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Article

IAP Regulation of Metastasis

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SUMMARY

Inhibitor-of-Apoptosis (IAP) proteins contribute to tumor progression, but the requirements of this pathway are not understood. Here, we show that intermolecular cooperation between XIAP and survivin stimulates tumor cell invasion and promotes metastasis. This pathway is independent of IAP inhibition of cell death. Instead, a survivin-XIAP complex activates NF-κB, which in turn leads to increased fibronectin gene expression, signaling by β1 integrins, and activation of cell motility kinases FAK and Src. Therefore, IAPs are direct metastasis genes, and their antagonists could provide antimetastatic therapies in patients with cancer.

INTRODUCTION

Metastasis, which is the dissemination of tumor cells to distant organs (Nguyen and Massague, 2007), heralds a nearly invariably fatal phase of epithelial malignancies, with few, if any, therapeutic options. This process reflects the acquisition of multiple molecular traits by tumor cells, including the ability to counteract the cell death program initiated by detachment from the extracellular matrix, or anoikis (Frisch and Screaton, 2001), proteolyze and migrate through basement membranes, invade blood or lymphatic vessels, and proliferate in unrelated microenvironments (Weigelt et al., 2005). The exact timing of these processes is unknown, and whether they reflect sequential adaptive changes (Scheel et al., 2007) or selection of specially endowed clones (Talmadge, 2007) has been debated. However, one invariable feature of the metastatic process is deregulated gene expression, which affects sequential stages of tumor cell invasion, organ tropism, and growth at distant sites (Nguyen and Massague, 2007). A potential loss of "metastasis-suppressor" genes may also contribute to this pathway (Stupack et al., 2006).

Although the molecular requirements of metastasis remain largely elusive, "outside-in" signaling initiated by ligation of integrin cell surface receptors (Ginsberg et al., 2005) with extracellular matrix proteins (Juliano et al., 2004) likely plays a pivotal role in this process. As an abundant constituent of the extracellular matrix, fibronectin binds multiple integrins (Cukierman et al., 2002), resulting in the activation of focal adhesion kinase (FAK) (Sieg et al., 2000), Src (Yeatman, 2004), and Akt (Irie et al., 2005), as well as modulation of small GTPases of the Rho family (Nobes and Hall, 1995). In response to these signals, cells remodel their actin cytoskeleton (Juliano et al., 2004), express matrix metalloproteinases (Han et al., 2006), become migratory (Livant et al., 2000), invade basement membranes (Gaggioli et al., 2007), and acquire the ability to resist apoptosis (Fornaro et al., 2003).

In this context, aberrantly increased cell survival is an invariable requirement of metastasis (Mehlen and Puisieux, 2006) and is typically contributed via deregulated expression of Bcl-2 (Cory and Adams, 2002) or inhibitor-of-apoptosis (IAP) (Srinivasula and Ashwell, 2008) cytoprotective proteins. However, some of these molecules, especially IAPs, have recently emerged as broader regulators of cellular homeostasis, with functions extending beyond apoptosis inhibition (Srinivasula and Ashwell, 2008). For instance, IAP family protein XIAP has been linked to the activation of multiple gene expression networks, including Smad/TGFβ (Birkey Reffey et al., 2001), JNK (Sanna et al., 1998), or NF-κB (Hofer-Warbinek et al., 2000; Lu et al., 2007), whereas survivin plays essential roles in mitosis, the cellular stress response, and developmental

SIGNIFICANCE

Metastasis is a hallmark of tumor progression, characterized by the dissemination of cancer cells to distant organs. Despite a better understanding of this process, which is often characterized by deregulated gene expression, antimetastatic therapies do not presently exist, and patients with disseminated disease have limited options. We now show that inhibitor-of-apoptosis (IAP) molecules function as direct activators of tumor cell motility and metastasis genes independently of their roles in cytoprotection. IAP antagonists are now being tested in early phase clinical trials, and these agents may provide first-in-class antimetastatic therapies for patients with cancer.

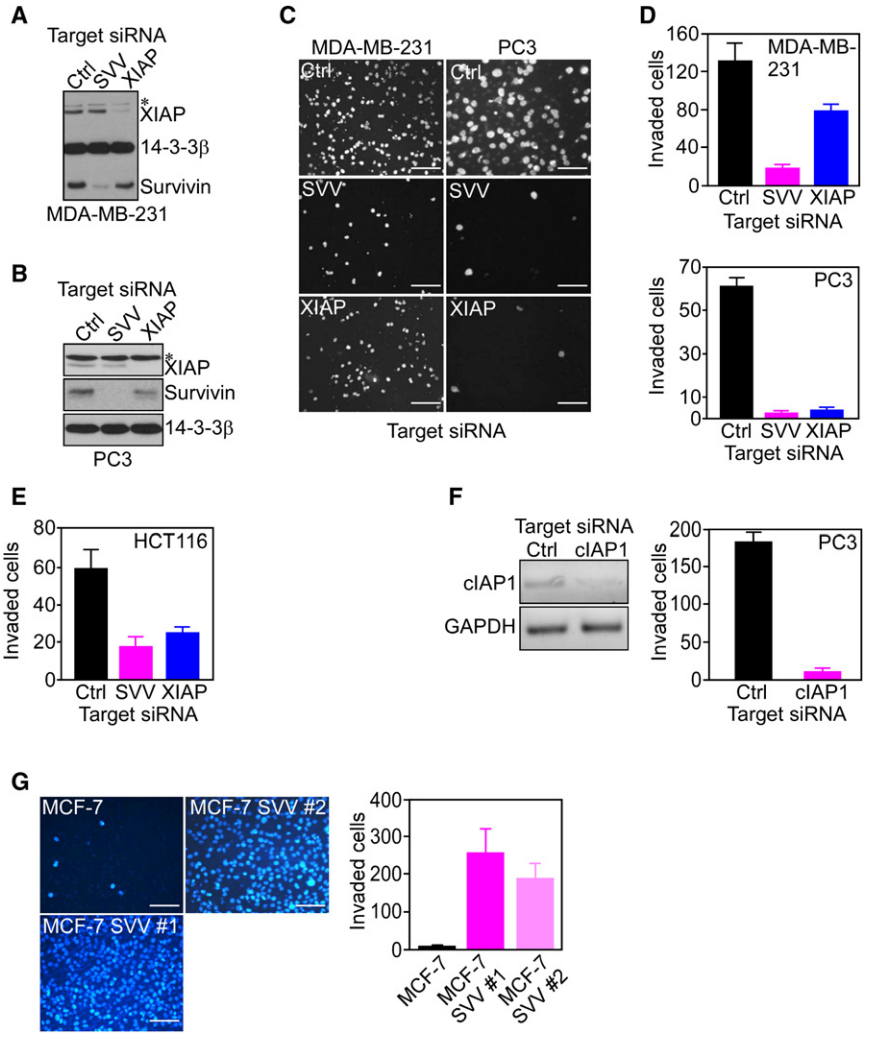

Figure 1. IAP-Mediated Tumor Cell Invasion

(A and B) Breast adenocarcinoma MDA-MB-231 (A) or prostate adenocarcinoma PC3 (B) cells transfected with control (Ctrl) or survivin (SVV)–directed or XIAP-directed siRNA were analyzed by western blotting after 48 hr. Asterisks indicate nonspecificity.

(C–E) The indicated siRNA transfected cell types were analyzed for invasion through Matrigel-coated Transwell inserts after 6 hr by DAPI staining (C) and were quantified (D and E). Scale bars, 200 μm (MDA-MB-231) and 100 μm (PC3).

(F) PC3 cells were transfected with control (Ctrl) or cIAP1-directed siRNA and were analyzed by PCR (left) and Matrigel invasion after 6 hr (right).

(G) MCF-7 or MCF-7 SVV cells (clones no. 1 and no. 2) were analyzed for Matrigel invasion after 6 hr by DAPI staining (left) and were quantified (right). Scale bars, 200 μm. For (D)–(G), data are the mean ± SD of duplicates of a representative experiment out of at least two independent determinations. See also Figure S1.

pathways of gene expression (Altieri, 2008). How these multiple functions of IAPs work together in cellular homeostasis is unclear, and the exact contribution of these noncytoprotective mechanisms, if any, to tumor progression has not been investigated. In this study, we investigated whether IAP signaling affected metastasis as a key determinant of unfavorable disease outcome.

RESULTS

IAP-Mediated Tumor Cell Invasion

To begin investigating a role of IAPs in tumor progression, we first silenced the expression of XIAP or survivin in invasive breast adenocarcinoma MDA-MB-231 (Figure 1A) or prostate adenocarcinoma PC3 (Figure 1B) cells. Transfection of these cells with XIAP- or survivin-directed small interfering RNA (siRNA) suppressed the expression of the intended IAP protein, but not vice versa, whereas a nontargeting siRNA had no effect (Figures 1A and 1B). Under these conditions, survivin or XIAP knockdown inhibited MDA-MB-231 or PC3 cell invasion through Matrigel inserts, compared with control transfectants (Figures 1C and 1D). Similar results were obtained with siRNA silencing of these IAPs in colorectal adenocarcinoma HCT116 cells, whereas a nontargeting siRNA had no effect (Figure 1E). In addition, siRNA knockdown of a different IAP, cIAP1, also abolished Matrigel invasion of PC3 cells (Figure 1F), suggesting that tumor cell invasion was a general property of multiple IAPs.

Next, we examined the specificity of this pathway, and we used clones of noninvasive breast adenocarcinoma MCF-7 cells stably transfected with survivin. These cells, designated MCF-7 SVV, express 2- to 3-fold increased survivin levels, compared with those of MCF-7 cells (Figure S1A available online), and thus are similar to more invasive cell types (Figure 1A). Compared with parental cultures, MCF-7 SVV cells did not exhibit changes in cell proliferation (Figure S1B), adhesion to fibronectin- or collagen-containing substrates (Figure S1C), remodeling of the actin cytoskeleton (Figure S1D), or expression of various integrins (Figure S1E). Instead, MCF-7 SVV cells became highly migratory on collagen-coated inserts, compared with parental cultures (Figure S1F), or MCF-7 cells transfected with empty plasmid (data not shown). Consistent with this finding, two independent clones of MCF-7 SVV cells showed markedly enhanced invasion through Matrigel-coated inserts, whereas nontransfected MCF-7 cells were not invasive (Figure 1G).

Confirming the specificity of IAPs in this response, siRNA knockdown of XIAP or survivin in MCF-7 SVV cells (Figure 2A) suppressed Matrigel invasion, whereas a nontargeting siRNA had no effect (Figure 2B). Conversely, cell viability was only minimally affected after XIAP (7%) or survivin (14.2%) silencing, compared with control transfectants (4.8%). cIAP1 knockdown also reduced Matrigel invasion of MCF-7 SVV cells from 61.5 ± 11.1 (control siRNA) to 11.3 ± 1.6 invaded cells per field ($p = 0.0003$; $n = 10$). As an independent approach, we transduced MCF-7