# Elucidating the Role of Osteosarcoma-Derived Exosomes and MicroRNAs in Reprogramming Skin Fibroblasts into Cancer-Associated Fibroblasts

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## INTRODUCTION

- Cancer-associated fibroblasts (CAFs) are essential components of the tumor microenvironment, promoting cancer progression through extracellular matrix remodeling, angiogenesis, and immune evasion.
- Osteosarcoma, an aggressive bone malignancy, has been shown to reprogram normal fibroblasts into CAFs via exosomal signaling.[1]
- Exosomes are extracellular vesicles that facilitate intercellular communication by transferring bioactive molecules, including microRNA (miRNAs).[2]
- miRNAs are small (~22 nucleotide), noncoding RNAs that serve as key post-transcriptional regulators of gene expression, influencing cell differentiation, apoptosis, and proliferation.[3]
- In the context of cancer, oncogenic miRNAs contribute to tumor progression by targeting tumor-suppressor pathways.[4, 5] Investigating these miRNAs within exosomal cargo provides insight into their role in osteosarcoma pathogenesis and potential therapeutic interventions.
- This study aims to explore the mechanism by which osteosarcoma-derived exosomes, particularly through their miRNA cargo, facilitate the transformation of normal human skin fibroblasts into CAFs.
- Our goal is to identify key miRNAs that may suppress tumor inhibitory pathways while enhancing pro-tumorigenic signals, leading to fibroblast reprogramming.

## METHODS

- Conditioned medium (CM) and exosomes were isolated from Saos-2 cultures and used to treat primary dermal fibroblasts, compared to untreated control (0% FBS).
- Cell proliferation was assessed using the AlamarBlue assay.
- Morphology was assessed and visualized using an inverted microscope.
- Fibroblast migration was tested using the scratch wound assay.
- Quantitative real-time PCR was performed to determine the levels of CAF-related genes, such as familial adenomatous polyposis (FAP), interleukin-6 (IL-6), vascular endothelial growth factor (VEGF), and the SRY-Box Transcription Factor 2 (SOX2).
- We also examined the expression of a panel of miRNAs previously associated with tumor progression, including miR-200a, miR-301, miR-223, miR-203, and miR-218.

## RESULTS

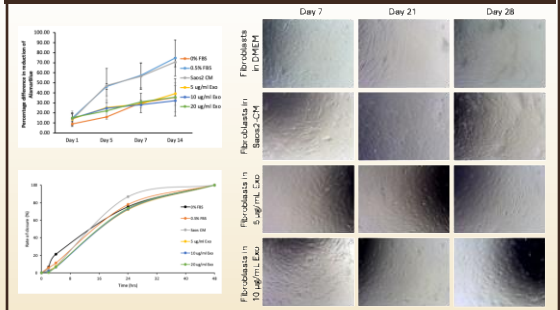


**Figure 1:** The proliferation and migration of human skin fibroblasts were analyzed following treatment with Saos-2 conditioned medium (CM) or exosomes. Fibroblast proliferation increased in response to Saos-2 CM, while migration remained unchanged. Additionally, fibroblasts exhibited a morphological shift from a spindle-shaped to a cobblestone-like appearance when cultured with Saos-2 CM or exosomes isolated from Saos-2 CM.

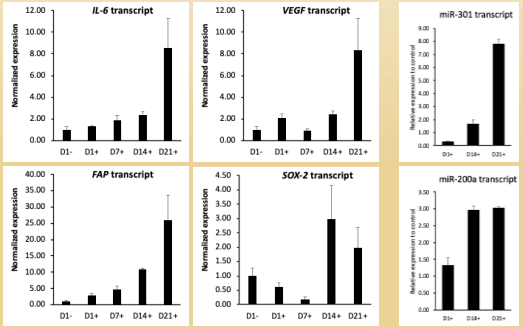


**Figure 2:**. Saos-2 conditioned medium enhances the gene expression of inflammatory IL-6, pro-angiogenic VEGF, and the cancer-associated fibroblast (CAF) marker FAP, along with SOX2, which plays a key role in fibroblast differentiation into CAFs. Additionally, it increases the expression of oncogenic miRNAs miR-301 and miR-200a.

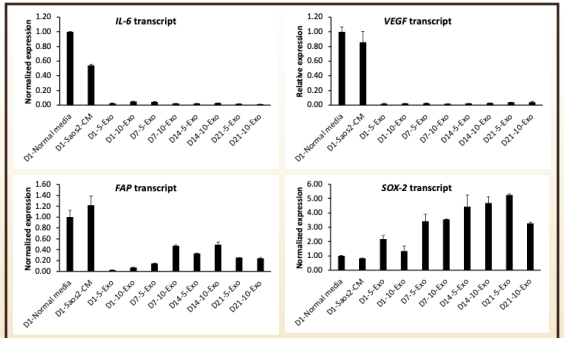


**Figure 3:**. Exosomes isolated from Saos-2 conditioned medium downregulate the gene expression of inflammatory IL-6, pro-angiogenic VEGF, and the cancer-associated fibroblast (CAF) marker FAP, while upregulating SOX2, which promotes fibroblast differentiation into CAFs.

## CONCLUSION

- Our findings suggest a novel mechanism by which osteosarcoma cells may reprogram dermal fibroblasts into cancer-associated fibroblasts (CAFs) through exosome-mediated delivery of oncogenic miRNAs.
- The upregulation of SOX2, FAP, IL-6, VEGF, and various miRNAs in fibroblasts exposed to Saos-2 conditioned medium provides evidence of a pro-tumorigenic shift in these cells.
- Understanding which specific miRNAs drive CAF activation could lead to the development of targeted therapies to disrupt tumor-fibroblast crosstalk within the tumor microenvironment.
- Future experiments will further explore the exosomal cargo, as it may contribute to fibroblast progression toward a carcinogenic phenotype.

## REFERENCES

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