UNIVERSITI TEKNOLOGI MARA

CELLULAR RESPONSES OF NORMAL HUMAN OSTEOBLASTS TO MULTIPLE ENVIRONMENTAL STRESSORS IN VITRO

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of the requirements for the degree of
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AUTHOR'S DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Teknologi MARA. It is original and is the results of my own work, unless otherwise indicated or acknowledged as referenced work. This thesis has not been submitted to any other academic institution or non-academic institution for any degree or qualification.

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ABSTRACT

Cells respond to environmental stress via the activation of various survival pathways and may possibly end with the initiation of cell death in order to eliminate damaged cells. The ability of cells to mount an adaptive or destructive response depends on the type and duration of the stress. The response to continuous orbital fluid shear stress (OFSS), moderate hypothermia (35°C) and moderate hyperthermia (39°) in this study demonstrated an anabolic effect on Normal Human Osteoblast (NHOst) cells where the cell metabolism, differentiation and proliferation was either promoted or retained. The anabolic effect correlated with an inhibition of osteoclast activity by reducing the RANKL/OPG ratio. In response to 3 days of OFSS, increase in NHOst mitochondrial metabolism and proliferation simultaneously prevented apoptosis. Meanwhile the increase in alkaline phosphatase (ALP) activity and osteocalcin (OCN) after recovery from OFSS suggested that NHOst function was promoted. The possible mechanism for the transduction of these anabolic signals might have been generated through the actin fibres of the cell's cytoskeleton. On the other hand, when NHOst were exposed to temperature stress for 1 h (acute), 12 h & 24 h (short) and 72 h (prolonged), cells responded by expressing heat or cold shock proteins according to hypo- and hyperthermia severity and exposure duration. Exposure to acute 1 h temperature stress lead to an overall reduction in NHOst metabolism, mRNA and protein expression. Overexpression of Rbm3 and Hsp70 promoted NHOst viability and proliferation in response to short and prolonged moderate hypo- and hyperthermia but not in severe exposure. Up regulation of Rbm3 was involved in the adaptation of NHOst survival while Cirbp was to inhibit NHOst survival. Despite NHOst were progressing in the cell cycle in response to moderate hypothermia, the percentage of NHOst undergoing apoptosis was slightly higher compared to NHOst under severe hypothermia. Both moderate and severe hypothermia showed apoptosis was activated via a caspase 3-independent pathway. Insignificant up regulation of caspase 8 and 9 under moderate hypothermia led to the activation of caspase 3, suggesting both extrinsic and intrinsic pathway was activated. Detachment of NHOst from the culture substratum in response to severe hyperthermia suggests that anoikis as a form of apoptosis was induced. The expression of ALP and OCN was dependent on the expression of Runx2. Meanwhile the overexpression of osterix showed that response to moderate hyperthermia in particular suggests that NHOst have the capability to mature. Prolonged exposure to moderate hypothermia promoted mineral deposition required for bone mineralization as the calcium nodules were slightly larger compared to control. In conclusion, continues exposure to OFSS and short term moderate hypo- and hyperthermia promote if not retains bone functionality *in vitro*.

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