Development of a DNA Microarray for Detection of Aneuploidy in Single Blastomeres

Dong Gui Hu, MD, M.Sc. (China)

Department of Obstetrics and Gynaecology
The University of Adelaide
The Queen Elizabeth Hospital
Woodville S.A., 5011, Australia

A thesis submitted to the University of Adelaide in fulfilment of the requirements for admission to the degree of Doctor of Philosophy

January 2004

Table of Contents

Abstract

Comparative Genomic Hybridisation (CGH) using metaphase chromosome spreads to screen all human chromosomes for aneuploidy in preimplantation embryos is hindered by the time required to perform the analysis. It takes at least three days to analyse a single cell using metaphase CGH, resulting in the need for the embryos to be cryopreserved rather than being able to be transferred in the maternal cycle that created them. Array CGH can also detect aneuploidy and requires much shorter hybridisation times. Microarrays manufactured to date are not purported to be able to analyse the very limited amount of genetic material (around 6pg) in a single cell; generally they require a DNA sample (0.5-1.0μg) far in excess of that contained in a single cell.

This thesis describes the development of a novel approach to the manufacture of a DNA microarray for CGH for the detection of aneuploidy in single cells. Human chromosome-specific libraries, which were depleted of repetitive sequences, were spotted on glass slides. Array CGH experiments were conducted on these arrays using either single male and/or single female lymphocytes. For the autosomes, the mean normalized ratios were all close to the expected ratio of 1.0 with overall 97% of the normalized ratios falling within the expected range 0.75-1.25. It was possible to deduce the correct copy number of X chromosomes in 93% of separate array CGH experiments, but the Y chromosome in only 29%. Array CGH was initially performed on a single fibroblast from each of three cell lines containing a specific chromosome aneuploidy (trisomy 13, 15 or 18) and in each case this method was able to obtain a diagnosis based on the fact that the aneuploid chromosomes gave the highest ratios (1.32, 1.27 and 1.27 respectively) with the ratios of all other chromosomes falling within the range 0.75-1.25. Finally, a small number of blastomeres removed from human cleavage-stage

embryos were analysed using this method. Results suggest that some blastomeres had a normal karyotype, whereas others were aneuploid or chaotic. This array CGH approach produces results within 30 hours, making it potentially more suitable for PGD aneuploidy screening than metaphase CGH.