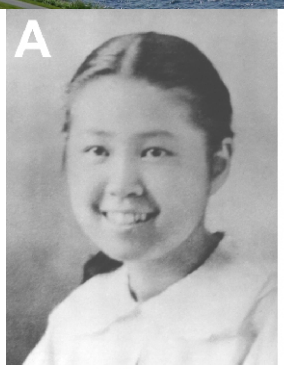
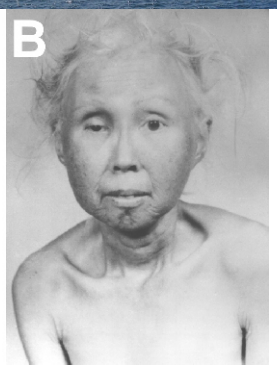
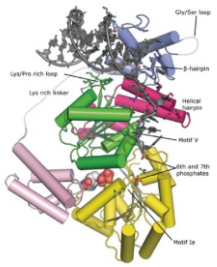
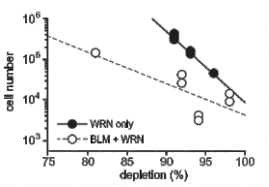
RECQ2016 - Partnering for Progress

3rd International Meeting
'RECQ Helicases in Biology and Medicine'









28 - 30 May 2016
Fred Hutchinson Cancer Research Center
Seattle, WA

Meeting Schedule

'RECQ2016 – Partnering for Progress'

3rd International Meeting on RECQ Helicases in Biology and Medicine

Saturday 28 May – Monday 30 May 2016, Fred Hutchinson Cancer Research Center Seattle, WA

Day 1: Saturday, May 28

All Attendees:

8:00 **FHCRC Pelton Auditorium: Registration opens/***Childcare room open until 6:00PM each day*

8:45 **Welcome/Introduction to *RECQ2016* + schedule/goals** – Ray Monnat

8:50 **Keynote 1: George Martin, UW** – ***'RECQ helicases: Their relevance for human disease research and the promise of translational science'*** (Intro: Ray Monnat).

9:30 **'RECQ101' - An Introduction to the RECQ syndromes, genes and proteins**
Nathan Ellis and Deborah Croteau - general intro to meeting topics and themes

10:25 **Coffee break — *all coffee breaks will be shared by all attendees***

10:40 **'An introduction to individuals and organizations working on behalf of RECQ syndromes'**

The Bloom's Syndrome Community*: *a Narrated History – Richard Gladstein
Bloom's Syndrome Foundation (Richard Gladstein)
Bloom's Syndrome Association (Paul Zaslaw)
Bloom's Connect (Sheryl Grossman)
Bloom Syndrome Foundation – Europe (Henri van den Hurk)

The Rothmund-Thomson Syndrome Foundation - John Kimmel/Lisa Wang

Werner Syndrome support networks - Fuki Hisama/Koutaro Yokote/Junko Oshima

11:30 **Poster Flash!** *All attendees* - 1 slide poster introductions by all poster presenters
Moderator: Vilhelm Bohr

12:00 **Group Photo/lunch** - **Pick up lunch to eat inside or outside if weather is good/Posters up**

CONCURRENT AFTERNOON SESSIONS

Scientific Meeting*: *Pelton Auditorium
Session Chair - Alessandro Vindigni

1:15 Ken Kitano - Structural mechanisms of human RecQ helicases WRN and BLM

1:35 Stephen Kowalczykowski – Visualizing RECQ activity to understand functions

1:55 Julia Sidorova - Class I histone deacetylases and WRN RECQ helicase: a collaboration that promotes resistance to replication stress

Family Meeting*: *Sze Conference Room

1:15 ***Family Sharing and Caring - Introduction***
John Kimmel/Lisa Wang

1:30 **Bloom's/RTS/Werner Syndromes in Depth**
Chris Cunniff/Lisa Wang/Fuki Hisama/ Koutaro Yokote/Junko Oshima
- diagnosis: features and findings
- molecular confirmation

***Scientific Meeting*: Pelton Auditorium**		***Family Meeting – continued*: Sze Conf Room**
2:15	Vincenzo Costanzo - A cell-free system to study replication, chromatin assembly and checkpoint response of repetitive DNA sequences.	**Bloom's/RTS/Werner Syndromes in Depth - continued** ▪ natural history/disease course/potential complications ▪ syndrome-specific similarities/differences
2:35	Alessandro Vindigni – RecQ helicase-dependent mechanisms of reversed fork processing and restart	
2:55	Aswin Mangerich – Functional interactions of WRN with PARP1 and poly(ADP-ribose)	
3:15	Weidong Wang – Bloom syndrome complex and Fanconi anemia core complex work together to protect genome stability	
3:35	**Coffee break/refreshments (All attendees)**	
4:05	Nathan Ellis – The SUMO-targeted ubiquitin ligase RNF4 regulates BLM helicase function in dormant origin firing	**4:05** ***Family Sharing and Caring sessions*** Patient, family, caregiver and foundation/ support group presentations followed by discussion, question and answer period.
4:25	Kristina Schmidt - Functional defects of variants of the Bloom's syndrome helicase BLM	
4:40	Junko Oshima - New progeroid syndromes - different diseases with common mechanisms?	
5:05	**Science Priorities Discussion** *Moderator:* Vilhelm Bohr	
6:00 on	**Poster Session/Hosted Reception with light buffet dinner/drinks (all attendees) Thomas-Weintraub atrium and cafe**	

Day 2: Sunday, May 29		
***All Attendees:* Pelton Auditorium**		
9:00 am	**Keynote 2: Jan Karlseder, Salk Institute** – ***'A telocentric view of RECQ helicase biology'*** (Intro: Vilhelm Bohr)	
CONCURRENT MORNING SESSIONS		
Scientific Meeting*: Pelton Auditorium** ***Session Chair - Deborah Croteau		***Clinical Focus presentations*: Sze Conf Room**
9:40	Patricia Opresko – Investigating Roles for RecQ Helicases in Telomere Replication	**9:40** ***Skin Manifestations of RECQ syndromes*** Dr. Moise L. Levy, Physician-in-Chief Pediatric/Adolescent Dermatology Dell Children's Medical Center, Austin, TX (talk followed by Q&A)
10:00	Joanna Groden – Coordinated regulation of a tri-serine cluster in the topoisomerase interaction domain of BLM controls chromosome breakage in human cells.	
10:20	Pavel Janscak - RECQ5 DNA helicase promotes MUS81-mediated resolution of late replication intermediates in mitosis	

10:40	**Coffee break/refreshments (All attendees)**	
Scientific Meeting*: Pelton Auditorium** ***Session Chair - Deborah Croteau		***Family Meeting*: Sze Conference Room**
11:00	F. Brad Johnson – Preclinical models indicate that augmenting Wnt pathway signaling can rescue telomere dysfunction	**11:00** ***Coping with Chronic Illness*** Dr. Emil Zakutny, RTSF Board Member, Licensed Marriage and Family Therapist Clinical Social Worker, Baldwin, NY (talk followed by Q&A)
11:20	Vilhelm Bohr – Roles for WRN protein in DNA repair and its regulation in breast cancer	
11:40	Henry Olson - RECQ5 promotes genomic stability by preventing RAD51-dependent homologous recombination	
12:00	**All attendees - Pick up lunch to eat inside or outside if weather is good/Posters viewing**	
CONCURRENT AFTERNOON SESSIONS		
Scientific Meeting*: Pelton Auditorium** ***Session Chair - Vilhelm Bohr		***Family Meeting*: Sze Conference Room**
1:30	Yilun Lu - Aging and Cancer: A molecular lesson from the human RECQ4 DNA helicase	**1:30** ***Clinical Focus topics-all patients/families:*** *Moderator:* Wang *Discussants:* Cunniff/Hisama/Kimmel/ Zaslaw/Gladstein/Grossman + addtional ▪ Registries as enablers of care and research ▪ Developing clinical care/screening guidelines ▪ Finding information and answers you need ▪ Additional topics from the floor ▪ Q&A ▪ Prepare 'Wish List' for Panel Discussion at 4:00
1:50	Carl Walkley – Understanding the role of Recql4 in normal and malignant disease: Insights from blood and bone development	
2:10	Sagar Sengupta - Role of RECQL4 in the maintenance of mitochondrial replication and genome integrity	
2:30	Deborah Croteau – RECQL4 in double strand break repair and senescence	
2:50	Corry Weemaes/Michiel Schoenaker - Immune system function in Bloom's syndrome	
3:10	Ray Monnat - BLM and WRN target G4 DNA to modulate transcription: clues to disease pathogenesis and therapy	
3:30	**Coffee break/refreshments (All attendees)**	
4:00	**All Attendees Panel Discussion: *RECQ helicase clinical care/research priorities*** *Moderator: Lisa Wang*	
5:00	***RECQ2016 Business Meeting***	**5:00** ***Patient/family free time***
6:00	**Reception/Banquet** (all registered attendees - ***meeting ID badge is required!***) **Burke Museum of Natural History and Culture, UW campus –** bus transportation will be provided leaving from and returning to the meeting site.	

Day 3: Monday, May 30

***All Attendees*: Pelton Auditorium**

9:00 **Keynote 3: Debbie Nickerson, UW** - ***'Next Generation Human Genetics and Genomics'***
(Intro: Ray Monnat)

CONCURRENT MORNING SESSIONS

Scientific Meeting:
Session Chair - Nathan Ellis

9:40 Giang Hong Nguyen - 'Small Molecules as Modulators of Bloom's Syndrome Biology'

10:00 Bob Brosh - RECQ1 Structure-Function Studies and WRN Helicase Small Molecule Inhibitors

10:20 Michel Lebel – Vitamin C treatment of Werner syndrome mouse models

Family/Foundation Meeting: Hutch B-suite/Sze Conference Room

9:40 ***Foundation/Support group meetings***

'Raising a Child with Bloom's syndrome'
'Living as an Adult with Bloom's syndrome' (Sze)
Hosts:
Leah Counts-Goldy for Bloom's Connect
Paul Zaslaw/Bloom's Syndrome Association

RTS Foundation meeting (B-suite)
Host: John Kimmel

10:40 **Coffee break/refreshments (All attendees)**

11:00 **All Attendees Panel Discussion: Clinical trials, translational priorities and RECQ futures**
Moderator: Ray Monnat

12:00 **Concluding remarks/Meeting end**

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RECQ2016

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Talk Abstracts

(in order of presentation)

Keynote Address 1: RECQ Helicases: Their relevance for human disease research and the promise of translational science.

George M. Martin, MD[1]

[1]Departments of Pathology and Genome Sciences, University of Washington

RECQ2016 is unique in bringing together, for the first time, basic scientists and clinicians with patients and families who are looking for answers to their questions about the nature of RECQ helicases and the three rare genetic disorders that result from autosomal recessive mutations at RECQ loci: Bloom Syndrome (mutations in the BLM or RECQL3/RECQ2 gene on chromosome 15q26.1), Rothmund-Thomson syndrome (mutations in the RECQL4/RECQ4 gene on chromosome 8q24.3) and Werner syndrome (mutations in the WRN or RECQL2 gene on chromosome 8p12).

The human RECQ helicases represent a relatively small sub-class of a much larger number of helicase enzymes that are vital partners of the DNA of all organisms. They have been described as functioning to “unpackage an organism's genes” (https://en.wikipedia.org/wiki/Helicase). The RECQ helicase proteins are characterized by a highly conserved sequence of amino acids known as the helicase domain, which is essential for ATP binding and hydrolysis to provide the energy needed to unwind double-stranded DNA, and in some instances DNA/RNA hybrids or RNA structures. Several human RECQ helicase proteins also contain other functional domains. The human RECQ helicases have been described as 'guardians of the genome' in light of their crucial roles in genome replication, repair and gene expression.

An important goal of our meeting is to provide patients, families and scientists with up-to-date information on the roles RECQ proteins play, how loss of function leads to disease, and what new therapies or disease preventions may be on the horizon. Since a large part of my own career has been devoted to the study of Werner syndrome, I will focus on this prototypic "segmental progeroid" or premature aging RECQ helicase syndrome to discuss these issues (GM Martin, Cell 120:523, 2005). Other speakers will address many of the same topics including new translational research for patients with Bloom and Rothmund-Thomson syndromes.

Finally, I hope to initiate discussions over the next three days on several important but neglected issues. The first is the need for research on potential environmental influences that may increase the vulnerability of heterozygotic carriers of RECQ disease mutations. One example in the case of Werner syndrome is occupational or therapeutic exposure to genotoxic agents (CE Ogburn et al., Human Genetics 101:121, 1997). This issue may also be important in Bloom and Rothmund-Thomson syndrome mutation carriers. The second issue is the need to identify genes or genetic variants with the potential to enhance or suppress the effect of disease-associated RECQ mutations (I Nielson et al. PLoS One Nov 20;8(11):e81015, 2013). A third issue is whether there are human “antigeroid syndromes” or conditions due to rare genetic variants that exhibit remarkably high degrees of physiological functioning (F Hisama, J Oshima and GM Martin, in Aging: The Longevity Dividend, Ed. by SJ Olshansky, GM Martin & JL Kirkland, Cold Spring Harbor Press, 2016). Could such alleles exist in RECQ or other genes? And if identified, how might we make practical use of this knowledge?

Structural mechanisms of human RecQ helicases WRN and BLM

Ken Kitano[1]

[1]Graduate School of Biological Sciences, Nara Institute of Science & Technology

The RecQ family DNA helicases Werner syndrome protein (WRN) and Bloom syndrome protein (BLM) play a key role in protecting the genome against deleterious changes. In humans, mutations in these proteins lead to rare genetic diseases associated with cancer predisposition and accelerated aging.

The RecQ helicases are distinguished from other helicases by possessing a signature domain referred to as the RecQ C-terminal (RQC) domain. Recently the 3D structures of the WRN and BLM RQC domains were determined, visualizing the unique functions of the RQC domain for recognizing, binding and unwinding DNA at branch points. A prominent hairpin structure (the β-wing) within the RQC winged-helix motif acts as a scalpel to induce the unpairing of a Watson-Crick base pair at the DNA duplex terminus. The RQC domain, therefore, functions as a novel "DNA zip-slider" domain to catalyze the direct strand separation of DNA duplexes.

References

[1] Kitano, K. (2014). Front. Genet., 5, 366.
[2] Kim, SY., Hakoshima, T., Kitano, K. (2013). Sci. Rep., 3, 3294.
[3] Kitano, K. et al., (2010). Structure, 18(2), 177.
[4] Sato, A. et al., (2010). J. Biochem., 148(4), 517.
[5] Kitano, K. et al., (2007). J. Biol. Chem., 282(4), 2717.

Visualizing RecQ helicases to understand functions

Stephen Kowalczykowski[1]

[1] Department of Microbiology & Molecular Genetics, University of California, Davis, CA

RecQ family of helicases is conserved in seemingly all organisms. In human, mutations in RecQ family helicases result in several genetic diseases: mutation of BLM results in Bloom syndrome, WRN in Werner syndrome, and RECQ4 in Rothmund-Thomson syndrome. Budding yeast has a single RecQ homologue, Sgs1, and bacteria possess the founding member of this family, the eponymous RecQ. The RecQ helicases are involved in both the first and last steps of homologous recombination (HR). In the initiation phase, BLM and WRN in human and Sgs1 in yeast, together with the DNA2 helicase/nuclease and RPA, catalyze resection of duplex DNA at a DNA break. At the end of homologous recombination, both BLM and Sgs1, together with Topoisomerase IIIα, RMI1, RMI2 and RPA proteins in human, or Top3, Rmi1, and RPA in yeast, promote a unique reaction that separates chromosomes linked by two Holliday junctions (HJs), without cutting them. This biological process is termed “dissolution”, and it unlinks two homologous DNA molecules that are topologically (but not covalently) linked through the HJs.

Dissolution is an elegant solution to separating linked chromosomes without cutting the DNA and without the resulting exchange of DNA segments that leads to crossing over and the subsequent deleterious biological consequences, such as chromosomal rearrangements or loss of heterozygosity. Loss of BLM function is typified by an increased frequency of sister chromatid exchanges and genome rearrangements. Dissolution requires a type IA topoisomerase, topoisomerase III, for the topological passage of parental strands located between two HJs and a motor protein, typically a homolog of BLM, for migrating the HJs. I will discuss the role of RecQ helicases in both the initiation and dissolution phases of chromosome maintenance by homologous recombination, and our progress on imaging the functions of RecQ helicases.

Class I histone deacetylases and WRN RECQ helicase: a collaboration that promotes resistance to replication stress

Keffy Kehrli[1], Michael Phelps[1], Pavlo Lazarchuk[1],Eleanor Chen[1], Ray Monnat, Jr. [1,2] and Julia M. Sidorova[1]

[1]Department of Pathology, University of Washington, Seattle, WA, 98195, USA
Department of Genome Sciences, University of Washington, Seattle, WA, 98195, USA

The human WRN RECQ helicase/exonuclease is mutated in Werner syndrome, a premature aging and cancer susceptibility disorder. We have previously demonstrated that WRN contributes to cellular resistance to replication stress, i.e. a condition of stalling, slowing or breakdown of replication forks due to exogenous or endogenous insults. In particular, WRN promotes replication fork reactivation after stalling due to hydroxyurea-induced nucleotide depletion. We now showed that this role of WRN at HU-stalled forks involves recruitment or retention of RAD51, a DNA strand exchange protein known for its role in homologous recombination. Interestingly, survival of WRN-deficient human fibroblasts after hydroxyurea-induced replication stress remains relatively high. This enabled us to perform a siRNA screen for genes required for survival of WRN-deficient but not wild type cells after hydroxyurea. We will describe the results of this screen and its follow-up studies.

In particular, we identified HDAC1 and HDAC2, members of the class I histone deacetylase family as two of the genes that were synthetically growth-suppressive with WRN deficiency after hydroxyurea challenge. Class I HDACs regulate DNA transcription, and have been also implicated in DNA repair and replication, though these roles are less well characterized. One function of HDAC1 and a closely related HDAC2 is deacetylation of new histones deposited onto DNA. HDAC1/2 act on nascent chromatin to remove acetyl groups from lysines 5 and 12 of the newly recruited histone H4. Using our DNA fiber assay, microfluidic-assisted Replication Track Analysis (maRTA), we showed that HDAC1 becomes important for recovery of replication forks after hydroxyurea in the absence of WRN, thus uncovering a previously unidentified function of HDAC1 in mitigating replication stress, and providing a novel connection between WRN and epigenetic modifiers. Interestingly, this role may not involve acetylation of histone H4 on lysines 5 and 12 by HDAC1. We hypothesize that HDAC1 may facilitate replication fork activity by promoting recruitment of critical non-histone proteins, and that WRN deficiency increases cell's dependence on this pathway. Our data suggest that both WRN and HDAC1 and 2 are independently recruited to forks and remain on newly replicated DNA after fork passage. A fraction of nuclear WRN can also be found associated with HDAC1 and HDAC2. We will discuss these and related data in the context of epigenetic mechanisms in stressed replication and cellular aging.

A cell-free system to study replication, chromatin assembly and checkpoint response of repetitive DNA sequences

Vincenzo Costanzo [1]

[1] IFOM – FIRC Institute of Molecular Oncology, Milan, Italy

RecQ helicases play essential roles in maintaining genome stability during replication. Different RecQ helicases found in higher eukaryotes preferentially bind substrates such as Holliday junctions, G-quadruplexes, DNA hairpins and stalled replication forks. These structures arise in the presence of damaged DNA. However, they can form spontaneously on chromosome loci containing tracts of repeated DNA sequences. Half of human genome is made of highly repeated DNA, which can frequently give rise to such structures. Mechanisms underlying replication of chromosome regions containing repetitive DNA replication are poorly understood. To study replication of defined chromosome loci containing repeated DNA we reconstituted replication of defined human chromosome segments using bacterial artificial chromosomes (BACs) in Xenopus laevis egg extract. Using this approach we characterized chromatin assembly and replication dynamics of highly repetitive alpha-satellite DNA present on centromeric DNA.

Proteomic analysis of centromeric chromatin revealed replication dependent enrichment of a network of DNA repair factors among which RecQ helicase proteins, replication pausing proteins Tipin-Tim and Msh2-6 complexes. We showed that some of these repair proteins were required for efficient centromeric DNA replication. However, contrary to expectations, the ATR dependent checkpoint monitoring DNA replication fork arrest in the presence of secondary DNA structures could not be activated on repetitive DNA due to inability of single strand DNA (ssDNA) binding protein RPA to accumulate on chromatin. Electron microscopy analysis of DNA and supercoil mapping revealed Topoisomerase I dependent DNA loops embedded in a protein matrix enriched for Smc2-4 proteins. This arrangement suppressed ATR signaling by preventing RPA hyper-loading. Suppression of spontaneous activation of the ATR dependent checkpoint facilitated replication of repetitive DNA. These findings have important implications on our understanding of repetitive DNA metabolism and under normal and stressful conditions. The availability of this experimental system will allow in depth characterization of the function of specialized enzymes such as RecQ helicases in the replication of repetitive DNA regions.

RecQ helicase-dependent mechanisms of reversed fork processing and restart

Saravanabhavan Thangavel[1], Matteo Berti[1], Denisse Carvajal[1], Chris Haddock[1], Jessica Jackson[1], Delphine Lemacon[1], Annabel Quinet[1], Massimo Lopes[2], and Alessandro Vindigni[1]

[1]Edward A. Doisy Department of Biochemistry and Molecular Biology, Saint Louis University, Saint Louis MO; [2] Institute of Molecular Cancer Research, University of Zurich, Switzerland.

Replication fork reversal is rapidly emerging as a pivotal mechanism of replication stress response to cancer chemotherapeutics. Although conceptually attractive, this mechanism implies a significant remodeling of stalled replication forks into four-way junction structures and the molecular determinants required fork reversed fork processing and restart are just beginning to be elucidated. We have identified the first two molecular mechanisms of reversed fork restart, which rely on the ATPase activity of two different human RecQ helicases. One depends on RECQ1 (alias RecQL), while the other requires the WRN ATPase and DNA2 nuclease activity. In the latter, the human DNA2 nuclease and WRN ATPase activities functionally interact to degrade the reversed arm of replication forks that have undergone fork reversal upon replication blockage. Of note, we find that reversed replication forks accumulate in RECQ1- and WRN-depleted cells also in unperturbed conditions suggesting that fork reversal is remarkably frequent when DNA replication faces intrinsic replication fork obstacles, and that RECQ1 and WRN have a conserved role in restarting reversed forks in unperturbed S-phase. Given the consolidated role of WRN at difficult-to-replicate regions (e.g, telomeres and fragile sites), we speculate that WRN is required to process reversed fork arising spontaneously at these genomic loci.

Functional interactions of WRN with PARP1 and poly(ADP-ribose)

Sebastian Veith[1], Andrea Schink[1],Matthias Mack[1], Alexander Bürkle[1], and Aswin Mangerich[1]

[1]University of Konstanz, Molecular Toxicology Group, Department of Biology, 78457 Konstanz, Germany

Poly(ADP-ribose) polymerase 1 (PARP1) and the RecQ helicase Werner syndrome protein (WRN) are important caretakers of the genome. They physically interact with each other and are both localized in the nucleus and in particular in the nucleoli. Both participate in various overlapping mechanisms of DNA metabolism, in particular genotoxic stress response and DNA repair [1]. Previously, we and others have shown in biochemical studies that enzymatic functions of WRN are regulated by PARP1 as well as by non-covalent poly(ADP-ribose)-WRN interaction [2-4]. Furthermore, pharmacological PARP inhibition as well as a genetic PARP1 ablation in HeLa cells alters the recruitment kinetics of WRN to sites of laser-induced DNA damage [5].

Here we report a novel role for PARP1 and poly(ADP-ribosyl)ation in the regulation of WRN's subnuclear spatial distribution upon induction of oxidative stress. We could verify previous reports that WRN is transiently released from nucleoli upon induction of oxidative stress, camptothecin (CPT) treatment, and laser-induced DNA damage in a time-dependent manner. While, CPT-induced translocation appears to be a PARP-independent process, our results reveal that upon H2O2-induced oxidative stress, PARP1 is essential for the translocation of WRN from the nucleoli to the nucleoplasm. PARP1 activity only partially contributes to WRN release from nucleoli, underlining the importance of a direct WRN-PARP1 interaction for subnuclear WRN redistribution. Furthermore, we identified a novel PAR-binding motif within the WRN sequence that is located in its RQC domain, which also harbors the binding site for PARP1 and is necessary for WRN's nucleolar localization under non-stress conditions. Currently, we are testing corresponding WRN mutants to analyze if this region is responsible for the PARP1-dependent release of WRN from nucleoli to sites of DNA damage. In conclusion, we provide novel insight into the role of PARP1 in WRN's
spatio-temporal regulation in the nucleus during the oxidative stress response.

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Bloom syndrome complex and Fanconi anemia core complex work together to protect genome stability

Chen Ling[1], Jing Huang[2], Yutong Xue[1], Dongyi Xu[1], Zhijiang Yan[1], Amom Ruhikanta Meetei[1], Rong Guo[1], Jinhu Yin[1], Masamichi Ishiai[3], Minoru Takata[3], Michael Seidman[2], and Weidong Wang[1]

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Bloom syndrome is caused by mutation in BLM, a member of the RecQ helicase family conserved from E.coli to human. We have previously reported isolation of three multiprotein complexes containing BLM from human HeLa cells and identification of their components (Meetei et al. MCB, 2003). These components include Topoisomerase 3a (Top3a), replication protein A, MLH1, RMI1 (BLAP75), RMI2 (BLAP18), and Rif1 (BLAP250) (Yin et al. EMBO J. 2005; Xu et al. G&D., 2008; EMBO J. 2010). Among them, BLM, Top3a, RMI1 and RMI2 form a complex, termed dissolvasome, which catalyzes dissolution of double-Holliday Junctions in vitro, and is essential to suppress crossover recombination in vivo (Xu et al. G&D, 2008). Conversely, Rif1 provides a new DNA binding interface for BLM, and is required for BLM to promote recovery of stalled replication forks. Interestingly, one of the BLM complexes, termed BRAFT (for BLM, RPA, FA, and Topoisomerase IIIa), contains components of the Fanconi anemia core complex. Fanconi anemia proteins, including the core complex, work in a DNA repair network to repair or bypass DNA interstrand crosslinks, a type of DNA damage that blocks DNA replication. Fanconi anemia resembles Bloom syndrome in genomic instability and cancer predisposition. Fanconi anemia has recently become an attractive model to study breast cancer susceptibility (BRCA) genes, as four FA genes, FANCS, FANCD1, FANCN, and FANCJ, are found to be identical to BRCA genes BRCA1, BRCA2, PALB2, and BRIP1.

BLM complex has been shown to specifically interact with FANCM of the Fanconi anemia core complex; and this interaction is required for targeting BLM to stalled replication forks, and for suppression of sister chromatid exchange (Deans and West, Mol. Cell, 2009), a hallmark feature of cells derived from Bloom syndrome patients. Structural analyses have revealed that the interaction between BLM complex and FANCM is mediated by RMI1 and RMI2. We identified specific mutations in RMI2 that disrupt this interaction (Hoadley et al. PNAS, 2012). Notably, cells expressing these RMI2 mutants display higher frequency of sister-chromatid exchange, and a defective Fanconi anemia DNA damage response pathway to DNA interstrand crosslinks. Our data suggest that the interaction between BLM complex and Fanconi anemia complex is required for cells to maintain genome stability in response to DNA damage.

The SUMO-targeted ubiquitin ligase RNF4 regulates BLM helicase function in dormant origin firing

Nathan A. Ellis[1], Wei-Chih Yang[2], Mary Yagle[1], Jianmei Zhu[2], Jing Huang[3], Alex Kwako[1], Michael Seidman[3], Michael J. Matunis[2]

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Background: Regulation of dormant origin firing under conditions of replication stress is poorly understood. The Bloom's syndrome DNA helicase BLM functions in maintaining replication fork stability, and BLM deficiency is associated with increased dormant origin firing. Because BLM sumoylation regulates BLM's function in ensuring homologous recombination (HR) repair at stalled forks, we hypothesized that ubiquitylation of sumoylated BLM by RNF4 may play a role in recovery from replication stress.

Results: To determine whether BLM is a substrate for RNF4, we used the his-tagged SUMO pull-down assay to measure the amount of SUMO-BLM under various conditions. We found that the levels of BLM sumoylation increased in response to replication stalling by hydroxyurea (HU) and to proteasome inhibition. Depletion of the RNF4 or SENP6 caused increased levels of BLM sumoylation. RNF4 directly interacts with BLM and can ubiquitylate sumoylated BLM in vitro. These data indicated that sumoylated BLM is an RNF4 substrate.

To determine whether RNF4 regulates BLM's function in HR repair at stalled forks, we evaluated HR protein accumulations in HU-treated and untreated cells and measured the frequency of sister chromatid exchanges (SCEs). Contrary to observations in irradiated cells, the numbers of RPA, RAD51, and gamma-H2AX foci in RNF4-depleted cells was similar to the numbers in control-depleted cells. Consistent with the RAD51 immunofluorescence results, the numbers of HU-induced and un-induced SCEs were similar in RNF4-depleted cells compared to control-depleted cells. On the other hand, although the numbers of BLM foci were similar in RNF4-depleted cells compared to control-depleted cells, excess focal BLM protein was detected by immunofluorescence. These data indicated that RNF4 regulates BLM retention at stalled forks without affecting HR repair. Consistent with a role in the replication stress response, RNF4 depletion caused an increase in collapsed replication forks and a decrease in activation of dormant origins following recovery from HU-induced replication stress, as determined by the DNA fiber assay. Co-depletion of RNF4 and BLM partially rescued these replication defects.

Conclusion: We conclude that sumoylated BLM blocks activation of dormant origins during replication arrest and RNF4 acts to remove sumoylated BLM from stalled replication forks to permit dormant origin firing. Our data suggest a pathway in which BLM sumoylation at stalled replication forks facilitates HR repair during which dormant origin firing is blocked. In the event of fork collapse, however, RNF4-mediated ubiquitylation leads to a proteasome-dependent degradation of BLM that relieves BLM's active inhibition of dormant origin firing.

Functional defects of variants of the Bloom's syndrome helicase BLM

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The Bloom's syndrome gene BLM encodes a 3'-5' DNA helicase that plays an important role in mediating DNA double-strand break repair by homologous recombination with noncrossover outcomes, and has been implicated in numerous other, less well-understood DNA metabolic processes. Amino acid substitutions at 11 residues in the helicase core of BLM are known to cause Bloom syndrome, and their biochemical characterization has provided valuable insight into BLM helicase function. Multiple genome sequencing projects have identified around 100 variants of uncertain significance (VUS) that cause amino acid changes throughout BLM. Using a novel yeast model system for screening BLM VUS, we have identified 8 new mutations in the human population that impair BLM function. Characterization of the cellular defects in human cell lines stably expressing these new alleles revealed that 5 are null alleles, and candidates for new Bloom's syndrome causing mutations, whereas 3 define a new class of hypomorphic

BLM alleles, which significantly impair BLM function in vivo, exhibiting increased levels of sister-chromatid exchanges and delays in the response to and repair of DNA damage, but do not cause Bloom's syndrome based on their allele frequency. We propose that these BLM alleles may be novel risk factors for developing cancer or other symptoms associated with Bloom's syndrome in otherwise healthy individuals. The N-terminal half of BLM extends from the helicase domain as a long disordered tail and its function as a site for interaction with a multitude of proteins and for posttranslational modification is only beginning to be understood. Using structural prediction algorithms and phylome analysis, we have identified putative functional motifs in the N-terminal tail and initiated the functional analysis of rationally designed point mutations and 17 BLM variants from the human population that are predicted to disrupt these motifs while leaving the other BLM functions intact. Finally, we present the construction and characterization of a cell line with a biallelic disruption of BLM just upstream of the helicase core using CRISPR/Cas9 mediated genome engineering.

New progeroid syndromes: different diseases with common mechanisms?

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The International Registry of Werner Syndrome (University of Washington, Seattle, WA; www.wernersyndrome.org) was established in 1988 with the original purpose of collecting classical Werner syndrome (WS) cases for positional cloning of the WRN gene (Science 1996; 272:258). Patients are referred from all over the world for the molecular diagnosis of Werner syndrome. Those who do not carry WRN mutations were operationally categorized as “atypical Werner syndrome” (AWS). Our Registry has expanded its scope from WS to the search for causative mutations and mechanisms responsible for the broader range of progeroid syndromes. As of May 2016, the International Registry has accumulated clinical data and cryopreserved biological materials from 151 WS patients with documented WRN mutations and 81 AWS cases. A combination of next generation sequencing, SNP arrays and candidate gene sequencing have successfully identified novel mutations in a small number of cases. Those AWS loci highlight major roles in DNA damage repair and response: POLD1 (DNA polymerase delta)(Hum Mut 2015; 36:1070), SPRTN (recruitment of translesional DNA polymerase eta)(Nat Genet 2014; 24:1239); LMNA (nuclear structure and chromatin interaction)(Lancet 2003; 362-440); MDM2 (an inhibitor of p53); and SAMHD1 (regulation of dNTP pools)(Am J Med Genet 2014; 164A:2510). Cases of a BSCL2 mutation responsible for Seip syndrome as well as mosaic trisomy 8 were also identified. These findings continue to support the concept of genomic instability as a major mechanism of biological aging.

Keynote Address 2: A telocentric view of RECQ helicase biology

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Telomeres are repetitive sequences at the ends of linear chromosomes that form protein-DNA complexes and evolved to protect the natural chromosome ends. The repetitive nature of telomeres, which consist of many kilobases of TTAGGG repeats, renders them sensitive to replication stress, G-quadruplex formation and recombination. All these events are controlled at least in part by members of the RecQ helicase family.

Werner Syndrome is a premature aging disease, where patients suffer from many symptoms of old age early in life. The syndrome is characterized by accumulative genome instability and patients are cancer prone. We found that the WRN helicase is required for replication of the telomeric G strand. The lack of WRN leads to fork stalling and telomere loss, which is responsible for the genome instability in the syndrome. Similarly, Bloom Syndrome patients are predisposed to cancer, but the BLM helicase plays an independent role at telomeres, likely by resolving late replicating intermediates, which are visible as BLM covered ultra-fine DNA bridges in anaphase.

BLM also regulates the alternative lengthening of telomeres pathway at telomeres (ALT), which allows telomere length maintenance independently of telomerase. Replication stress at telomeres, caused by the suppression of histone chaperones, led to induction of all characteristics of ALT, such as RPA recruitment, PML body formation, telomere association and recombination between short and long telomeres. Suppression of BLM counteracted these activities, suggesting a central role for BLM in the suppression of recombination of telomeres in primary cells.

Telomere protection is essential for the maintenance of genome instability. We discovered that mitotic arrest caused telomere deprotection, prompting us to hypothesize a novel mitosis duration checkpoint, where cells that cannot exit mitosis in time are removed from the population by inducing telomere deprotection and thereby a DNA damage response. The checkpoint prevents segregation failure and the associated genome instability, and is in part regulated by WRN and BLM.

Here we will discuss the roles of RecQ helicases in telomere replication, recombination and protection.

Investigating Roles for RecQ Helicases in Telomere Replication

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Telomeres are nucleoprotein structures that cap and protect chromosomes ends. Telomere dysfunction triggers cellular senescence which contributes to aging-related diseases, and chromosomal instability in premalignant cells which drives carcinogene-sis. In humans the telomeres consist of about 10 kb of tandem TTAGGG repeats and are coated by a complex of proteins termed shelterin. RecQ helicases WRN and BLM are proposed to function in dissociating alternate DNA structures during DNA replication and repair at telomeric ends. Failures in telomere replication induce telomere fragility and telomere loss, which are elevated in cells from Werner syndrome and Bloom syndrome patients.

We showed previously that WRN helicase prevents both spontaneous and genotoxic-induced telomeric DNA deletions and loss. Telomeres are highly susceptible to oxidative DNA damage and oxidative stress accelerates telomere shortening, although the precise mechanism is unknown. We are investigating roles for WRN in preserving replicating telomeres in the face of oxidative DNA damage. For this we developed a system to selectively induce singlet oxygen at telomeres, which primarily forms 8-oxoguanine when it reacts with DNA. Our approach uses fluorogen-activating peptides (FAPs) with high affinity for the fluorogen malachite green (MG), which only fluoresces when tightly bound to the FAP. We expressed FAP fused to telomere binding protein TRF1, and show the FAP-TRF1 fusion protein localizes exclusively to telomeres. The di-iodinated MG-dye derivative (MG2I) produces singlet oxygen upon FAP binding and excitation with far red light, making this a powerful system to selectively induce oxidative DNA damage at telomeres. We demonstrate cells expressing FAP-TRF1 exhibit rapid mobilization of XRCC1 DNA repair specifically to telomeres only upon addition of MG2I dye and excitation with red light. We are using this system to examine whether WRN is recruited to damaged telomeres, and whether WRN functions in preserving telomeres following oxidative damage.

Coordinated regulation of a tri-serine cluster in the topoisomerase interaction domain of BLM controls chromosome breakage in human cells.

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Bloom's syndrome (BS), an autosomal recessive human disorder caused by loss of function of the recQ-like helicase BLM, is characterized by proportional dwarfism and tumor susceptibility. Chromosomal instability in cells without BLM manifests in aberrant metaphases with quadriradial chromosome configurations, and multiple chromosome breaks and fragments, and an increased incidence of micronuclei in somatic cells. There is an increased frequency of inter- and intra-chromosomal recombination, rDNA instability and telomeric associations. The BLM helicase functions in the resolution of unusual DNA structures associated with replication stress and recombination, and the G4 quadruplex structures predicted to occur in G-rich DNA. BLM interacts in vitro and in vivo with several DNA repair factors including topoisomerases I, IIα and III that are part of the recombination, replication and transcription machineries and are critical for proper chromosomal segregation and repair of DNA damage.

Our studies show that the helicase activity of BLM and physical interaction of BLM and topoisomerase IIα (TOP2A) are key components of the pathway preventing chromosome breakage. BLM-TOP2A interaction in vitro results in reciprocal stimulation of their respective biochemical activities in resolving aberrant structures and relieving topological constraints in DNA. We have identified a novel tri-serine phosphosite cluster within the TOPO2A-interaction region of BLM critical for preventing chromosome breakage. Mutation of all three serines to alanines increases DNA damage, anaphase ultra-fine bridges (UFBs) and micronuclei formation, and disrupts co-localization of BLM and TOPO2A in vivo. These effects are reversed by mutation of these serines to phosphomimetic, aspartic acids. Our findings suggest a mechanism whereby phosphorylation regulates BLM/TOP2A interaction and the resolution of UFBs to prevent chromosome breakage and genomic instability.

RECQ5 DNA helicase promotes MUS81-mediated resolution of late replication intermediates in mitosis

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RECQ5 is unique amongst the RecQ DNA helicases for its ability to dismantle RAD51 nucleoprotein filaments that mediate homology search and strand invasion during homologous recombination and stabilize stalled replication forks. Our recent studies have provided several lines of evidence suggesting that RECQ5 cooperates with the MUS81-EME1 endonuclease in the processing of late replication intermediates at common fragile sites (CFSs) during early mitosis to facilitate faithful chromosome segregation. We have found that RECQ5 binds directly to MUS81 and stimulates cleavage of forked DNA structures by MUS81-EME1 in vitro. Upon entry of cells into mitosis, RECQ5 associates with CFSs in a manner dependent on MUS81 and is required for the appearance of MUS81-dependent chromatid breaks at CFSs following replication stress, a phenomenon termed "expression of CFSs". Depletion of RECQ5 leads to an increase in the frequency of chromosome bridges in anaphase and accumulation of DNA damage in the following G1 phase, a phenotype previously reported for MUS81-depleted cells. Importantly, RECQ5 depletion or mutations in its helicase or RAD51-interacting domains increased binding of RAD51 to CFSs upon replication stress. These mutations also compromised expression of CFSs and impaired chromosome segregation. Consistent with its ability to disrupt RAD51 filaments, RECQ5 was found to alleviate the inhibitory effect of RAD51 on 3'-ssDNA flap cleavage by MUS81-EME1 in vitro. Finally, we have found that expression of CFSs is dependent on phosphorylation of RECQ5 in early mitosis by CDK1. Together, our results suggest that RECQ5 disrupts RAD51 filaments formed on stalled replication forks at CFSs to facilitate their proper resolution by MUS81-EME1.

Preclinical models that indicate telomere dysfunction can be rescued via pharmacological augmentation of Wnt pathway signaling

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Telomeres can lose their ability to cap chromosome termini if critically shortened or if the proteins in the telomere-associated shelterin complex become dysfunctional. Such uncapping (e.g. secondary to partial telomerase deficiency in the rare disease dyskeratosis congenita) causes tissue pathology, but underlying mechanisms are not fully understood. Mice lacking telomerase (e.g. mTR-/- mutants, lacking the telomerase RNA template) provide a model for investigating pathogenesis. In such mice, after several generations of telomerase deficiency telomeres shorten to the point of uncapping, causing defects most pronounced in high-turnover tissues including intestinal epithelium. Intestinal mRNA and microRNA expression analyses in late- generation mTR-/- mutants revealed marked downregulation of genes in the Wnt pathway, normally involved in paracrine intercellular signaling. Wnt pathway gene expression was broadly diminished in crypt epithelia, including crypt base columnar (CBC) stem cells and Paneth cells, and in underlying stroma. The p53-dependent microRNA miR34a is involved in the downregulation because miR34a inhibition or deletion rescued Wnt pathway gene downregulation and pathology. The importance of the downregulation was revealed by rescue of crypt apoptosis and Wnt pathway gene expression upon treatment with Wnt pathway agonists, including R-spondin, lithium chloride, and CHIR99021. Remarkably, rescue was associated with reduced telomere- dysfunction induced foci (TIFs) and anaphase bridges, indicating that restoration of Wnt pathway signaling led to improved telomere capping. Rescue was associated with enhanced expression of several shelterins, including Trf2 and Pot1a, which are encoded by Wnt target genes, and may contribute to improved capping.

We next addressed whether the amelioration of pathology in telomerase-deficient mice provided by enhanced Wnt signaling might also apply to humans suffering from telomere dysfunction. We used CRISPR to edit the genomes of human iPS cell lines, derived from dermal fibroblasts, to create pairs of wild type or DKC1 mutant lines in an otherwise isogenic background. DKC1 encodes dyskerin, which is required for full telomerase activity, and DKC1 mutant cells thus display premature telomere shortening and uncapping. Differentiation of the iPS cell pairs into cultured intestinal organoids revealed defects in the DKC1 mutant organoids, including failure to maintain telomere length and capping, that could be rescued via enhanced Wnt signaling. Our findings indicate that a mutually reinforcing feedback loop exists between telomere capping and Wnt signaling, and that telomere capping can be impacted by extracellular cues and independent telomerase activity. These findings are distinct from previously suggested connections between Wnt and the catalytic component of telomerase, TERT,and moreover may provide new therapeutic approaches to diseases with underlying telomere dysfunction. Given that cells from people with Werner syndrome suffer fromtelomere dysfunction, we are now testing if pharmacological augmentation of Wnt signaling might improve telomere function in such cells.

Roles of WRN in DNA repair and its regulation in breast cancer

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We have studied the role of WRN in different DNA repair pathways and are finding that it participates in base excision repair and DNA double strand break repair. Recent work indicates that WRN may help determine the pathway choice between classical- and alternative nonhomologous end joining, and these results will be discussed.

We have examined expression patterns of RecQ helicases in a large cohort of breast cancer patients in the UK. RECQL4 and WRN expression correlated to the progression and the severity of the tumors. WRN is specifically degraded after camptothecin treatment in sensitive cells and this may be a biomarker for treatment options in breast cancer.

RECQ5 promotes genomic stability by preventing RAD51-dependent homologous recombination

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RECQ5 mutation and overexpression have both been associated with human cancer. RECQ5 has been implicated in repair of oxidative DNA damage, a critical pathway in which inherent redundancies may mask a key role for any single factor. Oxidative damage creates DNA nicks. By using CRISPR/Cas9 or CRISPR/Cas9D10A to target double-strand breaks (DSBs) or nicks to specific sites in the human genome our laboratory has shown that nicks can initiate homology directed repair (HDR) by an alternative pathway that is distinct from HDR at DSBs and that efficiently uses single-stranded DNA donors (Davis and Maizels, 2014). This alternative pathway is normally inhibited by RAD51, to prevent genomic instability at nicks.

To determine the functions of RECQ5 in HDR, we have assayed the effect of its depletion or overexpression at targeted nicks and DSBs. We found that depletion of RECQ5 inhibited HDR at both nicks and DSBs, by either single-stranded or duplex DNA donors. Conversely, overexpression of RECQ5 inhibited HDR at DSBs and HDR by dsDNA donors at nicks, but stimulated HDR by ssDNA donors at nicks. While RECQ5 associates with the moving transcription apparatus, we did not find that these activities of RECQ5 depended upon transcription of the target gene for recombination, or were affected by deletion of the domain of RECQ5 that interacts with RNA polymerase 2. Structure-function analysis did show that stimulation of HDR depended on the RECQ5 helicase ATPase activity, and the ability of RECQ5 to interact with RAD51. None of the effects of RECQ5 depletion or overexpression was evident in cells in which RAD51 filament formation had been inhibited by treatment with siRAD51 or siBRCA2.

We conclude that RECQ5 normally supports canonical HDR at both nicks and DSBs, to promote genomic stability. Somewhat paradoxically, RECQ5 overexpression has the unanticipated consequence of promoting genomic instability, apparently overriding the normally suppressive effect of RAD51 to enable nicks to initiate HDR. These results explain the genomic instability associated with both RECQ5 mutations and overexpression.

Aging and Cancer: A Molecular Lesson From the Human RECQ4 DNA Helicase

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RECQ4 belongs to the highly conserved RECQ family of superfamily 2 (SF2) DNA helicases, and is an essential enzyme for mammalian embryonic development [1]. RECQ4 mutations have been identified in patients suffering from Rothmund-Thomson syndrome, Baller-Gerold syndrome and RAPADILINO syndrome, with phenotypes ranging from developmental defects to premature aging and cancer predisposition [2]. In addition to the SF2 helicase domain, RECQ4 contains a unique N-terminus that resembles the essential yeast DNA replication initiation factor Sld2. Cellular and biochemical studies established RECQ4 as a component of the nuclear replication machinery that is important for faithfully duplicating the genome. Specifically, My laboratory discovered that human RECQ4 forms a chromatin-specific complex via this Sld2-like domain with the MCM2-7 replicative helicase complex [3].

In addition to nuclear DNA replication, RECQ4 also maintains mitochondrial DNA (mtDNA) copy number and the energy production capacity of mitochondria. In human cells, we showed that a subset of the RECQ4 proteins localizes to the mitochondria and interacts with the mitochondrial replicative helicase PEO1 to promote mtDNA synthesis [4]. Given that RECQ4 is a key component of the nuclear and mitochondrial replication machineries, deregulation of RECQ4 mitochondrial or nuclear localization is expected to have profound impacts to human health. We will present our new studies that identified post-translational modifications important for regulating RECQ4 protein-protein interactions and localizations. We will also discuss our recent published and new observations on how disease-associated RECQ4 mutations, including the most common RAPADILINO syndrome-associated RECQ4 mutation, c.1390+2delT, which produces a polypeptide lacking 44 amino acids (Ala420_Ala463) [5], lead to aberrant mitochondrial and nuclear localizations. Specifically, we found that Ala420_Ala463 deletion leads to defective interaction with p32, which is required to restrict RECQ4 mitochondrial localization [4]. Cells expressing this RECQ4 mutation accumulate excess RECQ4 in the mitochondria, and exhibit an increased PEO1 interaction, elevated mtDNA synthesis activity and Warburg effect [4]. Intriguingly, 40% of the individuals who were homozygous or compound heterozygous for this mutation were diagnosed with lymphoma at early age [5]. The unusually high number of lymphoma incidents found in these patients suggests that the aberrant mtDNA synthesis activity caused by a defective RECQ4-p32 interaction is responsible for cancer pathogenesis.

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Understanding the role of Recql4 in normal and malignant disease: Insights from blood and bone development

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The analysis of rare human cancer pedigrees has proven to be one of the most informative approaches to understanding human cancer. These studies have defined numerous genes fundamental to our understanding of cancer initiation and maintenance. These include genes such as p53 in Li-Fraumeni syndrome, Rb in hereditary retinoblastoma and Blm in Bloom's syndrome. These predispositions have provided critical insight into the molecular biology and genetics of human cancer. Rothmund-Thomson Syndrome (RTS, OMIM 268400) is a familial cancer predisposition syndrome associated with elevated rates of osteosarcoma (OS), hematological neoplasms and skeletal dysplasia. RTS is the third familial OS syndrome together with Li-Fraumeni and hereditary retinoblastoma. The cause of RTS has been defined as mutations in the RECQL4 gene. However, unlike the related Bloom and Werner syndromes RTS and the functions of RECQL4 are more poorly understood.

We have used mouse genetic models to understand the role of Recql4 in normal development and in the formation of bone cancer (osteosarcoma). These studies have demonstrated an essential function for Recql4 in blood cell homeostasis and that loss of Recql4 causes a rapid bone marrow failure syndrome. This phenotype was not modified by loss of p53 but could be rescued in vitro by use of an helicase inactive RECQL4, suggesting separable functions of Recql4 (see Smeets et al JCI, 2014 124(8):3551-65).

Of all the cell types impacted in RTS, osteoblastic cells appear particularly sensitive to the effects of RECQL4 mutation. In bone forming cells, we found that loss of Recql4 caused a low bone mass but it's loss alone did not lead to the development of cancer. Using OS predisposed cancer models, we surprisingly found that the deletion of Recql4 caused a delay in tumor onset. Upon further investigation we determined that complete loss of Recql4 was not seen in the tumor, demonstrating that the cells require some Recql4 function to be viable (see Ng et al PLoS Genet, 2015 11(4):e1005160).

These studies have resolved normal functions of Recql4 in blood and bone cell homeostasis but leave many questions unanswered about the role of RECQL4 mutations in the initiation of cancer. A parallel question that remains is why are some cell types particularly sensitive to RECQL4 mutation. I will discuss our ongoing studies seeking to address aspects of these outstanding questions.

Role of RECQL4 in the maintenance of mitochondrial replication and genome integrity

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Germline mutations in the nuclear RECQL4 helicase are associated with Rothmund Thomson syndrome (RTS). A subset of RTS patients is predisposed towards cancer. It has been shown that RECQL4 localizes to the mitochondrial nucleoid where it enhances the 5' to 3'polymerization and 3' to 5'exonuclease activities of Polymerase γ, thereby acting as an accessory factor during mitochondrial DNA replication. Mitochondrial genome sequencing in RTS patient cells have revealed incorporation of somatic mutations and polymorphisms in the mitochondrial genes coding for oxidative phosphorylation, tRNA, rRNA and the D-loop, which potentially can alter mitochondrial functions in the RTS patient cells.

To understand the mitochondrial functions that might be perturbed in the absence of functional RECQL4, we have studied the ultra structure of mitochondria, mitochondrial membrane permeability, mitochondrial ROS, mitochondrial mass, structure and functions of electron transport chain which subsequently effect the ATP production in the RTS patient cells and its complemented counterparts. Interestingly the loss of the mitochondrial functions of RECQL4 increases aerobic glycolysis which in turn led to an increased invasive capability in these cells. Altogether these studies will help to understand the functions of RECQL4 in the mitochondria and provide evidence about the interplay between the nucleus and mitochondria in the maintenance of genomic integrity.

RECQL4 in Double Strand Break Repair and Senescence

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Recently our lab analyzed the role for RECQL4 in all three major double strand break repair (DSBR) pathways: non-homologous end-joining, homologous recombination and alternative non-homologous end-joining. Using in vitro and in vivo DSBR assays we have quantified the relative contribution of RECQL4 in the various pathways. Defects in double strand break repair are known to contribute to premature cellular senescence. Cellular senescence refers to irreversible growth arrest of primary eukaryotic cells, a process thought to contribute to aging-related degeneration and disease. RECQL4-depleted cells display increased staining of senescence-associated β-galactosidase (SA-β-gal), higher expression of p16(INK4a) or/and p21(WAF1) and accumulated persistent DNA damage foci. We have mapped the region in RECQL4 that prevents cellular senescence to its N-terminal region and helicase domain. We further investigated senescence features in an RTS mouse model. Tail fibroblasts from Recql4 mice show increased SA-β-gal staining, increased DNA damage foci and increased senescence in tail hair follicles and in bone marrow cells. In conclusion, dysfunction of RECQL4 decreases DSBR capacity of human cells and increases the level of endogenous DNA damage and triggers premature senescence in both human and mouse cells, which may contribute to symptoms in RTS patients.

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Immunodeficiency in Bloom syndrome patients

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Bloom syndrome (BS) is an autosomal recessive disease, caused by mutations in the *BLM* gene. This gene codesfor BLM protein, which is involved in DNA repair. BS is clinically characterized by prenatal growth retardation, predisposition to malignancy, café-au-lait spots, butterfly-shaped erythema, and immunodeficiency. BLM function has been investigated regarding the early onset of malignancies, but the pathophysiology behind the immunodeficiency has not yet been elucidated. In the immune system, DNA repair is especially important for the development and maturation of the T and B cells. During B and T cell development DNA repair is required for the generation of the antigen receptor. In addition, B cells can undergo somatic hypermutation (SHM) to create a better affinity for their antigen and class switch recombination (CSR) to change the effector function of the antigen receptor. Both of these processes are dependent on DNA repair. Since BLM is involved in DNA repair, we aimed to study if BLM deficiency affects T and B-cell development and especially SHM and CSR processes. Clinical data of six BS patients were collected and immunoglobulin serum levels were measured at different time points over many years. In addition, we performed immunophenotyping and analyzed SHM and CSR in detail by analyzing IGA and IGG transcripts using next generation sequencing.

All patients had growth deficiency and recurrent ear infections and suffered from different (small) infections like bronchitis. These patients did not have severe infections.
The absolute number of T, B and NK cells were low, but still in the normal range. Remarkably, all BLM patients studied had a high percentage (20-80%) of CD4+ and CD8+ effector memory T cells. The serum immunoglobulin levels were low, but in the normal range. The percentage SHM was normal and we also did not observe differences in the targeting or repair of the SHM. However, CSR seemed affected since the BLM patient had more IgG1 and IgG3 transcripts, and an increase in the frequency of switch junction with short microhomology.
In conclusion, BS patients have low number of lymphocytes, but the immunodeficiency seems relatively mild since they have no severe or opportunistic infections. Most changes in the B cell development were seen in the CSR process, however further studies are necessary to elucidate the exact role of BLM in CSR.

The BLM and WRN RECQ helicases modulate human gene expression by targeting G-quadruplex DNA motifs

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Background: Bloom syndrome (BS) and Werner syndrome (WS) are rare autosomal recessive genetic instability and cancer predisposition syndrome caused by loss of function mutations in the *BLM* and *WRN* human RECQ helicase genes. In order to determine how altered gene expression might drive the pathogenesis of both RECQ syndromes, we analyzed mRNA and miRNA expression in primary fibroblasts from BS and WS patients and in BLM- and WRN-depleted control primary fibroblasts.

Results: Both BLM and WRN proteins bind and unwind G-quadruplex (G4) DNA substrates *in vitro*, and we identified significant enrichment in G4 sequence motifs near transcription start site and 5' ends of first introns of genes down-regulated in both BS and WS patient fibroblasts. This finding was more prominent in WS than in BS, and provides strong evidence that both BLM and WRN bind G4 DNA in human cells to modulate gene expression. The BLM and WRN RECQ helicases appear to bind a distinct subpopulation of G4 motifs in human cells, and modulate the expression of different sets of differentially regulated genes and miRNAs. Functional annotation of these differentially expressed genes and miRNAs has provided new and several unexpected insights into the time course of development of BS cellular and organismal pheno-types, and the role of gene and miRNA expression adaptation in the pathogenesis of WS.

My talk will place these results in perspective, and highlight the new and unexpected insights these analyses have provided into BS and WS disease pathogenesis and therapy.

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Keynote address 3: Next Generation Human Genetics and Genomics

Debbie Nickerson, University of Washington Department of Genome Sciences, Seattle WA.

Small molecules as modulators of Bloom's syndrome biology

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Bloom syndrome is a rare autosomal recessive disorder characterized by genetic instability and cancer predisposition, and caused by mutations in the Bloom's syndrome protein, BLM, a member of the conserved RecQ helicase family. A continuing aim of our research is to understand how a single loss of a gene could lead to the dramatic effects seen in Bloom patients. We and our collaborators have identified a network of mRNAs and microRNA that drive the many pathogenesis seen in BLM patients such as cancer and immune disfunction, using fibroblasts from individuals with Bloom syndrome. We also identified G4 motifs were enriched at transcription start sites and especially within first introns of differentially expressed mRNAs in Bloom syndrome compared with normal cells, suggesting that G-quadruplex structures formed at these motifs are physiologic targets for BLM.

Additionally, of critical importance is the development of a cellular model for the study of BLM as although cell lines lacking BLM exist, these exhibit progressive genomic instability that makes distinguishing primary from secondary effects of BLM loss problematic. We undertook a high throughput screen of a chemical compound library for small molecule inhibitors of BLM, and identified ML216, a potent inhibitor of the DNA unwinding activity of BLM. This compound showed cell-based activity and can induce sister chromatid exchanges with a strong selectivity for BLM in cultured cells. We will discuss the potential utility of these findings in BLM research.

RECQ1 Structure-Function Studies and WRN Helicase Small Molecule Inhibitors

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Helicases play major roles to maintain the genome by unwinding structured nucleic acids and remodeling proteins bound to DNA. Their prominence is marked by various cancers and genetic disorders that are linked to helicase defects. Helicase-dependent mechanisms help cells to cope with endogenous or exogenous stress to prevent chromosomal instability and maintain cellular homeostasis. Although considerable effort has been made to understand the functions of DNA helicases, the complexity of the DNA damage response leaves us with unanswered questions regarding how helicase-dependent DNA repair pathways are regulated and coordinated with cell cycle checkpoints and ongoing cellular events. We have been keenly interested in the molecular mechanisms of helicases that deal with replication stress, and more recently their involvement in mitochondrial DNA metabolism. Structure-function analyses and small molecule approaches have provided useful strategies for understanding how helicases of the RecQ and Fe-S cluster families operate to preserve genome integrity. Characterization of patient-derived or conserved missense mutants of FANCJ and RECQ1, respectively, has yielded new insights into the molecular and cellular functions of these helicases. Our discovery of a small molecule inhibitor of the WRN helicase defective in the accelerated aging disorder Werner syndrome provides new understanding of WRN-mediated pathways important for DNA repair and the replication stress response. Further studies may open the door to targeting helicases for therapeutic strategies.

Vitamin C treatment of Werner syndrome mouse models

Michel Lebel

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Werner syndrome is a premature aging disorder caused by mutations in a RecQ-like DNA helicase gene product. Interestingly, mice lacking part of the helicase domain of the WRN homologue develop several age-related phenotypes such as insulin-resistance, dyslipidemia, a pro-oxidant and pro-inflammatory status, a severe liver endothelial defenestration (a phenotype associated with old age), liver steatosis, aortic stenosis (hardening of the aorta) and cardiomyopathy. Such mutant mice exhibit a 15% decrease in their life expectancy compared to normal wild type mice. We found that the mutant protein in these mice is mislocalised in cells and is associated with the endoplasmic reticulum and the peroxisome organelles instead of the nucleus. These helicase mutant mice also exhibit an increase oxidative stress in the endoplasmic reticulum of different tissues, an abnormal metabolic profile and an increase in blood factors associated with inflammation and heart diseases, even before they exhibit the age-related symptoms. Vitamin C supplementation rescued the shorter mean life span of Wrn mutant mice and reversed several age-related abnormalities in the liver and cardiovascular system.

To better assess the impact of vitamin C on the health span of such mice, we crossed Wrn mutant mice with mice that cannot synthesize their own vitamin C (like humans) and performed different physiological measurements. Mice that cannot synthesize vitamin C has a deletion of the enzyme gulonolactone oxidase (from the Gulo gene) required for vitamin C synthesis and as such rely entirely on their diet to obtain the required amount of vitamin C to survive. The double mutant mice (referred as WrnΔhel/Δhel/Gulo-/- animals) showed a severe reduction in their life span (8 months instead of 24 months) with a minimum of vitamin C in drinking water (0.01% w/v). Although we did not detect a significant increase in reactive oxygen species in the tissues of these double mutant mice, we observed an increase in mitochondrial DNA mutation in the liver of these mice. Double mutant mice exhibit a shorter stature than wild type animals, hypogonadism, a severe decrease in bone density, earlier defenestration of liver endothelial cells than single Wrn mutant mice, and aortic stenosis. These phenotypes were reversed with a higher concentration of vitamin C (0.4% w/v) in drinking water. Finally, our results indicate that vitamin C improves the health span of double mutant mice.

Family Meeting Abstracts

CLINICAL FEATURES OF WERNER SYNDROME: DIAGNOSIS, NATURAL HISTORY, AND FUTURE DIRECTIONS

Fuki M. Hisama, M.D[1]and Koutaro Yokote, M.D[2]

[1]University of Washington, Seattle, WA
[2]Chiba University, Chiba, Japan

Werner syndrome (MIM 277700) is a rare, autosomal recessive disorder originally described in 1904 in German siblings with tight, scleroderma-like skin, and bilateral cataracts. The clinical features constitute a segmental progeroid syndrome, and include: a prematurely aged appearance, graying, thinning hair, skin changes, cataracts, high-pitched voice, diabetes, atherosclerosis, osteoporosis, hypogonadism, and increased risk of certain cancers. The International Werner Syndrome Registry at the University of Washington provides information and resources for health care professionals and scientists about this condition. Data on patients submitted to the Registry with >75 different pathogenic variants will be reviewed.

The Werner syndrome locus was mapped to chromosome 8p, and in 1996, the underlying cause of Werner syndrome was identified as mutations in the WRN gene, or RECQL2, encoding a member of the family of RecQ DNA helicases. The role of molecular testing in the diagnosis of Werner syndrome, and genetic counseling aspects will be discussed.

Although Werner syndrome occurs worldwide, the highest prevalence is in Japan, followed by Sardinia. Werner syndrome patients used to die in their forties due to atherosclerosis or malignant neoplasms, but their prognosis seems to have improved recently. According to the results obtained through a nation-wide survey in Japan, diagnostic criteria for Werner syndrome have been revised and therapeutic guidelines are being created. We will discuss the clinical features, and natural history of Werner syndrome patients in Japan, and what has been learned about the surveillance and management of this multi-system genetic condition.

Finally, we will discuss future directions of basic and clinical research, including potential novel therapies for Werner syndrome.

Skin manifestations of the RECQ syndromes

Dr. Moise L. Levy, Physician-in-Chief
Pediatric/Adolescent Dermatology
Dell Children's Center, Austin, TX

I have been involved with RTS patients since beginning my career in pediatric and adolescent dermatology, and have a strong interest in genetic skin disorders. Dr Lisa Wang and I worked closely during my (many) years at Texas Children's Hospital, and I have continued my affiliation with the advocacy group dedicated to RTS. I am a strong advocate of collaborative learning and know that this conference will provide many such opportunities from other clinicians, scientists, and patients/families. I look forward to the opportunity to attend.

As a medical educator, I feel obligated to enhance my expertise in such areas and to be able to extend such to those in the communities we serve.

Coping with Chronic Illness

Dr. Emil Zakutny, RTSF Board Member, Licensed Marriage and Family Therapist
Clinical Social Worker, Baldwin, NY

My interest in attending RECQ2016 is two-fold. Firstly, I will present a workshop on Coping with Chronic Illness for patients and families and other interested conference attendees. As a mental health clinician, I believe that given the biopsychosocial aspects of genetic disorders, it is important that patients and their families identify meaningful strategies for dealing with various psychosocial challenges associated with chronic disorders.

Secondly, as a RTS Foundation board member, I'm very interested in the latest research and potential implication(s) and/or application(s) for treatment, especially for the RTS syndrome. Staying current on the state of knowledge in this field is important in guiding my role as a RTS board member with regards to funding, the mission and direction of the RTS Foundation.

Bloom's Syndrome Patient & Family session (Monday 9:40, Sze Conference room)

Hosts:
Paul Zaslaw/Bloom's Syndrome Association
Leah Counts-Goldy for Bloom's Connect

This session will include two discussion threads, and will be held concurrently or as two groups, depending on attendee numbers and interest.

'Raising a Child with Bloom's syndrome'
Paul Zaslaw/Bloom's Syndrome Association

'Living as an Adult with Bloom's syndrome'
Leah Counts-Goldy for Bloom's Connect
Participants in this session will discuss strategies for coping with life's curve balls from diagnosis forward. Using the principle of a sturdy foundation, this session will delve into the Social and Identity Models of Disability, leaving time for interactive discussion and small group activities. This session is most appropriate for any conference participant. Please bring your creativity and life experience.

Poster Abstracts

Poster 1

Sumoylation of BLM modulates its functions in DNA damage response and replication fork stability

Christelle de Renty[1], Kelvin W. Pond[1], Mary K. Yagle[1], Nathan A. Ellis[1]

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Background: Genomic instability is a major driver of tumorigenesis. Among the many repair mechanisms ensuring genome integrity, homologous recombination (HR) is a high-fidelity repair pathway that is involved in the repair of DNA double-strand breaks (DSBs) and in the protection of stalled or damaged replication forks. The RecQ helicase BLM, which is mutated in Bloom's syndrome (BS), plays multiple roles in DNA replication and HR, and cells mutated for BLM are characterized by excessive HR. We previously found that BLM's functions in HR are regulated by post-translational modification by SUMO (small ubiquitin-related modifier). Specifically, cells that express a BLM protein that cannot be sumoylated (SUMO-mutant BLM or SM-BLM) exhibit excess DNA damage repair foci. We hypothesized that these excess repair damage foci result from increased replication fork failure and collapse. If this hypothesis is correct, then an analysis of replication dynamics on single DNA molecules should reveal increased collapsed forks and dormant origin firing.

Methods: We have previously developed cell lines that express normal BLM (BLM+ cells) and SM-BLM (SM-BLM cells), defective in sumoylation at lysines 317 and 331. Newly synthesized DNA was labeled in vivo by incorporation of the nucleoside analogs iododeoxyrudine (IdU) and chlorodeoxyuridine (CldU), in the presence or absence of hydroxyurea (HU), which stalls replication forks. Microfluidic capillary channels were used to stretch and align single DNA molecules on glass slides. The incorporated IdU and CldU was visualized by immunostaining. Measurements of the replication tracks were used to calculate parameters of the DNA replication dynamics, including replication fork velocity, replication fork and origin density, fork asymmetry, fork stability in presence of replicative stress, and the frequency of dormant origin firing.
Results: In untreated SM-BLM cells, replication fork velocity was decreased compared to BLM+ cells (0.7 kb/min vs. 1.3 kb/min) and replication fork density was increased (1.8 forks/Mb vs. 1.5 forks/Mb). Treatment of SM-BLM cells with 5 mM HU for 5 hrs significantly increased fork collapse from 6.4% to 21.6%, but treatment of BLM+ cells had only a minor effect on fork collapse from 3.6 to 5.2%. Dormant origin firing in response to HU treatment was also elevated in SM-BLM cells (4.3% of dormant origins firing) compared to BLM+ cells (0.6% of dormant origin firing). The replication dynamics of SM-BLM cells resembles those in BS cells as replication fork velocity is diminished (1.1 kb/min) and replication forks density is increased (2.1 forks/Mb). Fork collapse in BS cells after HU treatment is also elevated (17.2%). These results indicate that BLM sumoylation is required for normal fork progression in unperturbed cells and for fork stability in cells exposed to replication stre ss.

Conclusions: In cells that are unable to sumoylate BLM, replication forks stall and collapse more frequently, leading to increased dormant origin firing. Our results indicate that BLM sumoylation helps maintain normal DNA replication in both the presence and the absence of HU-induced replicative stress.

Poster 2

Bloom's Syndrome Association: an international patient and family support organization for persons affected by Bloom's syndrome

Paul Zaslaw[1]

[1]Bloom's Syndrome Association
http://www.bloomssyndromeassociation.org/

The landscape for Bloom's syndrome research, family support, and patient advocacy is dotted with organizations serving the various needs of distinct populations across the international Bloom's syndrome community. The Bloom's Syndrome Association is a bridge for communication and collaboration between these organizations and a clearinghouse for authoritative information about Bloom's syndrome.

The Association was chartered in 2012 as a nonprofit organization for charitable, scientific, and educational purposes. Since the launch of its website in 2014, the Association has attracted a diverse and balanced membership of individuals diagnosed with Bloom's syndrome; family members, friends, and other supporters; medical and scientific researchers; and healthcare providers. The Association has been vetted and listed as a family support and patient advocacy resource by a variety of trusted organizations, including the National Organization for Rare Disorders, National Center for Biotechnology Information, Genetic and Rare Diseases Information Center, and Genetics Home Reference.

This advocacy-themed poster will expound on the mission, activities, partnerships, and future plans of the Bloom's Syndrome Association, and illustrate the Association's distinct role in uniting all sectors of the international Bloom's syndrome community.

Poster 3

Nucleolar Functions of the BLM Helicase

Samir Acharya[1], Patrick Grierson[1], Larissa Tangeman[1], Michael McIlhatton[1] and Joanna Groden[1]

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Dysregulation of ribosomal DNA (rDNA) metabolism is a key player in cellular and organismal growth defects, cellular transformation and a primary cause of cellular senescence. It is correlated with genomic instability at the rDNA repeats and compromised DNA damage response across the genome including telomeres. Bloom's syndrome (BS), an autosomal recessive human disorder caused by mutation of the recQ-like helicase BLM, presents several characteristics of extreme nucleolar dysfunction: severe growth retardation, increased chromosomal instability and cancer susceptibility. Cells without BLM are characterized by elevated rDNA repeat instability, inter- and intra-chromosomal recombination, recombination of acrocentric chromosome arms where rDNA is found, chromosomal breakage and telomeric associations.

BLM localizes to the nucleolus and PML bodies, and functions in the resolution of unusual DNA structures associated with replication stress and recombination, and the G4 quadruplex structures predicted to occur in G-rich DNA (rDNA and telomeres). Our experiments test the hypothesis that BLM suppresses nucleolar dysfunction through its role in rDNA metabolism. Our data demonstrate that BLM is required for appropriate rDNA metabolism as well as telomeric repeat stability. BLM localizes to the nucleolus during active rDNA transcription and interacts directly with topoisomerase I, RNA polymerase I and other DNA repair proteins to facilitate rDNA transcription. We have identified domains in BLM critical for nucleolar targeting and interaction with topoisomerase I. Functionally, BLM and topoisomerase I display a reciprocal stimulation of their individual activities. Murine alleles generated using CRISPR technology carrying mutations of residues required to localize Blm to the nucleolus do not result in viable progeny. Our results implicate BLM in regulating key rDNA transactions in the nucleolus.

Poster 4

NSMCE2 is required for maturation of persistently stalled forks from a RAD51 bound intermediate to an induced double strand break

Kelvin W. Pond[1], Christelle de Renty[1], Mary K. Yagle[1], Nathan A. Ellis[1]

[1]Department of Cellular and Molecular Medicine and the University of Arizona Cancer Center, University of Arizona, Tucson, AZ

Background: DNA damage generated during replication is a major source of mutations and failure to repair this damage can cause genomic instability. Homologous recombination (HR) is a high-fidelity DNA repair process that rescues double strand breaks (DSBs) at stalled replication forks. Numerous HR proteins are regulated by sumoylation, but the mechanisms that control sumoylation and their roles in HR are poorly understood. Cells deficient in the E3 SUMO ligase NSMCE2 (the human ortholog of yeast MMS21) are sensitive to DNA damaging agents and have defects in HR. Our preliminary data indicated that BLM sumoylation is dependent on NSMCE2. Because BLM sumolyation is required to recruit RAD51 to stalled forks, we hypothesized that NSMCE2-deficient cells are defective in HR due to a defect in SUMO-BLM-dependent RAD51 recruitment.

Results: To test this hypothesis, we transfected HeLa cells with siRNAs specific to NSMCE2 and tested whether cells could recruit RAD51 to replication forks stalled by hydroxyurea (HU) treatment. Contrary to our hypothesis, we found that the amount of RAD51 protein that accumulated at stalled forks was greater in NSMCE2-deficient cells. Additionally, the amount of chromatin-bound single-stranded DNA binding protein RPA and the amount of nascent DNA detectable by BrdU incorporation was diminished by half in HU-treated NSMCE2-deficient cells. A corresponding reduction in DNA damage signaling was similarly observed as evidenced by lower levels of □-H2AX. In agreement with the low levels of □-H2AX, we observed a striking reduction of DSBs detectable by pulsed-field gel electrophoresis after extended treatment with HU. Consistent with previous reports, we found that the levels of HU-induced sister chromatid exchange were reduced. Moreover, sensitivity to PARP inhibitors was increased, together indicating that NSMCE2-deficient cells are unable to perform HR repair efficiently.

Conclusions: The hyper-accumulation of RAD51 at stalled forks we observed in NSMCE2-deficient cells suggests that sumoylation of one or more substrates by NSMCE2 is required for remodeling of stalled forks and subsequent repair by HR. NSMCE2-deficient cells are unable to unload RAD51, recruit nucleases to the replication fork, or both, locking the fork in a stable RAD51-associated replication repair intermediate. RAD51 has been proposed as a biomarker for HR proficiency in cancer. Our data show that high levels of RAD51 may not be sufficient to assess HR proficiency, and that RPA co-staining with RAD51 may distinguish HR-proficient and HR-deficient cancer cells.

Poster 5

International Registry of Werner Syndrome

Junko Oshima[1], Fuki M. Hisama[2], George M. Martin[1]

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The International Registry of Werner Syndrome (wernersydrome.org) was established in 1988 with the original purpose of collecting classical Werner syndrome (WS) cases for positional cloning of the WRN gene. The Registry also serves as a valuable resource for biological materials either derived from patients or developed within our Registry. Several therapeutic approaches are being explored that may have a direct influence on WS disease progression. We reported that mTOR signaling and basal autophagy are upregulated in WS cells and that long-term rapamycin treatment resulted in improved growth rate and a reduced accumulation of DNA damage foci (Aging Cell 2014, 13:573). Approximately 20% of clinically diagnosed WS cases do not carry WRN mutations; these cases are operationally categorized as “Atypical Werner Syndrome (AWS)”. Our Registry has expanded its scope from WS to the search for causative mutations and mechanisms responsible for the broader range of progeroid syndromes from all over the world.

A combination of next generation sequencing, SNP arrays and candidate gene sequencing have successfully identified novel mutations in a small number of cases. Newly found progeroid loci highlight major roles in DNA repair and replication: POLD1 (DNA polymerase delta)(Hum Mut 2015; 36:1070), SPRTN (recruitment of translesional DNA polymerase eta)(Nat Genet 2014; 24:1239); LMNA (nuclear structure and chromatin interaction); MDM2 (an inhibitor of p53); and SAMHD1 (regulation of dNTP pools)(Am J Med Genet 2014; 164A:2510). These findings continue to support the concept of genomic instability as a major mechanism of biological aging.

Poster 6

Pathological significance of telomere in a model mouse for Werner syndrome

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Werner syndrome (WS) is progeroid syndrome, which characterized by multiple features such as cataract, skin ulcer, diabetes mellitus, atherosclerosis, and malignancy resulted in premature death. WS is caused by the recessive mutation of WRN gene, a member of the RecQ family of DNA helicases. Accumulating evidence has indicated that Wrntm1Lgu, TercG4/G4 double-null mice showed small body, osteoporosis as well as short lifespan in a telomere shortening-dependent manner, suggesting that telomere length plays an important role in premature phenotype in model mouse for WS. In the present study, to reconfirm pathological significance of telomere in a WS model, we generated Wrntm1Lgu, Terttm1Fish double knockout mice and intercrossed those mice until G4 generation (TertG4/G4) to induce telomere shortening. Both TertG4/G4 single and Wrn-/-, TertG4/G4 double knockout mice showed significant shortening of telomere length in various organs. Wrn-/-, TertG4/G4 double knockout mice were normally born and grown without apparent abnormality in muscle, liver as well as bone. However, the double null-mice deteriorated fat atrophy in skin and testis degeneration, but not epidermis and dermis atrophy compared with those of TertG4/G4 single-null mice. These results suggested that telomere shortening by Tert deficiency is attributable to premature aging pathologies in WS models.

Poster 7

RECQL4 is required for normal skeletogenesis

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RECQL4 is a member of ATP-dependent RecQ DNA helicases. Its functions have been implicated in DNA replication, homologous recombination, DNA damage repairs, and the maintenance of telomere and mitochondrial DNA integrity. Mutations in RECQL4 are associated with three autosomal recessive conditions: Rothmund-Thomson syndrome (RTS), RAPADILINO, and Baller-Gerold syndrome (BGS). All three disorders have prominent skeletal abnormalities, including radial ray/thumb abnormalities, short stature, foreshortened limbs, absent patellae, osteopenia, and craniosynostosis. In addition, patients with RECQL4 mutations have a very high risk of developing osteosarcoma, the most common malignant bone cancer seen in children and adolescents. However, the molecular mechanisms underlying these findings are not fully understood.

To investigate the role of RECQL4 in skeletal development in vivo, we generated a mouse Recql4 conditional allele (Recql4fl/fl) by gene targeting. Recql4fl/fl mice were crossed with Prx1-Cre transgenic mice to inactivate Recql4 in mesenchymal progenitor cells. Prx1-Cre+;Recql4fl/fl mice displayed short forelimbs and hindlimbs, missing digits, and bilateral coronal suture synostoses. H&E staining of E18.5 Recql4 mutant growth plates showed reduced chondrocyte density and increased cell size. Brdu labeling and TUNEL assays in Recql4 mutant growth plates demonstrated reduced chondrocyte proliferation and increased chondrocyte death, respectively. γH2AX immunostaining of E18.5 growth plates showed significantly increased DNA damage in Recql4 mutant chondrocytes. Immunoblotting of forelimb tissues of E13.5 Recql4 mutant embryos demonstrated dramatically increased p53 serine15 phosphorylation and elevated p21 protein levels. Real-time RT-PCR revealed that transcripts of p53 target genes, including CDKN1A/p21 and Bax, were significantly up-regulated as well. Concurrent deletion of both Recql4 and p53 partially rescued the skeletal defects observed in Prx1-Cre+;Recql4fl/fl mutants.

We also analyzed postnatal bone development in Prx1-Cre+;Recql4fl/fl mutants. At 3 months of age, these mutant mice showed significantly smaller size, increased size of growth plate chondrocytes, and abnormal growth plate morphology. As demonstrated by μCT of distal femurs, these mutants had more than 50% reduction of bone volume fraction (BV/TV) and trabecular number (Tb. N), while trabecular separation (Tb. Sp) increased more than 2-fold, and trabecular thickness (Tb. Th) had no significant change. Bone histomorphometry showed similar findings as μCT analysis. In addition, there was more than 50% decrease in total osteoblast number per analyzed area, bone surface lined with osteoblasts, and osteoid surface with lining osteoblasts in mutant mice compared to littermate controls. However, mineral apposition rate in these mutant mice was unchanged. Osteoclast number and function were also not significantly changed compared to littermate controls.

In conclusion, inactivation of Recql4 in mouse mesenchymal progenitor cells led to foreshortened limbs, digit defects, small size, abnormal growth plates, decreased bone mass, and craniosynostosis, recapitulating the major skeletal findings in the RECQL4 spectrum of disorders. Our in vivo findings suggest that Recql4 is required for normal skeletal development that inactivation of Recql4 in mesenchymal progenitor cells causes strong activation of p53, and that Recql4 genetically interacts with p53 during skeletogenesis.

Poster 8

The yeast Hrq1 is a functional homolog of human RecQ4

Cody M. Rogers [1], Rakshin Kharwadkar[1], David G. Nickens[1], Yuichiro Takagi [2], and Matthew L. Bochman[1]

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Mutations in human RecQ4 are associated with cancer and premature aging. However, RecQ4 research is difficult because it is a fusion between a helicase and an essential DNA replication factor. Previously, we characterized Saccharomyces cerevisiae Hrq1, an enzyme homologous to the disease-linked helicase domain of RecQ4, and discovered that Hrq1 has two unusual activities: it uses its helicase activity to protect against DNA inter-strand crosslinks (ICLs) and acts structurally to protect against telomere addition (TA). Thus, HRQ1 joins PSO2 as the only yeast genes whose mutation specifically results in ICL sensitivity, and Hrq1 joins Pif1 as the only known TA inhibitors. In vitro, we have developed optimized methods to generate milligram levels of recombinant Hrq1 and RecQ4. These purified proteins are robust DNA-stimulated ATPases and 3'-5' helicases with similar unwinding kinetics and substrate specificities. Ongoing work focuses on structure-function investigations of these helicases, including N-terminal truncations of RecQ4, which more closely mirror Hrq1, and a fusion of S. cerevisiae Sld2 and Hrq1, which mimics RecQ4. Additionally, an in vitro system is being developed to determine the mechanism by which Hrq1 inhibits telomerase and if hRecQ4 functions in a similar manner. Overall, we are developing a comprehensive picture of how RecQ4 family helicases function to maintain genome integrity and why their dysfunction leads to disease.

Poster 9

Global and disease-associated genetic variation in the human RECQ helicase gene family

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RECQ helicase proteins play key roles in DNA metabolism and genome stability assurance in virtually all organisms. Heritable loss of function mutations in the human RECQ helicase genes WRN, BLM and RECQL4 cause, respectively, the cancer predisposition syndromes Werner syndrome, Bloom syndrome and Rothmund-Thomson syndrome and related disorders.

In order to better define genetic variation in the five human RECQ helicase genes, we systematically analyzed genetic variation in the human RECQ helicase genes using data from 60,706 unrelated individuals included in the Exome Sequencing Project (ESP), 1000 Genomes Project (1KGP) and Exome Aggregation Consortium (ExAC) datasets. We identified 968 unique, small (basepair-level) RECQ helicase gene mutations, across 17,605 potential mutation sites, in the five human RECQ genes. Among 968 variants, 210 were considered disease-associated or causative.

The direct counting of known, disease-causative variants yielded an aggregate carrier frequency of deleterious variants of 0.54% (or 1 in 185) for the WRN, BLM and RECQL4 disease-associated RECQ helicases, though no putative homozygous/ affected WS, BS or RTS individuals. Functional prediction algorithms correctly identified only 50% of the known disease-causing missense mutations as 'likely deleterious', emphasizing the need to experimentally confirm missense mutant functional predictions. We extended this general approach as well to the RECQL and RECQL5 genes that have not been associated with a heritable disease or syndrome by making use of experimental results and data from model organisms. Finally, among the disease-associated mutations in the WRN, BLM and RECQL4 genes, we identified 35 (or 17%) that might be candidates for exon skipping or stop codon read-through therapies to restore partial function.

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Poster 10

Cancer type-specific risk and tumor genomics in Werner syndrome

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Background: Werner syndrome (WS) is an autosomal recessive genetic instability and progeroid ('premature aging') syndrome, which is associated with an elevated risk of cancer. Despite the well-known association of cancer with WS, there has been no systematic analysis of the type-specific risk of cancer in WS relative to general population risk. Nor has there been genomic characterization of neoplasms arising in WS patients.

Cancer risk: We used a newly assembled study population of 189 WS patients with 248 reported neoplasms to define the spectrum of neoplasia in WS. The most frequent neoplasms in WS patients, representing 2/3 of all reports, were thyroid epithelial malignancies, malignant melanoma, meningioma, soft tissue sarcomas, leukemia and pre-leukemic conditions of the bone marrow, and primary bone neoplasms. The fold elevation in these neoplasms, calculated as standardized incidence and proportionate incidence ratios (SIR and SPIR, respectively) relative to Osaka Japan prefecture, ranged from 53.5-fold for melanoma of the skin (95% CI: 24.5, 101.6) to 8.9 (95% CI: 4.9, 15.0) for thyroid neoplasms.

Tumor genomics: We used autopsy tissue from four mutation-typed WS patients to characterize pathologic and genomic features of WS, and to determine genomic features of three tumors arising in two WS patients. Targeted capture and next generation sequencing of 234 genes related to cancer diagnosis, treatment and prognosis on the UW Oncoplex platform identified non-synonymous somatic SNVs in *KRAS* and *TP53* in both pancreatic adenocarcinomas, together with a stopgain SNV in *SMAD4* in one patient together with genomic regions of both pancreatic carcinomas also displayed evidence of potential copy number variation. None of these features distinguished either WS pancreatic carcinoma from sporadic pancreatic carcinomas arising in the general population.

Take-homes: These analyses provide new information on WS pathology and genomics; provide a first genomic characterization of neoplasms arising in WS.

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Poster 11

Exploiting the therapeutic potential of homologous recombination modulators for the treatment of Bloom's Syndrome

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Homology-directed DNA repair (HDR) is a universal mechanism of DNA repair that is crucial for the preservation of genomic integrity in most species. However, excessive and unwarranted utilization of this repair mechanism destabilizes the genome and causes severe pathologies. The human BLM helicase, mutated in Bloom's syndrome (BS) patients, is a crucial regulator of HDR. Cells isolated from BS patients are characterized by an increase in sister chromatid exchanges, a hallmark of unrestrained HDR. In a previous study, we reported the discovery of Sws1, a conserved regulator of HDR, and showed that its inactivation suppresses the cellular defects of the BLM helicase homolog, *rqh1* (PMID: 16710300). At this meeting I will report the discovery of additional conserved Sws1-interacting factors in yeast and humans that also rescue *rqh1* defects. Our findings further corroborate that attenuation of HDR alleviates defects of RecQ-family mutations. I will also discuss the development of our proprietary drug-discovery platform that has enabled us to identify potent inhibitors of components of the HDR pathway, and the efforts we are currently undertaking to develop these leads into a treatment from Bloom’s Syndrome.

Poster 12

RECQL4 promotes DNA end resection in repair of DNA double-strand breaks

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DNA double-strand break (DSB) is a major contributor to genome instability and cell death and it can be repaired via homologous recombination (HR) in S and G2 phases of cell cycle. HR repair is initiated with 5' DNA resection at broken ends, which generates 3' protruding single-strand DNA for RAD51-mediated strand exchange. In mammalian cells, 5' DNA resection occurs in two steps: the initial resection with MRE11-RAD50-NBS1 (MRN) and CtIP, and then the extensive resection by EXO1 or DNA2/BLM/TOP3/RMI1/2. However, the regulatory mechanisms of this process are largely unknown. Human RecQ helicase RECQL4 is associated with three genetic diseases: Rothmund-Thomson Syndrome (RTS), RAPADILINO and Baller-Gerold syndrome, as well as cancers. We previously reported that lack of RECQL4 induces persistent DNA damage and triggers senescence, which contributes to the RTS features in the mouse model. However, it is unclear how RECQL4 participates in DSB repair. RECQL4 is highly expressed in S phase, when HR repair dominates. Thus, we investigated the possibility that RECQL4 plays a role in HR repair. Here, we found that RECQL4 co-localizes with DSB marker γH2AX at laser-induced DSBs, and depletion of RECQL4 causes cell sensitivity to ionizing radiation. Significantly, loss of RECQL4 reduces 75% of HR repair and 60% of DNA resection in U2OS cells, suggesting that RECQL4 plays an unrecognized but crucial role in HR repair and DNA resection. To gain insight into the role of RECQL4 in DNA resection, RECQL4-interacting proteins were pulled down from irradiated cells and identified by mass spectrometry. RECQL4 forms a complex with DNA resection players including MRN, CtIP, EXO1, DNA2 and BLM. Further, we found that MRN recruits RECQL4 to DSBs and that the exonuclease of MRE11 regulates the retention of RECQL4 at laser-induced DSBs. In vitro, RECQL4 also stimulates the exonuclease activity of MRE11, which is required for DNA resection. Interestingly, RECQL4 interacts with CtIP via its N-terminal domain and promotes CtIP recruitment to the MRN complex at DSBs to initiate resect ion. Moreover, inactivation of RECQL4's helicase activity impairs DNA resection and HR repair, indicating an important role for RECQL4's unwinding activity in the process. Thus, we identified a crucial role for RECQL4 in HR repair whereby it promotes 5' DNA end resection through MRN-CtIP. These findings promote understanding the molecular mechanism of RECQL4-associated diseases.