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Stress-induced haematopoietic stem cell proliferation: new roles for p38α and purine metabolism.

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Abstract

In the bone marrow, Long Term Haematopoietic Stem Cells (LT-HSC) are normally maintained in a quiescent state. Following haematological stresses, such as infection, bone marrow transplantation or exposure to ionising radiation or cytotoxic drugs, LT-HSC are stimulated to proliferate in order to allow repopulation of mature haematopoietic cells. This entry into the cell cycle requires changes in the metabolism of the HSCs, however the pathways controlling these changes are not fully understood. In a recent issue of Cell Stem Cell, Karigane et al have shed new light on how this process is regulated. They have indentifed a new role for the p38 MAPK pathway regulating the transition of LT-HSCs from a quiescent to proliferative state. Proliferation requires DNA replication and thus a supply of nucleotides, and Karigane have uncovered that p38 regulates purine metabolism in HSCs via controling the espression of key enzymes in this metabolic pathway.

Haematopoietic stem cells (HSCs) are self-renewing cells that sustain the production of all blood and immune cell lineages throughout life by residing at the top of the haematopoietic hierarchy. They first arise early in embryogenesis and their site of action changes during development. In adult mammals, HSCs reside in the bone marrow within a complex microenvironment termed the HSC niche (1). They are defined by their ability to completely repopulate the entire blood system of a recipient, resulting in the formation of red blood cells, megakaryocytes, myeloid cells and lymphocytes, and in order to do this they are able to undergo asymmetric division where one daughter cell remains as a HSC whilst the other becomes committed to differentiation (2). In the bone marrow, to prevent exhaustion and to preserve their ability to self-renew, HSCs are normally maintained in a dormant or quiescent state, from which they can be rapidly awakened in response to haematological stress (3). How this is regulated is an important issue and in a recent issue of Cell Stem Cell Karigane et al have shed new light on how the p38 MAPK pathway promotes stress-induced proliferation in HSCs (4).

HSCs are maintained in quiescence through tight regulation of their metabolic state, which is controlled by both cell intrinsic and extrinsic factors. Long-term HSCs (LT-HSCs) keep rates of energy consumption low through anaerobic glycolysis, resulting in low levels of reactive oxygen species (ROS) production (5). Maintaining minimal levels of ROS is thought to be key for maintaining the self-renewing capacity of stem cells and preventing HSC exhaustion (6). ROS production is limited in quiescent HSCs through the actions of hypoxia inducible factor 1α (HIF1α), which drives cellular metabolism towards anaerobic glycolysis instead of mitochondrial respiration (6,7). HIF1α controls the activation of pyruvate dehydrogenase kinase 2 (Pdk2) and Pdk4, which maintain glycolysis whilst actively suppressing mitochondrial metabolism in quiescent HSCs (5).

Understanding the metabolic changes that underlie the switch from quiescence to proliferation could not only help to improve the methods and protocols for expanding and maintaining HSCs in vitro to prevent HSC exhaustion, but could also inform on how alterations in these cells underlie the development of stem cell diseases such as leukaemias (8). As quiescent cells have different energy requirements to active cells, changes in cellular metabolism are thought to be important in the transition from dormancy to cycling. This is reflected by the observation that whilst ROS levels are kept low in quiescent cells, elevating ROS production promotes HSC differentiation to enable repopulation (6).

The p38α MAPK pathway has been suggested to act downstream of ROS in HSCs (9,10). p38 MAPKs form a subgroup of MAPKs, of which 4 isoforms exist in mammalian cells and of which p38α has been the most intensely studied. Interest in p38α in immunity was first stimulated by the finding

that it was the cellular target of a group of pyridinyl imidazole drugs, such as SB203580, that could inhibit TNF production, however since then a wide variety of functions for p38α have been uncovered (11,12). In mice, p38α knockout is embryonic lethal due to problems with extra-embryonic and placental development, impacting on cardiovascular development in the embryo (13,14). Rescue of the extra-embryonic issues allowed p38α embryos to develop until term. One knockout study additionally reported an issue with erythropoiesis, however this was not cell autonomous as HSCs from these mice could restore haematopoietic development in recipient mice (15). Together these results suggest that p38α is not critical for HSC development and homeostasis. Several studies have however indicated roles for p38α in HSCs following stress. Treatment of HSCs in culture with buthione sulfoximine (BSO) to induce ROS production leads to activation of p38 and was linked to a decreased ability to maintain HSC quiescence (9). Significantly, mice with null mutations in ATM, Jak2 or FOXO3a show elevated levels of ROS in their HSC populations leading to a decreased repopulation capacity of these HSCs in adoptive transfer experiments in mice (9,10,16). The elevated ROS levels were linked to increased p38 activation, and long term treatment of the mice with SB203580 (which inhibits p38α and p38β) in vivo was able to restore the capacity of these HSCs to repopulate the HSC compartment in lethally irradiated mice (9,10). To investigate if p38α also played a similar role in wild type HSCs, serial transfer of LSK cells was used. In these experiments strong elevations in ROS were only apparent after the 3rd transfer, and this correlated to increased to p38α activation and a reduction in the capacity of the cells to repopulate the HSC compartment (9). Together these results suggest that when HSCs are exposed to high levels of ROS, p38α activation has a negative effect on the capacity of cells to maintain an HSC phenotype.

p38α is however activated in response to many other stimuli in addition to ROS and it is possible that it may have different functions in HSCs depending on the context or upstream stimuli. An early indication that this might be the case came from studies on the p38α activated kinase MK2, where bone marrow or LSK cells from mice deficient in MK2 showed a reduced capacity to repopulate the bone marrow relative to wild type cells (17). Work by Karigane et al has now uncovered a new mechanism by which p38 MAPK signalling regulates HSC metabolism in response to haematological stress, under conditions that are unlikely to be driven by ROS (4). In a series of experiments, they reveal a novel p38α dependent pathway that regulates the metabolic status of HSCs and promotes cellular proliferation via activating purine metabolism. After initially identifying p38α as the major p38 MAPK isoform expressed in HSCs, they analysed haematopoietic phenotypes in an inducible conditional p38α mouse knockout model. Global deletion of p38α induced by intra-peritoneal injection of tamoxifen was found to be ~99% effective in HSCs compared to wild-type controls, and under steady-state conditions the frequencies of all types of HSCs and progenitors analysed were

indistinguishable between p38αΔ/Δ and p38α+/+ mice. Importantly, compensatory increases in the other p38 isoforms was not observed in the p38αΔ/Δ animals.

In contrast to their findings under steady state haematopoiesis where p38α did not play a major role, p38α was found to be important in the response of HSCs to haematological stress, with loss of p38α equating to defective stem cell capacity. In competitive transfer of LSK cells into lethally irradiated mice, p38αΔ/Δ HSCs displayed lower donor-derived chimerism than p38α+/+ cells, despite the differentiation status being comparable between genotypes. This difference was enhanced when a 2nd serial repopulation experiment was performed. Furthermore, the stress resistance of p38αΔ/Δ mice in response to the chemotherapy drug 5-fluorouracil (5-FU), which induces proliferation of quiescent HSCs by eliminating differentiated hematopoietic cells, was compromised as shown by a greater than 50% increase in mortality compared to p38α+/+ control animals. Importantly, the defective function of p38αΔ/Δ HSCs in response to haematological stress was not attributable to altered homing, apoptosis or production of ROS.

p38α phosphorylation was found to be increased in all haematopoietic cell fractions in response to haematological stress following 5-FU injection of mice and in transplanted BM cells following BMT. Analysis of cell cycle progression in p38αΔ/Δ HSCs showed delayed cell division after BMT and, in agreement with this, wild-type cells incubated in the presence of the p38 inhibitor SB203580 showed dose-dependent reductions in cell proliferation. Gene set enrichment analysis showed that the defects in cell cycle progression and proliferation observed in p38αΔ/Δ LT-HSCs following bone marrow transplantation were associated with a significant reduction in proliferation and HSC-marker genes, indicating a key role for p38α in regulating cell cycle progression in HSCs in response to haematological stress.

In order for HSCs to shift from a quiescent state to proliferation under homeostatic conditions they are required to alter their metabolic activity, and accordingly the metabolic state of HSCs has been linked to control of cell cycle activity (5,18). To investigate potential mechanisms for the action of p38α in regulating stress-induced metabolic change, the authors performed mass spectrometry based metabolomic analysis of p38αΔ/Δ and p38α+/+ lineage negative, Sca1+, c-Kit- (LSK) cells isolated from naive mice or those that had undergone bone marrow transplantation. Of those metabolites that were upregulated in transplanted p38α+/+ LSK cells relative to cells at steady state, most showed lower levels in p38α deficient cells. Glycine and aspartic acid were exceptions to this and were upregulated to higher levels in transplanted p38αΔ/Δ relative to p38+/+ LSK cells. Both Glycine and aspartic acid can feed into purine metabolism and this, along with a reduced level of

allantoin (a product of purine catabolism) in p38αΔ/Δ cells, indicated that p38α was regulating purine metabolism during stress haematopoiesis. To establish the mechanism by which p38α regulates purine metabolism to induce HSC proliferation in response to haematopoietic stress, the authors analysed mRNA levels of enzymes controlling purine metabolism and found that both inosine-5-monophosphate dehydrogenase 2 (*Impdh2,* the rate-limiting enzyme regulating guanosine monophosphate production) and guanosine monophosphate synthetase (*Gmps,* which catalyses the final step in GMP synthesis) were significantly reduced in p38αΔ/Δ HSCs relative to p38α+/+ HSCs the day following transplantation. To provide further evidence for a role of p38α in purine metabolism the authors treated EML-C1 cells (a stem cell factor-dependent lympho-hematopoietic progenitor cell line) with the p38 inhibitor SB203580 and showed that this resulted in decreased GTP levels. Further confirmation of a role for Impdh in the HSC response to haematopoietic stress was achieved by employing the Impdh inhibitor mycophenolic acid (MPA), which dose-dependently blocked the proliferation and repopulation abilities of HSCs.

The decreased levels of *Impdh2* and *Gmps* transcripts in the p38αΔ/Δ cells would be consistent with a role for p38α in regulating the expression of these genes, either directly or indirectly. A search of open-access databases for proteins that are expressed in HSCs and which regulate the transcription of purine-related enzymes revealed the microphthalmia-associated transcription factor (Mitf) as a candidate transcription factor. Transcript analysis indicated that Mitf expression was more highly expressed in HSCs than in differentiated cells, and that its expression in p38αΔ/Δ HSCs was significantly lower than in wild-type cells following BMT. Further validation of Mitf as a target of p38α in HSCs was undertaken through the use of mice with the vitiligo-associated spontaneous point mutation in Mitf (Mitf vit/vit) that reduces its transcriptional activity. Mitf mutant cells displayed proliferation defects following BMT, along with significantly reduced expression of *Gmps* and *Impdh2*, the latter of which was consistently decreased steady state signifying a key role for Mitf in regulating basal *Impdh2* expression. The p38α-Mitf pathway was further validated by demonstrating that Impdh2 overexpression by retroviral transduction restored the short-term defective proliferation in p38αΔ/Δ LSK cells and Mitf vit/vit cells, as well as the long-term defects observed in Mitf mutant cells.

A key question is how p38 regulates Mitf transcripts in HSCs, as p38 is known to regulate both transcription and mRNA stability. In melanocytes, p38 has been reported to upregulate Mitf transcription via the transcription factor CREB (19,20). In line with this Karigane et al found that high concentrations of the p38α inhibitor SB203580 reduced CREB binding at the Mitf promoter in EML-C1 cells by approximately 50%. These results do suggest some interesting questions, including how

p38α regulates CREB in HSCs and if p38α also acts via additional mechanisms. While p38α can activate CREB dependent transcription, it does not directly phosphorylate CREB (21). Instead, p38α activates the CREB kinases MSK1 and MSK2, which phosphorylate CREB on serine 133 to increase its transcriptional activity. Both MSK1 and MSK2 are also activated by the MAPKs ERK1/2, and in many systems MSK1/2 integrate ERK1/2 and p38 inputs into CREB (22). How this affects HSCs and Mitf expression is unknown. Via the phosphorylation of MK2, p38α has well established roles in regulating the stability and/or translation of transcripts containing AU-rich regulatory elements (23), and given that MK2 has reported effects on HSC repopulation (17), post-transcriptional regulation of Mitf mRNA by p38α is a possibility. While the current study shows that Mitf transcript levels are regulated downstream of p38α signalling, p38α may have additional post-translational roles. Intriguingly Mitf has been found to be activated by p38α in osteoclasts via phosphorylation on serine 307 (24) although the relevance of this in HSCs remains to be determined.

Taken together, the elegant experiments reported by Karigane and co-workers describe a key role for a p38α-Mitf pathway in regulating purine metabolism and proliferation in response to haematopoietic stress, and highlight the importance of understanding how metabolic changes relate to HSC function. Whilst this work extends our knowledge of the dynamic roles of p38α in HSCs, a greater understanding of the mechanisms by which p38α is activated in response to haematological stress along with further details of exactly how p38α regulates Mitf in these cells is likely to lead to more efficient strategies to harness this pathway for therapeutic benefit. Given the recent identification of Mitf as a novel gene target regulated by treatment with the HIV protease inhibitor nelfinavir (25) it will be exciting to see whether modulating Mitf expression is a viable approach for manipulating HSCs during ex-vivo expansion or during leukaemia chemotherapy.

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