

A novel treatment strategy targeting both sarcoma cells and tumor-associated macrophages in soft-tissue sarcoma

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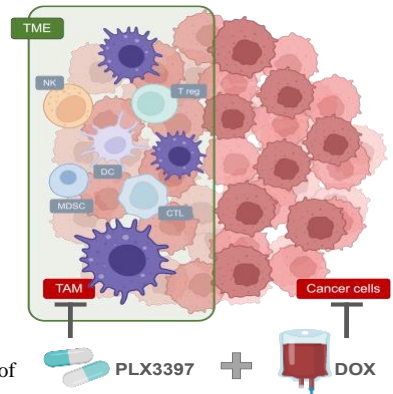
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INTRODUCTION: Drug resistance of malignant tumors are promoted by a variety of factors including cell cycle heterogeneity, anomaly microRNA expression, and aberrant expression or localization of drug transporter. Tumor-associated macrophages (TAMs), recruited by tumor-derived cytokines/chemokines including CSF-1, also promote drug resistance in major types of carcinomas. TAMs also promote angiogenesis, tumor invasion and metastasis, and immune suppression. Given the immune checkpoint inhibitors (ICI) are shown to be ineffective to bone and soft-tissue sarcomas, TAM-targeted drugs constitute currently promising therapy for sarcomas. TAM-targeted therapies appear to be promising in the preclinical trials for a variety of carcinomas. Our group previously reported the role of TAMs in osteosarcoma and confirmed the preclinical efficacy of TAM inhibition via blockade of CSF-1/CSF-1R axis. Specifically, PLX3397, a potent CSF-1R inhibitor, inhibited macrophage polarization toward M2-like phenotype, macrophage proliferation, and migration of TAMs. However, there is little evidence of TAM-targeted therapy in soft-tissue sarcomas. In this study, we explored the role of TAM in soft-tissue sarcomas and evaluated the efficacy of CSF-1R blockade in combination with conventional chemotherapy, aiming the development of novel therapeutic approach targeting both sarcoma cells and tumor microenvironment of soft-tissue sarcoma.



METHODS: A total of 19 patients with soft-tissue sarcoma, who were diagnosed and treated with neoadjuvant chemotherapy and surgical excision in Okayama University Hospital between 2006 and 2017, were investigated. Immunohistochemical staining (IHC) were performed with antibodies directed against CD168 and iNOS. CD163, CMAF, pSTAT1, CD8C and PD1. A mouse fibrosarcoma cell line (NFSa Y83) was transplanted subcutaneously into the back of 9- to 10-week-old C3H/HeJ mice at 1×10^6 cells (day 0), and four groups were evaluated: no treatment group (CONT), doxorubicin (DOX) group (4 mg/kg), PLX3397 (50mg/kg) group and DOX (4 mg/kg) + PLX3397 (50mg/kg) group. DOX was administered intraperitoneally on days 5, 12 and 19. PLX3397 was administered orally on days 5–20. Tumor was excised on day 21. The expression of CD45, F4/80, CD11b, CD80, CD206, CD3, and CD8a were evaluated using the flow cytometer (FCM), which were validated by IHC. All procedures for these experiments were approved by the Ethics Committee for Animal Experimentation in Okayama University, in accordance with the Basic Guidelines for the Conduct of Animal Experiments at Research Institutes and the university's internal regulations of Okayama University.

RESULTS: A total of 19 clinical samples were subjected to IHC biomarker analysis. The responses to the neoadjuvant chemotherapy were progressive disease (PD) in 3, stable disease (SD) in 14, and partial response (PR) in 2. The infiltration of CD163⁺ cells (M2-like macrophage) before and after chemotherapy were 6.7% and 13.5% (PD), 2.3% and 5.9% (SD), and 3.5% / 4.8% (PR), respectively. The infiltration of iNOS⁺ cells (M1-like macrophage) before and after chemotherapy were 3.1% and 1.2% (PD), 7.1% and 6.5% (SD), and 9.4% and 10.1% (PR), respectively. These results indicated that increased infiltration of M2-like macrophages and decreased infiltration of M1-like macrophages after chemotherapy are associated with poor drug response and prognosis. The mean tumor volume (mm³) in the CONTOL, DOX, PLX3397, and DOX+PLX3397 combined groups at Day 20 were 2138.4 ± 125.5 , 808.1 ± 96.3 , 1408.4 ± 182.6 , and 501.5 ± 158.9 , respectively. The tumor growth was significantly inhibited by DOX + PLX3397 treatment compared with CONTOL ($p=0.018$) and PLX3397 ($p=0.065$) treatments. The FCM analysis revealed that the mean cell percentages of pan-macrophage (CD45⁺CD11b⁺Gr-1⁺F4/80⁺ cells) were 80.6% (CONT), 80.0% (DOX), 82.1% (PLX3397), 72.3% (DOX+PLX3397), respectively. While the FCM analysis of the mean percentage of CD45⁺CD11b⁺F4/80⁺CD206⁺ cells (M2-like) were 99.5% (CONT), 99.5% (DOX), 99.6% (PLX3397), 96.3% (DOX+PLX3397), respectively, IHC revealed that the infiltration of CD163⁺ macrophages (M2-phenotype) in NFSa Y83 tumor were observed with 13.0% (CONT; range, 41 to 78 per HPF), 8.0% (DOX; range, 32 to 66 per HPF), 5.1% (PLX3387; range, 19 to 34 per HPF), 1.1% (DOX+PLX3397; range, 2 to 6 per HPF), respectively. While the FCM analysis revealed that the mean percentage of CD45⁺CD11b⁺Gr-1⁺F4/80⁺CD80⁺ cells (M1-phenotype) were 3.6% (CONT), 3.7% (DOX), 6.0% (PLX3397), 6.1% (DOX+PLX3397), respectively, IHC revealed that the infiltration rates of iNOS⁺ macrophages (M1-phenotype) were 4.1% (CONT; range, 3 to 6 per HPF), 3.8% (DOX; range, 2 to 5 per HPF), 15.1% (PLX3387; range, 11 to 17 per HPF), 21.7% (DOX+PLX3397; range, 22 to 35 per HPF), respectively. Interestingly, the mean percentages of CD8⁺ T cell (CD45⁺CD3⁺CD8⁺) were 4.8% (CONTOL), 7.9% (DOX), 4.0% (PLX3397), 14.8% (DOX+PLX3397), respectively. Overall, DOX+PLX3397 treatment showed strongest antitumor effect among the four treatment groups; the combined therapy decreased the infiltration of TAMs and increase of M1 macrophages and promoted infiltration of CD8⁺ T cells into the tumor microenvironment of soft-tissue sarcoma.

DISCUSSION: The anti-tumor effects of monotherapy of CSF-1R blockade and its combination with conventional chemotherapy for soft-tissue sarcoma were preclinically demonstrated in the study. These effects could be explained by the following findings; CSF-1R blockade decreased the infiltration of TAMs and also promoted their repolarization to M1 (anti-tumoral) phenotype from M2 (pro-tumoral). Of note, CSF-1R blockade resulted in the increased infiltration of CD8⁺ T cell, which was prominent in the tumors treated with combined therapy with conventional chemotherapy. Further investigations are warranted to clarify the molecular mechanisms underlying the efficacy of the combination therapy of CSF-1R inhibition and conventional chemotherapy.

SIGNIFICANCE/CLINICAL RELEVANCE: This study is the first to demonstrate the preclinical efficacy of combination therapy of conventional chemotherapy and TAM-targeted agent via blockade of CSF-1/CSF-1R axis against soft-tissue sarcoma. Since increased infiltration of TAMs was associated with drug resistance and poor survival outcomes in patients with soft-tissue sarcoma, this strategy targeting both sarcoma cells and tumor microenvironment of sarcoma could improve the prognosis of patients with soft-tissue sarcoma. Clinical trials of this novel therapy are awaited.