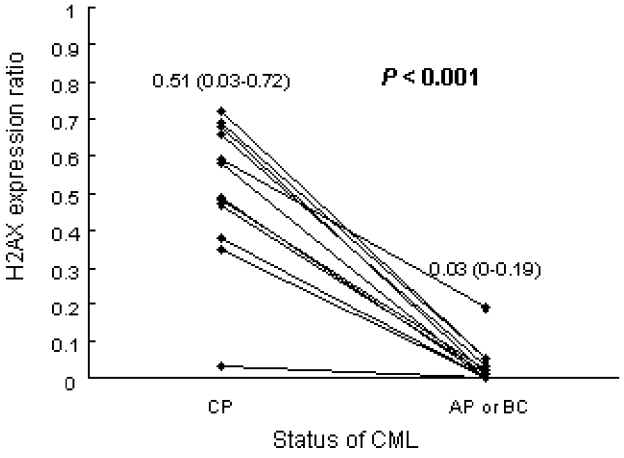
Material and methods: We evaluated the expression of γ H2AX protein in bone marrow samples by western blot from 65 CML patients and analyzed its prognostic significance.
Results: Western blot analysis revealed that the γ H2AX was undetectable in 20 (40.8%) of 49 cases in chronic phase and 12 (75.0%) of 16 cases in accelerated phase/blastic crisis. The levels of γ H2AX expression of the CML samples were significantly lower than that of the normal bone marrow controls ($P<0.001$). The levels of γ H2AX in the leukemia cells were markedly lower in the accelerated phase or blastic crisis at the initial presentation compared to that in the chronic phase ($P<0.05$, and $P<0.01$, respectively). Correlation analyses of γ H2AX levels and characteristics of patients demonstrated that the γ H2AX levels correlates well with peripheral blast counts ($P<0.05$). There was no correlation between γ H2AX levels and patient age, sex, white blood cell count, platelet count, hemoglobin, spleen size, percentage of eosinophils, or basophils. No differences in the cytogenetic response to interferon-α were observed according to the γ H2AX levels. The disease free survival or overall survival time was not significantly different according to expression levels of γ H2AX protein. Of 49 patients diagnosed as chronic phase, 12 patients progressed to the acute phase during the follow up period. In these patients, the levels of γ H2AX were markedly decreased with disease progression ($P<0.001$).



Conclusions: The γ H2AX protein was markedly down-regulated in a substantial proportion of CML. Down-regulation of γ H2AX protein was significantly associated with disease progression. These findings might lend additional insight into the molecular pathogenesis of CML.

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Quantitative trait locus analysis reveals two intragenic sites that influence O6-alkylguanine-DNA alkyltransferase activity in peripheral blood mononuclear cells

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The repair of specific types of DNA alkylation damage by O^6-alkylguanine-DNA alkyltransferase (MGMT) is a major mechanism of resistance to the carcinogenic and chemotherapeutic effects of certain alkylating agents. The levels of expression of MGMT in normal and tumour tissues are thus of interest in relation to the prevention and treatment of cancer. MGMT expression in a given tissue varies widely between individuals but the underlying causes of this variability are not known. Here we investigate the contribution of variation at the DNA level on intra-individual differences in MGMT activity in peripheral blood mononuclear cells (PBMC). First we use an expressed single nucleotide polymorphism (SNP) to demonstrate that the two MGMT alleles are frequently expressed at different levels in PBMC, suggesting that there is a genetic component of inter-individual variation of MGMT levels that maps close to or within the MGMT locus. Next, we show by quantitative trait locus analysis using intragenic SNPs that there are at least two sites influencing interindividual variation in MGMT activity in PBMC. One of these sites is characterized by an SNP at the 3′ end of the first intron and the second by two SNPs in the last exon. The latter two are in perfect disequilibrium and result both in amino acid substitutions; one of them, Ile143Val, affecting an amino acid close to the cysteine (145) residue at the active site of MGMT. In vitro assays did not reveal any influence of the amino acid substitutions on the activity of the protein on methylated DNA substrate, however, the Val^{143} variant was more resistant to inactivation by the MGMT inactivator O^6-(4-bromothenyl)guanine. The effect of analogue inhibitors on the variant MGMT is currently being investigated. Finally, the relationship between alleles at the two sites and MGMT expression levels allows the prediction of MGMT activity in individuals according to their genotype and we report the results from a case-control series suggesting a link between MGMT activity and lung cancer risk.

483 POSTER

Polymorphisms of DNA repair genes in the molecular pathogenesis of esophageal (Barrett) adenocarcinoma

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Background: To test the hypothesis that aberrations of DNA repair contribute to susceptibility for the progression of gastroesophageal reflux disease (GERD) to Barrett esophagus (BE) and esophageal adenocarcinoma (EADC), we studied the frequency of polymorphisms of selected DNA repair genes (XPC, XPD, XRCC1, XRCC3) in patients with GERD, BE and EADC enrolled in a 2-year prospective case-control study.
Materials and methods: Genomic DNA was extracted from blood samples (obtained with informed consent) of patients with GERD (n=126), BE (n=125), and EADC (n=56), defined according to strict clinicopathologic criteria. Controls comprised 95 healthy, asymptomatic individuals from the same geographic region. Polymerase chain reaction, restriction digestion and gel electrophoresis were used to identify wild-type and polymorphic variants of XPD (C22541A and A35931C), XRCC1 (C26304T and G28152A), XRCC3 (T18067C), and the poly (AT) insertion/deletion of XPC (PAT). Allelic frequencies were compared between cases (GERD, BE, EADC) and controls using logistic regression analysis to calculate age, gender, smoking and alcohol-adjusted odds ratios (OR) and 95% confidence intervals (CI).
Results: Genotype frequencies in controls were as predicted from Hardy-Weinberg equilibrium theory. Compared to controls, a large and statistically significant increased frequency for the XPC PAT homozygous variant genotype was seen in patients with EADC (OR 3.82; 95% CI 1.05–13.93). However, significantly reduced frequencies were seen for the XPD A35931C homozygous variant genotype in patients with EADC (OR 0.24; 95% CI 0.07–0.88), and for the XRCC1 G28152A homozygous variant genotype in patients with BE (OR 0.38; 95% CI 0.12–0.64) and GERD (OR 0.29; 95% CI 0.12–0.66).
Conclusions: 1) The contribution of DNA repair gene polymorphisms to the molecular pathogenesis of EADC is complex, with polymorphisms of nucleotide excision repair genes showing opposing effects (increased risk for XPC vs. a protective effect for XPD). 2) The protective effect of the homozygous variant of XRCC1 G28152A for GERD and BE suggests that base excision repair alterations may occur early in progression to EADC, possibly in response to endogenous oxidative or inflammatory DNA damaging processes, and suggests potential clinical application for this polymorphic marker in endoscopic Barrett surveillance programs.

Antimetabolites

484 POSTER

In vivo induction of resistance to gemcitabine results in amplification of ribonucleotide reductase as the major determinant

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Gemcitabine is a deoxycytidine analog with activity against several solid cancers. Deoxycytidine kinase (dCK) phosphorylates gemcitabine, which is required for its incorporation into DNA by DNA-polymerase. The drug can be inactivated by deoxycytidine deaminase (dCDA). The metabolite gemcitabine diphosphate, (dFdCDP) is an inhibitor of ribonucleotide reductase (RNR).
In most *in vitro* models resistance to gemcitabine was associated with a decreased dCK activity. In addition, RNR might be an important determinant of gemcitabine resistance. In all these models resistance was established using continuous exposure to gemcitabine with increasing concentrations, which is clinically not relevant.