the FEBS Journal

POSTER SESSIONS

CSI-01 – Cell Cycle & Checkpoints

SUN-001
Adaptations of the DNA replication licensing process in mouse embryonic stem cells

A. Kanellou[1], T. Giavridis[1], S. Taraviras[2], Z. Lygerou[1]
[1]*Medical School, Laboratory of General Biology*, [2]*Medical School, Laboratory of Physiology, University of Patras, Patras, Greece*

Mouse Embryonic Stem Cells (mESCs) have the ability to proliferate and self-renew indefinitely in culture and, when stimulated, to differentiate towards all three germ layers. They exhibit an unusual cell cycle which consists mainly of S phase cells, a short G1 phase and a lack of major checkpoint responses. Maintaining genome stability is pivotal for embryonic stem cells, as they give rise to all mature cell types. In order for committed cells to maintain genome integrity, DNA replication is restricted to only once per cell cycle. This is accomplished through the assembly onto chromatin of multisubunit protein complexes which license DNA for a subsequent round of replication. Replication licensing takes part in G1 phase and consists of the loading of the hexameric MiniChromosome Maintenance 2-7 (MCM2-7) complex onto chromatin, a step which is dependent on the licensing factor Cdt1. We previously showed that the loading of MCM2-7 onto chromatin takes place in two waves in live mammalian cells: upon mitotic exit and at the G1/S phase transition.

We are interested to understand the molecular mechanisms which ensure genome stability in mESCs, and govern DNA replication licensing, and how these are compared to differentiated and cancer cells. We show that mESCs have a very short G1 phase and move to S-phase with high synchrony following mitotic arrest. During S-phase, replication factories are visualized by immunofluorescence against the replication protein PCNA. They show characteristic early, middle and late S-phase localization, reminiscent of replication factory dynamics in differentiated and cancer cells. Cdt1 is specifically detected during the G1 phase of the mESC cell cycle and degraded following entry to S-phase. MCM2 and MCM7 can be immunodetected onto chromatin following mitotic exit and in S phase, and exhibit differential localization along the cell cycle. Following DNA damage by UV-irradiation in G1, Cdt1 is rapidly proteolysed, while changes in chromatin-loaded MCM proteins are evident. When mESCs are irradiated during mitosis, Cdt1 degradation is delayed until entry into G1, suggesting the Cdt1 is protected from proteolysis in mitosis. mESCs irradiated during mitosis or in G1 fail to progress to S-phase. We will be using functional live-cell imaging to assess licensing in mESCs by Fluorescence Recovery After Photobleaching (FRAP) of the GFP-tagged MCM protein subunits, through the cell cycle of uncommitted cells, or when cells are moving towards differentiation, with a concomitant lengthening of G1 phase.

Keywords: DNA replication licensing, mouse embryonic stem cells.

SUN-002
Analysis of the mechanism of DNA damage and replication arrest-induced histone mRNA decay

P. Panomwan, C. Smythe
Biomedical Science, University of Sheffield, Sheffield, UK

Histone mRNA decay (HD) is the process which ensures that histone mRNA is rapidly degraded following completion of DNA replication at the end of S-phase. Strict coordination between histone protein production and DNA replication is essential for the correct packaging of newly replicated DNA, as imbalances can lead to deleterious effects such as genomic instability. Interestingly, histone mRNA stability is controlled by the presence of a stem-loop structure at the 3′ end of histone mRNA, and a protein, HBP/SLBP (hairpin/stem loop binding protein) which specifically binds to this structure, plays a major role in histone mRNA metabolism. Importantly, previous studies have shown HD is also one functional target of an intra S-phase checkpoint activated when DNA synthesis is inhibited, ensuring that histone mRNA is rapidly destroyed when global DNA replication is blocked. Consistent with this, replication stress-induced histone mRNA decay is blocked in the presence of inhibitors of the PIKK family of checkpoint protein kinases, implicating PIKK family members in the regulation of this process. Therefore, we aim to utilise a proteomic approach to to identify HBP/SLBP-associated proteins and post-translational status in order to elucidate the detailed mechanism of checkpoint activated HD. We have established isogenic cell lines stably expressing functional, tagged HBP/SLBP by using the Flp-InTM T-RexTM Expression system. Our results indicate that isogenic Flp-In HeLa cell lines inducibly expressing tagged forms of SLBP under the control of a doxycycline promoter are a useful model system for the molecular analysis of SLBP function during replication stress.

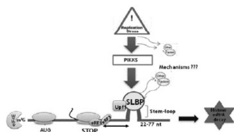

Fig. 1.

Keywords: hairpin/stem loop binding protein (HBP/SLBP), histone mRNA decay, Intra S-phase checkpoint.

Results: Allelic frequencies of the polymorphisms evaluated were in Hardy-Weinberg equilibrium. Statistically significant differences in the frequency of TT genotype of rs3918253 in *MMP9* (11.21% versus 6.83%, p = 2.50E-02, OR = 1.72, 95% CI 1.08–2.71) when compare cases versus controls, showing this SNP is a genetic factor to COPD susceptibility in Mexican population.Two SNPs were found associated in *MMP2*, the GG genotype of rs243864 (EP = 10.3% versus FS = 1.51%, p = 1.00E-10, OR = 7.44, 95% CI = 3.62–15.26) and the GG genotype of rs116643 (EP = 15.45 versus FS = 10.33, p = 2.50E-02, OR = 1.58, 95% CI = 1.07–2.34) suggesting also genetic susceptibility to COPD in the Mexican population. There was not associated with genetic susceptibility to COPD in the *MMP1* and *MMP12* gene polymorphisms.The haplotype consisting of the polymorphisms in the *MMP1* and *MMP12* genes, located in different positions of chromosome 11 were calculated. No haplotype blocks were found in high linkage disequilibrium with r2 > 80.

Conclusion: There are polymorphisms SNP-type in *MMP2* and *MMP9* genes were associated with genetic susceptibility to COPD in the Mexican population.

Keywords: COPD, MMP, polymorphisms.

SUN-178
Glutathione intake promotes longevity through the activation of SIR-2.1 and DAF-16 (FoxO) pathway in *C. elegans*

R. Cascella, E. Evangelisti, M. Becatti, C. Fiorillo, C. Cecchi
Department of Experimental and Clinical Biomedical Sciences, University of Florence, Florence, Italy

Oxidative stress has a prominent role in lifespan regulation of the living organisms. One of the endogenous free radical scavenger systems is associated with GSH, the most abundant nonprotein thiol in mammalian cells, acting as a major reducing agent and antioxidant defence. We have recently designed a series of novel S-acyl-GSH derivatives capable to prevent amyloid oxidative stress and cholinergic dysfunction in Alzheimer disease models, upon the increase of GSH intake. In this study we show that the longevity of wild-type N2 *Caenorhabditis elegans* strain was significantly enhanced by dietary supplementation with linolenoyl-SG (lin-SG) thioester with respect to ethyl ester of GSH, linolenic acid or vitamin E. RNA interference analysis and activity inhibition assay indicate that lifespan extension was mediated by the upregulation of SIR-2.1, a NAD-dependent histone deacetylase ortholog of mammalian SIRT1. In particular, lin-SG-mediated overexpression of sir-2.1 appears to be related to the DAF-16 (FoxO) pathway. Moreover, lin-SG derivative protects N2 worms from the paralysis and oxidative stress induced by Aβ/H_2O_2 exposure. Overall, our findings put forward lin-SG thioester as an antioxidant supplement triggering sirtuin upregulation, thus opening new future perspectives for healthy aging or delayed onset of oxidative-related diseases.

Keywords: Glutathione, longevity, oxidative stress.

SUN-179
GM-CSF-induced CCL17 expression in monocytes/macrophages is IRF4 dependent: a pathway of potential significance in inflammation

A. Achuthan, P.-Y. Lam, M. W. Chang, A. J. Fleetwood, D. C. Lacey, A. D. Cook, J. A. Hamilton
Melbourne Academic Centre, University of Melbourne, Parkville, Australia

Clinical trials in rheumatoid arthritis (RA) targetting the cytokine, granulocyte macrophage-colony stimulating factor (GM-CSF), are showing promise although its mode of action remains largely unknown. Once responsive cell target type to GM-CSF is monocytes/macrophages. Increased macrophage numbers in the synovial fluid from RA knee joints is highly correlated with the severeity of the disease. The interferon regulatory factor (IRF) family of transcription factors is important in controlling expression of genes involved in immune functions. Their key role in controlling gene expression in monocytes/macrophages has recently become a major focus of research.

We report here that GM-CSF induced IRF4 expression, while suppressing IRF8 in primary human monocytes. We found that the chemokine CCL17 expression was induced in GM-CSF-treated human monocytes and largely dependent on IRF4 transcription factor. Significantly, a recent study reported that synovial fluid from knee joints of RA patients had elevated levels of CCL17 as compared to healthy controls. Interestingly, CCL17 gene is clustered together with CCL22 and CX3CL1 on human chromosome 16q13. The transcriptional regulation of these three chemokines by GM-CSF in human monocytes and their role in inflammation will be presented and discussed.

Keywords: inflammation, monocytes, transcription factors.

SUN-180
Granzyme B/perforin system and serpinB9: impact on inflammation and insulin resistance in coronary atherosclerosis

H. O. El-Mesallamy[1], N. M. Hamdy[1], Y. A. Ibrahim[2], A. K. Al-Etriby[3], S. A. Sebak[2], **E. F. Sanad**[1]
[1]*Biochemistry Department, Faculty of Pharmacy, Ain Shams University*, [2]*Cardiothoracic Surgery Department, Kobry El-Kobba Military Hospitals*, [3]*Cardiology Department, Faculty of Medicine, Ain Shams University, Cairo, Egypt*

Background/Objectives: Pro-apoptotic protease granzyme B (GZB) and perforin (PRF) are the major mediators of cytotoxic T lymphocytes, the dominant immune cells accumulated in advanced atherosclerotic lesions. Protease inhibitor-9 (PI-9) or serpinB9, the only known inhibitor of human GZB, protects vascular cells against GZB-induced apoptosis. This study aims to explore the role of GZB, PRF and PI-9 in atherosclerosis through assessing their gene expression in peripheral leucocytes and atherosclerotic tissues as well as their contribution to atherosclerosis-related key regulatory processes.

Method: 69 patients with atherosclerotic coronary artery disease (CAD) divided into 26 non-diabetics and 43 diabetics were compared to 15 apparently healthy controls. 24 atherosclerotic tissues were compared to normal mammary arteries. Serum insulin, hsCRP and GZB levels were estimated by ELISA while mRNA expression levels of GZB, PRF and PI-9 were quantified by Taqman RT-PCR.

Results: Serum insulin, hsCRP and GZB levels were significantly elevated in atherosclerotic patients compared to control subjects ($P < 0.001$). There is a significant increase in GZB mRNA expression and a significant reduction in PI-9 mRNA in peripheral leucocytes and atherosclerotic lesions ($P < 0.001$). PRF mRNA levels increased significantly only in atherosclerotic tissues ($P < 0.001$). Also, diabetic patients showed lower levels of PI-9 mRNA in peripheral leucocytes and atherosclerotic tissues compared to non-diabetics ($P < 0.05$). Regression analysis revealed that GZB and PI-9 have opposite significant modulating effects on inflammation and insulin resistance markers. Interestingly, PI-9 mRNA expression in peripheral leucocytes was inversely contributed to CAD severity.

Conclusions: GZB and PI-9 are proposed key modulators for inflammation and insulin resistance in atherosclerosis. Low levels of circulating PI-9 mRNA might be a biomarker of CAD severity. Induction of PI-9 could be a novel therapeutic approach to attenuate atherosclerosis progression.

Keywords: Atherosclerosis, granzyme B, protease inhibitor 9.