mutation and hypermethylation of hMLH1 promotor. MSS tumours from patients < 50, were tested for K-ras mutations in codon 12/13.

Results: MSI-high tumours (75/524) were negative in at least one of the four MMR IHC staining (14%). MSI-high tumours negative in MLH1 IHC (n=37), contained in 16% the BRAF V600E mutation and in 27% hypermethylation of the MLH1 promotor. BRAF mutation together with MLH1 methylation was present in 20 % of the cases. Most of the tumours showed MSS and were positive in the four MMR IHC (83%), In 85 MSS and MMR positive IHC tumours (patients < 50), K-ras mutations were detected in 26 cases (30,5%). We identified six different K-ras mutations and most of the DNA changes (80%) occurred at the second base of codon 12 and codon 13 (21/26).

Conclusions: A combination of both MSI and IHC provides the most optimal selection for mutation analysis. MLH1 methylation analysis in MSI-H CRC is a valuable molecular tool to distinguish between HNPCC and sporadic MSI-H CRC and the detection of a BRAF V600E mutation further supports the exclusion of HNPCC. A somatic transversions G>T in K-ras is associated with a biallelic mutation of MYH and was found in 34%. A K-ras G>A transition is associated with MGMT epigenetic silencing in sporadic CRC lesions and was found in 46 %.

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Cholangiocarcinoma associated blood transcriptome

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Background: Risk factor of cholangiocarcinoma (CCA), a major cause of cancer death in the northeast of Thailand, is disclosed to be the chronic inflammation related to liver fluke (Opisthorchis viverrini) infection. Association of inflammatory cells, particularly the monocyte-macrophage, with pathogenesis of chronic inflammation diseases and cancer is repeatedly reported. Here we report the gene expression profiling of the circulating peripheral blood cells from CCA patients compared to those of healthy persons.

Material and Methods: RNA was extracted from heparinized blood using TRIzol reagent and purified by Purelink kit. Biotinylated cRNA was synthesized and probed with Oligonucleotide array, HG_U133 plus 2. RNA expression levels were quantitated by measuring the Fluorescence intensity using Affymetrix scanner 3000. Eight differential expressed genes between CCA and healthy subjects were validated by real-time PCR.

Results: Expression profiles of peripheral blood from 9 CCA and 8 healthy subjects were compared. Principle component analysis indicated 49.2% difference between transcriptome of peripheral blood from CCA and those from healthy subjects. Ingenuity Pathway Analysis indicated the involvement in immune response and cancer for blood transcriptome from CCA patients, and the association with immune and hematological development for blood transcriptome from healthy subjects. A gene set coordinately up-regulated by the existence of CCA was obtained. Eight up-regulated genes were verified in homogenates of peripheral blood cells from 18 CCA patients. The obtained gene profiles were grouped according to the pattern to be tumor signature of CCA.

Conclusion: Our findings demonstrated that peripheral blood cells expressed genes which responded to CCA. Blood cells from CCA patients expressed genes involving in cell proliferation, angiogenesis, chemotaxis and anti-apoptosis pathways which may support growth and progression of CCA. In addition, the unique patterns of blood transcriptome may be surrogate transcriptional markers for CCA.

Supported by the Thailand Research Fund (BRG4980015) and the Royal Golden Jubilee Ph.D. program to Subimerb C and Wongkham S.

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Relevance of breast carcinoma extracellular matrix composition in drug sensitivity

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A new view of tumors as functional tissue intimately connected with its microenvironment has recently been presented. It is well-established that tumor cells must reorganize the extracellular matrix (ECM) to facilitate communication and escape the homeostatic control exerted by the microenvironment. The identification of the tumor matrix composition represents an important step toward developing response prediction tests and toward the goal of increasing cancer cell sensitivity by modifying their interaction with the microenvironment.

A statistical analysis of microarray (SAM), performed on a dataset obtained from a cohort of breast carcinoma patients treated with docetaxel as neoadjuvant therapy, using sensitivity as supervised variable among a gene list enriched in ECM molecules, resulted in a list of 47 genes that consist of several collagens, laminin chains, matrix-associated proteins as SPARC, and proteolytic enzymes as ADAMs and Cathepsins significantly downmodulated in resistant tumors. Furthermore SAM method using as supervised variable the disease progression resulted in 2 genes significantly downmodulated in tumors with a residual disease major than 100%: PRSS22, a serine protease and CTSC, a cysteine protease that appears to be a central coordinator for activation of many serine proteases, and in 4 upmodulated genes among which serpinB5, an inhibitor of serine proteases, presented a fold increase of 13.4. Based on these data, we hypothesized that tumor protease levels through remodeling of mammary tissue, could play a critical role in drug response. To test this hypothesis, drug response of MDA-MB-361 breast carcinoma cells xenotransplanted in nude mice was evaluated in presence or absence of doxycicline, an inhibitor of collagenolitic enzymes. Taxol or doxorubicin activity was impaired in doxycicline treated mice by 73% and 61%, respectively. Evaluation of collagenases levels in animal plasma and xenotransplants showed that doxycycline significantly reduced both plasma and tumor enzymes level providing evidence that the blocking of tumor proteases expression leads to drug resistance.

The secreted proteolytic enzymes (from both the cancer and stromal environment) are belived to degrade the extracellular matrix, thus facilitating drug diffusion. (Partially supported by a grant from AIRC)

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Re-assessment of Smad-interacting protein 1 expression in human tumor tissues

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Smad interacting protein 1 (SIP1, also known as ZEB2) encoded by ZFHX1B is a member of ZEB family of transcriptional repressors. SIP1 has been reported to mediate epithelial-to-mesenchymal transition (EMT) in developmental processes and during tumor cell invasion and metastasis by attenuating E-cadherin expression. However, due to the lack of human SIP1-specific antibodies, the expression of SIP1 in human tumor tissues has never been studied. Hence, by immunizing BALB/c mice with a partial human SIP1 recombinant protein (aa 1-400), we generated two monoclonal antibodies, clones 1C9 and 6E5, with IgG1 and IgG2a isotypes, respectively. A squamous epidermoid carcinoma cell line A431 with Tet-on doxycycline-inducible wild-type SIP1 expression (A431/WTSIP1) was used to assess the specificity of generated MAbs. Using both antibodies, nuclear expression of SIP1 in A431/WTSIP1 cells maintained in the presences of doxycycline for 24 hours was detected by immunofluorescence. By using MAb 6E5 we evaluated the tissue expression of SIP1 in paraffin-embedded tissue microarrays consisting of normal and tumoral tissues of kidney, colon, stomach, lung, bladder, esophagus, uterus, rectum and liver. Interestingly, SIP1 mainly displayed a granular cytoplasmic expression and to a lesser extent nuclear localization. The examination of normal tissues revealed a strong expression of SIP1 in distal tubules of kidney, parietal cells of the basal layer of stomach and lobular hepatocytes, implicating a co-expression of SIP1 and E-cadherin. Squamous epithelium of the esophagus and surface epithelium of the bladder, colon and rectum were faintly stained. Normal uterus and lung tissues remained completely negative. By comparison with normal tissues, we observed SIP1 overexpression in renal cell carcinoma, stomach adenocarcinoma, squamous carcinoma of the lung and adenocarcinoma of the uterus. SIP1 was found to be down-regulated in hepatocellular carcinoma and bladder transitional cell carcinoma, and no differential expression was found between normal and tumor tissues of colon and rectum. To our best knowledge, this is the first immunohistochemical study of the expression of SIP1 in human cancers. Our finding that SIP1 is not exclusively localized to nuclei suggests that the subcellular localization of SIP1 is regulated in normal and tumor tissues. This observation adds another level of complexity to the control of EMT programs in tumors progressing towards metastatic state.