camptothecin. *dss1*⁻ mutant cells also showed wild type sensitivity to UV light and ionizing radiation. The fission yeast cells lacking *dss1*are unusual in that they are both cold sensitive and temperature sensitive for growth. *dss1*⁻ cells that are capable of growth at higher temperature can be readily isolated, and retain enhanced sensitivity to topo II targeting agents. These cells retain cold sensitivity for growth. Our results indicate that *Dss1*has separable functions that are important for DNA repair or growth. Current experiments are examining proteins that interact with Dss1, which may illuminate repair processes that require Brca2 in mammalian cells.

478 POSTER

Preclinical investigation of novel inhibitors of DNA dependent protein kinase

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The repair of DNA double strand breaks (dsb) is critical for the survival of cells exposed to ionising radiation or chemical agents such as topoisomerase II poisons. In mammalian cells non-homologous end-joining (NHEJ) is the main pathway for repair of DNA dsb in which DNA-dependent protein kinase (DNA-PK) is a major participant. DNA-PK deficient cells are hypersensitive to ionising radiation and some DNA-damaging anticancer drugs, and inhibition of DNA-PK therefore represents a potential strategy for radio- and chemo-sensitization.
The catalytic subunit of DNA-PK (DNA-PKcs) is a member of the phosphatidylinositol (PI) 3-kinase like kinase (PIKK) family of serine/threonine protein kinases. LY294002, a PI 3-kinase inhibitor also inhibits DNA-PKcs, and sensitizes tumour cells to ionizing radiation and dsb-inducing chemotherapeutics. NU7441 is a more potent and specific novel DNA-PK inhibitor (IC50 = 12 nM) developed from LY294002.
The cellular specificity NU7441 for DNA-PKcs was studied in V3 and V3-YAC cells, deficient and proficient in DNA-PKcs respectively. V3 cells were inherently more sensitive to ionising radiation and etoposide (a topoisomerase II poison) than V3-YAC cells and NU7441 increased the radiosensitivity of V3-YAC cells but not of V3 cells. NU7441 also potentiated etoposide cytotoxicity in V3-YAC cells but not V3 cells, confirming that DNA-PKcs is the cellular target of NU7441.
Exposure of the human colon cancer cell lines LoVo and SW620 to 1 μM NU7441 for 16hr did not effect cell survival but enhanced the cytotoxic effects of both etoposide and doxorubicin.

	% Survival		Dose mod[a]	% Survival		Dose mod[a]
	Etoposide (100 nM)	Etoposide + NU7441		Doxorubicin (10 nM)	Doxorubicin + NU7441	
LoVo	68	18	3.7	41	24	1.7
SW620	84	47	1.8	61	36	1.7

[a]Dose modification.

Plasma pharmacokinetic analyses performed following intravenous (i.v), intraperitoneal (i.p.) and oral (p.o.) administration showed 100% i.p. bioavailability and 33% p.o. bioavailability. Following i.p. administration peak plasma levels were 2.4 μg/ml, AUC was 150 μg/ml*min and the $T_{1/2}$ was 50 min. Administration of 10 mg/kg NU7441 i.p. daily for 5 days was well tolerated and did not cause significant weight loss. Tissue distribution studies conducted in SW620 xenograft bearing mice show that NU7441 was well distributed to the tumour and other tissues, where it was retained following clearance from the plasma. Levels of NU7441, commensurate with chemo and radiosensitization in vitro were maintained in tumour tissue for approximately 4hr.
These experiments demonstrate that the cellular effects of NU7441 are specific for DNA-PKcs and that the concentrations required for chemo and radiosensitization in vitro can be achieved in tumour xenografts following i.p. administration of well tolerated doses of NU7441.

479 POSTER

Impact of the DNA repair efficiency in the outcome of sarcoma patients treated with ET-743 (Yondelis)

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ET-743 (trabectedin, Yondelis) induces long lasting objective remissions and tumor control in a subset of patients (pts) with advanced pretreated sarcoma (SA). ET-743 cytotoxicity in experimental models directly correlates with efficient DNA repair. XPD and BRCA1 are involved in transcription-coupled nucleotide excision repair (NER) and in homologous recombination repair, while ERCC1 is involved in global genome NER. On this basis, we performed a retrospective study to correlate the polymorphisms of the XPD (Lys751Gln and Asp312Asn) and ERCC1 (C118T) endonucleases, as well as the mRNA expression levels of ERCC1, XPD and BRCA1 with the pts clinical outcome to ET-743 therapy. Paraffin embedded tumor samples obtained from the pts before treatment were analysed by quantitative RT-PCR in a blind manner by which the investigators were unaware of the clinical data. Fifty-three heavily pretreated pts were included in the study. The overall response (RR) rate in 45 evaluable pts was 11% (5 PRs) and 10 pts (22%; 5 PRs, 1 MR and 4 SD) achieved progression free survival $\geqslant$ 6 months (PFS6). Median survival was 17 months (22 pts still censored). The highest RRs were observed in pts homozygous for wild-type XPD Lys/Lys (20%) and Asp/Asp (19%) as compared to 5 and 7% in the heterozygous, and no responses in pts homozygous for variant genotype Gln/Gln and Asn/Asn. Additionally, pts harbouring high levels of ERCC1 and XPD mRNA expression have higher PFS6 rates of 32% and 25% vs 16% and 18% respectively. However, low levels of BRCA1 mRNA expression appear to increase both the PFS6 (35% vs 6%, [p=0.06]), and the median survival (19 vs 6 months, [p=0.04]) compared to those with high BRCA1 expression levels. Therefore, polymorphisms and expression levels of the DNA repair genes XPD, ERCC1 and BRCA1 may induce differential sensitivity to ET-743 in SA patients. These results merit further validation in a prospective setting in SA and other tumours.

480 POSTER

The ING family tumor suppressor genes enhance nucleotide excision repair

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Background: The ING1 (inhibitor of growth 1) tumor suppressor gene plays an important role in cellular stress response to ultraviolet (UV) radiation, such as cell cycle arrest, apoptosis, and DNA repair. Four additional related genes (ING2–5) have recently been identified and shown to possess tumor suppressive functions.
Materials and Methods: A host cell reactivation assay was used to study the DNA repair functions of ING proteins.
Results: We have previously shown that the ING1b gene enhances the repair of UV-induced DNA damage. Furthermore, sequencing of the ING1 gene in human cutaneous melanoma biopsies revealed that mutations of the ING1b gene are detrimental to DNA repair. In addition, we found that treatment with the histone deacetylase inhibitor trichostatin A resulted in an increase in DNA repair efficiency in cells overexpressing mutant ING1b gene to the level equivalent to cells-transfected with wild-type ING1b gene, suggesting that ING1b may activate histone acetylation. Local irradiation and immunofluorescence reveals that p33ING1b, together with the histone acetyltransferase p300, is expressed in the entire nucleus and is not localized to UV-induced lesions, suggesting that p33ING1b may facilitate acetylation of histones 3 and 4 upon UV irradiation, thus act as a chromatin accessibility factor. Moreover, melanoma patients that harbor ING1 mutations in the tumors may be at higher risk to die from the disease within 5 years (50%) compared to patients with no ING1 mutation (18%). We further demonstrate that ING2–5 proteins also enhance nucleotide excision repair of UV-induced DNA lesions.
Conclusion: Taken together, our data indicates that ING genes enhance nucleotide excision repair, which leads to increased genomic stability.

481 POSTER

Expression and prognostic significance of phosphorylated histone H2AX in chronic myelogenous leukemia

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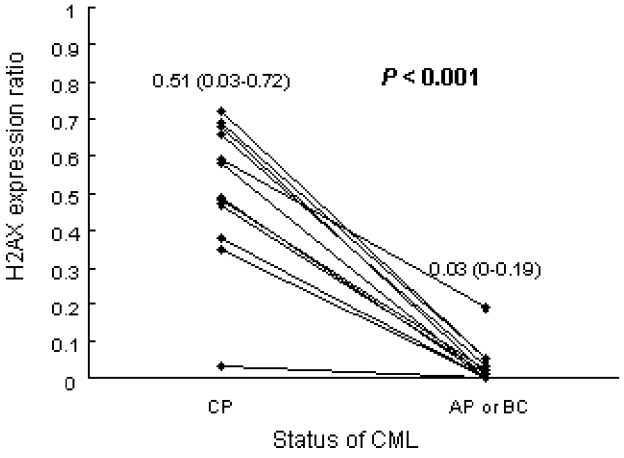
Background: H2AX function is essential for mammalian DNA repair and genomic stability. DNA double-strand breaks cause rapid phosphorylation of the histone H2AX (γ H2AX), which is associated with the recruitment of repair factors to damaged DNA. The progression of chronic myelogenous leukemia (CML) from chronic phase toward acute phase is generally accompanied by an increased Bcr-Abl in leukemic cells, with evidence of additional genetic and chromosomal abnormalities, suggesting a genetic instability in Ph1 cells. We hypothesized that the H2AX could also play a role in this process.

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.

482 POSTER

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.