The miR-423-5p/MMP-2 Axis Regulates Nerve Growth Factor-Induced Promotion of Chondrosarcoma Metastasis

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Research

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Abstract

Background: The effects of Nerve growth factor (NGF) in chondrosarcoma are not confirmed, although NGF is capable of promoting the progression and metastasis of several different types of tumors. Here we aim to explore the role of NGF in chondrosarcoma and elucidate how NGF acts.

Methods: Immunohistochemistry (IHC)-stained tissue samples from chondrosarcoma patients were stained with NGF and MMP-2 antibodies. Cell migration was examined by Transwell and wound healing assays. The expression levels of MMP-2, microRNA-423-5p (miR-423-5p) were measured by quantitative real-time polymerase chain reaction. MMP-2, FAK, and c-Src protein expression were examined by Western blot assays. The interaction between MMP-2 3′-UTRs and miR-423-5p binding site was explored by luciferase assay. We established the orthotopic *in vivo* model of chondrosarcoma lung metastasis to further investigate the promoting effects of NGF in metastatic chondrosarcoma.

Results: Here, we found that the levels of NGF and matrix metalloproteinase-2 (MMP-2) correlated with tumor stage in patients with chondrosarcoma. NGF facilitated MMP-2-dependent cellular migration in human chondrosarcoma JJ012 cells, while overexpression of NGF enhanced lung metastasis in a mouse model of chondrosarcoma. NGF promoted MMP-2 synthesis and cell migration by inhibiting miR-423-5p expression through the FAK and c-Src signaling cascades. NGF appears to be a worthwhile therapeutic target in the treatment of metastatic chondrosarcoma.

Conclusions: Our study has identified that NGF promotes MMP-2-dependent cell migration in human chondrosarcoma tissue by inhibiting miR-423-5p synthesis via the FAK and c-Src signaling cascades

Background

Chondrosarcoma is a common tumor of soft tissue and bone that occurs typically in cartilage-enriched bone (e.g., femur, tibia, or pelvis) (1, 2) and has a high propensity to metastasize to distant organs (1). High-grade chondrosarcomas are particularly prone to metastasize to the lungs (3, 4), so therapeutic strategies that delay or inhibit this phenomenon will improve patient survival. The metastatic process involves the secretion of proteolytic enzymes such as matrix metalloproteinases (MMPs) and cathepsins, capable of degrading the extracellular matrix and basement membrane (5, 6). Not only have significantly higher levels of MMP-2 expression been recorded in human chondrosarcoma specimens than in normal cartilage (7), but also, increasingly higher levels of MMP-2 expression in human chondrosarcoma cells stimulate their migratory and metastatic potential [8,9]. Notably, chemokine (C-C motif) ligand 3 (CCL3) has been found to increase MMP-2 expression in human chondrosarcoma cells and thus encourage their migratory abilities, while inhibition of MMP-2 expression abolishes this effect of CCL3 (7). It therefore seems reasonable to speculate that inhibiting MMP-2 expression be a useful therapeutic tactic in chondrosarcoma metastasis.

MicroRNAs (miRNAs) are involved in the cellular processes of different human diseases, including cancer, cardiovascular disease, and arthritic diseases (8-11) where they regulate different activities of the tumor

cell, including apoptosis, proliferation, angiogenesis, drug resistance and metastasis (12). The importance of miRNAs in tumorigenesis is underlined by the fact that they perpetuate the process by targeting key metabolic enzymes and protein messenger RNAs (mRNAs) (13). In particular, as regards lung cancer cellular metabolism, researchers have suggested that miRNA mimics or inhibitors of metabolic processes and gene regulatory events could improve overall survival in lung cancer (13). In recently, miRNAs levels have been suggested as serving as potential biomarkers and therapeutic targets in cancer (14). In addition, miRNAs are regulated in different roles of the tumor cell, (12). Interestingly, evidence suggests that suppressing miR-101 and MMP-2 expression in human chondrosarcoma cells inhibits chondrosarcoma metastasis to the lungs (15).

Nerve growth factor (NGF) plays a critical role in neuronal cell growth, apoptosis and differentiation (16). The binding of NGF to its receptor tropomyosin receptor kinase A (TrkA) activates intracellular signaling, immune cell proliferation, differentiation and survival (17). Several reports have suggested that NGF plays an integral part in the progression of several types of malignancies, such as ovarian, prostate, and liver cancers (18-20). NGF also mediates metastasis in several types of tumors (20-22). However, the role of NGF in chondrosarcoma is unknown. In this study, we found that NGF promotes chondrosarcoma metastasis *in vitro* and *in vivo*. NGF also promotes MMP-2-dependent migration and invasion by inhibiting miR-423-5p expression through the FAK and c-Src signaling cascades.

Materials And Methods

Materials

NGF, MMP-2, FAK, c-Src and β-actin antibodies were obtained from GeneTex International Corporation (Hsinchu City, Taiwan). The phosphorylated forms of FAK (p-FAK) and c-Src (p-c-Src) antibodies were purchased from Cell Signaling Technology (Danvers, MA, USA). MMP-2, FAK, c-Src and control ON-TARGET*plus* siRNAs were obtained from Dharmacon (Lafayette, CO, USA). Quantitative polymerase chain reaction (qPCR) primers and probes, as well as Taqman® One-Step PCR Master Mix, were supplied by Applied Biosystems (Foster City, CA, USA). Recombinant human NGF was obtained from PerpoTech (Rocky Hill, NJ, USA). All other chemicals used in this study were supplied by Sigma-Aldrich (St. Louis, MO, USA).

Cell culture

The human chondrosarcoma cell line JJ012 was kindly provided by Dr. Sean P. Scully (University of Miami School of Medicine, Miami, FL, USA), while the SW1353 chondrosarcoma cell line was obtained from the American Type Cell Culture Collection (Manassas, VA, USA). JJ012 cells stably expressing the NGF complementary DNA (cDNA) clone (JJ012/NGF cells) were established according to our previous method (23). Cells were cultured 50%/50% in Dulbecco's Modified Eagle Medium (DMEM)/alpha-minimum essential medium (α-MEM) medium, 10% fetal bovine serum (FBS) and antibiotics, then maintained in a humidified incubator at 37°C in 5% CO_2.

Cell migration assay

Chondrosarcoma cells were seeded into the upper chamber of Transwell plates (Costar, NY, USA), while NGF and pharmaceutical inhibitors were added to the lower chamber. After 18 h of incubation, migrated cells were fixed with 3.7% formaldehyde and stained with crystal violet, then counted manually under the microscope (24, 25).

Wound healing assay

The confluent chondrosarcoma monolayer was scratched by a fine pipette tip to create extended scratches in each well. Cells were then treated with the conditions as indicated, migratory activity was evaluated by microscopy after 24 h and the rate of wound closure was quantified (26).

Western blot analysis

After the indicated treatments, chondrosarcoma cells were lysed in RIPA buffer. The extracted proteins were resolved by SDS-PAGE and transferred to Immobilon® polyvinylidene fluoride (PVDF) membranes. Western blot analysis was performed using the methodology described in our previous reports (27-29).

mRNA and miRNA quantification

Total RNA was extracted from chondrosarcoma cells using TRIzol reagent and RNA concentrations were determined using a NanoVue Plus spectrophotometer (GE Healthcare Life Sciences; Pittsburgh, PA, USA). The M-MLV RT kit (Thermo Fisher Scientific; Waltham, MA, USA) and the Mir-X™ miRNA First-Strand Synthesis kit (Clontech; Mountain View, CA, USA) were used to perform reverse transcription of total RNA into cDNA. Quantitative real-time PCR (qPCR) analysis was performed according to our previous reports (30, 31).

Luciferase assay

The Human MMP-2 luciferase reporter plasmids containing wild-type or mutant sequences of the three prime untranslated region (3'-UTR) encompassing miR-423-5p binding sites were obtained from MDBio, Inc. (Taipei, Taiwan). Chondrosarcoma cells were transfected with the plasmids using Lipofectamine 2000 (Thermo Fisher Scientific; Waltham, MA, USA), then stimulated with NGF for 24 h. Luciferase activity was monitored using a luciferase assay kit (30, 32, 33).

Tumor xenograft study

Four-week-old male BALB/c nude mice (8 in each group) were bought from Taipei's National Laboratory Animal Center and orthotopically injected with JJ012 or JJ012/NGF cells (5×10^6, resuspended in 100 μL of medium containing 50% serum-free DMEM/α-MEM and 50% Matrigel), according to a previous protocol (23). Tumor growth in the tibiae was monitored each week by bioluminescence imaging using a Xenogen IVIS imaging system 200 (PerkinElmer, MA, USA). At 12 weeks, the mice were euthanized by CO_2

inhalation. The lungs were removed and fixed in 10% formalin for further analysis. All animal experiments satisfied the protocols specified by China Medical University's Institutional Animal Care and Use Committee (IACUC Approval No. 104-154-N).

Immunohistochemistry (IHC) staining

Mouse lung tissues or specimens from a human chondrosarcoma tissue array (Biomax; Rockville, MD, USA) were rehydrated and incubated with primary anti-NGF or MMP-2 antibodies, then treated with biotin-labeled secondary antibody. Finally, the slides were detected using the ABC Kit (Vector Laboratories, CA, USA) and photographed using the microscope.

Statistics

All values are presented as the mean ± standard deviation (SD). Significance testing on the difference between the groups was assessed by the Student's *t*-test and considered significant if the *p*-value was <0.05.

Results

NGF and MMP-2 levels are positively correlated in human chondrosarcoma tissue

NGF is associated with progression and survival in several cancer types (34, 35). MMP-2 reported to control migration and metastasis of chondrosarcoma (7, 36). IHC tissue array results revealed higher levels of NGF and MMP-2 expression in patients with higher grade chondrosarcoma than in those with lower grade disease; the levels of NGF and MMP-2 expression were reflected by tumor stage (Fig. 1A-C). These results are quantified in Figures 1D&E, which illustrate how the levels of NGF and MMP-2 expression were significantly higher in higher-stage tumors (IIA and IIB) than in lower-stage tumors (IA and IB). A positive correlation observed between MMP-2 and NGF staining intensity of human chondrosarcoma tissue (r^2 = 0.6, Fig. 1F) indicates that the levels of these proteins are associated with the progression of chondrosarcoma disease.

NGF promotes MMP-2-dependent migration via the FAK and c-Src pathways in chondrosarcoma

We first investigated the effects of NGF upon cell motility in chondrosarcoma cell lines SW1353 and JJ012. Treatment of cells with NGF promoted migration ability, according to Transwell and wound healing assay data (Fig. 2A-D). Next, we examined whether MMP-2 plays a role in NGF-regulated migration in chondrosarcoma. Stimulation of cells with NGF enhanced mRNA and protein synthesis of MMP-2 (Fig. 2E&F). Transfection of cells with MMP-2 siRNA diminished NGF-induced promotion of migration (Fig. 2G-I), implying that MMP-2 is critical to NGF-induced chondrosarcoma cell migration.

The FAK and c-Src signaling pathway plays a critical role in chondrosarcoma metastasis (37). Treating cells with a FAK inhibitor or c-Src inhibitor (PP2) significantly reduced NGF-induced stimulation of cell migration and MMP-2 production (Fig. 3A-F and 4A-F). Similar effects were observed when the

chondrosarcoma cells were transfected with FAK or c-Src siRNAs (Fig. 3A-G and 4A-F). NGF stimulation time-dependently promoted FAK and c-Src phosphorylation in both cell lines (Fig. 3H & 4H). Treating cells with a FAK inhibitor diminished NGF-induced c-Src phosphorylation (Fig. 4I), indicating that the FAK/c-Src signaling cascade regulates NGF-induced MMP-2 synthesis and chondrosarcoma cell migration.

The miR-423-5p/MMP-2 axis controls NGF-induced stimulation of chondrosarcoma cell migration

miRNA-associated regulation of MMP-2 expression is critical mechanism in the development, progression, migration and metastasis of cancer cells (38). Five online databases for miRNA target prediction indicated that the 3'-UTR region of MMP-2 mRNA contains 13 promising candidate miRNAs (Fig. 5A&B). Treatment of JJ012 cells with NGF (100 ng/ml) significantly reduced miR-423-5p expression (Fig. 5B) and at the concentrations of 30, 50, or 100 ng/ml, significantly inhibited miR-423-5p synthesis in both chondrosarcoma cell lines, in a concentration-dependent manner (Fig. 5C). Transfection of cells with miR-423-5p mimic significantly reduced NGF-induced stimulation of cell migration and MMP-2 mRNA expression in both chondrosarcoma cell lines (Fig. 5D-F). Next, we investigated whether FAK and c-Src signaling regulate NGF-induced suppression of miR-423-5p synthesis. FAK and c-Src inhibitors, and their respective siRNAs, all reversed NGF-induced inhibition of miR-423-5p expression (Fig. 5G&H). Analyses of the MMP-2 3'-UTR luciferase plasmids revealed that NGF increased luciferase activity of the wild-type, but not mutant, MMP-2 3'-UTRs (Fig. 5I&J). These results indicate that miR-16-5p controls MMP-2 expression by anchoring to the 3'-UTR region of the human *MMP-2* gene via the FAK/c-Src pathway.

Overexpression of NGF facilitates chondrosarcoma metastasis to lungs in mice

We used the orthotopic *in vivo* model of chondrosarcoma lung metastasis to further investigate the promoting effects of NGF in metastatic chondrosarcoma (23). JJ012 and JJ012/NGF cells were orthotopically implanted into the right leg tibia and tumor size was monitored each week by the IVIS system (Fig. 6A&B). Overexpression of NGF significantly increased tumor growth in the tibia (Fig. 6A&B). At 12 weeks, metastasis to the lung was significantly more likely with JJ012/NGF cells than with JJ012 cells (Fig. 6C&D). IHC results revealed significant increases in the levels of NGF and MMP-2 expression in the JJ012/NGF orthotopic model (Fig. 6E), confirming that NGF facilitates the metastasis of chondrosarcoma to mouse lung.

Discussion

Chondrosarcoma is a malignant bone neoplasm that constitutes almost one-third (~26%) of all bone cancers (39). Chemotherapy and radiotherapy have very limited effectiveness, so treatment with surgery is therefore the major management modality for chondrosarcoma. This malignancy is notorious for its aggressive clinical course and propensity to metastasize (40). An effective adjuvant therapy is urgently needed to suppress the metastasis of chondrosarcoma (1, 3). NGF plays an important role in tumor cell proliferation, migration and survival (34, 35). The effect of NGF in chondrosarcoma metastasis is uncertain. Our investigation has found that levels of NGF and MMP-2 expression are positively correlated with tumor staging in patients with chondrosarcoma. We also confirmed that NGF facilitates MMP-2-

dependent chondrosarcoma cell migration and metastasis by inhibiting miR-423-5p synthesis via FAK/c-Src signaling.

NGF acts not only on the central and peripheral nervous systems, but also on non-neuronal tissues and cancer cells (34). NGF plays a multi-functional role in the tumor environment, exerting effects on tumor cell proliferation, survival, apoptosis, angiogenesis and metastasis (34, 41, 42). Our study is the first to describe an association between NGF levels and tumor stage in chondrosarcoma tissue specimens. The evidence from us *in vitro* and *in vivo* results suggest that NGF facilitates chondrosarcoma metastasis. The binding of NGF with the neurotrophin receptor TrkA mediates NGF control in the development of cancer (43). Knockdown of TrkA receptor suppresses the progression of liver cancer (44) and a Trk receptor inhibitor antagonizes NGF-induced cell motility in prostate cancer (45). These results suggest that inhibition of the TrkA receptor reduces NGF-mediated cancer development. In this study, we did not examine TrkA receptor levels in patients with chondrosarcoma. Whether TrkA inhibition antagonizes NGF-mediated chondrosarcoma metastasis is yet to be confirmed.

Activation of the FAK/c-Src pathway is important in the regulation of different cellular functions (46, 47). This signaling pathway also regulates the expression of MMP-mediated cancer motility (48, 49). Here, our results show that NGF promotes phosphorylation of FAK and c-Src, while FAK and c-Src pharmacological inhibitors suppress NGF-induced promotion of MMP-2 expression and chondrosarcoma migration. This phenomenon is confirmed by similar effects observed with genetic siRNAs of FAK and c-Src. However, c-Src has been reported to be an upstream molecule of FAK, with the capacity to regulate cell motility (50, 51). Here, we found that a FAK inhibitor curtailed NGF-promoted phosphorylation of c-Src, indicating that FAK activates c-Src.

MiRNAs post-transcriptionally regulate gene expression (52). During tumor metastasis, aberrant miRNA expression mediates cancer cell migration and invasion (53). Our analysis of five open-source databases identified that 13 miRNAs potentially interfere with MMP-2 transcription. NGF significantly lowered miR-423-5p expression. We enhanced miR-423-5p levels in chondrosarcoma cells by transfecting them with a specific miR-423-5p mimic, which also reduced MMP-2 synthesis and the migratory capacity of the cells. miR-423-5p expression was negatively correlated with MMP-2 expression and cell migratory activity in chondrosarcoma cells. Thus, our evidence has identified novel antimetastatic properties of miR-423-5p.

Conclusion

In conclusion, our study has identified that NGF promotes MMP-2-dependent cell migration in human chondrosarcoma tissue by inhibiting miR-423-5p synthesis via the FAK and c-Src signaling cascades (Fig. 7). It appears to be worth targeting NGF expression in metastatic chondrosarcoma.

Abbreviations

NGF, Nerve growth factor; MMP-2, matrix metalloproteinase-2; CCL3, chemokine (C-C motif) ligand 3; miRNAs, MicroRNAs; mRNAs, messenger RNAs; TrkA, tropomyosin receptor kinase A; qPCR, quantitative polymerase chain reaction; cDNA, complementary DNA; DMEM, Dulbecco's Modified Eagle Medium; α-MEM, alpha-minimum essential medium; FBS, fetal bovine serum; PVDF, polyvinylidene fluoride; 3'-UTR, three prime untranslated region; IHC, Immunohistochemistry; SD, standard deviation.

Declarations

Ethics approval and consent to participate

The authors declare that the data supporting the findings of this study are available within the article. All animal experiments satisfied the protocols specified by China Medical University's Institutional Animal Care and Use Committee (IACUC Approval No. 104-154-N).

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author for reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

HET, SLL, and CHT conceived and designed the experiments. LAT, CYK, JFL, and CYL performed the experiments. HET, SLL, and LAT contributed to perform in vitro and in vivo experiments. LAT and CYL contributed to analyze experiment data. YCF and CHT contributed to the writing of the manuscript. All authors approved for publication.

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References

1. MacDonald IJ, Lin CY, Kuo SJ, Su CM, Tang CH. An update on current and future treatment options for chondrosarcoma. Expert Rev Anticancer Ther. 2019;19(9):773–86.
2. Chen PC, Cheng HC, Yang SF, Lin CW, Tang CH. The CCN family proteins: modulators of bone development and novel targets in bone-associated tumors. BioMed research international. 2014;2014:437096.
3. Chen JC, Fong YC, Tang CH. Novel strategies for the treatment of chondrosarcomas: targeting integrins. BioMed research international. 2013;2013:396839.
4. Ferguson JL, Turner SP. Bone Cancer: Diagnosis and Treatment Principles. Am Family Phys. 2018;98(4):205–13.
5. Fan TM, Roberts RD, Lizardo MM. Understanding and Modeling Metastasis Biology to Improve Therapeutic Strategies for Combating Osteosarcoma Progression. Frontiers in oncology. 2020;10:13.
6. Steeg PS. Targeting metastasis. Nature reviews Cancer. 2016;16(4):201–18.
7. Hsu CJ, Wu MH, Chen CY, Tsai CH, Hsu HC, Tang CH. AMP-activated protein kinase activation mediates CCL3-induced cell migration and matrix metalloproteinase-2 expression in human chondrosarcoma. Cell communication signaling: CCS. 2013;11:68.
8. Broderick JA, Zamore PD. MicroRNA therapeutics. Gene therapy. 2011;18(12):1104–10.
9. Si W, Shen J, Zheng H, Fan W. The role and mechanisms of action of microRNAs in cancer drug resistance. Clinical epigenetics. 2019;11(1):25.

10. Wojciechowska A, Braniewska A, Kozar-Kaminska K. MicroRNA in cardiovascular biology and disease. Advances in clinical and experimental medicine: official organ. Wroclaw Medical University. 2017;26(5):865–74.
11. Tavallaee G, Rockel JS, Lively S, Kapoor M. MicroRNAs in Synovial Pathology Associated With Osteoarthritis. Frontiers in medicine. 2020;7:376.
12. Tutar Y, Ozgur A, Tutar E, Tutar L, Pulliero A, Izzotti A. Regulation of oncogenic genes by MicroRNAs and pseudogenes in human lung cancer. Biomedicine pharmacotherapy = Biomedecine pharmacotherapie. 2016;83:1182–90.
13. Iqbal MA, Arora S, Prakasam G, Calin GA, Syed MA. MicroRNA in lung cancer: role, mechanisms, pathways and therapeutic relevance. Mol Aspects Med. 2019;70:3–20.
14. Mohammadi A, Mansoori B, Baradaran B. The role of microRNAs in colorectal cancer. Biomedicine pharmacotherapy = Biomedecine pharmacotherapie. 2016;84:705–13.
15. Tsai CH, Yang DY, Lin CY, Chen TM, Tang CH, Huang YL. Sphingosine-1-phosphate suppresses chondrosarcoma metastasis by upregulation of tissue inhibitor of metalloproteinase 3 through suppressing miR-101 expression. Molecular oncology. 2017;11(10):1380–98.
16. Lewin GR, Barde YA. Physiology of the neurotrophins. Annu Rev Neurosci. 1996;19:289–317.
17. Minnone G, De Benedetti F, Bracci-Laudiero L. NGF and Its Receptors in the Regulation of Inflammatory Response. International journal of molecular sciences. 2017;18(5).
18. Garrido MP, Torres I, Avila A, Chnaiderman J, Valenzuela-Valderrama M, Aramburo J, et al. NGF/TRKA Decrease miR-145-5p Levels in Epithelial Ovarian Cancer Cells. International journal of molecular sciences. 2020;21(20).
19. Chen WY, Wen YC, Lin SR, Yeh HL, Jiang KC, Chen WH, et al. Nerve growth factor interacts with CHRM4 and promotes neuroendocrine differentiation of prostate cancer and castration resistance. Communications biology. 2021;4(1):22.
20. Lin H, Huang H, Yu Y, Chen W, Zhang S, Zhang Y. Nerve growth factor regulates liver cancer cell polarity and motility. Molecular medicine reports. 2021;23(4).
21. Singh R, Karri D, Shen H, Shao J, Dasgupta S, Huang S, et al. TRAF4-mediated ubiquitination of NGF receptor TrkA regulates prostate cancer metastasis. J Clin Investig. 2018;128(7):3129–43.
22. McCaffrey G, Thompson ML, Majuta L, Fealk MN, Chartier S, Longo G, et al. NGF blockade at early times during bone cancer development attenuates bone destruction and increases limb use. Cancer research. 2014;74(23):7014–23.
23. Tzeng HE, Tang CH, Wu SH, Chen HT, Fong YC, Lu YC, et al. CCN6-mediated MMP-9 activation enhances metastatic potential of human chondrosarcoma. Cell death disease. 2018;9(10):955.
24. Lee HP, Wang SW, Wu YC, Lin LW, Tsai FJ, Yang JS, et al. Soya-cerebroside inhibits VEGF-facilitated angiogenesis in endothelial progenitor cells. Food Agr Immunol. 2020;31(1):193–204.
25. Hou CH, Lin FL, Hou SM, Liu JF. Cyr61 promotes epithelial-mesenchymal transition and tumor metastasis of osteosarcoma by Raf-1/MEK/ERK/Elk-1/TWIST-1 signaling pathway. Mol Cancer.

2014;13:236.

26. Liu JF, Lee CW, Tsai MH, Tang CH, Chen PC, Lin LW, et al. Thrombospondin 2 promotes tumor metastasis by inducing matrix metalloproteinase-13 production in lung cancer cells. Biochem Pharmacol. 2018;155:537–46.
27. Lee HP, Chen PC, Wang SW, Fong YC, Tsai CH, Tsai FJ, et al. Plumbagin suppresses endothelial progenitor cell-related angiogenesis in vitro and in vivo. J Funct Foods. 2019;52:537–44.
28. Lee HP, Wang SW, Wu YC, Tsai CH, Tsai FJ, Chung JG, et al. Glucocerebroside reduces endothelial progenitor cell-induced angiogenesis. Food Agr Immunol. 2019;30(1):1033–45.
29. Liu SC, Tsai CH, Wu TY, Tsai CH, Tsai FJ, Chung JG, et al. Soya-cerebroside reduces IL-1 beta-induced MMP-1 production in chondrocytes and inhibits cartilage degradation: implications for the treatment of osteoarthritis. Food Agr Immunol. 2019;30(1):620–32.
30. Yang YC, Chiou PC, Chen PC, Liu PY, Huang WC, Chao CC, et al. Melatonin reduces lung cancer stemness through inhibiting of PLC, ERK, p38, beta-catenin, and Twist pathways. Environmental toxicology. 2019;34(2):203–9.
31. Wang M, Chao CC, Chen PC, Liu PI, Yang YC, Su CM, et al. Thrombospondin enhances RANKL-dependent osteoclastogenesis and facilitates lung cancer bone metastasis. Biochem Pharmacol. 2019;166:23–32.
32. Su CM, Tang CH, Chi MJ, Lin CY, Fong YC, Liu YC, et al. Resistin facilitates VEGF-C-associated lymphangiogenesis by inhibiting miR-186 in human chondrosarcoma cells. Biochem Pharmacol. 2018;154:234–42.
33. Wu TJ, Lin CY, Tsai CH, Huang YL, Tang CH. Glucose suppresses IL-1beta-induced MMP-1 expression through the FAK, MEK, ERK, and AP-1 signaling pathways. Environmental toxicology. 2018;33(10):1061–8.
34. Aloe L, Rocco ML, Balzamino BO, Micera A. Nerve growth factor: role in growth, differentiation and controlling cancer cell development. Journal of experimental clinical cancer research: CR. 2016;35(1):116.
35. Jiang J, Bai J, Qin T, Wang Z, Han L. NGF from pancreatic stellate cells induces pancreatic cancer proliferation and invasion by PI3K/AKT/GSK signal pathway. J Cell Mol Med. 2020;24(10):5901–10.
36. Roomi MW, Kalinovsky T, Monterrey J, Rath M, Niedzwiecki A. In vitro modulation of MMP-2 and MMP-9 in adult human sarcoma cell lines by cytokines, inducers and inhibitors. Int J Oncol. 2013;43(6):1787–98.
37. Wang CQ, Huang YW, Wang SW, Huang YL, Tsai CH, Zhao YM, et al. Amphiregulin enhances VEGF-A production in human chondrosarcoma cells and promotes angiogenesis by inhibiting miR-206 via FAK/c-Src/PKCdelta pathway. Cancer letters. 2017;385:261–70.
38. Javadian M, Gharibi T, Shekari N, Abdollahpour-Alitappeh M, Mohammadi A, Hossieni A, et al. The role of microRNAs regulating the expression of matrix metalloproteinases (MMPs) in breast cancer development, progression, and metastasis. J Cell Physiol. 2019;234(5):5399–412.
39. Leddy LR, Holmes RE. Chondrosarcoma of bone. Cancer treatment research. 2014;162:117–30.

40. Gelderblom H, Hogendoorn PC, Dijkstra SD, van Rijswijk CS, Krol AD, Taminiau AH, et al. The clinical approach towards chondrosarcoma. Oncologist. 2008;13(3):320–9.
41. Romon R, Adriaenssens E, Lagadec C, Germain E, Hondermarck H, Le Bourhis X. Nerve growth factor promotes breast cancer angiogenesis by activating multiple pathways. Mol Cancer. 2010;9:157.
42. Su R, Su W, Jiao Q. NGF protects neuroblastoma cells against beta-amyloid-induced apoptosis via the Nrf2/HO-1 pathway. FEBS open bio. 2019;9(12):2063–71.
43. Micera A, Lambiase A, Stampachiacchiere B, Bonini S, Bonini S, Levi-Schaffer F. Nerve growth factor and tissue repair remodeling: trkA(NGFR) and p75(NTR), two receptors one fate. Cytokine Growth Factor Rev. 2007;18(3–4):245–56.
44. Luo T, Zhang SG, Zhu LF, Zhang FX, Li W, Zhao K, et al. A selective c-Met and Trks inhibitor Indo5 suppresses hepatocellular carcinoma growth. Journal of experimental clinical cancer research: CR. 2019;38(1):130.
45. Festuccia C, Muzi P, Gravina GL, Millimaggi D, Speca S, Dolo V, et al. Tyrosine kinase inhibitor CEP-701 blocks the NTRK1/NGF receptor and limits the invasive capability of prostate cancer cells in vitro. Int J Oncol. 2007;30(1):193–200.
46. Wang YH, Kuo SJ, Liu SC, Wang SW, Tsai CH, Fong YC, et al. Apelin Affects the Progression of Osteoarthritis by Regulating VEGF-Dependent Angiogenesis and miR-150-5p Expression in Human Synovial Fibroblasts. Cells. 2020;9(3).
47. Chen CY, Su CM, Huang YL, Tsai CH, Fuh LJ, Tang CH. CCN1 induces oncostatin M production in osteoblasts via integrin-dependent signal pathways. PloS one. 2014;9(9):e106632.
48. Zhu X, Wang K, Chen Y. Ophiopogonin. D suppresses TGF-beta1-mediated metastatic behavior of MDA-MB-231 breast carcinoma cells via regulating ITGB1/FAK/Src/AKT/beta-catenin/MMP-9 signaling axis. Toxicology in vitro: an international journal published in association with BIBRA. 2020;69:104973.
49. Chuang CY, Ho YC, Lin CW, Yang WE, Yu YL, Tsai MC, et al. Salvianolic acid A suppresses MMP-2 expression and restrains cancer cell invasion through ERK signaling in human nasopharyngeal carcinoma. J Ethnopharmacol. 2020;252:112601.
50. Ramirez-Ricardo J, Leal-Orta E, Garcia-Hernandez A, Diaz-Aragon R, Cortes-Reynosa P, Thompson-Bonilla R, et al. Role of Src/FAK in migration and invasion mediated by extracellular vesicles from MDA-MB-231 cells stimulated with linoleic acid. Medical oncology. 2021;38(4):40.
51. Olea-Flores M, Zuniga-Eulogio M, Tacuba-Saavedra A, Bueno-Salgado M, Sanchez-Carvajal A, Vargas-Santiago Y, et al. Leptin Promotes Expression of EMT-Related Transcription Factors and Invasion in a Src and FAK-Dependent Pathway in MCF10A Mammary Epithelial Cells. Cells. 2019;8(10).
52. Nugent M. MicroRNAs: exploring new horizons in osteoarthritis. Osteoarthritis cartilage. 2016;24(4):573–80.
53. Puppo M, Valluru MK, Clezardin P. MicroRNAs and Their Roles in Breast Cancer Bone Metastasis. Current osteoporosis reports. 2021.

Figures

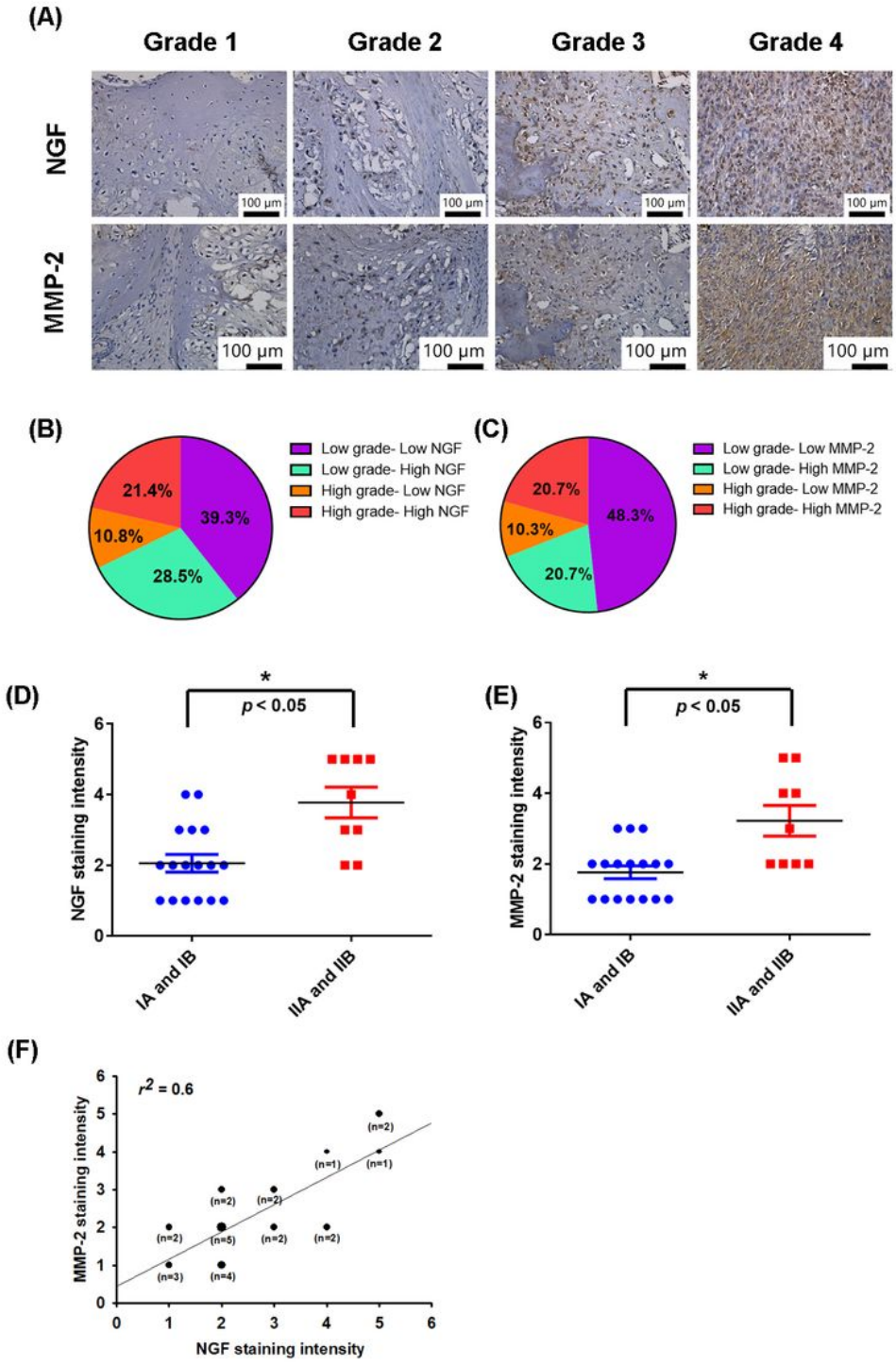

Figure 1

NGF and MMP-2 levels correlated with chondrosarcoma tumor stage. (A-E) Immunohistochemistry (IHC)-stained tissue samples from chondrosarcoma patients were stained with NGF and MMP-2 antibodies,

then photographed and quantified. (F) Levels of NGF and MMP-2 were positively correlated. * p < 0.05 compared with the early-stage (IA and IB) tumor group.

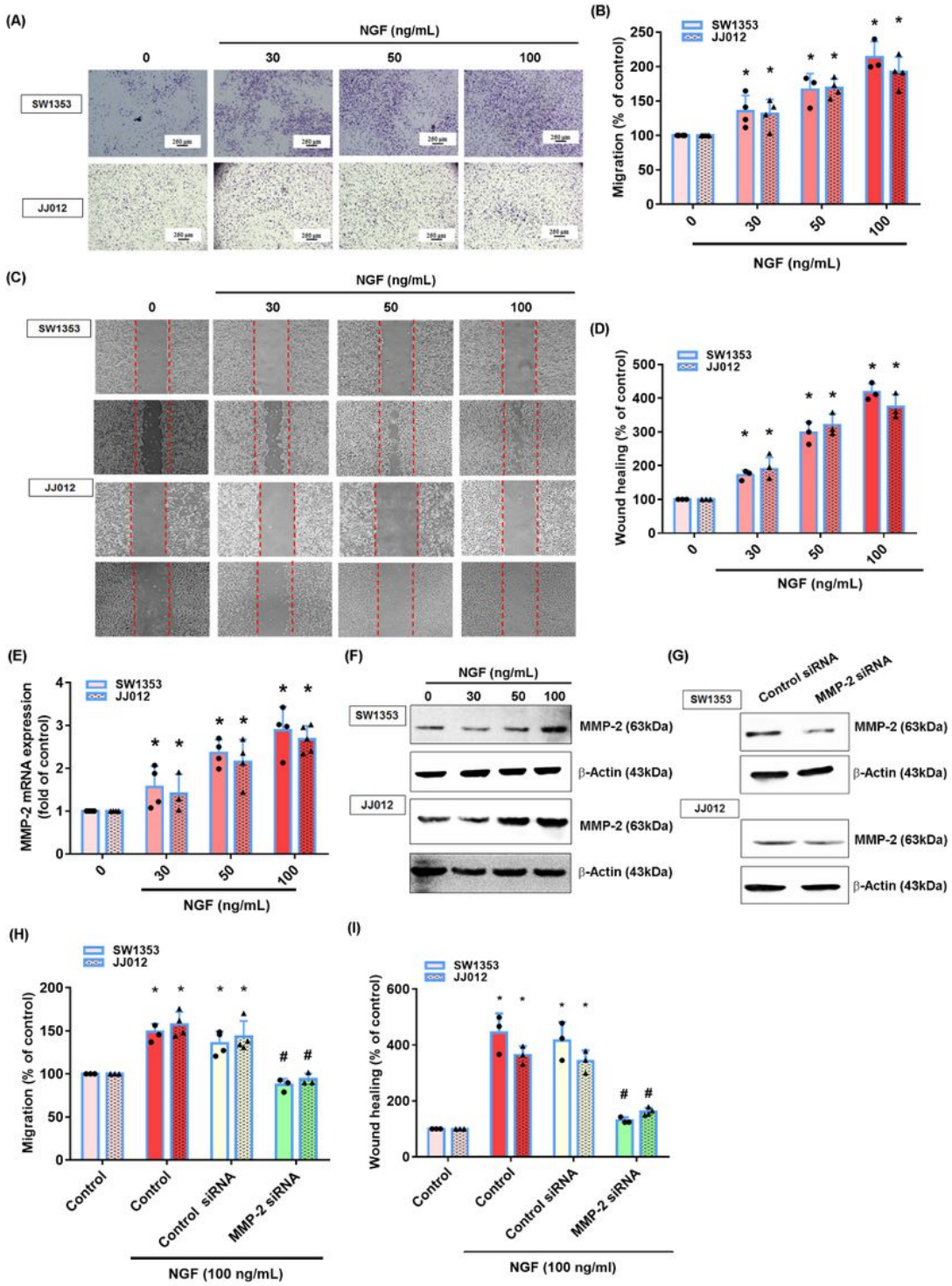

Figure 2

NGF promotes MMP-2-dependent cell migration in human chondrosarcoma. (A-D) Cells were incubated with NGF (30–100 ng/mL) and cell migration was examined by Transwell and wound healing assays. (E-F) Cells were incubated with NGF (30–100 ng/mL) and levels of MMP-2 mRNA and protein expression

were examined by qPCR and Western blot assays. (G-I) Cells were transfected with MMP-2 siRNAs then stimulated with NGF. Cell migration and MMP-2 expression levels were examined by Transwell, wound healing and Western blot assays. * $p < 0.05$ compared with the control group; # $p < 0.05$ compared with the NGF-treated group.

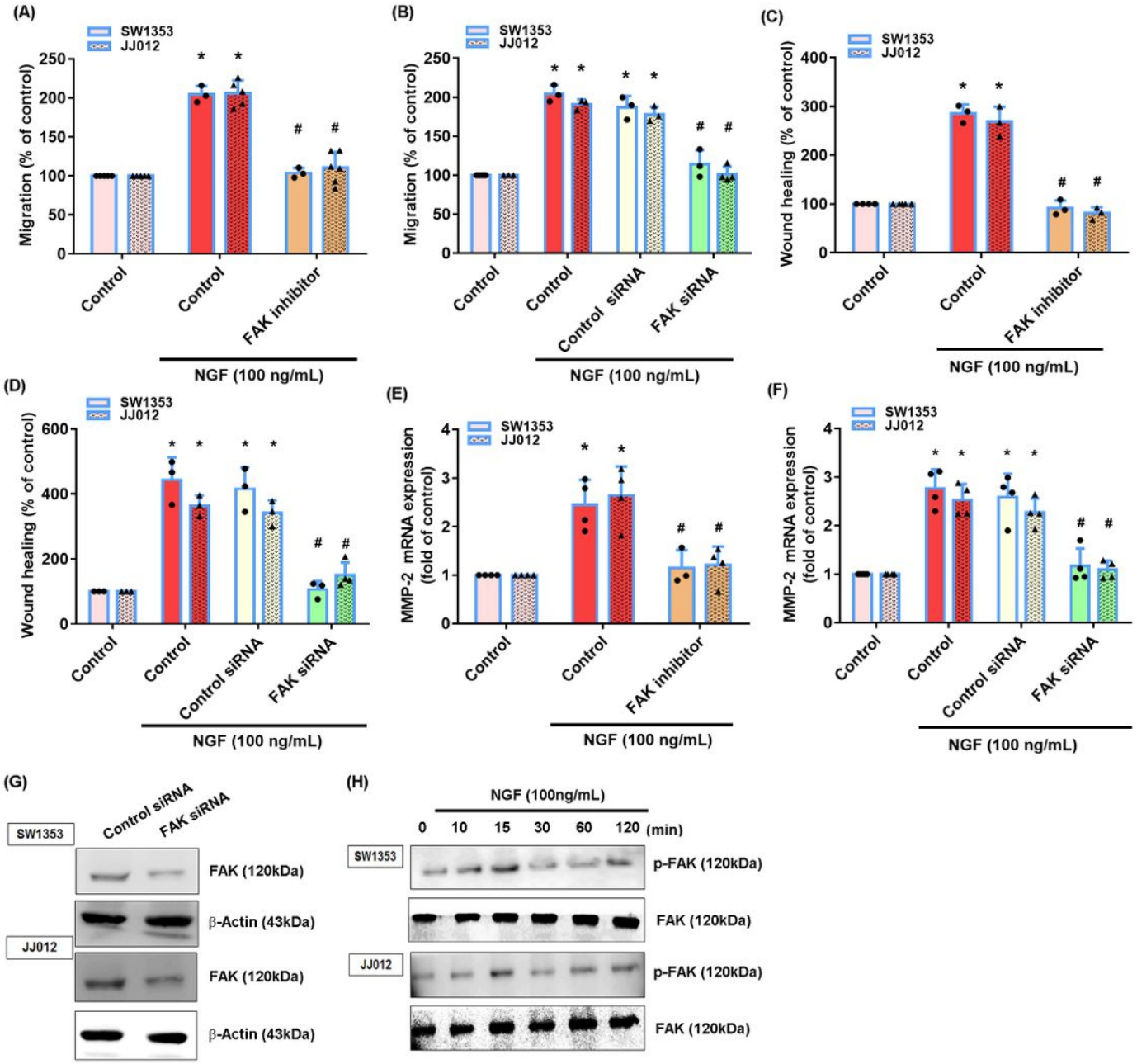

Figure 3

The FAK pathway mediates NGF-induced MMP-2 expression and cell migration. (A-F) Cells were pretreated with a FAK inhibitor or transfected with a FAK siRNA, then stimulated with NGF. Cell migration and levels of MMP-2 expression were examined by Transwell, wound healing and qPCR. (G) Cells were transfected with a FAK siRNA and FAK expression was examined by Western blot. (H) Cells were

incubated with NGF for the indicated time intervals; FAK phosphorylation was examined by Western blot. * p < 0.05 compared with the control group; # p < 0.05 compared with the NGF-treated group.

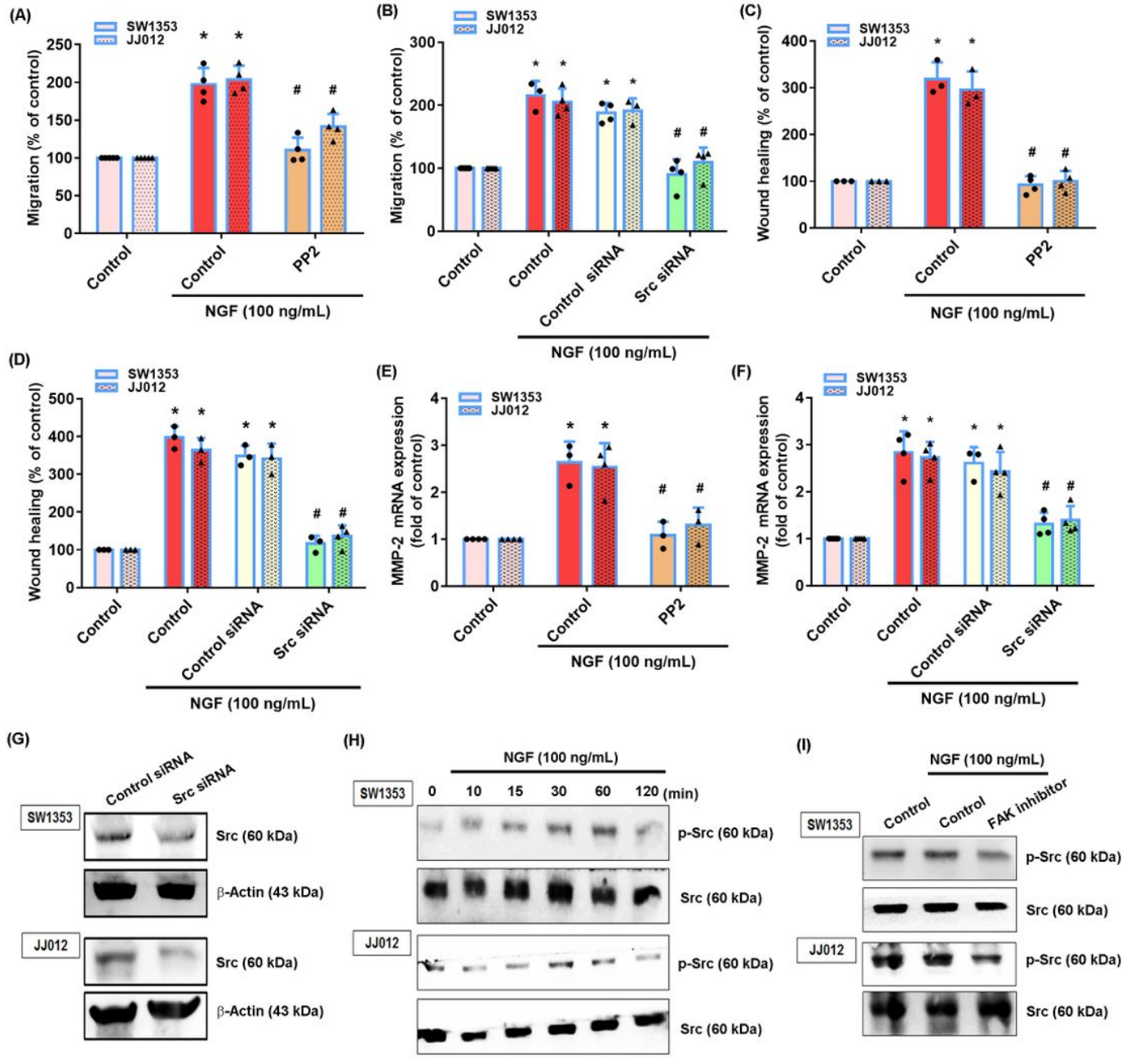

Figure 4

The c-Src pathway mediates NGF-induced MMP-2 expression and cell migration. (A-F) Cells were pretreated with a c-Src inhibitor (PP2) or transfected with a c-Src siRNA, then stimulated with NGF. Cell migration and levels of MMP-2 expression were examined by Transwell, wound healing and qPCR assays. (G) Cells were transfected with a c-Src siRNA and c-Src expression was examined by Western blot. (H) Cells were incubated with NGF for the indicated time intervals or pretreated with a FAK inhibitor

then stimulated with NGF; c-Src phosphorylation was examined by Western blot. * p < 0.05 compared with the control group; # p < 0.05 compared with the NGF-treated group.

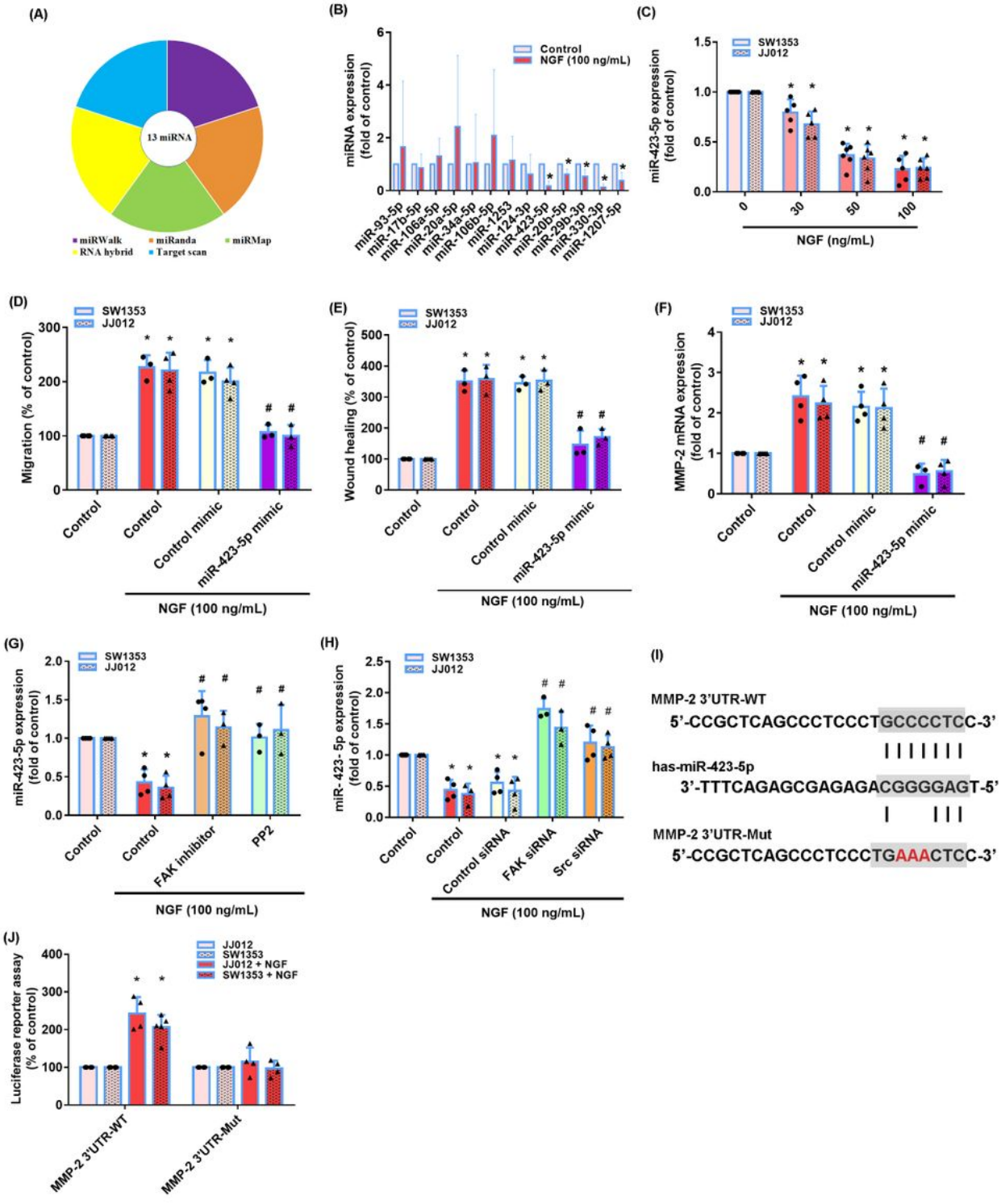

Figure 5

Inhibition of miR-423-5p mediates NGF-induced stimulation of MMP-2 expression and migratory activity of human chondrosarcoma cells. (A&B) MiRNA target prediction software was used to identify miRNAs that potentially bind to the MMP-2 3'-UTR. (C) SW1353 and JJ012 cells were incubated with NGF and

miR-423-5p levels were examined by qPCR. (D-F) Cells were transfected with miR-423-5p mimic then stimulated with NGF. Cell migration and MMP-2 expression levels were examined by Transwell, wound healing and qPCR. (G&H) Cells were pretreated with FAK and c-Src inhibitors, or an siRNA, then stimulated with NGF prior to qPCR analysis of miR-423-5p levels. (I) The wild-type and mutant MMP-2 3'-UTRs contain the miR-423-5p binding site. (J) Cells were transfected with 3'-UTR plasmids as indicated, then stimulated with NGF. Luciferase activity was examined. * $p < 0.05$ compared with the control group; # $p < 0.05$ compared with the NGF-treated group.

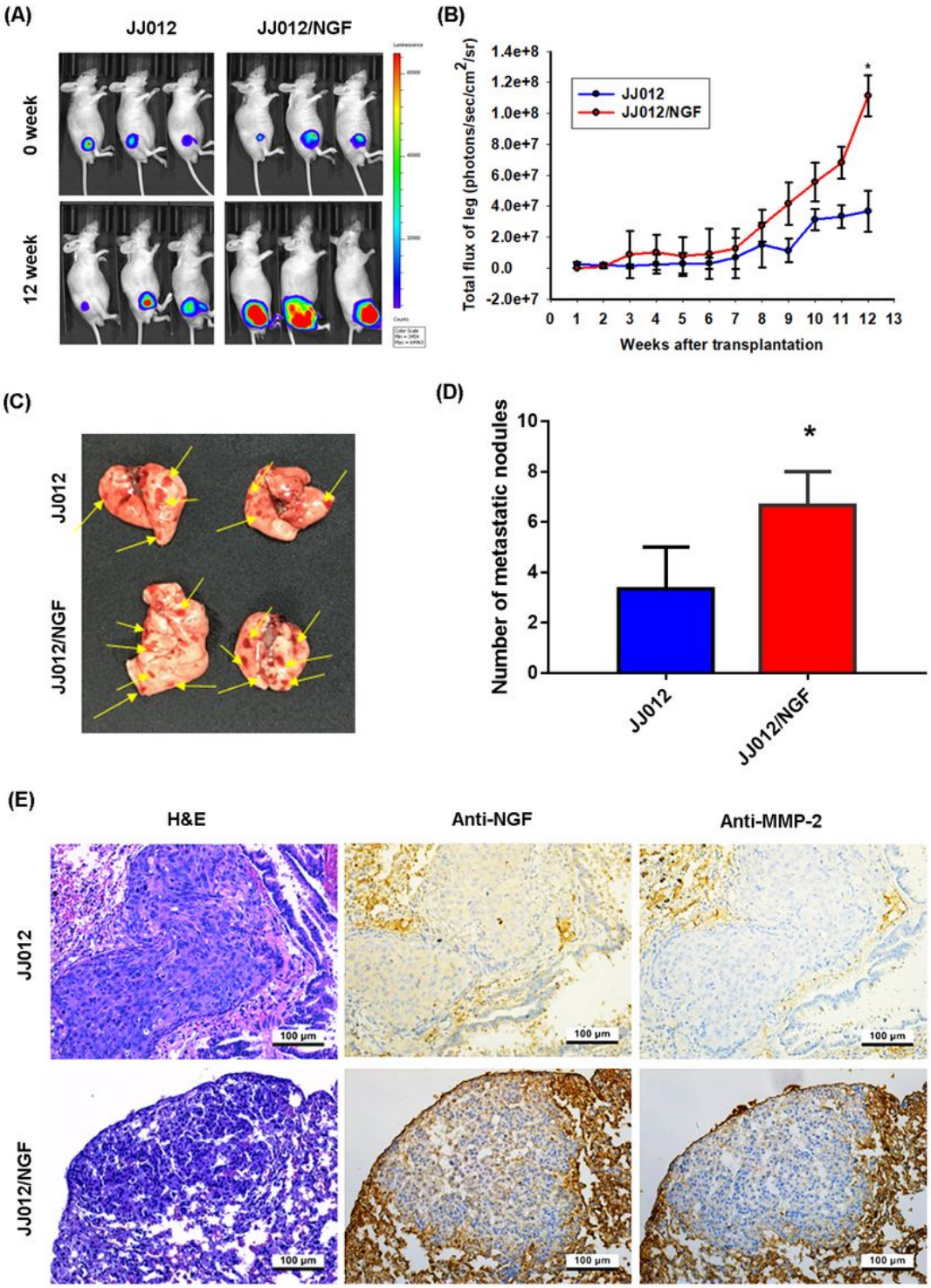

Figure 6

NGF promotes chondrosarcoma metastasis to lungs in vivo. (A-B) The mice were injected with JJ012 or JJ012/NGF cells. Lung metastasis was monitored by bioluminescence imaging at the indicated time intervals, then quantified by photon images. (C-D) After 12 weeks, the mice were humanely sacrificed and the lung tissue was excised, photographed and quantified. (E) Levels of NGF and MMP-2 expression in lung tumors were subjected to IHC analysis. * $p < 0.05$ compared with the control group; # $p < 0.05$ compared with the NGF-treated group.

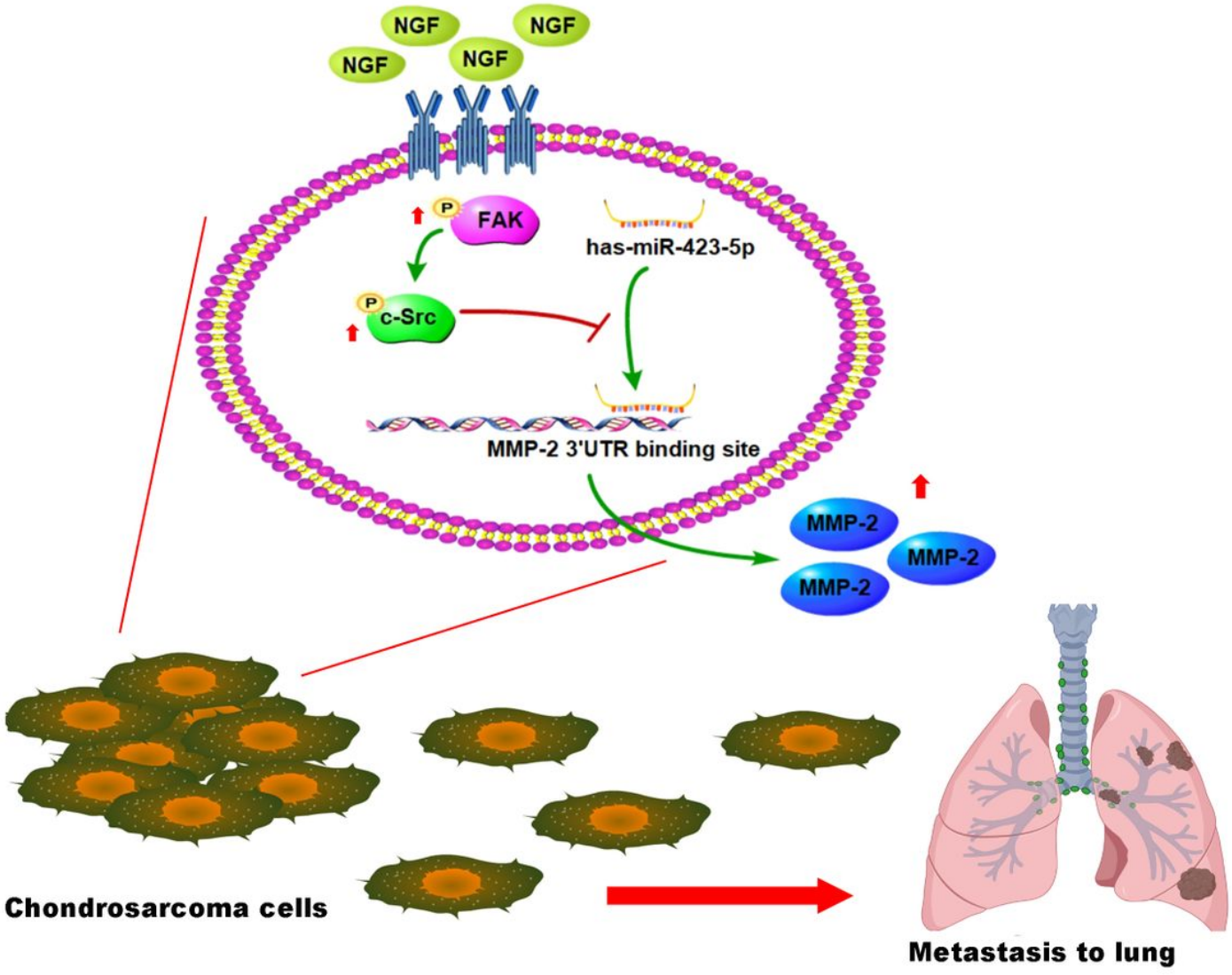

Figure 7

Schema illustrating the effects of NGF in chondrosarcoma metastasis. NGF facilitates MMP-2-dependent migratory activities of chondrosarcoma cells and metastasis by inhibiting miR-423-5p synthesis via the FAK and c-Src signaling cascades.