M059K, M059J) and that the activation of the PPARgamma pathway by its ligands - namely, 15-deoxy-delta12,14-prostaglandin J2 (PGJ2) and rosiglitazone - induces cell growth inhibition in these cell lines, with PGJ_2 being more effective. In this report, we further characterized the mechanisms of action of these two PPARgamma ligands by looking at induction of apoptosis, cell cycle arrest, and gene modulation. Treatment of glioblastoma cells with PGJ_2 and rosiglitazone resulted in a dose-dependent induction of apoptosis, which correlated with the sensitivity of the cells to each ligand. In the most responsive of our glioblastoma cell lines, namely M059K, we observed a reversible (with rosiglitazone) and irreversible (with PGJ_2) arrest in the G_2/M phase, accompanied by p21 up-regulation only with PGJ_2. After treatment with PPARgamma ligands, a consistent amount of M059K cells detach in a short time. Far more than the attached cells, the floating population is growth-arrested and undergoing apoptosis, as shown by cell cycle analysis and apoptosis detection through PARP cleavage and annexin-V positivity. Interestingly, in the floating population of the treated samples, we observed a dramatic down-regulation of PPARgamma and a concomitant up-regulation of RXRalpha and RARbeta, while in the attached population no substantial modulation of these genes was observed. PPARgamma down-regulation may be due to proteasome-dependent degradation following activation of PPARgamma by its ligands. Our results indicate that PPARgamma ligands exert an antiproliferative effect in glioblastoma cells through induction of apoptosis and cell cycle arrest in G_2/M phase. In addition, PPARgamma ligands might induce glioblastoma cells to differentiate, since RARbeta up-regulation is a marker of this phenomenon.This work was supported by Fondazione per l'Oncologia Pediatrica, Roma

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Establishment of a chemotherapeutic drug/gene expression database for the molecular pharmacology of cancer

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The potential of microarray technology for the identification of new drug targets is at an exploratory phase. Studies using the 60-cell line screen of the National Cancer Institute have revealed its usefulness in predicting mechanism of drug action. We have studied a panel of 42 cell lines of colon, lung and ovarian origin. The sensitivity of these cell lines to a variety of chemotherapeutic agents (N>30) has been measured using the MTT cytotoxicity assay and IC_{50} values determined. Gene expression analysis in the same cell lines was done using a 7000-element cDNA microarray platform. Relationships were established between genes and drugs across the entire panel of cell lines using a variety of statistical algorithms. Cluster analysis grouped the cell lines with respect to their tissue of origin and arranged the drugs with respect to their individual classes (e.g. antimicrotubule agents, topoisomerase inhibitors). Relationships between classes of chemotherapeutic drugs and gene expression patterns were established so groups of drug sensitivity or resistance genes could be identified. The expression of several potential drug targets was validated by quantitative "real time" RT-PCR. This approach has the potential to allow *in vitro* data from cancer cell lines to guide selection of candidate drugs within a class for clinical development and to identify new therapeutic targets.

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Growth suppressive effect and apoptosis induction by N-(4-hydroxy phenyl) retinamide in human osteosarcoma cells *in vitro*

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Osteosarcomas are the most common primary bone tumors and occurr mainly in children and young people between 10 and 20 years of age. Despite intensive treatment, including adjuvant chemotherapy, wide excision of tumors and amputation of the affected limbs, approximately half patients die within 5 years. The optimal schedule of therapy is still being investigated, as is the acquisition of novel active agents. Retinoids, the naturally occurring and synthetic analogues of vitamin A, have demonstrated therapeutic and chemopreventive activities. Among these, N-(4-hydroxyphenyl)-retinamide (4-HPR) (fenretinide) is a synthetic derivative and is being evaluated clinically in the prevention of the development of second primary tumors of breast and aerodigestive tract. The primary cellular target of this drug is unidentified as is the mechanism through which it kills tumor cells, by necrosis or apoptosis, and in this case by p53 or caspases dependent or independent pathways. In this study we analysed the growth suppressive effect of 4-HPR on human HOS (p53-positive) and MG-63 (p53-negative) osteosarcoma cell lines *in vitro* and the molecular mechanism of this response. Results show for the first time that 4-HPR (0.125 - 25μM) is active against human osteosarcoma *in vitro*, causing in it a significant dose- and time- dependent inhibition of cell survival, as determined by SRB and Trypan Blue exclusion tests, in each cell line. IC 50% was about 10 μM after 24hr of treatment in the HOS cells, which lowered to 6 μM after 72 hr of treatment, whereas the MG-63 cells were less sensitive to the drug, the IC 50% being about 10 μM only after 48 hr of treatment with 4-HPR. Programmed cell death by the drug was definitively documented here by the internucleosomal DNA fragmentation shown by the evidence of ladder after 48 hr of treatment in each cell line, independent of p53, the role of which as an apoptotic marker is not relevant here. By Western blot it was evident the dephosphorylation of pRb in each cell line treated with 4-HPR for 48 hr and up. This is the first report indicating that pRb may represent the cellular target for the molecular pathway carried out by fenretinide for the suppression of cell proliferation in osteosarcoma. Work supported by grants from Carisbo Foundation, Bologna, MIUR, CNR and University of Bologna (Funds for Selected Topics).

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Prognostic value of nuclear survivin expression in oesophageal squamous cell carcinoma

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Purpose: Survivin, a new member of the family of apoptosis inhibitors, is expressed almost exclusively in proliferating cells, above all in cancers. Subcellular localization and prognostic implications of the survivin protein have not yet been determined in oesophageal squamous cell carcinoma.
Patients and methods: Survival of 84 patients with oesophageal squamous cell carcinomas was correlated with the extent of immunohistochemical survivin expression in tumour cell nuclei. Tumours were scored positive when >5% cells stained positive. Patients were followed up for at least 5 years or until death.
Results: In normal oesophageal squamous cell epithelium some cytoplasmic survivin expression was detected in the basal cells, whereas proliferating cells showed nuclear staining of survivin. Nuclear expression of survivin was also detected in 67 cancers (80%). The mean survival for patients of this group (28 months, range 20-36) was significantly less than that for patients without survivin expression in the tumour cell nuclei (108 months, range 62-154, p=0.003). Using univariate analysis nuclear survivin expression (p=0.003), tumour depth (p=0.001), lymph node metastasis (p=0.003) and stage (p< 0.001) were the best predictors of survival. In contrast, cytoplasmic survivin staining was noted in 53 (63%) tumours and had no prognostic relevance.
Conclusion: The analysis of nuclear survivin expression identifies subgroups in oesophageal squamous cell cancer with favorable (survivin-) or with poor prognosis (survivin+). We suggest that the determination of nuclear survivin expression could be used to individualize therapeutic strategies in oesophageal squamous cell cancer in the future.

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Carbohydrate mimics bind to tumour cell surfaces and inhibit cell adhesion

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Lectin-carbohydrate interactions mediate cell recognition and are involved in cell adhesion. The binding avidity of branched, i.e. multiantennary oligosaccharides to lectins has been shown to be greater than of linear saccharides. We synthesised di- and triantennary galactosides and fucosides with a carbohydrate mimic as core; either bishydroxymethyl-furan (**1**) or trishydroxymethyl-cyclohexane (TMH, **2**).
To visualise cell surface carbohydrate-binding proteins, the diantennary carbohydrates were coupled via a Diels-Alder reaction (**1**) or directly (**2**) to