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The first Sino-German Symposium on DNA Repair and Human Diseases

ABSTRACT

The first Sino-German Symposium on DNA Repair and Human Diseases was held in the Capital Normal University, Beijing, China, from October 9th to 11th, 2010. It was initiated and organized by Xingzhi Xu and Zhao-Qi Wang with strong support from top scientists in the field from China, Germany and the United States. Financially, it was fully supported by the Sino-German Center for Science Promotion jointly founded by the National Natural Science Foundation of China (NSFC) and the Deutsche Forschungsgemeinschaft (DFG). This report summarizes 35 plenary lectures presented during this three-day symposium, with topics ranging from DNA damage checkpoint signaling, DNA repair, post-translational protein modifications in DNA damage response (DDR) to DDR in ageing and cancer. This symposium stimulated extensive discussions on science and potential collaboration among the 230 participants.

1. Introduction

Cells in our body are constantly under assault from exogenous factors (e.g. ultraviolet (UV) in the sunlight) and endogenous factors (e.g. free radicals generated from metabolic intermediates), and thus genomic DNA is damaged. Fortunately, the cell has evolved a set of highly efficient and complex DNA damage response (DDR) mechanisms, to arrest cell cycle progression, allowing time to repair damaged DNA, or to induce apoptosis if the damage is too severe to be repaired. There are two kinds of DNA repair: high fidelity and error-prone. Error-prone repair or defects in the high-fidelity repair will lead to gene mutation, accumulation of which would ultimately result in genome instability, which is a prominent feature of many human diseases, including ageing-related diseases and cancer. Apart from surgical removal, other core cancer treatments, such as chemotherapy and radiation therapy, adopts this mechanism to introduce DNA damage that is beyond repair to the tumor cells, hoping to trigger apoptosis. Therefore, research in the DNA repair and DDR is the key for us to understand how human diseases, particularly cancer and ageing-related diseases evolve and develop. Only based on these understandings can we improve current therapies and develop novel strategies. In summary, research on DNA repair and genomic stability is one of the most active and fruitful area in biomedical research.

With the common goal to communicate recent advances in DNA repair research and in identification of novel therapeutic targets of DNA repair factors, and to develop potential interdisciplinary collaborations, the first Sino-German Symposium on DNA Repair and Human Diseases was held at the Capital Normal University, Beijing, China, from October 9th to 11th, 2010. About 50 senior scientists from China, Germany, and the US as well as about 180 graduate students and postdoctoral trainees attended the meeting. This symposium was fully funded by the Sino-German Center for Science Promotion, a joint enterprise of the NSFC and DFG (www.sinogermanscience.org.cn). The scientists discussed the molecular mechanisms of DNA repair and its function in human disease development, such as ageing and cancer.

2. DNA damage checkpoints

A major focus of the meeting was on mechanisms that detect damaged DNA and transmit signals to halt cell cycle progression, induce apoptosis and adopt cellular metabolism to allow cells to cope with the damage. The underlying topic of the talks in this session was the close interplay between protein ubiquitylation and phosphorylation and how both processes co-operate to regulate checkpoint signaling processes. One striking example for this interplay arises from work on what is probably the best understood DNA damage checkpoint: the response to DNA double-strand breaks (DSBs) that is mediated by the ATM kinase. **Junjie Chen** (University of Texas MD Anderson Cancer Center, Houston) discussed models how both molecular processes co-operate to assemble signaling and repair complexes at sites of damage. Specifically, he discussed how the phosphorylation-dependent recruitment of the ubiquitin ligase RNF8 leads to localized protein ubiquitylation of histones and thereby recruits proteins that bind to poly-ubiquitin chains such as Rap80. Intriguingly, protein ubiquitylation can act in forms of signaling cascades, since ubiquitylated histones in turn recruit a second ubiquitin ligase, RNF168, which can further amplify the signal. A second related theme that was discussed by Chen is the role of the adaptor protein, TopBP1, which is a critical activator of the ATR kinase at stalled replication forks. TopBP1 contains multiple BRCT domains, which recognize phosphorylated serine and threonine residues, and therefore TopBP1 is thought to have a key role in assembling checkpoint complexes. Chen discussed recent findings that the BACH helicase, which was originally identified as an interactor of the BRCA1 tumor suppressor protein, binds to TopBP1 when phosphorylated and is required for loading of the single-strand binding protein RPA to chromatin.

Similarly, ubiquitylation is a key function of the Fanconi-Anemia (FA) complex, which mediates cellular responses to and repair of DNA interstrand cross-linking. Eight of the FA proteins form an ubiquitin ligase complex that mono-ubiquitylates two additional proteins, FANCI and FANCD2 (ID). **Jun Huang** (Zhejiang University, Hangzhou) described the role of a novel nuclease (FAN1) in this complex. FAN1 promotes repair of interstrand cross-links and is recruited to sites of damage in a manner that depends on mono-ubiquitylation of the ID complex, reiterating the theme that localized protein ubiquitylation serves to assemble checkpoint and repair complexes.

One further example was discussed by **Martin Eilers** (University of Würzburg, Würzburg). Previous work from several laboratories had suggested that down regulation of expression of the MYC oncoprotein is an important element of cell cycle arrest in response to DNA damage checkpoints, since forced MYC expression renders cells unable to halt cell cycle progression in response to DNA damage. Similarly MYC levels increase rapidly when cells recover from a checkpoint-mediated cell cycle arrest. MYC is continuously turned over by proteasome-mediated degradation and the FBW7 ubiquitin ligase plays a key role in this process. Eilers showed that FBW7 can target the amino-terminus of MYC, but that after DNA damage alternate ubiquitin chains are assembled on the amino-terminus that are inefficient in mediating MYC turnover. These chains are assembled by the beta-TrCP ubiquitin ligase, which interacts with MYC in a manner that depends on polo-like kinase 1 (PLK1). PLK1 has a key role in reactivating cell cycle progression after a DNA damage-mediated arrest, and these data suggest that stabilizing MYC is a downstream effector of PLK1 in this process.

3. DNA repair and mutagenesis

DNA repair and mutagenesis was a core topic in this meeting and several talks were on two processes, namely double-strand break repair (DSBR) and translesion DNA synthesis (TLS).

Markus Löbrich (Darmstadt University of Technology, Darmstadt) reported that DSBR consists of slow and fast repair components. It became clear that while non-homologous end joining (NHEJ) comprises the majority of repair in both G1 and G2, homologous recombination repair (HRR) only represents the slow repair component in G2. Interestingly, *ATM* and *Artemis* mutant cells are highly sensitive to ionizing radiation (IR) and defective in the slow repair component in both G1 and G2, whereas only their G2 repair portion is related to HRR. Indeed ATM and Artemis operate in the same pathway as BRCA2 and RAD51. Since DSBs located within heterochromatin are repaired with slow kinetics, it was speculated that ATM is specifically required for the repair of DSBs associated with heterochromatin. Further confirmation came from an observation that depletion of KAP-1 overrides the requirement for ATM for repair. Through studies of genetic interactions between *CtIP* and *Artemis*, Löbrich proposed a working model in which ATM phosphorylates both CtIP and KAP-1 in response to IR, which remodels heterochromatin and creates substrates for Artemis-mediated DSB end resection that leads to HRR.

George Iliakis (University of Duisburg-Essen, Essen) briefly reviewed the history of discovery of an alternative NHEJ pathway (A-NHEJ; or B-NHEJ, backup NHEJ) of DSB repair that operates as a backup to the standard DNA-PK dependent pathway of NHEJ (D-NHEJ; or C-NHEJ, canonical NHEJ). This presumed backup NHEJ was recently brought to the forefront by a series of discoveries for its involvement in IgH class switch and V(D)J recombination as well as the maintenance of the stability of chromosomes and telomeres. In experiments designed to characterize these pathways and to separate their roles in NHEJ, it was found that B-NHEJ requires DNA ligase III/XRCC1 instead of ligase IV/XRCC4. In addition, histone H1 and PARP-1 were also found to promote B-NHEJ. In order to further address the biological functions mediated by B-NHEJ, Iliakis' group took advantage of chicken DT40 cells and made conditional *LIG3* knockout lines as well as *LIG1* and *LIG4* knockout cells. It is expected that the investigation of combinations of DNA ligase mutations will shed light on the roles of different NHEJ subpathways in immune system development, DSBR and the maintenance of genomic stability.

Frank Grosse (Leibniz Institute of Age Research–Fritz Lipmann Institute, Jena) reported the characterization of the DNA/RNA helicase *DHX9*. Of particular interest to DNA repair are the physical and genetic interactions between DHX9 and the WRN helicase. While both helicases unwind DNA–RNA hybrids, WRN preferentially unwinds Okazaki fragment-like hybrids. Interestingly, DHX9 stimulates the above as well as other activities of WRN, including the 3′–5′ exonuclease activity on D-loop DNA and the resolution of a chicken-foot structure. Hence, it is speculated that DHX9 and WRN may cooperate in unwinding stalled replication forks.

Yun-Gui Yang (Beijing Institute of Genomics, CAS, Beijing) reported his attempt to search for *Nbs1*-extragenic-suppressor (NESR) factors repressing cellular lethality caused by *Nbs1* depletion in response to DSBs. The Nbs1/Mre11/Rad50 (MRN) complex possesses endonuclease activity, generating 3′-overhang ssDNA essentially required for HRR. Deletion of any member of the MRN complex leads to lethality at the cellular and embryonic levels. Strikingly they obtained viable *Nbs1* null ES cells by sequential gene targeting to disrupt both alleles and also derived its null fibroblasts. Interestingly, those cells display normal p53 activation and defective ATM/ATR signalling with only a mild reduction of HRR. These data suggest that the NESR factors may retain endonuclease activity required for the remaining HRR, and *Nbs1* depletion-induced lethality is independent of p53.

In his keynote speech, **Errol Friedberg** (University of Texas Southwestern Medical Center at Dallas, Dallas) summarized research advances in the last decade since the discovery of specialized DNA polymerases. To date nine specialized DNA polymerases have been discovered, belonging to A (Polθ, Polν), B (Polζ), X (Polλ, Polμ, Polσ) and Y (Polη, Polι, Polκ and Rev1) families. Of particular interest with regard to mutagenesis are Y-family polymerases because of their low fidelity and lack of proofreading activity. Friedberg suggested two future directions for the study of these polymerases. Firstly, do any of these polymerases have functions besides TLS? This question has been at least partially answered since some of them have been shown to participate in somatic hypermutation, class switch, NER and the repair of DSBs. Secondly, do any of these polymerases support TLS in living cells? And if so, why are vertebrates endowed with so many of them? Friedberg's group has made interesting inroads in both directions through characterizing Polκ. By using a phage λ *cII* reporter gene assay, it was found that *Polκ* null mice have an increased mutation frequency in an organ-specific manner, with predominantly GC transversions. It is interesting that the mouse *Polκ* promoter contains two binding sites for arylhydrocarbon receptor (AhR) proteins and the *Polκ* expression is induced by polycyclic aromatic hydrocarbons, among which benzo[*a*]pyrene diol epoxide (BPDE) is best characterized. Indeed, purified Polκ is able to efficiently bypass a variety of N^2G-BPDE derivatives. However, Friedberg effectively argued that other natural metabolic products, most likely cholesterol instead of BPDE derivatives, may be the cognate substrates for Polκ. Since cholesterol is the precursor for all steroid hormones, whose metabolic products include numerous polycyclic compounds, Polκ may play an important role in limiting spontaneous mutagenesis in an organ specific manner.

Caixia Guo (Beijing Institute of Genomics, CAS) found that Polκ is highly expressed in specific meiotic and post-meiotic cells with no ongoing DNA replication. This and other observations led her

to propose that Polκ is involved in the repair of strand-breaks. By a variety of other means, including adjusting the 365-nm pulsed nitrogen laser microirradiation to favor SSBs induction, Gou was able to show that Polκ physically interacts with XRCC1 and also colocalizes with XRCC1 after laser irradiation in a manner distinct from its recruitment to stalled replication forks. Since Polκ mutant is hypersensitive to H_2O_2, Guo has proposed that Polκ might play a role in SSBR as well as in TLS.

Polη is a well-characterized TLS polymerase specialized in bypassing UV-induced T<>T dimers with relative high accuracy; however, like other Y-family polymerases, Polη is highly distributive in DNA synthesis and may require other specialized polymerases to complete TLS. **Wei Xiao** (Capital Normal University, Beijing) attempted to assess the sequential assembly of native TLS polymerases after UV irradiation. It was found that REV1 and Polη are independently recruited to the UV-induced nuclear foci, whereas the recruitment of REV3, the catalytic subunit of Polη, is dependent on REV1 but independent of Polη. This finding is consistent with the two-polymerase or the polymerase switch model and helps to clarify issues related to the *in vivo* TLS assembly.

Cytosine methylation and its subsequent deamination can be a major source of mutagenesis. **Guoliang Xu** (Institute of Biochemistry and Cell Biology, CAS, Shanghai) found that the cytosine methyltransferase DNMT3a interacts with TDG and stimulates its glycosylase activity, whereas TDG suppresses the DNMT3a activity. The DNMT3a–TDG interaction was also found to play an unexpected role in cyclical DNA methylation in the transcriptionally active promoter. Recently, Xu has turned his attention to the biogenesis of 5-hydroxymethylcytosine, the sixth base of DNA.

Due to its extreme radioresistance, *Deinococcus radiodurans* has been considered an ideal model organism for the study of DNA repair. In this meeting, **Yuejin Hua** (Zhejiang University, Hangzhou) reported recent advances made by his team in the characterization of PprI. PprI appears to be a key transcriptional regulator in response to DNA damage and its targets include important radioresistance genes such as *recA* and *pprA*, as well as those newly identified through 2D gel and microarray analyses. With the identification of the PprI DNA-binding domain and its target sequences as well as its physical interaction with RNA polymerase, it is expected that molecular mechanisms of this regulatory network will soon be unveiled.

4. Post-translational modifications in DDR

Post-translational modifications (PTMs) of proteins are a major means to regulate cellular pathways. The dynamic and reversible nature of PTMs is particularly suitable for promptly initiating signaling events in response to genotoxic stress and properly terminating the signal after successful repair of DNA lesions. The use of sensitive detection methods such as mass spectrometry has led to an ever-increasing list of PTMs on DNA damage response proteins. It is a difficult task to understand the physiological functions of these PTMs. The presentations given by seven speakers on related topics in this meeting, however, gave the encouraging message that fruitful insights can be gained by our continuing efforts.

Ping-Kun Zhou (Beijing Institute of Radiation Medicine, Beijing) presented data from a quantitative phosphoproteomic analysis of *ATM*-deficient cells treated with a DNA-PKcs inhibitor. Several hundred phosphoproteins were identified, and among them were proteins whose phosphorylation levels were down-regulated by the DNA-PKcs inhibitor. Some of the potential DNA-PKcs targets identified by this study are proteins involved in mitotic progression, consistent with a recent report from the same group showing that inactivation of DNA-PKcs results in mitotic progression failure in response to DNA damage.

Haiying Hang (Institute of Biophysics, CAS, Beijing) reported a novel PTM mechanism regulating the function of RAD9, a subunit of the heterotrimeric 9-1-1 checkpoint clamp complex. Hang's group identified PRMT5, a protein arginine methyltransferase, as a RAD9-interacting protein. They went on to show that RAD9 is a methylated protein *in vivo* and PRMT5 can catalyze its methylation in vitro. Three arginine residues on RAD9 were identified as methylation sites by PRMT5. Mutating these residues to alanines leads to defects in survival against genotoxic treatment, checkpoint failures, and reduced CHK1 phosphorylation.

DNA damage response signaling is mainly driven by protein phosphorylation. Turning off the signal by protein phosphatases may just be as important as turning on the signal by kinases. **Xingzhi Xu** (Capital Normal University, Beijing) presented works from his group on phosphatases PP4 and PP6. The catalytic subunit of each phosphatase forms multiple complexes with a myriad of regulatory subunits, thereby acquiring additional specificity for substrate selection. Xu's group found that among the several known PP4 complexes in mammalian cells, the PP4c–PP4R2–PP4R3β complex is specifically required for dephosphorylating γ-H2AX generated during replication. They showed that PP6 also acts on γ-H2AX, with a partition of jobs between different PP6 complexes depending on the type of DNA lesions. PP6R1-containing complex dephosphorylates γ-H2AX after IR treatment, whereas PP6R2-containing complex targets γ-H2AX after camptothecin (CPT) treatment. Interestingly, using I-SceI-based DNA repair assays, they discovered that both PP4 and PP6 are required for homology-dependent repair.

Increasing evidences have implicated protein kinase CK2 in the regulation of DNA damage response. **Shuanglin Xiang** (Hunan Normal University, Changsha) described a CK2-mediated phosphorylation of a PCNA-binding protein TNFIP1. Phosphorylated form of TNFIP1 translocates from cytoplasm to nucleus and influences the transcription activity of AP-1 and NFκB. Hydroxyurea (HU) treatment stimulates the phosphorylation of TNFIP1, suggesting a potential link to DNA damage response.

Poly(ADP-ribosyl)ation is important for a number of nuclear events including DNA repair. **Zhao-Qi Wang** (Leibniz Institute for Age Research-Fritz Lipmann Institute, Jena) reported a novel role of poly(ADP-ribosyl)ation in intra-S-phase checkpoint. His group found that *Parp1*$^{-/-}$ and *Parg110*$^{-/-}$ cells defective in this modification are hypersensitive to HU, and are unable to fully activate CHK1 upon HU treatment. Furthermore, he showed that HU-induced CHK1 foci are dependent on poly(ADP-ribose) (PAR) and Chk1 binds to PAR *in vivo*. Inspecting *Chk1* sequence led to the identification of a novel PAR binding motif in CHK1. Mutation of this motif abolished CHK1–PAR interactions and resulted in intra-S-phase checkpoint defects.

Stefan Jentsch (Max Planck Institute of Biochemistry, Martinsried) presented a number of works elucidating the multi-facet roles of ubiquitylation and SUMOylation in DNA damage tolerance and repair. He showed that the *RAD6*-dependent DNA damage tolerance pathways, which involve PCNA ubiquitylation, are not necessarily coupled to S phase and remain fully functional when restricted to G2. Jentsch has also reported that the histone variant H2A.Z is important for initial processing of DSBs and SUMOylation of H2A.Z is required for tethering a persistent DSB to nuclear periphery. He discussed these findings and suggested that DSB relocation to the periphery results from a *RAD51*-dependent homology search process.

Berit Jungnickel (Friedrich Schiller University, Jena) introduced the special roles of DNA damage response machinery in the adaptive immune system of vertebrates, in particular, the involvement of *RAD6*-dependent PCNA ubiquitination pathways in somatic hypermutation. Her group has explored the possibility that p53 may influence somatic hypermutation through the PCNA

ubiquitination pathway. She showed that p53 inhibition leads to increased hypermutation in cell lines, and hypermutation is moderately increased in $p53^{-/-}$ mice.

5. DDR in cancer

Lisa Wiesmüller (University of Ulm, Ulm) started the session on DNA damage responses in cancer firstly summarizing data on two signalling nodes downstream of ATM: p53 and NF-κB. Whereas p53 inhibits HR via transcriptional repression of the *RAD51* gene and inhibitory physical interactions with the RAD51 protein itself, NF-κB stimulates HR via transcriptional activation of target genes involved in DNA strand exchange and stimulatory interactions with the end-processing machinery. Both factors have been associated with breast cancer development, supporting the critical role of HR dysfunction in the etiology of this disease. When analysing DSB repair activities in cells from individuals with defined or unknown mutations in breast cancer susceptibility genes, indeed, a shift from HR to error-prone pathways like single-strand annealing (SSA) and NHEJ became apparent. These data suggest that detection of distinct DSB repair activities can serve as a powerful tool for the identification of breast cancer susceptibility beyond the limits of genotyping.

Since DNA repair defects involving poly(ADP-ribose) polymerase (PARP-1) have been implicated in cerebellar medulloblastomas (MBs) development, **Wei-Min Tong** (Chinese Academy of Medical Sciences, Beijing) employed a model for MBs derived from *Parp-1* and *p53* deficient primitive neuroectodermal neoplasia (PNET) mouse with dysfunctional Shc/Ptc pathway. *Parp-1*-deficient pre-neoplastic cerebella showed a significant accumulation of DNA breaks during tumor progression. Different from postmitotic neurons, *Parp-1* deficient primary MB cells and tumors show a high expression of the HR genes *BRCA1* and *RAD51* in medulloblastoma but not of *Nbs1* or the NHEJ genes *Ku70* or *Ku80*, suggesting the overaction of the HR pathway during neuronal tumorigenesis.

Tiebang Kang (Sun Yat-sen University Cancer Center, Guangzhou) focused on the mechanisms underlying increased radiosensitivities, genomic instabilities and checkpoint signalling deficiency after hSSB1 depletion. Surprisingly, p21 is found to be down-regulated independently of p53, which is accompanied by a decrease of the cellular G1 fraction. Importantly, hSSB1 and p21 expression correlate in hepatocellular carcinoma (HCC) emphasizing the intricate links between DNA repair and ubiquitin-dependent degradation of key cell cycle regulators in tumorigenesis.

Advancing one step further to the clinical applications, **Bernd Kaina** (University of Mainz, Mainz) gave an overview of his work on the DNA repair and checkpoint mechanisms thwarting anticancer drug treatments of malignant gliomas and melanomas. Alkylating agents are first line therapeutic drugs inducing DNA lesions such as O^6-methylguanine, which is repaired by MGMT, a promising drug resistance marker in gliomas. Kaina demonstrated that O^6-methylguanine induced cell death is executed by activation of receptor and mitochondrial apoptotic pathways and involves mismatch repair driven DSB formation. He also showed that HR, but not NHEJ, effectively protects against genotoxicity and cell death in response to O^6-methylguanine. Complicating the picture, glioblastoma cell lines mutated in *p53* show better responses to chloroethylating but weaker ones to methylating anticancer drugs. On the other hand, melanoma cell lines with mutated *p53* display higher sensitivities to both types of therapeutic agents. Kaina proposed that the differential response in glioblastoma and melanoma cells could be explained by a dual role of p53 in apoptosis via cell death receptor regulation and repair gene activation.

Using *p53* mutated and wild-type human tumor cell lines as well as $p53^{-/-}$ and $p53^{+/+}$ mouse embryonic fibroblasts **Si-Qing Zhang** (Xiamen University, Xiamen) studied p53-independent cell death mechanisms in response to DNA damage induced by etoposide, cisplatin or MMS treatment. Starting from the initial observation that the Bcl-2 family member Bim is transcriptionally upregulated upon etoposide treatment, Zhang discovered a *p53*-independent DNA damage response pathway involving AKT kinase dependent phosphorylation of the transcription factor FoxO3a and downstream transcriptional induction of Bim. Clearly, with the majority of tumors showing a defect in the p53 signalling pathway, these findings shed light on potential new markers and targets for cancer therapies.

Starting from the notion that foci of the HR and SSA protein RAD52 mark sites of spontaneous DNA damage, in particular regions of single-stranded DNA, **Li-Lin Du** (National Institute of Biological Sciences, Beijing) used RAD52-specific ChIP-seq as a potential method to map so-called fragile sites in the *Schizosaccharomyces pombe* genome. Localization at defined HO-induced breaks and colocalization with RPA as well as signal intensification in mutants with defects in genome integrity maintenance confirmed the basic assumption. Du then found RAD52 binding at transcriptionally highly active genes suggesting that strong transcription activities generate vulnerable regions in the genome. Interestingly, his new approach also indicated a requirement for a H3-K56 deacetylase in maintaining replication fork stability.

Lars Zender (Helmholtz Centre for Infection Research, Braunschweig) addressed the issue of tumor cell clearance by the immune system. Employing different mouse models Zender showed that HCCs were formed as a consequence of oncogenic Nras (NrasG12V) expression in $p53^{-/-}$ liver progenitor cells but not in $p53^{-/-}$ hepatocytes. However, knockout of *p19Arf* or p19Arf knockdown in a $p53^{-/-}$ background de-repressed the formation of aggressive HCCs upon NrasG12V expression in hepatocytes. Also, Zender presented work on how the cellular senescence program and the immune system interact to suppress tumorigenesis in the liver.

6. DDR in ageing

Zhenyu Ju (Chinese Academy of Medical Sciences, Beijing) focused on cell intrinsic and extrinsic checkpoints in stem-cell ageing. To further define the importance of systemic regulating factors, Ju and colleagues transplanted bone or thymus into the kidney capsule of late-generation telomerase-deficient mice ("G3-mice") or wild-type mice. Strikingly, the systemic environment of young wild-type mice could regenerate the atrophic thymus from ageing G3-mice. Similarly, these experiments showed that decrease in B-lymphopoiesis in response to telomere dysfunction is mainly induced by alterations in the systemic environment. B-lymphopoiesis was also rescued when hematopoietic stem cells (HSCs) of G3 telomere dysfunctional mice were transferred into a wild-type environment. The group is currently analyzing whether alterations in the systemic environment can also induce permanent, cell intrinsic defects in HSCs, which may not be rescued by re-exposure to a wild-type environment. Taken together, not only telomere dysfunction seems to induce cell-intrinsic checkpoints but also alterations in the environment lead to age-associated stem cell failure.

Hartmut Geiger (University of Ulm, Ulm) reported on DNA damage mechanisms in HSCs in ageing. His group discovered that aged progenitor cells show increased survival after irradiation compared to young ones, while no survival difference is detected between aged and young HSCs. Likewise, studying the response of

aged hematopoietic stem and progenitor cells (HSPCs) in *in vivo* transplantation experiments, differences were detected in the susceptibility of hematopoietic progenitor cell population but not in stem cells. In summary, hematopoietic progenitor cells, but not stem cells, from aged mice are more resistant to DNA damage compared to cells from young animals. Thus the more likely cancer-initiating cell in age-related cancer could be a hematopoietic progenitor cell.

Yu-Sheng Cong (Beijing Normal University, Beijing) spoke about the implication of the essential caveolae component PTRF/cavin-1 in cellular ageing and cancer. Cellular senescence has been proposed to contribute to ageing and age-related pathologies including cancer. Cellular senescence may also act as an *in vivo* tumor suppression mechanism that prevents oncogenic transformation. PTRF was first identified as an RNA polymerase I and transcript release factor, and its essential functional role in caveolae biogenesis and function was recently demonstrated. Cong showed that PTRF is upregulated during cellular senescence and acts as a novel regulator of cellular senescence through the p53/p21 and caveolae pathways. On the other hand, they found that PTRF is downregulated in breast cancer cell lines and breast tumor tissues, with epigenetic silencing playing an important role. Overexpression of PTRF inhibits breast cancer cell proliferation whereas shRNA-mediated silencing extends replicative life span. Therefore, PTRF may be implicated both in ageing and in cancer, which further supports the concept that ageing and cancer may share common biological principles.

K. Lenhard Rudolph (University of Ulm, Ulm) addressed the role of DNA damage checkpoints in telomere dysfunction-induced ageing. Telomere shortening limits proliferative lifespan of human cells by activating the tumor suppressor protein p53, which induces either cell-cycle arrest (senescence) or apoptosis. *In vivo*, telomere shortening impairs stem cell function, organ maintenance and lifespan. Previously, Rudolph has shown that deletion of *p21/Waf1* in telomerase-deficient mice partially rescues the organism defects induced by telomere dysfunction. The group is currently analyzing the role of *p53/Puma*-dependent apoptosis in the context of ageing and telomere dysfunction. It remains to be seen whether p53 has additional functions beyond cell-cycle arrest and apoptosis in ageing tissues harboring dysfunctional telomeres.

Genomic instability is a well-known causal factor for carcinogenesis and was recently also identified as a major factor contributing to ageing. **Björn Schumacher** (University of Cologne, Cologne) reported on the role of DNA damage in ageing and longevity. Various skin cancer susceptibility and progeroid syndromes are linked to defects in nucleotide excision repair. Based on genome-wide comparative correlation analysis, Schumacher's group discovered striking similarities between progeroid mouse models and mice with extended longevity. They identified a response program to persistent DNA damage *in vivo* as well as in cultured cells. Low levels of persistent DNA lesions that interfere with RNA polymerase II-mediated transcript elongation lead to attenuation of somatotropic genes, which is linked to enhanced stress resistance and extended longevity. Sensing of low levels of persistent DNA damage by RNA polymerase II could therefore provide a mechanistic basis for hormesis and the shift from growth to somatic maintenance in ageing.

Jianwei Zhou (Nanjing Medical University, Nanjing) focused on ageing-related diseases in mice due to deficiency of the protein JWA/Arl6ip5. This protein is responsive to environmental stimuli such as heat shock and oxidative stress and acts as a regulator of XRCC1 in base excision repair. Using conditional knock-out mice, Zhou's group showed that JWA inactivation enhances the expression of cellular senescence markers p16 and p21 and induces an ageing-like phenotype *in vivo*. Furthermore JWA plays a role as a negative regulator of NF-κB and silencing of NF-κB with specific shRNA can antagonize cellular senescence resulting from JWA deficiency. Taken together, the results suggest that unrestricted NF-κB signaling due to loss of JWA may contribute to premature and normal ageing.

Alexander Bürkle (University of Konstanz, Konstanz) presented the design of an ongoing EU-funded multi-center study on biomarkers of human ageing, which he is directing, called MARK-AGE. Biomarkers of ageing have been defined as age-related changes in body function or composition that could serve as a measure of biological age. So far no single parameter has proven to yield a useful biomarker of ageing on its own, probably due to the multicausal and multi-system nature of ageing. The strategy, therefore, is to identify biomarkers of ageing which, as a combination of parameters with appropriate weighting, would measure biological age better than any marker in isolation. The largest group of subjects under MARK-AGE is randomly recruited age-stratified individuals from the general population, covering the middle-age range. A wide range of candidate biomarkers is tested in each of these individuals. Bioinformatics will be used in order to extract a robust set of biomarkers of human ageing.

7. Outcomes and perspectives

Scientific communication and exchange of ideas is the beginning of potential collaborations. This symposium is for the first time brought together scientists of China and Germany and also guest speakers of the US in the DNA repair field to discuss the current progress and future directions. This has provided such an opportunity in particular with respect to collaborations between Chinese and German scientists. To consolidate this possibility, the Sino-German Center for Science Promotion will provide funding to the invited Chinese and German attendees of this meeting for their bilateral joint proposals. As this report was being written, a few joint applications were on their way to the Sino-German Center for Science Promotion. Therefore, we have many reasons to believe that this successful symposium will efficiently promote bilateral collaborations and exchange of young scientists between Chinese and German laboratories, deepen the DDR research and ultimately transform the research findings into products for diagnosis and therapies of human disease.

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