary, in order to predict tumor response to combined chemotherapy we have to bear in mind that apoptotic cell death and angiogenesis are affected by hypoxia; that interactions of tumor cells with the extracellular matrix modulate intracellular signaling; and that expression of molecules involved in the regulation of apoptotic cell death can be modulated by microenvironmental factors. From an improved understanding of how the microenvironment exerts control over the genome in metastatic cells, multiple novel strategies have already emerged for restoring apoptosis sensitivity in cancer cells (Table 1).

6. Conclusion and future perspectives

Two related themes that are keys to the future of cancer treatment are emerging: prediction of therapy response and combination chemotherapy. The former takes into account the genetic make-up of a tumor and thus the molecules that are deregulated. In the latter, understanding in molecular detail the interactions of survival pathways and chemotherapeutics will allow the design of rational combinations of drugs. Synergistic drugs may include agents targeting specific gene products associated with apoptosis and modulators of the host microenvironment.

Adjuvant chemotherapies or hormone therapies are used as a preventive measure to extend the survival time of patients, but are associated with long-term morbidity and treatment failure. There is a need for markers that can predict treatment efficacy, since established diagnostic methods of histology and cytology have a low sensitivity with assessment based on cell morphology. Micrometastases and individual dormant cancer cells in tissues go unnoticed by pathologists. In fact, most efforts are concentrating on the accurate prediction of the outcome of primary treatment among patients with early-stage disease and on avoidance of unnecessary interventions causing risk to the patient (Borg et al., 2003).

Microenvironmental factors at the metastatic foci may affect the response of tumors to chemotherapy and condition drug resistance. A wide variety of genetic and epigenetic factors are involved as determinants. Much remains to be learned about how best to exploit these new potential therapies by matching the genetic lesions in cancers to the optimal agent. Better understanding of apoptosis mechanisms has led to many new strategies for restoring apoptosis sensitivity in cancer (Stenner-Liewen and Reed, 2003). The fact that cancer cells are more dependent on apoptosis suppression than normal cells enables us to develop new therapies that might improve clinical outcome.

On the other hand, therapeutic intervention addressed to the stroma may have limited effectiveness given the diverse growth potentiating factors that redundantly induce tumor–microenvironment interactions, and different pathways might be connected to the achievement of metastatic

activity (Cheng and Weiner, 2003). Therefore, the identification of adaptor proteins and protein–protein interactions connecting several metabolic pathways is necessary to therapeutic disruption of multiple signals, which include several functional pathways useful to metastasis development (España et al., in press).

Metastasis is the single catastrophic complication of cancer, and understanding the biology of the process should provide not only greater insight into normal cell behaviour, but also lead to new therapies designed to limit or prevent this cause of morbidity and mortality (Pantel and Brakenhoff, 2004). Our understanding of the cellular and molecular events is improving significantly, and the possibility of selective anti-metastatic therapy is becoming more realistic. By studying the molecular factors that affect the metastatic process, a better understanding of how these molecular factors function in metastasis will be obtained. This, in turn, will aid the development of molecularly targeted anti-metastatic treatments, and the identification of nodal points on multiple survival-signal pathways that might represent a useful target for anti-tumor drugs.

Acknowledgements

Work in the author's laboratory is supported by current or previous grants from the Spanish Government: FIS 99/0770, FIS 01/1469, FIS/PI041937; and by the European Community: MetaBre contract No. LSHC-CT-2004-506049. We thank Mr R. Rycroft for expert language advice.

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