explore whether or not radiotherapy can enhance the immune response to tumours. Time- and dose-response assays to ionizing radiation of various cell lines established from spontaneously-arising tumours are underway. These cells have also demonstrated ability to establish tumours *in vivo* when injected subcutaneously into host mice. Once these assays are complete, then *in vivo* tumours will be irradiated, and T cell proliferation will be evaluated as described above.
Results: In mice that received OT-II cells, little to no proliferation was observed in response to *neu*$^{OTI/OTII}$ x *DNp53* tumours. In contrast, mice that received OT-I and OT-II T cells demonstrated proliferation of OT-I cells within 3 days, while OT-II cells remained unresponsive. Approximately 20% of tumours showed a complete response. Histopathologic analysis demonstrated infiltration of tumour by T lymphocytes, despite strong expression of *neu*$^{OTI/OTII}$. In fact, residual lymphocytes can be identified in the mammary tissue at the tumour's original location, even after complete regression of the tumour.
Conclusions: Established tumours trigger a strong, potentially curative $CD8^+$ response, but appear to selectively evade $CD4^+$ cells. High *HER2/neu* expression is associated with more aggressive tumours. Subcutaneous reintroduction of cells isolated from a spontaneously-arising tumour into a host mouse results in establishment of tumour within a significantly shortened time period. Future directions include further characterizing the immune response to mammary tumours. This will include using ionizing radiation as an injury stimulus to determine whether the immune response to tumours is enhanced.

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Effect of retinoic acid on prostate cancer cells *in vitro*

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Background: Retinoic acid induces differentiation or/and growth arrest of cancer cells through regulation of the expression of several genes. Heparin Affin Regulatory Peptide (HARP) is a growth factor with high affinity to heparin, which plays a key role in the development of several types of cancer. In the present work we studied the effect of all-trans retinoic acid (ATRA) on the growth of epithelial prostate cancer cells LNCaP and the involvement of HARP in this effect.
Materials and methods: An antisense strategy for inhibition of HARP expression in the human prostate cancer cell line LNCaP was used to study the role of HARP on the effect of ATRA on cancer cell growth. The effect of ATRA on HARP expression was studied by a combination of Western analysis and RT-PCR.
Results: ATRA decreased LNCaP cell growth in a concentration-dependent manner. This effect seems to be mediated by HARP, since ATRA had no effect on LNCaP cells that did not express HARP. Moreover, ATRA significantly decreased HARP expression by LNCaP cells at both protein and mRNA level.
Conclusions: These data suggest that HARP is essential for the growth of human prostate cancer cells *in vitro* and that ATRA affects prostate cancer cell growth through an effect on the expression of HARP.

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Aberrant expression of neuropilin-1 and –2 in human pancreatic cancer cell

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Purpose: Neuropilin (Np)-1 and -2 are coreceptors for vascular endothelial growth factor (VEGF). This study was designed to assess their role in pancreatic ductal adenocarcinoma (PDAC).
Experimental design: We assessed Np-1 and Np-2 expression by real-time quantitative PCR in relation to the expression of VEGF ligands and receptors in pancreatic cancer cell lines and tissues.
Results: ASPC-1, CAPAN-1, and PANC-1 pancreatic cancer cells and tumor-derived, laser-captured pancreatic cancer cells exhibited higher Np-1 and Np-2 mRNA levels than VEGF receptor-1, -2, or -3 mRNA levels. Transfection of Np-1 and Np-2 cDNAs in COS-7 cells, and treatment with tunicamycin revealed that both proteins were glycosylated. Both proteins were expressed in pancreatic cancer cell lines, in the PDAC samples, and in acinar cells adjacent to the cancer cells. The normal pancreas was devoid of Np-1 immunoreactivity, whereas Np-2 immunoreactivity was present in the endocrine islets and in some acinar cells, but not in ductal cells.
Conclusions: The aberrant localization of Np-1 and Np-2 in the cancer cells in PDAC suggests that in addition to exerting proangiogenic effects, these coreceptors may contribute to novel autocrine-paracrine interactions in this malignancy.

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Effect of TNP-470 on the growth of melanoma in irradiated bed

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Background: The angiogenic inhibitor TNP-470, analogue of fumagillin, has been reported to inhibit the growth of some tumours cell lines. However, it is still unknown which effect of TNP-470 was obtained by the administration of this compound on melanoma growing in irradiated bed. The aim of study is to analyze the action of TNP-470 under condition of Tumour Bed Effect (TBE); it is with very poor bed angiogenic response.
Materials and methods: Twenty-four C57BL/6 male mice were assigned to four groups. Irradiation was carried out using a Telecobalt unit, at single dose of 60 Gy on posterior-right paw. B16-F10 melanoma 1mm^3 of solid tumour was then transplanted into the irradiated bed. TNP-470 was administered subcutaneously on the back of mice a single dose (30 mg/Kg) every other day during 14 days. After this time mice were sacrificed for tumour measurement and pathologic examination.
Results: Control group (A), only implant of tumour, the melanoma growth was nodular and presented great neoangiogenesis, with a mean volume of 2904 mm^3. Group B, irradiation and tumour implant, reached a volume of 225 mm3. Group C, tumour implant and TNP-470, the volume was of 377 mm^3. Group D, irradiation and tumour implant and TNP-470, got a final volume of 68 mm^3.
Conclusions: TNP-470 produces inhibition of tumour growth. Under TBE conditions (with tumours growing slower), this effect is additive.

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Oncogenesis of v-Src-transformed cells is associated with upregulation of mTOR signalling pathway

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The signalling mechanisms that are present in the cell play a major role in all phases of its life. However, asynchronous activation of some of these signals can commit cells to changes resulting in diseases such as cancer. A critical role in transmitting the signals is played by the Src family of tyrosine kinases. The activated Src protein is involved in the multiple mechanisms by which cells are transformed to the malignant phenotype.
To recognize some of them, we analysed, in cells transformed by Rous Sarcoma Virus (RSV), the mTOR-dependent signalling pathway, which is activated during malignant transformation and cancer progression in many human cancers. mTOR (mammalian target of rapamycin) is emerging as a central controller of cell growth and proliferation, which is mediated by its downstream targets implicated in translational control of gene expression. These are the repressor proteins 4E-BPs that regulate the activity of the "cap-binding protein", the initiation factor 4E (eIF4E), and S6 protein kinases (S6K) catalyzing phosphorylation of the ribosomal protein S6, a component of the 40S subunit of eukaryotic ribosomes.
We found that in RSV-transformed cells, the enhanced expression and activity of the v-Src oncoprotein correlated with increased levels of overall protein synthesis. Phosphorylation of 4E-BP1, ribosomal protein S6 and its physiological protein kinase, p70 S6K, were highly upregulated and inhibited by the mTOR specific inhibitor, rapamycin. Direct interactions between Src and p70 S6K were found by immunoprecipitation and pull-down assays of the p70 S6K with GST(src)SH2 and GST(src)SH3 fusion proteins. Inhibition of Src kinase activity resulted in decreased activity of the mTOR signalling pathway. Rapamycin, which is under intensive examination as a novel agent in cancer therapy, completely eliminated the colony formation in soft agar by the transformed cells.
These data provide evidence suggesting that mTOR is an obligatory mediator of the oncogenic signals from the v-*src* oncogene. The activated mTOR signalling pathway may promote the enhanced rapamycin-sensitive expression of specific proteins that are involved in malignant process induced by RSV transformation.
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