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NETs unleashed: neutrophil extracellular traps boost chemotherapy against colorectal cancer

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Chemotherapy, which primarily acts on cancer cells, can influence the tumor microenvironment and the recruitment and behavior of stromal cells. In this issue of the JCI, Li et al. explored the potent anticancer effect of the combination of a glutaminase inhibitor (CB-839) and 5-FU against PIK3CA-mutant colorectal cancer tumors. This chemotherapy treatment strongly induced the recruitment of neutrophils that formed neutrophil extracellular traps in cancer, which actively killed cancer cells by inducing apoptosis. This study substantially advances our understanding of the multifaceted role of neutrophils and NETs in the outcome of anticancer treatment.

CB-839 and 5-FU combination shrinks PIK3CA-mutant colorectal cancer tumors
The tumor microenvironment (TME) is now widely recognized as a crucial factor influencing the efficacy of chemotherapy. When chemotherapy is effective, it induces cell damage and death, which substantially impacts the TME, including the recruitment and behavior of inflammatory cells. High neutrophil infiltration is commonly linked to a poor response to chemotherapy in various human cancer types (1). However, exceptions have been observed in colorectal, gastric, and high-grade ovarian cancers, where increased neutrophil levels are associated with a better response to chemotherapy (2, 3). In this issue of the JCI, Li and colleagues focused on the impact of chemotherapy on colorectal cancer (CRC), specifically those cases with PIK3CA mutations, which account for 30% of CRC cases (4). The authors had previously demonstrated that PIK3CA mutations make CRC more dependent on glutamine and therefore the combination of a glutaminase inhibitor (CB-839) and 5-FU leads to notable regression of PIK3CA-mutant CRC in xenograft nude mouse models (5, 6). Building on this foundation, Li and colleagues aimed to investigate the cellular and molecular mechanisms underlying the potent anticancer effect of this drug combination, referred to as the combo drug (4).

Neutrophil extracellular traps modulate CB-839/5-FU combo antitumor effects
Focusing on innate immune cells, the authors showed that while neither macrophage nor NK cell depletion influenced chemotherapy response, neutrophil depletion blocked the efficacy of the combo drug on multiple CRC xenograft tumor models. Indeed, following the combo drug treatment, neutrophils were massively recruited into tumors and formed neutrophil extracellular traps (NETs). NETs are scaffolds of DNA with several cytotoxic enzymes and proteases that are released by neutrophils, and NETs have recently emerged as strong modulator of anticancer treatment (7). Even though NETs were previously described to protect cancer cells from chemotherapy and radiotherapy (8–10), Li, et al. established that NET digestion with DNase I strongly inhibited the combo drug efficacy in their models. The authors next investigated the molecular mechanisms involved. Interestingly, PIK3CA-WT tumors had much less infiltration of NET-forming neutrophils following the combo drug treatment. Taking advantages of these differences, the authors performed RNA-Seq on PIK3CA WT and mutant tumors and identified an enrichment of the neutrophil chemoattractant IL-8 in PIK3CA-mutant cancer cells. Even though IL-8 is specific to humans, it can also attract mouse neutrophils (11). Accordingly, KO of IL-8 in different CRC cell lines attenuated neutrophil recruitment and NET formation and counteracted the combo drug efficacy (Figure 1). In addition, the authors were able to show that the induction of IL-8 transcription following treatment with the combo drug was mediated by the transcriptional factor Nuclear factor (erythroid-derived 2)-like 2 (NRF2), which bound to the promoter region of the IL-8 gene. The authors also identified that the combo drug directly induced NETs, which was dependent on ROS production (Figure 1).

NET-associated Cathepsin G induces cancer cell apoptosis
To gain insight into how NETs participate in the shrinkage of CRC following the combo drug treatment, the authors turned to in vitro experiments and demonstrated that NETs, induced by the combo drug, directly induced the apoptosis of cancer cells. Similarly, the combo drug induced apoptosis in vivo, a process that was diminished following neutrophil depletion or DNase I digestion of NETs. The authors thus proposed that NETs may directly contribute to CRC cell death by inducing apoptosis. NETs are associated with granular proteins, including several proteases like Neutrophil elastase, Matrix MetalloProteinase 9, and Cathepsin G (CG) (12, 13). The authors first demon-
The dual role of neutrophils in chemotherapy outcomes raises new questions. While another study recently described that chemotherapy-induced IL-1β triggers NETs, which, in turn, promote chemoresistance against breast cancer lung metastasis (8), Li et al., demonstrate that the combo drug directly induces NETs to enhance chemotherapy efficacy against PI3KCA-mutated CRC. These two processes not only differ in triggering mechanisms but also in downstream effects. The first associates NETs with TGF-β activation, leading to epithelial to mesenchymal transition (EMT) and chemoresistance, while the second highlights the involvement of NETs in the direct promotion of cancer cell apoptosis via NET-associated CG and its effects on 14-3-3 protein degradation. In addition, different types of chemotherapy that were used in these study (e.g., AC chemotherapy for breast cancer versus the combo drug for CRC) might be responsible for the differences observed. These findings also challenge the belief that NETs primarily exert antiapoptotic effects (16), prompting critical questions about contextual determinants for NET behavior in cancer. Differences between “chemoresistant NETs” and “proapoptotic NETs” may rely on various factors, including NET-inducing agents, NET types, or targeted cells. Li et al. suggest that NETs induced by the combo drug differ from PMA-induced NETs, indicating the existence of distinct NET subtypes or compositions. Considering this heterogeneity, targeting specific NET types could provide a nuanced therapeutic approach. The mutational landscape of cancer may also regulate neutrophils’ different roles in chemotherapy responses.
The combo drug induces NETs specifically in PI3KCA-mutated CRC, suggesting a mutational influence. However, assessing whether these NETs enhance apoptosis of WT cells and exploring the regulation of the cell surface receptor RAGE by PI3KCA mutation remains unexplored.

The authors propose that the binding of NETs to CRC cells is crucial for preventing the washout of CG in the TME. Interestingly, Li and colleagues reveal that coiled-coil domain containing protein 25 (CCDC25), a previously described NET receptor (17), is not involved in NET-induced apoptosis, suggesting the involvement of possible alternative binding mechanisms. Indeed, unraveling the mechanisms by which NETs bind to cancer cells could lead to a more specific understanding of the cancer cell response to NETs. Notably, recent findings indicate that NETs can trap doxorubicin, therefore limiting chemotherapy efficacy (10) and that NETs can shield cancer cells from cytotoxicity mediated by CD8\(^+\) T cell natural killer (NK) cells (11). Consequentially, evaluating whether blood neutrophilia in patients correlates with heightened neutrophil recruitment in tumor tissue becomes a critical consideration.

While chemotherapy is conventionally associated with neutropenia, the preclinical models in Li, et al. (4) did not exhibit neutropenia. Instead, chemotherapy resulted in a substantial recruitment of neutrophils in the tumor tissue, a phenomenon observed by other research groups (7, 8). This outcome implicates that the recruitment of neutrophils to tumor tissues may contribute to what is traditionally labeled as neutropenia in the bloodstream. Consequently, evaluating whether blood neutrophilia in patients correlates with heightened neutrophil recruitment in tumor tissue becomes a critical consideration.

The absence of objective responses in patients treated with the combination drug prompts crucial questions. Despite the positive link between increased NET levels and extended progression-free survival in posttreatment tumor biopsies, understanding the reasons behind this lack of objective responses is essential. The complexities involved in translating promising preclinical results into successful clinical outcomes highlight the need to consider factors such as patient heterogeneity, tumor-specific characteristics, and sample sizes. Altogether, this elegant work by Li, et al. (4) not only elucidates the intricate interplay between NETs and PI3KCA-mutated CRC but also highlights the context-dependent nature of these interactions in shaping the response to chemotherapy.

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