Pentraxin 3 inhibits human osteosarcoma cells metastasis by suppressing MAZ through Stat3

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INTRODUCTION:

Pentraxin 3 (PTX3), also known as TNF-inducible gene 14 protein (TSG-14), exerts pleiotropic roles in inflammation, immune responses, and a myriad of cancers, but little is known about PTX3 in human osteosarcoma metastasis.

METHODS:

Using RNA sequencing technology, transfection with pcDNA3.1, pCMV6, or pcDNA3.0 vectors, Western blotting, flow cytometry, and Luciferase reporter, Luc, colony formation, and Boyden chamber assays, the effects of PTX3 on cell migration and invasion in human osteosarcoma cells were investigated.

RESULTS:

Expressions of PTX3 were higher in U2OS and MG-63 cells and lower in HOS and Saos-2 cells. Silence of PTX3 repressed migration and invasion in U2OS cells and overexpression of PTX3 accelerated migration and invasion in HOS cells (Fig. 1A-B). Recombinant human PTX3 (rhPTX3) increased migration and invasion of HOS cells (Fig. 1C-D). In siPTX3 U2OS cells, the heatmap showed that Myc-associated zinc finger protein (MAZ), correlated with the epithelial-mesenchymal transition pathway and osteosarcoma tissues, was downregulated. Knockdown of MAZ repressed migration and invasion in U2OS cells, while MAZ overexpression enhanced migration and invasion in HOS cells (Fig. 2A-B). Overexpression of MAZ rescued the inhibition of migration by PTX3-knockdown in U2OS cells, whereas knockdown of MAZ reversed the activation of migration by overexpression of PTX3 in HOS cells (Fig. 2C-D). MAZ promoter activity was decreased by knockdown of PTX3 in U2OS cells but increased by overexpression of PTX3 in HOS cells (Fig. 2E). Knockdown of PTX3 decreased Stat3 phosphorylation in U2OS cells but PTX3 overexpression increased it in HOS cells (Fig. 2F). While overexpression of Stat3 rescued the inhibition of MAZ expression and migration by knockdown of PTX3, Stat3 inhibitor (30 μM of C188-9) reversed the acceleration of MAZ expression and migration by overexpression of PTX3 in HOS cells (Fig. 2G-H). Moreover, in the tail vein injection experiments, PTX3-knockdown U2OS cells reduced pulmonary metastasis formation in immunodeficient (BALB/c) nude mice (Fig. 3).

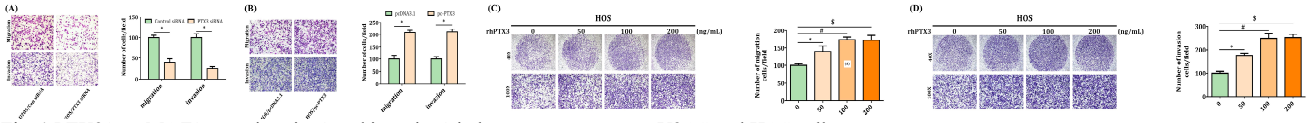

Fig. 1 PTX3↓ → MAZ↓ → migration↓ and invasion↓ in human osteosarcoma U2OS and HOS cells

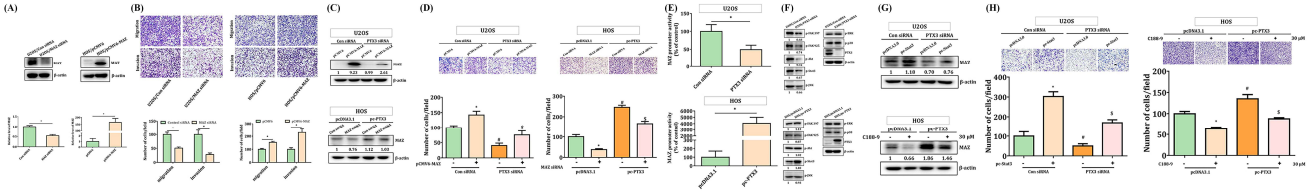

Fig. 2 PTX3↓ → Stat3↓ → MAZ↓ → migration↓ and invasion↓ in U2OS and HOS cells

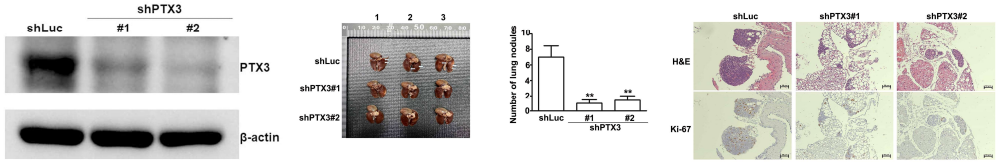

Fig. 3 PTX3↓ → pulmonary metastasis↓ in BALB/c nude mice

DISCUSSION:

PTX3, a soluble molecule produced by various cells in inflammatory sites, plays a role as a promoter or suppressor in multiple malignancies by involving energy metabolism, neovascularization, innate immune response, migration, invasion, and metastasis mechanisms. We demonstrated that overexpression of PTX3 is associated with enhanced migration and invasion in human osteosarcoma, participating in MAZ inhibition through Stat3.

CONCLUSION:

PTX3 inhibits migration and invasion of human osteosarcoma cells by suppressing MAZ via Stat3 and reduces pulmonary metastasis *in vivo*.

SIGNIFICANCE/CLINICAL RELEVANCE:

In human osteosarcoma, PTX3 suppression inhibits migration and invasion by suppressing MAZ through Stat3 and reduces pulmonary metastasis *in vivo*. This discovery illustrates the involvement of the PTX3-related MAZ pathway via Stat3 in human osteosarcoma metastasis.

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