Bone-derived signals activate PKA and promote resistance to AR-targeted therapies in prostate cancer.

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BACKGROUND: Bone metastasis (BM) in prostate cancer (PCa) is a milestone of disease progression closely associated with therapeutic resistance, largely due to tumor heterogeneity. Previous studies identified Protein Kinase A (PKA) as a central regulator of PCa-BM metabolism. Here, we investigate the role of PKA signaling in PCa–bone interactions and its contribution to treatment response.

METHODS: We performed transcriptomic analyses using publicly available datasets (GSE74685, SU2C-PCF, GSE32269, and Westbrooke et al.) to compare PCa samples across metastatic sites and treatment conditions. To model PCa–bone cell communication, we used a 24-hour indirect co-culture system with PCa cells (PC3/C42B) and osteoblast/osteoclast precursors (MC3T3/Raw264.7), integrating RT-qPCR, RNA-seq, and secretomic profiling (ESI-MS/MS) of conditioned media. PKA signaling was modulated using forskolin (FK; 1 µM) and H89 (10 µM). AR signaling was assessed via AR plasmid transfection and dihydrotestosterone (DHT; 10 nM) treatment. *In vivo* validation was performed using patient-derived xenografts (PDX) and cell line implants (intrafemorally-*i.f.* and subcutaneously-*s.c.*) in sham or castrated CB17 *SCID* mice. Statistical significance was determined by ANOVA and t-tests.

RESULTS: Secretomic analysis identified 65 proteins from MC3T3 and 38 from Raw264.7 cells upon co-culture with PC3 cells. Protein–protein interaction mapping revealed that bone-derived factors—including collagen and fibronectin—interact with PKA subunits and significantly activate PKA in PCa cells. *In vivo*, phospho-PKA substrate levels were elevated in PC3 xenografts growing i.f. compared to *s.c.*, confirming bone-induced PKA activation. Given the critical role of AR in PCa, we assessed how AR signaling impacts PKA activity in AR-overexpressing PC3 cells. Interestingly, DHT reversed FK-induced PKA activation. Similarly, castration of mice bearing C42B or MDA-PCa-PDX-183 bone xenografts led to increased PKA activity, as shown by IHC. Functionally, FK-mediated PKA activation increased the viability of C42B cells, MDA-PCa-PDX-183 and MDA-PCa-PDX-118b derived-organoids, and conferred resistance to enzalutamide in C42B cells ($P<0.05$). Integrated *in vitro*, *in vivo*, and *in silico* analyses revealed that PKA signaling drives expression of Osteopontin (SPP1), a known mediator of PCa bone progression. Notably, two BM patient subpopulations were identified—those with and without *SPP1* induction at the time of resistance to enzalutamide. Strikingly, IPA analysis showed a positive activation z-score for cAMP/PKA regulators in the SPP1-positive group.

CONCLUSIONS: Our findings demonstrate that the bone microenvironment activates PKA signaling in PCa cells through secreted factors, and that AR negatively regulates this axis. PKA activation promotes survival and resistance to AR-targeted therapy, positioning the PKA–AR interplay as a potential driver of therapeutic failure in bone-metastatic PCa.

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