ABSTRACT

THE ROLE OF HIF1α SIGNALING IN METAL-INDUCED TOXICITY

By

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Oxygen is critical for all aerobic organisms and they have well developed cell signaling systems in place to maintain homeostasis under oxygen deprivation. Hypoxia inducible factor 1α (HIF1α) is the major transcription factor that mediates the cellular response to hypoxia in higher eukaryotes. HIF1α protein undergoes proteolytic degradation under normoxia by the concerted activity of HIF prolyl hydroxylase (PHD) that modifies HIF1α and the Von hippel-lindaeu (VHL) protein that recognizes the hydroxyproline and instigates HIF1α's 26s proteosome dependent proteolysis. PHDs require oxygen and 2-oxoglutarate as substrates and iron and ascorbate as cofactors. Divalent metals such as cobalt and nickel and iron chelator desferoxamine act as hypoxia mimic by inhibiting PHDs. Global gene expression profiling in Hep3B cells revealed that cobalt, desferoxamine and hypoxia modulate the expression a battery of common genes. We hypothesized that divalent metal ions may cause toxicity to cells by their ability to aberrantly activate HIF1 signaling under normoxia. Using wild type and HIF1α null mouse embryonic fibroblasts, we showed that cobalt and hypoxia treatments induce similar HIF1α dependent gene expression patterns (i.e. cobalt exposure includes 'signature hypoxia genes'). For example, cobalt exposure

leads to elevated levels of genes with characterized hypoxia response elements, including many glycolytic enzymes, glucose transporters and the pro-death factors, NIX and BNip3. BNIP3 expression was only observed in the wild type cells and its increase had functional significance, leading to caspase-independent cytotoxicity. Moreover, shRNA that specifically reduced the levels of BNIP3 within the cell were shown to be protective against cobalt-induced cell damage. In contrast, cadmium treatment does not stabilize HIF1α protein and is more toxic to HIF1α null cells compared to wild type cells, and cadmium exposure induces apoptotic cell death characterized by chromatin condensation and caspase-3 activity. Cadmium treatment also causes increased oxidative stress, and cadmium induced toxicity could be prevented by co-treatment with N-acetyl cysteine and melatonin. The increased susceptibility of HIF1α -/- cells to cadmium-induced damage could be due, in part, to the fact that these null cells had elevated levels of ROS in the untreated condition and a compromised ROS scavenging system. Taken together, our results suggest that cobalt-induced cell damage is promoted through HIF1α stabilization, and subsequent transcriptional activation of BNip3 gene and cadmium-induced cytotoxicity is due to ROS generation and the compromised nature of the ROS scavenging system in the HIF1α -/- cells. This study suggests that HIF1 signaling is an important factor in the underlying pathology of injuries seen in workers exposed to metals, such as cobalt and cadmium, and understanding this connection is critical to our ability to cope with these injuries.