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Ceramide glycanase activities in human cancer cells.

Basu M, Kelly P, O'Donnell P, Miguel M, Bradley M, Sonnino S, Banerjee S, Basu S.

Author information

Abstract

Ceramide glycanase (CGase) activities have been detected in different human tumor cells (colon, carcinoma Colo-205; neuroblastoma, IMR-32; breast cancer lines, SKBr3 and MCF7). However, the level of enzymatic activity is lower in these cells compared to that present in other mammalian tissues reported before (Basu, M., Kelly, P., Girzadas, M. A., Li, Z., and Basu, S. Methods Enzymol. (in press)). The majority of CGase activity was found in the 100,000 g soluble supernatant fraction isolated from all these cell lines and tissues. Using the soluble enzyme, the requirement for optimum CGase activity was found to be consistent with previous observations found for rat and rabbit tissues (Basu, M., Dastgheib, S., Girzadas, M. A., O'Donnell, P. H., Westervelt, C. W., Li, Z., Inokuchi, J. I., and Basu, S. (1998) Acta Pol. Biochim. 42:327). The CGase activities from both Colo-205 and IMR-32 cells are optimum at a protein to detergent ratio of one. All the mammalian CGases, including human cancer cells, show an optimum pH between 5.5 and 5.8 in sodium acetate buffer. The CGase activities from cancer cells are found to be cation-independent; however, mercury, zinc, and copper ions seem to inhibit the enzyme activity substantially in both tumor cells lines. The mercury ion inhibition of CGase activities from all different sources indicates a possible structural homology in the CGase proteins. Radiolabeled substrates, labeled at the sphingosine double bond or at the 3-position of sphingosine without modifying double bond of sphingosine were used in this investigation. Both were active substrates with all enzyme preparations isolated from different cancer cells (apparent Km, 500 microM for nLcOse5[3H-DT]Cer and 350 microM for GgOse4[sph-3-3H]Cer with Colo-205 enzyme). Structural analogues of ceramide and sphingosine (L-PPMP. L-PDMP, alkylamines, and Tamoxifen) inhibited cancer cell CGase activities in vitro.

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