REVIEW

Contribution of p53 to Metastasis

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ABSTRACT The tumor suppressor p53 is lost or mutated in about half of all human cancers, and in those tumors in which it is wild-type, mechanisms exist to prevent its activation. p53 loss not only prevents incipient tumor cells from undergoing oncogene-induced senescence and apoptosis, but also perturbs cell-cycle checkpoints. This enables p53-deficient tumor cells with DNA damage to continue cycling, creating a permissive environment for the acquisition of additional mutations. Theoretically, this could contribute to the evolution of a cancer genome that is conducive to metastasis. Importantly, p53 loss also results in the disruption of pathways that inhibit metastasis, and transcriptionally defective *TP53* mutants are known to gain additional functions that promote metastasis. Here, we review the evidence supporting a role for p53 loss or mutation in tumor metastasis, with an emphasis on breast cancer.

Significance: The metastatic potential of tumor cells can be positively influenced by loss of p53 or expression of p53 gain-of-function mutants. Understanding the mechanisms by which p53 loss and mutation promote tumor metastasis is crucial to understanding the biology of tumor progression and how to appropriately apply targeted therapies. *Cancer Discov; 4(4); 405–14. ©2014 AACR.*

INTRODUCTION

Epithelial tumors initially arise as organ-confined lesions that eventually progress from the primary site to colonize distant secondary sites. These metastases are generally responsible for the lethality of tumors. The mechanisms by which tumor cells exit their primary site, intravasate into the bloodstream, extravasate into distal organs, and then establish growth in the secondary sites are not well understood.

Processes such as epithelial–mesenchymal transition (EMT) and the activation of proteases that degrade the basement membrane and extracellular matrix (ECM) have been implicated in the metastatic process (1). Although these processes likely contribute to the ability of a tumor cell to exit its primary site and enter the circulation, the genes responsible for mediating these initial steps are likely not solely responsible for metastasis. If a cell is to populate a tumor at a secondary site, it must also survive in the circulation for hours to days, extravasate out of the bloodstream and into the secondary organ, and grow and divide to populate the metastatic tumor (Fig. 1). The ability of a tumor cell to undergo this full metastatic program is thought to require a plethora of somatic mutations and changes in gene expression and metabolism that allow it to successfully complete each step. Some of these changes are likely required at certain steps of the metastatic process but not at others. Consequently, efforts are under way to establish model systems that accurately recapitulate all steps in human tumor metastasis and identify the required spectrum of changes necessary to mediate each step of the metastatic process.

One of the most intriguing potential master regulators of metastasis is p53, which directly controls the transcription of genes that are involved in canonical metastasis pathways, including cell adhesion, motility, invasion, EMT, stemness, ECM interactions, and anoikis (Fig. 2). p53 is a tumor-suppressor protein that has been dubbed the "guardian of the genome" because of its ability to induce senescence, cell-cycle arrest, or apoptosis when cells are exposed to various forms of stress, including DNA damage. The transcriptional activity of p53 leads to the activation of downstream target genes, including *CDKN1A*, *PCNA*, *GADD45*, *BAX*, *NOXA*, *MDM2*, and *miR-34a*, which are responsible for inducing cell-cycle arrest, DNA repair, senescence, or apoptosis. Therefore, loss of functional p53, which renders cells unable to engage apoptosis or senescence programs after exposure to cellular stress, contributes to tumor formation. Indeed, *TP53* mutation is associated with poor prognosis in many human tumors, including breast cancer (2).

Loss of p53 not only aids in tumor initiation and progression but also allows tumors to more quickly acquire a full repertoire of metastatic facilitators. p53 directly influences transcription of genes involved in metastasis (Fig. 2 and Table 1) by binding promoters of a variety of genes known to be involved in regulating cell motility and adhesion, processes that are important for metastasis (3). One particular study

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STATEMENT OF RELEVANCE

- A growing body of evidence demonstrates that wild-type p53 negatively regulates multiple stages of metastasis.
- Paradoxically, certain p53-mutant proteins that lack transcriptional activity drive gain-of-function activities with respect to metastasis.
- An understanding of the mechanisms by which loss of wild-type p53 and expression of gain-of-function p53 mutants influence metastasis will help us to accurately predict metastatic potential and appropriately tailor treatment regimens.

(3) used p53–wild-type (WT) or p53-null colorectal cancer cells that were treated with 5-fluorouracil (or vehicle) to determine the binding of transcriptionally active p53 to gene promoters on a global scale. Gene expression data revealed that decreased expression of some p53-activated genes and increased expression of other p53-repressed genes were significantly correlated with distant metastasis of breast tumors within 5 years of diagnosis, supporting a role for p53 in inhibiting metastasis in breast tumors (3). The Perou laboratory evaluated gene expression differences with and without doxorubicin in breast cancer cell lines that were isogenic for endogenous WT p53 or expressed p53-specific short hairpin RNAs (shRNA; ref. 4). The combined gene expression data were used to compile a list of genes that are regulated by p53, irrespective of the molecular classifiers that defined the breast cancer subtype. This *TP53* gene expression signature was significantly predictive of overall survival and relapse-free survival, suggesting that disruption of the p53 pathway in breast cancer is correlated with metastasis.

For cells to metastasize, they must be able to invade the surrounding tissue, breach the barrier of the basement membrane, and enter the circulation or lymphatic system (Fig. 1). For this to occur, cancer cells must invade through the stroma and its associated ECM. Studies have demonstrated that p53 deletion can alter cell polarity and morphologic features, resulting in increased migration in scratch wound-healing assays and three-dimensional matrices (5). p53 is thought to inhibit metastasis by transcriptionally regulating targets that are implicated in key metastasis pathways, including cell migration, EMT, stemness, ECM interactions, and anoikis.

p53 LOSS INFLUENCES CELL MOTILITY

The RHO family of small GTPases regulates cell migration and invasion. Loss of p53 leads to increased levels of GTP-bound (active) RHOA and activated ROCK, its main effector protein (5). These properties are not limited to fibroblasts, as similar observations were made in other cell types, including epithelial cancer cells (6). The signals that lead to the migratory and invasive phenotype converge on members of the Rho family, including RAC, CDC42, and RHOA, which control the actin dynamics that are fundamental to tumor cell invasion. The characteristic phenotypes by which tumor cells migrate are influenced by the balance of RAC and RHO proteins. When RAC predominates, cells acquire an elongated migratory phenotype typical of tumor cells with mesenchymal characteristics. Conversely, RHOA and ROCK promote contractility and rounded amoeboid migration phenotypes,

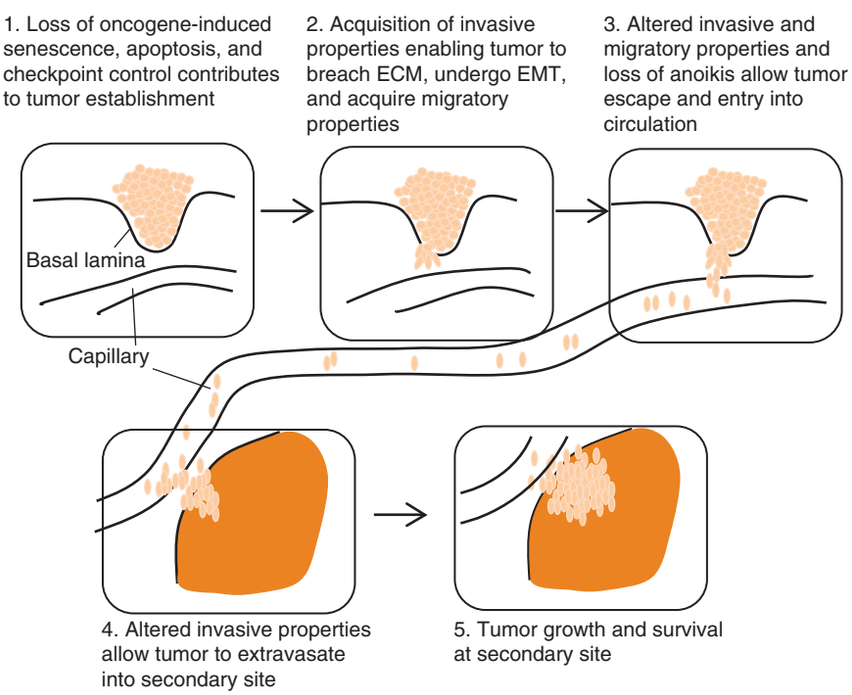

Figure 1. p53 loss affects several steps of the metastatic process. The loss of oncogene-induced senescence and apoptosis that results from the loss of p53 allows tumors to be established. The loss of checkpoint control enables p53-deficient tumors to continue cycling, creating a permissive environment for the acquisition of additional mutations. Theoretically, this could contribute to the evolution of a cancer genome that is conducive to metastasis (step 1). The loss of p53 results in gene expression changes (see Table 1) in tumor cells, leading to the acquisition of invasion properties that enable tumor cells to breach the ECM, undergo EMT, and acquire migratory capabilities (step 2). Altered invasive and migratory properties allow tumor cells to intravasate neighboring blood vessels, and loss of anoikis enables tumor cells to survive detachment from the ECM (step 3). Altered invasive and migratory properties allow tumor cells to extravasate into the secondary (metastatic) site (step 4) and proliferate (step 5).

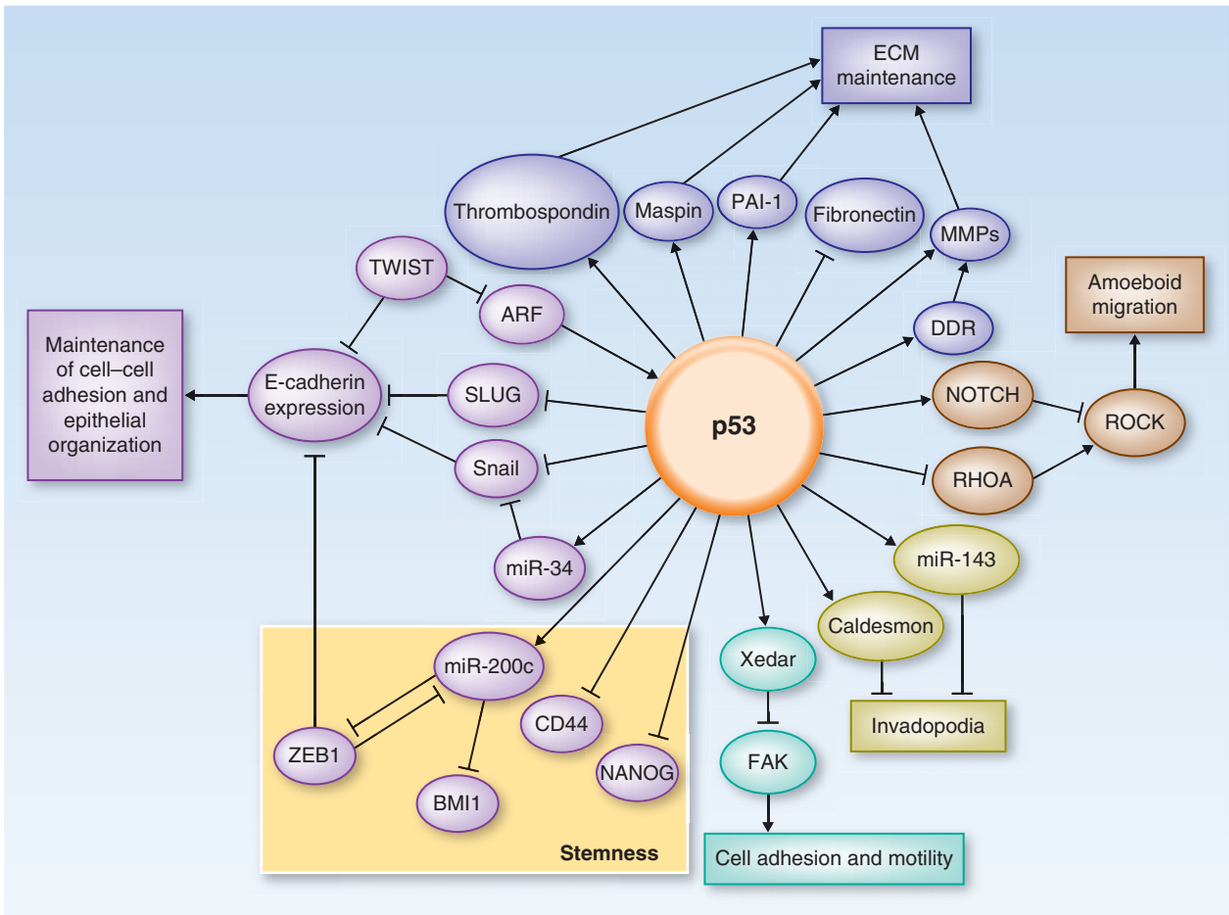

Figure 2. Metastasis pathways that affect, or are affected by, p53. p53 regulates the transcription of genes that are involved in pathways that negatively regulate tumor metastasis. The key pathways and pathway components in the metastatic cascade that are regulated by or converge on p53 are grouped according to color. MMP, matrix metalloproteinase.

which tumor cells likely use to migrate *in vivo* (5). Therefore, RhoA–ROCK signaling after p53 loss promotes amoeboid cell motility and invasion.

Loss of p53 cooperates with activated Ras in colonic epithelial cells to synergistically induce RHOA activity, resulting in increased cell motility in epithelial cells [ref. 6; this topic is discussed in greater detail in a review by Muller and colleagues (5)]. A list of p53-regulated genes that contribute to different steps of metastasis is shown in Table 1. As indicated in Table 1, direct regulation means that p53 has been shown to bind to the gene promoters (by gel shift assays or chromatin immunoprecipitation assays), whereas indirect regulation indicates that p53 signaling regulates the transcription factors that, in turn, control expression of the gene in question.

p53 directly regulates expression of KAI-1/CD82, a member of the tetraspanin or transmembrane 4 superfamily (7). KAI-1/CD82 suppresses cancer metastasis by inhibiting cell migration and invasion. Downregulation or loss of expression of KAI-1/CD82 is a frequent occurrence in clinically advanced cancers (8). KAI-1/CD82 has been shown to suppress cell migration, in part, through regulation of focal adhesion kinase (FAK) and its downstream targets, LYN and p130CAS (9). Activated FAK mediates cell invasion and metastasis in cancer cells, and high FAK expression is observed in highly aggressive cancers (10). The FAK promoter contains p53 binding sites, wherein p53 inhibits FAK transcription, and there is a high correlation between FAK overexpression and *TP53* mutations in a wide variety of human tumors (11). X-linked ectodermal dysplasia receptor (*XEDAR*), a member of the TNF receptor superfamily, is a p53 target gene that also negatively regulates FAK (12). In summary, p53 loss affects the expression of many genes whose protein products regulate cell motility, and this is expected to provide the tumor cell with enhanced metastatic potential.

EMT

EMT is the process by which epithelial cells undergo a coordinated cascade of signaling and transcriptional changes, resulting in the acquisition of a more mesenchymal phenotype (1). Epithelial cells line the cavities and surfaces of tissues and organs and are organized into sheets that are held together through several interactions, including tight junctions, adherens junctions, desmosomes, and gap junctions.

Table 1. List of genes implicated in the metastatic cascade that are direct or indirect targets of p53

Gene	Function	Activated or repressed by p53	Direct or indirect p53 target	Reference
KAI-1/CD82	Suppresses cell migration	Activated	Direct	(7)
XEDAR	Suppresses cell adhesion and migration	Activated	Direct	(12)
miR-200c	Suppresses EMT	Activated	Direct	(14)
MMP2	Interactions with ECM	Activated	Direct	(28)
DDR1	Interactions with ECM	Activated	Direct	(30)
PAI-1	Inhibits plasminogen and hence fibrinolysis	Activated	Direct	(24)
miR-34	Suppresses cell migration	Activated	Direct	(77)
Maspin	Interactions with ECM	Activated	Direct	(25)
PCDH7	Cell migration	Repressed	Direct	(3)
Vimentin	Mesenchymal marker	Repressed	Direct	(3)
CD44	EMT, stemness	Repressed	Direct	(19)
NANOG	Stemness	Repressed	Direct	(20)
CXCR4	Chemotaxis, cell migration	Repressed	Direct	(60)
FAK	Adhesion, motility, metastasis, survival signaling	Repressed	Direct	(78)
E-cadherin	Maintains epithelial integrity	Activated	Indirect	(17)
CTGF	Cell migration and adhesion to ECM	Activated	Indirect	(79)
Thrombospondin	Interactions with ECM	Activated	Indirect	(80)
Caldesmon	Interactions with ECM	Activated	Indirect	(37)
SNAI1	EMT	Repressed	Indirect	(16)
SNAI2/SLUG	EMT	Degraded	Indirect	(17)
MMP9	Interactions with ECM	Repressed	Indirect	(35)
MMP1	Interactions with ECM	Repressed	Indirect	(29)
SPARC	Cell migration (negative regulator)	Repressed	Indirect	(59)
Fibronectin	Interactions with ECM	Repressed	Indirect	(36)

Epithelial cells are polarized in an apical–basal orientation and are tethered to neighboring cells through intercellular junctions that permit only cohesive epithelial cell movement. The movement of these cells is further restricted by the underlying basement membrane, which allows only lateral movement within the epithelial layer (13). The transcriptional programs that mediate EMT are characterized by the loosening of cell–cell adhesion, loss of epithelial structural integrity, loss of cell polarity, and acquisition of a more motile, mesenchymal phenotype. In an orchestrated series of events in which cell–cell and cell–ECM interactions are altered, epithelial cells are released from their surrounding tissue, and their cytoskeletons are reorganized to allow them to move through that tissue (13). A transcriptional program is induced that maintains this acquired mesenchymal phenotype. Most EMT transcription factors are transcriptional repressors, including SNAIL, SLUG, ZEB1, and TWIST. They repress epithelial-specific genes, particularly molecules involved in stabilizing cell–cell junctions, such as E-cadherin, and upregulate components of the mesenchymal migratory machinery.

p53 functions to suppress metastasis, in part, by negatively regulating factors demonstrated to be important to initiating and maintaining EMT programs (3, 14). Overexpression of p53 in p53-proficient human mammary epithelial cells (HMEC) that have undergone EMT results in their reversion back to an epithelial phenotype (14, 15). Because EMT is an important step in the metastatic process, the ability of p53 to negatively regulate EMT may help to explain why p53-deficient tumors have a poor prognosis (2). p53 signaling affects SNAIL, SLUG, and TWIST levels to negatively regulate EMT. Activation of p53 in colorectal cells initiates the acquisition of a more epithelial phenotype through a process known as mesenchymal–epithelial transition (MET). In this case, p53 activates the expression of miR-34 to repress SNAIL expression. Indeed, miR-34 has been shown to be necessary for p53-dependent inhibition of tumor cell migration and invasion (16). In addition, p53 regulates expression of MDM2, which, in turn, degrades SLUG to enhance E-cadherin expression and oppose EMT (17). Because SNAIL and SLUG are master regulators of EMT, the ability of p53 to oppose their

function underscores the importance of p53 in maintaining an epithelial phenotype. TWIST has been shown to oppose p53 function, further supporting the idea that the loss of p53 is important for the ability of cells to undergo EMT. TWIST downregulates the ARF tumor-suppressor protein (Fig. 2), leading to the ubiquitin-mediated proteolysis of p53 by MDM2 (18). Thus, by indirectly antagonizing p53 function through ARF loss, TWIST promotes EMT.

EMT AND STEMNESS

Tumors are composed of a biologic hierarchy of cell types that consist of at least two distinct cell populations: undifferentiated cancer stem cells (CSC) or tumor-initiating cells (TIC) and their differentiated progeny. CSCs are thought to give rise to all tumor cell types through the process of self-renewal and differentiation. Such cells may be responsible for relapse and metastasis by giving rise to new tumors. EMT transcription factors not only induce EMT but also generate cells with the traits of CSCs, including self-renewal capabilities (13). For example, the EMT transcription factor ZEB1 negatively regulates miR-200 family members, which function to suppress expression of the polycomb protein BMI1. BMI1 supports the stem cell state in both cancer cells and embryonic stem cells. By suppressing miR-200 expression, ZEB1 promotes the stem cell state by enhancing BMI1 expression. p53 positively regulates miR-200c to inhibit both ZEB1 and BMI1. Thus, p53 loss results in failure to activate miR-200c, thereby promoting both EMT (through ZEB1 expression) and the stem cell state (through BMI1 expression; ref. 13). Loss of p53 in mammary epithelial cells has been shown to decrease expression of miR-200c and activate EMT, resulting in an increase in mammary stem cells and high-grade tumors (14).

p53 has also been shown to regulate stemness by directly repressing expression of CD44, a known stem cell marker and an important supporter of anchorage-independent growth and metastasis. In fact, growth-inhibitory and tumor-suppressive functions of p53 may depend on its ability to directly repress CD44 expression. In a study by Godar and colleagues (19), constitutive CD44 expression blocked p53-dependent apoptosis and rendered cells resistant to doxorubicin. These results link p53 loss to increased CD44 expression, which in turn promotes the expansion of TICs. p53 seems to play a similar role in embryonic stem cells; it represses the expression of NANOG, limiting the pool of pluripotent cells (20). In keeping with this finding, p53 loss expands the repopulating activity of tissue-specific stem cells (21).

INTERACTIONS WITH THE ECM

EMT is known to impart a migratory phenotype to tumor cells, but epithelial cells may also acquire the ability to migrate and invade the surrounding tissue without initiating the full EMT program (22). In these instances, only one or a few EMT markers may be activated. Indeed, in addition to its ability to control expression of EMT proteins, p53 can influence signaling pathways that modulate ECM, cell migration, and chemotactic responses that contribute to invasion and metastasis.

The predominant cell type in the stromal compartment is the fibroblast, which synthesizes, organizes, and maintains a three-dimensional network of glycoproteins and proteoglycans known as the ECM. Normal stromal fibroblasts and their ECM are believed to exert an inhibitory constraint on tumor growth and progression. However, major alterations occur in stromal fibroblasts and ECM during neoplastic transformation, giving rise to a permissive and supportive microenvironment for tumor growth and metastasis (23).

Abundant evidence has validated the importance of synthesis and deposition of ECM proteins, as well as their degradation by extracellular proteases in the invasive process (23). Moreover, p53 is known to regulate components of the adhesive machinery that contribute to cell motility and invasion through the stroma. p53 normally represses the transcription of plasminogen activators, which promote ECM degradation and cell invasion; thus, loss of p53 stimulates cell invasiveness. p53 induces the expression of at least two serpins: plasminogen activator inhibitor-1 (PAI-1; ref. 24) and maspin (25). PAI-1 inhibits the function of urokinase-type plasminogen activator, which initiates a cleavage cascade that ultimately results in plasmin activation (24). Plasmin degrades a wide variety of ECM proteins, such as fibrin, fibronectin, and laminin. Thus, PAI-1 induction results in ECM maintenance and metastasis inhibition; loss of p53 function results in a reduction of PAI-1, leading to increased metastatic potential. In a similar manner, p53 activates the expression of maspin. Although maspin is classified as a serpin, it does not use its protease inhibitor activity to inhibit migration or metastasis. Rather, maspin interacts with collagen types I and III and increases cell adhesion to the ECM; overexpression of maspin in a highly invasive mouse mammary tumor model inhibited tumor growth and metastasis (26).

Matrix metalloproteinases (MMP) are proteolytic enzymes that can disrupt the ECM, among other functions. They degrade the structural components of the ECM, allowing tumors to invade and metastasize. Cleavage of ECM proteins results in altered signaling by intracellular or transmembrane receptors that respond to ECM ligands, such as integrins. More recently, MMPs were found to have a more diverse role in multiple steps of tumor progression, including angiogenesis, cell growth and differentiation, apoptosis, and migration and invasion (27). MMPs are frequently upregulated in metastatic tumors, and their overexpression can result in invasive tumors by way of EMT induction (27). p53 regulates the expression of MMPs—specifically, MMP2 (28), MMP1 (29)—and the MMP1-inducing collagen receptor DDR1 (30). However, the regulation of MMPs by p53 is complex, as it upregulates MMP2 and DDR1 but downregulates MMP1 and MMP9. Given the roles of p53 as a metastasis suppressor, this upregulation of MMP2 and DDR1 is an apparent paradox because MMP2 and MMP1 upregulation is correlated with tumor stage (31), and MMP2 upregulation is correlated with lymph node metastasis (32). The reasons for this apparent discrepancy have not been specifically evaluated, but it is possible that the complexity of MMP functions in cancer progression is at the root of the paradox.

MMPs have both cancer-promoting and cancer-inhibiting functions; furthermore, these opposing effects are sometimes initiated by cleavage of the same substrates (27). MMPs may

inhibit metastasis by cleaving CXCL12, a chemokine that promotes breast cancer metastasis; indeed, MMP2 cleaves and inactivates CXCL12 (33), which may help explain the paradoxical upregulation of MMP2, a metastasis promoter, by p53. The multifaceted roles of MMPs in tumor progression are beyond the scope of this article, but have been reviewed by Egeblad and Werb (27) in detail. Clinically, loss of functional p53 is strongly correlated with the upregulation of MMP1, MMP2, and MMP9 and basement membrane dissociation (34), again illustrating the complexity of the regulation of MMP2 by p53 and suggesting that it is tumor stage–dependent. In soft-tissue sarcoma, p53 inhibits NF-κB–induced expression of MMP9 (35). p53 also activates the expression of the ECM component thrombospondin, which inhibits tumor growth by blocking angiogenesis, and p53 suppresses the expression of fibronectin, which promotes tumor growth (36).

Cells can interact with and move through the ECM by way of invadopodia, which are cell extensions that can trigger degradation of the ECM and basal membrane. p53 transcriptionally activates the gene that encodes caldesmon, the product of which inhibits podosome formation after oncogenic transformation (37). Furthermore, p53 regulates the transcription of miR-143, which may target components of the invadopodia formation machinery to inhibit podosome formation (5). These activities further support a role of p53 in metastasis.

ANOIKIS

Anoikis is a form of p53-dependent apoptosis that is triggered when epithelial cells detach from the ECM (38). Anoikis is thought to be a safeguard against metastasis because if cells cannot survive when detached from the ECM, they cannot complete the final steps of the metastatic cascade. In support of this concept, only a small fraction of clonal cancer cells survive to form metastatic lesions when they are injected directly into the circulation (39–41). Inhibition of p53 function in thyroid epithelial cells inhibits anoikis (42), and detached transformed fibroblasts undergo anoikis only if they express WT p53 (43). The ability of p53 to induce anoikis likely contributes to its role as a metastasis suppressor. Anoikis-resistant tumors exhibit increased metastasis and survival in circulation (44). Cell detachment activates a signaling cascade involving LKB1 and SIK1 (salt-inducible kinase 1), leading to p53 accumulation through SIK1-mediated phosphorylation of p53. p53 does not accumulate and anoikis is not observed when HMECs deficient in either LKB1 or SIK1 are detached from the substratum and grown in suspension culture (45). The LKB1–SIK1–p53 signaling pathway was shown not only to induce anoikis but also to suppress anchorage-independent growth, invasion, and metastatic potential (45).

MUTANT p53 HELPS DRIVE CELL MIGRATION AND INVASION

Despite abundant evidence that p53 suppresses metastatic processes, p53-null mouse tumors do not metastasize frequently or display invasive physiologic characteristics (5, 46), suggesting that p53 loss alone is insufficient to drive invasive cellular migration *in vivo*. Most *TP53* alterations are missense mutations in exons 4–9, which encode the DNA binding domain of the protein. Of the mutations in this domain, about 30% fall within six "hotspot" residues: R175, G245, R248, R249, R273, and R282. The introduction of *TP53* mutants (R175H or R273H) increases the incidence of highly metastatic carcinomas in mouse models (47, 48). In many human tumors, p53 is mutated such that it loses its ability to bind DNA and function as a tumor suppressor (49). However, a growing body of evidence suggests that these mutations give p53 a gain-of-function role in the context of tumorigenesis, invasion, and metastasis (5). In contrast to the ability of WT p53 to suppress mediators of EMT, p53 mutants can act through TWIST or SLUG to induce partial EMT-like conversions, which are indicated by E-cadherin suppression (5, 17). p53 mutants have been found on the promoters of target genes, including *EGR1* and *MSP*, suggesting that they may function as transcription factors (via the N-terminal transactivation domain) with their own set of target genes (50–52). However, because most p53 "hotspot" mutations occur in the DNA binding domain and ablate DNA binding, this conclusion likely does not indicate direct DNA binding by these mutants. The effects of mutant p53 on transformation may thus be due to nontranscriptional effects; however, studies have indicated that the p53 transcriptional domain is required for these effects (53), and it is therefore possible that mutant p53 translocates to non-p53 promoters through aberrant protein–protein interactions. These mutants may also exert their effects by modifying the function of other proteins, including the p53 family members p63 and p73 (5).

The Piccolo laboratory (54) has shown that oncogenic RAS and TGF-β cooperate with mutant p53 to form a mutant p53/p63 complex that serves to inhibit the function of p63 and targets two metastasis suppressors: Sharp-1 and cyclin G2 (54). Thus, tumors with oncogenic RAS and mutant p53, which are often observed in lung and pancreatic cancers, are poised for metastasis in the presence of TGF-β. However, the inhibitory interaction of p63 with mutant p53 was not enhanced in response to TGF-β in a more recent study by the Vousden laboratory (55), suggesting that not all cells that express mutant p53 are sensitive to these migratory effects induced by TGF-β (55). Nevertheless, the results of this study confirmed that mutant p53 can promote cell invasion and metastatic behavior by inhibiting p63. Furthermore, this study demonstrated that this motility and invasion is dependent on β1-integrin and EGF receptor signaling (55).

The Prives laboratory (56) recently found that mutant p53 can disrupt mammary tissue architecture to a more invasive phenotype by upregulating the mevalonate pathway. Furthermore, p53 mutations in human breast tumors were correlated with high expression of sterol biosynthesis genes, and mutant p53 was found to associate with sterol gene promoters via sterol regulatory element-binding protein transcription factors, which are critical for fatty acid and sterol biosynthesis. Because the mevalonate pathway is responsible for *de novo* cholesterol synthesis, the authors treated breast cancer cells with clinically approved statins to determine whether blocking this pathway inhibited the ability of mutant p53 to disrupt mammary architecture. Indeed, pharmacologic inhibition of the mevalonate pathway impaired anchorage-independent growth and caused extensive cell death in mutant p53-expressing cell lines. The growth of tumor xenografts in

nonobese diabetic/severe combined immunodeficient (NOD/SCID) mice was also impaired by statin treatment. These findings open the intriguing possibility that breast cancer cells bearing p53 mutations are addicted to the mevalonate pathway. Therefore, clinical inhibition of the mevalonate pathway through statin use may be an exciting therapeutic option for patients with p53 mutation–bearing tumors.

p53-mutant proteins are found on the promoters of certain target genes, including the sterol regulatory element-binding proteins EGR1 and MSP (56). Although the mutant p53 proteins do not directly bind DNA, they may contribute transcriptional activity to their DNA binding partners through their transactivation domains. Indeed, although mutant p53 proteins with a functional transactivation region can disrupt mammary architecture, mutant p53 proteins lacking functional transactivation domains cannot. This suggests that the oncogenic and metastatic effects of mutant p53 proteins may depend on their ability to influence transcriptional change (56). Indeed, mutant p53 may act more as a coactivator for other sequence-specific transcriptional factors binding to their own cognate sites (2, 56).

A popular cell line for studying breast cancer metastasis is the MDA-MB-231 cell line, which is triple-negative (negative for estrogen- and progesterone-receptor expression and *HER2* amplification) and mutant for *TP53*. This cell line was isolated from the pleural effusion of a patient with breast cancer and can therefore be thought of as a migratory subpopulation of a human breast tumor. Metastasis studies from the Massague laboratory using these cells revealed the deregulation of genes in subpopulations of tumor cells isolated from metastatic lesions in lung and bone (57, 58). This analysis revealed that several metastasis-associated genes that are negatively regulated by p53 were upregulated in the metastatic subpopulations that homed to the lungs and bones. These included *SPARC* (also known as osteonectin; ref. 59), *MMP1* (collagenase 1; refs. 29, 34), and *CXCR4* (60). These findings support the conclusion that p53 loss or mutation contributes to metastasis.

TP53 EXPRESSION AND MUTATION ARE CORRELATED WITH POOR PROGNOSIS IN BREAST CANCER PATIENTS

Breast cancer has been successfully used as a model disease system for investigating metastasis mechanisms. However, it is still not possible to accurately predict the risk of metastasis in individual patients; as a result, more than 80% of patients with breast cancer undergo adjuvant chemotherapy, even though only approximately 40% of treated patients relapse and ultimately die of metastatic disease. Clearly, in a significant proportion of patients, adjuvant therapy is unnecessary. New prognostic markers are urgently needed to identify patients who are at risk for metastasis.

Currently, large primary tumor size, lymph node metastasis, and poor histopathologic differentiation (grade) are clinical markers of poor prognosis. However, approximately one third of women with node-negative breast cancer develop distant metastases, and about one third of patients with node-positive breast cancer remain free of metastases 10 years after local therapy (61). Although several proteins have been found to have roles in tumor invasion, EMT, and metastasis, a clear set of prognostic molecular markers is needed. Identifying the molecules responsible for facilitating or abrogating the metastatic process will help classify patients into good or poor prognosis groups and aid in the design of treatment regimens. A significant effort is under way to identify these molecules and the pathways to which they belong.

TP53 is mutated in about 40% of all breast cancers (62). This rate varies among subtypes, with the highest frequency in basal-like (80%) and HER2-enriched (72%) subtypes and the lowest in the Luminal A (12%) and Luminal B (29%) subtypes (62). Moreover, when *TP53* mutation status is evaluated across the sub-subtypes of basal-like and triple-negative breast cancer, the *TP53* mutation status is correlated with the molecular subtype (i.e., basal-like) rather than with a common biologic feature defined by being triple-negative (63). Furthermore, basal-like tumors are reported to exhibit a higher frequency of complex mutations (deletions and insertions) leading to frequent lack of p53 protein, whereas luminal tumors tend to exhibit nucleotide substitutions that lead to the gain-of-function mutations discussed above (64). In addition, families with inherited *TP53* mutations exhibit increased frequencies of breast tumors (65).

Another mechanism by which tumors can inactivate the p53 pathway is by upregulating the expression or activity of MDM2, the negative regulator of p53. Indeed, 14% of Luminal A, 31% of Luminal B, 14% of basal-like, and 30% of HER2-enriched breast tumors have gain-of-function *MDM2* genetic aberrations (62). We mined The Cancer Genome Atlas (TCGA) database for the two most aggressive subtypes with the poorest prognosis (invasive basal-like and HER2-enriched) to catalog their frequencies of *TP53* and *MDM2* alterations at the genomic, mRNA, and protein levels. We found these alterations in 90% of basal-like and 80% of HER2-enriched breast cancers. Therefore, alterations of the p53 pathway are found in nearly all of the most aggressive and metastatic forms of invasive breast cancer.

Breast cancers with *TP53* mutations are high-grade tumors; they are particularly aggressive and have poor prognosis (66), which is an indication of distant metastasis. In a recent study, whole-genome sequencing was performed on a basal-like breast cancer, its corresponding brain metastasis, and a xenograft derived from the breast tumor. The breast tumor and brain metastasis were sequenced directly from the patient. Deep sequencing revealed that the metastasis harbored genetic aberrations that were not identified in the primary tumor, along with a significantly enriched subset of 20 mutations that were shared with the primary tumor. The xenograft and metastasis shared 16 of 20 genetic aberrations, suggesting that secondary metastases result from the outgrowth of a minority of cells from the primary tumor. A frameshift mutation in *TP53* was enriched in the xenograft relative to the primary breast tumor and brain metastasis (67). *TP53* mutational enrichment has also been found in lung, colon, and gastrointestinal carcinoma metastases and colon carcinoma circulating tumor cells (CTC; ref. 68). These findings indicate that *TP53* mutations precede metastasis, and that the subpopulation of tumor cells that is capable of metastasizing harbors *TP53* mutations. These findings underscore the importance of p53 as a master regulator of metastasis.

p53 AS A CLINICAL TARGET FOR METASTASIS PREVENTION

Because of the correlation between *TP53* mutation and poor prognosis, significant clinical and research efforts are under way to target p53 in a variety of tumor types. Several studies have demonstrated that restoring p53 function in established tumors leads to tumor regression (69–72). One of these studies showed that restoration of p53 in p53-null early-stage lung tumors had no effect, but its restoration in later stages caused tumor regression (69). The results of this study suggest that the p53 pathway is not engaged by low levels of oncogenic stimuli in early-stage lung tumors, but may become activated in later stages of tumorigenesis. Therefore, if metastasis is viewed as a late event in tumor progression, activating the p53 pathway in late-stage p53-deficient tumors may serve as an antimetastasis therapy. However, incomplete tumor regression was observed when p53 was reactivated in late-stage tumors (69).

Clinical and research efforts to restore the p53 pathway in human tumors are currently under way. One therapeutic strategy has been to develop small-molecule inhibitors of MDM2 to restore the function of the p53 pathway. Indeed, MDM2 is a negative regulator of p53 that is frequently overexpressed in tumor cells. One such MDM2 inhibitor, Nutlin-3, is currently being evaluated in a phase I clinical trial as a retinoblastoma treatment (73). The adenoviral delivery of WT p53 cDNA (Advexin) to tumor cells has also shown promise when combined with radiotherapy or chemotherapy in p53-deficient cancer cell lines and in phase III clinical trials (74).

Mutant p53 proteins are found at high concentrations in tumor cells relative to WT p53 (75). Thus, therapeutically inhibiting p53 mutants (but not WT p53) is an attractive strategy for rescuing the function of WT p53 in patient tumor cells that are heterozygous for a *TP53* mutation. Several small molecules, including CP-31398, STIMA-1, PRIMA-1, and MIRA-1, have been found to restore partial function to the p53 pathway by acting on mutant p53 (76). The binding of these small molecules to mutant p53 proteins may induce WT-like conformational changes in the DNA binding domains of p53 mutant proteins, restoring sequence-specific p53 transcription (76). Therefore, therapeutically targeting these p53-mutant proteins may not only ablate their prometastasis gain-of-function capabilities, but also restore some of the antiapoptotic and antimetastasis capabilities of WT p53. The high frequency of *TP53* mutations in triple-negative and serous ovarian tumors indicates the likelihood that this mutation is a shared driving event in these cancers and, therefore, these p53-targeted therapies may hold great promise for the future.

CONCLUSIONS

Loss of p53 is becoming increasingly appreciated as an important event in metastasis. In general, p53 loss seems to contribute to loosening of cell–cell junctions and disruption of epithelial cell integrity, contributing to the dissemination of cells from solid tumors. An abundance of *in vitro* and cellular evidence suggests that p53 does indeed contribute to at least some stages of the metastatic cascade (Fig. 1). However, the results of most *in vivo* studies indicate that these events alone are insufficient to generate invasive or metastatic tumors. In addition, mutant p53, through its gain-of-function activities, induces the metastatic phenotype both *in vitro* and *in vivo*.

Both loss of WT p53 and expression of mutant forms of p53 are associated with metastasis regulation. Distinguishing between tumors with loss of p53 and those with a mutated gain-of-function phenotype may help predict tumor behavior and aid clinicians in their prognostic, diagnostic, and treatment decisions. Tumors with loss of p53 may be more likely to acquire the ability to metastasize stochastically through faster growth, evolution, and the consequent acquisition of mutations and gene expression changes that facilitate metastasis, whereas tumors expressing p53 gain-of-function mutants may metastasize as a direct consequence of the mutation. In the future, it may be possible to restore the WT p53 pathway by therapeutically targeting mutant p53 proteins in tumors with heterozygous *TP53* mutations. Differentiating between these two types of p53 perturbations in human cancer may therefore help clinicians provide more accurate prognoses and tailor treatment regimens.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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