Structural Biology

Major Finding: Mutating a conserved motif in the activation loop of MEC1 (ATR) revealed the activation mechanism.

Approach: Cryo–electron microscopy, genetic studies, and biochemical analyses were combined in this study.

Impact: Understanding the activation mechanism of ATR may enable targeting of this checkpoint protein.

CHECKPOINT KINASE MEC1 (ATR) STRUCTURE REVEALS ACTIVATION MECHANISM

The protein kinase ATR, the *Saccharomyces cerevisiae* homolog of which is MEC1, is a checkpoint protein that causes cell-cycle arrest and promotes DNA repair upon detection of DNA damage or replication fork stalling, making ATR an attractive target in cancers deficient in DNA damage repair. Using cryo–electron microscopy along with genetic and biochemical studies, Tannous, Yates, and colleagues discovered mechanisms by which MEC1 can be autoinhibited or activated. Mutating a highly conserved phenylalanine residue (F2244) in the DFD motif of MEC1's activation loop to an alanine residue or a leucine residue boosted MEC1's basal activity by approximately tenfold and twentyfold, respectively. The activity of $MEC1^{F2244L}$ was minimally affected by known activators; combined with its high basal activity, $MEC1^{F2244L}$ was therefore considered constitutively active. This lack of dependence on activators for activity enabled $MEC1^{F2244L}$ to rescue cell proliferation and DNA repair otherwise observed in MEC1 activator–deficient cells. Cryo–electron microscopy analyses of wild-type or F2244L-mutant MEC1 bound to the MEC1 partner DDC2 (the human homolog of which is ATRIP) along with the nonhydrolyzable ATP analogue AMP-PNP and Mg^{2+}—in each case forming a dimer of Mg^{2+}- and AMP-PNP–bound MEC1–DDC2 heterodimers—enabled determination of the structures of these tetrameric complexes at 3.8-Å and 2.8-Å resolution, respectively. Close inspection of the near-complete atomic models generated using the cryo–electron microscopy results combined with further experiments revealed how the activating F2244L mutation or activator binding altered the conformation of the activation loop, freeing MEC1 from autoinhibition and enabling it to exert its kinase activity. In summary, this work uncovers critical structural insights that—given the conservation of the DFD motif between MEC1 and ATR—may be exploitable for developing ATR targeted therapies for cancers with DNA-repair defects. ■

Tannous EA, Yates LA, Zhang X, Burgers PM. Mechanism of autoinhibition and activation of $Mec1^{ATR}$ checkpoint kinase. Nature 2020 Nov 9 [Epub ahead of print].

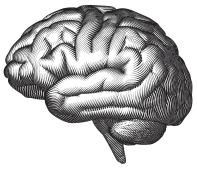
Metastasis

Major Finding: Myeloid cells of the central nervous system expressed a cytokine that suppressed antitumor immunity.

Concept: This cytokine, CXCL10, recruited PD-L1^{+}VISTA^{hi} myeloid cells that inhibited brain T-cell function.

Impact: This work elucidates the mechanism behind the metastasis-promoting qualities of brain myeloid cells.

CNS-NATIVE MYELOID CELLS EXPRESS CXCL10, PROMOTING BRAIN METASTASIS



The characteristics of the premetastatic niche—and, later, the metastatic microenvironment—play a critical role in whether disseminated tumor cells form metastases that take hold and grow, and recent evidence has suggested that central nervous system (CNS)–native myeloid cells and bone marrow–derived myeloid cells may be important mediators of metastasis in the brain. Guldner and colleagues further investigated these findings, discovering that IBA1^{+} myeloid cells could be found around and within human brain metastases of primary tumors from a variety of sites. This result was replicated in mice, in which it was confirmed that there were fewer IBA^{+} brain metastasis–associated myeloid cells in brains without metastases. Brain metastasis–associated myeloid cells exhibited a transcriptional profile unlike non–metastasis associated brain myeloid cells, with notable downregulation of the gene encoding the chemokine receptor CX3CR1, a finding confirmed in humans using publicly available datasets. In CNS-native myeloid cells, *Cx3cr1* knockout led to an interferon response and upregulation of *Cxcl10*, encoding a ligand of CX3CR1 that acts as an inflammatory cytokine. In turn, *Cx3cr1* knockout also increased the outgrowth of brain metastases in mice injected intracranially with cancer cells. Notably, *Cxcl10* expression was elevated in myeloid cells derived from human brain metastases compared with healthy human brain tissue, suggesting the relevance of these findings in patients. Mechanistically, CXCL10 recruited PD-L1^{+} CNS-native myeloid cells that also expressed the inhibitory immune checkpoint protein VISTA at high levels, leading to suppression of T-cell function that may explain the relationship between high CXCL10 expression and metastasis. Correspondingly, anti-VISTA or anti–PD-L1 treatment reversed immunosuppression in the brain and reduced brain-metastasis outgrowth. Collectively, these results identify key characteristics of the immune microenvironment that permit growth of brain metastases and suggest that targeting PD-L1 and VISTA signaling may be a useful strategy for treating these metastases. ■

Guldner IH, Wang Q, Yang L, Golomb SM, Zhao Z, Lopez JA, et al. CNS-native myeloid cells drive immune suppression in the brain metastatic niche through Cxcl10. Cell 2020;183:1234–48.E25.