AGING BIOLOGY AND LONGEVITY

P11-01
TELOMERE AND CELLULAR AGING REGULATION IN *S. CEREVISIAE*

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Telomeres are the physical ends of eukaryotic chromosomes. They are essential for chromosome integrity and genome stability. Functional telomeres potentate the continuous cell division and prevent the cells from undergoing cellular senescence. The replication of telomeric DNA is achieved by a specialized reverse transcriptase telomerase or homologous recombination in the absence of telomerase. Telomeres represent a heterochromatin domain that usually represses the expression of neighbor genes. In this presentation, I will present and discuss our recent studies on regulation of telomere replication/recombination, telomere heterochromatin formation and cellular replicative aging in budding yeast Saccharomyces cerevisiae.

P11-02
ER STRESS INDUCES EXPRESSION OF TELOMERASE REVERSE TRANSCRIPTASE

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Human telomerase is a ribonucleoprotein enzyme complex that is minimally composed of a RNA component (hTR or hTERC) and the telomerase reverse transcriptase (hTERT). Telomerase has fundamental roles in aging and in cancer. However, the pathways and molecular mechanisms regulating telomerase remain incompletely understood. Importantly, telomerase appears to have telomere-independent functions in a number of fundamental cellular processes. The endoplasmic reticulum (ER) is a cytosolic membrane network connected to the nucleus, mitochondria, and the plasma membrane. The ability to respond to perturbations in ER function (ER stress) is a fundamental important property of cells. In this report, we discovered that ER stress transiently activates the catalytic components of telomerase expression in human cancer cell lines and murine primary neural cells. Importantly, we show that depletion of hTERT sensitizes cells to undergo apoptosis under ER stress, whereas increased hTERT expression reduces ER stress-induced cell death independent of catalytically active enzyme or DNA damage signaling. Our findings establish a functional link between ER stress and telomerase, both of which have important implications in the pathologies associated with aging and cancer.

P11-03
YAP/TEAD-MEDIATED TRANSCRIPTION REGULATES THE CELLULAR SENESCENCE

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Transcription co-activator YAP plays an important role in the regulation of cell proliferation and apoptosis. Here, we identify a new role of YAP in the regulation of cellular senescence. We find that the expression levels of YAP proteins decrease following the replication-induced cellular senescence in IMR90 cells. Silencing of YAP inhibits cell proliferation and induces premature senescence. In additional experiments, we observe that cellular senescence induced by YAP deficiency is TEAD- and Rb/p16/p53- dependent. Furthermore, we show that Cdk6 is a direct downstream target gene of YAP in the regulation of cellular senescence, and the expression of Cdk6 is through the YAP-TEAD complex. Ectopic expression of Cdk6 rescued YAP knockdown-induced senescence. Finally, we find that down-regulation of YAP in tumor cells increases senescence in response to chemotherapeutic agents and YAP or Cdk6 expression rescues cellular senescence. Taken together, our findings define the critical role of YAP in the regulation of cellular senescence and provide a novel insight into a potential chemotherapeutic avenue for tumor suppression.

P11-04
ION CHANNELS AS EPIGENETIC REGULATORS: A MICRORNA PERSPECTIVE

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Alteration of MicroRNA (miRNA) in response to changes in environmental cues has been considered as one of the major mechanisms for epigenetic regulation of the cell. While most studies focus on the understanding of the role of miRNAs in regulating cellular responses to microenvironment changes, the mechanistic insight into how extracellular signals can be transduced into miRNA alterations in cells is still lacking. As membrane proteins, ion channels can respond to a wide variety of

extracellular signals. Interestingly, recent studies have shown that ion channels also exhibit changes in levels of expression and activities in response to changes of extracellular microenvironment. More importantly, alterations in expression and activation of ion channels have been shown to result in changes in miRNAs. In this talk, I will summarize the recent data demonstrating the ability of ion channels to transduce extracellular signals into miRNA changes and propose a potential epigenetic regulation mechanism that is mediated by ion channels. Evidence will be provided shedding new insights into epigenetic regulatory mechanisms underlying a number of physiological and pathological processes, including embryo development and cancer metastasis.

P11-05
LET-7 REGULATES CELLULAR LIFE SPAN BY REPRESSING P66SHC TRANSLATION

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The p66Shc adaptor protein is an important regulator of life span in mammals, but the mechanisms responsible remain unclear. Here we show that expression of p66Shc, p52Shc, and p46Shc is regulated at the post-transcriptional level by the microRNA let-7a. The levels of let-7a correlated inversely with the levels of Shc proteins without affecting Shc mRNA levels. We identified 'seedless' let-7a interaction elements in the coding region (CR) of Shc mRNA; mutation of the 'seedless' interaction sites abolished the regulation of Shc by let-7a. Our results further revealed that let-7a-repressed p66Shc, but not p52Shc or p46Shc, restrains senescence of human diploid fibroblasts (HDFs). In sum, our findings link let-7a abundance to the expression of p66Shc, which in turn controls the replicative life span of HDFs.

P11-06
REGENERATING CRYPTS RETAIN HYPERPROLIFERATING STATE BY SUPPRESSING GENOMIC SURVEILLANCE FUNCTIONS AT THE EXPENSE OF GENOMIC STABILITY

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It has been reported that cultured mammalian cells cannot sustain cell cycle arrest in a long run after the number of DNA double strand breaks drop to a relative low level and enter mitosis with DNA breaks. Here we aimed at assess the checkpoint activation and cell cycle restarting in process of intestinal crypt regeneration. Crypt regeneration of intestine was induced by a single dose of 12 Gy abdominal irradiation. γ-H2AX, 53BP1 were used a used as DNA repair surrogates to investigate the inherent ability of intestinal crypt cells to recognize and repair double-strand breaks. The Ki67 staining and 5-bromo-2′-deoxyuridine incorporation assay were used to study patterns of cell proliferation in regenerating crypts and ATM, p53, Check2 staining to study checkpoint activation and release. Apoptosis was evaluated by HE staining and Terminal deoxynucleotidyl transferased UTP nick end labeling. After reaching to very low levels after irradiation, the DSBs in crypt stem cells rose again in crypts underwent regeneration. A sudden rose of chromosomal bridges was also observed in this process. ATM–Chk2–p53 pathway was activated immediately after irradiation. Nevertheless, to our surprise, this genomic surveillance pathway was depressed during the regeneration phase despite the presence of a second wave of DNA damage, including DSBs and chromosomal bridges, in the cells in the regenerating crypts. Intestinal crypt cells can adapt to IR-induced DNA breaks before regeneration. This process in characterized by chromosomal in stability. It was switch to reliance on mitotic cell death rather than on cell cycle delay or apoptosis to eliminate the cells with severe DNA damage or CIN that would prevent cell division.

P11-07
CORRELATION OF GENETIC AND DIETARY/ LIFESTYLE DETERMINANTS TO CARDIOVASCULAR DISEASE RISK MARKERS WITH BLOOD TELOMERE LENGTH

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Telomeres at the ends of chromosomes are critical for maintaining chromosomal stability and cellular genomic integrity. Telomere length (TL) shortens with age and can be accelerated through oxidative stress. Epidemiologic studies have shown that average TL of peripheral blood or buccal DNA, asurrogate biomarker of telomere abnormality, is related to risk of various chronic diseases such as cardio vascular disease (CVD) and cancer, all of which increase in incidence with ageing. Dietary nutrients and genetic factors also influence TL; accelerate TL shortening in some cases. However, the link is not clear and the mechanism is unknown. Using a pilot study of about 500 human blood samples from the Singapore Chinese Health Study (SCHS) with well characterized dietary determinants and known risk markers of CVD already measured in the cohort (e.g., cholesterol levels in hypercholesterolaemia condition of CVD), we performed leukocyte TL association studies. We found TL of participants shortens with increasing chronological age, correlated with literature. Statistical analysis also reveals sexual dimorphism in TL where females had longer mean TL than males. Interestingly, smokers have a significantly shorter TL than non-smokers, which implies an increased exposure to oxidative stress. This is the first report ever in an Asian population with genetic homogeneity from the SCHS. More importantly, TL correlated inversely with cholesterol level and it is mainly with low density lipoprotein, suggesting the development of TL as an early risk marker of CVD. With the pilot 500 samples and employing arrays, chips, and molecular telomere biology techniques, I will discuss the link of TL to CVD risk markers and whether this link could be influenced by primary genetic (single nucleotide polymorphisms) and/or secondary (nutrition and dietary determinants) causes of CVD. Finally, I will

mention the possible mechanistic pathways involved and there after devise appropriate therapeutic agents against CVD risk.

P11-08
AGING RELATED DEFECTS IN LYSOSOMAL TRAFFICKING DISORDERS

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Lysosomal trafficking is being regarded as an important process in cellular homeostasis. This trafficking pathway has been involved in the biogenesis of lysosomes and lysosome-related organelles (LROs), modulation of signaling pathways and regulation of secretory lysosomes. It is known that several lysosomal trafficking complexes including AP-3, HOPS, ESCRTs, BLOCs participate in this process by mediating the trafficking of cargo proteins into lysosomes or LROs. Hermansky-Pudlak syndrome (HPS) is an autosomal recessive and genetically heterogeneous disorder characterized by oculocutaneous albinism, bleeding tendency and ceroid deposition which likely leads to deleterious lesions in lung, heart and other organs. Currently, nine genes in human and 15 genes in mouse have been identified as causative for HPS. Their pathological effects are attributable to the disrupted biogenesis of lysosomes and lysosome-related organelles (LROs) existing in multiple cell types or tissues. Aging-related defects exist mainly in lung fibrosis and neurodegeneration. Current understanding of the underlying mechanisms is focused on how the lysosomal functions are impaired in HPS to cause altered autophage.

P11-09
MARK4 IS A NEGATIVE REGULATOR OF MTOR

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The mammalian target of rapamycin (mTOR) is a central cell growth regulator. It resides in two protein complexes, which in mammals are referred to as mTORC1 and mTORC2. mTORC1, which is directly inhibited by rapamycin, promotes cell growth by stimulating protein synthesis and inhibiting autophagy. A wide range of extra and intracellular signals, including growth factors, nutrients, energy levels, and various stress conditions, regulates mTORC1. Dysregulation of mTORC1 contributes to many human diseases, including cancer, cardiovascular disease, autoimmunity, and metabolic disorder. In this study, we identified MARK4, an AMP-activated kinase-related kinase, as a negative regulator of mTORC1. In Drosophila S2 cells and mammalian cells, knockdown of MARK family member increased mTORC1 activity, whereas overexpression of MARK4 in mammalian cells significantly inhibited mTORC1 activity. Interestingly, MARK4 selectively inhibits mTORC1 activation by Rag GTPases, which are involved in amino acid signaling, but does not inhibit the effect of Rheb, which directly binds to and activates mTORC1. In addition, we found that MARK4 phosphorylates Raptor, a key component of mTORC1, and this phosphorylation may interfere with Raptor-Rag interaction. Our data demonstrate MARK4 as a new negative regulator of mTORC1.

P11-10
OXIDATIVE AND ENDOPLASMIC RETICULUM (ER) STRESS IN KIDNEY DISEASE AND AGING

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Various extrinsic stresses, including hypoxia and oxidative stress, trigger the progression of kidney diseases via the pathogenic alteration of these stress signals. Recent evidence emphasizes that the extrinsic stress-induced phenotypic changes are widely associated with endoplasmic reticulum (ER) stress and induction of ER stress signal, unfolded protein response (UPR). Oxidative stress induces unfavorable activation of the UPR pathway as well as hypoxia-induced HIF pathway and vice versa, indicating that regulation of the crosstalk of these stress signals is important for kidney homeostasis. We previously identified a microRNA, miR-205, which links to HIF and UPR pathways and contributes to tubular damages. miR-205 expression was markedly decreased by both oxidative and ER stress conditions in tubular cells. The suppression of miR-205 enhanced the cellular damages caused by these stresses in association with overwhelming oxidative stress, while miR-205 overexpression ameliorated them. Functional analysis of miR-205 showed that miR-205 regulated the expression of prolyl hydroxylase (PHD) 1, which degrades the transcription factors of HIF and UPR pathways. Upregulation of PHD1 by miR-205 inhibition altered the expression of the HIF and UPR target genes, anti-oxidant enzymes. Of note, these cellular phenotypic changes were associated with senescence phenotypes: arrest of cell proliferation and an increase in senescence-associated β-gal accumulation. These senescence phenotypes were mimiced by miR-205 inhibition. In conclusion, miR-205 modulates the cellular damage phenotypes against oxidative and ER stresses, and the subsequent cellular senescence in tubules, suggesting the contribution of miR-205 in the pathogenic aging caused by these stresses in tubules.

P11-11
TRF2 ATTENUATES DNA DAMAGE RESPONSE BY INCREASING THE EXPRESSION OF PPP2R2C, A GENE ENCODING A REGULATORY SUBUNIT OF PROTEIN PHOSPHATASE 2A

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The binding to telomeric DNA of TRF2 is essential to prevent the activation of DNA damage response (DDR) at chromosome ends. We previously reported that, in addition to telomeres, TRF2 binds to Interstitial Telomeric Sites (ITS) close to or within genes (Ye et al, 2010; Simonet et al, 2011). Among the genes containing an intronic TRF2-bound ITS, PPP2R2C encodes a regulatory subunit B gamma isoform of Protein Phosphatase 2A (PP2A). We reveal here that TRF2 overexpression leads to an increased occupancy of the ITS by TRF2 and an enhanced PPP2R2C transcription. How the binding of TRF2 to the intronic ITS regulates PPP2R2C expression is currently under investigation. Interestingly, the expression of both TRF2 and PPP2R2C increased upon genotoxic stress suggesting that, in addition to TRF2, PPP2R2C is involved in DDR regulation. Indeed, a reduced expression of PPP2R2C by RNA interference increases ATM and H2AX phosphorylation. We conclude that the level of TRF2 attenuates the global DNA damage response independently of an effect on telomere protection by directly targeting the expression of PPP2R2C and consequently increasing PP2A phopsphatase activity. These results suggest that TRF2 inhibits DDR both locally, where it binds e.g. at telomeres, and globally via the regulation of PPP2R2C and maybe other DDR modulating genes.

P11-12
REGγ DEFICIENCY PROMOTES PREMATURE AGING VIA A CK1Δ-MDM2-P53 PATHWAY

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Our recent studies suggest a role for the proteasome activator REGγ in the regulation of p53. However, the molecular details and *in vivo* biological significance of REGγ-p53 interplay remain elusive. Here, we demonstrate that REGγ deficient mice develop premature aging phenotypes that are associated with abnormal accumulation of CK1δ and p53. Antibody array analysis led us to identify CK1δ as a direct target of REGγ. Silencing CK1δ or inhibition of CK1δ activity prevented decay of Mdm2. Interestingly, a massive increase of p53 in REG$\gamma^{-/-}$ tissues is associated with reduced Mdm2 protein levels despite that *Mdm2* transcription is enhanced. Allelic p53 haplodeficiency in REGγ deficient mice attenuated premature aging features. Furthermore, introducing exogenous Mdm2 to REG$\gamma^{-/-}$ MEFs significantly rescues the phenotype of cellular senescence, thereby establishing a REGγ-CK1-Mdm2-p53 regulatory pathway. Given the conflicting evidence regarding the 'anti-aging' and 'pro-aging' effects of p53, our results indicate a key role for CK1δ-Mdm2-p53 regulation in the cellular aging process. These findings reveal a new model that mimics acquired aging in mammals and indicates that modulating the activity of the REGγ-proteasome may be an approach forintervention in aging associated disorders.

P11-13
SIRTUIN REGULATION OF AGING, METABOLISM, AND STEM CELLS

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The metabolic network is coordinately regulated in response to nutritional status to maintain homeostasis. Perturbed metabolic homeostasis is integral to the aging process and underlies many aging-associated diseases. Recent studies strongly suggest that metabolic enzymes are concertedly regulated via acetylation to allow coordination of the directionality and the rate of the metabolic flux upon changes in nutritional status. This mode of metabolic regulation is conserved evolutionarily and is regulated by the sirtuin family of deacetylase. SIRT3, a mammalian mitochondrial sirtuin, regulates the global acetylation landscape of mitochondrial proteins and triggers a metabolic reprogramming to reduce oxidative stress. SIRT3 regulation of oxidative stress has profound physiological relevance, such as stem cell maintenance and tissue homeostasis at an old age, and prevents many aging-associated diseases, including cancer, heart failure, and hearing loss. The SIRT3 regulatory program is suppressed with aging and, intriguingly, SIRT3 reactivation is an effective means of rejuvenation.

P11-14
LESSONS FROM THE WORM: HOW TO LIVE A LONG, HEALTHY LIFE?

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The nematode *C. elegans* has emerged as an excellent model organism for aging research due to its short lifespan and amenability to genetic manipulation. Many longevity genes and pathways initially identified in *C. elegans* were subsequently found to be evolutionarily conserved in mammals. In addition to the similarity at the genetic level, *C. elegans* also exhibit many aging phenotypes that resemble those found in higher organisms.
Aging can be modulated by both environmental and genetic factors. Food and temperature are the two major environmental factors that regulate lifespan. The past two decades have witnessed a rapid progress in our understanding of how genetic factors

affect lifespan. By contrast, relatively little is known about how environmental factors, particularly temperature, regulate longevity. We have recently begun to investigate how temperature affects lifespan. Both cold-blooded (worms, flies and fish) and warm-blooded (mice) animals live longer at lower body temperatures, highlighting a general role of temperature reduction in lifespan extension. We challenge a century-old view that cold temperature-dependent lifespan extension is a passive thermodynamic process by showing that it is actively regulated by genes. We are also screening for new genes involved in regulating temperature regulation of lifespan. Our work will lead to a better understanding of how environmental factors modulate aging.

Much of the current effort in aging research has been directed to investigating the mechanisms underlying lifespan regulation. Nevertheless, it should be noted that aging is in fact characterized by gradual, progressive declines in physiological functions of multiple tissues (i.e. functional aging), which ultimately lead to death. Yet, the mechanisms underlying functional aging remain largely elusive. As animals age, they exhibit a gradual loss in motor function with an unknown mechanism. Here we approach this question in *C. elegans* by functionally characterizing its aging nervous system and muscles. We find that the nervous system exhibits the first sign of functional aging followed by muscles. Pharmacological stimulation of neuronal activity can improve motor functions in aged animals. These data uncover a critical role for the nervous system in age-dependent motor function decline. Elderly humans also develop deficits in motor function, which represent one of the main risks for falling that leads to injury and mortality, but the underlying mechanisms are unclear. Given the high conservation of aging mechanisms between worms and other organisms, our results raise the possibility that a similar phenomenon may occur in mammals.

P11-15
SYSTEMS BIOLOGY OF AGING: NEW TRICKS TO THE OLD PROBLEM

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Many fundamental questions on aging are still unanswered or are under intense debate. These questions are frequently not addressable by examining a single gene or a single pathway, but can best be addressed at the systems level. In order to examine the systems level changes and regulations of aging, we examined aging and lifespan-related changes in the epigenome, transcriptome and phenome of model organisms (*C. elegans* and mouse) and human tissues (blood and brain), and inferred regulatory networks governing these changes. This allowed us to identify the links among different layers of systems changes, genes and pathways that modulate the aging process through dietary intervention and epigenetic modifications. We found that various changes through different input points often impinge on a common set of regulatory networks involving many combinatorial and feedback interactions.