BPH Corporate Ltd

BPH Corporate [ASX: BPH] ASX Announcement

16 September 2010

Companies Announcements Office
Australian Securities Exchange Limited
10th Floor, 20 Bond Street
SYDNEY NSW 2000

Dear Sir/Madam,

HLS5 presented at The Society for Hematology and Stem Cells

BPH Corporate Limited [ASX: BPH] is pleased to announce that researcher Dr Louise Winteringham from the Western Australian Institute for Medical Research (WAIMR) presented a poster at The Society for Hematology and Stem Cells (ISEH) 2010 in Melbourne yesterday. A copy of the poster presented, *HLS5, a novel ubiquitin E3 ligase, modulates levels of sumoylated GATA-1 and regulates erythroid progenitor cell differentiation,* has been attached for your information.

A brief summary of the poster is outlined below:

> *We have shown that Hls5 is able to regulate the levels of a critical erythroid transcription factor GATA-1. GATA-1 is required for the normal differentiation of red blood cells and its expression within these cells is tightly regulated. We present a novel mechanism for the regulation of GATA-1 by HLS5. Post-translational modification of GATA-1 by SUMO, allows HLS5 to bind and target it for degradation via the ubiquitin – proteasome pathway, Failure to regulate GATA-1 appropriately may contribute to leukemia and other blood related disorders.*

Dr Winteringham stated "this is a great opportunity to present our work at an international meeting to an audience of researchers and clinicians working in the field of hematology and stem cells".

BPH Corporate manages a strong portfolio of biomedical technologies emerging from research by leading Universities, Medical Institutes and Hospitals across Australia. BPH is working with WAIMR to develop and commercialise HLS5 which is part of BPH investee company Molecular Discovery Systems' portfolio of projects.

Yours sincerely,

David Breeze
Chairman

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HLS5, a novel ubiquitin E3 ligase, modulates levels of sumoylated GATA-1 and regulates erythroid progenitor cell differentiation

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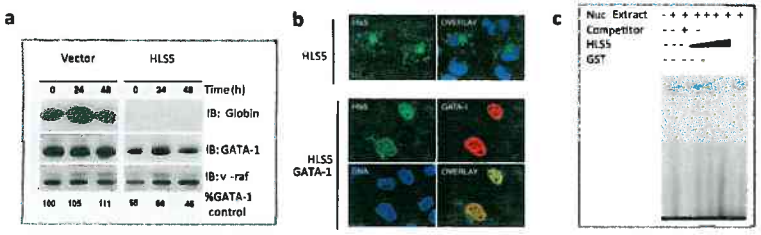
Abstract

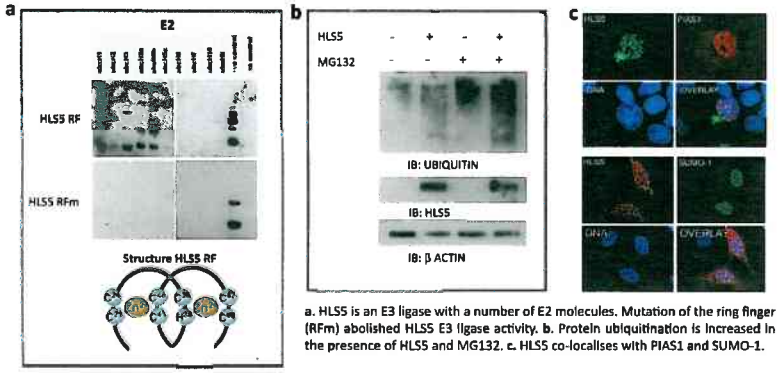
Lineage commitment of haemopoietic progenitor cells is controlled, in part, by transcription factors that regulate specific genes required for the formation of mature blood cells. De-regulation of transcription factors can result in leukemias or other blood disorders. GATA-1 is a key lineage-determining gene, essential for the differentiation of erythroid progenitor cells. GATA-1 regulation occurs at multiple levels including ubiquitination and sumoylation. HLS5 is a member of the RBCC family of proteins, which includes PML. 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. Here we show that HLS5 is a bona fide ubiquitin E3 ligase. HLS5 also interacts with several components of the intracellular sumoylation machinery. We show that HLS5 can reduce sumoylated proteins globally, indicating it may target these modified proteins for degradation. A new family of ubiquitin E3 ligases has been described which specifically mark sumoylated proteins for degradation viz Sumo-Targeted Ubiquitin Ligases (STUbL). We postulated that HLS5 may be a STUbL, capable of regulating sumoylated GATA-1. Recently it has been shown that sumoylated GATA-1 activates a specific sub-set of erythroid genes, including globin and heme biosynthesis genes critical for red cell maturation. Our data demonstrate that binding of HLS5 to sumoylated Gata-1 via a Sumo Interacting motif (SIM) results in increased GATA-1 ubiquitination and, as a consequence, levels of sumoylated GATA-1 are reduced substantially. Therefore, in addition to impeding GATA-1 DNA binding, HLS5 promotes ubiquitination of sumoylated GATA-1 for proteasomal degradation. These data indicate that HLS5 plays a pivotal role in determining haemopoietic lineage fate by modulating the levels and activity of sumoylated GATA-1.

HLS5 inhibits GATA-1



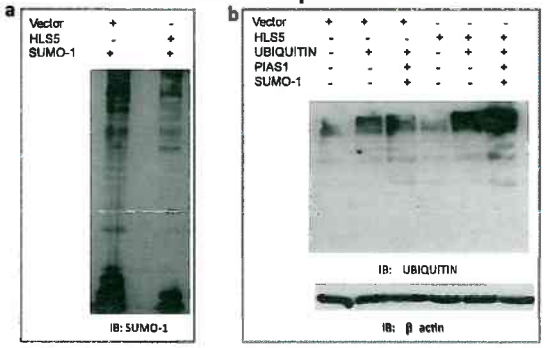
a. GATA-1 and β-globin protein are down-regulated in the presence of HLS5. b. HLS5 moves into the nucleus to associate with GATA-1. c. GATA-1 DNA binding is inhibited in the presence of HLS5.

HLS5 is a ubiquitin E3 ligase



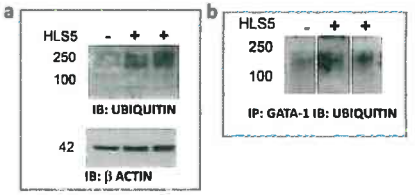
a. HLS5 is an E3 ligase with a number of E2 molecules. Mutation of the ring finger (RFm) abolished HLS5 E3 ligase activity. b. Protein ubiquitination is increased in the presence of HLS5 and MG132. c. HLS5 co-localises with PIAS1 and SUMO-1.

HLS5 decreases global sumoylaton, increases ubiquitination



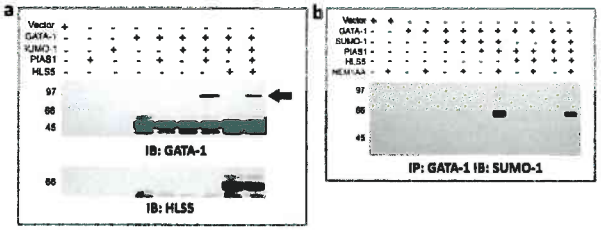
a. In the presence of HLS5 global sumoylation is reduced. b. Protein ubiquitination is significantly increased in the presence of HLS5 and in the presence of SUMO ubiquitination is increased further.

HLS5 increases GATA-1 ubiquitination in erythroid cells



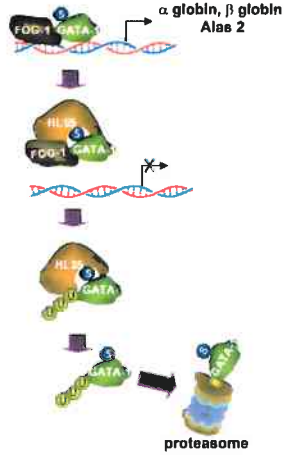
In an erythroid cell line (J2E) HLS5 a. increases ubiquitination. b. Increases GATA-1 ubiquitination.

HLS5 decreases sumoylated GATA-1



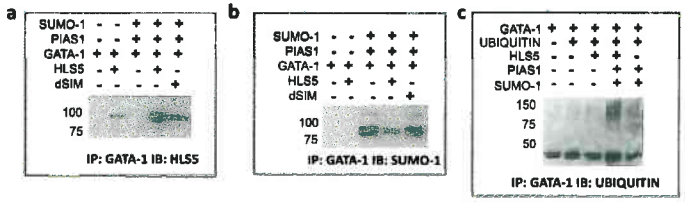
a. HLS5 decreases sumoylated GATA-1 (high molecular weight band) in the presence of PIAS1 and SUMO-1.b. GATA-1 immunoprecipitation confirms that HLS5 decreases the level of sumoylated GATA-1.

SUMO-targeted ubiquitination of GATA-1 by HLS5



GATA-1 and FOG-1 bind DNA, HLS5 can interact with sumoylated GATA-1 via its SIM. The ring finger of HLS5 functions as an E3 ligase to uniquitinate GATA-1 and target it for degradation.

HLS5 SIM is important in GATA-1 sumoylation



a HLS5 - GATA-1 binding is reduced when HLS5 SIM domain is deleted. However, binding is not affected when GATA-1 is not sumoylated. b. The Sumo Interaction Motif (SIM) of HLS5 is required for downregulation of sumoylated GATA-1. c. In the presence of HLS5 ubiquitination of GATA-1 is significantly increased.

WAIMR Western Australian Institute for Medical Research

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