also revealed negative regulation of gene sets involved in cell motility, cellular differentiation and growth pathways in BL cells lacking nuclear isoform c expression. These results suggest that NErbB-2 function modulates tumor growth and promotes a metastatic phenotype in TNBC. Furthermore, our clinical findings identified NErbB-2 as an independent predictor of shorter OS
(HR 2.54; 95% CI 1.22-5.28; P = 0.013), DFS (HR 2.91; 95% CI 1.44-5.87; P = 0.003), and DMFS (HR 2.59; 95% CI 1.20-5.60; P = 0.015) in 99 TN primary tumors. Our discoveries challenge the present scenario of drug development for personalized BC medicine that focuses on wild-type proteins, which conserve the canonical domains and are located in their classical cellular compartments, highlighting the potential of NErbB-2 isoforms as novel therapeutic targets and clinical biomarkers in TNBC.

Tumor Biology

HORMONE ACTIONS IN TUMOR BIOLOGY: FROM NEW MECHANISMS TO THERAPY

Osteoblast-Induced Prostate Cancer Cell Migration and Invasion Is Mediated Through TGF-β1/SMAD2 Signal Pathway and Blocked by 17α-Estradiol

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Prostate cancer (PCa) is curable if it is diagnosed and treated in localized and regional stage. However, PCa outcome is poor once it has distant metastasis. Approximately 70% to 100% of PCa deaths have bone metastasis, which may be associated with a specific bone microenvironment. In this study, we investigated the effect and molecular mechanism of osteoblast cells on stimulation of PCa cell migration and invasion and examined the effectiveness of 17α-estradiol on blocking osteoblast-induced PCa cell migration and invasion using in vitro cell analysis. PCa cells (PC3, LNCaP and DU145), osteoblast hFOB, kidney CV-1, breast tumor MCF-7 and liver cancer Huh-7 cells (ATCC) were cultured in RMPI-1640 or DMEM media supplemented with or without fetal bovine serum (FBS) at 37 °C in a 5% CO_2-humidified incubator. hFOB condition media (HCM) without FBS were collected at different times of hFOB cell culture. Transwell and wound-healing experiments were used to determine PCa cell migration and invasion. Cell migration and invasion in PC3, DU-145 and LNCaP PCa cells were markedly promoted by co-culturing hFOB osteoblast cells or HCM, but not by cells or condition media originated from kidney (CV-1), liver (Huh-7) and breast (MCF-7). Compared to other non-osteoblast cell conditioned media, HCM had much higher levels of several cytokines and chemokines including tumor growth factor (TGF) β1. Both HCM and TGF-β1 produced a dose- and time-dependent induction of PCa cell migration and invasion as well as SMAD2 phosphorylation without altering cell proliferation. These HCM and TGF-β1 effects were inhibited by a specific TGFβ receptor inhibitor, LY2157299, as well as by 17α-estradiol in a dose-dependent manner. Most intriguing, 17α-estradiol significantly inhibited the HCM and TGF-β1-induced PCa cell migration and invasion at very low nanomolar concentrations, presumably mediated through estrogen receptor β. These findings suggest that TGF-β1 is a major factor in mediating hFOB cell stimulation of PCa cell migration and invasion, and 17α-estradiol is a potential agent to block PCa cell bone metastasis, probably through inhibition of TGF-β1/SMAD2 signal pathway.

Tumor Biology

HORMONE ACTIONS IN TUMOR BIOLOGY: FROM NEW MECHANISMS TO THERAPY

Preclinical Evidence of the Efficacy of Lewis Y Car T Cells in Patient-Derived Models of Prostate Cancer

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Chimeric antigen receptor T (CAR T) cell therapy is an adoptive immunotherapy that has led to new treatments for lymphoma, leukemia, and other blood cancers; however, its efficacy for prostate cancer remains unproven. Here we report pre-clinical evidence of the efficacy of CAR T cell therapy against the Lewis Y antigen (LeY) using patient-derived models of prostate cancer. To assess the expression of LeY on prostate tumours, we performed immunohistochemistry on a cohort of 41 patient-derived xenografts (PDXs). Cytoplasmic and membrane expression were separately assessed and quantified, for each patient. Overall, 61% (25/41) of PDXs were positive for membrane LeY expression, of which 18 PDXs had greater than 50% membrane-positive cells, and considered most suitable to detection and stable binding by anti-LeY CAR T's. To determine the *in vitro* sensitivity to CAR T cytotoxicity, we selected 4 PDXs with high and 2 PDXs with low LeY expression using 3 androgen receptor (AR)-positive adenocarcinomas and 3 AR-negative tumors expressing neuroendocrine markers. Next we established organoids for *in vitro* co-culture assays where organoids were co-incubated with an equal number of anti-LeY+ CAR T cells or Empty vector control CAR T cells (Ev CAR T). Using time-lapse microscopy we reported destruction of organoids by LeY+ CAR T cells as indicated by their morphological collapse and uptake of propidium iodide from the culture medium; control Ev CAR T cells produced no cytotoxicity. Over the 48h assay, the level of target cell death of the LeY+ organoids was correlated to the intensity LeY surface expression. Target cell death mediated by the CAR T cells required perforin and granzyme B, as potent and highly specific small molecule inhibitors of perforin (SN34960) and granzyme B (C20) applied alone or in combination greatly decreased PI uptake, indicating organoid survival. Neither inhibitor adversely affected CAR T cell viability as measured by PI and Annexin V staining. This demonstrated canonical activation of granule exocytosis pathway by the CAR T cells, leading to organoid cell death. To assess CAR T cell efficacy *in vivo*, we selected one PDX