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The roles of osteoclasts and RUNX2 in the progression of prostate cancer bone metastases

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av Junchi Huang

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Avhandlingen baseras på följande delarbeten:

I. **Junchi Huang**, Eva Freyhult, Jan-Erik Damber, Karin Welén. *Osteoclasts directly influence castration-resistant prostate cancer cells.* Manuscript.

II. **Junchi Huang**, Malin Hagberg Thulin, Jan-Erik Damber, Karin Welen. *The roles of RUNX2 and osteoclasts in regulating expression of steroidogenic enzymes in castration-resistant prostate cancer cells.* Mol Cell Endocrinol. 2021 Sep 15;535:111380.

III. **Junchi Huang**, Mikael Montelius, Claes Ohlsson, Matti Poutanen, Jan-Erik Damber, Karin Welén. *RUNX2 as a mediator of castration resistant prostate cancer growth in the bone microenvironment.* Manuscript.

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The roles of osteoclasts and RUNX2 in the progression of prostate cancer bone metastases

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Abstract

Metastasis to the skeleton is the major cause of death from prostate cancer (PC). Patients with metastatic PC are treated with androgen deprivation therapy (ADT) to decrease testosterone levels and thereby inhibit further growth of the tumor. However, a castration-resistant prostate cancer (CRPC) inevitably develops, often with activated androgen receptor (AR) signaling due to increased sensitivity of the AR or intratumoral steroidogenesis enabling AR activation despite castrate levels of testosterone in the circulation.

In the skeleton, PC cells interact with the bone microenvironment. It is known that degradation of bone matrix, one effect of androgen deprivation, releases growth factors stimulating tumor growth. We have previously identified the role of osteoblasts, bone-building cells, in promoting tumor growth and intratumoral steroidogenesis. A direct effect of their balancing counterpart, the bone-degrading osteoclasts, has previously not been investigated.

In the present thesis, osteoclasts were found to stimulate proliferation and inhibit apoptosis of both osteolytic and osteoblastic CRPC cells in an in vitro co-culture model. Gene expression was affected by osteoclast co-culture, more extensively so in the osteolytic model, where for example DNA repair genes were upregulated by osteoclasts. In both cell lines, genes related to endoplasmic reticulum stress-induced apoptosis were downregulated by osteoclasts. Osteoclasts were also found to increase expression of the osteoblast transcription factor RUNX2 in the osteoblastic CRPC cell line, while the high levels in the osteolytic cell line was not affected. RUNX2 was found to promote expression of steroidogenic enzymes and the androgen regulated prostate-specific antigen (PSA) in CRPC cells co-cultured with osteoclasts.

To evaluate the importance of RUNX2 for CRPC growth in bone, RUNX2 was knocked-out (KO) in osteoblastic CRPC cells which then was implanted in the tibia of immune-deficient mice. Magnetic resonance imaging (MRI) was validated and used to give accurate size and location of intratibial CRPC xenografts. It was shown that RUNX2-KO CRPC cells grew slower and formed smaller intratibial tumors compared to control cells. Both expression of steroidogenic enzymes and PSA-expression was inhibited by depletion of RUNX2.

In conclusion, this thesis show that both osteoclasts and RUNX2 affect CRPC growth and steroidogenesis. Treatment for metastatic PC include further targeting of the AR axis, a strategy that may be counteracted by both RUNX2 and interaction with osteoclasts. In addition, the effect of targeted therapy towards cells with defect DNA repair, such as PARP-inhibitors, may be affected by the action of osteoclasts. Thus, the results of the present thesis suggest targeting osteoclasts or RUNX2 in combinatory therapeutic approaches to be investigated.

Keywords: *Castration-resistant prostate cancer, Androgen, Bone metastases, RUNX2, Osteoclasts*

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