CREATINE KINASE ISOENZYMNE BB IS ASSOCIATED WITH THE ESTROGEN RECEPTOR IN BREAST CANCER

Zarghami, N., Grass, L., Sutherland, D.J.A. and Diamandis, E.P., Department of Clinical Biochemistry, The Toronto Hospital and Sunnybrook Medical Centre, Toronto, Canada

The creatine kinase isoenzyme CK-BB is produced by many tumors but its clinical usefulness has not as yet been established. In order to examine the value of CK-BB isoenzyme as a prognostic indicator in breast cancer, we have initially developed a highly sensitive immunofluorometric procedure, based on enzymatically amplified time-resolved fluorometry with Tb$^{3+}$-chelates as labels and two monoclonal antibodies. One antibody was immobilized in polystyrene microtiter wells and the other was biotinylated. The immunocomplex was detected with a streptavidin-alkaline phosphatase conjugate using diflunisal phosphate as substrate. The dephosphorylated diflunisal forms highly fluorescent complexes with Tb$^{3+}$-EDTA which can be quantified with time-resolved fluorometry. The developed assay takes 2h to complete, is precise (CV's<10%) and highly sensitive, with a detection limit of <0.05 µg/L. Comparison with an enzymatic CK-BB assay for 166 breast tumor cytosols (determination of CK-B subunit after immunoinhibition of CK-M subunit) gave the following equation: y (TR-FIA method) = 0.71 x (enzymatic method) + 5.8, r = 0.83. Cross-reactivity of the CK-BB TR-FIA assay by CK-MM was not detectable at concentrations as high as 1000 µg/L. The cross-reactivity of CK-MB isoenzyme was 5% at 100 µg/L of CK-MB. The method was used to measure CK-BB activity in 166 breast tumor cytosols which were also measured for (ER) and progesterone (PR) receptors. Using a cutoff value of 10 fmol/mg protein for the receptors and 26 µg/L for CK-BB, we examined the possible association between receptors and CK-BB. We found a strong association between CK-BB and ER (P=0.001, chi-square test) and a weak association between CK-BB and PR (P=0.054). We conclude that CK-BB production by breast tumors is mediated by the action of the estrogen receptor.