P208 (ND)

Adipocyte accumulation-induced increased tumor burden can be abrogated by antagonization of PPARγ

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Background/Introduction: Obesity has been linked to cancer progression in primary tumors in the past decade, though the relationship to secondary tumor manifestations remains unclear. This is especially true for metastasis formation with skeletal involvement, which is to date an abundant cause of death in tumor patients.

Purpose: In our work, we aimed to elaborate whether accumulation of adipocytes in the bone marrow causes the bone niche to be preferable for tumor cell infiltration and if so, why, and how to treat it.

Methods: Obesity in mice, accompanied by a strong increase in adipocyte accumulation in the bone marrow, was achieved by implementing a high fat diet (HFD) into the tumor models. By utilizing imaging techniques (MRI, CT), histology (IF, histomorphometry/-chemistry) and molecular analysis (qPCR, proteome) in two murine species (mice, rats) and two metastasis setups (melanoma, breast cancer), we covered a broad spectrum of experimental approaches. The animals were subjected to tumor cell inoculation methods inducing bone metastasis.

Results: Both murine species showed increased soft tissue and osteolytic tumor burden when being obese. IF of human bone metastasis biopsies confirmed our previous observations by showing a correlation (R^2=0.6372) between proliferation and adipocyte signal. As treatment approach, we applied the PPARγ antagonist Bisphenol-A-diglycidylether (BADGE) as adipocyte differentiation and storage inhibitor to lean and obese animals. Obesity-induced increase in osteolytic tumor burden was significantly (p=0.006) decreased with treatment. This development was accompanied by a decrease in pro-tumorigenic, inflammatory, and osteoclastogenic markers as well as a normalization of bone remodeling parameters, indicating increasing bone strength.

Conclusion(s): In conclusion, our study could show that adipocyte accumulation induces an increase in bone tumor burden, which can be reversed by inhibiting PPARγ. This opens up a novel treatment approach in obese patients to prevent loss in bone strength and prolong survival.

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P209 (ND)

Involvement of FXR in the OPG/RANKL pathway of breast and prostate cancer cells

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Background/Introduction: Bone is the first and most common distant metastatic site for breast and prostate cancers. Such metastases complicate cancer management, induce considerable morbidities and decrease patient quality of life and survival. Osteomimetism is part of the complex process of osteotropism of cancer cells. Our recent clinical and experimental data highly support a relationship between the expression of the bile acid receptor FXR in breast cancer and the propensity of tumor cells to develop bone metastases. RUNX2 is involved in this process and, subsequently, promotes synthesis of bone proteins, such as osteopontin, osteocalcin and bone sialoprotein (Absil et al., BMC Cancer, 2020).

Purpose: In the present study, we examined the impact of cancer cells on the OPG-RANK-RANKL pathway by assessing RANKL and OPG expression in breast and prostate cancer cell lines and FXR involvement in their regulation.

Methods: OPG and RANKL protein expression levels were evaluated by immunofluorescence in breast (MCF7, MDA-MB-231) and prostate (LNCap, PC3) cancer cell lines exposed to FXR agonist (chenodeoxycholic acid, CDCA) and/or antagonist (lithocholic acid, LCA). FXR depletion was conducted using siRNA strategy.

Results: We showed that FXR activation by the CDCA agonist significantly increased OPG expression in breast (130%) and prostate (115%) cancer cells ($p<0.05$, Anova and post hoc Dunnet's test), but did not change RANKL levels. Moreover, FXR inhibitors used in combination with CDCA decreased OPG expression and had no effect on RANKL. Silencing RNA against FXR validated OPG and RANKL results in breast cancer cells.

Conclusion(s): Therefore, FXR in metastatic cells may play a role (i) in the balance between bone formation and resorption, and (ii) probably in the survival of cancer cells by stimulating the production of OPG, a decoy receptor for RANK and TRAIL pathways.

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P210 (ND)

Whole-Body Vibration (WBV) affects perichordal and endochondral bone development and patterning in zebrafish larvae

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Background/Introduction: Whole body vibrations (WBV) are a potentially harmful non-chemical pollutant that can have severe effects on developing embryos. This is especially relevant at early stages of embryonic development, when organisms are most susceptible to defects and abnormalities.

Purpose: In this study, we used zebrafish embryos and larvae, aged between 10 hours post-fertilization hpf and 5 days post fertilization (dpf) to decipher the effect of exposure to WBV on the bone tissue of a living organism, during bone development and maintenance.

Methods: A vibration platform was developed to expose zebrafish embryos and larvae to controlled levels of WBV. Zebrafish aged between 10hpf and 5dpf were exposed to WBV frequencies of 20Hz (Low frequency vibrations) for up to 4 days. Fish were either fixed immediately after WBV treatment and used to perform *In Situ Hybridization* ISH or raised to SL5.5-6.6mm prior to fixation to perform Alcian Blue/Alizarin Red staining. These experiments were approved by the institutional Animal Care Committee, in line with the CCAC guidelines.

Results: Despite their mildness, these treatments were sufficient to induce a wide array of skeletal defects in zebrafish larvae. Depending on the developmental stage at which the exposure to WBV was started, different skeletal elements of zebrafish larvae were affected. WBV also differentially affected perichordal and endochondral bones. In fact, fish exposed to WBV starting at 10hpf were missing their ural (perichordal) bones, while exposure to WBV starting at 4 and 5 dpf primarily affected the hypural (endochondral) bone. ISH revealed the WBV effect on the hypural bone development was independent of *sox9a* expression.