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OP7.205

The effects of histone deacetylase inhibitors on leukaemia differentiation

A. Cassar, KB. Theuma, P. Schembri-Wismayer
Department of Anatomy, Faculty of Medicine and Surgery, University of Malta, Msida

Cancerous mutation of lymphogenous or myelogenous cells results in an abnormal proliferation of white blood cells. Chemotherapy is the mainstay of treatment but unfortunately has its own harmful effects. An alternative method of treatment is differentiation. All-trans retinoic acid (ATRA) has been used to treat acute promyelocytic leukaemia (PML) successfully. PML represents only 3% of leukaemia, and ATRA does not differentiate other types of leukaemia at 100% efficiency. Gene expression is carried out by fine control of coiling and uncoiling of DNA around histones. Histone acetylase uncoils DNA, allowing for a more transcriptionally active chromatin, whereas histone deacetylase gives rise to a coiled, inactive chromatin. A histone deacetylase inhibitor will consequently give rise to a higher degree of active chromatin which should in turn further expose retinoic acid receptor-α (RAR-α) sites on the DNA for ATRA to take effect. To better improve differentiation, in these experiments ATRA is being used in combination with several histone deacetylase inhibitors (HDACIs) with the aim of giving rise to a higher degree of differentiation. Several HDACIs, such as sodium butyrate (NaBut), sodium valproate (NaVal) and trichostatin A (TSA) were tested on different leukaemic cell lines, prior to adding the differentiation inducer ATRA. Pre-treatment was intended to alter the chromosomal conformation in order to increase the possibility that ATRA binds to the RAR-α sites and subsequently promotes differentiation. The degree of differentiation was calculated by the NBT reduction assay with respect to amount of viable cells as detected using the MTT assay.When used individually, histone deacetylase inhibitors only slightly differentiate leukaemia cells. The HDACIs working together and used prior to ATRA resulted in a significant degree of differentiation of HL60 cells ($p<0.001$). Pre-treating HL60 cells with NaBut at 50nM and TSA at 20nM prior to adding ATRA has decreased the required dosages of the HDACIs compared to other publications. Similar results were achieved with other cell lines and different combinations of HDACIs used as pre-treatment.HDACIs do in fact seem to expose transcription sites allowing ATRA to act on RAR-α on the DNA and therefore progress differentiation. This will potentially increase the possibility of using ATRA treatment in differentiation therapy from one kind of leukaemia (PML) to a much broader spectrum.

OP7.206

Investigation of heat shock proteins as regulators of hematopoetic stem cell expansion

A. Abdul-Aziz[1], C. Saliba, P. Schembri-Wismayer[1]
[1]Department of Anatomy, Faculty of Medicine and Surgery, University of Malta, Msida

Introduction: The capability to self-renew is fundamental for stem cells to expand their numbers during development. Self-renewal and differentiation of stem cells are tightly regulated by intrinsic and extrinsic signals. Molecular chaperones and co-chaperones, especially heat shock proteins (Hsp), play a role in modulation of protein conformation, complex-formation and degradation. The function of Hsp, which are associated with stress response and tolerance, is well characterized in differentiated cells, while information on their role in stem cells remains scant. Recent studies suggest a central role for heat shock proteins in immature CD34+ hematopoietic stem cells (HSC). Understanding the role of Hsp in HSC self-renewal is a crucial step towards medical solutions such as stem cell transplants for blood malignancies.

Aim: To investigate the effect of Hsp stimulation on expansion of HSC.

Methodology: CD34+ cells were isolated from Umbilical cord blood, expanded in serum free medium supplemented with a cytokine cocktail and treated with a Hsp stimulator, Tex-OE® (an Opuntia extract) at doses of 5ppm, 10ppm and 15ppm for 6 days of expansion. Cells were then analysed by flow cytometry for expression of CD34 and CD133 as early HSC markers. Results are expressed as % increase in CD34+ and CD133+ population between the control (untreated) and treated samples.

Results: The % of CD133+ CD34+ cells is higher in treated than untreated samples and as well increases with higher doses of Tex-OE® up to 15ppm, beyond which any increase is unbeneficial. The increase in expression of these markers is statistically significant at 10ppm ($p<0.05$).

Conclusion: Our results demonstrate that Tex-OE® is able to maintain and expand a CD133+CD34+ HSC population, thus confirming that Hsp play an important role in HSC. The stem cell potential of these cells will be assayed using Human colony-forming assays and with further flow cytometric analyses.

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OP7.207

Effect of rib cage shape on spontaneous pneumothoraces

A. Casha[1], A. Manche[2], C. Giordimaina[2], M. Gauci[3], W. Wolak[4], K. Dudek[4], R. Gatt[4], J.N. Grima[5]
[1]Department of Anatomy, University of Malta, Msida; Department of Cardiac Services, Mater Dei Hospital, Msida, [2]Department of Cardiac Services, Mater Dei Hospital, Msida, [3]Department of Anaesthesia and Pain Management, Mater Dei Hospital, Msida; Department of Anatomy, University of Malta, [4]Metamaterials Unit, Faculty of Science, University of Malta, Msida, [5]Department of Chemistry and Metamaterials Unit, Faculty of Science, University of Malta, Msida

Introduction: Spontaneous pneumothoraces occur mostly in tall thin young males due to a congenital bulla bursting.

Aim: To demonstrate that spontaneous pneumothoraces occur in the apices because the tall thin rib cage shape predisposes any bulla present to rupture because of mechanical buckling.

Methodology: Rib cage measurements were taken from postero-anterior and lateral CXRs in 12 patients presenting with spontaneous pneumothorax and from 12 controls admitted with blunt chest trauma. Patients older than 30 years, females and non-Maltese were excluded. A finite element analysis of a normal lung model and a series of three similar lungs with a progressive reduction in antero-posterior depth were analysed to assess surface strain patterns on maximal inflation.

Results: Rib cage measurements showed that patients presenting with spontaneous pneumothorax are flatter in antero-posterior depth than controls (chest width to depth ratio 1.9 versus 1.7, $p=0.19$, ns). A series of finite element analyses of a model lung and lung models with reducing antero-posterior depth showed that buckling occurred near the apex in all cases but was more pronounced with progressive flattening of the apices.

Conclusion: Flat chested individuals have an increased risk of apical lung buckling due to increased stress leading to visceral pleural failure and rupture. Weakened pleura at an apical bulla will burst under these conditions. This means that apical congenital bullae are more likely to burst in tall thin individuals. This particular rib-cage shape predisposes to spontaneous pneumothorax.