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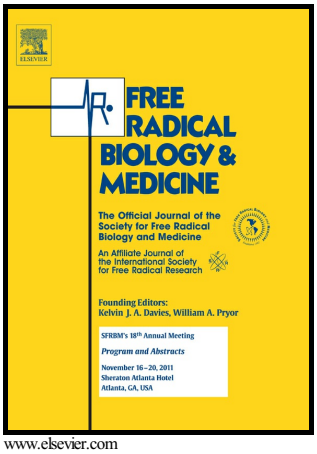

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Lack of mitochondrial DNA impairs chemical hypoxia-induced autophagy in liver tumor cells through ROS-AMPK-ULK1 signaling dysregulation independently of HIF-1α

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Abstract

Alterations in mitochondrial DNA (mtDNA) and autophagy activation are common events in tumors. Here we have investigated the effect of mitochondrial genome depletion on chemical hypoxia-induced autophagy in liver tumor cells. Human SK-Hep-1 wild-type and mtDNA-depleted (Rho) cells were exposed to the hypoxia mimetic agents $CoCl_2$ and deferoxamine (DFO). Up-regulation of HIF-1α, but not HIF-2α was observed. The expression of several HIF-1α target genes was also found. In human SK-Hep-1 and mouse Hepa 1-6 liver tumor cells, but not in the counterpart Rho derived lines, chemical hypoxia increased the abundance of autophagosomes and autolysosomes. In wild-type and Rho cells, chemical hypoxia induced down-regulation of HIF-1α-dependent autophagy inhibitors Bcl-2 and mTOR, whereas activation of AMPK/ULK1-mediated pro-autophagy pathway occurred only in wild-type cells. Chemical (compound C) and genetic (shRNA) inhibition of AMPK activation resulted in reduced autophagy. ATP levels were similar in both cell types, whereas constitutive and chemical hypoxia-induced reactive oxygen species (ROS) generation was lower in Rho cells. In wild-type cells, the antioxidant N-acetylcysteine blocked $CoCl_2$- and DFO-induced AMPK and autophagy activation, but not endoplasmic reticulum stress induced by $CoCl_2$. Enhanced Bax-α/Bcl-2 ratio and cell death was induced by hypoxia mimetic agents more markedly in wild-type than in Rho cells. Upon blocking autophagy activation with 3-methyladenine, DFO-induced cell death was partially prevented whereas that induced by $CoCl_2$ was increased, but only in wild-type cells. These results suggest that mitochondrial dysfunction associated with