RESEARCH HIGHLIGHT

Chromosomal instability drives immunosuppression and metastatic dissemination

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Chromosomal instability (CIN) contributes to tumor initiation, progression, and metastatic dissemination. In a recent issue of *Nature*, Li et al. characterized a cancer cell-heterologous mechanism through which CIN drives metastasis upon the rewiring of cGAS-STING1 signaling in cancer cells and the consequent establishment of local immunosuppression.

Chromosomal instability (CIN) accelerates the pace at which cancer cells acquire genomic alterations that foster oncogenesis.[1] CIN is indeed poorly tolerated in normal cells, but characterizes most human tumors, at least in part as moderate degrees of CIN provide developing neoplasms with a broad genomic substrate for accelerated evolution despite the existence of numerous cell-intrinsic and microenvironmental (including immunological) oncosuppressive mechanisms.[2] That said, excessive CIN levels also limit tumor progression, largely reflecting the acquisition of karyotypic configurations that are incompatible with survival by an expanding fraction of replicating cancer cells.[3] Importantly, chromosome missegregation (a common feature of CIN) is known to promote the formation of micronuclei, which are potent drivers of type I interferon (IFN) secretion and NF-κB signaling via cyclic GMP-AMP synthase (cGAS) and stimulator of interferon response cGAMP interactor 1 (STING1).[4] However, while robust, acute and ultimately resolving type I IFN responses as driven in cancer cells by a panel of therapeutic DNA-damaging agents including radiation therapy support the (re)establishment of immunosurveillance in support of immunological disease control,[5] chronic, indolent and non-resolving STING1 signaling as occurring downstream of CIN[6] or in the context of suboptimal therapeutic challenges[7] has consistently been associated with the establishment of an immunosuppressive tumor microenvironment (TME) coupled with the selection of immunoevasive malignant cell clones (including cancer stem cell-like clones) that drive disease progression and therapeutic failure.[8,9] Recent data from Li and collaborators demonstrate that CIN drives metastatic dissemination by favoring an endoplasmic reticulum (ER) stress response associated with type I IFN tachyphylaxis (desensitization) downstream of rewired cGAS-STING1 signaling, culminating with local and systemic immunoevasion.[10]

Li and collaborators set to interrogate the influence of the immune system on CIN-driven metastasis harnessing four different syngeneic mouse models of CIN^{high} neoplasms, namely, colorectal adenocarcinoma CT26, melanoma B16F10 as well as triple negative breast cancer (TNBC) 4T1 and EO771.LMB cells, some of which were optionally engineered to express kinesin family member 2B (KIF2B) or KIF2C (best known as MCAK) as a strategy to limit CIN. Moreover, all models were subjected to the CRISPR/Cas9-mediated deletion of *Cgas* or *Sting1*. These cells were assessed for their ability to form lung metastases once orthotopically transplanted or intravenously inoculated in immunocompetent syngeneic vs immunodeficient mice. Intriguingly, CIN increased metastatic potential only in immunocompetent settings, by a mechanism that depended on intact cGAS and STING1 functions, as assessed by both genetic and pharmacological approaches.[10]

Li and colleagues next explored the impact of CIN on TME composition. To this aim, primary 4T1 lesions established orthotopically in immunocompetent syngeneic BALB/c mice were collected and profiled by single-cell RNA sequencing (scRNA-seq). Of note, CIN^{high} 4T1 tumors displayed an immunosuppressive TME enriched for "M2-like" macrophages, granulocytic myeloid-derived suppressor cells (G-MDSCs) and dysfunctional T cells. Conversely, CIN^{low} 4T1 tumors as well as lesions formed by CIN^{high} *Sting1*$^{-/-}$ 4T1 cells exhibited a pronouncedly pro-inflammatory TME. Similar results were obtained by studying these neoplasms by multi-parametric flow cytometry,[10] indicating that the pro-metastatic effect of CIN involves the establishment of an immunosuppressive TME downstream of cGAS-STING1 signaling.

Such a heterologous mechanism was elucidated through ContactTracing, an analytical tool developed by the authors to map the interactions between TME components using scRNA-seq data. This elegant method analyzes effective ligand–receptor interactions by evaluating the availability of ligands released in the TME by donor cells and (1) the expression of their putative receptors on target cells, and (2) the activation of transcriptional responses in receptor-expressing target cells. By this approach, Li and co-workers identified a number of pro-metastatic and immunosuppressive factors underlying the interactions between CIN^{high} cancer cells and immune cells, including (but not limited to) apolipoprotein E (APOE), C-C motif chemokine ligand 2 (CCL2), C-X-C motif chemokine ligand 1 (CXCL1), interleukin 11 (IL11) and serine (or cysteine) peptidase inhibitor, clade E, member 2 (SERPINE2). Moreover, the unfolded protein response (UPR) downstream of ER stress turned out to be among the most differentially expressed pathways associated with CIN- and STING1-dependent ligands.[10]

Building on these observations, Li and colleagues provided several lines of evidence for a critical role for cGAS-STING1

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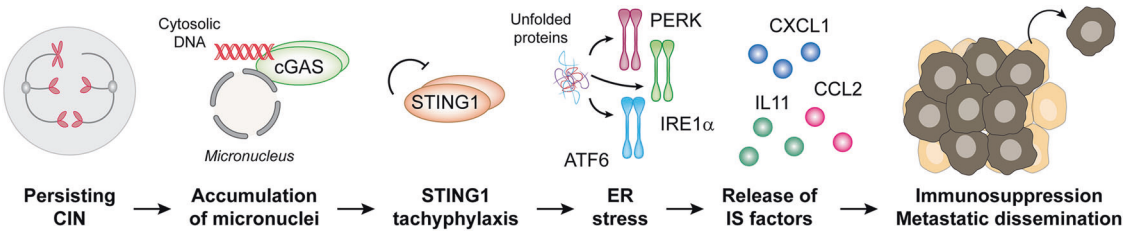

Fig. 1 CIN drives metastatic cancer dissemination through rewiring of cGAS-STING1 signaling. Persisting CIN in cancer cells results in a progressive reduction in the ability of micronuclei to drive productive type I IFN responses downstream of cGAS and STING1, a tachyphylactic response that involves ER stress and culminates with the establishment of an immunosuppressive (IS) TME that is permissive for disease progression in the context of metastatic dissemination. ATF6 activating transcription factor 6; CCL2 C-C motif chemokine ligand 2; CXCL1 C-X-C motif chemokine ligand 1; IRE1α (official name: ERN2) endoplasmic reticulum to nucleus signaling 2; IL11 interleukin 11; PERK (official name: EIF2AK3) eukaryotic translation initiation factor 2 alpha kinase 3.

signaling in the ER stress response[11] elicited during CIN-driven metastatic dissemination. First, the constitutive or pharmacological activation of cGAS-STING1 signaling in CIN^{high} cancer cells was not accompanied by the induction of a strong type I IFN response, as demonstrated by limited expression of canonical IFN-stimulated genes (ISGs). Second, genetic or pharmacological inhibition of STING1 abolished the response of CIN^{high} cancer cells to the ER stress inducer tunicamycin. Third, the genetic abrogation of the ER stress response, as imposed by the deletion of endoplasmic reticulum to nucleus signaling 2 (*Ern2*), activating transcription factor 6 (*Atf6*) or eukaryotic translation initiation factor 2 alpha kinase 3 (*Eif2ak3*), decreased the metastatic potential of CIN^{high} cancer cells when injected intravenously in immunocompetent mice. Moreover, analysis on non-immortalized IMR90 human lung fibroblasts subjected to consecutive rounds of STING1 pharmacological agonisms confirmed that prolonged cGAS-STING1 activation ultimately results in limited type I IFN activation.[10] This phenomenon, known as tachyphylaxis, was accompanied by enhanced expression of ER stress-related and NF-κB target genes and proteolytic degradation of STING1, which could all be prevented with a STING1 pharmacological inhibitor.[10]

Finally, Li and collaborators analyzed human TNBC samples by immunofluorescence microscopy, revealing an inverse correlation in the malignant cells between cGAS activation at micronuclei and STING1 levels.[10] In this setting, a cGAS^{high}STING1^{low} phenotype displayed poor lymphocytic infiltration and was associated with an unfavorable distant metastasis-free survival as compared to a cGAS^{low}STING1^{high} phenotype. In line with data obtained in the murine system, scRNA-seq data from eight human TNBC samples revealed a correlation between CIN levels (evaluated using CIN transcriptional signatures) and expression of ER stress-related (but not ISG) genes as well as an immunosuppressive orientation of the TME.[10] These data not only point to CIN as a negative prognostic factor for human TNBC, but also support the development of biomarker-driven therapeutic strategies based on the activation status of the cGAS-STING1 pathway.

Taken together, these findings support a model according to which CIN drives metastatic dissemination by promoting chronic, indolent STING1 signaling in cancer cells culminating with tachyphylaxis and ER stress (Fig. 1). Intriguingly, a signature of the so-called "epithelial-mesenchymal transition", a cell reprogramming process associated with stem-like properties,[12] was the top differentially expressed pathway identified by Li and collaborators with ContactTracing,[10] which is in line with previous findings by us and other suggesting that suboptimal type I IFN supports tumor progression at least in part upon selection of aggressive and treatment-resistant cancer stem cells. It will therefore be interesting to investigate whether stemness plays a role in CIN-driven metastatic dissemination. Despite this and other incognita, the recent findings from Li and collaborators add to a growing literature demonstrating that unsuccessful immune signaling in the TME fosters rather than restrains tumor progression.

REFERENCES

1. Bakhoum, S. F. & Cantley, L. C. *Cell* **174**, 1347–1360 (2018).
2. Vitale, I., Shema, E., Loi, S. & Galluzzi, L. *Nat. Med.* **27**, 212–224 (2021).
3. Janssen, A., Kops, G. J. & Medema, R. H. *Proc. Natl. Acad. Sci. USA* **106**, 19108–19113 (2009).
4. Vanpouille-Box, C., Demaria, S., Formenti, S. C. & Galluzzi, L. *Cancer Cell* **34**, 361–378 (2018).
5. Yamazaki, T. et al. *Nat. Immunol.* **21**, 1160–1171 (2020).
6. Bakhoum, S. F. et al. *Nature* **553**, 467–472 (2018).
7. Musella, M. et al. *Nat. Immunol.* **23**, 1379–1392 (2022).
8. Boukhaled, G. M., Harding, S. & Brooks, D. G. *Annu. Rev. Pathol.* **16**, 167–198 (2021).
9. Rodriguez-Ruiz, M. E. et al. *Oncoimmunology* **8**, e1655964 (2019).
10. Li, J. et al. *Nature* **620**, 1080–1088 (2023).
11. Chen, X. & Cubillos-Ruiz, J. R. *Nat. Rev. Cancer* **21**, 71–88 (2021).
12. Dongre, A. & Weinberg, R. A. *Nat. Rev. Mol. Cell Biol.* **20**, 69–84 (2019).

ADDITIONAL INFORMATION

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