IMMATURE NEUTROPHILS INHIBIT ANTI-TUMOR IMMUNITY AND IMPEDE CHECKPOINT BLOCKADE THERAPY IN BONE METASTASES

1Tao Shi*, 2Kaijie Liang, 2Yipeng Zhang, 2Xiaoyu Zhou, 2Baorui Liu, 2Jia Wei. 1Nanjing University Medical School Affiliated Drum Tower Hospital, Nanjing, China; 2The Comprehensive Cancer Centre of Nanjing Drum Tower Hospital, Affiliated Hospital of Medical School, Nanjing University; Clinical Cancer Institute of Nanjing University, Nanjing, China

Background Despite the important breakthroughs of immune checkpoint blockade (ICB) therapy in multiple primary cancers, responses of ICB therapy in bone metastases remain extremely poor, largely due to the highly immunosuppressive bone metastasis microenvironment. Tumor-associated neutrophils have recently been reported to have critical impact in the responses of cancer immunotherapies. However, the role of neutrophils within bone metastasis microenvironment is still unknown. Here, we aim to explore the impact of immature neutrophils during bone metastasis progression, and assess immunotherapies targeting neutrophils to improve ICB therapy in bone metastases.

Methods RNA-seq and mass-spectrum data of bone metastasis samples from publicly available databases and our center were obtained. Mouse bone metastasis models from gastric, breast and lung cancer were established, and in vitro treatment efficacy of DKK1 and/or PD-1 blockade was evaluated by micro-computed tomography (CT), micro-magnetic resonance imaging (MRI) and immunohistochemistry (IHC) staining. Impacts of DKK1 on the bone-tumor microenvironment were explored via in vivo immune analysis. The impact of DKK1 on the maturation, function and phenotype of neutrophils was analyzed via in vitro co-culture models.

Results The proportion of neutrophils within bone metastasis microenvironment of breast cancer model was gradually increased, and neutrophils gradually became immature (Ly6Ghigh, CD101-, CXCR2-, CXCR4+) during bone metastasis progression, with immature neutrophils occupied the most proportion (80%) at day 14. Meanwhile, analysis of bone metastasis samples from patients identified that DKK1 expression was significantly up-regulated in both mRNA and protein levels. DKK1 directly induced neutrophils to become immature in vitro, and immature neutrophils induced by DKK1 exhibited strong immunosuppressive capacity to inhibit the tumor-killing ability of CD8+ T cells in vitro co-culture models. Next, using multiple bone metastasis mouse models, we observed that DKK1 blockade with murine DKK1 antibody (mDKKN-01) significantly controlled the tumor burden of bone metastases and alleviated bone destruction. Results of flow cytometry and immunofluorescence staining on bone metastasis samples demonstrated immature neutrophils were effectively reversed, and both innate and adaptive anti-tumor immunity were improved after DKK1 blockade. Finally, reversion of immature neutrophils by DKK1 blockade remarkably enhanced the efficacy of PD-1 blockade in bone metastases, and complete response (CR) was observed with combined blockade of DKK1 and PD-1.

Conclusions Our study provides novel insights into the critical impact of the immature neutrophils in the immunosuppressive bone metastasis microenvironment. DKK1 is a potential immunotherapeutic target for bone metastases, and dual blockade of DKK1 and PD-1 is a promising combination immunotherapy strategy for patients with bone metastasis.

Ethics Approval Collection and analysis of tumor samples were approved by the Ethics Committee of Nanjing University Medical School Affiliated Drum Tower Hospital (2021–324-01). All animal experiments were approved by the Institutional Animal Care and Use Committee of Drum Tower Hospital (2020AE01064).

Consent Written informed consent was obtained from the patient for publication of this abstract and any accompanying images. A copy of the written consent is available for review by the Editor of this journal.

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