Tumor Biology

HORMONE ACTIONS IN TUMOR BIOLOGY: FROM NEW MECHANISMS TO THERAPY

Markers of Lymphocytic-Macrophagal Infiltration and Their Association With the Receptor Phenotype and Proliferative Activity of Tumor Tissue in Various Molecular-Biological Types of Endometrial Cancer

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Background and Aims: The last years were characterized by a shift from the former subdivision of endometrial cancer (EC) into two main types [1, 2] to modern molecular biological classifications of this disease [3-5]. The purpose of this investigation was an attempt to compare such prognostic indicators for EC as features of lymphocytic [6] and macrophage infiltration of tumor tissue with markers of its hormonal sensitivity (receptor phenotype) and the proliferation index Ki-67 [7], taking into account the molecular biological type of the disease. **Materials and Methods:** The study involved material from untreated patients with endometrial cancer (a total of 219 people). The average age of patients was close to 55-60 years. Using classification of Talhouk et al. [5] allowed to perform a search for POLE mutations, evaluate by IHC the expression of the oncoprotein p53 and MMR (mismatch-repair) proteins / MLH1, MSH2, MSH6 and PMS2/, and also identify the type of disease without a characteristic molecular profile (WCMP). The IHC method was also used to study the rate of estrogen (ER) and progesterone (PR) receptors, Ki-67 proliferative activity index, as well as the severity of macrophage-lymphocytic tissue infiltration of EC based on the analysis of the macrophage (CD68) and lymphocytic cells (cytotoxic CD8 and regulatory FoxP3) markers using reagents from Ventana and Dako. Statistical assessment of the relationships of the studied indicators was carried out by the Spearman rank correlation coefficient. **Results:** FoxP3 (in contrast to CD8 and CD68) positively and significantly correlates (ρ varies from 0.2895 to 0.3477) more often with ER, but not with PR. Ki-67 index in EC tissue positively and reliably correlates with FoxP3 both in the MMR-D and WCMP groups and in the combined cohort of EC patients. In the latter case, a similar relationship with Ki-67 extends to other studied markers of lymphocytic-macrophage infiltration, namely CD8 and CD68 (ρ 0,1746-0,3294). Only in the entire group of EC patients there is a positive rank correlation (0.4119!) between ER and PR expression. **Conclusions:** In patients with certain types of EC the connection between the estrogenic signal and PR induction is lost; it is especially noticeable in the MMR-D group, as exemplified by the negative correlation (-0.2951) of FoxP3 and PR expression. Taken together with existing data this indicates an important role of the endocrine component for differentiating separate groups of patients with EC, that may also be of practical importance. **References:** 1. Bokhman JV. *Gynecol Oncol* 1983; 15: 10-17. 2. Suarez AA et al. *Gynecol Oncol* 2017;144(2):243-249. 3. Murali R et al. *Lancet Oncol* 2014; 15: e268-278. 4.Berstein LM et al. *Future Oncol.* 2017 13(28):2593-2605. 5. Talhouk A. et al. *Cancer.* 2017;123(5):802-813. 6. Gargiulo P. et al. *Cancer Treat Rev.* 2016;48:61-8. 7. Kitson S. et al. *Mod Pathol.* 2017; 30(3): 459-468.

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Mechanism of Mutant p53 Using Three-Dimensional Culture on Breast Cancer Malignant Phenotype via SREBP-Dependent Cholesterol Synthesis Pathway

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In many cancers, including hormone sensitive tumors such as breast cancer, the "gain of function" caused by mutations in the tumor suppressor gene p53 plays an important role in the acquisition of malignant phenotypes and the regulation of cancer stem cell characteristics. However, its action of molecular mechanisms, particularly in vivo conditions, has not been fully clarified. Therefore, we focused on the "gain of function" of mutant p53 and the cholesterol biosynthesis pathway, especially the mevalonate(MVA) pathway, using breast cancer cells, and clarified the interaction between them and the relationship with cancer malignancy using 3D-culture. Here, we generated knock out and knock in breast cancer cell lines for p53 using CRISPR-Cas9 system, and then confirmed malignant morphological changes by 3D-culture model. We found that the introduction of mutant p53 was solely able to mediate the malignant transformation of cancer. Next, focusing on the relationship between cancer malignant transformation and lipid metabolism pathway, we investigated the role of the MVA pathway in malignant transformation by mutation p53. When investigating the effects of the addition of HMG-CoA inhibitors and isoprenoids, intermediate metabolites were important for malignant transformation during 3D culture. Furthermore, knockdown of SREBP2, which controls the MVA pathway, suppressed malignant phenotypes, so we proceeded with analysis of the interaction between mutant p53 and SREBP2. As the result, we found that mutant p53 and SREBP2 co-localize in the nucleus and consistently mutant p53 was associated with mevalonate pathway genes in parallel with binding pattern of SREBP2. Thus, our results provide the novel insight into the potential therapeutic targets for breast cancer with poor prognosis.

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Microtubule-Associated Protein 2 as a DHEA Binding Protein in Endometrial Cancer

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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.

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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.

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Novel Metformin Analogues for Treatment of Pancreatic Cancer