THE EFFECT OF MILD AND MODERATE HYPOTHERMIA ON NORMAL HUMAN OSTEOBLAST CELL CYTOSKELETON

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Downshift in normal body temperature induces cold shock then becomes catastrophic towards cells depending on the degree of hypothermic severity. Cells react to such insult by activating cellular response that modulates the cell cycle and cell cytoskeleton in order to prevent irreversible damages. In this study, we measured the cytoskeletal changes, DNA damage and expression of cold shock protein in normal human osteoblast cells treated with prolonged mild and moderate hypothermia. Aim was achieved by culturing osteoblast cells at 37°C (control), 27°C (moderate) and 35°C (mild) with optimum growth medium for 12h, 24h, 72h, and 5 days. Both cytoskeleton changes and DNA damage were viewed under confocal laser scanning microscope whereas cell viability was measured using MTS assay at respective time points. Level of Rbm3 mRNA was determined using Real-time PCR.

By day 5, reduction in tubulin intensity (Fig. 1a) with regards to cold shock was seen in both temperatures as compared to control. Conversely, significant increase in actin intensity (Fig. 1b) parallels with an increase in cell viability. Level of Rbm3 was significantly up-regulated (p < 0.001) by 24h. Furthermore, circularity index of osteoblast nuclei was lower than control indicating cells are able to spread and retain mobility [1] through actin. In addition, DNA damage was negative for both temperatures.

Up-regulation in Rbm3 directly correlates with the increased polymerization [2] of actin fibers giving better chances in cell survival. Although tubulin decline, mitotic spindles were not abruptly disrupted as Rbm3 were chaperonin the cells. Therefore, Rbm3 is important in maintaining cellular integrity during prolonged mild and moderate hypothermia.

Figure 1: Effect of mild and moderate hypothermia on (a) tubulin and (b) actin fibers. Cells were fixed and stained with (a) Anti-α-Tubulin-FITC and (b) Alexa Fluor 635 Phalloidin. Fluorescent intensity was analyzed using Leica QWin Pro software. Data obtained from 30 - 40 cells is shown as the mean ±SD of percentage change relative to Control (100%). * p < 0.001.