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Expression of the DNA damage repair gene p53r2 is predictive of survival of patients with lung cancer

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We have recently reported that expression of the gene RRM1 is predictive of survival for patients with surgically resected non-small-cell lung cancer (NSCLC) (J Clin Oncol 22: May 15, 2004). Patients whose tumors expressed high levels of the gene had longer overall (OS, p=0.011) and disease-free survival (DFS, p=0.002) than those with low levels of gene expression. RRM1 is the regulatory component of ribonucleotide reductase (RR), the dose-limiting enzyme in deoxynucleotide synthesis. In the same study, we found no correlation between patient outcome and the expression of the catalytic subunit of RR, RRM2. This is likely explained by RRM1-induced cell migration and metastasis suppression, a function of the gene that is independent of RRM2 and mediated through the PTEN pathway (Oncogene 22: 2135–2142, 2003). In addition, RRM1 is one of the molecular targets of gemcitabine, an agent active in the treatment of patients with advanced stage NSCLC. Patients with metastatic NSCLC receiving gemcitabine and cisplatin combination chemotherapy had better survival if RRM1 expression was low compared to those with high RRM1 expression (p=0.009, Clin Cancer Res 10: 1318–1325, 2004). Recently a second catalytic subunit of RR has been described (Nature 404: 42–49, 2000). It is encoded by the gene p53R2, which is induced by p53 upon DNA damage. p53R2 together with RRM1 forms a second RR that provides the deoxynucleotides required for DNA damage repair. Here we assessed if p53R2 expression is predictive of survival in patients with surgically resected NSCLC. In a prospective dataset of 77 patients, p53R2 expression was assessed by semi-quantitative RT-PCR. Corrected p53R2 expression, using the housekeeping gene 18SrRNA, ranged from 0.00 to 904.05 with a median of 20.20 (mean 84.31). Patients whose tumors had p53R2 values above the median had a significantly longer OS (p=0.014) and DFS (p=0.010) than those with values below the median. p53R2 expression was not associated with tumor stage, histopathology, performance status, smoking history, age, and gender. This better patient outcome suggests that NSCLCs with high levels of p53R2 expression have a less malignant phenotype possibly through lower overall genome damage.

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The caffeine derivative 1,3-dipropyl-7-methylxanthine selectively inhibits the ATM kinase and causes sensitisation to ionising radiation in vitro

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The methylxanthine caffeine is a weakly effective radiosensitiser and abrogates the G2/M cell cycle checkpoint. Caffeine is believed to exert these effects through the inhibition of the phosphatidylinositol (PI)-3 kinase-related kinases (PIKKs). The PIKKs (ATM, ATR and DNA-PK) respond to DNA double strand break (DSB) damage by signalling, *via* phosphorylation events, to key cell cycle and DNA-repair components. Mutation of ATM occurs in the human autosomal recessive disorder ataxia-telangiectasia (A-T), which is characterised by a hypersensitivity to ionising radiation (IR) and aberrant cell cycle control. It has therefore been proposed that inhibition of ATM activity could lead to cellular radio- and chemo-sensitisation. In an attempt to identify molecules more potent and selective than caffeine against ATM, we screened a panel of methylxanthine derivatives against the PIKK family *in vitro*. From this, we identified 1,3-dipropyl-7-methylxanthine (DPMX) as a potent ATP-competitive ATM inhibitor with an IC50 of 3 μM and a Ki of 2.7 μM. DPMX showed 20 to 100 times more potency for ATM over the other PIKKs. Cellular inhibition of ATM by DPMX was demonstrated by ablation of ATM dependant IR induced phosphorylation of serine-15 of p53 and threonine-68 of CHK2. DPMX (500 μM) significantly sensitised the human tumour cell lines HeLa and Lovo to IR (survival enhancement ratio at 2Gy of 2.8 fold and 4.4 fold respectively). DPMX produced no potentiation of the cytotoxic effects of IR in A-T derived cell lines. Treatment of HeLa and LoVo cells with DPMX resulted in the loss of IR induced cell cycle arrest whilst the cell cycle profiles of A-T cells were unchanged by the addition of DPMX. We conclude that DPMX is a specific inhibitor of ATM that can significantly enhance the cytotoxic effects of IR and is a useful tool for investigating the roles of ATM in the cellular response to DNA damage.

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Germline MYH mutations and risk of colorectal cancer

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Background: Colorectal cancer (CRC) follows a multistep progression from normal epithelium to adenoma to cancer, which is accompanied by an accumulation of genetic events. Three Base Excision Repair pathway (BER) proteins, OGG1, MTH and MYH are responsible for correcting 8oxoG-A mismatches that can arise as a result of DNA damage caused by oxidative processes. Recent studies have shown an association between biallelic germline mutations in the MYH gene and the development of colorectal polyps and cancer in an autosomal recessive inheritance pattern.
Objective: We have conducted a population-based study of colorectal cancer cases and (age and sex matched) healthy controls to determine the association between germline MYH mutations and the risk of developing CRC.
Methods: We tested cases and controls from the Ontario Familial Colorectal Cancer Registry (OFCCR) for two common germline mutations: Y165C and G382D. The entire coding region was screened in carriers of either or both of the two mutations. Denaturing high performance liquid chromatography and sequencing were used to detect all mutations.
Results: 29/1238 (2.3%) CRC cases and 21/1255 (1.7%) controls were heterozygotes for either Y165C or G382D, while 12/1238 (1.0%) CRC cases and none of the controls carried biallelic mutations. MYH germline mutation carriers (both monoallelic and biallelic) have an OR of CRC of 2.0 (95%CI, 1.2–3.4), compared to noncarrier cases, while carriers of heterozygous (monoallelic) mutations only have an estimated OR of 1.4 (95%CI, 0.8–2.5), compared to noncarrier cases. Importantly, both monoallelic and biallelic MYH mutation carrier cases are more likely than non-carrier cases to have a first or second degree relative affected with CRC, even when biallelic carriers are excluded from the analysis (p<0.003, Poisson regression with offset to correct for family size).
Conclusions: This population-based study shows an increased risk of CRC conferred by germline mutations in the MYH gene. The demonstration of increased numbers of first and second degree relatives with CRC in families of both monoallelic and biallelic MYH mutation carriers suggests a potentially important low penetrant risk associated with even the monoallelic genotype. Larger studies are necessary to accurately determine this risk and study the associated phenotypes caused by MYH germline mutations.
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Dss1, a homolog of the split hand/split foot malformation candidate gene, is required for cell survival following exposure to topoisomerase II targeting agents

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DNA topoisomerases (topos) are required for normal replication, transcription and chromosome segregation. The topo reactions include enzyme-mediated DNA cleavage, where the cleaved intermediate includes enzyme bound to DNA via a phosphotyrosyl linkage. Anti-tumor topo targeting agents interfere with the reaction resulting in accumulation of these covalent complexes. These covalent complexes can be converted into DNA damage that includes DNA strand breaks and protein covalently bound to DNA. We identified repair pathways that are required for cell survival following exposure to topo targeting agents using both fission and budding yeasts as model systems. We previously showed that repair of topo mediated DNA damage requires both homologous recombination and checkpoint control pathways. Fission yeast strains lacking nucleotide excision repair pathway are also hypersensitive to topo II targeting agents. Covalent complexes formed with topo II and DNA are formally similar to interstrand DNA crosslinks. To further examine this correspondence, we examined mutants that may be specifically defective in the repair of interstrand crosslinks. Yeast cells lack homologs of the genes mutated in Fanconi's anemia complementation groups and also lack both Brca1 and Brca2 (=FANCD1). However, both fission and budding yeast have homologs of the Dss1 gene, encoding a protein that interacts with Brca2. Mammalian *Dss1* has also been implicated in a heterogeneous limb development disorder split hand/split foot malformation. Mutations in the budding yeast homolog of *Dss1* (*SEM1*) do not confer sensitivity to DNA damaging agents, or to drugs targeting topo I or II. Fission yeast cells lacking *Dss1* are hypersensitive to topo II mediated DNA damage, although they have reduced sensitivity to