Contribution of the lab to drug development

744 ORAL

Bisphosphonates induce apoptosis in human lung adenocarcinoma cells through disruption of actin cytoskeleton and is suppressed by geranylgeranyl pyrophosphate

M. Shehata[1,2], J.D. Schwarzmeier[1,2], M. Hilgarth[1], R. Hubmann[2], R. Berger[2]. [1]*Vienna Univ., Internal Med. I, Vienna;* [2]*LBI.f.Cytokine Res., Internal Med. I, Vienna, Austria*

Recent results point to the beneficial effect of bisphosphonates (BP) in vivo in patients with breast cancer and in vitro studies revealed an inhibitory effect on tumour cell adhesion to bone matrices. We also reported that BP could induce apoptosis in human osteosarcoma cells in vitro. However, the mechanisms underlying these effects are not clearly understood. There are indications that the mevalonate pathway of cholesterol biosynthesis might be modulated by BP. Therefore, we investigated the effects of a potent bisphosphonate (alendronate) on lung adenocarcinoma cells (A549) which have high requirements of mevalonate for cell growth.

Exposure of A549 cells to 1–100 μM alendronate resulted in growth inhibition and cell death by apoptosis. This was confirmed by XTT and 3H-thymidine assays, Hoechst fluorochrome, Annexin V and propidium iodide staining. The typical DNA fragmentation and laddering were demonstrated by agarose gel electrophoresis. Before the onset of apoptosis, cells showed marked morphological changes. Initially epithelioid, the cells retracted, became round, detached and fragmented. These morphological changes were associated with actin filament disruption, demonstrated by FITC-labelled phalloidin. Cytochalasin D (an inhibitor of actin filament polymerization) and perillic acid (an inhibitor of isoprenylation) induced similar effects on the tumour cells. Isoprenoids, particularly, geranylgeranyl pyrophosphate (GG-PP, 20 μM) effectively retained cell morphology and rescued the cells from death by apoptosis. A549 cells treated with alendronate under nonadherent condition in poly-HEMA coated plates also underwent apoptosis and were rescued by GG-PP confirming a direct effect of alendronate on the tumor cells which might be adhesion independent.

The results demonstrate that bisphosphonate (alendronate) induce apoptosis in tumour cells. The mechanism might be due to interference with the mevalonate pathway of cholesterol biosynthesis. Consequently, depriving the cells of the isoprenoids required for activation of small G proteins (such as Rho, Rac, Rab) which are essential for the maintenance of the actin cytoskeleton and cell survival. These results may also explain the beneficial effect of bisphosphonates in reducing bone metastasis and skeletal complications of tumours.

745 ORAL

p73 and *p53* genes expression are mutually exclusive in MCF7 cells treated with adriamycin and vincristine

M. Vayssade, M.L. Le Bihan, S. Tourpin, J.C. Sabourin, J. Bénard, J.C. Ahomadegbe. *Institut Gustave Roussy, 94805 Villejuif, France*

P53 is instrumental in the response of tumor cells to anti-cancer drugs *via* transcriptional regulation of its target genes, such as *p21*. *P73* gene, the first *p53*-related gene described, encodes 2 distinct polypeptides p73α and p73β differing at their C termini. When overexpressed in p53-deficient cells, p73 can induce p21 expression or leads to apoptosis. Adriamycin (ADR) and vincristine (VCR) both induce apoptosis; however, unlike ADR, VCR toxicity has been described as p53-independent. We hypothesized that p73 could be involved in cellular response to VCR. p53, p73 and p21 gene expression (mRNA) and protein levels were measured in MCF7 treated with ADR and VCR at doses leading to similar toxicity.

Results: Roughly, there is an increase in p53 and p21 mRNA levels in MCF7 after exposure to ADR or VCR. Nuclear accumulation of p53 and p21 was also found. Surprisingly, a 2- to 5-fold decrease was observed in both p73α and p73β mRNA levels in treated MCF7 at doses inducing p53 overexpression. In contrast, no change was detected in p73 expression in treated MCF7 at lower drug doses and not overexpressing p53.

Conclusions: 1) p73 cannot mediate VCR toxicity, and unlike previously reported data, VCR can induce apoptosis *via* a p53-dependent pathway; 2) p53 overexpression parallels down-regulation of p73, suggesting that the transcription of these 2 homologous genes could be regulated in an opposite manner.

supported by Ministère de l'Enseignement & de la Recherche, France

746 ORAL

Taxane induced apoptosis occurs independently of AP-1 and acidic sphingomyelinase activation

C. Belka, P. Marini, V. Heinrich, W. Budach, A. Lepple-Wienhues, F. Lang, E. Gulbins, M. Bamberg. *Departments of Radiation-Oncology and Physiology, University Tuebingen, Germany*

Introduction: Taxanes are potent inducers of p53 independent apoptotic cell death. The pathways involved in taxane mediated cell death are not analyzed in greater detail. The activation of stress pathways including AP-1 or acidic sphingomyelinase were shown to be involved in apoptosis induction in response to different stimuli. We tested in how far taxane induced apoptosis involves activation of AP-1 or acidic sphingomyelinase (ASM).

Material and Methods: Paclitaxel and docetaxel induced activation of JNK1 or p38 kinase was tested using in vitro kinase assays. Activation of AP-1 and ATF-2 transcription factor activity was analyzed using EMSA. ASM activity was determined by an immunoprecipitation enzyme assay. AP-1 action was blocked by transient expression of a dominant negative c-jun mutant (TAM67). Apoptosis induction was analyzed by Hoechst staining.

Results: Taxanes induced a rapid activation of JNK1 and p38 kinase followed by induction of AP-1/ATF2 containing transcription factor complexes. In parallel, taxanes induced activation of acidic sphingomyelinase. Overexpression of TAM67 did not interfere with taxane induced apoptosis. No differences in apoptosis induction between ASM deficient and ASM expressing cell was detectable.

Conclusion: Taxanes are potent inducers of cellular stress responses. However, the activation of acidic sphingomyelinase or AP-1 are not involved in the regulation of the apoptotic response.

747 ORAL

Cisplatin resistance in human tumor cell lines is associated with DNA polymerase beta expression but not with thymidilate synthase expression

W. Dempke[1], W. Voigt[1], H.J. Schmoll[1], B.T. Hill[2]. [1]*Martin-Luther-University Halle, Hematology/Oncology, Halle, Germany;* [2]*Centre de Recherche Pierre Fabre, Division de Cancerologie, Castres Cedex, France*

Introduction: Factors generally implicated in the expression of cisplatin resistance include alterations of drug transport, modified intracellular drug inactivation, differential DNA damage, tolerance of that damage and/or its repair, reduced folate metabolism and altered oncogene expression. DNA polymerase Beta is known to be involved in the "short patch" repair pathway following cisplatin-induced DNA damage. In addition, thymidylate synthase (TS) provides the sole source of de novo thymidylate required by repair enzymes in removing platinum-DNA adducts. Therefore, the main objective of this study was to examine whether a correlation between cisplatin resistance and DNA Polymerase Beta/TS expression excists.

Methods: In a panel of eight human ovarian carcinoma cell lines IC50 values were determined following exposure to cisplatin in vitro. In addition, the DNA Polymerase Beta activity was measured using the methodology described by Mivechi and Dewey (1984). TS activity was determind according to the method by Roberts (1966).

Results: A significant positive correlation was found between cisplatin resistance and DNA Polymerase Beta expression ($r = 0.841$, $p < 0.01$, $n = 8$). In contrast, cisplatin resistance did not correlate with TS levels in the tumor cell lines examined ($r = 0.130$, $p > 0.05$, $n = 8$).

Conclusion: The observation that cellular resistance to cisplatin is strongly correlated with the repair enzyme DNA Polymerase Beta adds weight to the proposal that enhanced DNA repair significantly contributes to the development of cisplatin resistance.