

High Mobility Group Box 1 (HMGB1) Phenotypic Role Revealed with Stress

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High mobility group box 1 (HMGB1) is an evolutionarily ancient protein that is present in one form or another in all eukaryotes. It fundamentally resides in the nucleus but translocates to the cytosol with stress and is subsequently released into the extracellular space. HMGB1 global knockout mice exhibit lethal hypoglycemia, whereas tissues and cells from conditional knockout or knock-in mice are born alive without apparent significant functional deficit. An aberrant response to targeted stress in the liver, pancreas, heart or myeloid cells is consistent with a protective role for HMGB1 in sustaining nuclear homeostasis and enabling other stress responses, including autophagy. Under some conditions, HMGB1 is not required for liver and heart function. Many challenges remain with respect to understanding the multiple roles of HMGB1 in health and disease.

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HIGH MOBILITY GROUP BOX 1 (HMGB1) AND STRESS

HMGB1 translocates from the nucleus under stress conditions (1). Within the nucleus, it serves as a highly abundant and conserved protein, binding and bending DNA, promoting access to various transcriptional factors within chromatin and inhibiting apoptosis (2). These roles are redundant because knockout animals are born alive, albeit with a markedly limited longevity. In initial reports, knockout of the protein was associated with the generation of a metabolically crippled animal that dies in the early postnatal period with hypoglycemia and, in inbred strains, is late embryonic lethal (3). HMGB1 is thus essential for life. HMGB1^{-/-} mice die shortly after

birth because of downregulation of the glucocorticoid receptor and inability to use glycogen stored in the liver (3). In contrast, glucose administration prolongs survival of HMGB1^{-/-} mice, which die before reaching sexual maturity (3). Double knockout of HMGB1 and HMGB2 in mice or zebrafish embryos shows a significant deficiency in Wnt signaling and posterior digit development (4). Both endogenous and exogenous HMGB1 are required for pre-implantation embryo development in the mouse (5). As expected, injection of HMGB1 siRNA into the zygote increases apoptosis (5). In addition, overexpression of HMGB1 in cardiac tissue by transgenic knock-in methods significantly increases animal survival and protects mice against myocardial infar-

tion by enhancing angiogenesis and cardiac function (6). The metabolic causes of this syndrome are unclear but have been variously attributed to alteration in gluconeogenesis within the liver or, alternatively, a defect in essential neonatal autophagy.

We and others have studied the selective ablation of HMGB1 in targeted tissues and found that, without stress, these animals appear to live normally without health impairments. This result suggests that HMGB1 available *in trans* is sufficient to provide its regulatory function to knockout cells, or alternatively, that it is redundant without stress within individual cells for basal metabolic functions. In contrast, an aberrant response to targeted stress is observed in the liver, pancreas or myeloid cells, consistent with a protective role for HMGB1 (7–9) as well as unpublished results of studies on natural killer and dendritic cells. Interestingly, a recent study from RF Schwabe and colleagues indicates that HMGB1 is not required for autophagy, mitochondrial function and organ function *in vivo* (10). In this study, the authors created mice with HMGB1 conditional knockout in the liver and heart, and the protein expression level of HMGB1 after gene depletion in livers

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(Hmgb1^{Ahep} mice) decreased by 72%. Although the precise mechanism remains to be fully elucidated, Hmgb1^{Ahep} mice exhibit normal mitochondrial structure, function and glucose metabolism under normal conditions (10). Induced stress needs to be assessed in RF Schwabe's models in both the heart (for example, myocardial infarction or contusion) or the liver (for example, ischemia/reperfusion (I/R), acetaminophen or tumorigenesis).

In 1999, Haichao Wang and colleagues (11) made breakthrough progress in uncovering the extracellular role of HMGB1 in inflammation and infection. They demonstrated that HMGB1 functions as a late lethal mediator in sepsis, a systemic inflammatory response syndrome resulting from microbial infection. In 2002, Marco Bianchi and colleagues indicated that HMGB1 is a damage-associated molecular pattern (DAMP) molecule and demonstrated that HMGB1 released from necrotic cells, but not apoptotic cells, triggers the inflammatory response (12). We now know that HMGB1 can be actively secreted by immune cells or passively released by dead, dying or injured cells (13). Extracellular HMGB1 has multiple functions and is involved in several processes such as inflammation, immunity, migration, invasion, proliferation, differentiation, antimicrobial defense and tissue regeneration (14). Notably, the extracellular activities of HMGB1 depend on its form (for example, reduced or oxidized, full-length or cleaved, single or partner), receptor types (for example, positive or negative) and downstream signaling (15,16).

HMGB1 AND AUTOPHAGY

Although autophagy inhibition prevents HMGB1 release, secretion and degradation, HMGB1 itself regulates autophagy at multiple levels (17–25). For example, nuclear HMGB1 functions as a transcriptional cofactor, regulating the expression of heat shock protein β -1 (26), which in turn sustains dynamic intracellular trafficking during autophagy and mitophagy (26). Cytosolic HMGB1 competes with B-cell lymphoma 2 (BCL2) for

interaction with Beclin1/Atg6 by forming intra-molecular disulfide bridge (C23/45) of HMGB1, which in turn promotes generation of Beclin1-mediated autophagosomes (27). The interaction between HMGB1 and Beclin1 is positively regulated by unc-51-like kinase 1 (ULK1) (28), mitogen-activated protein kinase (MAPK) (27) and nucleus accumbens-1 (NAC1) (29), but negatively regulated by p53 (30), α -synuclein (31) and lysosomal thiol reductase (32). Extracellular HMGB1 in its reduced form promotes autophagy through binding to the receptor for advanced glycation end products (RAGE) (19), which may contribute to lactate production and glutamine metabolism to sustain tumor growth (33). HMGB1-mediated autophagy increases chemoresistance in cancer cells such as colon cancer, pancreatic cancer, osteosarcoma, leukemia, gastric cancer, retinoblastoma and ovarian cancer (22,28,34–41). In addition, HMGB1-mediated autophagy *in vitro* or *in vivo* prevents polyglutamine aggregates in Huntington disease (42), systemic inflammation during sepsis (9,43), *N*-methyl-D-aspartate-induced excitotoxicity (44) and hepatic I/R injury (45,46) and sustains T-cell survival in myositis (47). Microtubule-associated protein light chain 3 (LC3) is now widely used to monitor autophagy. Interestingly, Hmgb1^{Ahep} mice crossed with GFP-LC3 mice exhibits GFP-LC3 puncta formation in liver tissue after starvation for 24 h, suggesting an HMGB1-independent role in the regulation of autophagy *in vivo* (10). Of note, overexpression of GFP-LC3 *in vivo* or *in vitro* may lead to easy incorporation of LC3 into protein aggregates, which is independent of autophagy. For example, GFP-LC3 puncta are observed in hepatocytes from mice bearing an Atg5 depletion or Atg7 liver-specific knockout (48–50). Thus, GFP-LC3 mice present some limitations in the interpretation of LC3 puncta formation and localization.

HMGB1 AND MITOCHONDRIA

HMGB1 expressed in human umbilical vein endothelial cell mitochondria is important for fission and fusion processes

occurring during human endothelial cell *Toxoplasma gondii* infection (51). Recombinant human HMGB1 has been shown to exert cytotoxic activity on malignant tumor cells by promoting the formation of giant mitochondria (52). We demonstrated that HMGB1 is required for mitochondrial quality control and ATP production in immortalized mouse embryonic fibroblasts and cancer cells, which may have elevated HMGB1 expression (26). The metalloproteinase Zmpste24 is responsible for diverse human progeroid syndromes, which are characterized by signs of premature aging (53). Zmpste24^{-/-} mice exhibit altered metabolic pathways, including mitochondrial dysfunction and aberrant HMGB1 expression (53). Conditional knockout of HMGB1 in the liver accelerates mitochondrial injury and reactive oxygen species and alanine aminotransferase (ALT) production (8) at 6 h of liver I/R injury. However, there is no alteration of ALT level at 90 min of liver I/R injury with conditional knockout of HMGB1 in the liver (9). Thus, intracellular HMGB1 may have a time-dependent role in the stress response to liver I/R injury. Intracellular HMGB1 in general is an antiapoptotic protein in yeast and mammalian cells in response to several apoptotic stimuli such as ultraviolet radiation, CD95 or tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) ligation as well as Bax activation (2,54). In addition, gene chip microarray studies demonstrate that knockout of HMGB1 in mouse embryonic fibroblasts significantly impairs metabolic pathways, including those involved in fructose, mannose, galactose, glycolysis and purine metabolism (55). These findings suggest a role of HMGB1 in the regulation of mitochondrial structure and function.

CONCLUSION AND FUTURE RESEARCH DIRECTIONS

Several genetic animal models of HMGB1 have recently been created to examine the physiological and pathological roles of HMGB1 in health and disease. The phenotype of HMGB1 condi-

tional knockout mice is complex and even paradoxical. The understanding of the tissue-specific role of HMGB1 remains largely unknown. Most models, particularly under stress conditions, require further characterization to determine which features of human disease they include. In addition, further insight into the mechanisms and contributions of the key events of the HMGB1 signaling pathway hold the keys to furthering our understanding of HMGB1 function.

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DISCLOSURE

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