High mobility group box 1 promotes endothelial cell angiogenic behavior in vitro and improves muscle perfusion in vivo in response to ischemic injury.

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Abstract

OBJECTIVES: The angiogenic drive in skeletal muscle ischemia remains poorly understood. Innate inflammatory pathways are activated during tissue injury and repair, suggesting that this highly conserved pathway may be involved in ischemia-induced angiogenesis. We hypothesize that one of the endogenous ligands for innate immune signaling, high mobility group box 1 (HMGB1), in combination with autophagic responses to hypoxia or nutrient deprivation, plays an important role in angiogenesis.

METHODS: Human dermal microvascular endothelial cells (ECs) were cultured in normoxia or hypoxia (1% oxygen). Immunocytochemical analysis of HMGB1 subcellular localization, evaluation of tube formation, and Western blot analysis of myotubule light-chain 3I (LC3I) conversion to LC3II, as a marker of autophagy, were conducted. 3-Methyladenine (3MA), chloroquine, or rapamycin were administered to inhibit or promote autophagy, respectively. In vivo, a murine hind limb ischemia model was performed. Muscle samples were collected at 4 hours to evaluate for nuclear HMGB1 and at 14 days to examine endothelial density. Perfusion recovery in the hind limbs was calculated by laser Doppler perfusion imaging (LDPI).

RESULTS: Hypoxic ECs exhibited reduced nuclear HMGB1 staining compared with normoxic cells (mean fluorescence intensity, 186.9 ± 17.1 vs 236.0 ± 1.6, P = .01) with a concomitant increase in cytosolic staining. HMGB1 treatment of ECs enhanced tube formation, an angiogenic phenotype of ECs. Neutralization of endogenous HMGB1 markedly impaired tube formation and inhibited LC3II formation. Inhibition of autophagy with 3MA or chloroquine abrogated tube formation, whereas its induction with rapamycin enhanced tubing and promoted HMGB1 translocation. In vivo, ischemic skeletal muscle showed reduced numbers of HMGB1-positive myocyte nuclei compared with nonischemic muscle (34.9% ± 1.9% vs 51.7% ± 2.0%, P < .001). Injection of HMGB1 into ischemic hind limbs increased perfusion recovery by 21% and increased EC density (49.2 ± 4.1 vs 34.2 ± 3.4 ECs/high-powered field, respectively; P = .02) at 14 days compared with control hind limbs.

CONCLUSIONS: Nuclear release of HMGB1 and autophagy occur in ECs in response to hypoxia or serum depletion. HMGB1 and autophagy are necessary and likely play an interdependent role in promoting the angiogenic behavior of ECs. In vivo, HMGB1 promotes perfusion recovery and increased EC density after ischemic injury. These findings suggest a possible mechanistic link between
autophagy and HMGB1 in EC angiogenic behavior and support the importance of innate immune pathways in angiogenesis.

Published by Mosby, Inc.

PMID:21944908[PubMed - indexed for MEDLINE]

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