extracellular domain of plasma membrane integrin αvβ3 (PJ Davis et al., Physiol Rev 101:319-352, 2021). Induction of apoptosis in glioblastoma xenograft with chemically modified tetrac (P-bi-TAT) has yielded 90% in volume of grafts that continues after discontinuation of tetrac. In the present study, we show that human glioblastoma xenograft shrinkage in response to P-bi-TAT is associated with local appearance of phagocytic monocytes and clearance of apoptotic debris (efferocytosis). Primary culture xenograft of glioblastoma cells (GBM 052814, kindly provided by the University of Pittsburgh Medical Center, Department of Neurosurgery) and U87-luc (ATCC, Manassas, VA) xenografts were generated in 5-member groups of nude mice for each tumor cell type and for controls. Five days post-implantation, injection of animals was begun with PBS (control) or P-bi-TAT (10 mg/kg body weight). Injection was continued X21 days and animals were then maintained off-treatment for an additional 21 days. Tumors were harvested, formalin-fixed and slide-mounted, then analyzed by TUNEL assay for apoptosis and by anti-CD68 staining for monocytic macrophage content. Histologic analysis (H&E staining) was also carried out. TUNEL analysis and histopathology of both xenograft models revealed more than 90% apoptotic change with 21-days of P-bi-TAT treatment ($P < 0.001$) and persistence of 40% apoptotic change 3 weeks post-discontinuation of drug ($P < 0.001$ vs. end of treatment change). By H&E histology and CD68 analysis, monocytes accounted for more than 90% of the viable cells after 3 weeks' drug treatment. Sixty percent of the end-of-treatment monocyte population persisted 3 weeks after discontinuation of P-bi-TAT ($P < 0.001$). Histology revealed negligible cell debris after 3 weeks of drug treatment and at 3 weeks post-discontinuation of P-bi-TAT. Thus, the anticancer/pro-apoptotic action of tetrac-containing P-bi-TAT is associated with efferocytosis that contributes to the frank tumor shrinkage that results from P-bi-TAT treatment of human glioblastoma xenografts. This is the first documentation of efferocytosis regulated from the thyroid hormone analogue receptor on tumor cell integrin αvβ3.

Tumor Biology

HORMONE ACTIONS IN TUMOR BIOLOGY: FROM NEW MECHANISMS TO THERAPY

Cholecalciferol Mediates Apoptosis in Siha Cervical Cancer Line via Autocrine Mechanisms

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Cervical cancer disproportionately affects low-resource countries and is a significant health burden in South Africa. Pre-clinical studies have demonstrated numerous anti-cancer actions of vitamin D metabolites. Here, the anti-cancer action of the vitamin D precursor, cholecalciferol, was investigated in a high-grade cervical cancer cell line, SiHa. SiHa cell cultures were treated with a range of cholecalciferol doses (26 nM, 104 nM, 260 nM and 2600 nM) for 72 hours. Cell count and viability were assessed by crystal violet and trypan blue assays, respectively. Apoptotic cell death was investigated by flow cytometry, which measured mitochondrial membrane potential ($\Delta\Psi m$), phosphatidylserine (PS) externalisation, effector caspase activation and the expression of DNA damage markers. Additionally, brightfield microscopy and transmission electron microscopy (TEM) were respectively used to characterise morphological and ultrastructural features of apoptosis. Expression of the vitamin D metabolising system (VDMS) – consisting of cholecalciferol activating (CYP2R1 and CYP27A1), calcidiol activating (CYP27B1) and calcidiol inactivating (CYP24A1) enzymes, and the vitamin D receptor (VDR) – was assessed by qPCR and Western blots. Data were analysed using a one-way ANOVA and Bonferroni post-hoc tests and $p < 0.05$ was considered statistically significant. Significant decreases in cell count ($p = 0.011$) and cell viability ($p < 0.0001$) were identified in SiHa cells treated with 2600 nM cholecalciferol. Furthermore, biochemical markers at 2600 nM treatment were significant for apoptosis, and included decreased $\Delta\Psi m$ ($p = 0.0145$); increased PS externalisation ($p = 0.0439$); terminal caspase activation ($p = 0.0025$); and nuclear damage ($p = 0.004$). Moreover, biochemical apoptosis was corroborated by classical apoptotic features observed by brightfield microscopy and TEM. Additionally, a significant increase in CYP2R1 gene ($p < 0.0001$) and protein ($p = 0.021$) expression, and a converse significant decrease in CYP27B1 gene ($p = 0.003$) and protein expression ($p = 0.031$) were observed at 2600 nM cholecalciferol treatment. Furthermore, significant increases in VDR gene ($p = 0.033$) and protein ($p = 0.04$) expression, and CYP24A1 gene ($p < 0.0001$) and protein ($p = 0.0274$) expression were observed at 2600 nM cholecalciferol. In summary, high-dose cholecalciferol treatment of SiHa cervical cancer cells inhibits cell growth, induces apoptosis, and furthermore, upregulates CYP2R1 and VDR expression. Taken together, these findings suggest that autocrine activation of cholecalciferol to calcidiol may mediate VDR signalling of cell growth inhibition, and apoptosis in SiHa experimental cultures.

Tumor Biology

HORMONE ACTIONS IN TUMOR BIOLOGY: FROM NEW MECHANISMS TO THERAPY

Clinicopathologic Characteristics of Thyroid Nodules Positive for PTEN Mutations on Preoperative Molecular Testing

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Somatic and germline mutations of *PTEN* tumor suppressor gene are associated with follicular-pattern thyroid tumors and *PTEN* Hamartoma Tumor Syndrome (PHTS). The incidence of cancer in thyroid nodules positive for *PTEN* mutations on fine-needle aspiration (FNA) is not well defined. The aim of this study was to characterize diagnostic and phenotypic features of thyroid nodules with preoperatively detected *PTEN* mutations and their impact on management. Thyroid nodules with *PTEN* mutations on ThyroSeq v3 GC testing of FNA and core needle biopsy specimens from November 2017 to July 2020 were identified from the ThyroSeq Molecular Database. Demographic and