3rd Alberta Genomic Instability and Aging Conference

PROGRAM AND ABSTRACT BOOK

Monday 7 June – Tuesday 8 June, 2010
Health Sciences Centre
Health Research Innovation Centre
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ALBERTA GENOMIC INSTABILITY AND AGING CONFERENCE 2010

June 7 – 8, 2010

The University of Calgary
Health Sciences Centre, Faculty of Medicine

Monday, June 7 – Theatre Three

8:15 am Registration Desk opens – Theatre Three Lobby

Morning Session

8:45 am Welcome and opening remarks – Susan Lees-Miller

Chair – Jennifer Cobb

9:00 am Curtis Harris, NCI-NIH
Inflammation and colon cancer: Interaction of the p53, microRNA and inflammatory cytokine pathways

9:40 am Chris Williamson, U of Calgary
ATM-deficiency sensitizes Mantle Cell Lymphoma cells to PARP-1 inhibitors

10:05 am Roy Golsteyn, U of Lethbridge
Identifying the molecular pathways that lead to checkpoint adaptation in human cancer cells

10:30 am Coffee break

Chair – Michael Weinfeld

11:00 am Ismail Ismail, U of Alberta
A role of polycomb group proteins in the DNA damage response

11:25 am Laura Williamson, U of Calgary
Estrogen induced transcription leads to replication associated DNA double strand break formation

11:50 am Jody Filkowski, U of Lethbridge
Role of non-mutational mechanisms in cancer cell drug resistance

12:15 noon Lunch – HRIC Atrium

Monday, June 7 – Theatre Three

Afternoon Session

Chair – Mark Glover

2:00 pm John Rouse, U of Dundee
Cutting remarks on new regulators of DNA repair

2:40 pm Mireille Tittel-Elmer, U of Calgary
The MRX complex regulates sister chromatin cohesion during replication stress

3:05 pm Charles Leung, U of Alberta
Structural basis for interactions between TopBP1 and the BACH1 helicase in DNA replication checkpoint control

3:30 pm Coffee break

Chair – Tara Beattie

4:00 pm Nasser Tahbaz, U of Alberta
Novel roles of polynucleotide kinase

4:25 pm Slava Ilnytskyy, U of Lethbridge
Epigenetic mechanisms of genome stability: a story of two generations

Evening

5:00 pm Poster Session – HRIC Atrium

6:30 pm Conference Dinner – HRIC Atrium

Tuesday, June 8 – Theatre Three

Morning Session

Chair – Roy Golsteyn

9:00 am Jan Karlseder, Salk Institute
Telomere driven epigenetic changes as a cause for cellular senescence

9:40 am Tara Beattie, U of Calgary
Characterization of human telomerase reverse transcriptase (hTERT) mutations associated with idiopathic pulmonary fibrosis

10:05 am Sitar Shah, U of Calgary
AgeING and other stories uncovered in *Caenorhabditis elegans*

10:30 am Coffee break

Chair – Ebba Kurz

11:00 am Heather Smith, U of Calgary
ING1b is transcriptional misregulated in premature aging syndrome, Hutchinson-Gilford progeria syndrome

11:25 am Gernot Neumayer, U of Calgary
DNA damage response regulated by the spindle assembly factor TPX2

11:50 am Andrey Golubov, U of Lethbridge
UVC and X-ray- induced genome instability in bystander *Arabidopsis thaliana* plants

12:15 pm Michael Weinfeld, U of Alberta
Polynucleotide kinase/phosphatase – a potential target for therapy

12:40 pm Closing remarks

12:45 pm Bag Lunch

ABSTRACTS

ORAL PRESENTATIONS

Inflammation and colon cancer: Interaction of the p53, microRNA and inflammatory cytokine pathways

Curtis Harris

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The p53 pathway is an intrinsic monitor and response pathway of telomeric attrition involved in cellular aging and senescence. Cellular senescence is tumor suppressive that can be activated by p53 in cancer cells. We are studying the molecular mechanisms of cellular senescence in normal and malignant human cells and the role of the telometric multiprotein complex, shelterin, that includes TRF2 and POT1 (1-3). Our ongoing studies have revealed that p53 and its endogenous isoforms regulate both specific microRNAs and TRF2 expression as mechanisms of replicative senescence. In addition, POT1 isoforms are functionally diverse in both maintaining telomeric integrity and preventing p53-dependent senescence induced by telomeric shortening. A switch in the expression patterns of p53 isoforms, Δ133Np53 and p53 beta, is also associated with the transition of benign to malignant human cancers.

Chronic inflammation and deregulation of microRNAs have roles in human carcinogenesis (4-7). In addition to our mechanistic and genetic studies, we are investigating the expression of microRNAs and inflammatory genes as cancer biomarkers of diagnosis, prognosis, and therapeutic outcome (8-11). We are especially interested in developing prognostic classifiers of early stage cancer.

1. Fujita K, Mondal AM, Horikawa I et al. (2009). p53 isoforms Delta133p53 and p53beta are endogenous regulators of replicative cellular senescence, *Nat Cell Biol* 11: 1135-1142.
2. Yang Q, Zheng YL, and Harris CC (2005). POT1 and TRF2 cooperate to maintain telomeric integrity, *Mol Cell Biol* 25: 1070-1080.
3. Yang Q, Zhang R, Horikawa I et al. (2007). Functional diversity of human protection of telomeres 1 isoforms in telomere protection and cellular senescence, *Cancer Res* 67: 11677-11686.
4. Hussain SP, Hofseth LJ, and Harris CC (2003). Radical causes of cancer, *Nat Rev Cancer* 3: 276-285.
5. Tlsty TD and Coussens LM (2006). Tumor stroma and regulation of cancer development, *Annu Rev Pathol* 1: 119-150.
6. Croce CM (2009). Causes and consequences of microRNA dysregulation in cancer, *Nat Rev Genet* 10: 704-714.
7. Schetter AJ, Heegaard NH, and Harris CC (2010). Inflammation and cancer: interweaving microRNA, free radical, cytokine and p53 pathways, *Carcinogenesis* 31: 37-49.
8. Schetter AJ, Leung SY, Sohn JJ et al. (2008). MicroRNA expression profiles associated with prognosis and therapeutic outcome in colon adenocarcinoma, *JAMA* 299: 425-436.
9. Schetter AJ, Nguyen GH, Bowman ED et al. (2009). Association of inflammation-related and microRNA gene expression with cancer-specific mortality of colon adenocarcinoma, *Clin Cancer Res* 15: 5878-5887.
10. Seike M, Goto A, Okano T et al. (2009). MiR-21 is an EGFR-regulated anti-apoptotic factor in lung cancer in never-smokers, *Proc Natl Acad Sci U S A* 106: 12085-12090.
11. Yanaihara N, Caplen N, Bowman E et al. (2006). Unique microRNA molecular profiles in lung cancer diagnosis and prognosis, *Cancer Cell* 9: 189-198.

ATM-deficiency sensitizes Mantle Cell Lymphoma cells to PARP-1 inhibitors

Williamson CT[1,2], Muzik H[2,3], Turhan, AG[4], ZamÒ A[5], O'Connor MJ[6], Bebb DG[2,3] and Lees-Miller SP[1,2,3]

[1]Department of Biochemistry and Molecular Biology, [2]Southern Alberta Cancer Research Institute, [3]Department of Oncology, University of Calgary, Calgary, Alberta, Canada. [4]Université Paris Sud, CHU de Poitiers, France. [5]Dipartimento di Patologia, University of Verona, Italy. [6]KuDOS Pharmaceuticals, Cambridge, UK.

Poly-ADP ribose polymerase-1 (PARP-1) inhibition is toxic to cells with mutations in the breast and ovarian cancer susceptibility genes *BRCA1* or *BRCA2,* a concept, termed synthetic lethality. However, whether this approach is applicable to other human cancers with defects in other DNA repair genes has yet to be determined. The *Ataxia-Telangiectasia Mutated (ATM)* gene is altered in a number of human cancers including Mantle Cell Lymphoma (MCL). Here, we characterize a panel of MCL cell lines for ATM status and function and investigate the potential for synthetic lethality in the presence of small molecule inhibitors of PARP-1. We show that Granta-519 and UPN2 cells have low levels of ATM protein, are defective in DNA damage-induced ATM-dependent signaling, are radiation sensitive and have cell cycle checkpoint defects: all characteristics of defective ATM function. Significantly, Granta-519 and UPN2 cells were more sensitive to PARP-1 inhibition, than were the ATM-proficient MCL cell lines examined. Furthermore, the PARP-1 inhibitor olaparib (previously known as AZD2281/KU-0059436) significantly decreased tumour growth and increased overall survival in mice bearing subcutaneous xenografts of ATM-deficient Granta-519 cells, while producing only a modest effect on overall survival of mice bearing xenografts of the ATM-proficient cell line, Z138. Our results suggest that PARP inhibitors have therapeutic potential in the treatment of MCL and that the concept of synthetic lethality extends to human cancers with alterations in *ATM*.

Identifying the molecular pathways that lead to checkpoint adaptation in human cancer cells

Philip Kubara, Sophie Kernéis-Golsteyn, Elfriede Dyck, Roy Golsteyn

Dept of Biological Science, University of Lethbridge, Lethbridge, AB, Canada

Cytotoxic treatments that damage DNA are the cornerstone of cancer treatments. Cells with damaged DNA induce a checkpoint that causes them to arrest in the cell cycle. Cancer cells are able to escape the checkpoint and enter mitosis with damaged DNA, a process known as checkpoint adaptation. The molecular pathways that lead to checkpoint adaptation are not known.

We use mechanical shake-off to isolate and compare cells that have undergone checkpoint adaptation (non-adherent, mitotic cells) to those that are under a DNA damage checkpoint (adherent, interphase cells). We treat human cell lines with clinically relevant cancer drugs, such as alkylating agents, Topo I inhibitors, Topo II inhibitors or cisplatin. These cytotoxic agents cause distinct types of DNA damage, yet, they induce a reproducible sequence of molecular events including gamma histone H2AX induction, phosphorylation of Chk1 on ser345 and cyclin B1 accumulation. Starting by 48 hours after treatment, cells enter mitosis with their DNA still damaged. Chk1 inactivation by dephosphorylation appears to be closely linked to checkpoint adaptation because changing the timing of entry into mitosis also changes the timing Chk1 dephosphorylation. Our data will help improve the outcome of cytotoxic treatments because we will understand better how cells respond to damaged DNA.

A role of polycomb group proteins in the DNA damage response

Ismail Hassan Ismail, Christi Andrin, Darin McDonald & Michael J Hendzel

Department of Oncology, Faculty of Medicine and Dentistry, University of Alberta, 11560 University Avenue, Edmonton, Alberta, Canada.

Polycomb group proteins (PcG) are major determinants of cell identity, stem cell pluripotency, and epigenetic gene silencing during development. The polycomb repressor complex 1, which contains the RING finger proteins BMI-1, RING-1, and RING-2, functions as an E3-ubuiquitin ligase, ubiquitylating H2A histones on lysine 119. This ubiquitylation of H2A is thought to stabilize PcG-mediated gene repression. Ubiquitylation of histone H2A and the conserved site in the histone H2A variant, H2AX, have recently been implicated in the DNA damage response (DDR). Here we define a novel role for the PcG protein in the DDR. Using microscopic techniques we dissect the function of the PcG in the DNA double strand break pathway. We also, shown the cellular depletion of PcG proteins increase cellular sensitivity to radiation. These data uncover an unexpected link between the polycomb and the DDR pathways, and suggest a novel role for PcG in maintaining genomic stability.

Estrogen induced transcription leads to replication associated DNA double strand break formation

L.M. Williamson and S.P. Lees-Miller

Dept. Biochemisty and Molecular Biology, Southern Alberta Cancer Research Institute, University of Calgary, Alberta, Canada

Reduced expression and mutations in several DNA double strand break response proteins confers an increased risk of breast cancer. This increased risk is likely due to an inability of cells to efficiently manage DNA damage within the dynamic breast tissue. One possible source of this damage is the presence of hormones, specifically estrogen. Metabolism of estrogen to its catechol metabolites has previously been shown to result in an accumulation of oxidized bases and abasic sites. In this study we show that treatment of breast cancer cells with 17β-estradiol results in an accumulation of DNA repair foci that are representative of replication associated DNA damage. However, in contrast to the previously demonstrated model, these foci are not mediated by estrogen metabolites and are dependent on ERα mediated transcription. We also demonstrate that γH2AX is enriched at the estrogen inducible promoter of trefoil factor-1 suggesting the damage occurs at transcription sites. Furthermore, γH2AX foci are dependent on topoisomerase II β, an enzyme involved in transcriptional regulation of estrogen inducible genes. Here we propose a novel model of replication associated DNA damage that is mediated by topoisomerase II β induced double strand break formation occurring in response to estrogen stimulation.

Role of non-mutational mechanisms in cancer cell drug resistance

Jody Filkowski and Olga Kovalchuk

Biological Science Department, University of Lethbridge, Lethbridge, Alberta, Canada

Causes of cancer drug resistance are currently believed to be linked to drug-induced random mutation events or non-mutational epigenetic alterations of gene function. Here, we examined the role of non-mutational mechanisms in development of a multidrug-resistant phenotype of MCF-7 human breast cancer cells induced by doxorubicin (DOX) and cisplatin (cisDDP). Both cell lines are characterized by extensive alterations in gene-specific cytosine methylation. A detailed analysis revealed that the acquisition of the drug-resistant phenotype of DOX and cisDDP cells was characterized by three common mechanisms: dysfunction of genes involved in estrogen metabolism, apoptosis, cell-cell contact, DNA repair. Another non-mutational mechanism of gene expression control is mediated via microRNAs. How they might be involved in cancer cell drug resistance remains largely unexplored. The results of our study demonstrate that DOX and cisDDP cells exhibit a considerable dysregulation of the miRNAome profile. We show that miR-451 regulates the expression of multidrug resistance 1 (mdr1) gene. Furthermore, we demonstrate that miR-345 and miR-7 target the human multidrug resistance-associated protein 1 gene (mrp1). These results provide evidence that non-mutational mechanisms are an important feature of drug resistant cells and may be a crucial contributing factor to development of breast cancer drug resistance.

Cutting remarks on new regulators of DNA repair

John Rouse

MRC Protein Phosphorylation Unit, College of Life Sciences, University of Dundee, Dundee DD1 5EH, Scotland, UK.

DNA inter–strand crosslinks (ICLs) are highly toxic because they block the progression of replisomes. The Fanconi Anemia (FA) proteins, encoded by genes that are mutated in FA, are important for ICL repair. The FA core complex catalyses the mono–ubiquitination of FANCD2 and this event is essential for several steps of ICL repair. Furthermore, loss of FANCD2 mono–ubiquitination is observed in most FA patients. However, how mono–ubiquitination of FANCD2 promotes ICL repair is unknown. Here I describe a novel and highly conserved DNA repair protein that interacts with, and is recruited to sites of DNA damage by, the mono–ubiquitinated form of FANCD2. This at least partly explains how FANCD2 ubiquitination controls DNA repair.

The MRX complex regulates sister chromatin cohesion during replication stress

Mireille Tittel-Elmer[1], John Petrini[2], Jennifer A. Cobb[1]

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The Mre11-Rad50-Xrs2 (MRX) complex is important for the maintenance of genomic integrity and is recruited to forks during pauses in replication. Moreover, one essential function of the MRX complex is to provide architectural support to DNA during repair, meiotic recombination and telomere maintenance. The structure of the MRX complex indicates it can bridge two strands of duplexed DNA and serve as a long-range tether, becoming 'critical glue' between sister chromatids. Cohesion between sister chromatids is coupled to fork progression and the cohesin complex holds replicated chromosomes together from their synthesis until the onset of anaphase. Here we show that deficiency in the cohesin complex in combination with the absence of Mre11 during replication stress leads to an additive loss of cohesion between newly synthesized daughter strands and correlates with a dramatic loss of cell viability. This function is further characterized using a structurally defective Rad50 allele. In all, our data indicate that the MRX and cohesin complexes both contribute to the maintenance of sister chromatid cohesion (SCC) at stalled replication forks but independently of each other. One critical role for the MRX complex in preserving chromosome integrity involves supporting the architecture of newly synthesized daughter strands during pauses in replication, preventing fork-associated damage.

Structural basis for interactions between TopBP1 and the BACH1 helicase in DNA replication checkpoint control

M. Glover, C. Leung

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Human TopBP1 plays a critical role in the DNA replication stress response, where it mediates key protein-protein interactions through its multiple BRCT domains. Here we show that the BRCA1-associated helicase, BACH1, specifically associates with TopBP1 in a phosphorylation-dependent manner and participates in the replication stress response. This interaction involves the C-terminal tandem BRCT repeats of TopBP1 and catalyzes a novel structural transition in TopBP1. Structure/function analyses reveal the structural basis for the specific interaction of these proteins.

Novel roles of polynucleotide kinase

Nasser Tahbaz, Michael Weinfeld

Department of Oncology Cross Cancer Institute University of Alberta

Human Polynucleotide kinase phosphatase (hPNK) possesses both DNA 5'-kinase and 3'-phosphatase activities. hPNK contains a forkhead-associated (FHA) domain, and two independent kinase and phosphatase domains. The functional domains are responsible for the generation of the repair-competent DNA ends (5'Phosphate and 3'OH) following DNA damage.
Our biochemical (Western blot and Immunoprecipitation), and immunoflourecsence microscopy analysis indicates that functionally active full-length hPNK is present in mitochondria. In addition, down-regulation of hPNK results in the decrease of both mitochondrial and nuclear signals. Immunoprecipitation of hPNK from purified mitochondria, pulled-down mitochondrial resident enzymes and proteins including mitofilin, prohibitin and pol G. While association with mitofilin could demonstrate how hPNK is imported into mitochondria, interaction of hPNK with prohibitin is the first direct evidence indicating that the DNA repair machinery in mitochondria indeed is associated with the nucleoids and thus with the inner membrane of mitochondria.

Epigenetic mechanisms of genome stability: a story of two generations

Slava Ilnytskyy, Andrey Golubov and Olga Kovalchuk

Biological Science Department, University of Lethbridge, Lethbridge Alberta, Canada

While modern cancer radiation therapy has led to increased patient survival rates, the risk of radiation treatment-related complications is becoming a growing problem. Radiation poses a threat to the exposed individuals and their progeny. It causes genome instability that is linked to carcinogenesis. Radiation-induced genome instability manifests as elevated delayed and non-targeted mutation, chromosome aberration and gene expression changes. Its occurrence has been well-documented in the directly exposed cells and organisms. Radiation is known to cause a wide variety of indirect effects. It can affect the unexposed progeny of the pre-conceptually exposed animals and humans. Genome instability has been implicated in the latter phenomena. Yet, the mechanisms by which it arises remain obscure. We hypothesized that epigenetic alterations play leading roles in the molecular etiology of the radiation-induced genome instability. Epigenetic changes comprise cytosine DNA methylation, histone modifications and small RNA-mediated events. We analyzed the roles of aforementioned epigenetic changes in the transgenerational radiation-induced effects using animal models. Based on the results of deep sequencing of germline short RNA pools we will present new and compelling evidence that epigenetic changes (DNA methylation and short RNA pool changes) are important for the molecular etiology of the transgenerational radiation effects.

Reduced histone biosynthesis and redistribution of chromatin marks during replicative aging arising from a chronic damage signal at human telomeres

Roderick J. O'Sullivan[1], Stefan Kubicek[2], Stuart L. Schreiber[2,3] and Jan Karlseder[1]

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During replicative aging of primary cells morphological transformations occur, the expression pattern is altered and chromatin changes globally. Here we show that chronic damage signals, likely caused by telomere processing, impact expression of histones and lead to their depletion. Interrogation of the abundance and cell cycle expression of histones and histone chaperones revealed defects in histone biosynthesis during aging. Simultaneously, epigenetic marks were redistributed across the phases of the cell cycle and the DNA damage response (DDR) machinery was activated. The age-dependent reprogramming affected telomeric chromatin itself, which was progressively destabilized during aging, resulting in a boost of the telomere associated DDR signal with each successive cell cycle. We propose a mechanism where changes in the structural and epigenetic integrity of telomeres impact core histones and their chaperones, enforcing a self-perpetuating pathway of global epigenetic changes that ultimately leads to senescence.

Characterization of human telomerase reverse transcriptase (hTERT) mutations associated with idiopathic pulmonary fibrosis

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Telomeres are protective nucleoprotein structures found at the ends of linear chromosomes that play a crucial role in maintaining genomic stability in cells. Telomeres consist of tandem DNA repeats that shorten after every round of cell division. Telomere length can be maintained by the enzyme telomerase, a ribonucleoprotein reverse transcriptase, which minimally consists of a telomerase reverse transcriptase (TERT) catalytic subunit and a telomerase RNA (TR). Telomerase functions by catalyzing the *de novo* synthesis of telomeric DNA onto the ends of linear chromosomes. Shortened telomeres and abnormal telomerase activity have been associated with a variety of human diseases. Recently, several human TERT (hTERT) mutations have been identified in a subset of patients with idiopathic pulmonary fibrosis (IPF), a fatal lung disease. These patients have also been shown to exhibit shorter telomeres compared to age-matched controls; however, the underlying mechanism(s) behind this phenotype is still unclear. In this study, we used a combination of biochemical and cellular techniques to characterize the functional effects of three IPF-associated hTERT mutations: hTERT V144M, R865C and R865H. *In vitro*, telomerase activity assays and nucleic acid binding assays demonstrated that each hTERT mutation affects telomerase activity and hTERT-DNA and/or hTERT-hTR interactions in a unique manner. We expanded on these observations by using human cells to determine how these hTERT mutants may influence telomerase and telomere biology. Together, we show that V144 and R865 are important amino acids in hTERT, and mutating these residues result in a defect in telomerase function. Interestingly, we have identified hTERT amino acids that when mutated can uncouple the senescence checkpoint from telomere elongation, suggesting an additional role for hTERT in the cell. Our study has provided some insight into how these hTERT mutations may contribute to the development of IPF; however, further studies will be required to better understand all of the factors involved in disease onset and pathogenesis. This may ultimately lead to the development of novel therapeutic strategies for IPF.

AgeING and other stories uncovered in *Caenorhabditis elegans*

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The INhibitor of Growth (ING) family of proteins is involved in multiple cellular processes such as growth regulation, DNA repair and apoptosis. ING proteins are activated by stress, such as ionizing radiation, which also leads to the activation of p53 and subsequently apoptosis.

Mammals have five ING genes and three are found in *C. elegans*. My research focuses on the characterization of *C. elegans ing-3*, the gene with the highest sequence identity to mammalian *ING3*.

Previous experiments from our lab have shown that *ing-3* mutant worms exhibit a decrease in apoptosis when exposed to radiation. Initially it was thought that *ing-3* may function in concert with *cep-1/p53*, yet my results indicate that *ing-3* does not affect the downstream target of *cep-1/p53*, and therefore the two genes function in a parallel apoptotic pathway.

Our lab has recently found other *ing-3* phenotypes. *ing-3* mutants have a weak kinker phenotype. Under electron microscopy, a subset of neuronal cells in *ing-3* mutants show an increase in condensed heterochromatin, which might be linked to the kinker movement of *ing-3*. Finally, the *ing-3* mutant has a lifespan of 25% longer than wild type, and is dependent upon *daf-16*, the main regulator of lifespan in *C. elegans*, involved in the insulin/IGF pathway.

Studying the functional role of INGs in *C. elegans* development will increase our knowledge about the role(s) they play in cancers and cancer therapy.

ING1b is transcriptional misregulated in premature aging syndrome, Hutchinson-Gilford progeria syndrome

Heather Smith, Xijing Han, Xioalan Feng, Karl Riabowol

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ING1 is a type II tumour suppressor and is important regulating cellular growth, apoptosis and senescence. ING1 contains a highly conserved 50 amino acid domain called the *lamin interacting domain* (LID), named for its ability to bind to lamin A. Lamin A is a structural protein essential for maintaining proper nuclear lamina integrity. Lamin A is also important for regulating transcription and replication. Lamin A has a de novo mutation in HGPS resulting in an improperly processed protein retaining a farnesyl group called progerin.
ING1b is unable to interact with progerin in HGPS fibroblasts under endogenous conditions. ING1b mislocalizes to the cytoplasm from nucleus in HGPS, indicating that lamin A anchors ING1b in the nucleus. ING1b protein levels were found to be decreased in HGPS primary skin fibroblasts and HGPS B-cell lymphocytes suggesting that the decrease is may be preserved among cell types. ING1b expression is effected at the transcriptional level. The decrease in ING1b protein levels were seen in a corresponding level to the decrease in ING1b mRNA expression.

DNA damage response mediated by the spindle assembly factor TPX2

Gernot Neumayer[1], Hoa Thi Le[1], Su Yeon Shim[1], Jiang Wang[1], Cecilia Lundin[3], Camille Belzil[1], Mathieu Chansard[1], Oliver Gruss[2], Susan Lees-Miller[1], Thomas Helleday[3] and Minh Dang Nguyen[1]

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The Microtubule (MT)-associated protein TPX2 mediates spindle assembly and mitosis through activation of Aurora A kinase and Microtubule nucleation near the chromosomes. In interphase, TPX2 is actively imported in the nucleus, hypothetically to prevent abnormal spindle assembly in the cytosol. However, TPX2 must have defined nuclear functions. We now report that TPX2 plays a key role during DNA damage response (DDR): TPX2 prevents the hyper-amplification of DDR triggered by the Mediator of DNA damage Checkpoint 1 (MDC1), the Ataxia Telangiectasia Mutated (ATM) kinase, the DNA dependent protein kinase (DNA-PKcs) and their substrate, the histone variant H2AX.
After phosphorylation by ATM or DNA-PK, phospho-H2AX (p-H2AX or γ-H2AX) serves as an early molecular signal to amplify the DDR. However, if the p-H2AX signal is excessive or not turned off on time the consequences could be as deleterious as the absence of response. We found that cells lacking TPX2 overreact to DNA lesions triggered by ionizing radiation (IR): they over-amplify the p-H2AX signals induced by DNA-PKcs and ATM, resulting in alterations in recruitment of 53BP1, sustained RAD51 foci, increased accumulation of DNA lesions and, ultimately, increased apoptosis. Conversely, cells overexpressing TPX2 have reduced levels of p-H2AX after IR and exhibit a radio-resistant DNA synthesis (RDS) phenotype. Thus, TPX2 levels appear to dictate whether the cell suppresses DDR to favor cell cycle progression, or maintains the DDR at a level appropriate for cell cycle arrest, repair and maintenance of genomic stability.

UVC and X-ray-induced genome instability in bystander *Arabidopsis thaliana* plants

Andrey Golubov, Viktor Titov, Youli Yao and Igor Kovalchuk

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We recently found that non-irradiated tissue of irradiated plants have changes in genome stability. We hypothesized that such changes can be triggered by signal that can be transmitted through phloem or air. To test whether the signal can be airborne, we used transgenic Arabidopsis thaliana plants carrying in the genome luciferase gene serving as a substrate for homologous recombination. Cells in which recombination events took place are visualized in CCD luciferase camera after application of luciferine. Recombination events then are scored and recombination frequency calculated. In the experimental set-up we planted two groups of plants in Petri dish and covered one group with either aluminium or lead cover and irradiated the second group with either UVC (7,000 ergs) or X-ray (5 Gy). In another set of experiments we placed two pots, one with irradiated and one with non-irradiated plants in sealed plastic bag for 4 days and scored recombination frequency in 7 days. We found that both groups of plants, grown on Petri dish or in plastic bags, irradiated and non-irradiated had higher recombination frequency. We concluded that irradiated plants exchange warning signals that could promote rearrangements in plant genome.

Polynucleotide kinase/phosphatase – a potential target for therapy

Gary K. Freschauf[1], Todd R. Mereniuk[1], Rajam S. Mani[1], Feridoun Karimi-Busheri[1], Agnieszka Ulaczyk-Lesanko[2], Dennis G. Hall[2], and Michael Weinfeld[1]

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There is increasing interest in targeting DNA damage repair enzymes by small molecule inhibitors as a means to increase the sensitivity of cells to ionizing radiation and genotoxic chemotherapeutic agents. Our lab has focused attention on polynucleotide kinase/phosphatase (PNKP), a DNA strand break repair protein that generates the 3'-hydroxyl and 5'-phosphate termini required by DNA polymerases and ligases to complete strand rejoining. We have identified an imidopiperidine compound, 2-(1-hydroxyundecyl)-1-(4-nitrophenylamino)-6-phenyl-6,7a-dihydro-1H-pyrrolo[3,4-b]
pyridine-5,7(2H,4aH)-dione (A12B4C3), that effectively inhibits the 3'-phosphatase activity of PNKP. The compound appears to be fairly specific since it does not inhibit the phosphatase activity of T4 phage polynucleotide kinase or the well-known eukaryotic protein phosphatases, calcineurin and protein phosphatase-1. A12B4C3 sensitized A549 human lung adenocarcinoma cells to ionizing radiation and the topoisomerase 1 poison, camptothecin, but not the topoisomerase II poison, etoposide. Importantly, it failed to sensitize cells already depleted of PNKP by shRNA, implicating PNKP as the primary cellular target of inhibition. *In vitro* and cellular studies of the mechanism of action of A12B4C3 revealed that (i) the compound acts as a non-competitive inhibitor by disrupting the secondary structure of PNKP, and (ii) the compound slows the rate of repair of radiation-induced DNA single- and double-strand breaks. As a single agent, a PNKP inhibitor may be effective if PNKP is shown to participate in synthetic lethal partnerships with other proteins. Our preliminary data indicates synthetic lethal partnerships of PNKP with poly(ADP-ribose) polymerase and DNA polymerase β.

ABSTRACTS

POSTER PRESENTATIONS

POSTER #1

Structural dynamics of the NHEJ XRCC4/DNA Ligase IV/polynucleotide kinase-phosphatase DNA repair complex.

R. Daniel Aceytuno, Ross A. Edwards, Ryan Au, J.N. Mark Glover

Department of Biochemistry, University of Alberta, Alberta, Canada.

Non-homologous end-joining (NHEJ) is the dominant mechanism for repair of DNA double-strand breaks (DSBs) in higher eukaryotes. Inefficient repair of DSBs can generate chromosomal translocation substrates and lead to genetic instability. These events are often oncogenic, as is common in hematological malignancies.

NHEJ mediates strand rejoining by the concerted action of its core machinery, in which XRCC4/Ligase IV catalyzes the final repair step. XRCC4/Ligase IV specifically ligates free 5'-phosphate and 3'-hydroxyl DNA termini. However, DNA strand breaks commonly form with 5'-hydroxyl and 3'-phosphate termini, and polynucleotide kinase-phosphatase (PNKP) is the primary enzyme recruited to restore ligatable ends. PNKP is directed to the DNA ends by an interaction between its forkhead-associated (FHA) domain and a canonical phosphothreonine motif in the XRCC4 C-terminal tails.

We have used small-angle X-ray scattering (SAXS) to generate static reconstructions of the XRCC4/Ligase IV heterodimer and XRCC4/Ligase IV/PNKP complex. Additionally, we have defined the conformational variability of this complex and its components. The results show significant flexibility in the system, but suggest a secondary interaction between PNKP and XRCC4/Ligase IV that may be crucial for coordinating DNA end-processing and repair. Targeting these interactions for disruption may lead to the development of new chemo and radiosensitizing agents.

POSTER #2

Genome instability and G2/M regulation by Cdk1 inhibitory phosphorylation in *Drosophila*

Joseph Ayeni, Oindrila Mukharjee, Stanley Tiong, and Shelagh Campbell

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Regulation of G2/M progression by Cdk1 inhibitory phosphorylation is critical for genome stability in eukaryotes, because premature Cdk1 activation can cause mitotic catastrophe and apoptosis. This mechanism involves a switch-like cycle of inhibitory phosphorylation and dephosphorylation of Cdk1 on T14 and Y15 residues by Wee1 related kinases and $Cdc25^{stg}$ phosphatases, respectively. *In vitro* studies have suggested differences in catalytic properties of Y15-phosphorylated and dual T14- and Y15-phosphorylated Cdk1 isoforms, however little is known about how these differences affect cells during development, or their regulation by two different types of Cdk1 inhibitory kinases found in metazoans: Wee1 and Myt1.

To address these issues, we made Gal4-inducible VFP-tagged transgenic strains expressing wild type Cdk1 and two different Cdk1 phospho-inhibition mutants. In one, tyrosine-15 is mutated to phenylalanine (Cdk1F); in the other, both tyrosine-15 and threonine-14 were mutated to phenylalanine and alanine (Cdk1AF). Expression studies indicate that differences in Cdk1 inhibitory phosphorylation differentially affect *Drosophila* developmental G2/M regulation, cell proliferation and apoptosis during Drosophila wing imaginal development. We also observed intriguing localization differences among different Cdk1 phospho-isoforms, implicating Y15 Cdk1 inhibitory phosphorylation in a novel mechanism for regulating G2/M progression.

POSTER #3

Sodium salicylate as a novel inhibitor of topoisomerase II

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Topoisomerase IIα (topo II) is a ubiquitous enzyme involved in DNA replication and transcription, as well as in the separation of sister chromatids prior to mitosis. Anthracyclines, such as the commonly used chemotherapeutic doxorubicin, are a class of topo II poisons that stabilize covalent topo II-DNA cleavable complexes resulting in the accumulation of DNA double-strand breaks. We have previously reported that pretreatment of human lymphoblastoid cells with the hydroxyl radical scavenger, N-acetyl cysteine, attenuates doxorubicin-induced DNA damage signaling through the ATM protein kinase. In the present study, using a human breast cancer cell line, we investigated the effects of additional hydroxyl radical scavengers on doxorubicin-induced DNA damage and cytotoxicity. Using MCF-7 cells, we observed that doxorubicin triggers autophosphorylation of ATM and the ATM-dependent activation of multiple downstream effectors. Furthermore, this doxorubicin-induced DNA damage signaling was attenuated by pretreatment of cells with sodium benzoate or sodium salicylate, and, to a lesser extent, N-acetyl cysteine. These effects were independent of doxorubicin-generated reactive oxygen species as similar results were observed in cells treated with 5-iminodaunorubicin, a non-redox cycling analog of doxorubicin. As sodium salicylate is widely used as an anti-inflammatory and analgesic, as well as being a major metabolite of acetylsalicylic acid, long-term use of which is associated with a decreased risk of cancer, we investigated the mechanism underlying the observed effects of cellular pretreatment with sodium salicylate. The attenuated damage signaling observed in cells pretreated with sodium salicylate is accompanied by a significant decrease in doxorubicin-induced DNA double strand breaks. The attenuated DNA damage signaling is not attributable to inhibition of cyclooxygenase or NF-κB, as pharmacological inhibitors of these pathways had no effect on doxorubicin-induced DNA damage signaling. Furthermore, we have determined that pretreatment of cells with sodium salicylate results in a decrease in doxorubicin-stabilized topo II-DNA cleavable complex formation *in vivo*. Using an *in vitro* topo II-DNA cleavage assay, we have determined that sodium salicylate is an inhibitor of topo II catalytic activity, a novel mechanism of action for salicylates. Pretreatment of cells with sodium salicylate also results in cell cycle arrest at G2/M, consistent with other established inhibitors of topo II. Importantly, pretreatment of cells with sodium salicylate attenuates doxorubicin cytotoxicity, suggesting that further study in animal models of the effects of salicylate administration on doxorubicin sensitivity are warranted.

POSTER #4

Age dependent microsatellite instability in *Arabidopsis thaliana*

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Plant development consists of the initial phase of intensive cell division followed by continuous genome endoreduplication, cell growth and elongation. The maintenance of genome stability under these conditions is the main task performed by DNA repair and genome surveillance mechanisms. Our previous work showed that the rate of homologous recombination repair in older plants decreases. We hypothesized that this age-dependent decrease in the recombination rate is paralleled with other changes in DNA repair capacity. We analyzed microsatellite stability using transgenic plants that carry the non-functional β-glucuronidase gene disrupted by microsatellite repeats. We found that microsatellite instability increased dramatically with plant age. Analysis of total polymerase activity using partially purified protein extracts showed an age-dependent decrease in activity and an increase in fidelity. Analysis of the steady-state level of *Msh2*, *Msh6* and *Msh7* RNA showed an age-dependent increase. An *in vitro* repair assay showed lower efficiency of non-homologous end joining in older plants, paralleled by an increase in Ku70 expression and a decrease in Rad51. Thus, we assume that the more frequent involvement of non-homologous end-joining in strand break repair and less efficient end-joining repair may be the main contributors to the observed age-dependent increase in microsatellite instability.

POSTER #5

Nse5 regulates sumoylation of Mms21 targets

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The Structural Maintenance of Chromosomes (SMC) family of proteins provides structural support and organization to DNA, and is crucial in the maintenance of genomic integrity. While other SMC family members such as the cohesin and condensin complexes have been well-studied, relatively little is known about the Smc5-Smc6 complex. In addition to the Smc5 and Smc6 proteins, this complex consists of 6 other Non-Smc Elements (Nse), all of which are essential proteins. Importantly, Nse2 (Mms21) is an E3 SUMO ligase. Nse5 has never been studied in detail in *Saccharomyces cerevisiae*, however a high throughput yeast 2 hybrid screen reported it to interact with components of the SUMO modification pathway. We have identified two putative SUMO-Interacting Motifs (SIMs) in Nse5 and have detected a genetic interaction between a temperature sensitive Nse5 mutant allele (*nse5-ts)* and a SUMO-deficient Mms21 allele (*mms21-11)*, suggesting a role for Nse5 in the SUMO pathway. Sumoylation of Smc5 and yKu70, which are normally sumoylated by Mms21, is abolished in Nse5 mutant cells. Finally, *nse5-ts/siz1Δ/siz2Δ* triple mutants, like *mms21-11/siz1Δ/siz2Δ* triple mutants, display synthetic lethality. We propose a role for Nse5 in recruiting the SUMO E2 conjugating enzyme Ubc9 through its SIMs.

POSTER #6

Inhibition of the tumour supressor BRCA1 as a novel therapeutic strategy

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Here we explore the possibility of Breast Cancer Associated 1 (BRCA1) as a possible therapeutic target. As a key component of the DNA damage response, germline mutations in BRCA1 have been identified as a major initiator of hereditary breast and ovarian cancers. Conversely, in many sporadic breast cancers, BRCA1 is fully functional and may play a key role in acquired resistance to chemotherapies due to its important role in DNA repair and checkpoint control. The ability to impair these pathways by inhibiting BRCA1 in cancer cells may prove to be a useful means to sensitize tumours to DNA damaging agents. Because BRCA1 deficiencies are only associated with a marked increased risk for cancers in the breast and ovarian tissues, highly specific BRCA1 inhibitors might selectively target these tissues, thereby reducing off-target side effects. Currently, the inhibition of ssDNA repair via PARP1 inhibitors, in addition to the already impaired ability of $BRCA1^-$ tumours to repair dsDNA breaks, have shown to be very effective. In $BRCA1^+$ cancers, a combination of PARP1 and BRCA1 inhibitors may prove to be equally as effective. We use Fluorescence Polarization (FP) and streptavidin pull-down competition assays to locate and verify novel inhibitors of BRCA1.

POSTER #7

A role for Ezh2 in the DNA damage response pathway

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Polycomb group proteins (PcG) are chromatin associated proteins involved in stem cell maintenance and gene repression. Enhancer of Zeste Homolog 2 (Ezh2) is a histone methyltransferase associated with polycomb repressive complex 2 (PRC2) and is best known for the trimethylation of histone 3 lysine 27 (H3K27me3). Overexpression of Ezh2 correlates with aggressive and metastatic tumor profiles, but whether or not this results directly from PRC2-dependent changes in gene regulation remains unknown. In this study, we examine the potential involvement of EZH2 in the DNA damage response (DDR) pathway. GFP labeled Ezh2 is recruited to laser induced DNA damage with similar kinetics as other recognized DDR proteins. Consistent with its function as a DDR protein, knockdown of Ezh2 causes increased sensitivity to gamma-irradiation and reduced ability to repair double-stranded-breaks. Collectively, this data implicates EZH2 in the DDR pathway and PcG in the repair of double-strand-breaks.

POSTER #8

A potential role for microRNA-34a in Hutchinson-Gilford progeria syndrome

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Hutchinson-Gilford progeria syndrome (HGPS) is a rare genetic disorder characterized by several indices of premature aging, such as muscle and skin atrophy, cardiovascular complications and cataracts among others. Mutating Lamin A is known to result in HGPS but the molecular pathways affected by this mutation, which transduce the HGPS phenotype, are still unknown. MicroRNAs (miRNAs) are small and non-coding RNA molecules. They are important gene regulators in cellular pathways such as proliferation, apoptosis and senescence. MicroRNA-34a (miR-34a) was reported to inhibit cell proliferation, induce apoptosis and induce senescence depending on cell type. MiR-34a has also been implicated in both replicative senescence and stress induced premature senescence. Using a real time RT-PCR method we find that miR-34a is expressed at higher levels in fibroblasts from HGPS patients compared to fibroblasts from normal control donors. Transfection of miR-34a inhibited progeria cell proliferation while inhibiting endogenous miR-34a using miRNA inhibitors increased cell proliferation. Western blot analysis showed that E2F-1, a critical positive cell cycle regulator, was downregulated by the transfection of miR-34a. These results indicate that miR-34a may inhibit progeria cell proliferation through down-regulation of E2F-1, which might be involved in the formation of abnormal proliferative characteristics seen in HGPS.

POSTER #9

Phosphorylation of Pho23 by the Hog1 MAP kinase modulates the function of the yeast Rpd3L histone deacetylase complex

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The yeast Rpd3L histone deacetylase (HDAC) complex is highly conserved and plays critical roles in modifying the chromatin substrates appropriate for gene transcription, DNA replication and repair. We previously demonstrated that Pho23, an Ing1-related protein, is a component of the Rpd3L complex and is required for the normal function of this complex. Here, we have examined whether Pho23 is phosphorylated and whether such modification is involved in the regulation of the Rpd3L HDAC complex function. Our evidence indicates that Pho23 is phosphorylated, and that this phosphorylation is induced upon osmotic stress. Furthermore, our genetic evidence suggests that this phosphorylation event is mediated by the osmotic stress-activated Hog1 MAP kinase. In addition, we have mapped this phosphorylation site to Ser 234, and generated a mutant strain lacking this site; and we have shown by qRT-PCR that the expression of the salt-inducible and Rpd3-dependent genes, HSP12 and CTT1, were significantly reduced (> 3-fold) in the mutant strain, as compared to the WT strain, following osmotic stress treatment. These results support a model of a novel regulatory mechanism for Rpd3L-HDAC complex, in which the Hog1 MAP kinase phosphorylates Pho23 at Ser^{234}, rendering the complex functional to mediate activation of gene transcription.

POSTER #10

A biochemical assay to study protein recruitment to DNA double-strand breaks

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DNA integrity is continually challenged by exposure to DNA damaging agents. A major form of damage is double-strand breaks (DSBs), which if mis-repaired are mutagenic or cytotoxic. Radiation-induced DSBs, characterized by base lesions, abasic sites or single-strand breaks in close proximity to DSB termini, exhibit diverse types, yield and spatial orientation and hence require intense co-ordination of repair.

Non-homologous end joining (NHEJ), the predominant DSB repair pathway in mammalian cells, involves break recognition, repair factor recruitment, processing and ligation of the DNA. Although the 'core' components of NHEJ have been widely studied, little is known about the order of recruitment or structural conformations adopted during repair. Less is known about the interplay between different repair pathways or the resolution/ dissociation of repair factors.

We have developed a biochemical assay to study the interaction of repair factors with modeled radiation-induced DSBs. Synthetic biotinylated oligonucleotides are incubated with extracts from unirradiated or irradiated cells. Protein recruitment is detected by western blot and mass spectrometry to study the effect of DNA structure on protein recruitment. Through transient knock-down of repair factors, the requirement for NHEJ factors can be assessed, and insights gained into possible hierarchies of protein recruitment and interchangeability of repair proteins in the absence of classic NHEJ.

POSTER #11

Function of the Artemis endonuclease in the repair of complex double-strand DNA breaks

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The induction of double-strand breaks (DSBs) with associated base lesions and abasic sites, termed complex DSBs, are characteristic of ionising radiation (IR) exposure, and are refractory to repair by the classic non-homologous end joining and base excision repair pathways. Artemis, responsible for hairpin opening of coding joints in V(D)J recombination, has been implicated in the repair of a subset of IR-induced breaks that are believed to be complex, as increased numbers of residual DSBs are observed in Artemis null cells following IR.

Using a biochemical approach we have directly investigated the role of the Artemis endonuclease activity to cleave overhangs from oligonucleotides with 8-oxo-7,8-dihydroguanine (8-oxoG) or abasic sites at defined positions in close proximity to the termini. We find that Artemis exhibits an activity to process a small subset of complex termini that is dependent upon overhang length and orientation. Furthermore, Artemis is retarded when 3' terminal blocking groups, 8-oxoG or abasic sites are positioned within three to five bases either side of the cleavage site. Consequently, we suggest that Artemis processing of complex DSBs is a minor role and propose a hierarchy of complex DSB repair which is dependent upon the co-ordination of different repair pathways.

POSTER #12

Protein phosphatase 6 interacts with the DNA-dependent protein kinase catalytic subunit and dephosphorylates γ-H2AX

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The catalytic subunit of the DNA-dependent protein kinase (DNA-PKcs) plays a major role in the repair of DNA double-strand breaks (DSBs) by nonhomologous end joining (NHEJ). We have previously shown that DNA-PKcs is autophosphorylated in response to ionizing radiation (IR) and that dephosphorylation by a protein phosphatase 2A (PP2A)-like protein phosphatase (PP2A, PP4, or PP6) regulates the protein kinase activity of DNA-PKcs. Here we report that DNA-PKcs interacts with the catalytic subunit of PP6 (PP6c) and PP2A (PP2Ac), as well as with the PP6 regulatory subunits PP6R1, PP6R2 and PP6R3. Consistent with a role in the DNA damage response, silencing of PP6c by small interfering RNA (siRNA) induced sensitivity to IR and delayed release from the G_2/M checkpoint. Furthermore, siRNA silencing of either PP6c or PP6R1 led to sustained phosphorylation of histone H2AX on serine 139 (γ-H2AX) after IR. In contrast, silencing of PP6c did not affect the autophosphorylation of DNA-PKcs on serine 2056 or that of the ataxia-telangiectasia mutated (ATM) protein on serine 1981. We propose that a novel function of DNA-PKcs is to recruit PP6 to sites of DNA damage and that PP6 contributes to the dephosphorylation of γ-H2AX, the dissolution of IR-induced foci, and release from the G_2/M checkpoint *in vivo*.

POSTER #13

Mechanism of action of an imidopiperidine inhibitor of human polynucleotide kinase/phosphatase

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The small molecule, 2-(1-hydroxyundecyl)-1-(4-nitrophenyl-amino)-6-phenyl-6,7a-dihydro-1*H*-pyrrolo[3,4-b]pyridine-5,7(2*H*,4a*H*)-dione (A12B4C3), is a potent inhibitor of the phosphatase activity of human polynucleotide kinase/phosphatase (PNKP) *in vitro*. Cellular studies revealed that A12B4C3 sensitizes A549 human lung cancer cells to the topoisomerase I poison, camptothecin, but not the topoisomerase II poison, etoposide, in a manner similar to siRNA against PNKP. A12B4C3 also inhibits the repair of DNA single and double-strand breaks following exposure of cells to ionizing radiation, but does not inhibit two other key strand break repair enzymes, DNA polymerase beta or DNA ligase III, providing additional evidence that PNKP is the cellular target of the inhibitor. Kinetic analysis revealed that A12B4C3 acts as a non-competitive inhibitor, and this was confirmed by fluorescence quenching, which showed that the inhibitor can form a ternary complex with PNKP and a DNA substrate, i.e. A12B4C3 does not prevent DNA from binding to the phosphatase DNA binding site. Conformational analysis using circular dichroism, UV-difference spectroscopy and fluorescence resonance energy transfer all indicated that A12B4C3 disrupts the secondary structure of PNKP. Investigation of the potential site of binding of A12B4C3 to PNKP using site directed mutagenesis pointed to interaction between Trp^{402} of PNKP and the inhibitor. A12B4C3 is thus a useful reagent for probing hPNKP cellular function and will serve as the lead compound for further development of PNKP-targeting drugs.

POSTER #14

Age-dependent changes in DNA repair in radiation-exposed mice

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Ionizing radiation is one of the most potent DNA damaging agents. As such it is used for the therapy of various cancers. Various previous publications suggest that younger and older cells have a different response to ionizing radiation. To test whether this hypothesis is sustained, we analyzed the response of three age groups of animals, 14-day-old, two-month-old and 18-month-old mice, to IR. Animals received 1 Gy whole-body irradiation and spleen tissues were taken for the analysis at 6 and 96 h post irradiation. We found that exposure to 1 Gy of IR affects several DNA repair pathways, such as NHEJ, HR and BER in the age-dependent manner. Old animals had the largest increase of gamma-H2AX loci and of Atm1 expression as well as the decrease in the expression of Ku70 and an increase in the expression of Rad51 proteins. No significant changes in Pol ε and Pol β in response to IR were detected in oldest animals, suggesting predominance of short-patch, error-prone BER pathway in ageing spleen. We also found that old animals had the lowest efficiency of the DNA repair.

POSTER #15

Aurora kinase inhibition and cell survival in pediatric leukemia

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Introduction: Overexpression of Aurora kinases has been shown to induce a high level of genomic instability that favors the development of aggressive and metastatic tumors. Increased levels of Aurora kinases have been found in pediatric acute lymphoblastic leukemia. We are investigating targeted Aurora kinase inhibitors as potential therapeutic agents for the treatment of these children. **Methods:** Cells were incubated with increasing concentrations of the Aurora kinase inhibitor AT9283. Cell survival was measured and IC_{50} values and combination indices were calculated. Alterations in cell signaling pathways and survival proteins were measured by Western blot analysis. **Results:** AT9283 inhibited the growth of the cell lines with a 10 fold variation in IC_{50} within cell lines. There was a corresponding increase in the number of cells displaying a polyploid phenotype, an effect of Aurora kinase inhibition. Changes in the expression pro-apoptotic proteins and the activation of signaling molecules such as AKT and MEK1/2 were detected. Aurora kinases synergized with HDAC inhibition. **Conclusions**: AT9283 significantly decreases the growth and survival of leukemia cells. Drug combination studies demonstrate the potential of HDAC inhibition to synergize with this agent, providing preclinical data for the formulation of an effective clinical trial.

POSTER #16

Role of prolactin on DNA damaging and chemotherapeutic resistance

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Prolactin, a peptide hormone, is associated with increased risk for breast cancer. We discovered that prolactin also induces breast cancer cell resistance to DNA damaging chemotherapeutic drugs. This involves the master cancer chaperone heat shock protein-90 (Hsp90), of which, HSP90alpha was identified by our laboratory as a prolactin regulated gene in breast cancer cells. We hypothesize that there is cross- talk between the prolactin pathway and the DNA damage response. Our studies showed that when breast cancer cell lines are treated with the Hsp90 inhibitor 17AAG and doxorubicin, there is a decrease in ataxia-telangiectasia mutated protein (ATM) and also phospho (active) - ATM levels, indicating there could be association between Hsp90 and active ATM. ATM plays a critical role in the DNA damage response. 17AAG causes a proteosomal degradation of Hsp90 client proteins and by using a proteasome inhibitor MG132 with 17AAG we were able to inhibit degradation of phospho- ATM. We have ongoing in vivo study to determine the effect of prolactin on latency and tumor volume in xenografts of DNA damaged tumor cells. These results indicate that prolactin may cause chemotheraputic resistance by cross-talk with the DNA damage response.

POSTER #17

Pathogen influence on genome stability

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Plant pathogens are one of the significant stresses encountered by plants. Two types of plant pathogen interactions are possible based on presence of pathogen specific resistance (*R*) genes. If an *R* gene is present against an invading pathogen, it leads to incompatible interaction. Absence of such an *R* gene results in compatible interaction, evident by disease symptoms. A novel systemic signal was identified during compatible interactions which lead to increased somatic and meiotic recombination in infected plants. A transgenerational change in epigenetic status of host plants was observed. The progeny of pathogen infected plant possess higher DNA methylation and the distribution of heterochomatin and euchromatin is altered. DNA methylation level of Leucine rich repeat regions of plant resistance genes is decreased. To investigate possible role of small RNA species in establishment of these transgenerational changes, DICER-like mutants were used. Homologous recombination frequency was analyzed in *dcl2*, *dcl3* and *dcl4* following compatible pathogen infection. Results indicate that dcl4 mutant is unable to report increase in recombination frequency following pathogen infection. Hence, function of *DCL4* might be pivotal for establishment of transgenerational changes following pathogen infection.

POSTER #18

Identification and characterization of p54(nrb), a novel topo II interacting protein

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DNA topoisomerase IIα (topo II) is a nuclear enzyme charged with the task of resolving the topological complexities of DNA during cellular replication and division. Topo II is absolutely essential to the survival of organisms, and, given this critical role, it is the target of a number of widely used anti-tumour chemotherapeutics. Despite its essential nature, investigations into its functional regulation remain limited. Previous work has suggested that protein-protein interactions could play an important role in the modulation of topo II activity. To identify novel topo II-interacting proteins, we generated a cell line stably expressing FLAG-tagged, wild-type topo II under the control of a tetracycline-inducible promoter. This exogenously expressed FLAG-tagged topo II protein was selectively captured and immunopurified using an anti-FLAG affinity gel and novel interacting proteins were identified using an unbiased mass spectrometric approach, gel-enhanced LC-MS/MS analysis. Among the novel putative protein partners identified was p54/nrb, a multifunctional nuclear protein recently implicated in double-strand break repair and radioresistance. Following verification of the topo II/p54 interaction, we have undertaken characterization of the topo II/p54 interaction and its impact on topo II catalytic activity.

POSTER #19

Structural and functional investigations of TopBP1 BRCT4/5 domains

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The checkpoint response to replication stress is critical for maintaining the faithful replication of DNA. At the apex of the replication stress checkpoint response pathway is the ATR kinase, which phosphorylates a number of proteins at the replication fork to regulate downstream processes such as DNA damage repair, cell cycle control, replication fork stabilization and fork restart. ATR activity requires activation by TopBP1, a protein that contains eight conserved BRCT domains that function in protein-protein interactions. Here we present the crystal structure of TopBP1 BRCT4/5 domains, which reveals an unusual packing between the tandem BRCT domains. We further provide evidence for DNA binding activity *in vitro*, which may be important for TopBP1 localization and processivity during replication stress.

POSTER #20

Epigenetic aspects of acquired cancer cell chemo- and radio-resistance

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A number of studies indicate a substantial involvement of epigenetic mechanisms in drug-resistant cancer cells, including changes in DNA methylation patterns. In the present study, we first examined the alteration in epigenetic mechanism in drug-resistant MCF-7 human breast cancer cells induced by doxorubicin (DOX) and cisplatin (cisDDP). Both of these drug-resistant cell lines displayed similar pronounced changes in global epigenetic landscape showing loss of global DNA methylation. Ionizing radiation is another important treatment modality. Therefore, the next step of our study was aimed to analyze and compare the resistance of the MCF-7, MCF-7/DOX and MCF-7/cisDDP cells to radiation. We noted that chemoresistant cells were able to withstand killing by radiation and do not undergo radiation-induced cell cycle arrest and apoptosis. Additionally, chemoresistant cells had altered capacity to repair radiation-induced damage. Importantly, modification of the epigenetic status of chemoresistant lines by 5-aza-2′-deoxycytidine improved their radiation sensitivity. These results provide the evidence that epigenetic changes are an important feature of cancer cells with acquired drug-resistant phenotype. Modification of epigenetic status may be used to increase their sensitivity to chemo- and radiotherapy.

POSTER #21

The XLF C-terminal region is required for DNA binding and interaction with Ku70/80 in vitro but not for repair of double-strand breaks *in vivo*

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DNA double strand breaks (DSBs) are one of the most detrimental DNA lesions in the cell. DSBs can be induced by ionizing radiation (IR) and in mammalian cells these DSBs are primarily repaired by the non homologous end-joining pathway (NHEJ) which involves several core proteins including the DNA-PK complex which is composed of the Ku70/80 heterodimer and the DNA-PKcs catalytic subunit; the XRCC4-DNA ligase IV complex; and the XRCC4-like factor (XLF) which stimulates XRCC4-DNA ligase IV mediated DNA end-joining. Here we show that the C-terminal region of XLF, which is predicted to be unstructured, is required for the DNA-dependent interaction between XLF and the DNA-PK complex in cell extracts and for interaction with purified Ku70/80 in pull-down assays. Interestingly, the highly conserved penultimate amino acid F298 is absolutely required for the interaction between XLF and Ku as well as for DNA binding in electrophoretic mobility shift assays. However, C-terminal truncation of XLF or mutation of F298 does not significantly alter the kinetics of gamma-H2AX foci resolution following IR *in vivo*. These results suggest that although the C-terminal region of XLF is important for DNA binding and interaction with Ku in vitro these interactions may not be necessary for double-strand break repair *in vivo*.

POSTER #22

Determining the synthetically lethal partners of PNKP and its potential role in directed cancer therapies

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Synthetic lethality arises when the combination of two non-essential protein disruptions in the same cell is lethal. This phenomenon has been shown to occur between proteins involved in DNA repair and much attention has been focused on associations between PARP and BRCA. Recent findings have shown that synthetically lethal relationships also occur between proteins not directly involved in DNA repair.

We have shown that DNA polymerase β-PNK and PARP-PNK double knockdowns are synthetically lethal, the latter finding agrees with data from Turner *et al.* (2008). Furthermore, we have shown that co-disruption of PNKP and DNA-PK is not synthetically lethal. *We therefore hypothesize that in synthetically lethal relationships involving PNKP, the function of PNKP in DSBR is critical.*

This is important because the *pnkp* gene is found near the telomeric region of chromosome 19q, an area commonly deleted in oligodendrogliomas, and to a lesser extent oligoastrocytomas and epithelial ovarian cancers.

We are also currently undertaking an siRNA-based screen of ~9000 genes to determine synthetically lethal relationships between PNKP and non-DNA repair proteins. This will allow us to determine if synthetically lethal relationships with PNKP exist outside the realm of DNA repair proteins.

POSTER #23

Do murine Piwi proteins play a role as epigenetic regulators in the molecular etiology of IR induced transgenerational genome instability?

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Parental exposure was documented to result in a significant (6-8 fold) increase in the risk of leukemia in children. The exact molecular mechanisms of transgenerational carcinogenesis have yet to be elucidated, although there is support for it being an epigenetically-induced phenomenon. Epigenetic alterations include cytosine DNA methylation, histone modifications, and small RNA -mediated events. Our study is focused on the protein component of a novel small RNA pathway (piRNA pathway) and its response to genotoxic stress (IR). The Piwi proteins of the of the Argonaute protein family play a integral role in piRNA pathway that is essential for gametogenesis, silencing of selfish genetic elements, and maintaining germline genome integrity in male mice. Our recent studies utilizing western immunoblotting and immunohistochemistry analysis have shown that expression of murine Piwi proteis is altered in response to genotoxic stress exposure (IR). IR induced aberrant expression of key proteins intimately involved in a small RNA pathway that is essential for paternal germline genome stability may play a key role in germline epigenetic alterations that can affect future generations. Further investigation into such germline epigenetic regulators may play a vital role in understanding radiation carcinogenesis, which, although widely studied, still remains enigmatic.

POSTER #24

DNA damage response regulated by the spindle assembly factor TPX2

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The Microtubule-associated protein TPX2 is required for mitotic spindle assembly and cell cycle progression. During interphase, TPX2 is actively imported into the nucleus. The function of TPX2 in the nucleus is unknown. We now report that TPX2 plays a key role in the DNA damage response (DDR) to Ionizing radiation (IR).

Inability to amplify and regulate a proper DDR results in cancers. Molecular amplification of the DDR is mediated by a complex composed of the Mediator of DNA damage Checkpoint 1 (MDC1), the Ataxia Telangiectasia Mutated (ATM) kinase and/or the DNA dependant protein kinase (DNA-PK). This complex triggers phosphorylation of histone H2AX (γ-H2AX when phosphorylated) after IR. The amplification of γ-H2AX is essential to promote cellular resistance to DNA damage. We found that TPX2 associates with MDC1, ATM and DNA-PK after IR and, critically, dampens the MDC1/ATM/DNA-PK-dependent phosphorylation of H2AX. Overexpression of TPX2 leads to decreased γ-H2AX signals and a disrupted intra-S-phase checkpoint whereas cells with decreased levels of TPX2 over-amplify γ-H2AX signals and exhibit repair defects and increased apoptosis after genomic insults. Thus, our study identifies a first function for TPX2 in the nucleus and a novel regulator of DDR.

POSTER #25

Localization of Dpb11^{TopBP1} and its effects at stalled replication forks and unfired origins of replication

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Dpb11^{TopBP1} is a protein that performs a dual function: it promotes the assembly of the DNA replication complex, and it enables full activation of Mec1-Ddc2ATR-ATRIP kinase in response to the replication checkpoint and DNA Double-Strand-Breaks (DSBs) in the budding yeast Saccharomyces cerevisiae. One question in the field is how Dpb11 is recruited to either forks or sites of DNA damage as well as how it contributes to downstream checkpoint activation events. Using Chromosomal Immunoprecipitation (ChIP) and Co-Immunoprecipitation (CoIP), I plan to track the changing localization and interaction partners of Dpb11TopBP1 as the replication checkpoint is activated by Hydroxyurea (HU), as well as if these interaction partners differ from when the cell is challenged with an irrepairable DSB. I have performed some mutagenesis of tagged dpb11 alleles to determine the regions responsible for replication fork recruitment. I plan to perform further mutations to better characterize this region. I also plan to compare the kinetics of its recruitment in Wt cells and dpb11 alleles in cells with alleles of the replication checkpoint and DNA damage response pathway to determine the hierarchy of Dpb11, Mec1-Ddc2 and the checkpoint clamp 9-1-1's recruitment to stalled forks and damaged sites. These experiments should provide a comprehensive understanding of the role of Dpb11TopBP1 in its checkpoint function under different stimuli.

POSTER #26

Epigenetic response to γ-irradiation and aging in Eukaryotes

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Epigenetic alterations are given increasing consideration in their roles in health and disease (Jirtle & Skinner, 2007). Recent data from the Kovalchuk's lab show that ionizing radiation influences epigenetic profiles of various cell-types in animal models. Some indication exists that radiation sensitivity is age-dependent, however mechanistic understanding is so far missing (Richardson, 2009). In contrast to animals, plants are much more resistant to ionizing radiation (Hase, 1999), and only recent publications indicate an effect of plant senescence on stress responses (Zinn, 2010, Taulavuori, 2010). Thus, the overall goal of this study is to get a mechanistic understanding of the epigenetic response and genome stability in aging plants and humans and their consequences on tolerance of γ –irradiation. This will include comparison of responses in human cell culture (human lung fibroblasts, WI-38) and plant cell suspension culture (derived from *Arabidopsis* cultivar Columbia). Epigenetic and genetic responses will be assessed on the level of gene expression, microRNAome, global and locus-specific DNA methylation, histone modifications, homologous recombination frequency and polymerase fidelity. We predict that this approach will allow correlation of epigenetic changes with changes in the level of DNA damage and repair as a function of age in irradiated human and plant cells.

POSTER #27

Radiation-induced bystander effects alter molecular mechanisms *in vivo*

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The bystander effect is a phenomenon in which non-irradiated cells respond to signals from directly irradiated neighbouring cells, producing genome destabilization and carcinogenesis. This study investigated the effects of ionizing radiation on male and female *Rattus norvegicus* bystander liver tissue. miRNAs have been shown to play a role in translational suppression. Using miRNA microarray analysis we found miRNA differences between male and female rat liver in a control treatment against a head exposed treatment (1Gy) at 96-hour and 14-day post irradiation. Putative targets of the miRNA showing changes in expression, and other associated proteins present within each exposure group were analyzed using Western Blotting. Results indicate an increase in miR-25, and a decrease in the expression of its known putative target, Bim. We also observed a significant decrease in the levels of Ape1 and an increase in SUFU, in bystander liver tissue of head-exposed female animals. In contrast, we noted a significant decrease in DNA repair in bystander liver tissue of head exposed males. Our analysis revealed that the observed molecular changes may account for altered levels of apoptosis and DNA repair in bystander liver tissue.

POSTER #28

Influence of pathogenic bacterial determinants on genome stability of exposed and distal cells

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We hypothesized that exposure to a pathogen's components would result in a decrease in genomic stability of the indirect "target" organs: intestine, liver, spleen of mice upon exposure to DNA, RNA or LPS extracted from heat-killed bacteria. We analyzed whether exposure to heat shock-killed bacteria and bacterial components such as DNA, RNA, proteins and lipopolysaccharides (LPS) results in genome instability by analyzing the levels of DNA damage, repair and homologous recombination (HR). The animal groups were given either control tap water, or water with the whole heat-killed bacteria. Other animal groups received DNA, RNA, protein or LPS in the water supply, respectively, for a period of 14 days. A second cohort with similar treatment plan was run in a form of a 'stop experiment' whereby animals received contaminated water for 14 days followed by clean tap water for another 14 days. We analyzed formation of γH2AX foci in liver and spleen cells to identify any significant alterations in the DNA damage within the test tissues for all treatments. We also studied the levels of DNA repair proteins in the exposed tissues. The results and the model of the influence of bacterial determinants on genome stability will be presented.

POSTER #29

ATM-deficiency sensitizes Mantle Cell Lymphoma cells to PARP-1 inhibitors

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Poly-ADP ribose polymerase-1 (PARP-1) inhibition is toxic to cells with mutations in the breast and ovarian cancer susceptibility genes *BRCA1* or *BRCA2,* a concept, termed synthetic lethality. However, whether this approach is applicable to other human cancers with defects in other DNA repair genes has yet to be determined. The *Ataxia-Telangiectasia Mutated (ATM)* gene is altered in a number of human cancers including Mantle Cell Lymphoma (MCL). Here, we characterize a panel of MCL cell lines for ATM status and function and investigate the potential for synthetic lethality in the presence of small molecule inhibitors of PARP-1. We show that Granta-519 and UPN2 cells have low levels of ATM protein, are defective in DNA damage-induced ATM-dependent signaling, are radiation sensitive and have cell cycle checkpoint defects: all characteristics of defective ATM function. Significantly, Granta-519 and UPN2 cells were more sensitive to PARP-1 inhibition, than were the ATM-proficient MCL cell lines examined. Furthermore, the PARP-1 inhibitor olaparib (previously known as AZD2281/KU-0059436) significantly decreased tumour growth and increased overall survival in mice bearing subcutaneous xenografts of ATM-deficient Granta-519 cells, while producing only a modest effect on overall survival of mice bearing xenografts of the ATM-proficient cell line, Z138. Our results suggest that PARP inhibitors have therapeutic potential in the treatment of MCL and that the concept of synthetic lethality extends to human cancers with alterations in *ATM*.

POSTER #30

Estrogen induced transcription leads to replication associated DNA double strand break formation

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Reduced expression and mutations in several DNA double strand break response proteins confers an increased risk of breast cancer. This increased risk is likely due to an inability of cells to efficiently manage DNA damage within the dynamic breast tissue. One possible source of this damage is the presence of hormones, specifically estrogen. Metabolism of estrogen to its catechol metabolites has previously been shown to result in an accumulation of oxidized bases and abasic sites. In this study we show that treatment of breast cancer cells with 17β-estradiol results in an accumulation of DNA repair foci that are representative of replication associated DNA damage. However, in contrast to the previously demonstrated model, these foci are not mediated by estrogen metabolites and are dependent on ERα mediated transcription. We also demonstrate that γH2AX is enriched at the estrogen inducible promoter of trefoil factor-1 suggesting the damage occurs at transcription sites. Furthermore, γH2AX foci are dependent on topoisomerase II β, an enzyme involved in transcriptional regulation of estrogen inducible genes. Here we propose a novel model of replication associated DNA damage that is mediated by topoisomerase II β induced double strand break formation occurring in response to estrogen stimulation.

POSTER #31

XRCC4 is phosphorylated at multiple sites *in vivo*

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Nonhomologous end joining (NHEJ) is a major pathway for the repair of DNA double-strand breaks (DSBs) in higher eukaryotes. XRCC4 plays a crucial role in the NHEJ pathway, acting as a scaffold protein that recruits other NHEJ proteins to DSBs. Proteins required for NHEJ in human cells include the DNA-dependent protein kinase (DNA-PK), XRCC4 and DNA ligase IV. DNA-PK, which is composed of a catalytic subunit (DNA-PKcs) and a DNA-binding protein, Ku70/80, first binds to the DSBs and the XRCC4-DNA ligase IV complex is subsequently recruited. We previously reported that XRCC4 is a substrate of DNA-PK *in vitro*. Two major DNA-PK phosphorylation sites (Ser-260 and Ser-318) in XRCC4 as well as several minor sites were identified by mass spectrometry *in vitro.* Serine to alanine substitution of these sites reduced the ability of DNA-PK to phosphorylate XRCC4 *in vitro* by at least two orders of magnitude (Yu et al, DNA Repair, 2003). We raised phosphospecific antibodies to both Ser-260 and Ser-318 of XRCC4 and show that XRCC4 Ser-318 is phosphorylated *in vivo*, that phosphorylation was induced by irradiation (IR) and that phosphorylation was dependent on both DNA-PKcs and ATM. XRCC4 has also been reported to be phosphorylated by protein kinase CK2 on threonine 233 and this phosphorylation has been shown to promote its interaction with the forkhead associated (FHA) domain of the end-processing enzyme polynucleotide kinase/phosphatase (PNKP) (Koch et al, EMBO, 2004). To examine Thr-233 phosphorylation *in vivo*, we raised a phosphospecific antibody. We show that phosphorylation of XRCC4 on Thr-233 occurs in untreated cells and is not induced by IR. Phosphoproteomics studies have also revealed XRCC4 phosphorylation sites suggesting that XRCC4 is highly phosphorylated *in vivo*. Studies to determine the effects of phosphorylation on XRCC4 function are underway.

POSTER #32

Phosphorylation of polynucleotide kinase phosphatase (PNKP) by ATM and DNA-PK

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Human polynucleotide kinase/phosphatase (PNKP) is a dual specificity 3'-DNA phosphatase/5'-DNA kinase that is required for multiple DNA repair pathways including base excision repair, DNA single-strand break repair and DNA double strand break repair via nonhomologous end joining (NHEJ). One of the essential players in NHEJ is the DNA-dependent protein kinase (DNA-PK), the activity of which is essential for NHEJ. PNKP is thought to be recruited to DSBs via its interaction with the NHEJ protein XRCC4 and *in vitro* studies have suggested that DNA-PKcs is required for the function of PNKP in NHEJ, making PNKP a potential substrate for DNA-PK *in vivo*. Here, we show that PNKP is phosphorylated by both DNA-PK and the related protein kinase, ATM *in vitro*. We have identified two *in vitro* phosphorylation sites, serine 114 and serine 126, and have shown that these sites are phosphorylated *in vivo* in response to IR and UV. IR-induced phosphorylation of PNKP on serine 114 was ATM-dependent, whereas serine 126 phosphorylation was dependent upon both ATM and DNA-PK. Interestingly, UV induced much greater phosphorylation levels of serine 126, while the level of serine 114 phosphorylation remained comparable to that induced by IR. Both UV induced events were ATM and DNA-PK dependent. Mutation of the two *in vivo* phosphorylation sites to a phospho-mimic, but not a phospho-mutant, caused a decrease in the *in vitro* PNKP kinase activity, indicating that phosphorylation may serve to regulate PNKP activity. This study identifies PNKP as a novel target for both DNA-PK and ATM.