status (wild type, deleted, mutated). Western and northern blot analysis were performed to evaluate the protein and mRNA expression of p53, p21, Mdm2, Bax with or without the presence of proteasome inhibitor, MG-132. Transient transfection and luciferase assay was performed to confirm a transcriptional activity of p53. Results: A marked induction of mRNA expressions of Mdm2, Bax and p21 was detected in wild-type p53 expressing cells after the treatment with both B[a]P and 1-NP, but not in either p53-negative or mutant cells. The induced mRNA levels of the p21 did not result in proportional p21 protein increase, indicating the possibility of post transcriptional regulation of the protein. Transcription from the wild-type p21 promoter was markedly induced by PAHs in p53 wild type cells but not in p53 deleted cells. In addition, luciferase activity was not affected by p21 promoter in which p53-binding site is truncated. By the addition of MG-132 to B[a]P treatment, both p21 and p53 protein levels were increased, however, the increase in p21 protein levels was significantly larger than the increase in p53 protein levels. On the other hand, increase in p21 protein was only modest by the addition of MG-132 to 1-NP treatment. B[a]P treatment increased the level of ubiquitinated p21. Cell cycle arrest was more obviously seen by the treatment with 1-NP than by the treatment with B[a]P. Conclusions: These results suggested that the p21 product is degraded by the ubiquitin-proteasome system induced by B[a]P. We conclude that B[a]P-induced p53 protein is transcriptionally active. However, rapid degradation of p21 protein by the ubiquitin-proteasome system may induce a blockade of p53-induced cell cycle arrest, resulting in genomic instability.

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Effectiveness of a new derivative of retinoic acid as differentiating agent on human neuroblastoma cells

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Purpose: Among the compounds that have been explored as differentiating agents, retinoic acid is one of the most potent in the regulation of proliferation and differentiation in neoplastic cells. In the present study we have explored the effect of IIF, (pat.PTC/IT99/00299) a new derivative of retinoic acid, as differentiation inducer in the human neuroblastoma cell line TS12.

Methods: Neuronal differentiation was assessed by means of morphological and cytochemical parameters, i.e. neurite outgrowth, tyrosine hydroxylase (TH) expression and acethylcholinesterase specific activity. The effect of the drug on cell growth was assessed by clonogenic assay.

Results: Treatment with IIF resulted in induction of morphological differentiation, as manifested by the appearance of neurite extension. TH expression was induced by the drug: following RT-PCR on mRNA from neuroblastoma cells, TH mRNA was detectable only in treated cultures but not in control ones. Treatment with IIF induced also a marked increase of acetylcholinesterase activity. Moreover clonogenic efficiency showed the growth inhibitory effect induced by the drug.

Conclusions: These results demonstrate the effectiveness of the new derivative of retinoic acid IIF as differentiation inducing agent on neuroblastoma cell line TS12.

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RBC CR1 in tumour patients: an implication in anaemia?

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Purpose: Tumour patients often suffer from an anaemic condition whose causes are not always completely clear. Increased levels of circulating immune complexes (cIC) are frequently present in these patients. The receptor responsible for cIC clearance is complement receptor 1 (CR1), present also on red blood cells (RBC). The aim of this study was to investigate the presence of RBC CR1 modifications in tumour subjects, as well as the possibility that they are in some way linked to the anaemic condition.

Methods: Patients (age 47-81) affected by breast, lung and colon cancer from stage 1 to 4, were studied; healthy donors, age 19-83, were used as controls. All subjects were submitted to blood withdrawal. Sera were employed for determination of cIC levels by ELISA; RBC were separated from leukocytes, and CR1 expression was evaluated at flow cytometry. The number of CR1+RBC was calculated and correlated to cIC sera levels, subject age and haematological condition.

Results: In tumour patients RBC number was decreased with respect to controls. CR1 expression was also significantly diminished, in a greater proportion than RBC number decrease; on the contrary, cIC levels were significantly increased, especially in the over-60 year old group. The over-60 control subjects also showed increased cIC levels, without CR1+RBC number modifications, and the values were comparable to those found in the under-60 patients. cIC-CR1+RBC correlation was negative in patients, indicating that the CR1 reduction accompanies the cIC increase.

Conclusion: A loss of RBC, in relation with the increased serum cIC level, is suggested as an adjunctive mechanism responsible for both the marked CR1+RBC reduction and anaemia in neoplastic patients; however, the considerable diminution of CR1+RBC (compared with the entire RBC population) also suggests an impaired CR1 production or its augmented proteolysis. CR1 decrease could be involved in the maintenance of high cIC sera levels in these patients (insufficient cIC removal), as well as in the appearance of anaemic condition consequent to the possible elimination from the circulation of cIC-carrying RBC.

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Regulation of vimentin mrna by 12-o-tetradecanoylphorbol 13-acetate (TPA) and all-transretinoic acid (RA) associated with in vitro invasive activity of hep 3b human hepatocellular carcinoma cells

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Purpose: Vimentin is a protein that assembles to form intermediate filaments, one of the major cytoskeletal structures in mammalian cells. Increased expression of vimentin is associated with increasing cancer grade, dedifferentiation, decreased cell-to-cell adhesion, motility, invasion, metastasis, drug resistance and poor prognosis in some cancers. We have reported that the vimentin mRNA was regulated by tumor promoter, TPA and differentiation agent, RA in several cancer cell lines. In this study we found that up- or down-regulation of vimentin mRNA by TPA or RA could modulate the invasive potential of human hepatoma cell line, Hep 3B in vitro.

Methods: To elucidate the role of vimentin gene expression of Hep 3B cells by TPA or RA, we evaluated the vimentin mRNA levels by Northern blot hybridization. Matrix metalloproteinases (MMP-2,-9) and urokinase plasminogen activator (uPA) activities were evaluated using substrate zymography in addition to in vitro invasion assay of Hep 3B cells.

Results: TPA (1-100 nM) treatment showed marked induction of vimentin mRNA up to 48 hrs with a dose- and time-dependent maner and then decreased its level. On the other hand, RA (0.1-10 uM) treatment showed a time-dependent gradual decrease of mRNA level. There was no change of MMP-2, MMP-9 and uPA activities in conditioned medium with concomittent treatment of TPA or RA on zymographic findings. TPA (0.1 uM) treatment significantly enhanced in vitro invasion of Hep 3B cells as much as 2 times, and RA (0.1-10 uM) inhibited the invasion with dose-dependent manner (33.4%, 71.9% respectively) ($p<0.05$).

Conclusion: These results suggest that regulation of vimentin mRNA may be related to invasiveness of Hep 3B human hepatocellular carcinoma cells by controlling cellular motility.

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Inhibition of cathepsin A activity in melanoma cell lines by lactacystin

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Purpose: In recent years considerable attention has been paid to the antitumor activity of the proteasome specific inhibitor, lactacystin. It inhibits the proteasome-mediated degradation of numerous key regulatory proteins which are involved in various cellular processes such as cell division, apoptosis, NF-kB activation, and MHC class I antigen presentation. The ability of the lactacystin to arrest cell cycle progression and induce apoptosis in various tumor cells suggests to their potential use in cancer therapy. Recently we showed, that lactacystin metabolite, b-lacton inhibited the activity