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POSTER ABSTRACTS

651.MULTIPLE MYELOMA AND PLASMA CELL DYSCRASIAS: BASIC AND TRANSLATIONAL

Disruption of DNA-PK-Mediated Cgas Retention on Damaged Chromatin Potentiates Doxorubicin-Induced Cgas/Sting-Dependent Anti-Multiple Myeloma Activity

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Multiple myeloma (MM) is an incurable plasma cell malignancy characterized by genomic instability. MM with multidrug resistance or extensive extramedullary plasmacytoma are difficult to manage and often treated with DNA damage-inducing chemotherapies, including topoisomerase II inhibitor doxorubicin. However, the efficacy of this treatment is still limited and requires further improvement. Targeting DNA damage response (DDR) may offer an opportunity for effective treatment of MM. In particular, in combination with DNA damage-inducing agents, this strategy has shown to improve chemo-radiotherapies in part by cGAS-STING-mediated innate immune response, which could be directly activated by an elevated level of cytosolic DNA due to DNA repair inhibition. However, as cGAS is primarily sequestered by chromatin in the nucleus, it remains unclear how cGAS is released from chromatin and translocated to the cytoplasm upon DNA damage, leading to cGAS-STING activation.

Here, after testing the potential of several DDR inhibitors in sensitizing MM cells (MMCs) to doxorubicin, we found that inhibition of DNA-dependent protein kinase catalytic subunit (DNA-PKcs), a key component of classical non-homologous end joining (c-NHEJ) repair, using DNA-PKcs inhibitor NU7441, synergistically enhanced doxorubicin-induced anti-MM effect by activation of cGAS-STING pathway, the combination index values at different combination doses were all less than 0.85. Knocking out either *cGAS* or *STING* in ARP-1 cells significantly reduced not only the transcription of *IFNB1* and multiple *ISGs* but also the proportion of NU7441/doxorubicin-induced apoptotic cells. Stably overexpressing *STING* in U266 and RPMI-8226 cells significant upregulated the *IFNB1* and *ISGs* levels, and made MMCs more susceptible to NU7441/doxorubicin-induced inhibition of cell proliferation. Despite a strong type I interferon (IFN) response induced by activation of cGAS-STING, apoptosis of MMCs caused by activated cGAS-STING was mediated in part by IRF3-NOXA-BCL2 axis, which is independent of type I IFN response. In addition to inducing intrinsic apoptosis of MMCs, activation of cGAS-STING signaling by NU7441/doxorubicin in MMCs also induced M1 polarization of macrophages (Mφs), which increased M1 marker CD86 of Mφs but decreased M2 marker CD163 and CD206, and reduced the IL-10 concentration secreted by Mφs, thus suppressing tumor-protecting potential of M2-like Mφs and improving bortezomib-induced apoptosis of MMCs.

Mechanistic studies showed that the activation of cGAS-STING signaling was not due to the defective c-NHEJ following continuous DNA-PKcs inhibition, because NU7441 did not enhance the damaged DNA accumulation after doxorubicin induction detected by γH2AX immunoblot analysis and alkaline comet assay. Besides, deletion of *XRCC4* in ARP-1 cells to directly impair c-NHEJ did not enhance doxorubicin-induced activation of cGAS-STING signaling, further excluding a role for impaired c-NHEJ. Instead, we found that cGAS was mainly localized in the nucleus and chromatin-bound in MMCs under mock treatment, and doxorubicin induced DNA damage and mobilized inactive cGAS into cytoplasm. Both mass spectrometry data and immunoblotting of cGAS immunocoprecipitates showed that cytoplasmic cGAS was associated with DNA-PK complex and nucleosomal histone H2A-H2B heterodimer, and DNA-PKcs inhibition promoted cGAS release from cytoplasmic chromatin fragments and increased the amount of free cytosolic cGAS and DNA, activating cGAS-STING. Disruption of cytoplasmic cGAS-nucleosome binding by mutating cGAS R236 or R255 sites to E abolished the effect of NU7441 on doxorubicin-induced activation of cGAS-STING signaling.

Analysis of Datasets MMRF CoMMpass and GSE2658 showed that MM patients with higher *DNA-PKcs* expression showed poorer prognosis than those with lower expression. Additionally, the *DNA-PKcs* expression was elevated with MM disease

progression according to Datasets GSE5900, GSE13591 and MMRF CoMMpass, these indicated a possible involvement of DNA-PKcs in MM progression and provided a basis for targeting DNA-PKcs in MM treatment.
Taken together, our study suggests that DNA-PKcs may help maintain the cGAS sequestration in damaged chromatin. Inhibition of DNA-PKcs may consequently disrupt this sequestration, inducing activation of cGAS-STING signaling and improving the efficacy of doxorubicin in treating MM.

Disclosures No relevant conflicts of interest to declare.

Figure 1. The model depicting the molecular mechanism by which DNA-PKcs inhibition, in combination with doxorubicin, activates the cGAS-STING pathway in MM cells, thus evoking anti-MM effects

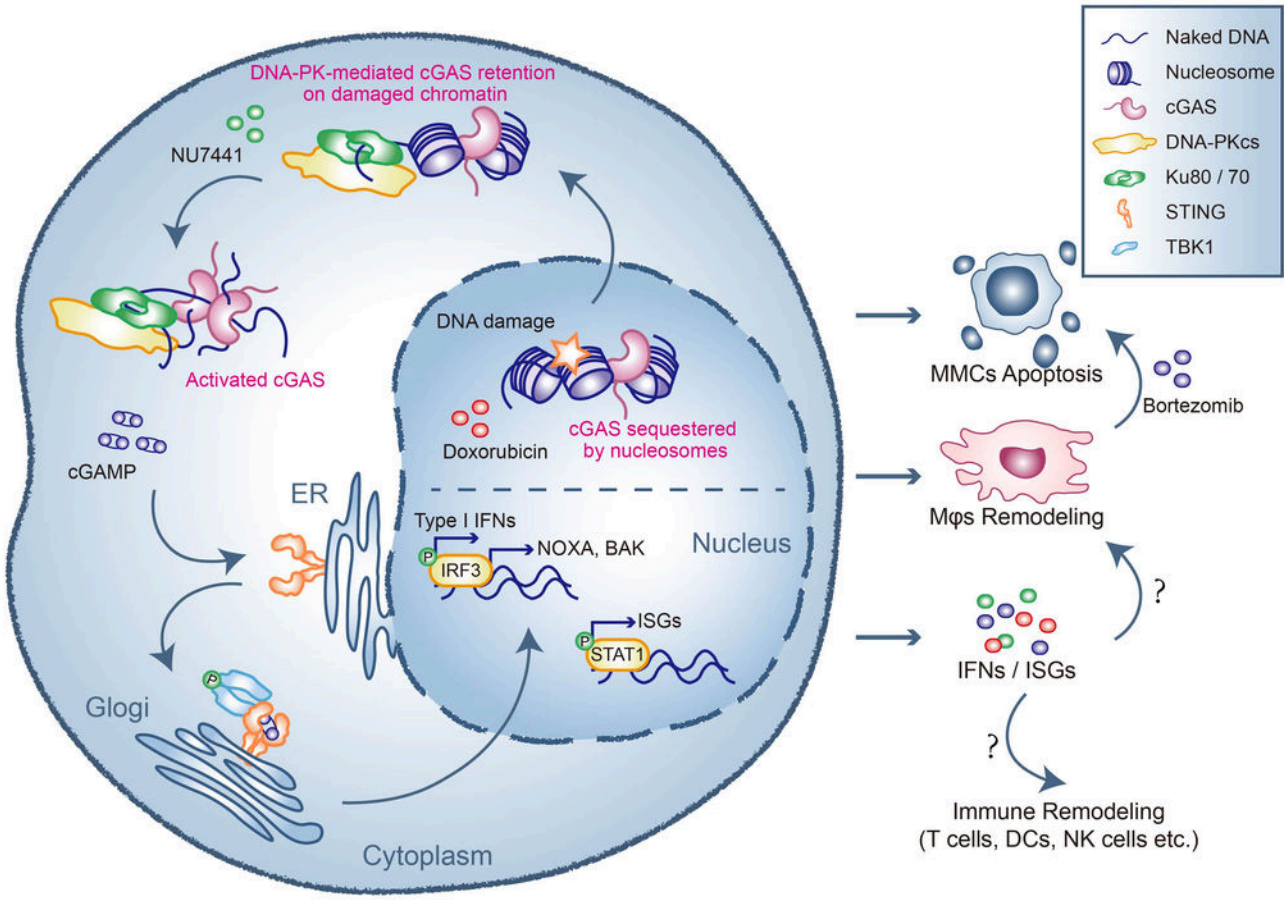

Figure 1

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