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HAART to heart: cardiac mitochondrial dysfunction from NRTIs

AIDS treatment has been revolutionized by highly active antiretroviral therapy (HAART). However, patients often suffer from serious side effects, including lactic acidosis, hepatic steatosis, myopathy, cardiomyopathy, peripheral neuropathy, pancreatitis and lipodystrophy syndrome. These disorders have been associated with mitochondrial toxicity caused by the nucleoside reverse transcriptase inhibitors (NRTIs) used in this treatment. In this issue, **Lewis *et al***[1] (p. 972) explored the role of a mitochondrial deoxynucleotide carrier (DNC) in NRTI uptake and toxicity. The authors expressed transgenic human DNC in murine myocardium. They treated animals with either a HAART regimen that included NRTIs to simulate AIDS therapy, or a second, NRTI-sparing regimen as a control. Cardiac and mitochondrial structure and function were then examined by magnetic resonance imaging, echocardiography, electrocardiography, transmission electron microscopy and measurement of plasma lactate levels. Cardiac mitochondria from the DNC transgenic (Tg) hearts had reduplicated cristae and amorphous deposits, but no apparent dysfunction. However, cardiomyopathy (CM) occurred in Tg animals treated with HAART that contained NRTIs. The CM features included increased left ventricle (LV) mass and volume, LV dilation, increased heart rate variability, hyperlactatemia and mitochondrial destruction. In contrast, HAART regimens without NRTIs caused no cardiac changes. These data suggest that the mitochondrial dysfunction in patients treated with HAART regimens that contain NRTIs may be causally related to myocardial expression of DNC. Future studies with DNC transgenic animals may help further clarify mechanisms of NRTI toxicity to mitochondria and how the effects may be prevented or ameliorated in human patients.

Reference

1 Lewis W, Haase CP, Miller YK, *et al*. Transgenic expression of the deoxynucleotide carrier causes mitochondrial damage that is enhanced by NRTIs for AIDS. Lab Invest 2005;85: 972–981.

Mesenchymal potential of lung fibroblasts

Mesenchymal stem cells (MSCs) have been isolated from adult bone marrow, peripheral blood, adipose tissue, articular synovium, amniotic fluid and various fetal tissues. These MSCs share common characteristics, including a fibroblastic-like morphology, long-term proliferation *ex vivo* and the capacity to generate the three types of mesenchymal-derived tissue: bone, cartilage and fat tissue. The ease of culture and genetic manipulation of MSCs indicates their potential usefulness in the repair of diseased tissues of mesenchymal origin. Most MSC studies focus on the administration of bone marrow-derived cells; the therapeutic utilization of resident MSCs in other adult tissues has been limited. Although bone-marrow-derived MSCs are currently under investigation for the treatment of lung diseases, lung MSCs might be more appropriate. However, the presence of MSCs in adult lung had not been yet documented. In this issue, **Sabatini *et al***[1] (p. 962) analyzed the mesenchymal phenotype and differentiation properties of fibroblast-like cells isolated from normal human bronchial tissue and compared them to fetal lung and adult bone-marrow-derived MSC progenitors. The adult bronchial cells, similar to fetal lung and bone marrow fibroblasts, were able to differentiate along the adipogenic, osteogenic and chondrogenic mesenchymal pathways when cultured under appropriate conditions. While the resident MSCs in adult lung are believed to be involved in the maintenance and repair of injured tissues, their existence may offer potential therapeutic value. For example, bronchial MSCs might be useful for the manipulation of airway remodeling following acute injury, the correction of acquired or genetically determined mesenchymal cell disorders, and possibly even the delivery of transgenic proteins to other airway structural cells.

Reference

1 Sabatini F, Petecchia L, Tavian M, *et al*. Human bronchial fibroblasts exhibit a mesenchymal stem cell phenotype and multilineage differentiating potentialities. Lab Invest 2005;85:962–971.

Validating microarray platforms for gene profiling of human tissue

Biomedical research is deriving great benefit from global transcription profiling of genes, through the use of massive microarray systems. These microarrays can be generated through robotic printing of cDNA on a chemically modified glass surface,

by *in situ* synthesis of oligonucleotides on a substrate, or by ink-jet technology on a hydrophobic glass support. Microarrays may be obtained from corporate suppliers, or, in the case of cDNA microarrays, can be produced by individual research groups or core facilities. Artefacts which can degrade data obtained from spotted cDNA microarrays include inaccuracies in nucleotide sequences of given 'spots', lack of cross-validating sequences for each gene being analyzed, and inaccuracies in the optical array of the spots. Oligonucleotide arrays can provide for more uniform DNA concentrations and oligonucleotide lengths, but are not currently feasible for local manufacture. Regardless of which microarray platform is utilized, findings obtained from global gene transcription profiling need verification from independent methods such as Northern blotting or quantitative reverse transcription PCR (RT-PCR).

Systematic comparison between spotted microarrays and synthesized oligonucleotide arrays has been performed using RNA isolated from cell lines, and using commercial reference RNA. However, a systematic examination of microarray performance using RNA from human clinical specimens has not been performed. **Schlingemann *et al***[1] (p. 1024) analyzed gene expression in a set of human head and neck squamous cell carcinomas, using two commercial platforms: a spotted cDNA microarray made from a large commercial set of 70-mer nucleotide probes (Operon Human Genome Oligo Set Version 2.1 and Version 2.1 Upgrade, Cologne, Germany), and an array produced by *in situ* synthesis of sets of multiple 25-mer oligonucleotides per gene (Affymetrix HG-U133A array, Santa Clara, CA, USA). Six carcinoma samples were compared either to healthy control mucosa ($n=4$) or to lymph node metastases ($n=2$). In the 70-mer probe microarray, two replicate two-color hybridizations were performed per patient, one performed with inverse assignment of fluorophores (dye swap) in order to obtain a normalized $\log_2$ ratio of gene expression. Comparisons of gene expression were made for 4425 genes represented on both platforms, and expression ratios were confirmed on a subset of genes using quantitative RT-PCR.

There was a high degree of reproducibility of data within each microarray platform. Across platforms, similar profiles of relative gene expression were obtained using either the Operon or Affymetrix system. On average, absolute log ratios of gene expression profiles obtained on the long-oligonucleotide platform (Operon) were lower than the corresponding values measured with the short-oligonucleotide platform (Affymetrix). However, the differential expression patterns of genes were highly similar when measured by the two platforms. Measured differences between carcinoma samples and their comparison groups, if any, favored genes engaged in ion binding being identified on the Operon platform (70-mer), and genes involved in lipid metabolism being identified on the Affymetrix system (25-mer). Regardless, for a small subset of genes, differential expression measurements were successfully verified by quantitative RT-PCR.

This validation of global gene expression profiling using clinical tissue specimens is a critically important step in application of microarray technology to the study of human cancer. At a time when there is intense interest in determining the genomic expression profiles of human cancers, one may conclude that similar but not necessarily identical results will be obtained using different microarray platforms. However, this paper underscores the fact that microarrays should be considered a screening tool, and that expression of individual genes should be verified by independent methodologies.

Reference

1 Schlingemann J, Habtemichael N, Ittrich C, *et al*. Patient-based cross-platform comparison of oligonucleotide microarray expression profiles. Lab Invest 2005;85:1024–1039.