



BioPharmica Limited

Bridging Biotechnology Borders

BioPharmica (ASX: BPH) ASX Announcement

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Hls5 to be presented at the 51st ASH Annual Meeting

BioPharmica Limited is pleased to announce that Dr Louise Winteringham from the Western Australian Institute for Medical Research (WAIMR) has been invited to present at the American Society of Hematology (ASH) in New Orleans on the 7 December 2009.

WAIMR combines the Royal Perth Hospital, Sir Charles Gairdner Hospital, Fremantle Hospital and the University of Western Australia 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 for when these conditions emerge.

BioPharmica is working with WAIMR to develop and commercialise Hls5 which will be part of the Molecular Discovery Systems IPO on the Australian Securities Exchange.

Molecular Discovery Systems will be a company dedicated to Hls5, the Anti-Mitotic Drug Discovery Development Program along with its current high content screening facility. Under a planned 'spin off' process, BioPharmica (ASX: BPH) shareholders will receive a share at no cost in Molecular Discovery Systems for every listed BioPharmica share that they hold five days after the date that shareholder approval of the spin off is obtained, and this is expected to take place late December 2009.

The following abstract outlines how Hls5, a novel ubiquitin E3 ligase, modulates levels of sumoylated GATA-1.

ASH Abstract 2009

Hemopoietic lineage commitment is controlled, in part, by transcription factors that regulate specific genes required for the formation of mature blood cells. Differentiation along particular hemopoietic lineages is dependant not only on the presence of particular transcription factors, but also on appropriate concentrations - altering transcription factor levels can force cells into different hemopoietic pathways. Transcription factors undergo numerous post-translational modifications and are controlled spatially via sub-cellular localisation. De-regulation of transcription factors can result in leukemias, or other blood disorders. GATA-1 is an example of a key lineage-determining gene, essential for erythropoiesis. Increasing GATA-1 levels promotes maturation along the erythroid pathway, whereas reducing GATA-1 concentrations favours myelopoiesis.

GATA-1 regulation occurs at multiple levels including transcription, translation and post-translational modifications such as phosphorylation, acetylation, ubiquitination and sumoylation. Although GATA-1 ubiquitination modifies the protein for proteasomal degradation, the effect of adding small ubiquitin-like modifier (Sumo) to GATA-1 is unclear. Several examples of hemopoietic differentiation plasticity have been observed. We reported a lineage switch by erythroleukemic J2E cells which spontaneously developed a monoblastoid phenotype. Two genes (Hls5 and Hls7/Mlf1) were isolated from this lineage switch with potential lineage-determining features. Hls5 is a member of the RBCC (Ring finger, B-box, Coiled-coil) family of proteins, which includes PML.

BioPharmica Limited ACN 95 912 002
PO Box 317, North Perth Western Australia 6906
14 View Street, North Perth Western Australia 6006
admin@biopharmica.com.au www.biopharmica.com.au
T: +61 8 9328 8366 F: +61 8 9328 8733

Ectopic expression of Hls5 impedes erythroid differentiation by reducing GATA-1 levels, and suppressing hemoglobin synthesis. Significantly, Hls5 relocates from the cytoplasm to associate with GATA-1 in the nucleus, where it interferes with DNA binding and transactivation of GATA-1. Several members of the RBCC family are ubiquitin E3 ligases, catalysing the final step in the ubiquitination process - these molecules play a vital role in regulating the levels of target proteins. Here we show that Hls5 is a bona fide ubiquitin E3 ligase, in partnership with several ubiquitin E2 enzymes. The Ring finger is critical for Hls5 ligase activity as mutation of key residues within the Ring finger ablates catalytic activity. Interestingly, a yeast 2 hybrid screen for Hls5 interactors identified Ubc9 and Pias1, which act as E2 and E3 enzymes in the sumoylation cascade. Co-immunoprecipitation, BRET and co-localization experiments confirmed the Hls5 association with Ubc9 and Pias1. Moreover, Hls5 binds Sumo-1 (but not Sumo-2 or 3), and co-localizes with Sumo-1 in discrete nuclear bodies. Thus, Hls5 interacts with several components of the intracellular sumoylation machinery. Hls5 can also reduce sumoylated proteins globally, indicating it may target these modified proteins for degradation. Recently, a new family of ubiquitin E3 ligases has been described which specifically mark sumoylated proteins for degradation. These Sumo-targeted ubiquitin ligases (STUbL) are found primarily in yeast, and only one mammalian STUbL has been identified. We postulated that Hls5 may be a STUbL, capable of regulating sumoylated GATA-1. Our data demonstrate that while Hls5 is able to bind GATA-1 via the B-box and Coiled-coil domains, it preferentially associates with sumoylated GATA-1 through a canonical Sumo interacting motif (SIM). This results in increased GATA-1 ubiquitination and, as a consequence, levels of sumoylated GATA-1 are reduced substantially. Since mutation of the lysine necessary for Sumo attachment does not affect GATA-1 transactivation, sumoylation may act as a prelude to ubiquitination and protein turn-over. We propose, therefore, that GATA-1 mediates transcription of target genes, and is subsequently sumoylated by Pias1 and Ubc9 – addition of Sumo moieties to GATA-1 enhance binding to Hls5, which in turn impedes GATA-1 DNA binding, and promotes ubiquitination for proteasomal degradation. This model is consistent with decreased levels of GATA-1 in erythroid cells ectopically expressing Hls5, and with the original isolation of Hls5 as a potential lineage-determining gene involved with the erythroid to monoblastoid lineage switch. Thus, Hls5 is a novel STUbL which plays a role in hemopoietic lineage commitment by modulating GATA-1 activity and content.

For more information contact:

David Breeze
BioPharmica Limited
Tel : +61 9 9328 8366

Natalie Papadopoulos
Capture Media - WAIMR
Tel : +61 8 9388 9280

Yours sincerely,



David Breeze
Chairman