CCN3 promotes prostate cancer bone metastasis by modulating the tumor-bone microenvironment through RANKL-dependent pathway

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Abstract

Bone metastasis in patient with advanced-stage prostate cancer, the most commonly diagnosed malignancy in Western countries, increases the risk of intractable bone pain. The nephroblastoma overexpressed (NOV/CCN3) gene, a member of the CCN gene family, is responsible for the secretion of CCN3, a matrix-associated protein involved in many cellular functions. However, the role of CCN3 in prostate cancer metastasis to bone is poorly understood. CCN3 was found to be highly expressed in bone metastasis patients and positively correlated with malignancy in human prostate cancer cells. Prostate cancer conditioned medium (CM)-induced osteoclast differentiation was inhibited by neutralizing antibody against CCN3. Specifically, CCN3 was found to induce osteoclastogenesis through the receptor activator of NF-κB ligand (RANKL)-dependent pathway, and the focal adhesion kinase (FAK)/Akt/p38/NF- B signal pathway was found to be involved in CCN3-mediated receptor activator of NF-κB (RANK) expression and RANKL-dependent osteoclastogenesis. In contrast, osteoblasts were observed to play an important role in osteoclast differentiation by paracrine manner, with treatment of osteoblasts with CCN3 found to change the RANKL (osteoclastogenesis)/OPG (anti-osteoclastogensis) ratio. Compared with parental PC3 cells, highly invasive PC3-I3 cells markedly enhanced osteoclast activity and bone metastasis in vivo. These results all indicate that CCN3 can be used as a novel therapeutic target in the prevention of bone metastasis of prostate cancer.

Results

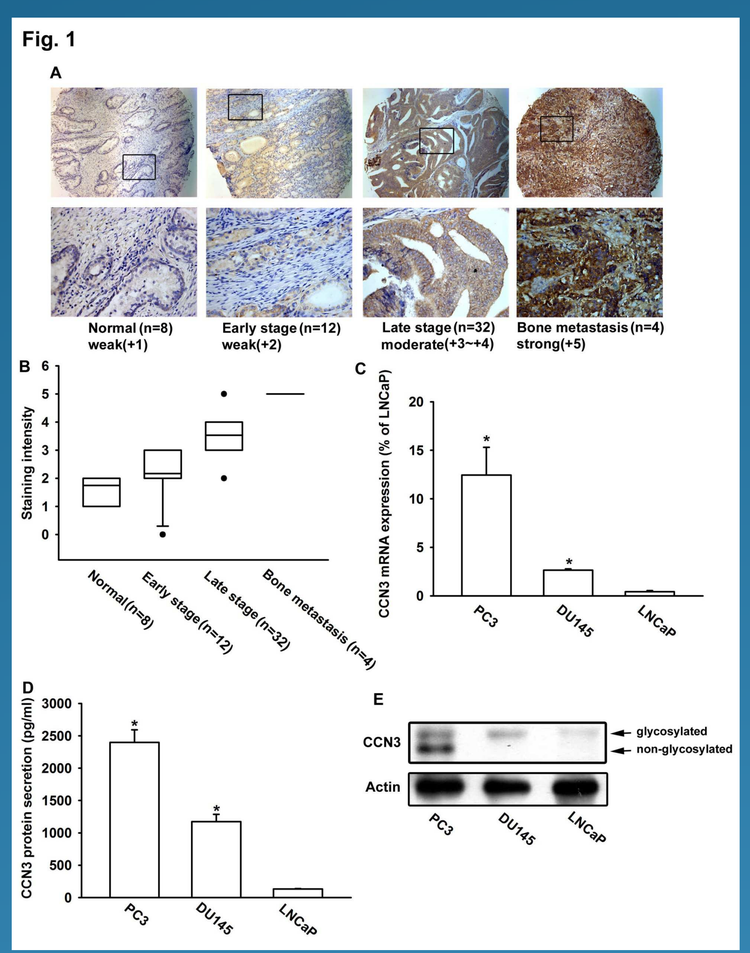

Fig.1 CCN3 is highly expressed in human bone metastases of patients with prostate cancer.

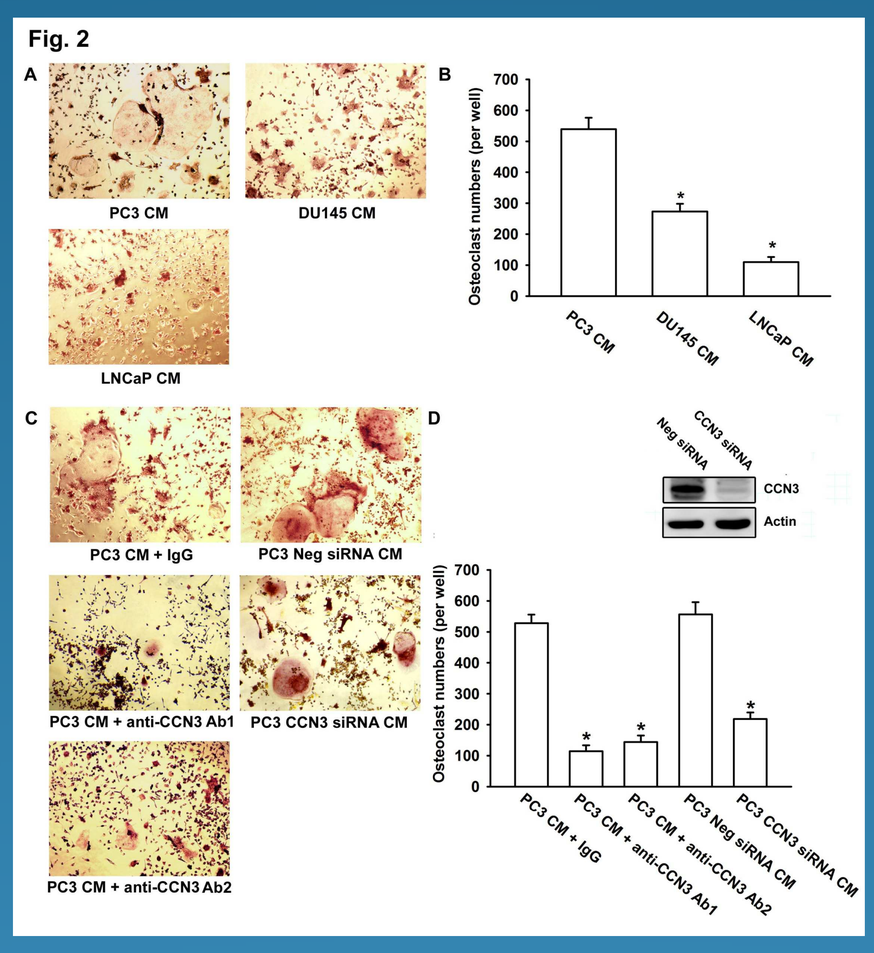

Fig.2 CCN3-neutralizing antibody inhibits prostate cancer conditioned medium (CM)–induced osteoclast formation.

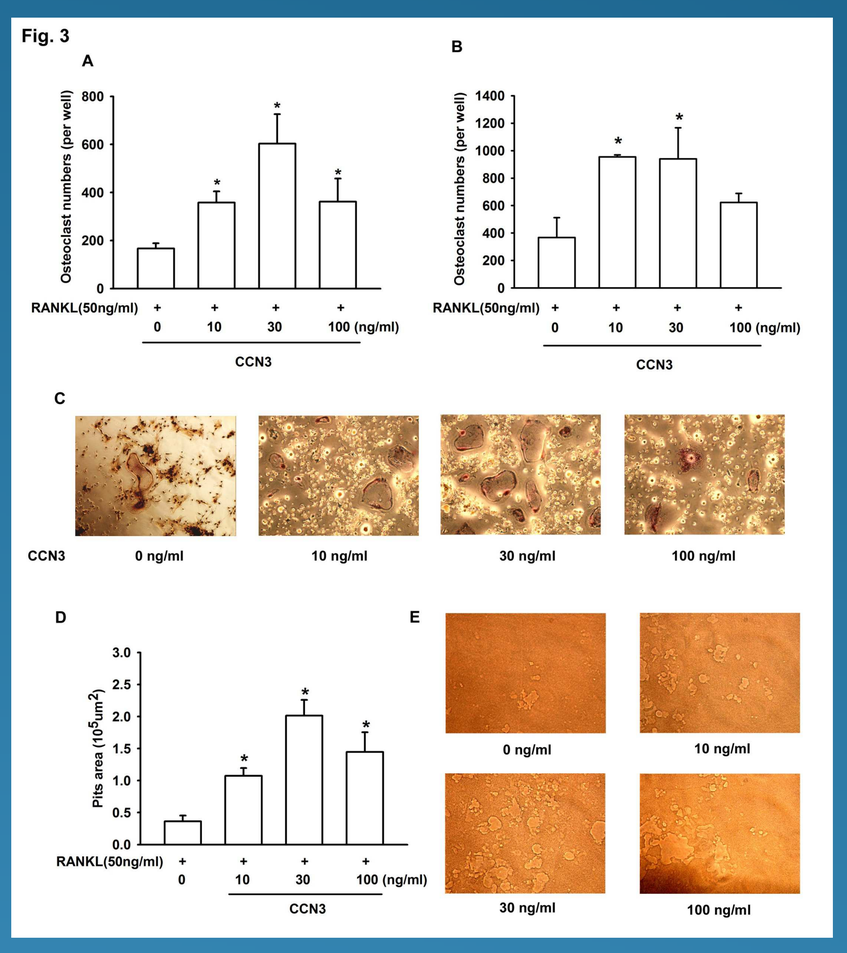

Fig.3 CCN3 synergizes with RANKL to promote osteoclast formation in RAW 264.7 cell cultures and rat bone marrow cultures.

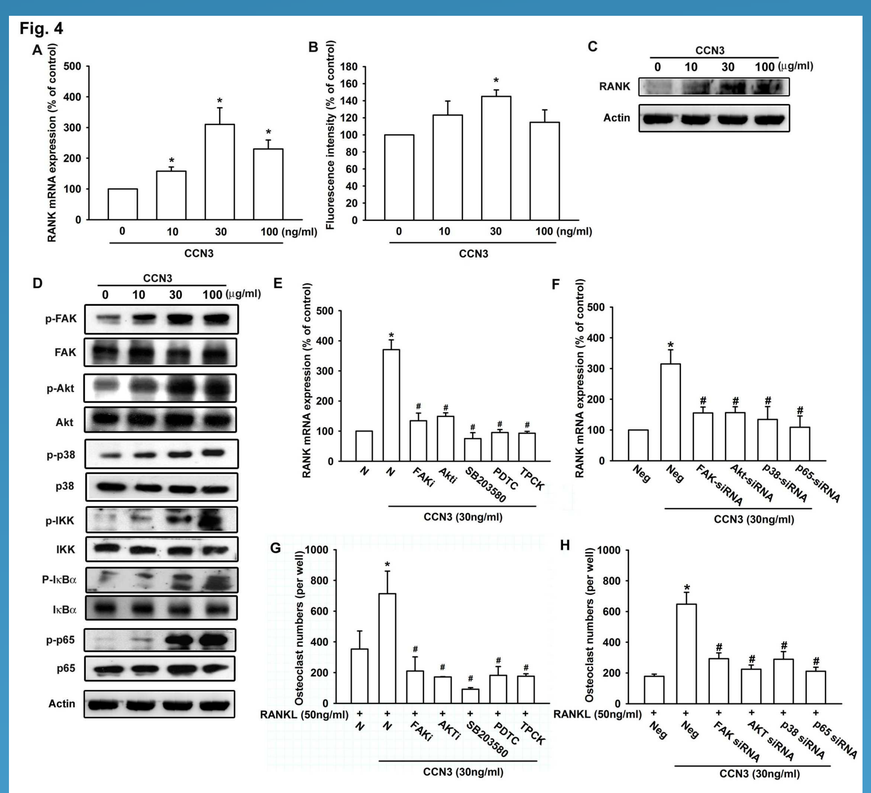

Fig.4 CCN3 increases RANK mRNA and protein expression in RAW 264.7 cells.

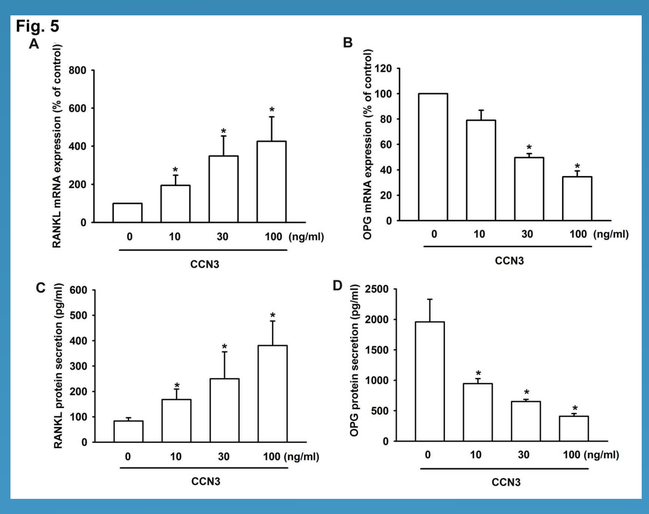

Fig.5 CCN3 affects RANKL/OPG expression in osteoblasts.

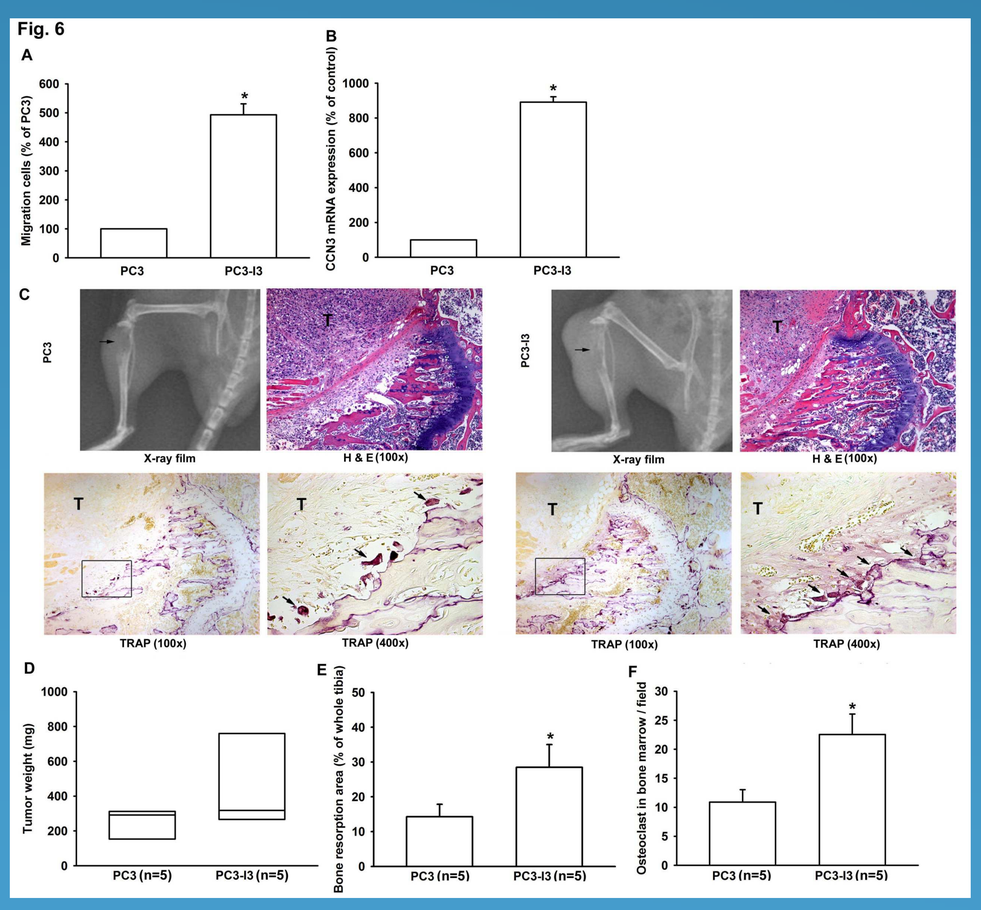

Fig.6 CCN3 overexpression in PC-3 cells increases osteoclast formation and bone metastasis *in vivo*.

Conclusion

CCN3 increases RANKL-dependent osteoclastogenesis through FAK/Akt/p38/NF-κB signal pathway in macrophage. On the other hand, CCN3 also affects RANKL/OPG expression in osteoblasts subsequently induces osteoclastogenesis. Finally, a pro-resorptive microenvironment conducive to promote prostate cancer bone metastasis was established through osteoclast formation.