Single Cell Aneuploidy Analysis by High Resolution Comparative Genomic Hybridization (CGH) after Whole Genome Amplification reveals Maternal Cell Contamination in the Original Products of Conception Cultures.

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Introduction

Comparative genomic hybridization (CGH) is an alternative molecular cytogenetic technique that can characterize unbalanced and often unrecognizable G-banded cytogenetic material in a one-step global screening procedure. It has the distinct advantage of providing a genome wide search without any prior information of the chromosomal aberration in question. CGH analysis provides information on the origin of gains and losses of chromosomal material and maps these imbalances to their position on the source chromosome. Since CGH analysis is DNA based, culturing the specimen and the availability and quality of metaphase spreads are not considerations. Thus, even non-viable tissues are amenable to analysis by CGH. These factors make CGH an attractive technique for application in clinical cytogenetic analysis especially for samples where a complete and detailed karyotype cannot be obtained by conventional methods.

It has been suggested that the efficiency of assisted reproductive techniques could be improved if chromosomally normal embryos could be identified and preferentially selected for transfer to the mother. To this end, infertility centers now perform aneuploidy screening by fluorescence in situ hybridization (FISH) using chromosome specific probes. However, only a limited number of chromosomes can currently be assessed and usually by employing more than one hybridization on the same cell. Since CGH is a DNA-based technique, it offers the potential for total aneuploidy screening in single human cells. The DNA content of a single cell is however only about 7pg and this amount is insufficient for many molecular biological assays, including CGH. There is therefore a requirement to perform whole genome amplification (WGA) in a manner that ideally permits unbiased amplification across the genome and also yields adequate quantities of DNA for any downstream analyses. Recent advances in WGA techniques have now made comprehensive aneuploidy screening of single cells a distinct possibility. We have used CGH to detect deletions and duplications in the range of 2–5MB. Since the smallest autosome is in excess of 50MB, CGH analysis provides a very powerful and sensitive method for detecting whole chromosome aneuploidy.

We have been using WGA coupled with CGH to successfully perform aneuploidy analysis of single cells derived from cell cultures of known karyotype. In this report we highlight two interesting single cell cases, from our larger series, that were diagnosed as normal female by WGA-CGH analysis but were revealed to derive from a POC culture that was karyotyped as 47,XY,+16. Given our success with the rest of the series, we considered maternal cell contamination as a feasible explanation for the discrepancy.

Materials and Methods

Whole Genome Amplification (WGA): Single cells were extracted from two different products of conception (POC) cultures that were initially obtained for clinical cytogenetic analysis. The POC cultures were part of a larger IRB approved study assessing aneuploidy analysis of single cells. All cultures were de-identified and blinded (with respect to the cytogenetic diagnosis) before being transferred to the research laboratory. Single cells were lysed and then subject to WGA using Sigma-Aldrich's Genome-Plex® kit. This specific kit uses random fragmentation of the genome followed by ligation with oligonucleotide adaptors which then serves as primers for the ensuring PCR reaction.

Comparative genomic hybridization (CGH): WGA-DNA was labeled using nick-translation. CGH probes were prepared and washed as described by Kallioniemi et al (1994) and Levy et. al (1998). The florescence ratios (green/red) for at least 10 of each autosome and 7 of each sex chromosome were obtained per slide. The CGH profiles were compared to a dynamic standard reference interval based on an average of normal cases, as described by Kirchhoff et al. [1998]. The dynamic standard reference intervals are wide at regions known to produce unreliable CGH profiles. The standard reference interval was scaled automatically to fit the individual test case. The mean ratio profile of each case with 99.99% CI was compared to the average ratio profile of the normal cases with similar confidence intervals. A positive finding was considered when the confidence intervals of the patient profile and normal averaged profile did not overlap. Digital image analysis was performed using a Cytovision™ Probe system and high resolution CGH software (Applied Imaging Corp, Santa Clara, CA). CGH results were compared to the karyotype as determined by conventional cytogenetic analysis.

Fluorescence in situ hybridization (FISH): 16 CEP and X/Y CEP were purchased from Abbott Molecular (Vysis). FISH studies were performed according to the manufacturer's instructions.

Quantitative fluorescence-polymerase chain reaction (QF-PCR): QF-PCR was carried out by amplification of polymorphic short tandem repeats (STR) markers using fluorescence-labeled primers, followed by quantitative analysis of the allele peaks. Markers on X chromosome were used to asses the male cells in the cultured DNA sample.

Results

WGA-CGH analysis on single cells obtained from POC showed a normal female CGH profiles for the first case (**Figure 1A**) and a female profile with a 7p gain for the second case (**Figure 1B**). These results were inconsistent with the cytogenetic analyses that revealed a 47 XY +16 karyotype for both cultures. We considered maternal cell contamination (MCC) as a feasible explanation for the discrepancy since MCC is a common occurrence in POC cultures. FISH analysis on residual POC cultures was performed using probes specific for the X and Y centromeres. The FISH studies revealed a high proportion of female cells in both cultures, 99% for case 1 and 94% for case 2 (**Figure 2**). Tri-color FISH was performed using probes specific for chromosomes 16 (Aqua), X (Green) and Y (Red) and revealed the presence of trisomy 16 cells only in male cells while all female cells were disomic for chromosome 16 (**Figure 3**). The hypothesis was further supported by QF-PCR studies that showed two alleles for a highly polymorphic marker on the X chromosome thus confirming the presence of female DNA.

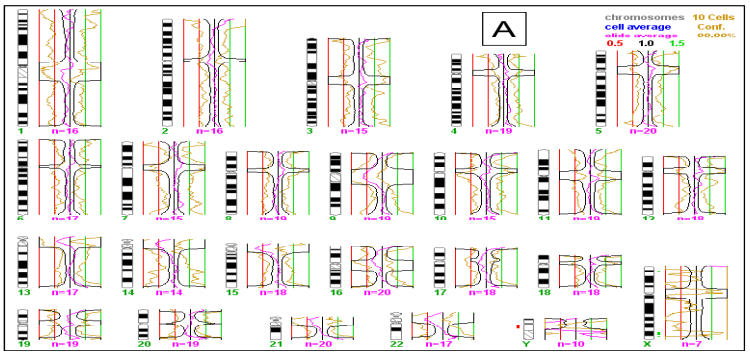
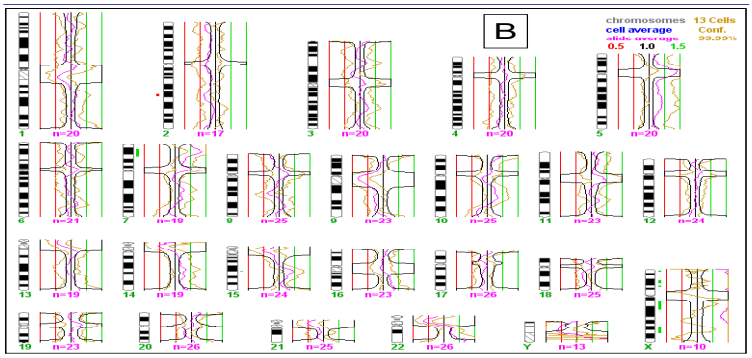


Figure 1: CGH profile showing a normal female profile for the first case (**A**) and a female profile with a 7p fain for the second case (**B**)



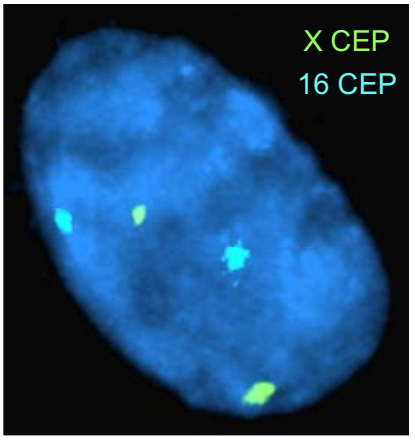


Figure 2: FISH image of an interphase nucleus showing two X CEP signals (i.e female) and two signals for the CEP 16 probe (i.e. disomy 16).

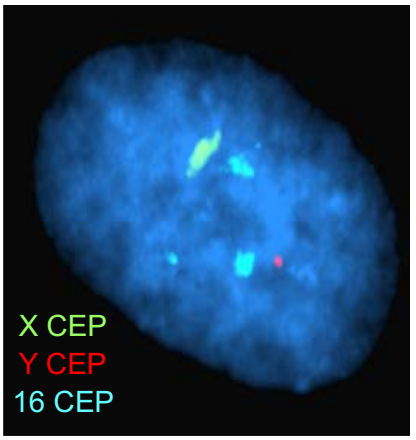


Figure 3: FISH image of an interphase nucleus showing one X CEP signal and one Y CEP signal (i.e male) and three signals for the CEP 16 probe (i.e. trisomy 16).

Conclusions

These cases illustrate the value of WGA as a means to performing single cell aneuploidy analysis. Importantly, this report highlights the dangers of a diagnosis based on a single cell. This is particularly applicable to preimplantation genetic diagnosis where mosaicism is an issue and fetal cells from maternal circulation where maternal cell contamination is a problem.