Title: **Novel regulatory mechanism of cytoskeletal dynamics via Runx2 in metastatic breast cancer cells**

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Objectives: Bone metastasis is a significant contributor to breast cancer mortality with no current treatment available. Aberrant expression of the master regulator Runx2 facilitates bone metastasis. Recently, Runx2 has been shown to enhance autophagy, a process of degradation of damaged organelles and proteins into subunits for biosynthesis. During autophagy, autophagosomes traffic along microtubules (MTs). Acetylation of α-Tubulin subunit (Ace-α-Tub) stabilizes MTs and prevents it from buckling under the forces of vasicular trafficking. High levels of Ac-α-Tub and autophagy are independently linked with breast cancer metastasis. We found that Runx2 promotes Ace-α-Tub and MTs stability. However, questions regarding the mechanism of Runx2 mediated- Ace-α-Tub and MTs stability remain. We hypothesize that high levels of autophagy and Ace-α-Tub will have worse overall survival in bone metastatic patients and inhibition of autophagy and MTs stability will block bone metastatic tumor growth.

Methods: Using patient biopsies, bone-derived breast cancer cells, gene and tumor arrays, we examined autophagy, Ace-α-Tub, and dynamics of MTs polymerization. We evaluated the protein levels of autophagy marker microtubule associated light chain protein (LC3B) and Ace-α-Tub levels by western blot and immunofluorescence approaches.

Results: The tumor array showed a significant association between high levels of Runx2 and autophagy, while patient biopsies showed increased Ace-α-Tub staining in bone metastatic samples compared to primary tumors. Profiling of 168 autophagy and cytoskeletal-related genes revealed that the mRNA levels of majority of these genes were unchanged with Runx2 silencing. Furthermore, no significant changes were observed in the mRNA and protein levels of regulators of Ace-α-Tub (HDAC6, SIRT2, and ATAT1) with Runx2 silencing. Additionally, expression of WT and transcriptionally inactive mutant of Runx2 increased Ace-α-Tub and MT polymer mass, while a C-terminal deletion mutant failed to recapitulate these findings. These results indicate a novel transcriptional independent regulation of Ace-α-Tub via Runx2. We tested whether Runx2 alters activity or subcellular localization of regulators of Ace-α-Tub via protein-protein interaction. Inhibition of HDAC6 activity increased Ace-α-Tub and autophagy, while SIRT2 silencing resulted in minimal changes. We used Leptomycin-B to suppress protein shuttling between the nucleus and cytoplasm and found enhanced Ac-α-Tub in both control and Runx2 silenced cells.

Conclusion: Our results show that Runx2 alters localization and activity of the regulators of Ace-α-Tub. Our studies suggest that autophagy, Runx2, and Ac-α-Tub levels may help stratify bone metastatic patients for effective treatments with FDA approved MTs targeting agents.