## DMM-09 Plasma membrane cholesterol homeostasis is essential for preventing oxidative damage in wild-type Chinese hamster ovary cells

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**Introduction:** The interrelation between plasma membrane cholesterol and cellular oxidative damage is unclear yet. Our recent study suggests that plasma membrane cholesterol appears to be a regular barrier to intracellular oxygen concentration. The generation of reactive oxygen species (ROS) is greatly depended on tissue oxygen concentration. In this study, we hypothesize that the abnormal of plasma membrane cholesterol would augment ROS production and increase the vulnerability of the cells to oxidative stress.
**Method:** To test this hypothesis, we investigated intracellular oxygen concentration, ROS production and apoptotic cell death in normal and mutant Chinese hamster ovary (CHO) cells defective in cholesterol synthesis and metabolism. Those cells were exposed to different oxidants such as xanthine/xanthine oxidase, hydrogen peroxide and menadione, etc. Plasma membrane cholesterol was detected with microenzymatic assay and filipin-staining confocal microscopy. Intracellular oxygen concentration was measured with 4-oxo-2,2,6,6-tetramethylpiperidine-$d^{16}$-1-$^{15}$N-oxyl ($^{15}$N-PDT) in presence of gadolinium complex. The spectrum of $^{15}$N-PDT was recorded with X-band electron paramagnetic resonance (EPR) spectroscopy. Oxygen concentration was simulated by the conversion of the EPR line width of $^{15}$N-PDT. ROS was trapped with 5-(diethoxyphosphoryl)-5-methyl-1-pyrroline-N-oxide (DEPMPO) and detected with X-band EPR spectroscopy. DNA fragmentation was detected by agarose gel electrophoresis and ELISA cell death kit.
**Results:** After treated with the oxidants, the high cholesterol cells had lower intracellular oxygen concentration and higher superoxide level and apoptotic cell death rates than normal cholesterol cells. It is interesting that the low cholesterol cells had higher intracellular oxygen concentration and apoptotic cell death rates than the normal cholesterol cells. The low cholesterol cells showed different levels of superoxide under the treatments of xanthine/xanthine oxidase, hydrogen peroxide and menadione respectively.
**Conclusion:** Plasma membrane cholesterol homeostasis might play an important role in cellular oxidative damage.

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## DMM-10 Expression studies of 24 genes on mouse chromosome 17, towards the identification of the gene(s) responsible for adolescent idiopathic scoliosis

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**Introduction:** Adolescent idiopathic scoliosis (AIS) is a spine deformity of unknown etiology which occurs commonly in adolescents. Our laboratory has recently identified a locus associated with adolescent idiopathic scoliosis on chromosome19 by linkage analysis of seven families. This region of chromosome 19 originally encompasses approximately 2 megabases of DNA, further fine mapping has now narrowed this stretch to about 800 kilobases that contain 23 genes or putative genes. This study aims to identify the genes that are expressed in the tissues involved in the phenotypic manifestation of the disease i.e., bone, cartilage, muscle and tendons. This knowledge will help reduce the number of candidate genes we need to screen for causative mutations in our patients. As the region of human chromosome 19 of interest is in synteny on mouse chromosome 17, comparative genomics will be used to that effort.
**Methods:** 1) Bioinformatics is used to identify the human genes on 19p13.3 that have mouse homologues. Primers are designed to amplify cDNAs from the murine sequences. RNAs are extracted from mouse bone, trachea, and muscle. 2) RT-PCR is performed to assay for gene expression in these tissues. Beta-actin is used as positive control for RT-PCR and reference for gene expression while liver and kidney RNAs are used as neutral control. 3) The cDNAs amplified from the RT-PCR are also printed on microarrays and are hybridized to fluorescent cDNA synthesized from RNA of the various tissues. The resulting signals are quantified using a laser scanner and the Quantarray software.
**Results:** 17 of the 23 genes on the locus identified have homologues on mouse chromosome 17. All of these 17 genes are expressed in the mouse tissues examined i.e. bone, muscle, trachea, kidney and liver when assayed by RT-PCR. Microarray experiments revealed that three of the genes studied have high expression in muscle, one in bone and one in trachea.
**Conclusion:** These experiments help prioritise the genes to focus on, in our search for the AIS candidate gene.
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