Vitamin D_3 influence on breast cancer associated fibroblasts

Vitamin D_3 plays an important role in modulation of cancer cells by regulating the growth, progression or metastasis of breast cancer. Vitamin D_3 is also crucial in modulation of fibroblasts in fibrosis. However, the effect of vitamin D_3 on breast cancer associated fibroblasts (CAFs) has not been elucidated.

The aim of this study was to investigate the influence of vitamin D_3 on breast cancer associated fibroblasts. To achieve this goal, the following approaches were used: 1) healthy mice and mice bearing well-characterized breast tumors (4T1, 67NR, and E0771) were fed with vitamin D_3-normal, -deficient or -supplemented diet and treated with calcitriol, or 2) breast CAFs, isolated from tumor tissues from female patients with different plasma vitamin D_3 levels were stimulated with calcitriol *ex vivo*.

Vitamin D_3-supplemented diet which led to high vitamin D_3 metabolite plasma level, resulted in the development of lung fibroblasts (NFs) at the metastatic site of 4T1 tumor with reduced activation features (low level of αSMA, PDGFRβ, TNC). On the other hand, 4T1-bearing mice deficient in vitamin D_3 and treated with calcitriol developed activated NFs at the metastatic site (increase in αSMA, PDPN and TNC levels). Moreover, in mice with E0771 tumors, high plasma vitamin D_3 metabolite levels and calcitriol administration triggered the development of CAFs with the same phenotype (PDPN+, TNC+). The increase in PDPN and TNC could be associated with enhanced activation and pro-tumoral properties in CAFs in the context of immunosuppression and promotion of metastasis. In CAFs derived from patients with vitamin D_3 deficiency, stimulation with calcitriol resulted in predominant anticancer effects, both in terms of immunosuppression and promotion of metastasis by CAFs (decrease in levels of CCL2, TNC, IDO1, and MMPs). A similar effect was observed when triple negative breast cancer cells (MDA-MB-231) were treated with the medium from calcitriol-stimulated CAFs (decrease in OPN and ZEB1 levels).

Vitamin D_3-rich diet, calcitriol treatment *in vivo*, or calcitriol stimulation *ex vivo* may lead to anti- and pro-tumoral CAFs activity. Considering the inconclusive effects of vitamin D_3 or calcitriol, further studies are needed to clearly establish the validity of vitamin D_3 supplementation in various types of breast cancer.