Promoting Cancer Stemness and Metastasis in Osteosarcoma: Role of Advanced Glycation End-Products and its Receptors

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INTRODUCTION: Osteosarcoma is an extremely aggressive bone cancer with poor prognosis. Osteosarcoma cells are highly mobile and have ability to invade and spread to other organs and tissues, such as the lungs, making it difficult to treat. One of the reasons for the poor prognosis of osteosarcoma is the presence of cancer stem cells (CSCs) within the tumor. Targeting CSCs has been suggested to be a promising strategy to prevent or inhibit metastasis in osteosarcoma. Nε-(1-Carboxymethyl)-L-lysine (CML), an advanced glycation end-product (AGE), has been implicated in age-related diseases, such as diabetes, atherosclerosis, and Alzheimer's disease. CML is believed to play a role in the development and progression of cancers. CML may promote cancer cell proliferation, invasion, and survival by binding to and activating the receptor for AGE (RAGE) signaling. The role CML-RAGE axis in osteosarcoma progression remains unclear. Here, we investigated the impacts of CML on migration, invasion, and stemness of osteosarcoma and the molecular mechanism involved using methods of in vitro, in vivo, and human tumor samples.

METHODS:

A commercial human osteosarcoma tissue microarray [40 samples with stage I, II, and IV; median age (range) of patients: 26 (11-64)] was used to test the expression of CML and RAGE. Human osteosarcoma cell lines MG63 and U2OS cells were cultured. The concentrations at 5-50 μM of CML were used for in vitro experiments. Male non-obese diabetic (NOD)/severe combined immunodeficiency (SCID) mice with or without streptozotocin (STZ)-induced hyperglycemia with high AGE/CML levels were used for in vivo tumor xenograft experiments (n=6/group) (IACUC approval has been obtained).

RESULTS: Both CML and RAGE were highly expressed in human osteosarcoma tissues with advanced stage from human tumor tissue microarray staining (Figure 1A, representative images; Figure 1B, IHC positive staining % for stage I, II, and IV, $p<0.0001$, $n=40$). In cell model, two osteosarcoma cell lines (MG63 and U2OS) treated with CML increased the formation of tumor spheres (Figure 1C, MG63 cells, a 2.3-fold increase at 25 μM; a 2.7-fold increase at 50 μM, $p<0.05$, $n\geq3$) and the protein expression of cancer stemness markers (Figure 1D, representative blots; MG63 and U2OS cells, quantification by densitometry, $p<0.05$, $n\geq3$). Additionally, CML treatment induced migration and invasion (Figure 1E, representative images; MG63 and U2OS cells, the 1.25-2.0-fold increases at both 25 and 50 μM, $p<0.05$, $n\geq3$), as well as epithelial-mesenchymal transition (EMT) process (Figure 1F, representative blots; MG63 and U2OS cells, quantification by densitometry, $p<0.05$, $n\geq3$) in osteosarcoma cells. CML treatment activated the RAGE expression and downstream signaling pathways such as NF-κB (Figure 1G, representative blots; MG63 and U2OS cells, quantification by densitometry, $p<0.05$, $n\geq3$). Pretreatment with a neutralizing RAGE antibody blocked CML-induced NF-κB activation and stemness characteristics ($p<0.05$, $n\geq3$), indicating that CML promotes osteosarcoma cell migration, invasion, and stemness through RAGE-mediated NF-κB signaling. In a mouse model of tumor xenograft combined with STZ-induced high CML level for osteosarcoma metastasis, the subcutaneous tumor growth was not affected, but the number of nodules in the lungs (Figure 1H, representative photos; Figure 1I, nodule count, a 5-fold increase in STZ group, $p<0.05$, $n=6$) and the CML levels in the bloods and lungs ($p<0.05$, $n=6$) were significantly increased in STZ-treated mice compared to the control mice.

DISCUSSION AND CONCLUSION:

One of the reasons for the poor prognosis of osteosarcoma is the presence of CSCs within the tumor. CML may promote cancer cell growth, migration, and stemness, although the underlying mechanism remains unclear. RAGE activation in cancer cells promotes cytokine and growth factor production, tumor growth, angiogenesis, and survival, triggers various signaling pathways and is involved in metastasis. The role CML-RAGE axis in cancer stemness of osteosarcoma remains unclear. In this study, our findings demonstrated the metastatic ability of osteosarcoma cells could be enhanced through the upregulation of the CML-RAGE axis. CML treatment induced the EMT process and activated the RAGE-mediated NF-κB signaling. These findings reveal that the CML-RAGE axis plays a significant role in promoting stemness and metastasis of osteosarcoma cells. The CML-RAGE axis may be a potential therapeutic target for osteosarcoma progression.

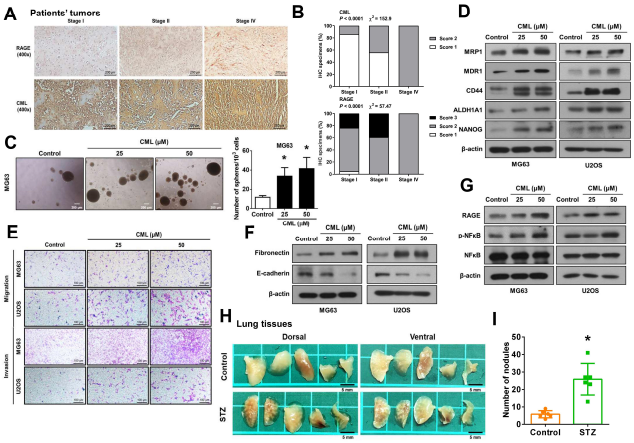

Figure 1. Advanced glycation end-product (AGE)-Receptor for AGE (RAGE) axis contributes to the malignancy of osteosarcoma cells via activation of cancer stemness characteristics. (A,B) The CML and RAGE stains in patients' tumor tissues were shown. (C-G) The in vitro sphere formation (C) and cancer stem cell marker expression (D), migration and transwell invasion (E), EMT markers (F), and RAGE/NFκB expression (G) were shown. (H,I) The in vivo xenograft mouse model with or without streptozotocin (STZ)-induced high CML levels for tumor lung metastasis (H) and number of nodules (I) was shown. In vitro: results are shown as mean±SD of at least three independent experiments. In vivo: Tumor nodules were counted in lung tissue. Results are shown as mean±SD (n=6 mice/group). *, $p < 0.05$, compared to the control.