Metastasis

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MALAT1 protects dormant tumor cells from immune elimination

Zhibin Zhang & Judy Lieberman

Cancer cells seed distant tissues and remain dormant before re-entering the cell cycle to form metastases. How tumor dormancy is maintained and how cells exit dormancy is poorly understood. A study shows that the lncRNA *MALAT1* reactivates dormant cancer cells by upregulating serpin protease inhibitors in tumor cells to evade CD8$^+$ T cells.

Metastasis that can arise even decades after surgical resection of primary tumors is the leading cause of cancer-related death. To improve treatment, understanding the features of the tumor cell and the tumor microenvironment (TME) that initially maintain dormancy and then allow cancer cells to proliferate and establish macrometastases is critical[1]. *MALAT1*, an abundant, evolutionarily conserved, ~7–8-kb long noncoding RNA (lncRNA) that localizes to nuclear speckles and is overexpressed in cancer cells, has long been linked to poor prognosis and metastasis in multiple types of cancer. In nuclear speckles, *MALAT1* binds to the speckle proteins nucleolin and nucleophosmin and acts as a regulator of transcription and splicing, although mechanistic understanding of this process is still lacking[2]. Despite many studies that have associated the expression of *MALAT1* in human tumor cells with proliferation, migration and metastasis, whether *MALAT1* promotes or suppresses metastasis is still a matter of debate because genetic ablation of *Malat1* in mice suppresses metastasis in a genetically engineered mouse model of breast cancer[2–4], an inconsistency that might be explained by a function of *Malat1* in non-tumor cells.

The authors of a paper in the current issue of *Nature Cancer* had previously identified a region of *Malat1* (bases 1040–2137) in a mouse genetic screen to uncover mediators of metastatic reactivation in breast cancer[5]. In the current study, Kumar et al.[6] found that genetic deletion of *Malat1* in mouse 4T1 triple-negative breast cancer completely blocked lung metastasis after intravenous tumor inoculation. By contrast, the deletion had no effect on in vitro proliferation, migration or invasion, suggesting that it may instead affect the TME. In fact, *Malat1*-proficient and -deficient breast tumors grew identically in athymic nude mice lacking T cells, pointing toward a role for T cells.

However, *Malat1* expression also increased mammosphere formation by 4T1 cells, and knockout or knockdown of *Malat1* blocked both in vivo primary tumorigenesis and spontaneous metastasis. The authors confirmed that a similar enhancement of sphere formation, primary tumor initiation and metastasis occurred in mouse colorectal (CT26), lung adenocarcinoma (LLC) and melanoma (B16F10) cell lines. These results indicated that *Malat1* expression mediates both tumor-cell-intrinsic metastasis-initiating properties and tumor-cell-extrinsic T cell control of latent tumors. Consistent with this, transcriptome sequencing (RNA-seq) of *Malat1*-proficient and -deficient 4T1 cells showed that *Malat1*-expressing cells were enriched for genes that act in WNT–β-catenin signaling (linked to cancer stem cell properties) as well as in inflammatory cytokine and chemokine signaling (linked to tumor immunity).

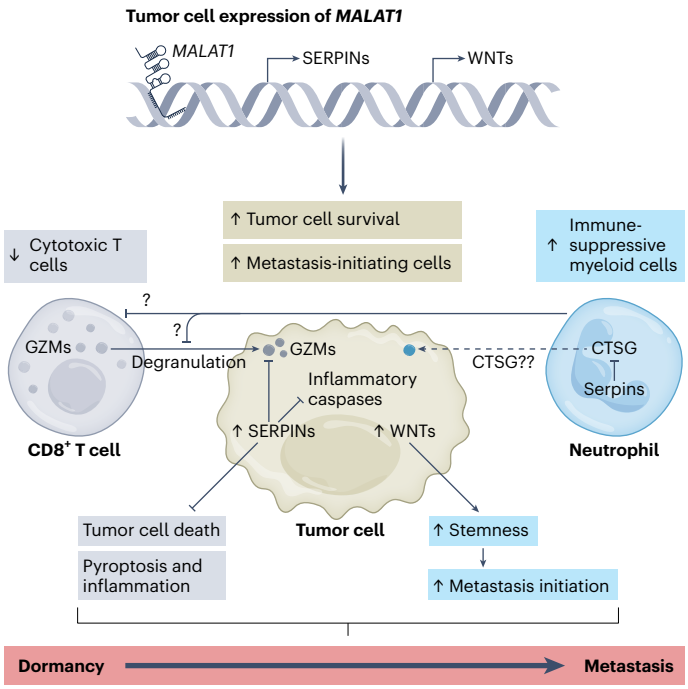

Fig. 1 | *MALAT1* upregulates serpins to block CD8$^+$ T cell-triggered death to promote breast cancer cell metastasis. *MALAT1* increases the expression of WNTs and serpins in metastatic tumor cells. WNTs promote stemness of tumor cells, key to initiating metastasis, and serpins block CD8$^+$ T cell killing of tumor cells. Serpin proteases inhibit the activity of neutrophil serine proteases, including cathepsin G (CTSG), killer cell death-inducing granzymes (GZMs) and inflammatory caspases, which activate pyroptosis and other forms of programmed cell death.

Next, the authors profiled the TME and found that the lungs of mice inoculated with *Malat1*-knockout 4T1 cells had many tumor-associated CD8$^+$ T cells and few neutrophils as compared to *Malat1*-expressing tumors. Furthermore, CD8$^+$ T cell depletion enabled knockout 4T1 cells to metastasize. In contrast, neutrophil depletion suppressed lung metastasis by *Malat1*-expressing 4T1 cells. These studies suggested that dormant tumor cells are actively inhibited from becoming macrometastases by CD8$^+$ T cells and that *Malat1* expression by the tumor allows dormant tumors to reactivate by suppressing CD8$^+$ T cell antitumor function, perhaps mediated at least in part by neutrophils.

Because *Malat1* functions as a transcriptional regulator binding to DNA, the authors next used chromatin isolation by RNA purification (ChIRP) and RNA-seq to identify *Malat1*-bound genes whose expression *Malat1* altered in the tumor. Although thousands of *Malat1* DNA-binding sites were found, only few of the genes involved were differentially expressed in *Malat1*-expressing cells. Prominent among the genes that *Malat1* upregulated were multiple members of two gene families: WNT and SERPIN, which encode a large family of serine and caspase protease inhibitors.

Serpin proteases are known to protect neutrophils and cytotoxic T cells from their own granule proteases (neutrophil elastase and cathepsin G, cytotoxic T cell granzymes) that could kill them if they leaked from specialized granules into the cytosol[7]. SERPINB1, which inhibits the neutrophil serine proteases, also inhibits the inflammatory caspases to prevent inflammatory cell death, called pyroptosis[8]. Pyroptosis, activated by cytotoxic T cell granzymes during cytotoxic lymphocyte attack, sensing of invasive infection or danger signals and other mechanisms, can potently suppress the growth of primary tumors[9], and consequently some tumors overexpress serpins to escape immune killing. For instance, SERPINB9, an inhibitor of granzyme B, is commonly amplified in tumors, and high SERPINB9 expression correlates with poor tumor cell killing by chimeric antigen receptor (CAR) T cells[10]. Hence the authors next probed whether this family of *MALAT1*-regulated genes might explain how *MALAT1* enables dormant tumors to resist T cell control at colonized sites.

SERPINA3G and SERPINB6B were the most highly expressed serpins downregulated by *Malat1*-knockout in 4T1 cells. Expression of *Serpinb6b*, but not *Serpina3g*, in *Malat1*-deficient 4T1 cells increased metastases after intravenous injection and conferred metastatic capability to non-metastatic 4T07 cells. SERPINB6 inhibits cathepsin G and the most abundant granzyme in mouse $CD8^+$ T cells, granzyme A[11,12]. In neutrophils, SERPINB6 blocks death by inhibiting cathepsin G cleavage of gasdermin D (GSDMD), which triggers highly inflammatory pyroptosis and netosis[12]. In fact, cells expressing SERPINB6B were resistant to $CD8^+$ T cell killing, and on the basis of these data, the authors concluded that *MALAT1* induction of SERPINB6B enables tumor cells to escape dormancy by conferring resistance to cytotoxic T cell killing.

The authors posited that SERPINB6B inhibition of cathepsin G in tumor cells is responsible for 4T1 resistance to T cell killing and metastatic capability; however, further evidence in support of that hypothesis is needed. They confirmed that 4T1 cells express GSDMD, which can be activated by either inflammatory caspases or cathepsin G, and showed that *Gsdmd* knockdown in nonmetastatic *Malat1*-deficient 4T1 cells restored their metastatic capability, supporting a role for GSDMD-dependent pyroptosis. However, it is unlikely that cathepsin G gets into tumor cells during T cell attack. The likely alternative is that granzyme A, which cytotoxic T cells deliver into target cells when they degranulate, kills dormant cells, but granzyme A protease activity and consequently cell death is blocked by *Malat1*-induced SERPINB6B. Although 4T1 cells grown in vitro only weakly express GSDME, which can be activated during CTL killing by granzyme B or caspase-3[13], one cannot rule out activation of the GSDME pathway in vivo because *Gsdme* expression could be induced in the tumor by exposure to T cell-generated interferon-γ (IFNγ)[14]. *Malat1* expression in neutrophils infiltrating the lung could also increase neutrophil half-life by inducing SERPIN6B6 and inhibiting cathepsin G-mediated neutrophil death, increasing myeloid cell suppressor cells and the risk for metastasis, as seen in *Malat1*-knockout mice.

Administration of *Malat1* antisense oligonucleotides (ASO), which knocked down both *Malat1* and *Serpinb6b* in vivo, strongly suppressed lung metastases in mice intravenously injected with 4T1 cells, increased T cells and reduced neutrophil lung infiltration, mimicking the effect of injecting *Malat1*-knockout 4T1 cells. There was, however, no significant effect of adding checkpoint inhibitors to *Malat1* ASO in this model of checkpoint-inhibitor-resistant cancer. Although the authors concluded that the therapeutic effectiveness of suppressing *Malat1* was dependent on SERPINB6B, they only showed that it was associated with reduced SERPINB6B. Nonetheless, this study identifies an interesting mechanism that disrupts tumor cell dormancy at metastatic sites and a potential strategy for preventing or treating metastatic cancer by suppressing *MALAT1* or inhibiting serpins to sensitize tumor cells to immune killing (Fig. 1).

Lastly, transcriptomic and proteomic analysis of human breast cancer databases indicated that SERPINB6 expression is increased in breast cancer and linked to poor prognosis, suggesting that the results in mice might be applicable to human breast cancer. However, because the granzyme, serpin and gasdermin variants and their substrates and activators differ somewhat between mice and humans, the precise molecular pathways in the two species might not be identical. Similarly, different serpins, granzymes or gasdermins might be important in controlling metastasis in different tumor subtypes or could be idiosyncratic to specific tumors. For example, in human tumors, granzyme A cleaves and activates GSDMB[15], a gasdermin that is not present in mice. However, the broad upregulation of serpins by *MALAT1* suggest that this lncRNA might use serpin induction as a general strategy for inducing metastasis in many tumors.

This study raises many questions. How are *MALAT1* binding sites determined and what is the mechanism behind its downstream effects on transcription and splicing? How does *MALAT1* reshape the TME and modulate the recruitment of immunosuppressive neutrophils and $CD8^+$ T cells? Because *MALAT1* has broad effects on multiple genes, it is likely that it may activate other pathways that contribute to awakening tumor dormancy. In addition, it remains unaddressed how *MALAT1* modulates neutrophil numbers and immunosuppression, how neutrophils suppress T cell immune control and whether serpin inhibition of cathepsin G and possibly other primary neutrophil proteases is key to their role in metastasis. Because *MALAT1* upregulates many other serpins, it could be interesting to explore whether this protein regulates other forms of protease-dependent cell death, including apoptosis. Although the authors interpreted tumor cell lactate dehydrogenase (LDH) release as an indicator of pyroptosis, LDH release is not specific to pyroptosis but rather is a general property of all forms of cell death in the time frame assayed in this study.

Although further mechanistic investigations are needed to clarify the underlying molecular basis of how *MALAT1* expression in human cancer cells modifies the tumor cell and immune cells that control it to allow dormant tumor cells to reawaken and form macrometastases, the current study postulates an interesting mechanism and lays the groundwork for future discoveries.

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Competing interests

The authors declare no competing interests.