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.

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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.

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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.

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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.

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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