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Dehydroepiandrosterone (DHEA) is an androgen secreted by the adrenal glands, but its binding affinity for the androgen receptor is very low. DHEA is transformed into androstenedione by 3β-hydroxysteroid dehydrogenase (HSD) and then into testosterone by 17β-HSD type 5, or into estrone by aromatase. DHEA is also converted into androstenediol by 17β-HSD type 1. Therefore, DHEA is considered to play an important role as a precursor hormone for sex steroid hormones. We performed a search for a protein having an amino acid sequence homology to the DHEA binding site of 17β-HSD type 1, and found that microtubule-associated protein 2 (MAP2) binds to DHEA (Laurine E et al., J Biol Chem. 2003). MAP2 expression is necessary for neurite extension and cessation of cell division. MAP2 is known to suppress migration and invasion and affect the assembly, stabilization, and bundling of microtubules in melanoma cells, but the function of MAP2 in endometrial cancer has not been clarified. In this study, we investigated the expression of MAP2 and its association with DHEA in order to clarify the direct non-receptor action of DHEA in endometrial cancer. We employed frozen and formalin-fixed paraffin-embedded (FFPE) tissues of 35 endometrial cancer tissues (G1, n=12; G2, n=10; G3, n=9; Serous, n=4). Hormone concentrations were measured by liquid chromatograph-tandem mass spectrometer from the frozen sample, and immunohistochemistry of MAP2 was performed using FFPE tissues. We also examined MAP2 immunoreactivity using 59 normal endometrial tissues (proliferative phase, n=33; secretory phase, n=26) of FFPE tissue microarray slides. MAP2 immunoreactivity was found in the cytoplasm of endometrial cancer cells, and the MAP2-positive rate was significantly higher in type 1 (G1 and G2) than in type 2 (G2 and G3). The cell proliferation marker Ki-67 index was significantly lower in the MAP2-positive group. MAP2 was also detected in the glandular epithelial cells of the normal endometrium. The MAP2-positive rate was lower in the proliferative phase than in the secretory phase. Furthermore, the concentration of DHEA in the cancer tissue was significantly higher in the MAP2-positive group than in the MAP2-negative group. MAP2 is known to act on the stability of microtubules and is thought to be involved in the suppression of proliferation and infiltration in cancer cells. It was suggested that DHEA is involved in the stabilization of MAP2 and suppresses the progression of cancer in a hormone receptor-independent manner.

Tumor Biology

HORMONE ACTIONS IN TUMOR BIOLOGY: FROM NEW MECHANISMS TO THERAPY

Molecular Genetics in a Cohort of Patients With Concurrent PTC and Melanoma

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PTC and melanoma are known to harbour common mutations, but this has not been extensively investigated. Targeted therapies for BRAF and PD-L1 have been used for melanoma and there are ongoing clinical trials for use of PD-L1 inhibitors in PTC but its utility is uncertain. Additionally, many of these patients have multiple cancers, so, whether they have a tumour predisposition syndrome is also unclear. Both germline and somatic mutations in BRCA1-associated protein 1 (BAP1) are associated with a wide spectrum of tumours. We hypothesized that a common genetic link may be present in our cohort of patients who have both PTC and melanoma.

The aim of this study was to elucidate molecular genetics, specifically BRAF, NRAS, KRAS, KIT using OncoFocus Mass Array System as well as expression of PD-L1 and BAP1, using a standard antibody (SP263) and C-4 respectively, in an Australian cohort with concurrent PTC and melanoma.

In our cohort of 21 patients (43% females, all Caucasian), melanoma was diagnosed about 8 years prior to PTC (50.3 ± 18.3 vs. 58.6 ± 12.8 years). The most common mutation was BRAFV600E seen in 88% of PTC, followed by NRAS mutation in 12% of PTC. Majority of the PTC (68%) stained negative for PD-L1. There was no significant association between PD-L1 tumour status and clinicopathologic outcomes. Interestingly, majority of multifocal, bilateral and both bilateral and multifocal PTC were PD-L1 negative (85%,69% and 69% respectively, $P<0.05$); only extrathyroidal extension was found to be associated with positive (≥1%) PD-L1 staining (83.3 vs.30.8; $p=0.057$). Regarding melanoma, clinicopathologic and mutation data were obtained for 15/21 patients and 8/15 patients respectively. Superficial spreading type of melanoma was present in 50% patients. The BRAFV600E and NRAS mutation were present in 3/8 patients each, and 2/8 patients had no mutations. PD-L1 staining was negative in 7/12 (58%) of melanoma tissues. Of the 5 cases that stained positive for PD-L1, 4 were at >25%, a much higher degree of staining compared to PTC group. Among 7 patients where data were available for both tissues, concordant mutations were found in only 2 patients (both BRAFV600E). In addition, 11 of the 21 patients had at least one other cancer apart from PTC and melanoma. Nine of the 11 patients who had more than one cancer were BRAF positive. BAP1 staining was retained in the majority of PTCs and melanoma tissues, indicating no loss of BAP1 protein.

PTC and melanoma both share molecular markers including BRAF, NRAS, PD-L1 as shown in our cohort. This is the largest study describing the mutation status of both PTC and melanoma. It is also the only study describing the PD-L1 and BAP1 expression in PTC and melanoma. BRAFV600E was the most common mutation. Majority of the PTC and melanoma stained negative for PD-L1. BAP1 expression was retained in both either PTC and melanoma tissues thus making presence of BAP1 tumour predisposition syndrome unlikely.

Tumor Biology

HORMONE ACTIONS IN TUMOR BIOLOGY: FROM NEW MECHANISMS TO THERAPY

Novel Metformin Analogues for Treatment of Pancreatic Cancer

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Pancreatic ductal adenocarcinoma (PDCA) is a leading cause of cancer death in the US. Patients diagnosed with PDCA generally present with advanced disease with poor prognosis and limited treatment options. African American patients have higher incidence and mortality of PDCA than Caucasian American or any other ethnic group. Different factors have been attributed to contribute to this health disparity, among them higher incidence of Diabetes Mellitus type 2. To address the need for new therapeutic approaches, we note epidemiologic reports that patients with diabetes mellitus-type 2 treated with the biguanide drug metformin, but not other antidiabetic drugs, have a reduced risk of PDCA and an increased survival rate among those with PDCA. The main physiologic effect of metformin is to lower blood glucose and reduce hyperinsulinemia associated with insulin resistance. In the cell, metformin stimulates AMP-activated protein kinase (AMPK) that in turn inhibits mTORC1 which integrates signals from an array of intracellular pathways to regulate cell growth. Recent clinical trials describe modest antiproliferative effects from use of neoadjuvant metformin, but no significant clinical benefit occurred when metformin was dosed at glycemic control levels in patients with advanced cancers. These findings suggest that development of more potent anticancer analogues of metformin may help to boost clinical benefit and patient survival. Hence, we have designed new biguanide analogues of metformin, and screening of these compounds in preclinical PDCA models show that selected analogues are more efficacious in blocking tumor progression than parental metformin at lower doses. Using proliferation assays *in vitro*, PDCA cells (Panc 1, MIA Paca-2) were treated 72-hrs with metformin or analogues, and greater dose-dependent inhibition of PDCA cell proliferation was found with analogues as compared to metformin ($P<0.001$). Further, apoptosis was also markedly induced by metformin analogues as compared to parental metformin ($P<0.01$). Antitumor effects of metformin are attributed in part to activation LKB1-AMPK pathways and downstream blockade of mTOR signaling, which is often increased in PDCA cells. Using PDCA cells treated *in vitro* with analogues for 24-hrs, we find that analogues induce AMPK phosphorylation and suppression of mTOR signaling, thus blocking protein synthesis and tumor proliferation. With an *in vivo* PANC 1 xenograft model in nude mice, lead metformin analogues given by oral gavage daily significantly inhibited tumor progression over 28-days as compared to appropriate controls ($P<0.0001$). Our findings show that selected metformin analogues have potent anticancer activity in preclinical PDCA models and may have promise as new targeted therapeutics for patients afflicted with this deadly disease. [Funded by NIH/NCI R21CA176337 and NIH/NCI U54 CA143930]

Tumor Biology

HORMONE ACTIONS IN TUMOR BIOLOGY: FROM NEW MECHANISMS TO THERAPY

Nuclear ErbB-2-Induced Transcriptome Drives Triple Negative Breast Cancer Growth

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Triple negative breast cancer (TNBC) refers to tumors that do not express clinically significant levels of estrogen and progesterone receptors, and lack membrane overexpression or gene amplification of ErbB-2 tyrosine kinase receptor. Transcriptome and proteome heterogeneity of TNBC poses a major challenge to precision medicine. Gene expression analyses have categorized TNBC into distinct molecular subtypes. Up to 78% of clinical TNBCs belong to the basal-like (BL) subtype. Here we found ErbB-2 in an unanticipated scenario: the nucleus of TNBC (NErbB-2). Our study on ErbB-2 alternative splicing, using a PCR-sequencing approach combined with RNA interference, revealed that BL TNBC cells express the canonical ErbB-2 (WTErbB-2), encoded by transcript 1, and the non-canonical isoform c, encoded by alternative transcript 3 (T3). The latter was not previously reported in normal or malignant cells. To characterize the isoform c we designed siRNAs targeting T3 (T3 siRNAs), which silenced up to 93% of said isoform. Transfection of T3 siRNAs into BL cells expressing only isoform c or both isoform c and WTErbB-2 was sufficient to decrease cell proliferation. Intratumoral injections of T3 siRNAs into mice bearing BL TN tumors also blocked *in vivo* growth. To explore whether isoform c growth-promoting effect is due to its functions as a transcriptional regulator, we performed RNA-seq in BL cells expressing only this isoform. We identified a set of genes differentially regulated in BL cells where we evicted isoform c from the nucleus, as compared to control cells. In the up-regulated group, we found enrichment of pro-apoptotic and tumor suppressor genes and in the down-regulated one, genes involved in proliferation and stemness. We used gene set enrichment analysis (GSEA) to identify the biological processes associated with these isoform c-regulated genes. We found a pronounced enrichment of gene sets related to apoptosis, activation of DNA damage pathways and cell cycle arrest in response to eviction of nuclear isoform c. GSEA