mechanisms of malignant transformation and for the development of novel diagnostic and immunotherapeutic approaches in gynecologic oncology.

The aim of our study was to identify and characterize on the molecular level the ovarian cancer antigen MX35, which is recognized by monoclonal antibody MX35 (mAb) in 90% of human epithelial ovarian carcinomas. MAb MX35 was developed from mice immunized with fresh ovarian carcinoma cells and selected by extensive analysis of normal and malignant tissues and cell lines. Despite the use of humanized MX35 antibody and Fab2 fragments of mAb MX35 in several clinical trials in patients with ovarian cancer, the MX35 antigen has not been identified so far.

By screeneing an expression cDNA library from a MX35 positive ovarian cancer cell line (OVCAR-3) with MX35 mAb we identified the sodium-dependent phosphate cotransporter NaPi2b (SLC34A2, Napillb) as a likely candidate. NaPi2b is a membrane sodium-dependent phosphate cotransporter, which is involved in the regulation of inorganic phosphate metabolism and the maintenance of phosphate homeostasis. The identity of NaPi2b as MX35 antigen was further validated and confirmed based on the following experiments and results: 1) MX35 mAb specifically recognized largest extracellular loop of recombinant NaPi2b, 2) Co-typing of a panel of cancer cell lines showed a good correlation of SLC34A2 RNA expression as determined by RT-PCR and MX35 antigen cell surface expression as determined in a mixed hemadsorption assay using mAb MX35; 3) Selective down-regulation of SLC34A2 gene expression by RNA interference resulted in loss of mAb MX35 binding to MX35-expressing human cancer cells; 4) Recombinant NaPi2b proteins blocked binding of MX35 mAb to ovarian cancer tissues in immunohistochemistry.

In conclusion, we have identified sodium-dependent phosphate transporter Napi2b as a new ovarian cancer marker and a potential target for immunotherapy of cancer. Membrane transporter molecules, such as NaPi2b, represent a new family of potential cell surface targets for immunotherapy of cancer with monoclonal antibodies.

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TWIST1 overexpression is associated with nodal invasion and male gender in primary colorectal cancer

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Background: TWIST1 is a bHLH transcription factor that has been involved in tumor progression and metastasis in several cancer types, although no evidence has been provided yet on its implication in colorectal carcinogenesis.

Matherials and methods: To elucidate the involvement of TWIST1 in colorectal cancer we have examined the expression pattern of TWIST1 mRNA in 54 colorectal cancer biopsies compared to each respective adjacent normal mucosa by real-time reverse transcriptase PCR (RT-PCR) methodology.

Results: TWIST1 mRNA was found significantly overexpressed in cancer tissues compared to non tumorous colon mucosa ($p<0.0001$). Western Blot analysis was performed in some representative cases where TWIST1 mRNA levels in tumoral tissues were markedly increased. We observed that in all of these cases the protein levels were higher in tumoral tissues than in normal colon mucosa. Moreover, we have investigated the clinical relevance of TWIST1 overexpression. Receiver operating characteristic (ROC) curves analysis demonstrated that TWIST1 mRNA levels are significantly increased in patients with nodal invasion with the patient gender.

Conclusions: These findings provide the first evidence of the up-regulation of TWIST1 mRNA in colorectal cancer, suggesting its crucial role in the malignant progression of this disease.

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Investigation of melanoma progression and identification of novel prognostic markers using comprehensive tissue microarrays

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There is a clear need to improve our understanding of the molecular pathogenesis of melanoma in order to develop more effective prevention strategies, define new prognostic markers and to identify new molecular targets for therapy. The aims of this study were to firstly, develop a tissue microarray that includes all stages of melanoma development, secondly, investigate changes in the expression of key proteins during melanoma progression and thirdly, identify novel prognostic markers in primary melanomas by analysing the correlation between protein expression and patient outcome. The proteins chosen for investigation were B-catenin, bcl-2, and galectin-3 due to their central role(s) in adhesion, apoptosis and control of proliferation respectively.

A series of tissue microarrays which included 51 benign naevi, 27 dysplastic naevi, 54 in-situ melanomas, 312 primary melanomas and 64 metastatic melanomas were constructed in order to provide an efficient method of evaluating the expression of proteins by immunohistochemistry at various stages of melanoma progression. The collection of detailed clinicopathologic data for all patients with primary melanoma was undertaken in order to allow correlation of protein expression with several clinical parameters including site of melanoma and survival.

Changes in the expression of all 3 proteins during melanoma progression were seen. A significant fall in B-catenin, bcl-2 and galectin-3 expression between primary and metastatic melanomas and a rise in B-catenin and galectin-3 expression between naevi and dysplastic naevi were found. Correlation of protein expression with clinicopathologic data demonstrated that low nuclear galectin-3 expression was associated with poor survival (log-rank $p=0.0004$) and was an independent marker of poor prognosis (Hazard Ratio for death for low nuclear galectin-3 = 8, 95% CI 1.01-64, $p=0.05$).

These data reveal significant differences in expression of key proteins during melanoma progression and suggest that galectin-3 is a novel prognostic marker in primary melanoma.

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Vitamin D suppresses tumour growth and enhances cyto-toxicity of chemotherapeutic agents in cholangiocarcinoma

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Background: Cholangiocarcinoma (CCA) is a fatal cancer, poor prognosis and lacks effective therapy. Although CCA is rare worldwide, it is the most common cause of cancer death in people of the north-eastern Thailand where the incidence of CCA is highest in the world. Although surgery is potentially curative in selected patients, failure is usually occurred due to recurrence. Adjuvant or neo-adjuvant therapy by chemotherapeutic drugs has been shown to improve local control, provide palliation and prolong survival in various cancers; however, this is uncommon for CCA owing to its poor response to therapy. In the present study, we investigated the effects of a vitamin D_3 and its analog, on growth of CCA cell lines and growth of tumor in NOD-scid-Jak3 knockout mice. The possibility of using combination of vitamin D_3 or its analog and chemotherapeutic drugs to enhance the efficacy of anti-cancer drugs is demonstrated.

Materials and Methods: Vitamin D_3 or analog 0.5 and 1.0 μM were added in the culture of CCA cell lines for 24, 48 and 72 h. Viable cells were determined using MTT assay. CCA cells were inoculated subcutaneously to NOD-scid-Jak3 knockout mice. A group of 5 mice were injected intraperitoneally with vitamin D analog (10 μg/kg body weight) or buffer everyday for 20 days before being sacrificed.

Results: vitamin D_3 or analog inhibited cell proliferation in a dose and time dependent manner. Adding vitamin D_3 or analog to chemotherapeutic drugs (5-FU, mitomycin C and Pacitaxcel) significantly increased the effectiveness of anti-cancer drugs. Intraperitoneal injection of vitamin D analog significantly reduced tumor size without changing body weight and level of serum calcium comparing with those of the control group.

Conclusion: vitamin D or its analog effectively controls growth of CCA cells in vitro and in vivo. Using vitamin D or its analog as an adjuvant therapy to enhance cyto-toxicity of chemotherapeutic drugs may be an encouraged approach to increase the effectiveness of chemotherapy and improve prognosis in patients with CCA.

Co-supported by Research Strengthening Grant from BIOTEC-NSTDA, Thailand and the Royal Golden Jubilee Ph.D. program to Seubwai W and Wongkham S.