On the other hand, the peak of fluorescence degree of a nutrient medium showed 2−10 times higher when compared to the fluorescence degree of a nutrient medium with a cell. We observed the existence of PpIX in the culture medium, however degree was different. We think that 5-ALA-induced PpIX formed by the brain tumor cells leaks out to the outside of the tumor cells.
Conclusions: Each brain tumor cell generated PpIX by the 5-ALA, and 5-ALA-induced PpIX was leaked out to the outside of brain tumor cell.

366 POSTER

Mechanism of the initiation of DNA methylation de novo by small RNA

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DNA methylation is an important epigenetic mechanism that assigns and maintains gene expression profile and thus enables cell differentiation, allelic exclusion and other key phenomena. We investigate possible role of small interfering RNA and microRNA in the DNA methylation de novo.
Human, mouse and rat sequences of siRNA – in all 599 sequences – were extracted from database "siRNA Database and Resources for RNA Interference Studies", http://www.rnainterference.org/Sequences.html. Human, mouse and rat sequences of mature miRNA – in all 1083 sequences – were extracted from database miRBase, http://microrna.sanger.ac.uk/.
We discover only 14.36% siRNA sequences and 20.68% mature miRNA sequences, containing none of 5′-CG-3′ dinucleotides or 5′-CNG-3′ trinucleotides. 5′-CG-3′ frequency amounts to 2.89% in siRNA sequences and 2.39% in mature miRNA sequences. This level exceeds more than twice the average genomic frequency of 5′-CG-3′ dinucleotides, that makes up 1% in human or mouse genome, and 1.2% in rat genome. 5′-CNG-3′ frequency amounts to 6.29% in siRNA sequences and 6.49% in mature miRNA sequences. Nevertheless, the 5′-CNG-3′ or 5′-CG-3′ frequency should theoretically make only 4.41% in random human DNA sequence, though this frequency appeares to be in reality much less as a result of 5-methylcytosine hypermutability.
Thus, 5′-CG-3′ and 5′-CNG-3′ sites are discovered in siRNA and miRNA sequences more often than they should be found in random sequence. This circumstance is evidence of an important biological purpose of 5′-CG-3′ dinucleotides and 5′-CNG-3′ trinucleotides in siRNA and miRNA sequences.
In our opinion, complexes of small RNA and Argonaute protein scan nucleotide sequence of DNA strands while RNA polymerase II is untwisting DNA molecule during the transcription. Recognition and binding of complementary site in DNA by siRNA leads to recruiting of DNA methyltransferases that methylate de novo cytosine in 5′-CG-3′ dinucleotides and 5′-CNG-3′ trinucleotides of DNA, which appeared to be bound with similar sites in the siRNA sequence. Histone deacetylase and histone methyltransferase are also attracted to DNA site, which was recognized by small RNA. They delete active chromatin marks. Several genes can be switched off simultaneously when they contain the motif, which is recognized by small RNA. We suppose that gene modules (elementary units of cell differentiation network) contain miRNA genes, which are activated in certain moments for the purpose of stable epigenetic repression of other gene modules that complete their mission in course of cell specialization or are responsible for other differentiation directions.

367 POSTER

Statistical correlations around the transcription initiation site in the DNA sequences of human promoters

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Background: Genetic control sites, such as promoters, generally have a characteristic consensus sequence. We have been interested in variation about the consensus sequence, especially correlations, i.e. the tendency of a particular base at one position to be associated with a particular base at another. This work addresses correlations in the region of the transcription initiation site, and extends our analysis of correlations upstream of that site in the region of the TATA motif (ESMO 2006, abstract 113P).
Methods: A dataset of 1975 promoters recognised by human RNA polymerase II was assembled from the Eukaryotic Promoter Database. Many of these promoters are of interest in oncology and the dataset includes sequences for the promoters of genes for growth factors (e.g. GM-CSF, erythropoietin, various interleukins) oncogenes and tumour viruses among others. For the 30-base sub-sequences from positions −19 to +10 relative to the transcription start, the consensus sequence was derived. The sequences were coded numerically and a correlation analysis performed. A principal components analysis enabled those promoters with the most similar sequences to be grouped taking account of the correlations.
Results: The consensus sequence was observed to be ggggg gc(c/g)cg ggggg cggca ttgcg gccgg. There were numerous statistically significant correlations, and 51 of these were greater in absolute value than 0.103 and thus very highly significant ($P < 0.000005$). As many as 38 of these correlations were positive and the rest negative. Almost half the correlations concerned bases in the range −2 to +6, i.e. at the initiation site or the first few transcribed bases. For example, a purine (an A or a G) at position −1 was associated with a purine at position 0, an A or a T at position 1 was associated with a C or a G at position 2. Almost all the highly significant correlations concerned bases separated by a few positions at most.
Conclusion: We have already shown significant correlations in the DNA sequences of human promoters associated with the TATA box; we now show comparable correlations at the transcription initiation site. Thus the variation among the sequences is seen not to be random. Principal components analysis allows groups of promoters with similar sequences to be defined, which may have similar functional properties.

368 POSTER

Genetic instability at 9p21 and its significance as prognostic indicators in liver fluke related cholangiocarcinoma

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Cholangiocarcinoma (CCA) is the highest incidence cancer in Northeast Thailand. CCA is caused by liver fluke, Opisthorchis viverrrini, infection resulting in genetic alterations. Loss of heterozygosity (LOH) and microsatellite instability (MSI) are the phenotypes of genetic instability caused by the abnormalities of tumor suppressor and DNA mismatch repair (MMR) genes. We investigated LOH and MSI on the chromosomal region 9p21-pter in 94 CCA patients using 6 microsatellite markers and determined the association between microsatellite alterations and clinicopathological parameters. A total of 59 out of 94 cases (62.8%) showed LOH in one or more loci. LOH was found most frequently at D9S157 (36.1%), D9S286 (34.2%) and D9S1752 (34%). MSI was found in 50 of 94 cases (53.2%) at one or more loci. Fine mapping at 9p21-pter showed a distinctive region of common loss, a region between D9S157 and D9S1752, indicating the existence of putative tumor suppressor genes that is likely to play important roles in the development of CCA. Tumor suppressor genes located at 9p21 are cyclin-dependent kinase inhibitor 2A (CDKN2A)/p16INK4A, CDKN2A/p14ARF, CDKN4B/p15INK4B, MTAP and interferon beta-1 (IFNB1). Nuclear factor 1 (NF1B) and endophilin-1 are located at D9S286 and D9S157 of chromosomal regions 9p24 and 9p22, respectively. Patients with LOH at D9S288 ($P = 0.022$) and D9S286 ($P = 0.043$) showed more blood vessel invasion while patients with LOH at D9S161 exhibited more lymphatic invasion than those without ($P = 0.015$). Moreover, patients who demonstrated LOH at D9S171 showed a poor prognosis ($P = 0.0296$). Our studies suggest that genetic alterations of tumor suppressor genes and DNA mismatch repair genes are involved in carcinogenesis and pathogenesis of liver fluke related CCA and genetic instability of 9p21 is of value as prognostic indicators in this cancer.

369 POSTER

Mismatch repair proteins and clinicopathologic factors in colorectal cancer

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Background: Microsatellite instability due to defective mismatch repair proteins (MMRP) is one of the major pathways for carcinogenesis in colorectal cancer (CRC). The impact of these proteins in prognosis is not well defined. The aims of this study were the evaluation of abnormal MMRP prevalence and its relationship with some clinical and pathologic factors.
Materials and Methods: In our study 350 patients with CRC were immunostained for DNA mismatch repair proteins (MMRP) including hMLH1, hMSH2, hMSH6 and PMS2. Patients with at least one abnormal above factors considered in abnormal MMRP group. Clinical factors such as sex, tumor site (colon or rectum), family history of CRC and vital status (alive or dead) is considerd. Pathologic factors including grade, T and N stage in tumor specimen were examined.
Results: Totally 350 patients with median age of 51 (20 to 94) were evaluated. One hundred ninety five patients were male and 151 were female. The site of tumor in 270 patients was colon and in 68 were

rectum. Among 329 patients with known family history, 132 (40.1%) had positive FH. The overall rate of abnormal immunostaining for hMLH1, hMSH2, hMSH6 and PMS2 were 4.4%, 7.2%, 3.5%and 4.9% respectively. Overall 44 patients (12.7%) had at least one abnormal MMRP staining. Abnormal MMRP were not significantly associated with histopathologic factors including T stage, N stage and grade. There was no difference in MMRP staining as a result of whether patients were male or female except PMS2 that was significantly more abnormal in male ($p = 0.083$). Abnormal staining of MMRP were seen further in colon than in rectum that was significant for hMLH1 ($p = 0.044$). Patients with family history of CRC had more abnormal staining that was significant for hMSH2 ($p = 0.061$). There was no difference in MMRP staining according to vital status.
Conclusion: Our results suggest that abnormal MMRP is associated with clinical factors such as family history of CRC but not with pathologic factors. Abnormal MMRP is more important pathway for carcinogenesis in colon than rectal cancer.

370 POSTER

Two pathways of carcinogenesis in patients with colorectal cancer less than 45 years old

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Background: Colorectal cancer (CRC) arises from a complex series of molecular changes that involve at least two different pathways. These include microsatellite instability (MSI) pathway and chromosomal instability (CIN) pathway. The aim of this study was the determination of predominant pathway involved in carcinogenesis of patients with CRC less than 45 years old with and without family history (FH) of CRC.
Materials and Methods: In our study surgical pathology specimens of 108 patients with CRC less than 45 years old were immunostained for DNA mismatch repair proteins (MMRP) including hMLH1, hMSH2, hMSH6 and PMS2. Beta-catenin and P53 were also examined for CIN pathway.
Results: Totally 108 patients with median age of 40 (20−45) were evaluated. Fifty seven patients were male and 51 were female. The site of tumor in 84 patients was colon and in 14 were rectum. Among 96 patients with known family history, 33 (34.4%) had positive FH. The overall rate of abnormal immunostaining were MLH1 8.3%, MSH2 18.5%, MSH6 8.3%, PMS2 11.1%, P53 74.1% and beta catenin 35.2%. Meanwhile abnormal staining for hMSH2 and hMSH6 were significantly more seen in patients with positive family history ($p = 0.008$ and $p = 0.032$ respectively). Patients with positive FH for CRC had significantly more abnormal MMRP (54.5% vs. 20.6%, $p = 0.001$) and less positive p53 (54.5%vs. 81%, $p = 0.006$) than patients with negative FH. Patients with early T, N stage tumor had at least one more abnormal MMRP than advance T, N stage ($P = 0.050$ for T and $P = 0.030$ for N stage). Among different factors abnormal hMSH2 had significant association with lower cancer related death ($P = 0.060$). Patients with rectal cancer had more abnormal MMRP than patients with colon cancer but not significantly (35.7% vs. 29.8%, $p = 0.655$) and positive p53 staining for rectal and colon cancer were 71.4% and 72.6% respectively. Both in colon and rectal cancer patients with negative family history had more prevalent positive p53 (80.4% vs. 56.7%, $p = 0.022$ for colon and 81.8% vs. 33.3%, $p = 0.099$ for rectal cancer).
Conclusion: Our study indicates that even in CRC less than 45 years old, the main pathway for carcinogenesis in patients with negative family history is CIN, but in positive family history MSI is as effective as CIN. However main pathway in both colon and rectal cancer is CIN.

371 POSTER

Activation of signalling pathways by increased expression of HC GP-39 in brain tumors

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Background: The aim of this research is determination and characterization of potential molecular markers for human brain tumors and their possible interaction with main signal pathways in eukaryotic cells. Such knowledge is necessary not only for understanding the tumorigenesis, but also the mechanisms of normal brain functioning.
Materials and Methods: Diffrentially expressed genes were determined by Serial Analysis of Gene Expression (SAGE); gene expression have been analysed by Northern hybridization.
Results: The comparison of 9 glioblastoma and 5 human adult normal brain SAGE-libraries revealed 129 genes with >5-fold differences, 44 of them met the criteria for genes overexpressed in tumors. The majority of these genes are related only to a few functional groups: genes encoding proteins involved in angiogenesis, immune response, extracellular matrix, drug-resistance, and several genes are related to the mitogen-activated protein kinase cascades: CD74, EGFR, CTGF, IGFBP5, IGFBP7, and IGFII. We found unusual processing of IGF-2 primary transcript in meningiomas and ependimomas, anomaly expression of IGF-II may contribute towards tumorigenesis. Increasing of IGF-I gene expression was not found in glioblastomas. It is possible to suppose that glial tumor development is activated by some other way. C hitinase 3-like 1 gene encoding human cartilage glycoprotein-39 (HC gp-39 or YKL-40) was among the most upregulated genes and as was shown recently, it initiates cellular responses very similar to those elicited by IGF-1: activates both extracellular signal-regulated kinase (ERK) – and protein kinase B (AKT)-mediated signalling cascades, which are associated with the control of mitogenesis. Both proteins act synergistically with respect to their growth-stimulating activity; both suppress the cytokine-induced secretion of MMPs.
Conclusions: Since deregulation of the IGF system and HC-gp39 is a frequent pattern in tumors, IGFs/IGFBPs/HC-gp39 should be included in the panel of tumors markers used for histopathological diagnosis and serological surveillance procedures in various malignancies. Novel antisense and iRNA strategies targeting components of IGF-axis and HC-gp39 may offer additional options for treatment of malignant gliomas.

372 POSTER

Role of hepatocyte growth factor/c-met signaling in regulating urokinase plasminogen activator on invasiveness in human hepatocellular carcinoma: a potential therapeutic target

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Background: Hepatocyte growth factor (HGF), its transmembrane tyrosine kinase receptor (c-Met) and urokinase type plasminogen activator (uPA) is a key protein in the plasminogen activation system, which plays a proteolytically important role in the invasion and metastasis of various types of cancers. However, the mechanisms by which HGF/c-Met signaling mediates cancer progression and metastasis are unclear.
Methods: this study was designed to investigate the roles of HGF/c-Met in tumor progression and metastasis in HepG2 and Hep3B hepatoma cell lines.
Results: Treatment with HGF increased c-Met phosphorylation in a dose-dependent manner. Activity of c-Met phosphorylation was peak at 1 to 3 minutes later after HGF treatment and then declined. HGF enhanced the protein level and the activity of uPA in HepG2 and Hep3B cells and also uPAR protein level increased in a HGF dose dependent manner. HGF increase cell invasion through matrigel. A monoclonal antibody against human uPA receptor, mAb 3936, inhibited HGF-mediated tumor cell invasion in a dose dependent manner. Down-regulation of uPA using uPA-shRNA induced a decrease in in vitro cell invasion in HepG2 cells.
Conclusions: These results suggest that HepG2 and Hep3B cells express functional c-Met, which may provide a target for a therapeutic basis to interfere with metastases of cancer cells by inhibiting uPA system-mediated proteolysis.

373 POSTER

Role of manganese superoxide dismutase on growth and invasive properties of human estrogen-independent breast cancer cells

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Background: Manganese superoxide dismutase (MnSOD) is known to play a role in cancer. MnSOD exerts a tumor suppressive effect in estrogen-dependent human breast cancer cells. In the present study we investigated the in vitro role of MnSOD in the growth of some aggressive and highly metastatic estrogen-independent breast cancer cells, i.e. MDA-MB231 and SKBR3 cells.
Experimental procedures: This in vitro study used estrogen-dependent and estrogen-independent breast cancer cell lines. Antisense RNA strategy was used to inhibit MnSOD expression and to study consequence on breast cancer cell growth and invasiveness.
Results: We show that estrogen-independent cells expressed a significantly higher basal MnSOD level compared to estrogen-dependent human breast cancer cell lines (MCF-7 and T47D). For MDA-MB231 cells, the high MnSOD level was accompanied by an overproduction of