Conclusions: For a defined type of genetic alteration, in our model, a defined type of tumoral pathway is associated, given a possibility to cure the animal only with surgery or associated with a specific type of recurrence. Future research is necessary to explore the possibility of understanding further the MSI tumor response to surgery.

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Heparin affin regulatory peptide signaling in human endothelial cells

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Background: Heparin affin regulatory peptide (HARP) is an 18 kDa secreted growth factor that has a high affinity for heparin and a potent role on tumor growth and angiogenesis. We have previously reported that HARP is mitogenic for different types of endothelial cells and also affects endothelial cell migration and differentiation. In the present work, the signaling pathways involved in the migration of human umbilical vein endothelial cells (HUVEC) induced by HARP were studied.
Materials and methods: A combination of immunoprecipitation, Western blot analyses and small interfering RNA technology was used in order to determine HARP receptor in HUVEC, as well as the downstream signaling pathways that lead to increased endothelial cell migration and tube formation *in vitro*.
Results: Receptor protein tyrosine phosphatase β/ζ (RPTPβ/ζ), which has been characterized as a receptor for HARP in neuronal cell types, is also expressed in HUVEC. HARP signaling through RPTPβ/ζ leads to activation of src kinase, FAK, PI3K and Erk1/2. Sodium orthovanadate, chondroitin sulfate-C, PP1, wortmannin, LY294002 and U0126 inhibit HARP-mediated signaling and HUVEC migration and differentiation. RPTPβ/ζ suppression using siRNA technology interrupts HARP-induced intracellular signals and HUVEC migration and differentiation. Finally, a peptide that corresponds to the last 25 aminoacids of the carboxy terminus of HARP seems to inhibit the effects of HARP on HUVEC.
Conclusions: These results establish the role of RPTPβ/ζ as a receptor of HARP in HUVEC and elucidate the HARP signaling pathway in human endothelial cells.

189 POSTER

Ionizing radiation affects cellular translation machinery

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There is increasing evidence that translation plays an important role in the regulation of gene expression under a wide variety of conditions, such as response to hormones, growth factors and nutrients. Furthermore, it has been well documented that alterations in the expression level of translation initiation factors can be involved in tumorigenesis or tumor progression. In addition, there is data substantiating that different forms of stress, such as DNA damage or nutrient withdrawal, reduce general translation while promoting the production of particular proteins. We hypothesized that cellular stress in the form of ionizing radiation induces changes in gene expression by modifying the process of translation. These changes might, in turn, play a role in the radiation-induced gene expression. Here, we report that radiation influences not only the signaling cascades that control translation, but also the translation machinery itself.
To determine whether radiation has specific effects on the translation machinery, human U251 glioblastoma cells were exposed to 6 Gy and proteins critical to translation were evaluated. We found that, while the key rate-limiting molecule eIF4E did not change after irradiation, a marked elevation of both expression and phosphorylation of the eIF4E inhibitor 4E-BP1 was observed. Upon phosphorylation, 4E-BP1 releases eIF4E and allows the formation of the initiation complex, in which eIF4G and Mnk-1 also take part. Interestingly, these proteins were also upregulated by radiation. Moreover, radiation also augmented the expression of both p70 S6 kinase and S6, which further promote translation initiation. Because the PI3K/AKT/mTOR pathway has been implicated in translation control, we investigated whether it was involved in the response of the translation machinery to radiation. Pre-treatment of cells with the mTOR inhibitor rapamycin, the PI3 kinase inhibitor LY294002, and the AKT inhibitor perifosine were able to abolish the effects of radiation in all cases.
Taken together, these data suggest that radiation affects translation by modulating eIF4E binding partners and thus controlling the availability of eIF4E for translation initiation. These results suggest an additional mechanism through which radiation may exert control of gene expression.

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Target identification and validation in pancreatic cancer

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Pancreatic cancer is the fourth commonest cause of death from cancer. Its incidence in Europe is approximately 30,000 patients per year. Most patients are diagnosed at an advanced stage and will die within a year. Thus, early diagnosis and treatment of pancreatic cancer remain great oncologic challenges.
The objectives of this study were:
1. The identification of genes differentially expressed in ductal pancreatic adenocarcinoma (PDAC) relative to both normal pancreas and chronic pancreatitis
2. The generation of diagnostic/therapeutic antibodies specific for the identified gene products
3. The development of an orthotopic model of human pancreatic cancer in SCID mice for the *in vivo* validation of said gene products.

In order to attain such objectives we followed a Functional Genomics approach to target discovery. Total RNA was prepared from 2 normal pancreas, 2 chronic pancreatitis and 12 PDAC samples. Biotinylated cRNA was hybridized to Affymetrix HGU133A chips, which can detect 21,744 transcripts. GeneSpring software was used to obtain a list of 116 genes overexpressed in PDAC samples. A subset of 52 genes was selected from this list after the following criteria: lack of previous association to PDAC, accessibility of the gene product to antibodies, and potential role in cancer development. Overexpression of 24 of these genes was confirmed by quantitative RT-PCR. Specific rabbit polyclonal antibodies and scFv fragments from a phage library were generated and tested by Western blotting or ELISA to confirm gene overexpression at the protein level. In order to establish an *in vivo* model of PDAC, SCID mice were injected at the pancreas head with a cell suspension of the cell line Capan-1.
Four weeks after injection mice were sacrificed and tumor establishment was evaluated. Upon histopathological examination, mice injected with Capan-1 developed orthotopic well-differentiated PDAC with hepatic and peritoneal metastasis. The characteristic stromal proliferation surrounding the tumor was also observed. We have combined the use of microarrays, Q-RT-PCR and Western blotting to identify changes in gene expression associated to PDAC. We intend to exploit the detection of these gene products as a diagnosis method for this type of cancer.
In addition, the *in vivo* model described provides us with a means to test the relevance of said gene products in the development of pancreatic adenocarcinoma, and therefore to evaluate their potential use as therapeutic targets.

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Evaluation of the T cell response to mammary tumours using a novel transgenic mouse model

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Objectives: To characterize the immune response to mammary tumours, to define the mechanisms by which tumors evade rejection by tumour-specific T cells, and to explore the potential of radiotherapy in enhancing the immune response to these mammary tumours.
Materials and methods: A transgenic mouse mammary tumour model in which tumour formation is driven by expression of a modified *HER2/neu* oncogene ($neu^{OTI/OTII}$) was developed. $CD8^+$ (OT-I) and $CD4^+$ (OT-II) T cell epitopes were added to *HER2/neu*, thereby allowing $neu^{OTI/OTII}$ to be recognized by specific TcR-transgenic $CD8^+$ and $CD4^+$ T cells. Transgenic mice expressing $neu^{OTI/OTII}$ and a dominant-negative *p53* transgene (DN*p53*) spontaneously develop mammary carcinomas. $CD8^+$ and $CD4^+$ T cells are activated when exposed to cells expressing $neu^{OTI/OTII}$.
Using this model, naïve OT-I or OT-II TCR-transgenic T cells labeled with CFSE were adoptively transferred into tumour-bearing $neu^{OTI/OTII}$ x DN*p53* mice. $CD4^+$ and $CD8^+$ T cell proliferation was serially evaluated by monitoring reduction in CFSE intensity. Mice were euthanized at appropriate time points. Sera was collected for immune analysis, and tumour was isolated for histopathologic analysis, protein and nucleic acid analysis, and potential establishment of novel cell lines. Preliminary radiotherapy experiments have been initiated with the ultimate goal to

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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