Chromosome Analysis of Single Cells by High Resolution Comparative Genomic Hybridization after Whole Genome Amplification Using a Random Fragmentation Approach.

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Introduction

The preparation of prometaphase/metaphase spreads is an essential part of chromosome analysis. Most samples received for cytogenetic study do not have a significant number of actively dividing cells that can be arrested in the prometaphase/metaphase stage of the cell cycle and thus require cell culturing. Single fetal cells derived from a 2–3 day old embryo or from the maternal circulation can therefore not be karyotyped in the traditional way. Fluorescence in situ hybridization (FISH) with chromosome specific probes can enumerate individual chromosomes in an interphase cell and it is now possible through a process of combinatorial labeling to produce 24 differentially colored human chromosome probes. However, due to the overlapping three dimensional spatial arrangement of the chromosomes in an interphase cell, the total number of chromosomes that can be enumerated at once is limited. Comparative genomic hybridization (CGH) is a DNA based cytogenetic technique that allows for the identification of chromosomal gains and losses without the need for cell culturing. Imbalances in chromosomal material are detected in a single-step scan of the entire genome. Since it is not feasible to culture and perform cytogenetic analysis on single cells, CGH presents itself as an attractive technique for identifying aneuploidy 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 provides 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.

In this report, we utilized high resolution CGH for the identification of chromosomal abnormalities in single cells. Future application of this method would provide cytogenetic analysis of fetal cells derived from maternal circulation during pregnancy for prenatal diagnosis as well as of single cells obtained from 2–3 day old embryos for preimplantation diagnosis.

Materials and Methods

Whole Genome Amplification (WGA): Single cells were extracted from 36 random specimen cultures that were initially obtained for clinical cytogenetic analysis. All cultures were de-identified and blinded (with respect to the cytogenetic diagnosis) before being transferred from the clinical laboratory to the research laboratory. The study was approved by the IRBs of Columbia University and the Mount Sinai School of Medicine. 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% confidence was compared to the average ratio profile of the normal cases with similar confidence intervals (CI). 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.

Results

WGA followed by CGH was performed on single cells obtained from 36 random specimen cultures. The specimens analyzed included normal males, normal females, various trisomies (4, 10, 11, 13, 14, 16 and 21), an unbalanced translocation and an iso-22 chromosome (**Table 1**). There were no false negatives and the correct sex and diagnosis was made in 36/36 cases at 99% confidence, in 35/36 cases at 99.9% confidence and in 33/36 cases at 99.99% confidence (**Table 1**). Certain artifactual abnormalities were observed in addition to the true abnormality in some cases and the impact of these still needs to be determined in a larger test series. In the two cases highlighted in light blue in **Table 1**, the original cultures were reinvestigated and shown to be contaminated by maternal cells. The single cells extracted for WGA from these two separate trisomy 16 cultures were most likely female and thus the single cell diagnosis of the cells analyzed can be considered to be correct. In the three cases highlighted in dark blue in Table 1, the true abnormality was evident at 99% confidence for all three cases, but as the confidence intervals were tightened, the true abnormalities were not apparent. **Figure 4** demonstrates this phenomenon for case 36.

Specimen #	Karyotype	Expected CGH Result	Observed CGH Result (99.99% CI)	Correct Sex Determination
1	47,XY,+21	Trisomy 21 Male	Trisomy 21 Male	Yes
2	47,XY,+16	Trisomy 16 Male	Trisomy 16 Male	Yes
3	46,XX	Normal Female	Normal Female	Yes
4	46,XY	Normal Male	Normal Male	Yes
5	69,XXX	Normal Female	Normal Female	Yes
6	47,XY,+16	Trisomy 16 Male	Trisomy 16 Male	Yes
7	46,XX,i(22q)	Trisomy 22 Female	Partial Trisomy 4q Female Partial Trisomy 5q	Yes
8	47,XX,+11	Trisomy 11 Male	Partial Trisomy 11q Male	Yes
9	47,XY,+21	Trisomy 21 Male	Trisomy 21 Male	Yes
10	46,XX	Normal Female	Normal Female	Yes
11	47,XX,+13	Trisomy 13 Female	Trisomy 13 Female Partial deletion 5p	Yes
12	46,XY	Normal Male	Normal Male	Yes
13	47,XX +10	Trisomy 10 Male	Trisomy 10 Male	Yes
14	47,XX +14	Trisomy 14 Female	Trisomy 14 Female	Yes
15	46,XX	Normal Female	Normal Female	Yes
16	46,XX	Normal Female	Normal Female	Yes
17	47,XX +21	Trisomy 21 Female	Trisomy 21 Female	Yes
18	47,XY,+4	Trisomy 4 Male	Partial Trisomy 4q Male	Yes
19	46,XX	Normal Female	Normal Female	Yes
20	46,XX	Normal Female	Normal Female	Yes
21	47,XY,+16	Trisomy 16	Normal Female	No/Yes
22	46, XY	Normal Male	Normal Male	Yes
23	46,XX	Normal Female	Normal Female	Yes
24	46, XY	Normal Male	Normal Male	Yes
25	46, XY	Normal Male	Normal Male	Yes
26	46, XX	Normal Female	Normal Female	Yes
27	46, XX	Normal Female	Normal Female	Yes
28	46, XY	Normal Male	Normal Male	Yes
29	46,XX,der(8)t(8;20)(p21.1;q13.1)mat	Partial Monosomy 8p	Partial Monosomy 8p	Yes
30	47,XY,+16	Trisomy 16 Male	Partial Trisomy 7p	No/Yes
31	47,XY,+16	Trisomy 16 Male	Trisomy 16 Male	Yes
32	47,XY,+21	Trisomy 21 Male	Trisomy 21 Male	Yes
33	46,XY	Normal Male	Normal Male	Yes
34	45,X	Monosomy X	Normal Female	Yes
35	46,XX	Normal Female	Normal Female	Yes
36	47,XX,+22	Trisomy 22 Female	Normal Female	Yes

Table 1: Summary of WGA-CGH results (99.99% CI) for 36 Specimens

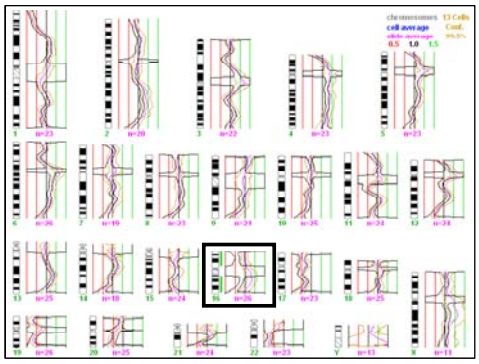

Figure 1: CGH profile showing a male profile with Trisomy 16 for Case 3

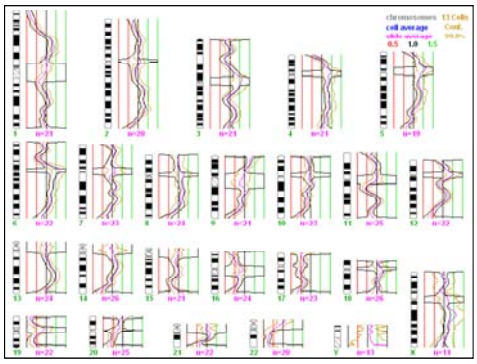

Figure 2: CGH profile showing a normal female profile for Case 3.

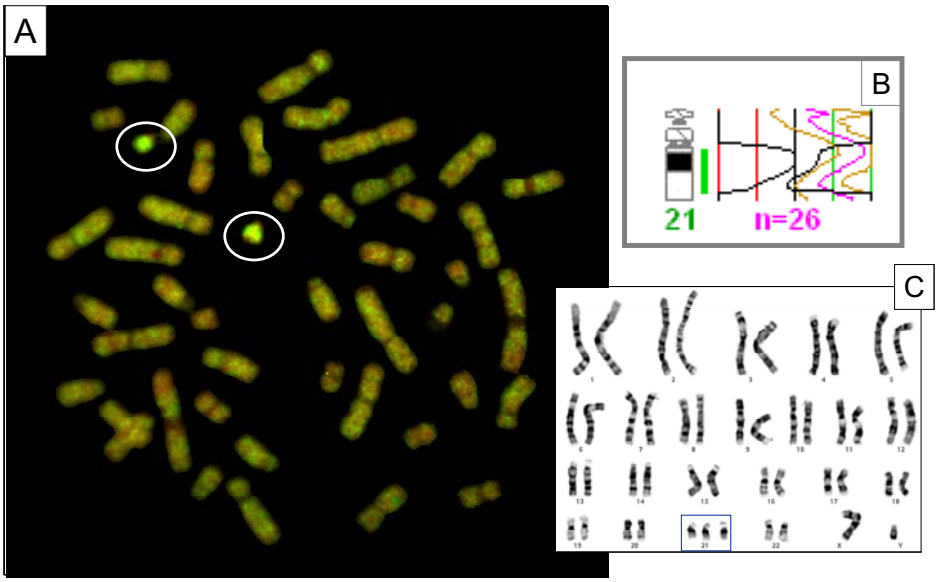

Figure 3: Single Cell analysis of a trisomy 21 culture [A] Gain of chromosome 21 can be visualized in dual FITC/Texas Red image as green intense regions (Circles). [B] CGH Ratio profile showing gain of chromosome 21. [C] Original trisomy 21 karyotype.

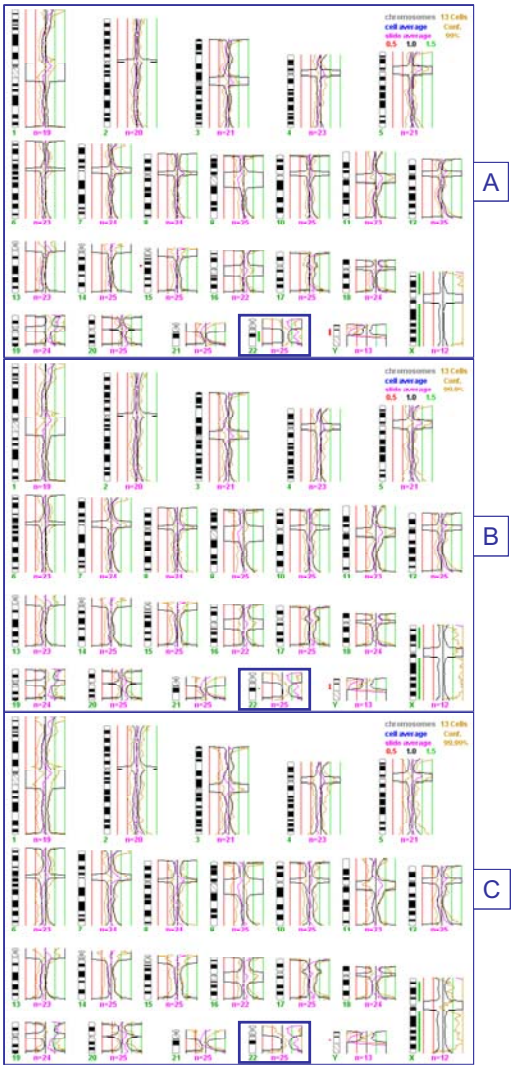

Figure 4: CGH ratio profiles demonstrating how the gain of chromosome 22 (box) becomes less evident as the confidence intervals (CI) are tightened. [A] 99% CI. [B] 99.9% CI. [C] 99.99% CI.

Conclusion

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. WGA followed by CGH allows for total aneuploidy screening and this approach offers the potential for avoiding the unwitting transfer of embryos carrying fatal chromosomal errors. Further assessment of sensitivity and specificity as well as the appropriate confidence intervals is required before such methods can be adapted for clinical application.