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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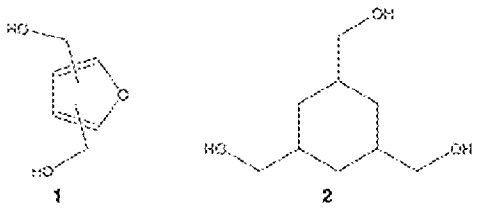
Lectin-carbohydrate interactions mediate cell recognition and are involved in cell adhesion. The binding avidity of branched, i.e. multiantennary oligosaccharides to lectins has been shown to be greater than of linear saccharides. We synthesised di- and triantennary galactosides and fucosides with a 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



1 2

biotin. Reaction with avidin-FITC revealed discrete staining patterns on epithelial tumour cell lines, while normal human fibroblasts showed no staining. The influence of the synthetic carbohydrates upon the adhesion of B16F1 and B16F10 mouse melanoma cells to different extracellular matrix proteins (ECM) was then investigated. The adhesion of the highly metastatic B16F10 cells to fibronectin, vitronectin or laminin was inhibited by the galactosides, while the low metastatic B16F1 cells showed only marginal sensitivity. The greatest effects were 80% inhibition by 1,3 (Gal)2-TMH on fibronectin and 70% by 3,4 (Gal)2-furan on laminin at 40 mM each. Lower concentrations enhanced binding of both cell lines to ECM. Zymography of serum free conditioned medium showed reduced expression and activation of matrix metalloproteases in B16F10 cells incubated with 3,4 (Gal)2-furan. The consequence of this is apparent in a reduced migration of these cells through matrigel. These synthetic di- and triantennary carbohydrate mimics are therefore versatile tools which can be modified to influence processes of adhesion and migration and in addition can be derivatised with fluorescent labels for diagnostic purposes.

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Endothelin a receptor blockade with atrasentan does not change PSA secretion in prostate cancer cell lines

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In the United States, it is estimated prostate cancer will account for 30% (189,000) of new cancer cases in men, with 30,200 deaths from the disease this year. Worldwide, it is estimated 204,000 men die each year of prostate cancer. Endothelin-1 (ET-1) has been identified as a potentially important factor in the pathophysiology of advanced prostate cancer. The effects of ET-1 appear to be mediated primarily through the endothelin-A (ETA) receptor, as the ETB receptor gene, EDNRB, is frequently hypermethylated. Atrasentan (ABT-627), developed by Abbott Laboratories, is a potent, ETA-selective receptor antagonist. In phase II clinical trials of men with advanced, hormone-refractory disease, atrasentan treatment significantly delayed time to disease progression in evaluable men, and delayed time to prostate specific antigen (PSA) progression. In some men, there was a drop in PSA following exposure to atrasentan. It is our hypothesis that the decline in PSA is evidence of anti-tumoral activity rather than a direct effect of atrasentan on PSA secretion by prostate cancer cells. To test this hypothesis *in vitro*, two human, PSA-secreting, androgen sensitive prostate cancer cell lines, LAPC-4 and LNCaP, were studied. In LNCaP cells, ETA receptor expression is very low, and the ETB receptor gene is hypermethylated. In LAPC-4 cells, saturation by 125[I]-ET-1 demonstrates high affinity binding (Kd 1.3 nM, 1200 binding sites/cell). LAPC-4 also has hypermethylation of EDNRB. Both LNCaP and LAPC-4 cells were exposed to atrasentan, or an ETB receptor antagonist (A192621) across a wide range of concentrations (10-6-10-10 M) for 24 hours. Neither atrasentan nor an ETB antagonist changed PSA concentrations in the media of LNCaP or LAPC-4, while addition of dihydrotestosterone, a positive control, produced a significant increase in PSA. These data suggest that changes in serum PSA in men treated with atrasentan are not due to a direct effect of that agent on the secretion of PSA from prostate cancer cells, but may result from an antitumoral activity.

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Image guided proteomics for molecular target discovery: a human giloma study

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Gliomas are the most common type of brain cancer and glioblastoma multiforme (GBM) is the most malignant form. Classic MRI appearance of GBM reflects the tumor's heterogeneous pattern of hypercellularity, necrosis, neovascularity, and invasion. Image guided proteomics may help elucidate mechanisms responsible for the imaging patterns and pathologic variability seen in GBM and offers a novel technique for discerning new molecular targets. In this study, image-guided protein expression profiling was performed on human GBM specimens. Samples were taken from MRI Gd-enhanced (CE) and non-enhanced (NE) regions of each tumor during surgical resection. Proteins were isolated from the fresh-frozen tissue samples followed by proteomic mass spectral analysis. Protein expression profiles of enhanced regions differ from non-enhanced regions. The NE regions across the four tumors and from different NE regions within the same tumor are markedly homogeneous. In contrast, no common protein profile can be determined from the CE regions. Protein expression profiles were further characterized by 2D-gel electrophoresis for mass spectrometric protein identification. This first use of MRI-guided proteomics in human tumors identified a protein fingerprint that correlates with spatial contrast enhancement patterns in solid tumors. Specific proteins identified by this method to account for the different patterns may prove useful as diagnostic markers and/or therapeutic targets.

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The cyclin-dependent kinase inhibitor cyc202 is effective in human leiomyosarcoma (LMS) cell lines in combination with doxorubicin

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The cyclin-dependent kinase (CDK) inhibitor CYC202 (R-roscovitine) is believed to exert its anticancer effects by inhibition of CDK types 1 and 2 and by inhibition of phosphorylation of pRb (retinoblastoma protein), thus preventing cell cycle progression. For this study 2 human LMS cell line models have been used: SK-UT-1 and SK-LMS-1. Both cell lines are mutant for p53, SK-UT-1 is pRb deficient, whilst SK-LMS-1 is pRb wild-type. Flow cytometric analysis for DNA cell cycle measurements using propidium iodide showed that when both cell lines were treated with DOX for 24h a G2M cell cycle arrest was induced. Treatment with CYC202 (20uM) in combination with DOX showed complete abrogation of the G2M arrest at 24h, with CYC202 treatment alone showing a G1 arrest for both cell lines. When SK-LMS-1 cells were treated with CYC202 a decrease in pRb phosphorylation was apparent, an effect that was further enhanced when CYC202 was combined with DOX, seen at 48h. For SK-UT-1 cells no p53 induction was seen following treatment with CYC202, DOX or the drugs in combination at 48h. SK-LMS-1 cells showed induction of p53 following treatment with DOX and in combination with CYC202 in the same experiments. In both cell lines there was no induction of either p21waf-1 or $p27^{kip-1}$ following treatment with DOX, CYC202 singly or in combination. The SK-UT-1 cell line shows relatively high levels of cyclins D and E which, following treatment with CYC202 remained unaltered after 6h. Cytotoxicity testing (MTT assay) showed an overall additive effect between DOX and CYC202 (used simultaneously) in SK-LMS-1 cells, but there was a suggestion of some synergy seen for the SK-UT-1 cells. CYC202 is effective in tumours showing a variety of molecular abnormalities such as human LMS which are very chemorefractory in nature. It may also be a useful agent when used in combination with chemotherapy.

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Cytochrome P450 1B1 (CYP1B1) is expressed in human colon adenocarcinomas, but its expression is not limited to the malignant epithelial cells

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Cytochrome P450 monooxygenases (CYPs) are the protein products of a superfamily of genes. CYPs have great diversity of expression and activity. They are involved in activation and detoxification of endogenous and exogenous compounds. CYP1B1 has been reported as present in humans only in tumours, being absent from the corresponding normal tissues; thus, CYP1B1 may represent a target for selective anticancer drug development. This study aimed to validate CYP1B1 as a target by looking at its expression in human colon cancer biopsy material. Immunohistochemical methods were used to investigate the expression pattern of CYP1B1 in human colon adenocarcinomas, in order to determine its potential role in drug metabolism. Sections (5um) of formalin-fixed and paraffin-embedded malignant human colon adenocarcinomas from fifty patients were used. These originated from tumours representing a range of stages, grades, and pa-