Microarray-based studies of cell death, a method development and application study

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ABSTRACT

This PhD dissertation consists of three articles. The studies were conducted at the Molecular Diagnostic Laboratory, Department of Clinical Biochemistry, Skejby Sygehus.

At present, the biological processes leading to type 1 diabetes mellitus are not fully understood, and the molecular mechanisms leading to beta-cell death are limited.

Our approach in this study was to develop a printed oligonucleotide microarray for the study of beta-cell apoptosis. In another study we investigated cell death and changes in gene expression in a macrophage cell line, induced by the heavy-metal bismuth.

60-mer oligonucleotide probes representing 575 genes were printed on the microarray, and complete protocols for the printing, labelling, hybridisation, and washing were developed.

The microarray was tested both internally and against the commercial Affymetrix arrays. Our data showed that the internal (within chip) reproducibility was high and that the additional variance due to external replication was negligible. A weighted linear regression showed that the majority of the oligonucleotide probes reacted in a predictable manner in response to changes in target concentration.

Comparison of the relative gene expression showed a high concordance between the two array platforms. There was a clear relationship between the correlation and quality filtering of the data, e.g. when low intensity data and data associated with genes exhibiting low variation in the relative gene expression was excluded, the correlation increased accordingly.

Bismuth is used in drugs for the treatment of *H. pylori* associated peptic ulcers and is progressively used as a substitute for lead and mercury in several industrial applications.

The present cell culture study demonstrated that bismuth is accumulated in lysosomes in a time and dose dependent manner with a corresponding decline in the number of viable cells. Analyses of microarray data showed that bismuth induces gene expression patterns similar to hypoxia induced stress. The induction of Bnip3 and/or intracellular acidification may be candidates for bismuth induced cell death independent of caspase-3 activation.