p63, Sharp1, and HIFs: Master Regulators of Metastasis in Triple-Negative Breast Cancer

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Abstract

Metastasis is the most significant cause of cancer-associated morbidity and mortality but remains poorly understood. Recent work revealed that metastasis of aggressive triple-negative breast cancers is suppressed by Sharp1, a factor that promotes degradation of hypoxia-inducible factors (HIF) and blunts HIF-induced malignant cell behavior. Cancer Res; 73(16): 4978–81. ©2013 AACR.

The growth of overt metastases is the most deadly and least understood attribute of cancer (1). In an ideal scenario, markers should be used to distinguish lesions with high probability to develop clinically relevant macrometastases from those that will remain indolent, and then well-tolerated antimetastatic treatments should provide benefit in the high-risk adjuvant setting. Unfortunately, reality is very far from this ideal scenario. Cancer cell dissemination from a primary tumor occurs remarkably early in tumor development, but predicting which, if any, of these cells will generate an overt metastasis is currently impossible. As such, the clinical management of the metastatic patient remains a largely unmet challenge in oncology (2).

Metastasis is frequently described as the sequential execution of multiple steps; during the dissemination phase, cancer cells locally invade the tissues surrounding the primary tumor, travel through the vasculature, and land in distant organs. In some tumors, such as breast cancer, disseminated cells may remain dormant, yet viable, for decades. During the following colonization phase, few disseminated cells adapt to or get reprogrammed by the new microenvironment to empower the capacity to grow into secondary tumors (1). However, metastasis is also an extremely inefficient process. Out of many millions of disseminated cells, only a tiny fraction will develop, if ever, into a clinically relevant metastasis (1). The molecular nature of metastasis inefficiency is only partially understood. Intriguingly, recent work suggests that a critical limiting step for colonization is the transition from a mesenchymal to an epithelial state (reviewed in ref. 3). Moreover, specific gene products must also be in place to ensure that disseminated epithelial cells would die or become dormant in a foreign microenvironment.

The emergence of p63 as a key metastasis suppressor gene in epithelial tumors has shed light on some of these issues (4–8). Tp63 is a member of the p53 family, abundantly expressed in the basal layer of stratified epithelia, where it controls epithelial integrity, growth, or stemness potential (9). p63 comes in different flavors. The gene Tp63 can be transcribed from two promoters, giving origin to two different isoforms at the N-terminus, a longer version (named TAp63) and an amino-deleted isoform (ΔN-p63). Moreover, alternative splicing produces three additional isoforms at the C-terminus, dubbed α, β, and γ isoforms (9). We and others recently reported that TAp63α is inhibited in metastatic cells; restoration of TAp63α function impairs lamellipodia formation and TGFβ-induced migration in vitro and severely opposes metastatic dissemination of injected aggressive breast and skin cancer cells in immunocompromised mice (4, 7). The notion that TAp63 bears metastasis-suppressive properties has been also validated genetically. When combined with p53 inactivation, 90% of the tumors emerging in TAp63 knockout mice metastasized (8).

p63 activity can be effectively monitored by signatures of p63-regulated genes, such as expression of just two genes, Sharp1 and CyclinG2 (4, 5). In human breast cancer datasets, application of this "p63-minimal signature" efficiently stratifies tumors according to metastatic risk that is lower in tumors retaining higher expression of Sharp1 and CyclinG2 (4). How TAp63 is regulated in aggressive breast cancer cells remains an open question and may entail transcriptional or microRNA-dependent regulations, as well as posttranslational modifications of TAp63 proteins (9). The best-understood mechanism by which TAp63 activity can be attenuated is through mutation of p53, one of the most frequent lesions in human cancers (4, 7). Indeed, mutation of p53 does not necessarily lead to loss of p53; in contrast, hot-spot mutations hitting the p53 DNA-binding domain often cause expression of a stable, yet transcriptionally deficient mutant-p53 protein. Far from being functionally “dead,” mutant-p53 protein is endowed with new functions not present in wild-type p53 to an extent that expression of mutant-p53 is associated with poor prognosis in human tumors and to development of aggressive tumors in mouse models (10). One of these gain-of-function attributes is the capacity to form a complex with p63, limiting p63
transcriptional activity. Then why do tumors not mutate \textit{TP63} directly? We can only speculate that inactivation of p63 may favor cancer cell dissemination, but because p63 also is required for cell survival, this would doom the same cells to an early death. In contrast, \textit{p53} mutations may be more advantageous than \textit{TP63} inactivation for efficient metastasis because it would allow tumors to catch "two pigeons with the same stone." Mutant-p53 is selected in primary tumors because it increases cell growth, survival, and chemoresistance through a variety of mechanisms (10). This would waive the requirement of p63 for cell survival, while at the same time disabling p63 metastasis-suppressing effects. If proved, this hypothesis would lend support to the notion that mechanisms involved in metastasis regulation may actually not be selected for such a purpose, but actually emerge as "byproducts" of mutations and other mechanisms that confer increased fitness to the primary tumor (11).

But how does TA\textit{p63} inhibit metastasis in specific tumor subtypes? Our group focused on the role of \textit{Sharp1} as a candidate metastasis suppressor in what is considered the terr\textit{a incognita} of mammary tumors, i.e., the triple-negative breast cancer (TNBC; ref. 12). These tumors are indeed a distinct clinical entity from other mammary tumors as they are merely defined by lack of expression of estrogen receptor, progesterone receptor, and HER2. TNBC represents a riddle for oncologists, as patients with TNBC cannot be treated with endocrine therapy or other available targeted therapies (12). In addition, TNBCs are very heterogeneous, including both indolent and extremely metastatic subtypes (12). To shed light on the biology of TNBC, we validated the prognostic value of \textit{Sharp1} and \textit{CyclinG2} expression in a cohort of genetically profiled TNBC primary tumors. Next, we interrogated patient datasets for association between \textit{Sharp1}/\textit{CyclinG2} and expression of more than 250 gene sets, each denoting the activity of a specific biologic process (e.g., secretion, growth, apoptosis) or an individual signaling pathway (e.g., TGF-\beta, Wnt, Notch, BRCA; ref. 6). The idea behind this approach is the "guilt-by-association" paradigm, whereby the function of a differentially expressed gene (or gene set) may be inferred by its effects on known pathways, as visualized by changes in the levels of its target genes. Strikingly, not only did this approach confirm previous links with TGF-\beta activity and mutant-p53 status (4, 6), but it also revealed an unexpected connection with hypoxia-inducible factors (HIF).

This bioinformatic prediction was intriguing, as activation of HIFs represents a final common event in the pathogenesis of a variety of tumors (13). HIFs are transcription factors that drive the cellular adaptation to hypoxic conditions by promoting the tumor's angiogenesis and reprogramming cancer cell metabolism toward alternate metabolic pathways that do not require oxygen (14). In addition, HIFs promote drug resistance, epithelial-to-mesenchymal transition, survival, stemness, and migration (13). This multifaceted HIF transcriptional program likely contributes to compelling evidence in human cancer, namely, the association of increased levels of HIF proteins with tumor aggressiveness, relapse, metastasis, and mortality (15).
What is behind the inverse correlation between Sharp1/Cyclin62 expression and HIF activity? Strikingly, we found that Sharp1 is robustly associated with HIF-1α at the endogenous protein level in different TNBC cell lines, and functionally, Sharp1 acts as a potent HIF inhibitor in TNBC (6). Raising Sharp1 level in aggressive TNBC cells causes inhibition of HIF-dependent transcriptional activities and HIF-dependent cell migration. This correlates with the effects of Sharp1 in vivo, as primary tumors emerging from Sharp1-expressing cells displayed severely reduced local invasion and development of distant metastases than parental cells that are rescued by coexpression of HIF-1α. Thus, Sharp1 activation recapitulates some of the key metastasis-suppressive properties of TAp63 in TNBC (6). Of note, Sharp1 expression specifically requires TAp63α, as depletion of ΔNp63 has no effect on Sharp1 stability (6). The requirement of Sharp1 as an HIF inhibitor was further experimentally validated by loss-of-function assays, as siRNA-mediated depletion of Sharp1 promotes HIF-dependent transcription and HIF-driven cell migration (6).

Two lines of evidence support the role of Sharp1 as limiting factor of HIF-induced malignancy in human TNBC. First, expression of Sharp1 is inversely correlated with HIF transcriptional activity, as judged by immunohistochemistry for CAIX, a robust target of HIF; second, an unbiased genetic signature build with Sharp1-regulated genes does have prognostic value for metastasis propensity of TNBC, but this is completely contained in the prognostic value of a HIF signature (6). Collectively, these results suggest that HIF is epistatic to Sharp1 in regulating TNBC malignancy.

Of particular interest is the mechanism by which Sharp1 opposes HIF activity. HIFs are heterodimeric proteins consisting of an O2-sensitive α (1α and 2α) subunit and a stable β subunit. At low oxygen levels, HIF heterodimers activate transcription of their targets genes, whereas in normoxia, HIFs are hydroxylated by prolyl-hydroxylase (PHD) and marked for proteasomal destruction by von Hippel–Lindau (VHL)-mediated ubiquitination. However, despite the overarching relevance of O2 levels in HIF regulation, this should not be associated with ubiquitination. However, despite the overwhelming evidence that Sharp1 opposes HIF activity, the mechanism by which Sharp1 regulates HIF is not fully understood.

Intriguingly, there is also evidence suggesting that Sharp1 can affect the most critical and rate-limiting step of metastasis, if not to shrink them. It is thus critical to test whether Sharp1 can affect the most critical and rate-limiting step of metastasis, that is, the colonization phase (1). Indeed, many "antimetastatic" targets and agents have foundered in the face of clinical reality.

In a previous publication, we monitored the capacity of Sharp1 and HIF to suppress metastatic spreading (6). This translates, in the clinical setting, to a "dissemination-preventing" set up. However, at the time of diagnosis, the patient with cancer already harbors a large number of disseminated cancer cells. Thus, an aspect that deserves further investigation is whether Sharp1 might also oppose the growth of overt metastases, if not to shrink them. It is thus critical to test whether Sharp1 can affect the most critical and rate-limiting step of metastasis, that is, the colonization phase (1). Indeed, many "antimetastatic" targets and agents have foundered in the face of clinical reality.

In conclusion, these findings unveil a new mechanism to control HIF stability within TNBC cells that may be exploited for the development of new diagnostic tools as well as new drugs for primary or adjuvant therapy.

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No potential conflicts of interest were disclosed.

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