



BioPharmica Limited

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BioPharmica (ASX: BPH) ASX Announcement

Genes Identified in a Hemopoietic Lineage Switch Influence Transcription

Dr Louise Winteringham from the Western Australian Institute for Medical Research (WAIMR) presented the attached poster at the American Society of Hematology (ASH).

The poster outlines some of the scientific findings that were published by the respected journal "Blood". In the publication the WAIMR research team report their important findings concerning the role of HLS5 in controlling gene regulation and differentiation of leukemic cells. <http://bloodjournal.hematologylibrary.org/>

WAIMR combines the Royal Perth Hospital, Sir Charles Gairdner Hospital, Fremantle Hospital and the University of Western Australia (UWA) and aims to uncover the genetic and environmental causes of a range of diseases. The ultimate goal is to prevent disease developing and to create improved treatments if these conditions do emerge.

BioPharmica manages a strong portfolio of biomedical technologies emerging from research by leading Universities, Medical Institutes and Hospitals across Australia. BioPharmica is working with WAIMR to develop and commercialise HLS5.

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Yours sincerely,

David Breeze
Chairman

Genes Identified in a Hemopoietic Lineage Switch Influence Transcription

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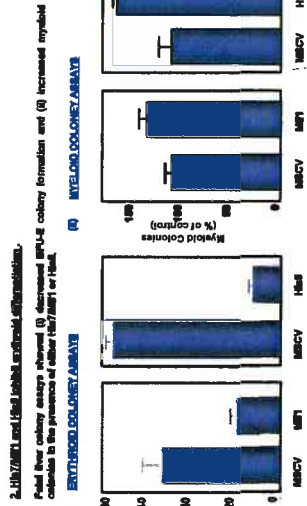
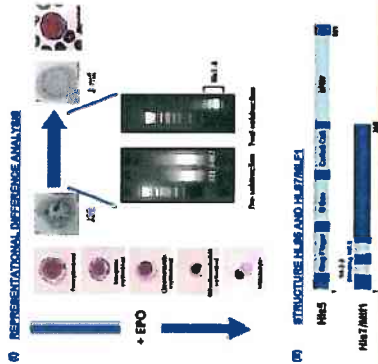
The University of Western Australia

Abstract

The JZE erythroid/oblast cell line responds to erythropoietin (Epo) by morphological maturation and hemoglobin synthesis. However, on rare occasions, these cells have been observed to undergo lineage switch and display features of macroblastoid cells which do not respond to Epo. An array of 74 genes was identified in the macroblastoid variant HNSG Herpetic Invasor Switch (HIS) 5 and 7. HIS 5 and 7 are members of the HNF1A family of transcription factors, which includes HNF1A, HNF1B, HNF1C, HNF1D, HNF1E, HNF1F, HNF1G, HNF1H, HNF1I, HNF1J, HNF1K, HNF1L, HNF1M, HNF1N, HNF1O, HNF1P, HNF1Q, HNF1R, HNF1S, HNF1T, HNF1U, HNF1V, HNF1W, HNF1X, HNF1Y, HNF1Z, HNF1AA, HNF1AB, HNF1AC, HNF1AD, HNF1AE, HNF1AF, HNF1AG, HNF1AH, HNF1AI, HNF1AJ, HNF1AK, HNF1AL, HNF1AM, HNF1AN, HNF1AO, HNF1AP, HNF1AQ, HNF1AR, HNF1AS, HNF1AT, HNF1AU, HNF1AV, HNF1AW, HNF1AX, HNF1AY, HNF1AZ, HNF1BA, HNF1BB, HNF1BC, HNF1BD, HNF1BE, HNF1BF, HNF1BG, HNF1BH, HNF1BI, HNF1BJ, HNF1BK, HNF1BL, HNF1BM, HNF1BN, HNF1BO, HNF1BP, HNF1BQ, HNF1BR, HNF1BS, HNF1BT, HNF1BU, HNF1BV, HNF1BW, HNF1BX, HNF1BY, HNF1BZ, HNF1CA, HNF1CB, HNF1CC, HNF1CD, HNF1CE, HNF1CF, HNF1CG, HNF1CH, HNF1CI, HNF1CJ, HNF1CK, HNF1CL, HNF1CM, HNF1CN, HNF1CO, HNF1CP, HNF1CQ, HNF1CR, HNF1CS, HNF1CT, HNF1CU, HNF1CV, HNF1CW, HNF1CX, HNF1CY, HNF1CZ, HNF1DA, HNF1DB, HNF1DC, HNF1DD, HNF1DE, HNF1DF, HNF1DG, HNF1DH, HNF1DI, HNF1DJ, HNF1DK, HNF1DL, HNF1DM, 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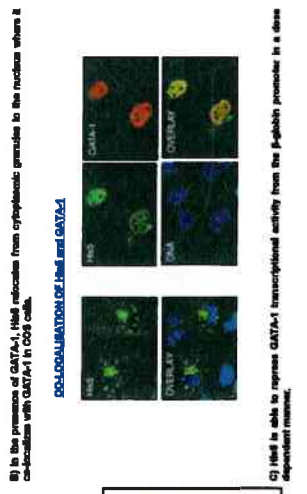
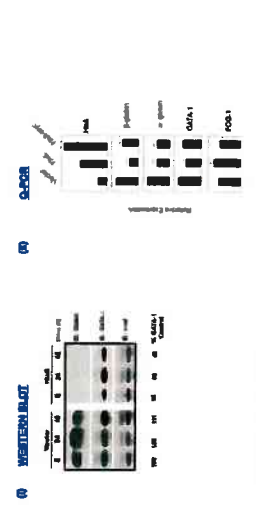
Background

1. Hemopoietic Differentiation Assay (HDA) identified differentially expressed genes.
 (a) An RNA was performed against the Epo responsive JZE cell line and its erythroid derivative J-nd.
 (b) Two of the J-nd's identified, Hsf1 and Hsf2 were novel genes.

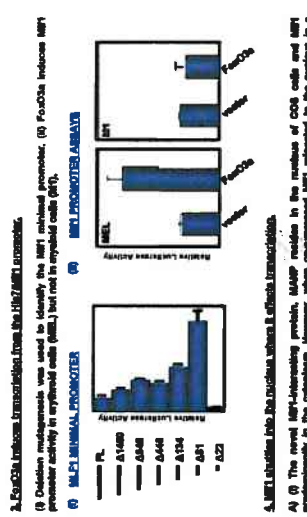
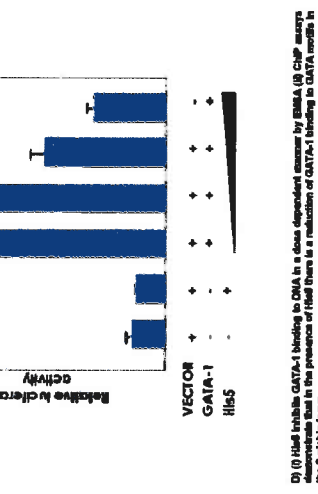


Results

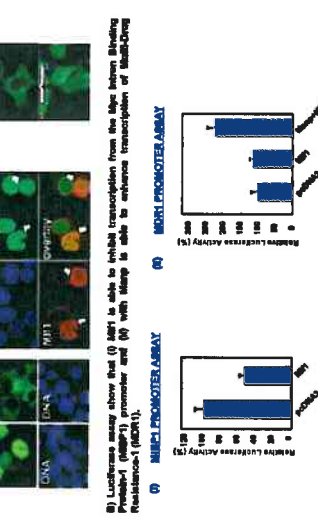
1. Hsf1 inhibits alpha-globin.
 JZE cells expressing Hsf1 (a) produce less globin and GATA-1 protein than vector controls and (b) have decreased expression of the GATA-1 target genes, alpha-globin.



2. Hsf1 inhibits GATA-1 binding to DNA. (A) EMSA shows Hsf1 inhibits GATA-1 binding to DNA. (B) ChIP assay shows Hsf1 inhibits GATA-1 binding to DNA in JZE cells.



3. Hsf1 inhibits beta-globin. (A) Bar graph shows beta-globin mRNA levels in JZE cells. (B) Western blot shows beta-globin protein levels in JZE cells.



Summary

Hsf1 and Hsf2 were identified as genes up-regulated in an erythroid to myeloid lineage switch. Both genes inhibit erythropoiesis by affecting transcription of Hsf1 target genes. Hsf1 is able to down-regulate beta-globin by inhibiting activity of the transcription factor GATA-1. Hsf2/HSF2 is able to disturb between the cytoplasm and the nucleus where it is able to affect transcription of a number of genes associated with hemopoiesis.

References

1. Lalonde JN, Endersby R, Majewski I, Beaumont J, Kobelke S, Winterringham LN, Klinken SP. Hsf1 and Hsf2 are novel transcription factors that repress erythropoiesis. *J Biol Chem* 2004; 279: 10445-10452.
2. Lalonde JN, Endersby R, Majewski I, Beaumont J, Kobelke S, Winterringham LN, Klinken SP. Hsf1 and Hsf2 are novel transcription factors that repress erythropoiesis. *J Biol Chem* 2004; 279: 10445-10452.
3. Lalonde JN, Endersby R, Majewski I, Beaumont J, Kobelke S, Winterringham LN, Klinken SP. Hsf1 and Hsf2 are novel transcription factors that repress erythropoiesis. *J Biol Chem* 2004; 279: 10445-10452.
4. Lalonde JN, Endersby R, Majewski I, Beaumont J, Kobelke S, Winterringham LN, Klinken SP. Hsf1 and Hsf2 are novel transcription factors that repress erythropoiesis. *J Biol Chem* 2004; 279: 10445-10452.