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BISPHOSPHONATES INDUCE APOPTOSIS IN TUMOUR CELLS BY INACTIVATION OF RhoA GTPase AND DISRUPTION OF ACTIN CYTOSKELETON

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Recent data indicate that bisphosphonates (BP) may have a beneficial effect in reducing bone metastasis *in vivo*. However, the mechanism underlying this effect is not clarified. Here we show that aminobisphosphonates directly induce apoptosis in several tumour cell types and that inhibition of RhoA GTPase is a critical step in BP-induced apoptosis. Prior to the onset of apoptosis, cells retracted, became round and fragmented. Immunofluorescence staining using phalloidin and anti-vinculin antibodies revealed that these changes were associated with loss of filamentous actin and focal adhesions. Similar effect was observed upon exposure to *Botulinum* C3 exoenzyme, a specific inhibitor of RhoA. Phorbol ester (TPA), which activates RhoA, enhanced membrane ruffling and induced translocation of RhoA from the cytosol to the ruffling areas. Pre-exposure of the cells to BP inhibited TPA-induced membrane ruffling and prevented RhoA translocation. Results were confirmed by western blotting analysis. Geranylgeranyl pyrophosphate (GGPP), a product of the mevalonate pathway and essential for RhoA activation, rescued the cells from death by apoptosis and restored responsiveness to TPA.

The results demonstrate that inactivation of RhoA and disruption of actin cytoskeleton are critical steps in the mechanisms leading to BP-induced apoptosis in malignant cells and point to the beneficial effect of bisphosphonates in cancer therapy.

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Significant growth inhibition of human lung cancer cells both *in vitro* and *in vivo* by the combined use of a cyclooxygenase 2-specific inhibitor, JTE-522, and conventional anticancer agents

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The present study reports that a COX-2-specific inhibitor JTE-522 (provided by Japan Tobacco INC. Tokyo, Japan) inhibits both *in vitro* and *in vivo* growth of human lung cancer cells as a single agent. Furthermore, the adjunct use of JTE-522 is shown to significantly enhance treatment efficacy of conventional anticancer drugs not only *in vitro* but also *in vivo* without causing any noticeable side-effects. Indeed, IC_{50} values of various anticancer agents *in vitro* were reduced by up to 70%, while the combination therapy of JTE-522 with taxotere and vinorelbine inhibited tumor growth *in vivo* by 65% and 55%, respectively. Taken together, these findings suggest that the use of a specific COX-2 inhibitor in the treatment of lung cancer may be promising, especially because of its enhancement of the treatment efficacy of conventional anticancer agents without compromising quality of life.

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Helenalin triggers a CD95 death receptor and FADD-independent apoptosis which is not abrogated by overexpression of anti-apoptotic Bcl-2 proteins

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Proper tissue homeostasis requires apoptosis. Thus, defects in apoptosis signaling pathways contribute to carcinogenesis and chemoresistance. It is therefore of importance to find agents which restore the ability of tumor cells to undergo apoptosis. We found that the natural product helenalin (10-50 μM) dose- and time-dependently induces a caspase-dependent apoptotic cell death in leukemia Jurkat T cells. Apoptosis occurres even in the absence of the CD95 death receptor or the adapter molecule FADD (Fas associated death domain), which is required for caspase-8 and caspase-10 recruitment to death receptors. Although helenalin triggers outer (cytochrome c release into the cytosol) and inner (dissipation of the mitochondrial electrochemical gradient) mitochondrial membrane permeabilization, overexpression of the mitochondria-protecting anti-apoptotic proteins Bcl-2 and Bcl-x_L failed to confer resistance towards helenalin. In contrast, activated peripheral blood mononuclear cells, sensitive to other cytotoxic drugs like etoposide and staurosporine, do not undergo apoptosis upon helenalin treatment (10-50 μM).

Thus, helenalin is a promising experimental chemotherapeutic agent possibly pointing to new strategies to overcome apoptosis resistance due to defects within the death receptor or due to overexpression of anti-apoptotic Bcl-2 proteins.

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c-myc Amplification and Enhancement of the Sensitivity to Cytosine Arabinoside – in Vitro and in Vivo Study in Four Sublines Established from a Pulmonary Adenocarcinoma.

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Four sublines with different growth characteristics in vitro were established from a primary tumor of pulmonary adenocarcinoma. 88-2T cells were small in cell size, and proliferated in a monolayer with migrating ability. 88-2 cells proliferated in a monolayer without migrating ability. 88-2FA cells were large spindle-like cells. 88-2F cells proliferated in floating cell aggregates. The four sublines also demonstrated obvious differences in vivo. 88-2T and 88-2 tumor revealed an adenocarcinoma, resembled the findings of original primary tumor. On the other hand, 88-2F revealed a large cell carcinoma resembled those of lymph-node metastasis. 88-2FA was composed of signet ring cells alone. In addition, there were differences in oncogenes, with line 88-2F alone exhibiting twelve-fold amplification of c-myc. Furthermore, it is interesting that 88-2F subline only showed strong sensitivity to Ara C. (conclusion) The study demonstrate that human lung cancer has a tumor heterogeneity within the primary tumor; c-myc amplification may carry an important role for changing drug sensitivity to Ara C and metastatic ability of the cancer cells; and these newly established sublines would provide an excellent in vitro-in vivo human