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A Mixture of *t,t* Conjugated Linoleic Acid (*t,t* CLA) isomers induces Apoptosis for Osteosarcoma MG-63 Cells

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The anticarcinogenic activity of a mixture of *trans,trans* conjugated linoleic acid isomers (designated as *t,t* CLAs) was investigated in human osteosarcoma cell line MG-63, with references to *c*9,*t*11 CLA and *t*10,*c*12 CLA The *t,t* CLA isomers consisted of *t*7,*t*9; *t*8,*t*10; *t*9,*t*11; *t*10,*t*12; *t*11,*t*13; and *t*12,*t*14 CLA. The cells were cultured with different concentrations (0~40M) of *t,t* CLAs, *c*9,*t*11 CLA, *t*10,*c*12 CLA or linoleic acid, complexed with bovine serum albumin and adapted to serum-free medium. Cell growth, cell cycle distribution, apoptosis, and cell membrane lipid composition were determined. As compared to reference treatments, *t,t* CLAs effectively induced a cytotoxic effect on MG-63 cells in a time- and concentration-dependent manner. Addition of 40 M *t,t* CLAs led to a concomitant decrease in growth of MG-63 cells (77% inhibition). Flow cytometric analysis revealed an increased proportion of apoptotic cells with low DNA content (sub G0/G1) and a marked loss of cells from the G0/G1 phase of the cell cycle. To elucidate the pathway linked with the *t,t*CLA-induced apoptosis, the effect of *t,t*CLAs on induction of apoptosis- related proteins was evaluated. The level of Bax protein was increased, whereas the Bcl-2 expression was reduced. Moreover, upon *t,t* CLA treatment, the cytochrome *c*was released from mitochondria into the cytosol and, then, activation of caspase-3 led to the cleavage of poly (ADP-ribose) polymerase (PARP). Finally, characteristic morphological changes confirmed the apoptosis execution. Supplementation with *t,t* CLAs also altered cell membrane composition by decreasing the linoleic and arachidonic acid contents of membrane phospholipids. Thus, taken together, all these findings suggest the possibleactivation of a mitochondria-mediated apoptosis pathway enhancing the anti-tumor effect of *t,t* CLAs in this type of cancer.