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14th International Workshop on Ataxia-Telangiectasia ATW2012

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Abstract

The fourteenth international Ataxia-Telangiectasia Workshop 2012 (ATW2012) (www.atw2012.com) on ataxia-telangiectasia (AT) and the role of the ataxia telangiectasia mutated (ATM) gene in DNA repair, neurological disease, cancer and related topics was held from February 07 to 11, 2012 in Delhi, India. The international ATW2012 meeting reported the latest advances in ATM research as well as potential therapeutic treatments for A-T. The meeting was attended by a productive mix of scientists, ranging from those prominent in the initial characterization of the underlying genetic defect to young scientists just entering the field. In broad terms, three main themes were discussed at the meeting: first, a wealth of new details emerged on DNA damage signaling/repair mechanisms for which ATM is a critical element; secondly, important functions for ATM in previously unrelated cellular pathways were identified; and thirdly, new physiological effects and potential therapeutic treatments related to A-T were presented. This report summarizes below a sampling of the many interesting results from the meeting.

Keywords

ATM; DNA repair; Chromatin structure; Cancer; Neurological diseases

1. Introduction

Ataxia telangiectasia (A-T) is a rare genetic disease that affects multiple organ systems and is characterized by neurodegeneration immunodeficiency, genomic instability, radiation sensitivity and cancer predisposition. A-T is caused by recessive mutations in the ataxia telangiectasia mutated (ATM) gene, which plays a pivotal role in regulating the cellular response to DNA damage caused by endogenous stresses (linked to metabolism) as well as exogenous agents like ionizing radiation (IR), environmental chemicals and viruses. Due to the critical roles of ATM in regulating cellular responses to DNA damage, research related

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to understanding of its involvement in DNA repair and the consequences of defective repair is important to a number of human diseases including neurodegenerative disorders, cancer, aging, etc. and has been extensively pursued throughout the world.

The meeting started with a keynote address by Michael B. Kastan (Duke University, Durham, NC), who spoke on Ataxia-Telangiectasia: bedside to bench to bedside. Dr. Kastan described a new role for ATM specifically in regulating mitochondrial homeostasis through clearance of damaged mitochondria by autophagy (mitophagy), suggesting that A-T should be considered, at least in part, as a mitochondrial disease. This talk was followed by sessions that focused on: (1) DNA Damage Response and Repair; (2) Cancer and Genomic Instability and; (3) Neurodegenerative Diseases.

Yosef Shiloh (Tel Aviv University, Tel Aviv, Israel) discussed a cardinal question in A-T research: tying ATM functions to cerebellar degeneration. He argued that this symptom may represent a general function ATM performs of streamlining the cellular responses to various genotoxic stresses. This function extends beyond ATM's documented role in regulating the response to one major DNA lesion – the double-strand break.

2. DNA damage responses and repair

2.1. Chromatin structure and DNA damage response

The critical role that chromatin structure plays during DNA repair in mammalian cells has begun to be explored. Yeast studies have shown that DNA packaging into chromatin regulates every stage of its repair process as well as cell survival afterwards. Based on the fact that repair proteins are highly conserved, new information regarding the role of chromatin structure in mammalian cells was presented. Penny Jeggo (University of Sussex, Brighton, UK) described the role of KAP1 in remodeling heterochromatin after DNA damage. KAP1 was previously shown to be an ATM substrate, being phosphorylated at S824 after DNA damage. She illustrated that S824 phosphorylation on KAP1 disrupted the interaction between KAP1 and CHD3, a component of the Nucleosome Remodeling and Deacetylase (NURD) chromatin remodeling complex, thus promoting histone acetylation and relaxation of the heterochromatin to facilitate DNA repair. This suggests that the restructuring of heterochromatin through regulation of nucleosome remodeling can also promote DNA repair. Along this same line, Dipanjan Chowdury (Harvard Medical School, Boston, MA) discussed that PP4C dephosphorylates KAP1 at S824 and regulates its role in chromatin compaction and gene expression. In addition, he showed that CHK2-mediated phosphorylation of another KAP1 residue, S473, played a role in enforcing the G2/M checkpoint after IR exposure and that PP4C dephosphorylates KAP1 at S473 to facilitate checkpoint recovery. Brendan Price (Dana Farber Cancer Institute, Boston, MA) described a new histone variant, histone H2A.Z, exchanged onto the nucleosomes at DSBs by p400 chromatin remodeling ATPase to form open chromatin domains, making these competent for DNA DSBs repair.

Jessica Tyler (MD Anderson Cancer Center, Houston, TX) discussed how Asf1 mediated H3 K56 acetylation drives reassembly of chromatin after DNA repair and surprisingly found that the resetting of chromatin was required to turn off the checkpoint response and the

completion of DNA repair was not sufficient for checkpoint recovery in budding yeast. She showed that the histone chaperone CAF-1 contributes to this chromatin assembly after DNA repair and that there is a transient patch of H3 K56 acetylation at the site of DNA repair due to chromatin assembly. Pandita's group (UT Southwestern Medical Center, Dallas, TX) has identified a chromatin modifying factor called MOF (Males absent on the first), a histone acetyltransferase specific for K16 of H4, essential for cellular viability and the DNA damage response (DDR). He reported that MOF is constitutionally phosphorylated; however, phosphorylation also increases post irradiation. Cells with normal ATM function, show an increase in MOF phosphorylation at T392, however, in cells deficient for ATM, the post irradiation increase in phosphorylation is delayed. The specific role of MOF phosphorylation and H4K16 acetylation on DNA DSB repair pathway choice is under investigation. Clayton Hunt (UT Southwestern Medical Center, Dallas, TX) discussed the significance of MOF and H4K16ac in T-cell development and genomic stability.

Junya Kobayashi (Kyoto University, Kyoto, Japan) reported that Nbs1 binds to RNF20, which ubiquitinates H2B. RNF20 knockdown cells had less Brca1 and fewer Rad51 foci, but the foci of ATM, γ -H2AX were normal. Simon Bekker-Jensen (University of Copenhagen, Copenhagen, Denmark) discussed the effect of ATM on HERC2 phosphorylation for RNF8 binding. DSBs trigger sumoylation of HERC2 and RNF168 in a PIAS4 (SUMO ligase) dependent manner. HERC2 sumoylation at damaged chromatin promoted its retention on damaged chromatin via a positive feedback mechanism.

2.2. Mechanisms of DNA DSB repair

Organisms have evolved two main pathways for DNA DSB repair: non-homologous end joining (NHEJ) and homologous recombination (HR). The DNA DSB repair pathway choice is dictated by inhibitory relationship between NHEJ and HR. Markus Lobrich (Darmstadt University of Technology, Germany) described characterization of a fast initial phase followed by a slow second phase of DSB repair as measured by disappearance of γ -H2AX foci post-IR exposure. He reported that the fast phase consisted of NHEJ-mediated repair, in both G1- and G2-phases of the cell cycle, whereas the slow phase consisted of CtIP-dependent HR. Jean Gautier (Columbia University, New York) studied the resection of DSBs in S-phase and in M-phase using *Xenopus* egg extracts. He found that the two-step mechanism of resection in yeast was evolutionarily conserved in S-phase *Xenopus* extracts, with the initiation of resection depending on MRN-CtIP followed by processive and extensive resection mediated by other nucleases and helicases. Stephen Meyn (University of Toronto, Toronto, Canada) argued that the MRN complex is important for the recruitment of ATM to DSBs, but not essential for maintaining the kinase at those sites.

Altaf Wani (Ohio State University, Columbus, OH) demonstrated that the recruitment of ATM and ATR at UV-induced lesions depends on protein-protein interactions of the kinases with XP-C and perhaps DDB2 proteins that directly bind to the DNA lesions. Suresh Bhaumik (Southern Illinois University, Carbondale, IL) discussed the mechanisms of transcription coupled DNA damage repair. Peter M. Burgers (Washington University, St Louis, MO) reported that the activation of yeast ATR (Mec1) depends on two aromatic

residues present in unstructured loops of the two activators TOPBP1 (Dpb11) and Rad9 (Ddc1).

The most common and physiologically relevant type of DSBs that occur in eukaryotic cells arise in the S-phase due to replication fork-related damage. Junjie Chen (MD Anderson Cancer Center, Houston, TX) discussed functional redundancy among these enzymes, showing that DSBs are generated during the S-phase when both SMARCAL1 and AH2 are depleted. David Cortez (Vanderbilt University School of Medicine, Nashville, TN) also described biochemical evidence showing that SMARCAL1 bound Holliday Junctions, and promotes branch migration of junctions – an activity that he proposed functions in regulating replication fork regression in response to DNA damage at replication forks. K. Muniyappa (Indian Institute of Science, Bangalore, India) discussed the mechanistic aspects of DNA replication fork reversal catalyzed by RuvA proteins.

The choice between NEHJ and HR repair was discussed by David Chen (UT Southwestern Medical Center, Dallas, TX). He showed that the NHEJ factor Ku80 rapidly binds to DSBs and inhibits recruitment of the HR factors. John Murnane (University of California, San Francisco, CA) reiterated that there was a differential processing of DSBs at telomeres versus interstitial sites in the chromosome.

Chromosome mis-segregation during mitosis, which induces chromosomal-breaks leading to nonreciprocal translocation events was discussed by Rene Medena (University Medical Center Utrecht, The Netherlands). He used monastrol, an inhibitor of the Eg5 kinesin motor protein that is necessary for bipolar spindle formation, to block cells at mitosis. Sherif El-Khamisy (University of Sussex, Brighton, UK) described that the Tdp1 deficiency resulted in an accumulation of ssDNA breaks. Tdp1 binds to UBEz1, a SUMO-conjugating enzyme that simulates Tdp1 at K111. Mutation of K111 in Tdp1 leads to more ssDNA breaks and ATM did not affect the interaction between Tdp1 and ssDNA breaks.

2.3. Oxidative stress and DNA damage repair

Several studies suggest that ATM protein kinase is involved in responses to oxidative stress as well. Tanya Paull (University of Texas, Austin, TX) discussed how ATM kinase promoted redox homeostasis by acting as a direct sensor of reactive oxygen species (ROS). Benjamin Chen (UT Southwestern Medical Center, Dallas, TX) reported that cells lacking DNA-PK also exhibited an increase in ROS, similar to cells lacking ATM. He further showed that cells lacking DNA-PKcs exhibited increased levels of activated (S1981 phosphorylated) ATM, and that this effect was specific to DNA-PKcs but independent of its NHEJ functions. B.S. Dwarakanath (Institute of Nuclear Medicine and Allied Science, Delhi, India) described that transient stimulation of glycolysis in cells with inhibitors of oxidative phosphorylation (e.g. DNP) showed enhanced DNA repair and better survival, which correlated with elevated levels of hexokinase II. Marian Carlson (Columbia University, New York, NY) described a role of ATM activation after oxidative stress and AMPK phosphorylation, thus underscoring the interface among DDR, ATM, and AMPK.

Sorab Dalal (Tata Memorial Centre, Mumbai, India), using FRET analysis, reported that 14-3-3 γ localized to centrosomes and that depletion of 14-3-3 γ resulted in a significant

increase in centro-some number, whereas loss of Cdc25C reverted this phenotype, suggesting that 14-3-3 γ plays a role in regulating centrosome duplication. Daman Saluja (Delhi University, India) described that p53 utilized Sin3B to regulate its transcriptional repression of heat shock genes as well as genes involved in regulation of cell cycle and apoptosis.

Simon Powell (Memorial Sloan-Kettering Cancer Center, New York, NY) highlighted the functional significance of Chk2-dependent S988 phosphorylation in BRCA1. Kienan Savage (Queen's University, Belfast, UK) reported that the association of BRCA1 with the transcription and splicing factor BCLAF1 was stimulated after DNA damage. Bin Wang (MD Anderson Cancer Center, Houston, TX) discussed functions of various components of the BRCA1 A complex that includes BRE, NBA1/MERIT40, BRCC36, CCDC98/Abra1, RAP80. Roberto Chiarle (Harvard Medical School, Boston, MA) reported that translocations occurred within the same chromosome and around the break site, suggesting the presence of chromosome territories.

The significance of oxidative stress-induced DNA damage repair in the nervous system was described by Sankar Mitra and Muralidhar Hedge (University of Texas Medical Branch at Galveston, TX). They detailed new links between heterogeneous ribonucleoproteins (hnRNP) during repair of DNA damage after oxidative stress and how oxidative stress-induced DNA damage is repaired in the nervous system. Sachin Katyal (St Jude Children's Research Hospital, Memphis, TN) described interconnections between tyrosyl DNA phosphodiesterase 1 and ATM during nervous system development. S. Nayler (Queensland Institute of Medical Research, Herston, Australia) reported that A-T iPS cells that failed to arrest in G2/M after IR exposure and are hypersensitive to IR. Gene expression analysis revealed that 500 genes involved in mitochondrial function and biogenesis were upregulated in AT mutant cells.

3. DNA repair and cancer

Proteins involved in DNA repair could act as oncogenes or tumor suppressors, perhaps through regulation of transcription. Kum Kum Khanna (Queensland Institute of Medical Research, Herston, Australia) reported that the Rad51 level was higher in brain metastases of breast cancers than in matched primary tumors, and that such overexpression promoted metastases and resistance to PARP inhibitors. In prostate tumors, Rad9 is overexpressed as discussed by Howard Lieberman (Columbia University, New York). He proposed that Rad9 acts as an oncogene and its high expression in prostate cancer is causally related to, at least, human prostate cancer growth. Thomas Ludwig (Ohio State University, Columbus, OH) highlighted the role of the RING finger (and polyubiquitylation activity through BARD1) of BRCA1, which is dispensable for tumor suppression, while the phosphopeptide binding activity of the BRCA1 BRCT repeats is essential for tumor suppression.

Michael White (UT Southwestern Medical Center, Dallas, TX) classified a large collection of lung cancer cell lines into 12 archetypes based on gene expression and copy number variation profiles and then screened exemplars of each archetype for susceptibility to siRNA or chemicals. The results raised the possibility of identifying unique cancer sensitivities

within specific molecular archetypes. Shantanu Choudhury (Institute of Genomics and Integrative Biology, Delhi, India) revealed that the metastasis suppressor protein NME2 acts as a transcriptional regulator of several genes, particularly as an inhibitor of vinculin, important for focal adhesion formation. Loss of NME2 in advanced lung cancer or by experimental manipulation upregulates vinculin and promotes cell invasion and establishment of metastases.

The telomerase enzyme, which maintains the telomere length is often found reactivated in tumor cells. Jerry Shay (UT Southwestern Medical Center, Dallas, TX) described how genome-wide human shRNA screening is being exploited to dissect pathways involved in telomerase action. During the initial approach, the Shay group knocked down candidate DNA repair proteins and demonstrated that many of the predicted factors such as ATM and Mre11 rescued cells from growth arrest. Thus, this novel genome-wide screening system may allow the identification of additional pathways and genes involved in telomerase action. Stephen Goff (Columbia University, New York, NY) recently developed genetic selections for virus-resistant mutant cells, which are being exploited for the selection to screen cDNA expression libraries or RNAi knockdown libraries for genes affecting retroviral vector transduction. Kristoffer Valerie (Virginia Commonwealth University, Richmond, VA, USA) discussed how targeting the DNA kinase could be a novel strategy for radiosensitizing glioblastoma multiforme in mice. David Boothman (UT Southwestern Medical Center Dallas, TX, USA), discussed a novel marker of genomic instability, which is ATM mediated IGF-I-sCLU expression. Nobuo Horikoshi (UT Southwestern Medical Center Dallas, TX) shed light how proapoptotic protein DAXX carboxyl terminal domain confers resistance to DNA damage. Walter N Hittelman (UT MDACC, Houston, TX) described how color-coding methodology provides unique opportunities for visualizing critical factors underlying genetic instability during tumor progression and therapy response.

Luis Parada (UT Southwestern Medical Center, Dallas, TX) discussed the origin of glioblastoma multiforme, and showed that it is derived from cancer stem cells in a mouse model with heterozygous NF1^{-/+} p53 mutations that underwent LOH leading to glioblastoma multiforme. He challenged the prevailing dogma that all cancers were clonal and hence all progeny cells identical. He showed that cells with distinct genotypes were present in tumor as illustrated by angiogenesis proficient cells. He favored the cancer stem cell idea, leading to a tumor cell hierarchy.

Anindya Dutta (University of Virginia, Charlottesville, VA) reported that the identification of microDNAs consisting of free circles of DNA in the embryonic mouse brain. Cancer cells have much higher levels of these micro DNAs. In general these are CG rich sequences, and in cancer cells they are about 200, 400, 600, 800 bp sizes. They came from genic areas, CpGs, exons and 5'-UTRs.

4. Neurodegenerative diseases

Defects in DNA damage signaling can result in human syndromes in which neurologic disease is a main feature. In the mature nervous system, DNA repair is also essential to prevent DNA damage arising from endogenous sources including oxidation and

transcription-associated DNA damage. Ari Barzilai (Tel Aviv University, Tel Aviv, Israel) described his new studies on defective vascular function observed in the retina of Atm deficient mice. His results revealed that glia cell abnormalities and altered vasculature might be important components of the neurological defects in A-T. Richard Gatti (UCLA School of Medicine, Los Angeles, CA) presented the latest promising results from his search for small molecule read-through (SMRT) drug-like molecules. These drugs are being developed to suppress the ATM non-sense mutations that occur in a high percentage of A-T patients and prematurely terminate the synthesis of functional ATM. Karl Herrup (Rutgers University, New Brunswick, NJ) presented data regarding epigenetic changes modulated by ATM. Using Atm deficient mice, his group identified alterations in histone deacetylases and histone methylation patterns in the nervous system, which suggested that ATM could influence the epigenetic profile of neural tissue that might be relevant to the brain pathology in A-T.

Peter McKinnon (St. Judes Childrens' Research Hospital, Memphis, TN) presented studies indicating the ATR activator, TopBP1 (Topoisomerase II-binding protein 1) was a key factor involved in preventing DNA strand breaks during replication stress in early neural progenitor and stem cells. Zhao-Qi Wang (Leibniz Institute for Age Research, Jena, Germany) presented additional data showing links between NBS1 (the gene mutated in the Nijmegen Breakage syndrome) and ATR. Martin Lavin (Queensland Institute of Medical Research, Herston, Australia) described the function of Senataxin, the gene mutated in the A-T-related syndrome ataxia with oculomotor apraxia 2 (AOA2).

In considering the clinical basis of A-T, Howard Lederman (John Hopkins University, Baltimore, MD) presented new findings indicating that A-T pathology is associated with increased levels of inflammation, which could contribute to the clinical spectrum of this disease. He presented details about widespread telangiectasia involvement in multiple organ systems and increased interstitial lung disease in A-T patients. Thomas Crawford (Johns Hopkins University, Baltimore, MD) discussed the key stages in the progression of A-T, which involves an initial decline in function beginning around 5–15 years of age, followed by a relatively stable phase, emphasizing that A-T was not an ingravescent disease.

Burcin Uygungil (from Howard Lederman's group at John Hopkins University, Baltimore, MD) discussed the utility of live vaccines for A-T patients and reported that vaccination records for about 300 A-T patients showed no adverse events.

In summary, the consensus of the participants in the five-day workshop was that the meeting was, very successful in disseminating, the current state of knowledge concerning the molecular mechanisms underlying genomic DNA damage repair, particularly with reference to the role of ATM and its impact on genomic instability, cancer and neurodegenerative disorders. The workshop not only generated new ideas to stimulate basic scientists to further elucidate the precise mechanisms underlying DNA repair, but also presented the clinical investigators with an increased basis with which to translate the research findings into clinical therapies for A-T patients. Given the intimate relationship between ATM and DNA damage signaling/repair, these advances will likely improve the management of many patients with cancer, genetic disorders and neurodegenerative diseases.

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