Dual pathways of p53 mediated glucolipotoxicity-induced apoptosis of rat cardiomyoblast cell: activation of p53 proapoptosis and inhibition of Nrf2-NQO1 antiapoptosis

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Purpose: Reactive oxygen species (ROS), driven by excessive levels of glucose and free fatty acids, appears to induce cell apoptosis. However, the underlying molecular mechanism of this process remains unclear in cardiac myocytes. We investigated the glucolipotoxicity effects of high glucose and palmitic acid (C16:0) on the rat cardiomyoblast cell line (H9c2) focusing on tumor suppressor p53.

Methods: Cultured H9c2 rat cardiomyoblasts were exposed to palmitate and/or to an elevated glucose concentration for 18 hours. Cell apoptosis and ROS generation were measured. Western blotting was used to study p53, NQO1 (NAD(P)H dehydrogenase [quinone] 1), Nrf2 (NF-E2-related factor 2), cleaved caspase-3, and β-actin. Chromatin immunoprecipitation assay was used to assess p53-ARE (antioxidant responsive elements) interaction.

Results: Only the glucolipotoxic condition of 30mM glucose in combination with 250μM palmitate resulted in significant generation of ROS and upregulation of p53 which caused to an increased cleavage of caspase-3. On the other hand, the expression of Nrf2 showed increased tendency while the expression of NQO1 was decreased. NAC (N-acetyl-L-cysteins) and pifithrin-α, an inhibitor of p53, abrogated glucolipotoxicity-induced ROS generation and p53 expression. Chromatin immunoprecipitation analysis revealed that p53 interacted ARE-containing promoter of NQO1. Upregulated p53 counteracted the Nrf2-induced transcription of ARE-containing promoter of NQO1 gene and led to decrease in NQO1 expression.

Conclusion: We demonstrated that the elevated p53 mediated glucolipotoxicity-induced apoptosis of rat cardiomyoblast cell through dual pathways: stimulating pro-apoptosis signaling as well as suppressing anti-apoptosis pathway of Nrf2-NQO1 signaling.

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