

[PB2-3] [04/21/2000 (Fri) 10:30 ~ 11:30 / [1st Fl, Bldg 3]]

Effects of pomegranate (*Punica granatum*) polyphenol fractions on proliferation of estrogen-dependent (MCF-7) and estrogen-independent (MDA-MB-231) human breast cancer cells

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Polyphenol-rich fractions, consisting of flavonoids and tannins, were extracted from the seed oil, pericarp, unfermented and fermented juice of the pomegranate, *Punica granatum*. The different fractions were incubated in individual well plates with both estrogen-dependent (MCF-7) and estrogen-independent (MDA-MB-231) human breast cancer cells for 48 hours. At that point, cell viability was assessed with the MTT assay. The fermented juice exerted the strongest overall anti-proliferative effect in both the MCF-7 and MDA-MB-231 lines. The second strongest in both lines was from the aqueous pericarp extract. The unfermented juice also exerted significant anti-proliferative activity of the MCF-7 cells, but only mild anti-proliferative activity in the MDA-MB-231 cells. Overall, the effect in the MCF-7 lines for all pomegranate materials was more pronounced than that for the MDA-MB-231. Polyphenol fraction isolated from the pomegranate seed oil failed to have anti-proliferative effect in either of the assays at the concentrations employed.

[PB2-4] [04/21/2000 (Fri) 10:30 ~ 11:30 / [1st Fl, Bldg 3]]

Characterization of The EF hand loop region of ATX

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Autotaxin (ATX) is a recently described member of the nucleotide pyrophosphatase and phosphodiesterase (NPP) family of proteins with potent tumor cell motility-stimulating activity. Like other NPPs, ATX is a glycoprotein with peptide sequences homologous to the catalytic site of bovine intestinal alkaline phosphodiesterase (PDE) and the loop region of an EF-hand motif. The PDE active site of ATX has been associated with the motility-stimulating activity of ATX. In this study, we have examined the roles of the EF-hand loop region and of divalent cations on the enzymatic activities of ATX. Ca⁺⁺ or Mg⁺⁺ were each demonstrated to increase the PDE activity of ATX in a concentration dependent manner, whereas incubation of ATX with chelating agents abolished this activity, indicating a requirement for divalent cations. Lineweaver-Burke analysis indicated that addition of these divalent cations increases reaction velocity predominantly through an effect on V_{max}. Three mutant proteins, Ala740-, Ala742-, and Ala751-ATX, in the EF hand loop region of ATX had comparable enzymatic activity to wild type protein. A deletion mutation of the entire loop region resulted in slightly reduced PDE with normal motility stimulating activity. However, the PDE activity of this same deletion mutant remained sensitive to augmentation by cations, strongly implying that cations exert their effect by interactions outside the EF hand loop region.

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The cellular expression of ATX is correlated with an invasive phenotype of breast tumor cells