The use of FISH technique to examine chromosomal realignment in leukemic bone marrow samples

Newcastle University
Northern Institute for Cancer Research

Introduction

- Acute lymphoblastic leukaemia (ALL)=most common cancer in children.
- Cytogenetics (the study of chromosomes) has identified 3 distinct genetic subtypes of ALL; high hyperdiploidy (50-65 chromosomes), low hypodiploidy (30-39 chromosomes) and near haploidy (23-29 chromosomes).
- Previous research shows near haploid patients have poor prognosis, this project focuses on this small subgroup of patients
- Within near haploids, patients possess 1 of 3 clone types; haploid, doubled up, or both
- Leukemic cells are characterised by the fact they have missing chromosomes. The cells divide, halving their genetic content, and then attempt to compensate for the missing chromosomes by doubling up. The cell now can't undergo mitosis as a normal cell would, as there are insufficient chromosomes.

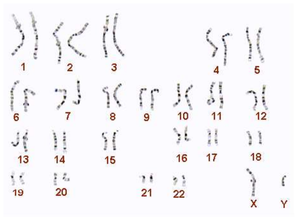

Figure 1: karyotype of healthy cell(1)

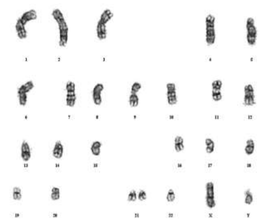

Figure 2: karyotype of near haploid cell

Aims & Objectives

1. To determine whether any haploid patients had hidden doubled up clones
2. To determine whether any doubled up clones had hidden haploid clones
3. Using this data to determine a correlation with survival

Method

- Prepare fixed cell samples (centrifuge and resuspension of pellet into fresh fix) & add to slides
- Prepare probe in a 1:10 dilution and load onto cover slips
- Seal the round edge of cover slips with rubber solution and insert slides into hybrite and leaving overnight to incubate at 37oC
- On day 2, after removing from hybrite, place slides into SSC, Wash1 then Wash2 solution
- Finally, use DAPI (counterstain) on cover slip to make the chromosomes visible under a fluorescent microscope
- View under microscope and count signals produced

Results

- From the data produced, it can be said that 6 patients (out of the 43) had a hidden clone in their karyotype which we identified by carrying out FISH technique
- There was a 23.5% decrease in haploids, 3.33% decrease in doubled ups and 23.1% increase in both clonal groups

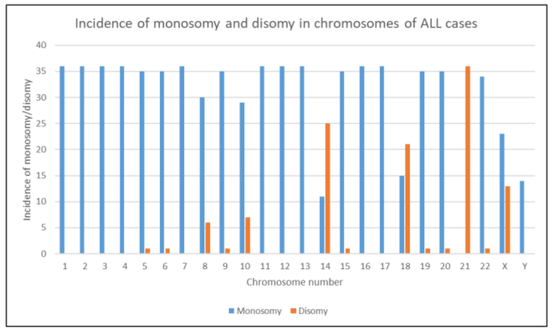

Figure 3: bar chart to show incidence of monosomy and disomy in haploid cases (left)

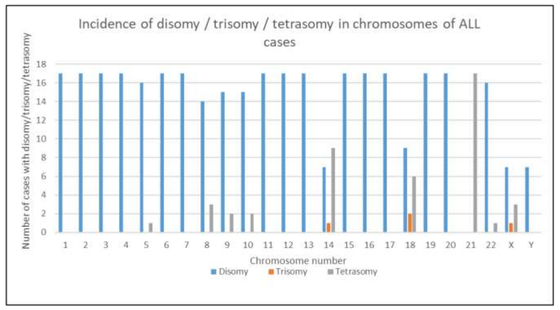

Figure 4: bar chart to show disomy/trisomy/tetrasomy in doubled up cases (left)

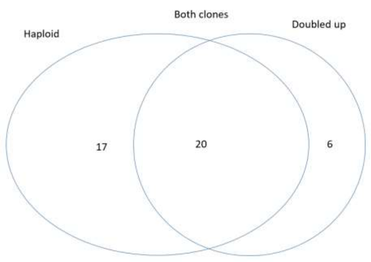

Figure 5: a Venn diagram to depict the patient cohort presence of clones (above)

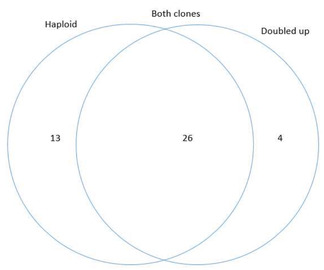

Figure 6: a Venn diagram to depict the patient cohort of clones following further FISH examination

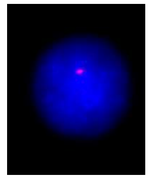

Figure 7: FISH using CEP18 (probe used to bind to centromere of chromosome 18) showing 1 copy of Chr18 is present, ie monosomy

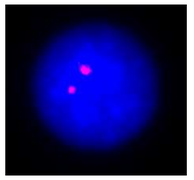

Figure 8: FISH using CEP18 showing disomy (2 copies of chromosome 18)

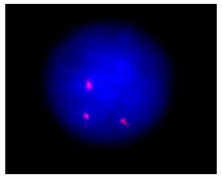

Figure 9: FISH using CEP18 showing trisomy (3 copies of chromosome 18)

Discussion & Conclusion

- It can be concluded from the findings that some of the patients did possess a hidden clone, with them switching from just haploid to both/doubled up and vice versa
- The results indicate that those patients who possess a hidden clone often result in poor prognosis (lower chance of survival), compared to those who don't

Future work

- The findings from this research could indicate that hidden clones are an element of the karyotype to look out for- now being aware of the likelihood of poor prognosis, researchers can look out specifically for the hidden clones in order to rule this out and if there is a hidden clone found, then to start treatment straightaway in order to increase the chances of survival

References

1. Chromosome Disorder Outreach, Inc. (2019). *Introduction to Chromosomes - Chromosome Disorder Outreach Inc*. [online] Available at: https://chromodisorder.org/introduction-to-chromosomes/ [Accessed 22 Jul. 2019].

Charlotte Stephenson (b7009290- BSc Biomedical Sciences)
c.stephenson@newcastle.ac.uk
Supervisor: Professor Anthony Moorman, Dr Tom Creasey, Wolfson Childhood Cancer Research Centre, Northern Institute for Cancer Research, Newcastle University

With special thanks to George Henderson and George Brown Endowment funds for funding my research scholarship. To my supervisor Dr Creasey for guiding me through my research, and to Professor Moorman, for making this opportunity possible.