TERF2 GENE EXPRESSION IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKAEMIA

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DISSERTATION SUBMITTED IN FULFILLMENT OF THE REQUIREMENT FOR THE DEGREE OF MASTER OF BIOTECHNOLOGY

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ABSTRACT

Advances in the understanding of cell biology in childhood acute lymphoblastic leukemia (cALL) have led to sophisticated risk-stratification based therapeutic interventions targeting individual patients. Despite these advances, approximately 10-20% of children with ALL still relapse. Thus, identification of new prognostic indicators will allow for better targeted treatment. *TERF2*, one of the main components of the shelterin complex (telosome), has been found to be over-expressed in a variety of epithelial malignancies, including lung, skin and breast cancer. This study aims to investigate the level of expression of *TERF2* in pediatric ALL and in different cALL patient subgroups and its potential as a prognostic marker in such patients.

Diagnostic bone marrow samples were obtained from 72 paediatric patients treated at the University Malaya Medical Centre (UMMC) under the Malaysia-Singapore ALL 2003 study protocol. Expression of *TERF2* was measured via real time quantitative PCR using cDNA synthesized from the samples described above. Results were standardized using *B2M* transcripts as the internal control and HL60 as the calibrator. Relative quantification of gene expression was calculated by using the delta delta Ct method.

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TERF2 was significantly upregulated in leukemia samples compared to control samples obtained from bone marrow cells and peripheral blood of 'normal' patients and those with non-lymphoblastic malignancies ($p<0.05$). The range of expression varied amongst the cALL patients. Thus a sequential *TERF2* analysis was performed according to the different cALL subgroups. Over expression of *TERF2* was observed in the *TEL-AML1* subgroup - associated with a good response to chemotherapy- whilst *TERF2* was underexpressed in the *BCR-ABL* subgroup which is associated with a poorer response to treatment.

Our study suggests that overexpression of *TERF2* provides a protective advantage to the chromosomes which ironically, is to the detriment of blast survival. Indeed, the cALL subgroup TEL-AML which overexpresses *TERF2* is associated with a good prognosis whilst the reverse is true for patients with *BCR-ABL1* positive disease. It protects the telomere and subsequently promotes chromosomal stability, thus allowing the cell to undergo normal cell death with viable *ATM* and *p53* signaling pathways. We hypothesize that the ability of the blasts to maintain normal cell cycle and undergo apoptosis allows the different chemotherapy agents which act at various stages of the cell death pathway to be effective thus minimizing the rate of relapse. We conclude that over-expression of *TERF2* results in better treatment outcome in cALL patients. Larger sample size and protein level studies are needed to validate the results of this finding.

ABSTRAK

Kemajuan dalam pemahaman biologi sel dalam leukemia limfoblastik akut (LLA) telah melahirkan rawatan canggih berdasarkan stratifikasi risiko dan penargetan individu. Walaubagaimanapun, sekitar 10-20% kanak kanak dengan LLA tidak sembuh sepenuhnya, malah mendapat penyakit ini semula.Oleh yang demikian, penemuan penanda prognostik yang baru akan membantu rawatan ini supaya. *TERF2*, adalah salah satu komponen utama dari kompleks shelterin (telosome) di mana tahap ekspresi dijumpai tinggi dalam pelbagai penyakit berkaitan epitel, termasuk paru-paru, kulit dan kanser payudara. Penyelidikan ini dijalankan dengan tujuan untuk mengetahui tahap ekspresi *TERF2* dalam sub-kumpulan pesakit LLA dan potensinya sebagai penanda prognostik bagi pesakit tersebut.

Sampel sumsum tulang diagnostik diperolehi daripada 72 pesakit kanak-kanak yang dirawat di Pusat Perubatan Universiti Malaya (PPUM) di bawah Protokol Penyelidikan LLA Malaysia-Singapura 2003. Ekspresi *TERF2* ditentukan melalui 'real time PCR' (dipendekan daripada istilah Bahasa Inggeris – polymerase chain reaction) dengan menggunakan komplemen deoksiribonukleat acid (cDNA) (dipendekan daripada istilah Bahasa Inggeris – complement deoxyribonucleic acid) daripada sampel yang dinyatakan di atas. Keputusan telah diselaraskan dengan menggunakan transkrip *ß2M* sebagai kawalan dalaman dan HL60 sebagai kalibrator. Kuantifikasi relatif ekspresi gen ditentukan dengan menggunakan kaedah delta delta C_T.

Ekspresi gen *TERF2* adalah tinggi dalam sampel leukemia berbanding dengan sampel kawalan yang diperolehi daripada sel sumsum tulang dan darah periferi daripada penderma yang normal dan mereka dengan penyakit lain yang berkaitan dengan hemoglobin ($p < 0.05$). Tahap ekspresi adalah amat berbeza antara pesakit LLA. Oleh yang demikian, analisis *TERF2* yang seterusnya dijalankan mengikut sub kumpulan yang berbeza. Ekspresi *TERF2* yang sangat tinggi dijumpai dalam subkelompok LLA, *TEL-AML1* – yang mempunyai tindak balas lebih baik terhadap kemoterapi. Sementara itu ekspresi rendah didapati pada sub kumpulan *BCR-ABL1* yang mempunyai tindak balas kurang baik kesan buruk terhadap rawatan kemoterapi.

Penyelidikan kami menunjukkan bahawa expresi *TERF2* yang tinggi memberikan kesan perlindungan terhadap kromosom dan sebaliknya kesan buruk kepada sel-sel kanser. Kesimpulannya, kumpulan *TEL-AML1* yang mempunyai ekspresi *TERF2* yang tinggi dikaitkan dengan prognosis yang baik sedangkan sebaliknya adalah benar untuk pesakit dengan penyakit positif *BCR-ABL1*. Ia melindungi telomer dan kemudian membantu kestabilan kromosom, sehingga membolehkan sel untuk mengalami kematian sel normal. Kami berhipotesis bahawa kemampuan sel kanser untuk mengekalkan kitaran sel normal dan menjalani apoptosis membolehkan agen kemoterapi yang berbeza untuk bertindak pada pelbagai tahap kematian sel pusat. Ia membolehkan rawatan lebih efektif dan meminimumkan kadar penyakit berulang. Kami menyimpulkan bahawa ekspresi tinggi *TERF2* memberi kesan rawatan yang lebih baik pada pesakit LLA. Saiz sampel yang lebih besar dan kajian pada tahap protein diperlukan untuk mengesahkan hasil kajian ini.

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LIST OF ABBREVIATIONS

1. ALL – acute lymphoblastic leukemia
2. ALT- alternative lengthening of telomeres
3. AML – acute myeloid leukemia
4. ANCOVA-analysis of co variance
5. BCR-ABL- breakpoint cluster region V-abl Abelson murine leukemia viral oncogene homolog 1
6. BLM- Bloom syndrome protein
7. cDNA- complementary deoxyribonucleic acid
8. CML- chronic myloid leukemia
9. CNS – central nervous system
10. CT value – critical threshold value
11. DMSO- dimethyl sulfoxide
12. DNA - deoxyribonucleic acid
13. DNA-PK DNA-dependent protein kinase
14. dNTP- deoxyribonucleotide
15. dsDNA-double stranded deoxyribonucleic acid
16. EDTA- ethylenediamine tetra-acetic acid
17. EFS- event-free survival
18. ERCC1- DNA excision repair protein
19. ETS – expressed tagged sequence
20. FISH- fluorescence in situ hybridization
21. HBSS- Hanks balance salt solution
22. HL60- Human promyelocytic leukemia cells
23. HOX11- TALE homeoproteinsHRC57
24. HRX- Histone-lysine N-methyltransferase
25. hTERT- Human telomerase reverse transcriptase

26. JMML – Juvenile myelomonocytic leukemia
27. LYL1- lymphoblastic leukemia derived sequence 1
28. MLL- mixed-lineage leukemia -
29. MYC – v-myc myelocytomatosis viral oncogene homolog (avian)
30. OFT- Oncogene Fusion Transcript
31. PARP- poly (ADP-ribose) polymerase 1
32. Ph- Philadelphia
33. POT1- protection of telomere 1
34. RAP - repressor activator protein 1'
35. RNA – ribonucleic acid
36. RQ-PCR – Real-time polymerase chain reaction
37. RT-PCR – reverse transcription polymerase chain reaction
38. SYBR-Green- Synergy Brands, Inc. Green
39. TAL1- T-cell acute lymphocytic leukemia 1
40. *TERF2* – Telomere Repeat Binding Factor2
41. TERT – Telomerase reverse-transcriptase
42. TIN- interacting nuclear protein
43. U.S. – United States
44. WBC – white blood cells
45. *β2M*- beta-2-microglobin