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.

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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