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-15N-oxyl (15N-
PDT) in presence of gadolinium complex. The spectrum of 15N-PDT was recorded with X-band electron paramag-
netic resonance (EPR) spectroscopy. Oxygen concentration was simulated by the conversion of the EPR line width
of 15N-PDT. ROS was trapped with 5-(diethoxy phosphoryl)-5-methyl-1-pyrroline-N-oxide (DEPMPO) and de-
tected 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 cho-
lesterol cells. The low cholesterol cells showed different levels of superoxide under the treatments of xanthine/x-
anthine 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 com-
monly in adolescents. Our laboratory has recently identified a locus associated with adolescent idiopathic scoliosis
on chromosome 19 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 syn-
thesized 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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