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Identification of cytotoxic mediators and their putative role in the signaling pathways during docosahexaenoic acid (DHA)-induced apoptosis of cancer cells.

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Abstract

Docosahexaenoic acid (DHA), an important w-3 fatty acid exhibits differential behavior in cancer cells of neural origin when compared to that in normal healthy astrocytes. Treatment of C6 glioma and SH-SY5Y cell lines and primary astrocytes, representing the neoplastic cells and normal healthy cells respectively, with 100 μM DHA for 24 h showed significant loss of cell viability in the both the cancer cells as determined by MTT assay, whereas the primary astrocytes cultures were unaffected. Such loss of cell viability was due to apoptosis as confirmed by TUNEL staining and caspase-3 activation in cancer cells. Proteomic approach, employing 2-dimensional gel electrophoresis (2DE), difference gel electrophoresis (DIGE), and MALDI-TOF-TOF analysis identified six proteins which unlike in the astrocytes, were differently altered in the cancer cells upon exposure to DHA, suggesting their putative contribution in causing apoptosis in these cells. Of these, annexin A2, calumenin, pyruvate kinase M2 isoform, 14-3-3ζ were downregulated while aldo keto reductase-1B8 (AKR1B8) and glutathione-S-transferase P1 subunit (GSTP1) showed upregulation by DHA in the cancer cells. siRNA-

reductase 1B8 (AKR1B8) and glutathione S-transferase P1 subunit (GSTP1) showed upregulation by DHA in the cancer cells. siRNA mediated knockdown of AKR1B8 and GSTP1 inhibit DHA-induced apoptosis confirming their role in apoptotic process. Furthermore, western blot analysis identified upregulation of PPARα and the MAP kinases, JNK and p38 as well as increased ROS production selectively in the cell lines. Results suggest that DHA selectively induces apoptosis in the neural cell lines by regulating the expression of the above proteins to activate multiple apoptotic pathways which in association with excess ROS and activated MAPKs promote cell death.

KEYWORDS:

2D gel electrophoresis; Apoptosis; Astrocytes; Cancer; Docosahexaenoic acid; ROS

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