Cancer Research

Lulling the Cancer Cell into an Eternal Sleep

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A hallmark of metastasis is the ability of cancer cells to undergo the epithelial-to-mesenchymal transition to invade and disseminate to distal sites. More recently, the case has been made that the critical last step in metastasis is dependent on the ability to undergo reversion to an epithelial phenotype in a process known as the mesenchymal-to-epithelial transition (MET). It is this transition in the metastatic cascade that researchers are focusing on clinically to treat disseminated disease. Shinde and colleagues identified spleen tyrosine kinase (SYK) as a critical mediator of MET that facilitated the removal of P-bodies during autophagy. Remarkably, pharmacologic inhibition of SYK inhibited the clearance of P-bodies and autophagy in preclinical models of metastasis, arresting cancer cells in an indefinite dormant state and preventing tumor cell colonization and thus the establishment of aggressive metastatic disease.

See related article by Shinde et al., p. 1831

Although significant gains have been made in treatment of localized cancer, resulting in improved overall survival, there have been few therapeutic advances for the treatment of metastatic disease. Thus, the diagnosis of metastatic disease is still associated with poor survival. Specifically, data from the NCI Surveillance, Epidemiology, and End Results Registries (SEER) highlights that patients diagnosed with localized disease have excellent 5-year survival, whereas less than 20% of patients who have metastasis at the time of diagnosis survive after 5 years (1). Notably, these numbers have not changed between 2005 and 2015, and in some types of cancer, 5-year survival has declined, highlighting a dearth of progress in the treatment of metastatic cancers. In breast cancer specifically, there has been a little improvement in the outcomes of patients with metastatic disease over the last 30 years (1).

Nevertheless, strides have been made in understanding the mechanisms regulating metastatic disease progression, resulting in a number of potential therapeutic strategies that have shown success in the preclinical setting. Unfortunately, successful translation to the clinic has not occurred. In fact, some of the more promising preclinical leads have resulted in no significant improvements in outcome, whereas others stimulated tumor growth (1). The reasons for this discordance between preclinical and clinical data is varied and nuanced but one area of recent focus has been the limitation of the current clinical trial protocols. The design of clinical trials has been slow to evolve to capture the therapeutic potential of the approaches being modeled at the preclinical level. This disconnect is a result of confounding factors such as trial design, time of treatment, patient selection, and drug combinations that are difficult to model and test in the course of standard clinical trial designs. As our understanding of metastasis and the design of clinical trials are evolving in parallel, there is hope to better capture a drug's potential to inhibit metastatic disease (1).

Metastasis is the result of a cascade of events: seeding, dormancy, and colonization. Each of these steps presents a unique therapeutic opportunity if the underlying driver mechanisms are properly validated and therapeutically targeted. By the time of diagnosis, the seeding phase has often already occurred. Thus, the majority of treatment strategies are designed to target cells in the dormancy and colonization phases (1). This approach has been successful in preclinical models, and in this issue of *Cancer Research*, Shinde and colleagues contributed to this body of work with an elegant approach targeting processes that facilitate the transition from dormancy to colonization (2).

Specifically, the authors focused on a cancer cell's plasticity or ability to switch cell states to adapt to their surroundings and their dependency on autophagy to undergo this process. The epithelial-to-mesenchymal transition (EMT) has long been studied as an integral mechanism for the spread of metastatic disease (3). It is recognized as integral for local invasion, and dissemination of tumor cells and the mechanisms regulating it are increasingly well understood. Recently, it has become clear that the ability of a given cancer cell to transition back or undergo the mesenchymal-to-epithelial transition (MET) may confer a greater propensity for that cell to emerge from dormancy and colonize distal sites (4, 5). An integral component of MET is a cell's epithelial to mesenchymal plasticity (EMP). Shinde and colleagues provided further evidence that MET and EMP are required for cancer cell metastasis–elucidated mechanisms governing this plasticity. From these findings, they proposed a novel therapeutic strategy with notable preclinical success in breast cancer models of metastasis presented.

Their work began with the validation of a well-described regulator of EMT and metastasis, TGFβ. TGFβ is a cytokine that regulates proliferation and differentiation of cells via signaling through the SMAD family of transcription factors. In normal cells, TGFβ acts as a tumor suppressor but it has been well described to give cell oncogenic properties in the setting of more aggressive cancers as a result of changes in the tumor microenvironment, notably through interactions with integrins and growth factors, something researchers within this field deem the "TGFβ paradox" (3). Upon oncogenic stimulation, TGFβ signaling induces both transcriptional changes and the expression of

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doi: 10.1158/0008-5472.CAN-19-0853

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specific miRNAs in the tumor cell that promote events associated with EMT such as cell migration and invasion (3). These properties have led many researchers to pursue TGFβ as a drug target for patients with metastatic cancer and successes in preclinical studies have resulted in early-stage clinical trials. In a previous study, the authors elegantly added to this body of work, demonstrating that a model of metastasis induced by exposure to TGFβ is capable of undergoing MET upon withdrawal of the TGFβ stimulus (4). In this study, the authors suggest that it is this capacity to undergo MET and a cell's EMP that is critical for metastatic progression, building on the burgeoning suspicion within the field that this reversible plasticity is necessary for the progression of disease at metastatic sites.

Previous work showed that this plasticity may come from the accumulation of RNA-processing complexes called P-bodies that were necessary for EMT and that autophagy-mediated removal of P-bodies facilitated MET (6). In this previous study, the authors demonstrated that P-body accumulation was stimulated by TGFβ. In their new research, Shinde and colleagues demonstrate that a critical mediator of P-body removal, and thus MET, is spleen tyrosine kinase (SYK). Much like the paradox that exists for TGFβ signaling, SYK and autophagy have been described to be both tumor suppressive and oncogenic, depending on the cellular context (7, 8). Thus, it follows that perhaps the three are linked in their ability to drive metastasis and to that end, Shinde and colleagues demonstrate that a cell's capacity to undergo the MET, and thus successful colonization and metastasis, was indeed dependent on autophagy and specifically SYK-mediated clearance of P-bodies during autophagy.

The authors used a highly sensitive peptide substrate array to identify SYK as a critical mediator of this process. They highlight that SYK was downregulated at the transcriptional level in the context of TGFβ-induced EMT, but that the remaining pool remained more active. They employed another novel and highly sensitive approach, a qPCR-based kinase array, to confirm this observation. More generally, these complementary approaches should prove quite useful to the field when seeking to confirm the often observed disconnect between protein expression and functional activity of proteins in the cell. Upon this observation, the authors inhibited SYK using a combination of chemical and genetic tools. This work validated SYK's critical role in the clearance of P-bodies and found that its inhibition resulted in the accumulation of P-bodies and the inhibition of autophagy and MET. The authors used a clinically approved SYK kinase inhibitor, R406 or fostasmatinib to demonstrate this in both *in vitro* and *in vivo* model systems. Notably, SYK inhibition did not inhibit metastasis by inducing apoptosis but rather by maintaining cells that have undergone EMT in a dormant, mesenchymal state.

Throughout their work, Shinde and colleagues emphasized the idea that it was the maintenance of mesenchymal cells in their dormant state versus their attempted eradication that made this work novel and potentially therapeutically beneficial and viable. Metastatic disease is more often than not resistant to multiple standard-of-care agents used to treat the primary tumor (1, 9). A commonly accepted hypothesis is that these metastatic cells acquire the drug-resistant phenotype as they are reprogrammed through the EMT process. Thus, it follows that short of being able to specifically and safely target these cells for cell death while in the dormant state, maintaining this cell population in this otherwise asymptomatic state and preventing colonization is a viable therapeutic strategy. The authors demonstrated this successfully in preclinical models with a kinase inhibitor that is, notably, clinically approved (10). This may help accelerate the translation of these findings to the clinic for the treatment of metastatic cancers. Specifically, in breast cancer, where cells are notably dormant for extended periods of time, this treatment strategy may represent a new form of long-term treatment to prevent late recurrence, a common clinical problem for patients with breast cancer. In addition, fostamatinib may be an ideal candidate for translation as it is already approved for the long-term treatment of rheumatoid arthritis and has a low toxicity profile. However, not discussed within this paper is the potential for dormant cells to acquire mechanisms to overcome this selective pressure over time and successfully reengage metastatic programs over time. Only time will tell whether this type of preventative approach is indeed successful, but Shinde and colleagues certainly demonstrate that with proper clinical trial design and collaboration, it is an approach worth studying.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Received March 13, 2019; revised March 13, 2019; accepted March 14, 2019; published first April 15, 2019.

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