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

clinicopathologic data were obtained through retrospective chart review. We identified 49 *PTEN* mutation-positive nodules from 48 patients. Patients were 57 years old on average (range 14-88) and 80% female. Cytology was predominantly indeterminate (73% atypia of undermined significance, 18% follicular neoplasm). There were 18 (29%) frameshift, 6 (10%) splice site, and 39 (62%) single nucleotide variant *PTEN* mutations. Fourteen (29%) nodules had two *PTEN* mutations, 5 (10%) had copy number alterations, and single cases had concurrent *BRAF K601N*, *EZH1*, and *NRAS* mutations. Surveillance was pursued for 27 (56%) and surgery for 21 (44%) patients (16 lobectomies, 5 total thyroidectomies). There were 14 follicular adenomas (FA), 4 oncocytic FA's, 1 oncocytic hyperplastic nodule, and 1 encapsulated follicular variant papillary thyroid carcinoma (EFVPTC). The EFVPTC had two low-frequency *PTEN* mutations, *PTEN* locus loss, an *NRAS* mutation, and was a low-risk tumor with capsular but no angiolymphatic invasion. Four (8.3%) patients had confirmed or suspected PHTS, all with multiple nodules. Two had surgery finding no malignancies (2 FA). One PHTS patient had a prior thyroidectomy for a *MET* mutation-positive nodule that was follicular carcinoma. On US, the mean nodule size of patients who had surgery was larger than the surveillance group (3.2 cm vs. 2.3 cm, p=0.02) but there was no difference in TI-RADS level (p=0.54). There was no difference in mean nodule size (3.5 cm vs. 2.6 cm, p=0.35) or TI-RADS level (p=0.81) between PHTS and non-PHTS patients. Among surveillance patients, follow-up US was done at 1 year in 13/19 (68%) and 2 years in 3/6 (50%) of eligible cases. Only 1/19 (5%) underwent repeat FNA for increased nodule size. No thyroid malignancy was found with a mean of 1.75 years of follow-up (range 1.00-2.78). The EFVPTC patient had no recurrence after 1.05 years of follow-up. In summary, thyroid nodules with isolated somatic *PTEN* mutations are primarily benign and can be safely followed with serial imaging. Nodules with multiple *PTEN* mutations were only associated with malignancy when accompanied by an additional *NRAS* mutation. About 8% of patients with *PTEN* mutations may be PHTS patients who may be at greater risk for malignancy.

Tumor Biology

HORMONE ACTIONS IN TUMOR BIOLOGY: FROM NEW MECHANISMS TO THERAPY

Comparative Analysis of Different International Criteria (ACMG-AMP vs. TENGEN) Applied to Classification of Missense Germline Allelic Variants in Patients With Multiple Endocrine Neoplasia Type 1 or Suspected to this Syndrome.

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Context: Multiple endocrine neoplasia type 1 (MEN1) is a rare autosomal dominant genetic syndrome caused by germline pathogenic allele variants (PAV) in the *MEN1* tumor suppressor gene, which predispose *MEN1* carriers to the increased risk of several endocrine neoplasms throughout life. The *MEN1* gene (11q13), contains 10 exons encoding the MENIN protein. About 600 different PAVs have been reported, with 25% of them being missense variants. Of value, the definition of pathogenicity can be challenging, especially for missense variants. Thus, international guidelines for improving the classification of allele variants were recently defined by the ACMG-AMP (2015). Recently, applying ACMG-AMP criteria with inclusion of clinical features the TENGEN French group suggested modifications aiming to refine the classification of variants in MEN1 syndrome. **Objective:** To classify missense allelic variants found in the *MEN1* gene by the ACMG-AMP guideline using VARSOME and by the TENGEN group to support a comparative analysis of the results obtained with these two methodologies (ACMG-AMP; TENGEN). **Methods:** the classification of 16 different missense allele variants identified in 17 index cases with or suspected to MEN1 syndrome was conducted according to ACMG-AMP criteria using the VARSOME software followed by the analysis defined by the TENGEN group. **Results:** Of the 16 variants, 6 were new, 1 was recurrent (2 unrelated index cases) and 9 of them occurred in codons with previous reports of different amino acid exchanges in the same region. Differences observed in the classification by ACMG-AMP and TENGEN were: pathogenic variant (6% vs. 65%); probably pathogenic (88% vs. 12%) and variants of uncertain significance (VUS) (6% vs. 23%). The four VUS classified by TENGEN (one of them for ACMG-AMP) were of sporadic cases without clinical diagnosis of MEN1 (2, for one MEN1-related tumor in early age; 1, for suspected MEN1) or with high risk of phenocopy (1, HPT + acromegaly). **Conclusion:** The difference observed in the classification of the pathogenicity of these variants, especially due to the higher occurrence of VUS in TENGEN, indicates that the criteria adopted by ACMG-VARSOME would have to be refined for clinical features. By other side, TENGEN apparently reinforce the classification of pathogenicity in cases with clinical diagnosis of MEN1 and reduce the definition of pathogenicity to variants found in MEN1-suspected cases without clinical criteria for the MEN1 diagnosis. These protocols apparently need to be investigate, validated and, probably, improved in other cohorts to reduce risks of misinterpretations and classifications that can, lately, interfere in genetic counseling and in the clinical management of patients. Finally, long-term outcome of cases classified as VUS, functional studies and, familial segregation may reinforce the initial impressions obtained with TENGEN classification.

Tumor Biology

HORMONE ACTIONS IN TUMOR BIOLOGY: FROM NEW MECHANISMS TO THERAPY

Comprehensive Analysis of Clinical Features in Index Cases With Multiple Endocrine Neoplasia Type 1 Refine the Risk Rate for Detection of Mutation Distinguishing Negative-Mutation (Phenocopies) and Positive-Mutation Cases.

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