ABSTRACTS

18th Annual International Ataxia-Telangiectasia Workshop

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The DOUBLE-STRAND BREAK RESPONSE: MOBILIZATION BY ATM, WITH A LITTLE HELP FROM ITS FRIENDS

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The protein whose function is lost in patients with ataxia-telangiectasia (A-T) is the homeostatic serine-threonine kinase, ATM. ATM's most documented function is mobilizing the DNA damage response (DDR) in response to DNA double-strand breaks (DSBs). Our and other labs have shown how ATM-mediated phosphorylation allows the recruitment of numerous proteins from various cellular circuits to temporary service with the broad DDR network. ATM initiates an extensive phosphorylation cascade that is accompanied by additional protein post-translational modifications, most notably those induced by the ubiquitin family. The linkage between ATM-mediated phosphorylation and protein ubiquitylation is exemplified by an ATM target we discovered recently - ubiquilin 4 (UBQLN4), a proteasome chaperone. However, we previously found in a phosphoproteomic screen that only 65% of DSB-induced phosphorylations are dependent on ATM. We have now carried out a more comprehensive screen aimed at revealing the crosstalk and share of the phosphorylation load among the three major PI3 kinase-like protein kinases (PIKKs) that take part in responding to genotoxic stress: ATM, ATR and DNA-PK. Following this screen with in-depth validation of many substrates using selected reaction monitoring (SRM), we uncovered redundant as well as non-redundant roles of the PIKKs following DSB induction, and provide evidence of other kinases functioning downstream and alongside the PIKKs. Interestingly, dissection of the DSB response in A-T cells, which are devoid of ATM, points to a certain degree of ATR- and DNA-PK-dependent compensation for ATM absence; apparently these PIKKs fill in to provide some of the missing protein phosphorylations. This observation adds another dimension to our understanding of the A-T phenotype. The data also shed light on the intricate relationships between the three PIKKs in regulating the DDR, contributing to our understanding of mechanisms of potential cancer treatment with PIKK inhibitors.

BRCA1 Haploinsufficiency Is Masked by RNF168-Mediated Chromatin Ubiquitylation.

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BRCA1 functions at two distinct steps during homologous recombination (HR). Initially, it promotes DNA end resection, and subsequently it recruits the PALB2 and BRCA2 mediator complex, which stabilizes RAD51-DNA nucleoprotein filaments. Loss of 53BP1 rescues the HR defect in BRCA1-deficient cells by increasing resection, suggesting that BRCA1's downstream role in RAD51 loading is dispensable when 53BP1 is absent. Here we show that the E3 ubiquitin ligase RNF168, in addition to its canonical role in inhibiting end resection, acts in a redundant manner with BRCA1 to load PALB2 onto damaged DNA. Loss of RNF168 negates the synthetic rescue of BRCA1 deficiency by 53BP1 deletion, and it predisposes BRCA1 heterozygous mice to cancer. BRCA1+/-RNF168-/- cells lack RAD51 foci and are hypersensitive to PARP inhibitor, whereas forced targeting of PALB2 to DNA breaks in mutant cells circumvents BRCA1 haploinsufficiency. Inhibiting the chromatin ubiquitin pathway may, therefore, be a synthetic lethality strategy for BRCA1-deficient cancers.

RECOMBINATION AND REPAIR AT STALLED MAMMALIAN REPLICATION FORKS

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Replication fork stalling at sites of abnormal DNA structure or following collision with transcription complexes is an important contributor to genomic instability. However, our understanding of the repair mechanisms engaged at stalled mammalian replication forks is limited. We adapted the *Escherichia coli* Tus/*Ter* replication fork barrier to induce site-specific replication fork stalling and homologous recombination (HR) on a mammalian chromosome. We found that the Fanconi anemia/BRCA pathway of homologous recombination mediates conservative repair of Tus/*Ter*-stalled forks, channeling processed forks into canonical "short tract" HR pathways. However, in cells lacking BRCA/HR functions, aberrant replicative responses become dominant at the stalled fork. In *BRCA1* mutant cells, an aberrant fork restart mechanism leads to the formation of ~10 kilobase tandem duplications, producing a unique rearrangement signature which plays a role in fostering *BRCA1*-linked tumorigenesis.

This presentation will address the mechanisms that regulate stalled fork repair in normal physiology and in the context of cells lacking *BRCA* or other HR genes. These mechanisms appear to differ significantly from those governing repair of a conventional chromosomal double strand break.

Charting the genetic architecture of the DNA damage response.

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The orchestration of DNA repair is of fundamental importance to the maintenance of genomic integrity and tumor suppression. DNA damage must be detected in the context of the varied chromatin landscape, its presence must be communicated throughout the cell to alter many ongoing processes, and the machinery that will mend the lesion must be recruited to the damage site. In my presentation, I will discuss our recent efforts in mapping genome maintenance pathways using genome-scale CRISPR/Cas9 screens in human cells. I will highlight how these screens can be used to identify new genome stability factors, characterize drug responses and provide new insights into the genetic architecture of the genome stability network by identifying potentially actionable synthetic lethal genetic interactions. I will argue that somatic genetic screens in human cells are powerful tools to study the DNA damage response and its integration within other cellular pathways.

A novel CDK5-PP4 phospho-signaling cascade regulates genome stability during cell cycle

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DNA damage response (DDR) is attenuated in mitotic cells, and fully activated when cells enters G1. The regulatory switch from 'inactive' state in mitosis to 'active' state in G1 remains unclear. Protein phosphatase 4 (PP4) dephosphorylates 53BP1, a prominent DDR mediator, in late mitosis, thereby relieving 53BP1 from mitotic inhibition, and allows its recruitment to DNA lesions in G1. Timely dephosphorylation of 53BP1 is critical for genome integrity, as premature recruitment of 53BP1 to DNA lesions in mitosis impairs mitotic fidelity. Here we elucidated the underlying mechanism of kinetic control of 53BP1 phosphorylation in mitosis. We observed that CDK5, a kinase primarily functional in post-mitotic neurons, is active in mitotic non-neuronal cells, and directly phosphorylates PP4R3□, the PP4 regulatory subunit that recognizes 53BP1. Using cells expressing an analog-sensitive (AS) CDK5 variant, we showed that specific inhibition of CDK5-AS in mitosis abrogated PP4R3□ phosphorylation, thereby abolishing its interaction with 53BP1, dephosphorylation of 53BP1 and ultimately prevented localization of 53BP1 to damaged chromatin. Our results establish CDK5 as a novel regulator of 53BP1 recruitment.

Targeting DNA damage repair in cancer therapy

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DNA double strand breaks (DSBs) are repaired by nonhomologous end joining (NHEJ) and homologous recombination (HR) pathways in mammalian cells. It is speculated that which pathway to use for DSB repair is mainly controlled by end resection process. This repair pathway choice is important for tumor response to PARP inhibition, which is now accepted therapeutic strategy for cancer patients carrying BRCA mutations. While BRCA1 promotes end resection and therefore favors HR repair, 53BP1 inhibits end resection and engages NHEJ pathway for DSB repair. We and others showed previously that RIF1 is a major downstream effector of 53BP1 and participates in 53BP1-dependent inhibition of end resection. Interestingly, while RIF1 accumulation at DSBs is antagonized by BRCA1 in S and G2 phases, the translocation of BRCA1 to damage sites in G1 cells is inhibited by RIF1, indicating that 53BP1-dependent pathway and BRCA1 counteract each other in a cell cycle-dependent manner. We showed that this cell cycle-dependent regulation is in part regulated by BRCA1-dependent inhibition of 53BP1 phosphorylation in S/G2 phase cells, which requires the E3 ubiquitin ligase activity of BRCA1. Besides RIF1, another DNA damage repair protein PTIP could also act downstream of 53BP1 and counteract BRCA1 function in DNA repair. We discovered that a nuclease SNM1C/Artemis associates with PTIP and functions to prevent end resection and HR repair. In addition, we and others demonstrated that REV7/MAD2L2 acts downstream of RIF1 and inhibits HR repair. Therefore, it is believed that 53BP1 controls RIF1-REV7 and PTIP-Artemis to promote NHEJ and suppress HR repair. We and others recently uncovered another 53BP1-binding protein, NUDT16L1 (also called Tudor Interacting Repair Regulator, TIRR), which associates with 53BP1 and regulates 53BP1 localization to DNA damage sites. We are now further investigating the regulation of DSB repair pathways and damage-induced checkpoint control. In addition, we are performing genome wide CRISPR/Cas9 screens and have identified RNASEH2 deficiency as potential biomarker for ATR inhibitor (ATRi)-based therapy. Moreover, we showed that ATRi could potentiate radiation-induced anti-tumor immune response. Therefore, these studies reveal the interplays between DNA damage repair and multiple cellular processes, which will help improve therapeutic outcome for cancer patients.

Deregulation of Mitophagy by Ubiquitin-like Proteins in Ataxia-Telangiectasia

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Defective mitophagy has been implicated in the pathogenesis of many neurodegenerative diseases including Ataxia-Telangiectasia (A-T). However, the mechanism(s) underlying defective mitophagy in these diseases and A-T remains unclear. Notably, we have recently identified the constitutively elevated ISG15 (**I**nterferon-**S**timulated **G**ene **15**) pathway, an antagonist of the canonical ubiquitin pathway, as a prospective mediator of defective mitophagy in A-T. Our results have revealed that in A-T cells, aberrantly expressed ISG15 conjugates (ISGylation) inhibit ubiquitin-dependent protein degradation and mitophagy, the latter of which is regulated by ubiquitin-dependent signaling through the ubiquitin E3 ligase Parkin. This empirical evidence led to our hypothesis that *the constitutively elevated ISG15 ligation pathway inhibits mitophagy and contributes to neurodegeneration in A-T.* While testing this hypothesis, we have identified another ubiquitin-like protein SUMO (**S**mall-**U**biquitin-like **Mo**difier) as a novel player in the regulation of mitophagy in normal cells. Furthermore, our results have revealed that ISG15 attenuates the SUMO pathway in A-T cells. Deregulation of the SUMO pathway is also implicated in the pathogenesis of several common neurodegenerative diseases and hence is emerging as an attractive drug target for treatment. We have thus identified ISG15 and SUMO as novel potential targets for the development of much-needed therapies for A-T *(US patent 9,599,626)*. I shall present these novel results for the first time at the 18th Annual International Ataxia-Telangiectasia Workshop, and my views on the plausible mechanism by which deregulated ubiquitin/ISG15/SUMO pathways
may inhibit mitophagy in A-T.

The HP1β Chromo Shadow Domain assists BRCA1 recruitment by promoting H2A ubiquitination.

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Chromatin modifications are associated with DNA damage repair, replication and transcription. DNA double strand break (DSB) repair depends upon DNA associated proteins and their post-translational modifications. The heterochromatin protein (HP1β) has three functionally distinct domains, chromodomain (CD), hinge region (HR), and chromoshadow domain (CSD). HP1β is recruited to DSB sites, but what domains are critical in repair is not known. Expression of the CSD domain in endogenously HP1β depleted cells rescued DNA repair by restoring DNA repairosme formation and resection, whereas CD domain expression did not. Both HP1β domains recruit to DNA DSBs but the CD domain is recruited at highest levels nearest the break site whereas CSD recruitment is enriched -1Kbp from the DSB site independent of transcription status. CSD is recruited to DSBs through chromatin assembly factor 1A (CHAF1) protein after DNA damage and this interaction assists homologous repair. HP1β depletion also decreased BRCA1 recruitment to DSBs and affected the normal symmetric distribution of BRCA1 around the DSB, which CSD expression could restore. In addition, HP1β interacts with RING1A, a component of the polycomb repressor complex that ubiquitinates histone H2A at lysine 118-119 in irradiated cells to promote BRCA1 foci formation and homologous recombination (HR). Present data suggest the HP1β CSD domain is essential for DNA repair by HR.

ATM suppresses ataxia by preventing toxic NHEJ

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Topoisomerase 1 (TOP1) regulates torsional stress generated in double-stranded DNA during replication, transcription and chromatin remodeling. Aberrant TOP1 function has been implicated in many neurological diseases including ataxia telangiectasia (A-T), spinocerebellar ataxia with axonal neuropathy 1 (SCAN1). To determine the pathobiology of TOP1 we have generated an inducible mouse model that expresses a $Top1^{T720A}$ mutation, which leads to increased DNA damage via Top1-cleavage complex formation. Expression of this mutation throughout the nervous system causes broad perturbation of neurogenesis resulting from widespread DNA damage. In the $Top1^{T720A}$ cerebellum, granule neuron ablation and a significant loss of oligodendrocytes and cerebellar interneurons occurs. However, coincident inactivation of disease-relevant genes, including ATM or TDP1 in the context of $Top1^{T720A}$ resulted in profound disruption of cerebellar development, with substantial disruption of Purkinje cell organization and ataxia after ATM inactivation. Remarkably, substantial rescue of cerebellar development occured in the $(Top1^{T720A};Atm)^{Nes\text{-}cre}$ mutants after simultaneous inactivation of NHEJ, by Lig4 loss. Thus, a key function of ATM is the prevention of toxic NHEJ. Our data provide critical insight into topoisomerase pathobiology and illuminates the etiologic bases for multiple spinocerebellar ataxias by explaining the molecular bases for A-T and further, the connections between A-T and SCAN1.

Mitochondrial and nuclear DNA replication instability in disease

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Ataxia-telangiectasia (A-T) is a rare neurodegenerative disease that results in poor coordination, a weakened immune system, and a high likelihood of developing cancer caused by mutations in the ATM gene, short for “ataxia-telangiectasia mutated”. ATM acts in oxidative stress sensing outside the nucleus and metabolic reactions in the mitochondria linked to the neurological defects in AT patients. ATM furthermore controls the DNA damage response, cell cycle progression, and DNA replication stability in the nucleus, which are functions that are likely to contribute to the cancer predisposition when ATM is mutated. ATM is regulated by MRE11, a nuclease that when defective leads to an ataxia-like disorders, and ATM in turn regulates MRE11 nuclease activity. MRE11 plays a critical role at DNA replication forks, where it facilitates the restart of stalled forks, however also causes detrimental fork degradation in cells that lack functional BRCA1/2 or Fanconi Anemia tumor suppressor genes. We will be presenting nuclear and mitochondrial roles of DNA replication stability genes, including RAD51C and BRCA2, as well as novel mouse models defective in defined DNA replication reactions. We will discuss the importance of distinct DNA replication structures on the phenotypic outcomes of derailed DNA replication reactions *in vitro* and *in vivo*, that are defined at least in part by the cellular compartment where these reactions occur, and which include inflammation, genome instability, Fanconi Anemia and cancer.

Neuromodulation and cell engineering to replace damaged brain circuitry

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A major challenge for regenerative medicine is the reconstruction and modulation of neural circuits that are damaged due to injury or degeneration. Neuromodulation shows promise to strengthen spared pathways or stimulate novel neural circuits to restore motor and sensory function. In our laboratory, intraspinal and intracortical stimulation of damaged motor circuitry leads to Hebbian plasticity and the limited recovery of hand function following spinal cord injury. Currently we are exploring semi-noninvasive methods to electrically activate motor circuitry combined with transplantation of stem cell-derived excitatory interneurons to generate restorative connections following chronic injury. Data indicates that electrical fields have the potential to shape migration patterns and integration of transplanted cells within motor columns of the spinal cord. These results suggest that that neurostimulation combined with cell engineering is a promising approach for neural circuity modulation or repair. This work supported by a grant from Wings for Life and The Neilsen Foundation.

Defective endoplasmic reticulum (ER)-mitochondrial signaling in A-T

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Evidence has been provided for an intrinsic mitochondrial dysfunction in A-T cells; for a new role for ATM in regulating mitochondrial function and mitophagy and for a role for ATM in mitochondrial redox sensing. Developmental lethality of a *Drosophila* ATM mutant is rescued by Ronnel (an organophosphate) by inhibiting the function of mitochondria. ATM is essential in mitochondrial radiation responses and senescence control by the lysosomal-mitochondrial axis is modulated by ATM activity. Loss of ATM induces mitochondrial dysfunction and compromises mitophagy due to NAD+ insufficiency and ATM mediates spermidine-induced mitophagy via PINK1 and Parkin regulation in human fibroblasts. More recently it has been shown that ATM is activated by ATP depletion and regulates mitochondrial function through NRF1.What emerges from all these studies is evidence for a defect in mitochondrial function and mitophagy in A-T cells and as a consequence accumulation of abnormal mitochondria which would impact adversely on function of normal mitochondria. Bearing in mind the mitochondrial abnormalities in A-T cells together with our earlier results providing evidence for a defect in intracellular release of Ca2+ in A-T cells exposed to ionizing radiation, we determined whether there might be a defect in endoplasmic reticulum (ER)-mitochondrial signaling in these cells. Here we provide evidence for such a defect and show that ATM plays a key role in controlling Ca^{2+} release from the ER and transfer to the mitochondrion. Furthermore we investigated a correction strategy that normalizes ER-mitochondrial dynamics which has potential for treatment of patients with A-T and possibly other neurodegenerative disorders .

The Role of ATM in Cerebellar Structure and Function

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An aberrant response to DNA lesions is implicated in many human brain degenerative disorders. Various types of DNA lesions activate a cellular process known as the DNA damage response (DDR). Mutations affecting the proteins involved in the DDR can lead to severe genomic instability syndromes, which involve varying degrees of sensitivity to genotoxic stress, and also to tissue degeneration, cancer predisposition, and premature aging. One of the key components of the DDR is the protein ATM, which is inactivated in the genomic instability disorder ataxia-telangiectasia (A-T).

We found that cerebellar cultures grown from *Atm−/−* mice had disrupted network synchronization, atrophied astrocytic arborizations, reduced autophagy levels, and higher numbers of synapses per neuron than wild-type cultures. Chimeric circuitries composed of wild-type astrocytes and *Atm−/−* neurons were indistinguishable from wild-type cultures. Adult cerebellar characterizations confirmed disrupted astrocyte morphology, increased GABAergic synaptic markers, and reduced autophagy in

Atm−/− compared with wild-type mice. In addition to astrocytes, microglia also play an essential role in the etiology of A-T. Microglia derived from *Atm−/−* mouse cerebellum were severely impaired in phagocytosis, secretion of neurotrophic factors, and mitochondrial activity, an impairment that might imply on an apoptotic process. Interestingly, no microglial impairment was detected in *Atm−/−* cerebral cortex. Vascular impairments were detected specifically in the cerebellum but not in the cerebral cortex of *Atm−/−* mice. Metabolomics analyses revealed alterations in metabolites that could cause DNA damage, oxidative stress, cell death, vascular leakage and microglia-mediated inflammation specifically in the cerebellum but not in the cerebral cortex. Our findings shed new light on the factors and processes that make the cerebellum so vulnerable to ATM deficiency.

Possible Role of Transflammation in Ataxia-Telangiectasia Syndrome

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Ataxia-Telangiectasia Syndrome (ATS) is due to a deficiency of ATM, the protein which coordinates the cellular response to double strand breaks. This disorder causes genome instability, which increases the risk for cancer in these patients. In addition, ATS is associated with a chronic DNA damage response (DDR) that incites a low level of inflammatory response. We speculate that this low level of cell-autonomous innate immune activation may play a significant role in the cancer risk of these individuals. Specifically, we find that activation of cell-autonomous innate immune signaling triggers a series of events that increases DNA accessibility, in a setting of increased generation of oxidative and nitrosative stress, further increasing the risk of genomic injury. Activation of NFKβ and IRF3 cause global changes in the expression of epigenetic enzymes that increases activating histone marks, and erases suppressive histone marks. In addition, inducible NO synthase translocates to the nucleus, binds to and S-nitrosylates polycomb complex and NURD, to inhibit their suppressive effects on chromatin. Furthermore, a glycolytic shift supplies to the nucleus more citrate, which serves as a precursor for acetylcoA, thereby supplying the substrate for the increased histone acetylation occurring during this process. The effect of cell-autonomous innate immune signaling to increase DNA accessibility, and thereby increase the risk of cellular transformation is termed “transflammation”. Understanding the role of this process in ATM may be useful in preventing malignancies in these patients.

ATM, DNA Damage and Cerebellar Ataxia

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Ataxia telangiectasia (AT) is a rare, primarily childhood-onset autosomal recessive neurodegenerative disorder that causes cerebellar ataxia with telangiectasia, cancer, immune deficiency, and an acute sensitivity to ionizing radiation. AT is caused by mutations in the *ATM* gene, which encodes a serine/threonine kinase that is recruited and activated by DNA double-strand breaks and phosphorylates key proteins to initiate activation of the DNA damage checkpoint. Several other autosomal recessive ataxias result from impaired DNA repair, including single- and double-strand break (DSB) repair and base excision repair. Furthermore, ataxia-causing mutations in genes coding for key proteins in SSB repair and those responsible for base excision repair also lead to defective mtDNA, whereas mutations in polymerase gamma (*POLG*) and the mtDNA helicase (*Twinkle/TWNK*) lead to impaired replication of the mtDNA. This is important because mitochondrial dysfunction is a major cause of ataxia. In spinocerebellar ataxia type 3 (SCA3), an autosomal dominant ataxia, the mutant ATXN3 protein with an expanded polyglutamine tract deactivates PNKP, which results in impaired transcription-coupled DNA repair, in contrast to normal ATXN3 which activated PNKP. GWAS identified three DNA repair loci, *FAN1, PMS2* and *PRM2B*, as significant modifiers of the age of onset in polyglutamine expansion SCAs. These observations suggest that Purkinje cells (PCs) have selective vulnerability to impaired DNA repair. PCs are one of the largest neurons in the brain and the sole output neurons of the cerebellar cortex. Thus, PCs are energy demanding and exposed to constant oxidative stress. PCs also receive robust glutamatergic excitatory signals from the parallel fibers and climbing fibers, triggering a rapid influx of Ca(2+) through voltage-gated calcium channels and a delayed release of Ca(2+) from intracellular stores. PCs, as well as neuronal and glial cells associated with PCs, in adults are terminally differentiated cells with negligible regenerative capacity, allowing for the accumulation of DNA damages. While further studies are warranted to elucidate pathogenic links between defective DNA repair and ataxia phenotype, impaired DNA repair may be considered as a potential therapeutic target in degenerative ataxia.

ATR in mitochondrial function and metabolism

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DNA damage response (DDR), including DNA repair, cell cycle checkpoints, apoptosis, and gene transcription, is vital for genomic stability. ATR is an essential gene due to its important role in damage signaling in response to DNA single strand breaks and stalled replication forks. ATR is mutated in human Seckel syndrome, characterized by dwarfism, microcephaly and intellectual disabilities, which suggest a role for ATR in non-nuclear functions. We find that ATR is located in many membrane-associated subcellular organelles, prominently in mitochondria. ATR deletion disturbs mitochondrial functions prior to the DDR, and alters the mitochondrial membrane potential and mitochondrial dynamics. Moreover, ATR deleted cells contain imbalanced mitochondrial proteins and exhibit dysfunction of mitochondrial metabolism accompanied by impairment of respiration generating high ROS and misregulated metabolic signaling pathways. Thus, ATR plays, beyond its well-known DDR function, an important role in cell metabolism and mitochondrial functionality.

DNA damage repair by homologous recombination is decreased in human pluripotent stem cell derived differentiated cells, astrocytes and dopaminergic neurons

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Stem cells have the dual ability to self-renew over the lifetime of the organism and differentiate into multiple cell lineages. Various factors, including reactive oxygen species (ROS), which accumulate during differentiation and over the stem cell lifespan, can cause DNA damage. Further, differentiation dependent changes in chromatin structure and transcriptional alterations can also affect genomic integrity by altering the DNA damage response (DDR) and repair capacity. Our studies indicate that pluripotent stem cell derived differenced cells, astrocytes and dopaminergic neurons exhibit increased residual damage after exposure to DNA damaging agents as determined by γ-H2AX or 53BP1 foci formation and increased S-phase-specific chromosomal aberration. Reduced homologous recombination (HR) repair in differentiated cells was suggested by decreased HR related foci formation of RAD51 and BRCA1. Differentiated cells also had increased fork stalling after DNA replication stress. Treatment of cells with a Nitric oxide donor (NOC-18), which induces stem cell differentiation, had no effect on double-strand break (DSB) repair by non-homologous end joining but reduced DSB repair by HR, further suggesting that HR is impaired in differentiated cells.

The impact of ATM mutations on cancer genomes and response to therapy

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There are several lines of phenotypic and genomic data to suggest that there are crucial differences in the biology of tumors arising in the context of alterations affecting 'upstream' DNA damage response (DDR) genes compared to BRCA1- and BRCA2-associated cancers. For the *ATM* gene, where bi-allelic germline mutations are causative of the disease ataxia-telangiectasia, the presence of only a single pathogenic germline variant predisposes to breast cancer. ATM-associated breast cancers are *TP53* wild-type and lack the genomic features observed in BRCA1- and most of the BRCA2-associated cancers. Despite these recognized differences, at present, defects affecting these genes are being therapeutically exploited with a 'one size fits all' approach, where the efficacy of PARP-inhibitors are being tested in patients with alterations affecting genes 'upstream' or 'downstream' in the homologous recombination (HR) DNA repair pathway. Loss of function of 'upstream' and 'downstream' genes results in DNA repair defects that are fundamentally distinct. Upstream versus downstream can be robustly identified through genomic computational analyses and predict distinct vulnerabilities to specific therapeutic agents. When ATM-associated breast cancers develop, there is usually no loss of heterozygosity of the remaining wild-type *ATM* allele, in contrast to what has been documented in BRCA1- and BRCA2-associated breast cancers. Indeed, across multiple cancer types, for example in NSCLC or prostate cancer, loss of one of the *ATM* alleles is usually found. Whether ATM-linked cancers are sensitive to PARP-inhibitors remains controversial. Whilst there are limited data suggesting that ATM-related cancers are sensitive to PARP-inhibition, preclinical data have shown that *ATM* homozygous mutant cells, which are exquisitely sensitive to ionizing radiation, are only mildly sensitive to PARP-inhibitors. In fact, ATM-heterozygous cancers are sensitive to radiation treatment and there is the possibility that radiation with ATR or DNA-PK inhibition might amplify the sensitivity. The sensitivity of CHEK2-associated cancers to specific therapeutic strategies is even less well characterized. However, preclinical models demonstrate that CHEK2-deficient cells are not significantly sensitive to PARP-inhibitors. ATM- and CHEK2-heterozygous state is observed to occur somatically as well as via germline inheritance. Thus, alterations in ATM and other upstream components of the DDR produce distinct patterns to their genomic landscapes and distinct sensitivities in response to therapies compared with classical HR defective cancers.

A novel role for ZEB1 in promoting NHEJ

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DNA double strand breaks (DSBs) are the most mutagenic and cytotoxic insults to the genome. DSBs are repaired through homologous recombination (HR), which is predominant in S and G2 phases, and also by error prone non-homologous end joining (NHEJ), which is active in all cell cycle phases. In cancer cells, heightened activity of these repair pathways has been linked to radioresistance. The transcriptional repressor ZEB1 is a well-established driver of the epithelial-to-mesenchymal transition (EMT) in both normal development and tumor progression .We have recently discovered: 1) ZEB1 physically interacts with 53BP1; 2) both factors co-localize at IRIF; 3) ZEB1 is required for NHEJ and, surprisingly, attenuates HR; 4) ZEB1 down-regulation enhances radiosensitivity in an ATM-independent manner; 5) ZEB1 is selectively recruited to DSBs at euchromatic (transcriptionally active) genomic regions; and 6) ZEB1 depletion results an increase in IR-mediated chromosomal aberrations. Overall our data establish a novel link between the EMT factor ZEB1 and DBS repair. Elucidating the mechanistic basis for this new pathway will significantly advance our understanding of radioresistance and accelerate the development of new treatment approaches.

Sensitizing cancer stem cells to radio- and chemotherapy

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Despite recent advances in precision radiotherapy technologies, tumour recurrence remains a significant clinical hurdle. It is imperative to determine the causes of, and find novel strategies to treat, recurrent disease. It is becoming increasingly evident that the spread of primary cancer can be initiated at a very early stage of cancer development by a small population of unique cancer cells, termed cancer stem cells (CSCs). There is an intrinsic difference in the DNA damage response between CSCs and other cells in the tumor, in particular with regards to their tolerance of DNA damage. Targeting of these CSCs has emerged as a priority area in cancer therapy. We are interested in targeting Triple-negative breast cancer (TNBC), the subtype enriched for cancer stem-like cells, associated with genomic instability/aneuploidy and the poorest prognosis. We have identified a new DNA damage-induced kinase, predominantly expressed in TNBCs that mediates resistance of CSCs to radio- and chemotherapy by altering the kinetics of DNA repair. By exploiting this knowledge, we aim to improve existing breast cancer management by specifically targeting the CSCs present in the heterogeneous tumor population to reduce morbidity and mortality from breast cancer.

Exonuclease 5 interacts with the BLM helicase complex and protects the genome from replication stress

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Exonucleases are key components of the DNA repair machinery and are involved in important steps of DNA replication. Their action on free DNA ends contributes to high fidelity proof reading during DNA synthesis that suppresses mutation rates. Mutations during DNA replication are often accumulated due to hindrances in replication tracts such as interstrand cross-links (ICL) or unusual quaternary DNA structures. Here we report that human Exonuclease V (EXO5) protects the genome by acting on single strand DNA during repair of DNA ICL damages as well as during DNA replication restart. An ATR mediated phosphorylation of EXO5 promotes an interaction with BLM helicase to carryout its function at stalled replication forks. The disruption of BLM-EXO5 interaction by mutation of the EXO5 (T88) ATR phosphorylation site significantly decreases clonogenic survival as well as damage induced EXO5 foci formation. We show here that the EXO5-BLM complex is a novel combination of exonuclease-helicase that acts on stalled forks and protects the genome from damage during stalled replication fork restart, and hence may be a potential therapeutic target for cancer therapy.

[4Fe-4S] cluster-containing human exonuclease V acts as a novel replication fork restart factor

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As most cancer mutations arise from replication, error-free DNA replication fork repair and restart are of critical importance for cancer etiology and therapeutic susceptibilities. Yet, damaged and stalled forks occur frequently and must be both repaired as well as accurately restarted through the action of nucleases, helicases, and other replication factors. Here, we identified human exonuclease V (hExo5) as the newest and perhaps least understood component of the replication fork restart pathway. hExo5 is a 5' to 3' single-strand DNA (ssDNA) exonuclease that does not cut blunt-end double-strand DNA (dsDNA) and circular DNA. To understand how hExo5 functions with DNA mechanistically, we solved the crystal structures with and without DNA substrates. The structures showed hExo5 contains an [4Fe-4S] cluster domain that discriminates ssDNA from dsDNA and exhibits a structural core similar to PD-(D/E)XK superfamily active site arrangements in DNA2 and Cas4 nucleases. Moreover, residue Y221F substitution near the active site abolishes the nuclease activity. hExo5 with ssDNA bound structure reveals Y221 interacts with a DNA phosphate group to critically position the DNA substrate for cutting. Interestingly, upon DNA binding, a helix-turn-helix motif moves out of the active site to allow ssDNA treading through the [Fe-S] cluster domain. Furthermore, our nuclease assay indicates hEXO5 is working together with BLM and RPA70NAB. Together, we propose an integrated model for how hExo5 functions at the replication fork.

ATM and regulation of protein homeostasis in human cells

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The Ataxia-Telangiectasia mutated (ATM) protein is a key regulator of checkpoint activation and homologous recombination in response to DNA double strand breaks (DSBs). In addition, ATM can be activated independently of MRN and DNA through direct oxidation, and ATM acts a redox sensor for oxidative stress in human cells. We have isolated and characterized separation-of-function mutants for each of the pathways and have investigated the effects of these changes on ATM phosphorylation events in response to a variety of stress conditions, in order to determine how these distinct pathways each contribute to the overall ATM response. One notable consequence of loss of the oxidative stress pathway is the appearance of widespread protein aggregation in human cells, which is interesting considering the common links between neurodegenerative disorders and defects in protein homeostasis. Our current studies investigate how each activation pathway affects protein stability and aggregation, and the mechanisms underlying these outcomes. These and other topics of ATM regulation will be discussed.

An ATR dependent mechanism that suppresses dormant origin firing during unperturbed DNA replication

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DNA damage-induced signaling by ATR and CHK1 kinases inhibits DNA replication, stabilizes stalled and collapsed replication forks, and mediates the repair of multiple classes of DNA lesions. We and others have shown that ATR kinase inhibitors, three of which are currently undergoing clinical trials, induce excessive origin firing in unperturbed replicating cells, indicating that ATR kinase activity limits replication initiation in the absence of damage. However, the origins impacted, and the underlying mechanism(s) have not been described. Here we show that unperturbed DNA replication is associated with a low-level of ATR and CHK1 kinase activities. Inhibition of these activities induces dormant origin firing at sites of ongoing replication throughout S-phase. We show that ATR and CHK1 kinase inhibitors induce RIF1 Ser2205 phosphorylation which disrupts an interaction between RIF1 and PP1 phosphatase. This interaction localizes PP1 to dormant origins where it dephosphorylates key CDC7 kinase substrates and thereby inhibits the assembly and activation of the replicative helicase. This is the first mechanism identified that limits origin firing during DNA replication in unperturbed cells.

Caspase-2 regulates S-phase checkpoint activation to facilitate DNA repair

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Despite being the most evolutionarily conserved caspase, the role of caspase-2 in apoptosis has been difficult to unravel. In fact, accumulating evidence suggests that caspase-2 has non-apoptotic roles and may even regulate cell division. Loss of caspase-2 is known to increase proliferation rates but how caspase-2 is regulating this process is currently unclear. We show that in the absence of caspase-2, cells exhibit numerous S-phase defects including delayed exit from S-phase and impaired ATR activation, leading to increased aneuploidy and DNA damage following S-phase arrest. One of the functions of ATR is to stabilize DNA replication forks and we have noted that caspase-2 deficient cells have a much higher frequency of stalled replication forks. These functions appear to be independent of the pro-apoptotic function of caspase-2 because blocking caspase-2-induced cell death had no effect on cell division. We propose that these functions of apoptosis and cell division are regulated by different caspase-2 signaling complexes. We recently reported that DNA damage induces the assembly of two distinct activation platforms for caspase-2: a cytoplasmic platform that is RAIDD-dependent but PIDD-independent, and a nucleolar platform that requires both PIDD and RAIDD as well as the nucleolar resident protein, nucleophosmin (NPM1). Inhibition of NPM1 impaired caspase-2-dependent inhibition of cell growth suggesting that it is the nucleolar PIDDosome that is responsible for the cell cycle effects of caspase-2. Thus, our data supports a model where the cytoplasmic complex induces apoptosis and the nucleolar PIDDosome is required to regulate cell cycle events to facilitate DNA repair.

Nucleolin and the genome instability associated with G4 DNA

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A significant increase in genome instability is associated with the conformational shift of a guanine-run-containing DNA strand into the four-stranded G4 DNA. Until recently, the mechanism underlying the recombination and genome rearrangements following the formation of G4 DNA *in vivo* has been difficult to elucidate but has become better clarified by the identification and functional characterization of several key G4 DNA-binding proteins. Mammalian nucleolin NCL is a highly specific G4 DNA-binding protein with a well-defined role in the transcriptional regulation of genes with associated G4 DNA-forming sequence motifs at their promoters. The consequence of the *in vivo* interaction between G4 DNA and nucleolin in respect to the genome instability has not been previously investigated. We show here that G4 DNA-binding is a conserved function in the yeast nucleolin Nsr1. Furthermore, we demonstrate that the Nsr1-G4 DNA complex formation is a major factor in inducing the genome instability associated with the co-transcriptionally formed G4 DNA in the yeast genome. The G4-associated genome instability and the G4 DNA-binding *in vivo* requires the arginine-glycine-glycine (RGG) repeats located at the C-terminus of the Nsr1 protein. Nsr1 with the deletion of RGG domain supports normal cell growth and is sufficient in *in vitro* G4 DNA binding as well as protection from the cytotoxicity of a G4 ligand TMPyP4. However, this deletion results in abrogation of *in vivo* binding to the co-transcriptionally formed G4 DNA and in severe reduction in the G4-assicated genome instability. Our data suggest that the interaction between Nsr1 with intact RGG repeats and G4 DNA impairs genome stability by precluding the access of G4-resolving proteins and obstructing replication.

Ataxia-Telangiectasia Mutated is located in cardiac mitochondria and impacts oxidative phosphorylation

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The absence of Ataxia-Telangiectasia Mutated protein kinase (ATM) is associated with neurological, metabolic and cardiovascular defects. The protein has been associated with mitochondria and its absence results in mitochondrial dysfunction. Furthermore, it can be activated in the cytosol by mitochondrial oxidative stress and mediates a cellular anti-oxidant response through the pentose phosphate pathway (PPP). However, the precise location and function of ATM within mitochondria and its role in oxidative phosphorylation is still unknown. We show that ATM is found endogenously within cardiac mitochondria under normoxic conditions and is consistently associated with the inner mitochondrial membrane. Acute *ex vivo* inhibition of ATM protein kinase significantly decreased mitochondrial electron transfer chain Complex I-mediated oxidative phosphorylation rate ($p=0.0024$) but do not decrease coupling efficiency or oxygen consumption rate during β-oxidation. Chemical inhibition of ATM in rat cardiomyoblast cells (H9c2) significantly decrease the excited-state autofluorescence lifetime of enzyme-bound reduced NADH and its phosphorylated form, NADPH (NAD(P)H; 2.77 ± 0.26 ns compared to 2.57 ±0.14 ns in KU60019-treated cells). This suggests an interaction between mitochondrial ATM and the electron transfer chain in the mitochondria, and may have an important role in oxidative phosphorylation in terminally differentiated cells such as cardiomyocytes.

THE ROLE OF SINGLE-STRANDED DNA BREAKS AND PARYLATION IN A-T AND ATLD PHENOTYPES

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Ataxia-Telangiectasia Mutated (ATM) responds to both DNA double-strand breaks as well as to oxidative stress. Many reports have shown that the levels of reactive oxygen species (ROS) are high in cells from A-T or ATM deficient cells. Oxidative stress has been proven to have a significant role in the cellular and clinical phenotype in A-T. Our recent studies also showed that loss of ATM activation induced by oxidative stress results in deficiencies in mitochondrial function and protein aggregation.

The MRN complex, which is composed of Mre11, Rad50, and Nbs1, is essential for DNA repair and ATM activation after double strand breaks (DSBs) in mammals. Our recent studies also showed that the MRN complex is dispensable for the ATM activation induced by oxidative stress. However, Mre11 mutation can result in A-T like disorder (ATLD), whose clinical phenotype is significantly similar to A-T, including neuronal degeneration.

Recently, many studies have been published about ATM function as an important sensor of reactive oxygen species in human cells. However, it is unclear to what extent ATM dysfunction is associated with the accumulation of intracellular ROS and whether this contributes to development of disease pathology. Also, the relationship between Mre11 mutations in ATLD and the role of ROS in the clinical phenotype of A-T are unclear. Here, we discuss these questions in light of recent results. Cells expressing mutant ATM, which is non-functional in response to oxidative stress, showed higher levels of single-strand breaks (SSBs) in DNA. Cells, in which ATM was depleted or mutant Mre11 (ATLD-3) was expressed, showed higher oxidative-stress dependent aggregates and it is rescued by treatment with the PARP inhibitor, veliparib. These results suggest that ROS-dependent SSBs and Parylation is closely associated with A-T and ATLD phenotypes.

The Importance of Lysine Acetyltransferase Complexes in the Repair of DNA Double Strand Breaks

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Histone post-translational modifications (PTMs) regulate all chromatin/DNA-based cellular processes like transcription, replication and DNA repair. Proper deposition, reading, and removal of these marks are therefore critical for the maintenance of genome integrity, and are often deregulated in cancer. The histone acetyltransferase complex NuA4/TIP60, through its catalytic subunit KAT5, acetylates nucleosomes on histones H4 and H2A(.X/.Z) and is also able to incorporate histone variant H2A.Z into chromatin. NuA4/TIP60 is essential for stem cell maintenance and renewal, regulates p53- and Myc-driven transcription and plays an important role in the repair of DNA double-strand breaks (DSBs). We have performed experiments in yeast and human cells to demonstrate that NuA4/TIP60 is recruited to DSBs by the MRX(N) complex and spreads during DNA end resection to favor repair by homologous recombination (HR), cooperating with another important acetyltransferase complex, SAGA, during this process. In addition, we have found that NuA4/TIP60 also directly block the action of factors involved in the other main DSB repair pathway, non-homologous end-joining (NHEJ). Furthermore, we have now dissected the function of KAT5 chromodomain, which has been suggested to recognize the H3K9me3 mark in chromatin during the DNA damage response (DDR), leading to ATM activation through its direct acetylation. Surprisingly, the determined structures of yeast and human KAT5 chromodomain fail to show formation of a hydrophobic cage necessary for recognition of methylated lysines. Sensitive in vitro screen for recognition of an extensive array of histone marks on peptides and recombinant nucleosomes (Epicypher) also failed to identify specific binding. We generated isogenic cell lines expressing point mutants in human and yeast KAT5 (Tip60/Esa1) chromodomain, corresponding to the key hydrophobic residues and reported PTMs (Kac, Yph), potentially involved in DNA repair. Phenotypic analysis and *in vitro* acetylation assays with purified wild-type and mutant NuA4/TIP60 native complexes indicate that the chromodomain is essential for cell viability and crucial for acetylation of the H4 tail, but not the H2A tail, in the context of nucleosomes/chromatin, but not free histones. Altogether, these data indicate that KAT5 chromodomain assists NuA4/TIP60 binding to nucleosomes, directing the acetyltransferase catalytic site towards the H4 tail, irrespective of any PTM present on the nucleosome.

Pre-existing H4K16ac levels in euchromatin drive DSB repair by homologous recombination

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The homologous recombination (HR) repair pathway maintains genetic integrity after DNA double-strand break (DSB) damage and is particularly crucial for maintaining fidelity of expressed genes. Transcribed chromosomal regions differ from gene-poor regions in terms of chromatin modifications and new chromatin marks are added or removed by the DNA damage response machinery. Histone H4 acetylation on lysine 16 (H4K16ac) is associated with transcription but how pre-existing H4K16ac directly affects DSB repair is not known. To answer this question, we used CRISPR/Cas9 technology to introduce I-SceI cleavage sites, with or without repair pathway reporter cassettes, at defined locations within gene-rich (high H4K16ac/euchromatin) and gene-poor (low H4K16ac/heterochromatin) regions. We show that the frequency of DSB repair by HR is higher in gene-rich regions. In addition, using site-specific DSBs induced by CRISPR/Cas9, we find that repair protein factors involved in HR were effectively recruited and assembled during S-phase in gene-rich regions containing higher levels of H4K16ac. Interestingly, artificially targeting H4K16ac at specific locations using gRNAs/dCas9-MOF increases enrichment of HR proteins and HR frequency specifically in euchromatin not heterochromatin. Furthermore, higher levels of H4K16ac in transcribed regions before DSB induction correlate with increased accumulation of RNA polymerase ll (RNAPII) and the Cockayne syndrome B protein (CSB) after DNA breakage. Finally, inhibition/depletion of RNAPII or CSB leads to decreased recruitment of HR factors at DSBs. Altogether, these results indicate that the pre-existing H4K16ac status at specific locations directly influences the repair of local DNA breaks, favoring HR in part through the transcription machinery and ensuring error-free repair in the expressed regions of the genome.

LONG NONCODING RNA IN NEURO-INFLAMMATION AND NEUROLOGICAL DISORDERS

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Neuro-inflammation is central to neuronal dysfunction and is associated with variety of neurological disorders, including Alzheimer's disease, Parkinson's disease, ataxia, and many others. Neuro-inflammation may be induced in response to a variety of factors including infection, traumatic brain injury, toxic metabolites, autoimmunity, oxidative stress, and DNA damage. The microglial cells, the resident macrophages present in central nervous system, play key roles to neuro-inflammation. Emerging studies suggest that non-coding RNAs (ncRNAs) are vital players in immune response and inflammation. Recently, we discovered that lncRNA HOTAIR plays critical roles in immune response and inflammation in macrophages as well as in microglial cells. HOTAIR expression is induced in macrophages and microglial cells upon stimulation with lipopolysaccharide (LPS, component of gram-negative bacterial cell wall). Transcription factor NF-κB activation and hence expression of cytokines and pro-inflammatory genes are critical to inflammation. Our studies demonstrate that HOATIR is required for the activation of NF-κB and hence regulates the expression of cytokines and pro-inflammatory genes expression (IL-6, iNOS, and others). HOTAIR regulates NF-κB activation via facilitating the degradation of IκBα. Thus, HOTAIR appears to be a critical player in inflammation and neurological disorders. Beyond HOTAIR, based on RNA-seq analysis, we also identified several novel lncRNA critical to inflammatory response. Overall, our studies demonstrate that lncRNA play critical roles in neuro-inflammation and potentially linked to neurological disorders.

DNA2 NUCLEASE DEFICIENCY RESULTS IN LARGE AND COMPLEX DNA INSERTIONS AT CHROMOSOMAL BREAKS

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Insertions of mobile elements, mitochondrial DNA and fragments of nuclear chromosomes at DNA double strand breaks (DSBs) threaten genomic integrity and are common in cancer. Insertions of pieces of other chromosomes at VDJ loci can stimulate antibody diversification. The origin of insertions of chromosomal fragments and the mechanisms that prevent such insertions remain unknown. Here we found the first mutant, lacking evolutionarily conserved Dna2 nuclease, that shows frequent insertions of ~0.1-1.5 kb long sequences into DSBs with many events carrying multiple DNA fragments joined together. Sequencing of ~500 DNA inserts revealed that they originate from Ty retrotransposons, ribosomal DNA and from throughout the genome with preference for fragile regions such as origins of replication, R-loops, centromeres, telomeres or replication fork barriers. Inserted fragments are not lost from their original loci and therefore represent duplications. These duplications depend on nonhomologous end-joining (NHEJ) and Pol4. We propose a model in which alternative processing of DNA structures arising in Dna2-deficient cells can result in the release of DNA fragments and their capture at DSBs. This work demonstrates a new mechanism of genome instability and a new function of Dna2 nuclease in restricting sequence duplications.

A NEW HDAC4 ROLE, INDUCED BY DEXAMETHASONE, IMPROVES AUTOPHAGY IN A-T CELLS

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In the past years, published papers assessed a chief role of the ATM-HDAC4 pathway in chromatin shape of A-T cells and subsequent gene expression outcome. Based on these evidences we have tried to test whether dexamethasone (dexa) administration could counteract the lacking of ATM in HDAC4 regulation. Surprisingly we discovered that in A-T fibroblasts, dexa is able to further accumulate whole HDAC4 in the nucleus regardless of its phosphorylation status**.** In this form, HDAC4 is able to participate in the HIF1-alpha mediated DDIT4 (DNA-damage-inducible transcript 4) gene and protein upregulation. DDIT4 in turn participate in dexa induced metabolic signalling by mTOR1 modulation, and the observed slightly improvement of the autophagy process (a well-established impaired biological process in A-T) is possibly triggered also by the AMPK-ULK1 axis.

R-LOOPS AND ABERRANT RNA SPLICING IMPACT CEREBELLAR PURKINJE CELLS IN GENOME INSTABILITY-INDUCED NEURODEGENERATION

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Genome stability is critically important for human health, particularly that of the nervous system. This is apparent from inherited DNA repair deficiency syndromes that manifest neurodegeneration, neurodevelopmental disorders or brain tumors. Defects in genome maintenance are also implicated in broader neurologic health issues, including age-related degenerative events that mar cognitive ability and impact quality of life. However, the underlying bases for pathology associated with DNA repair deficiency remains mostly unknown. We generated mouse models of chronic genome instability via combined deficiency of ATM and APTX or ATM and PARP1. These double-knockout (dKO) mice develop progressive motor coordination defects resulting in severe ataxia. Despite no overt neurodegenerative histology, *in vivo* awake recordings demonstrated dysfunction of synaptic transmission in the dKO cerebellum, including decreased average firing rate and irregular firing of Purkinje cells (PC). RNA-seq analysis demonstrated a marked reduction in expression of specific genes, with many linked to known ataxia syndromes such as *Itpr1* and *Grid2*, which are crucial for synaptic modulation and excitatory neurotransmission respectively. Multivariate analysis of transcript splicing showed pronounced splicing defects including intron retention in PC-associated genes, but not in granule cell-associated genes in the dKO cerebellum. R-loops, three-stranded nucleic acid structures, linked to genome instability, can derail transcription via aberrant splicing or transcription pausing. Notably, substantial R-loop levels were apparent in the dKO cerebellum from ~1 month of age. Finally, nonsense-mediated mRNA decay-related gene expression pathways were also increased in the dKO cerebellum, likely accounting for loss of gene expression identified by RNA-seq. Taken together, our study suggests persistent DNA damage induces neurodegeneration by perturbing key cerebellar homeostatic genes via R-loop formation leading to aberrant RNA splicing and subsequently nonsense-mediated mRNA decay.

NOVEL FUNCTION OF ATM EXPLAINS METABOLIC PROBLEMS IN ATAXIA TELANGIECTASIA

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Despite being the subject of intense investigation, the exact mechanism by which ATM mutations cause clinical manifestations of AT are poorly understood. Here we demonstrate that ATM kinase phosphorylates and potentiate the activity of a cystine/glutamate antiport pump. In the absence or inhibition of ATM, glutamate accumulates and the cell is not able to mount a normal glutathione response to radiation or chronic endogenous stresses. Accumulated glutamate is metabolized in the mitochondria by glutaminolysis and this chronic activity may be the basis for the dysfunctional mitochondria reported for AT cells. To demonstrate the clinical consequences of this dysregulation we find that ATM null mice develop diabetes following pancreatic islet cell accumulation of glutamate. High levels of glutamate are known to be toxic to islet cells and we observe a progressive loss of glucagon producing alpha cells and a decreased number of insulin producing beta cells. The absence of glucagon correlates with increased fat deposition in the liver that is observed in AT patients. Interestingly, N-acetyl-cysteine (NAC) treatment completely prevented this phenomenon in ATM null mice. NAC circumvents the cystine/glutamine pump to provide the rate-limiting cysteine needed to combine with glutamate and glycine to produce glutathione, a potent antioxidant. Further, the identified cystine/glutamine antiport system is known to be key for T-cell proliferation, endothelial cell integrity and is expressed on Purkinje cells, all implicated in AT phenotypes. Ongoing work is to evaluate how ATM regulates this antiport system in cell and mouse models.

New regulators of DNA Topoisomerase 1-induced DNA damage and repair

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DNA Topoisomerase 1 (Top1) is essential as it regulates DNA supercoiling to enable faithful transmission of our genetic information to the offspring. However, Top1 is toxic when selectively trapped on the DNA (Top1-DNA cleavage complexes; Top1cc) by the anticancer drugs like camptothecin (CPT) and its clinical derivatives, which are used to treat solid tumors including colon, lung and ovarian carcinomas. Therefore, DNA must be under constant surveillance and repair mechanisms for genome maintenance against such pathological DNA lesions. A key repair enzyme for Top1cc is tyrosyl-DNA phosphodiesterase (TDP1), which hydrolyzes the phosphodiester bond between the DNA 3'-end and the Top1 tyrosyl moiety and has been implicated for the repair of such lesions both in the nucleus and mitochondria of cells. A homozygous mutation of human tyrosyl-DNA phosphodiesterase 1 (TDP1) is responsible for the neurodegenerative syndrome, spinocerebellar ataxia with axonal neuropathy (SCAN1). The fine-tuning of their activities is governed by posttranslational modifications, which have the ability to alter the proteins' function. We discovered that protein arginine methyltransferase (PRMT5), the major arginine methyltransferase catalyzing symmetrical dimethylarginines (SDMA) modifications is a molecular determinant for Top1cc repair. While in our earlier studies we have established the critical role Poly(ADP-ribose) polymerases (PARP1) in Top1-associated DNA breaks repair.

EXPLORING POTENTIAL BIOMARKERS PREDICTIVE OF RESPONSE TO INHIBITORS TARGETING CELL CYCLE CHECKPOINT PROTEINS ATR AND CHK1

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The cell cycle checkpoint proteins ataxia-telangiectasia-mutated-and-Rad3-related kinase (ATR) and its major downstream effector checkpoint kinase 1 (CHK1) prevent the entry of cells with damaged or incompletely replicated DNA into mitosis when the cells are challenged by DNA damaging agents, such as radiation therapy or chemotherapeutic drugs. This regulation is particularly evident in cells with a defective G1 checkpoint, a common feature of cancer cells, due to p53 mutations. In addition, ATR and/or CHK1 suppress replication stress by inhibiting excess origin firing, particularly in cells with activated oncogenes. Those functions of ATR and CHK1 make them ideal therapeutic targets. ATR and CHK1 inhibitors have been developed and are currently used either as single agents or paired with radiotherapy or a variety of genotoxic chemotherapies in preclinical and clinical studies. However, to date only limited efficacy has been noted in clinical trials. Originally, p53 was thought to be a biomarker predictive of response to CHK1 inhibitors when combined with radiotherapy and chemotherapy; however, a clinical study suggested that p53 status was not associated with treatment outcome with CHK1 inhibitors. More recent preclinical studies suggest that ATR and CHK1 inhibitors can be used as single agents to target cancer cells with a high level of replication stress. The discovery of new biomarkers to guide the use of these agents will significantly improve their efficacy. Our recent genome-wide loss of function screen by a high-throughput Decode Pooled shRNA library revealed that reduced tumor suppressor PPP2R2A expression increased sensitivity to a CHK1 inhibitor. PPP2R2A is a B regulatory subunit of protein phosphatase 2 (PP2A). Reduced PPP2R2A expression occurs in over 40% of non-small cell lung cancer (NSCLC), as a result of loss of heterozygosity of the PPP2R2A coding region. Given that reduced PPP2R2A expression is associated with a poor prognosis, treating PPP2R2A deficient cancer represents an unmet clinical need. Thus, the objective of our study is to establish molecular basis for treating NSCLC with PPP2R2A deficiency by ATR and CHK1 inhibitors. In this presentation, I will provide the evidence to support the role of PPP2R2A in guiding the use of ATR and CHK1 inhibitors via regulation of oncogene-induced replication stress. NSCLC with α deficiency in PPP2R2A can be specifically targeted by ATR and CHK1 inhibition. Thus, PPP2R2A represents a new biomarker to identify treatment sensitive patient populations to ATR and CHK1 inhibitors.

ATM REGULATES CARDIAC MITOPHAGY

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Ataxia Telangiectasia (A-T), a recessive genetic disease due to a decrease or absence of Ataxia Telangiectasia mutated protein kinase (ATM), has a strong association with development of insulin resistance, type 2 diabetes and cardiovascular disease. In light of the importance of mitochondrial dysfunction in cardiovascular disease and our recent findings regarding the impact of inhibition of ATM on mitochondrial function as well as indications of changes in mitophagy in $ATM^{-/-}$ mice, we investigated the role of ATM in cardiac mitophagy using male Wistar rats.

Methods: Hearts were perfused *Ex vivo* (n=6-9/group) ± the specific ATM inhibitor, KU60019 (3 uM) or insulin (10 nM) prior to mitochondrial isolation. Markers of mitophagy (Pink, Parkin, LC3, TOM 70 and P62 SQSTM1 as well as the fission-associated protein Drp-1, were determined by western blotting.

Results: Perfusion of the heart with KU60019 lowered mitochondrial ATM levels. Furthermore, mitochondrial associated Parkin was upregulated accompanied by lower expression of TOM 70 but no accumulation of Pink was observed. However, the LC3 II/I ratio was lower, as was the association with P62SQSTM1, indicating that low mitochondrial ATM resulted in inhibition of mitophagy and the formation of autophagosomes. These effects were counteracted by insulin. In addition, KU60019 treatment lowered mitochondrial association of the fission protein Drp-1.

Conclusion: Chemical inhibition of myocardial ATM resulted in loss of mitophagy at the level of formation of the autophagosome as well fission of mitochondria. This could lead to defective mitochondria not being removed leading to mitochondrial dysfunction and would be exacerbated by insulin resistance.

LAMIN A/C INTERACTOME MODULATED BY DEXAMETHASONE IN A-T CELLS

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In the present study, we show some preliminary results about the nucleoplasmic lamin A/C dynamics in A-T fibroblasts after dexamethasone treatment (dexa). We demonstrated that dexa could alter the lamin A/C interaction with Lap2-alpha but the typical downstream effectors of this pathway, Rb-E2F1 as well as target genes, are unaffected. Therefore, we performed a high-throughput LC-MS/MS analysis of the proteome obtained by lamin A/C immunoprecipitation of A-T and WT samples treated or not with dexa. Apart the structural interactor proteins, some lamin A/C partners have been defined as dexa modulated and they have been verified by PLA assay. Also by the adopted strategy, the lacking of the Rb-E2F1 pathway was confirmed in the studied cellular model.

RIBONUCLEASE H2 IS REQUIRED FOR THE MAINTENANCE OF GENOMIC INTEGRITY DURING NEUROGENESIS AND PREVENTION OF BRAIN TUMORS

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Maintenance of genomic integrity is crucial during nervous system development and its failure leads to various neurological diseases. Ribonuclease H2 (RNaseH2) is a genome surveillance factor that protects DNA by removal of RNA-DNA hybrids and ribonucleotides incorporated into the DNA during replication. Mutations in *Rnaseh2* are linked with Aicardi-Goutieres syndrome (AGS), a childhood disorder characterized by severe brain dysfunction and neuroinflammation. Although the central nervous system is severely affected in AGS, available mouse models of RNaseH2 do not recapitulate the disease symptoms. Here we investigate how brain-specific deletion of RNaseH2 affects the nervous system. Contrary to previous findings, we show that loss of RNaseH2 in the mouse brain profoundly affects early neurogenesis and results in activation of interferon-responsive genes and neuroinflammation. RNaseH2-deleted brain shows cerebellar atrophy due to the loss of cerebellar interneurons and granule neuron precursors. This cell loss is rescued by deletion of p53, but these mice rapidly develop medulloblastoma tumors. Interestingly, p53 activation in RNaseH2-deleted brains is not solely dependent upon ATM kinase as deletion of *Atm* did not rescue the cerebellar phenotype. In fact, coincident deletion of *Atm* and *Rnaseh2* resulted in worsened cerebellar phenotype with mice developing ataxia, indicating ATM suppresses the genome damage after RNaseH2 inactivation. Taken together, our data provide new insight into understanding the contribution of RNaseH2 in preventing neurological disease.

DYNAMICS OF ATM, ATR AND THEIR SUBSTRATES IN COMPLEX DNA LESIONS

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Ataxia Telangiectasia Mutated (ATM), Ataxia Telangiectasia and Rad3 related (ATR) and DNA-dependent protein kinase (DNA-PK) are the major PIKK protein kinases that act in the cellular response to DNA damage by phosphorylating serine/threonine residues in a large number of proteins. ATM and DNA-PK were found to be recruited to double-stranded breaks, whereas ATR is recruited by binding to single-stranded DNA coated with RPA proteins. Once recruited to DNA lesions PIKK protein kinases interact and phosphorylate dozens of proteins to orchestrate the checkpoint control and the maintenance of genomic integrity. However, little is known about the order and the kinetics of recruitment and removal of these kinases to the sites of complex DNA damages with respect to the dynamics of their substrates. Here, we measured, clustered and modeled the kinetics of recruitment and dissociation of ATM and 70 DNA repair proteins to laser-induced DNA damage sites in HeLa cells. Our results reveal two consecutive waves of recruitment of PIKK protein kinases and determine their precise coexistence with most of their substrate proteins shedding light on unknown aspects of the repair of complex DNA lesions.

MRE11 complex control and the A-T phenotype

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ATM acts in the molecular orchestration of damage detection and repair and its interface with replication rescue. Consequently, A-T patients and ATM-deficient cells are hyper-sensitive to DNA-damaging agents used in cancer therapy, such as PARP inhibitors, as well as endogenous DNA lesions that can lead to neurodegeneration. We are investigating structures and mechanisms of MRE11 complex that activate ATM and act in repair pathway choice. As part of this work, we are making inhibitors to test the role of protein partners of the MRE11 complex that modulate its recruitment and impacts in the presence and absence of functional ATM. The emerging results suggest that MRE11 complex and its actions in the control of repair pathway choice provides a window into AT patient defects and strategies for intervention.

ATM loss during germ cell development results in deregulated double-strand break formation and copy number variation

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Gonadal dysgenesis is frequently observed in A-T patients, although it is often overlooked due to the severity of the pediatric disease. Mice with genetically engineered mutations have provided a valuable resource to assess the role of ATM during germ cell development. Both males and females are infertile due to defects during meiotic prophase I and show evidence for unrepaired DNA double-strand breaks (DSBs) introduced by the SPO11 protein. Several years ago, we reported that the number of meiotic DSBs is controlled by ATM. Levels of SPO11-oligonucleotide complexes, by-products of meiotic DSB formation, are elevated tenfold in spermatocytes lacking ATM. Moreover, *Atm* mutation renders DSB formation sensitive to genetic manipulations that modulate SPO11 protein levels. Further, ATM also shapes DSB distributions. We proposed that ATM restrains SPO11 via a negative feedback loop in which kinase activation by DSBs suppresses further DSB formation. More recently, we examined the consequence of ATM loss by analysis of meiotic DSB hotspots. We observed deletions both at individual hotspots and between hotspot pairs, as well as insertions of sequences from other hotspots. Thus, deregulated DSB formation during meiosis resulting from loss of ATM leads to copy number variation at genomic positions.

Multiple repair mechanisms employed by BLM helicase to maintain genome integrity

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The mammalian genome codes for five related RECQ-like 3'–5' DNA helicases: RECQL1, BLM, WRN, RECQL4 and RECQL5, all of which have been demonstrated to act upon a number of topologically different DNA structures in vitro. BLM is mutated in Bloom syndrome (BS); a rare autosomal recessive disorder that is typified by proportional dwarfism, sun-sensitive facial erythema, skin pigmentation abnormalities, immunodeficiency, infertility and an increased predilection to develop both lymphoid and epithelial-derived tumors. A unique cellular feature, commonly used in the molecular diagnosis of BS, is around 10-fold increase in the frequency of sister chromatid exchanges (SCEs), which is thought to arise as a consequence of uncontrolled homologous recombination (HR) during S and G2 phases of the cell cycle. Thus BLM helicase is thought to maintain genome stability and is classified as a caretaker tumor suppressor.

While the broad contours of BLM functions are known, precise molecular mechanisms how BLM affects DNA repair on the chromatin remains comparatively unexplored, especially for damages induced by double strand breaks (DSBs). In the present study a site-specific cleavage system has been utilized to determine the key determinants essential for the recruitment and subsequent functions of BLM at annotated DSBs spanning the entire genome. BLM recruitment is dependent on the presence of NBS1, MRE11 and ATM. While ATM activity is essential for BLM recruitment in early phase, it is dispensable in late phase when MRE11 exonuclease activity and RNF8-mediated ubiquitylation of BLM are the key determinants. Interaction between polyubiquitylated BLM and NBS1 is essential for the helicase to be retained at the DSBs. The helicase activity of BLM is required for the recruitment of HR and c-NHEJ factors onto the chromatin in S- and G1-phase, respectively. During the repair phase, BLM inhibits HR in S-phase and c-NHEJ in G1-phase. Consequently, inhibition of helicase activity of BLM enhances the rate of DNA alterations. Thus BLM utilizes its pro- and anti-repair functions to maintain genome stability.

TARGETING THE HIGH MOBILITY GROUP BOX 3 PROTEIN SENSITIZES CHEMORESISTANT OVARIAN CANCER CELLS TO CISPLATIN

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Ovarian cancer is the leading cause of death from gynecological cancer and is the fifth most common cause of cancer-related death in women. Chemotherapeutic treatment of ovarian cancer includes the use of DNA interstrand crosslink-inducing agents and/or DNA double-strand break-inducing agents. Unfortunately, the majority of patients fail to maintain a response to treatment, in part due to drug resistance, resulting in a poor survival rate. Thus, new approaches to overcome this resistance are warranted. Here, we found that cisplatin sensitivity can be restored in cisplatin-resistant ovarian cancer cells by targeting the High Mobility Group Box 3 (HMGB3) protein. The HMGB proteins have been implicated in ovarian cancer, and HMGB3 can be up-regulated (up to 20-fold) in cancer cells, making it a potential selective target for therapeutic intervention. We found that HMGB3 depletion in cisplatin-sensitive and cisplatin-resistant cells resulted in transcriptional down-regulation of the DNA damage response kinases, ATR and CHK1, attenuating the ATR/CHK1/p-CHK1 DNA damage-signaling pathway. Further, we found that HMGB3 was associated with the promoter regions of *ATR* and *CHK1*, suggesting a new role for HMGB3 in transcriptional regulation. Finally, DNA content analysis indicated that HMGB3 depletion significantly increased apoptosis in A2780/CP70 cells following cisplatin treatment. Taken together, our results indicate that HMGB3 depletion attenuated cisplatin resistance in human ovarian cancer cells, increasing tumor cell sensitivity to cisplatin.

ATM suppresses ataxia by preventing toxic NHEJ after aberrant topoisomerase I activity.

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Topoisomerase 1 (TOP1) regulates torsional stress generated in double-stranded DNA during replication, transcription and chromatin remodeling. Aberrant TOP1 function has been implicated in many neurological diseases including ataxia telangiectasia (A-T), spinocerebellar ataxia with axonal neuropathy 1 (SCAN1) and autism, amongst others, making understanding TOP1 biology of paramount importance. To determine the pathobiology of TOP1 we have generated an inducible mouse model that expresses a $Top1^{T720A}$ mutation, which retards religation without affecting rates of cleavage, leading to increased Top1-cleavage complex formation. Expression of this mutation throughout the nervous system causes broad perturbation of neurogenesis with decreased brain size, resulting from widespread DNA damage. In the $Top1^{T720A}$ cerebellum, granule neuron ablation and a significant loss of oligodendrocytes and cerebellar interneurons occurs. However, coincident inactivation of ATM in the context of $Top1^{T720A}$ [$(Top1^{T720A};Atm)^{Nes\text{-}cre}$] resulted in profound disruption of cerebellar development, with significant disruption of Purkinje cell organization and ataxia. Remarkably, substantial rescue of cerebellar development occurred in $(Top1^{T720A};Atm)^{Nes\text{-}cre}$ mutants after simultaneous inactivation of NHEJ, by Lig4 loss. Thus, prevention of toxic NHEJ is a key function of ATM in response to specific DNA lesions. Our data provide critical insight into topoisomerase pathobiology and illuminates the etiologic basis for A-T.

Mitochondrial DNA replication fork protection by the Fanconi Anemia pathway suppresses inflammation and disease

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Maintenance of the genome integrity is important for prevention of diseases such as cancer. ATM is a key DNA damage response kinase which regulates hundreds of down stream proteins including MRE11. In the nucleus, ATM and MRE11, along with others such as RAD51C, are crucial DNA repair proteins involved in homologous recombination. Additionally, RAD51C is essential in protecting stalled DNA replication forks from degradation by MRE11. ATM, MRE11, and RAD51C are all localized to the mitochondria. However, there is no strong evidence for the existence of mitochondrial homologous recombination and the mitochondrial oxidative stress response functions of ATM are less understood.

Here, we show that RAD51C is required for protecting mitochondrial DNA replication forks stalled upon oxidative damage to suppress mitochondrial genome instability, as seen by loss of nascent mtDNA and mitochondrial genome over-production in RAD51C patient cells. Mitochondrial fork protection defects result in delayed repair of 8-oxo-G lesions, driving TFAM-DNA binding and increasing mtDNA replication, revealing an unexpected mechanism for acute *de novo* mtDNA synthesis upon mtDNA damage. Importantly, stalled mitochondrial replication forks are degraded by MRE11, which results in activation of the cGAS inflammation pathway. The collective results establish a molecular pathway for replication stability in the mitochondria important for suppression of inflammation and explains how defects in genome maintenance proteins cause diverse hallmarks of cancer.

An AMPK-regulated energy pathway that directs ATM localization to the peroxisome

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Peroxisomes are autonomously replicating organelles that play key roles in several metabolic pathways. They do not contain DNA, hence all peroxisomal proteins needed for the function of these organelles are encoded in the nucleus, synthesized in the cytoplasm, and delivered to peroxisomes via specialized import receptors. In the process of carrying out their metabolic functions, peroxisomes generate significant amounts of reactive oxygen species (ROS). Thus, cells must maintain sufficient numbers of peroxisomes for metabolism while avoiding excess production of ROS that can cause oxidative damage.

We previously reported the PEX5 peroxisome import receptor binds ATM and localizes it to the peroxisome, where in response to ROS, this kinase induces autophagic degradation of peroxisomes (pexophagy) (Zhang et al *Nat. Cell Biol*. 2015).

We have now identified a new linkage between ATM and AMP-activated kinase (AMPK), which we find participate in a signaling cascade targeting the PEX5 peroxisome import receptor to regulate peroxisome homeostasis. In response to oxidative stress, ATM is activated and phosphorylates PEX5 at serine 141 (S141). ATM phosphorylation of PEX5 at S141 promotes mono-ubiquitination of PEX5 tethered to peroxisomes, providing a recognition site for the p62 adapter protein, which targets peroxisomes to autophagosomes via LC3 binding to induce pexophagy. Conversely, under conditions of energy stress, AMPK is activated, and this kinase phosphorylates PEX5 at a second site, serine 279 (S279). PEX5 phosphorylation at S279 is independent of S141 phosphorylation, and has a different functional consequence. AMPK phosphorylation of PEX5 at S279 blocks the interaction between the PEX5 import receptor and the peroxisomal docking protein PEX13, thus preventing delivery of ATM (and other cargo) to the peroxisome. These data provide a new model for regulation of peroxisome homeostasis, with ATM and AMPK signaling in response to oxidative or energy stress regulating destruction vs preservation of peroxisomes, respectively, via phosphorylation of the PEX5 import receptor at S141 or S279 to promote, or inhibit, ATM-mediated pexophagy.

Mitochondrial dysfunction and inhibition of mitophagy in mantle cell lymphoma lacking ATM

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Ataxia telangiectasia mutated (ATM), a critical DNA damage sensor, is frequently altered in human cancers including mantle cell lymphoma (MCL). Previous investigations demonstrated that loss of ATM protein is linked to accumulation of nonfunctional mitochondria and decreased mitophagy, in both murine thymocytes and in Ataxia-telangiectasia (A-T) cells. However, the role of ATM in cancer cell mitochondria and mitophagy is unknown. Here, we provide evidence that FCCP-induced mitophagy in MCL and other cancer cell lines is dependent on ATM but independent of its kinase function. While MCL line Granta-519 cells possess single copy and kinase dead ATM and are resistant to FCCP-induced mitophagy, both Jeko-1 and Mino cells are ATM proficient and induce mitophagy. Stable knockdown of ATM in Jeko-1 and Mino cells conferred resistance to mitophagy and was associated with reduced ATP production, oxygen consumption, and increased mitochondrial ROS; reflecting dysfunctional mitochondria. Neither loss of ATM kinase activity in primary B cell lymphomas nor inhibition of ATM kinase in MCL, A-T and HeLa cell lines mitigated FCCP or CCCP-induced mitophagy suggesting that ATM kinase activity is dispensable for mitophagy. These investigations were extended in primary cells from patients with lymphoma. Collectively, these data establish a non-canonical role of ATM in cancer.

MRE11 UFMylation promotes ATM activation

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A proper DNA damage response (DDR) is essential to maintain genome integrity and prevent tumorigenesis. DNA double-strand breaks (DSBs) are the most toxic DNA lesion and their repair is orchestrated by the ATM kinase. ATM is activated via the MRE11-RAD50-NBS1 (MRN) complex along with its autophosphorylation at S1981 and acetylation at K3106. Activated ATM rapidly phosphorylates a vast number of substrates in local chromatin, providing a scaffold for the assembly of higher-order complexes that can repair damaged DNA. While reversible ubiquitination has an important role in the DSB response, modification of the newly identified ubiquitin-like protein UFM1 and the function of UFMylation in the DDR is largely unknown. Here, we found that MRE11 is UFMylated on K282 and this UFMylation is required for the MRN complex formation under unperturbed conditions and DSB-induced optimal ATM activation, homologous recombination-mediated repair and genome integrity. A pathogenic mutation MRE11(G285C) identified in uterine endometrioid carcinoma exhibited a similar cellular phenotype as the UFMylation-defective mutant MRE11(K282R). Taken together, MRE11 UFMylation promotes ATM activation, DSB repair, and genome stability and potentially serves as a therapeutic target.

Regulatory mechanisms of transcription-coupled DNA repair

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Genomic DNA is continuously challenged by a range of damaging agents. A wide variety of DNA lesions in active and inactive genes are removed by two types of nucleotide excision repair (NER), classified as either global genomic (GG) or transcription-coupled (TC) NER. Lesions in transcriptionally inactive or silent areas of the genome are repaired by GG-NER, while TC-NER removes lesions from the coding sequence of active genes. Both NER subpathways are fundamentally identical except for their mechanisms of damage recognition. The damage recognition step has been implicated in the differential kinetics for these two repair processes. TC-NER functions at a faster rate than GG-NER, as damage is quickly recognized in TC-NER. However, hoe the DNA lesion is promptly recognized in TC-NER is not clearly understood. To address this, we performed a series of experiments in yeast and found that the DNA lesion recognition factor in TC-NER, Rad26 (homologue of human CSB), is recruited to active coding sequences in response to DNA damage through elongating RNA polymerase II. However, Rad26 does not recognize DNA lesions in the absence of elongating RNA polymerase II or active transcription. Subsequent to Rad26 recruitment to the DNA lesion, elongating RNA polymerase II is disassembled through the degradation of its largest subunit, thus facilitating DNA repair factors to access the lesion through Rad26 for repair. Further, Rad26 plays an important role in altering chromatin structure to facilitate transcription as well as DNA repair. Like Rad26, another NER factor, namely Rad14, is also involved in facilitating transcription. Together, our results provide an important insight as to how Rad26 is preferentially delivered to DNA damage sites in active, but not inactive, genes to stimulate repair. Next, we analyzed whether, like NER, the repair of highly toxic DNA double-strand breaks (DSBs) is also coupled to transcription and found preferential DNA DSB repair in active genes, thus supporting the existence of transcription-coupled DSB repair. Active genome repair was found to be regulated by an optimal level of histone H2B ubiquitylation as well as histone sumoylation and methylation. Histone acetylation by NuA4 histone acetyltransferase also facilitates DNA repair as well as transcription, and TOR (target of rapamycin) has been found to promote NuA4 targeting to active genes via the 19S proteasome subcomplex. Active genome repair is also regulated by a histone chaperone, FACT (Facilitates Chromatin Transcription), via its fine-tuning by ubiquitin- proteasome system. Collectively, our results provide significant mechanistic insights into transcription-coupled DNA repair.

Ufmylation signaling and ATM activation

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The ataxia-telangiectasia mutated (ATM) kinase, an upstream kinase of the DNA damage response (DDR), is rapidly activated following DNA damage, and phosphorylates its downstream targets to launch DDR signaling. However, the mechanism of ATM activation is still not completely understood. We report that UFM1 specific ligase 1 (UFL1), an ufmylation E3 ligase, is important for ATM activation. UFL1 is recruited to double strand breaks by the MRE11/RAD50/NBS1 complex, and monoufmylates histone H4 following DNA damage. Monoufmylated histone H4 is important for Suv39h1 and Tip60 recruitment. We further showed that the Hippo kinase STK38 acts as a reader for histone H4 ufmylation and promotes ATM activation. These findings reveal that ufmylation of histone H4 by UFL1 is an important step for amplification of ATM activation and maintenance of genomic integrity.

A Novel mechanism of RAG-mediated chromosomal translocations in lymphoid cancer

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Chromosomal translocations are considered as one of the major causes for generation of lymphoma and leukemia. RAG complex (recombination activating genes) is a sequence specific endonuclease which cleaves at recombination signal sequence (RSS) and is responsible for antigen receptor diversity. RSS consists of conserved heptamer and A/T-rich nonamer, separated by 12/23 bp spacer. Recent studies have shown that RAG complex can also act as structure specific endonuclease. Misrecognition of targets, due to structure and sequence specific nuclease activity of RAGs could lead to translocations involving non-antigen receptor loci. Activation-induced cytidine deaminase (AID), expressed in germinal B cells, is responsible for C to U or ^{5m}C to T conversions, thus generating single nucleotide mismatch of either U:G or T:G. Upon performing whole genome analysis of around 2000 chromosomal translocations associated with lymphoid cancer patients, we observed a significantly high frequency of cryptic nonamers near CpGs and translocation breakpoint region. Importantly, in more than 65% of the reported translocations, breakpoints possess adjacent to CpG sites and nonamers. Using biochemical and cellular assays, we show that RAGs can efficiently bind to cryptic or canonical nonamers present in selected regions and cleave at single nucleotide mismatches (U:G or T:G) generated to mimic deamination of CpG. Thus, we propose a novel mechanism for the generation of lymphoid specific chromosomal translocations, wherein RAGs bind to a cryptic nonamer, leading to cleavage at an adjacent mismatch generated due to deamination of a methylated CpG site or unmethylated cytosine.

Kinase independent ATM-Parkin interaction drives mitochondrial autophagy in mantle cell lymphoma

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ATM, a DNA damage sensor has been recently implicated to have non- nuclear role owing to its presence in extra-nuclear compartments including lysosomes and mitochondria. Loss of ATM protein has been shown to be associated with defective mitophagy and preservation of nonfunctional mitochondria. However, the mechanism of ATM association in mitophagy is elusive. Multiple proteins interact with ATM either for mitochondrial localization or in the initial phase of mitophagy. Mechanistically, ATM interacts with the E3 ubiquitin ligase Parkin and contribute in mitophagy via stabilizing Parkin. ATM knockdown in HeLa cells resulted in proteasomal degradation of GFP-Parkin which was rescued by the proteasome inhibitor, MG132 suggesting that ATM-Parkin interaction is important for Parkin stability. LC-MS study identified HSP90-Tom70 interaction is critical for extra-mitochondrial ATM transport inside mitochondria. Inhibition of HSP90 by 17-AAG abrogated both HSP90-ATM interaction as well as mitochondrial ATM transport in MCL cell lines. Finally, we show that neither loss of ATM kinase activity in primary B cell lymphomas nor inhibition of ATM kinase in MCL, A-T and HeLa cell lines mitigated FCCP or CCCP-induced mitophagy suggesting that ATM kinase activity is dispensable for mitophagy. Malignant B-cell lymphomas without detectable ATM, Parkin and Pink1 were relatively resistant to mitophagy. Therefore, our study provide the first molecular evidence of ATM's role in mitophagy in MCL and other B-cell lymphomas.

Physiologically Important Small Molecules as Regulators of RecA function

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The bacterial RecA protein plays a pivotal role in the repair of stalled replication forks, double-strand break repair, homologous recombination, and the SOS response. RecA is regulated at many levels. The expression of the *recA* gene is regulated by LexA within the SOS response network. The biological activities of RecA protein is regulated by the action of a number of accessory proteins, including RecF, SSB, RecO, RecR, DinI, RecX, RdgC, PsiB, and UvrD. All of these proteins typically exert their actions on the assembly and/or activity of RecA nucleoprotein filaments through positive or negative regulatory mechanisms. A variety of accessory proteins have since been found to play key roles in the regulation of RecA homologues (RadA/Rad51) in archaeans and eukaryotes. Given these findings, we surmised that additional physiologically relevant non-protein factors might affect recombination reactions carried out by RecA proteins. Towards this end, our previous studies revealed that the second messenger cyclic di-AMP plays an important role in the negative regulation of RecA protein function as well as *recA* gene expression. To further inform our understanding of the regulation of RecA function by endogenous small molecules, the potential roles of the plasma membrane components were examined. Our recent work supports the notion that anionic phospholipids of the bacterial plasma membrane may be physiologically relevant, as they provide a scaffold for RecA storage and regulate recombinational DNA repair and the SOS response. I will present our results in both of these areas and discuss their implications.

Mechanisms of intrachromosomal deletion rearrangements, and other chromosomal break repair outcomes

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Chromosomal rearrangements can result from aberrant end joining (EJ) repair that ligates distal ends of two DSBs on the same or different chromosomes. Such rearrangements could potentially be mediated by either canonical non-homologous EJ (C-NHEJ) or Alternative-EJ (ALT-EJ), which leads to distinct mutational patterns at the rearrangement junction. However, the relative role of the C-NHEJ pathway in rearrangement formation and its fidelity during EJ repair has been controversial. To address this, we developed a novel reporter assay that measures a 0.4 Mbp rearrangement that is formed by EJ between two Cas9-induced DSBs. We find that ATM deficiency causes an increase in the frequency of the rearrangement, as well as a marked shift towards rearrangement junctions that show hallmarks of C-NHEJ. In addition, several C-NHEJ factors (i.e., XRCC4, XLF, and KU70) are required for the increase in rearrangement frequency caused by ATM kinase inhibition. These results indicate that the relative contribution of the C-NHEJ to rearrangement formation is magnified by ATM kinase deficiency. Strikingly, we also found that rearrangement junctions from C-NHEJ-deficient cells showed a loss of EJ events without insertion/deletion (indel) mutations. Thus, we sought to further examine the frequency and mechanism of EJ without indel mutations, by developing an assay for such EJ. Indeed, we find that EJ without indels is robust, and absolutely requires XRCC4, XLF, and KU70. To further distinguish between EJ events mediated by C-NHEJ and ALT-EJ, we generated variants of this assay with 1-4 nucleotides (nts) of microhomology. We found that C-NHEJ is also required for EJ events that use 1-2 nucleotides of terminal microhomology but dispensable for EJ events that utilize >2 nts of terminal microhomology. In contrast, Alt-EJ factors (e.g. CTIP and POLQ) are required for EJ events that use 4 nts of microhomology that are embedded from the edge of a DSB. Taken together, these findings indicate that C-NHEJ is required for repair of DSBs without causing indels, but can be prone to forming chromosomal rearrangements, particularly in ATM-deficient cells.

NOVEL PATHOGENIC ATM VARIANTS IN INDIAN ATAXIA TELANGIECTASIA PATIENTS

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Pathogenic variants in the ATM gene are responsible for the autosomal recessive neurodegenerative disorder, ataxia telangiectasia (A-T). Mutations in various ethnic groups have been described and there seem to be no hotspots.
In the present study, A-T patients from 41 Indian families were assessed for their clinical phenotypes and ATM haplotype analysis, and screened for ATM mutations.

Thirty seven distinct haplotypes were observed in 41 unrelated families comprising of 21 homozygous and 16 heterozygous haplotypes. Pathogenic ATM variants were identified in 33 out of 41 families, Elon of these were aberrant splicing, 5 truncations, 13 frameshifts, 2 missense, and one large genomic deletion spanning exon 17-63 and the other a deletion of 193nt of exon 11. Recurring haplotype and associated variant c.5631_5635delinsA was observed in 7 families of North Indian origin, suggestive of a possible founder effect in Indian A-T patients.

Of the 33 ATM variants identified, 29 were novel unreported variants, thereby suggesting that the profile of mutations in the India subcontinent is unique. The present data adds to the multitude of unique ATM mutations identified worldwide.

INVESTIGATION OF THE CEREBELLAR DEGENERATION IN ATAXIA-TELANGIECTASIA USING MOUSE MODELS WITH COMBINED Atm AND Setx GENOTYPES

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Ataxia-telangiectasia (A-T) is an autosomal recessive, genome instability disorder caused by loss or inactivation of the ATM protein kinase. A-T is characterized primarily by progressive cerebellar atrophy, which begins with loss of Purkinje, basket and granule cells. ATM is a homeostatic kinase that mobilizes the vigorous cellular network responding to DNA double-strand breaks, and is also involved in responding to other DNA lesions, and in various metabolic pathways. Many symptoms of A-T can be explained by ATM's role in the DSB response, but the origin of the cerebellar degeneration in this disease is still debated. We are studying this aspect of A-T using mouse models. Atm-deficient mice recapitulate many of the symptoms of the human disease but their cerebellar phenotype is extremely mild and has been reported inconsistently. Using an array of fine behavioral tests, we were able to show that these mice do develop a distinct, progressive neuro-motor phenotype, which is discernible at 7 months of age and by 11 months of age leads to abnormal gait pattern. Our strategy to exacerbate this phenotype is to combine Atm loss with deficiency of another enzyme involved in maintenance of genome stability. In view of the extensive transcription in Purkinje cells, we added to Atm deficiency lack of the senataxin (Setx) protein. SETX is a DNA helicase that is involved in the resolution of DNA:RNA hybrid loops (R-loops) – a frequent event in highly transcribing cells and a source of genome instability. Notably, SETX is recruited to sites of DNA damage in an ATM-dependent manner and SETX loss in humans causes ataxia with oculomotor apraxia 2 (AOA2) – a disorder characterized primarily by cerebellar degeneration. We obtained all possible genotypic combinations of the Atm and Setx null alleles. Importantly, the double-knockout mouse (Atm-/-//Setx-/-) is born alive (at sub-Mendelian ratio) and, surprisingly, its neuro-motor phenotype is not significantly different from that of the Atm-/- mouse. .

ISG15 Inhibits Mitophagy in Ataxia-Telangiectasia

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Deregulation of the mitophagy pathway, the selective degradation of damaged mitochondria *via* autophagy, is a common underlying cause of neurodegeneration in several neurodegenerative diseases, including Ataxia-Telangiectasia (A-T). However, the mechanism underlying defective mitophagy remains unclear. Previously, our lab has demonstrated that constitutively elevated ISG15 (**I**nterferon-**S**timulated **G**ene **15**) inhibits polyubiquitylation of cellular proteins in A-T. Literature reports reveal that the ubiquitin E3 ligase Parkin appends polyubiquitins onto mitochondrial fusion proteins mitofusins 1 and 2 (Mfn1 and Mfn2) among many other proteins on depolarized mitochondria, targeting them to the proteasome for their degradation prior to the initiation of mitophagy. Because the ISG15 pathway inhibits ubiquitin-dependent signaling and ubiquitylation of mitochondrial proteins is a necessary committed step for mitophagy, we examined whether elevated ISG15 inhibits polyubiquitylation of mitochondrial targets as a mechanism to explain the defective mitophagy observed in A-T cells. Our results have revealed that both mitophagy and polyubiquitylation of mitochondrial proteins are, in part, restored in ISG15-silenced A-T cells. These results suggest that ISG15-mediated defective polyubiquitylation of mitochondrial proteins, an essential initiation signal for mitophagy, may be an underlying cause of defective mitophagy in A-T cells. These findings open up new perspectives for understanding the pathogenesis of and may provide a potential therapeutic target for the neurodegenerative symptoms seen in patients with A-T.

Mice expressing ATM refractory to acute activate is viable, in contrast to kinase-dead ATM

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ATM kinase is activated by DNA strand breaks to orchestra DNA damage responses by phosphorylating is downstream targets. Germline inactivation of ATM causes Ataxia-Telangiectasia (A-T) syndrome that is characterized by cerebellus degeneration, and variable degrees of primary immunodeficiency and increased risk for lymphomas and leukemia. Mice lacking ATM protein expression recapitulates the immunodeficiency and lymphoma risk of A-T patients. In contrast to the normal development of ATM null mouse models, we and others found that mice expressing kinase-dead ATM died in the uterus with severe genomic instability. This finding explains why over 90% of A-T patients carry truncating/frameshift ATM mutation with little or no protein expression and also suggests that inactive ATM protein can further impair genomic stability beyond the loss of ATM kinase-dependent signaling. To understand how ATM activation impairs the physiological function of ATM, we generated two novel mouse models with truncating mutation (R3047X) in the FATC domain or point mutation (R3008H) in the Kα10 helices. Both the FATC domain and Kα9 and Kα10 helixes are conserved in all PI3 kinase-related protein kinases and implicated in kinase activation. R3047X mutation affects the stability of ATM while the R3008H mutation does not affect ATM stability *in vivo*. Purified ATM-R3008H cannot be activated by the MRN complex in a DNA dependent manner or by H2O2. Correspondingly, $Atm^{R3008H/R3008H}$ cells fail to phosphorylate KAP1 and other targets upon radiation or by ROS and cannot establish G2/M nor G1/S checkpoint upon DNA damage. $Atm^{R3008H/R3008H}$ lymphocytes display classical defects in T cell development and B cells immunoglobulin class switch recombination. But in contrast to the embryonic lethality of $Atm^{KD/KD}$ mice, $Atm^{R3008H/R3008H}$ mice are small, but viable with spontaneous craniofacial abnormalities. Surprisingly, despite defects in both DNA double-strand breaks and in checkpoints, $Atm^{R3008H/R3008H}$ mice have delay onset lymphomas. Preliminary cell biological analyses suggest that R3008H mutation results in a faster exchange of ATM at the DNA damage sites and partially rescue the delayed exchange of ATM-KD. Those findings might provide one explanation for the broad spectrum of clinical presentations of A-T patients. We will discuss the implication of these findings in ATM activation and the structural function of ATM in DNA repair and physiological function of ATM.

RAD52 AND RAD59 ANNEALING ACTIVITY SUPPRESS DNA BREAKS REPAIR VIA MUTAGENIC BREAK-INDUCED REPLICATION

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Break-induced replication (BIR) mechanism of single-end DNA double-strand break (DSB) repair involves extensive and highly mutagenic DNA synthesis and promotes genome instability. BIR is not involved in the repair of two ended DSBs but the mechanisms limiting its usage at such DNA breaks are largely unknown. Using several recombination assays we demonstrate that proteins with single-strand DNA annealing activity, Rad52 and Rad59, regulate the choice between gene conversion and BIR in repair of two ended DSBs. Annealing deficient *rad52*-R70A cells and to a lesser degree *rad59* mutant cells increase the usage of BIR associated with the loss of one part of the broken chromosome. We propose decreased ability to engage second end of a DSB in recombination in the annealing mutant cells results in increased usage of mutagenic BIR.

Autoregulation of Budding Yeast ATR and the Different Dynamics of ATM- and ATR-dependent Phosphorylation of γ-H2AX

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Even a single unrepaired double-strand break triggers an extended G2/M cell cycle arrest in budding yeast. Arrest is maintained for 12-15 h (6 normal cell division times) until cells adapt and resume cell cycle progression, despite the continuing presence of the resected chromosome break. Activation of the DNA damage checkpoint depends on Mec1^{ATR} and its partner Ddc2^{ATRIP}. Deletion of PP2C phosphatases that are required to dephosphorylate Rad53^{CHK2} and other components of the checkpoint kinase cascade prevents adaptation. We mutated the 9 SQ/TQ sites in Mec1 and found that S1964A is adaptation-defective. Both by mass spectrometry and a phospho-specific antibody, we found that phosphorylation of S1964 does not occur until ≥ 3 h and peaks at 9 h, whereas Mec1 phosphorylates Ddc2, Rad9^{TOPBP1}, Rad53 within an hour. Mec1-dependent S1964 phosphorylation occurs in *trans* in the absence of Tel1^{ATM}. These results suggest that extinguishing the DNA damage checkpoint depends on a delayed autophosphorylation of Mec1.

Checkpoint adaptation also parallels the damage-dependent degradation of Ddc2^{ATRIP}. Mec1 phosphorylation of three SQ/TQ sites in Ddc2 facilitate phosphorylation by another kinase. We identified two additional sites, S173 and S182, whose phosphorylations are required for adaptation.

Both Mec1^{ATR} and Tel1^{ATM} phosphorylate histone H2A (termed γ-H2AX) over 100-kb region around a site-specific DSB. In G1-arrested cells, where 5' to 3' resection of DSB ends is blocked, only Tel1 is active. By deleting yKu70, we restored Exo1-dependent resection in G1-arrested cells and then used chromatin immunoprecipitation to analyze the kinetics and extent of γ-H2AX of each kinase, separately. The patterns of modification of Mec1 and Tel1 are distinct, suggesting that different mechanisms are used to reach H2A histones around the break. We considered four modes of phosphorylation propagation: (a) 3D diffusion of a kinase initially bound to the break, (b) looping of chromatin to bring a kinase tethered to the break to distant sites along the chromatin, (c) directed sliding of a kinase along the chromatin, and (d) 1D diffusion of a kinase along the chromatin. Using Bayesian model selection, we determined that the activity of Mec1 is best described by looping while Tel1 is best described by directed sliding.