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

G. Bepler, S. Sharma, Z. Zheng. *H. Lee Moffitt Cancer Cancer & Research Institute, Thoracic Oncology Program, Tampa, FL, USA*

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

C.J. Richardson, G.C.M. Smith. *KuDOS Pharmaceuticals Ltd, Cambridge, UK*

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

M. Croitoru[1], S. Cleary[1,2], N. Di Nicola[1], M. Manno[1], M. Aaronson[3], M. Redston[4], M. Cotterchio[3], J. Knight[1], R. Gryfe[1,2], S. Gallinger[1,2]. *[1]Samuel Lunenfeld Research Institute, Toronto, Canada; [2]Department of Surgery Mount Sinai Hospital, Toronto, Canada; [3]Ontario Familial Colorectal Cancer Registry, Toronto, Canada; [4]Brigham and Women's Hospital, Department of Pathology, Boston, United States*

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

M. Malik, J. Nitiss. *St. Jude Children's Research Hospital, Molecular Pharmacology, Memphis, USA*

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

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.

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Preclinical investigation of novel inhibitors of DNA dependent protein kinase

M.A. Batey[1], Y. Zhao[1], H.D. Thomas[1], G.C.M. Smith[2], D.R. Newell[1], N.J. Curtin[1]. [1]*Northern Institute for Cancer Research, Newcastle, UK;* [2]*KuDOS Pharmaceuticals, Cambridge, UK*

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.

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

A. van Oosterom[1], R. Maki[2], M. Taron[3], R. Sciot[1], J.M. Fernandez Sousa-Faro[4], J.C. Tercero[4], J. Jimeno[4], R. Rosell[3]. [1]*UZ Gasthuisberg, Oncology, Leuven, Belgium;* [2]*MSKCC, Oncology, New York, United States;* [3]*Hospital Univ. Germans Trias i Pujol, Oncology, Badalona, Spain;* [4]*PharmaMar, Scientific Development, Madrid, Spain*

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.

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The ING family tumor suppressor genes enhance nucleotide excision repair

E.I. Campos[1], W.H. Kuo[1], M.Y. Chin[1], D.L. Mitchell[2], G. Li[1]. [1]*University of British Columbia, Medicine, Vancouver, Canada;* [2]*University of Texas, Carcinogenesis, Smithville, USA*

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.

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

J. Cheong[1], H. Jeung[2], J. Kim[2], J. Lee[2], S. Lee[1], J. Hahn[1], Y. Ko[1], Y. Min[1]. [1]*Yonsei University College of Medicine, Department of Internal Medicine, Seoul, Republic of Korea;* [2]*Yonsei University College of Medicine, Clinical Research Center, Seoul, Republic of Korea*

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.