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Phosphorylation of S6K1 by Casein kinase 2 regulates its subcellular localization

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The ribosomal protein S6 kinase (S6K) belongs to the AGC family of Ser/Thr protein kinases which includes the protein kinase C's, protein kinase B's, SGKs, and 90 kDa ribosomal S6 kinases. Two forms of S6K have been identified (S6K1 and S6K2). Both kinases are activated in response to mitogenic stimuli and nutrients via PI3-K and mTOR signaling pathways. Biochemical and genetic studies provided the evidence for the involvement of S6K in the regulation of cell growth, size and proliferation. It is believed that conformational changes induced by multiple S/T phosphorylations open the structure of S6K1, making domains available for protein-protein interactions.
In this study we describe the identification of Casein Kinase 2 (CK2) as a physiological binding partner of S6K1. Screening of a HeLa cDNA library with an activated version of S6K1 (T412D mutant) bait construct allowed us to isolate three clones corresponding to beta subunit of Casein Kinase 2 (CK2). The specificity of interaction between S6K1 and both CK2 alfa and beta subunits was further confirmed in mammalian cells using immunoprecipitation studies with transiently overexpressed and native proteins.
Bioinformatic analysis of S6K1 sequence revealed three potential phosphorylation sites for CK2. The localization of CK2 phosphorylation site was narrowed down to the N-terminal region of S6K1 with the use of deletion mutants in *in vitro* kinase assay. The N-terminal region contains only two Ser/Thr sites and one of them, Ser17, is in the CK2 phopshorylation motif. Mutation analysis of S17 clearly showed that it is the major *in vitro* phosphorylation site for CK2. Fluorescent microscopy study indicated that phosphorylation mimicking mutant of S6K1 (S17E) doesn't translocate to the nucleus in serum stimulated cells. Treatment of cell with nuclear export inhibitor Leptomycin B demonstrated that S6K1 S17E mutant accumulates in the nucleus.
These results indicate that nuclear import of S17E mutant is not affected while the export is significantly enhanced. In summary, this study shows for the first time that S6K1 interacts with and is phoshorylated by CK2 in mammalian cells. The phosphorylation of S6K1 at S17 enhances its nuclear export, causing the accumulation of S6K1 in the cytoplasm.

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Extracellular acidosis as a mechanism of neurotensin-stimulated growth in pancreatic carcinoma cells

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Background: Advanced pancreatic cancer is inevitably linked with high mortality. The peptide neurotensin (NT) acts as a growth factor on pancreatic tumor cells via the G-protein-coupled receptors NTR1/3, triggering signaling pathways affecting proliferation and possibly other intracellular responses.
Material and methods: The pancreatic tumor cell lines BxPC-3, PANC-1, MIAPaCa-2 and the NTR-positive colonic tumor cell line HT-29 were used to study NT-induced Ca^{2+}- and pH-responses in spectrofluorimetry (Fura-2, BCECF). Proliferation was assessed with MTT-assays and the expression of NTR1 quantitated in flow-cytometry (Euroclone B-N6).
Results: Stimulation of NTR1/3 in the pancreatic cancer cell lines BxPC-3 and PANC-1 using the stable analog lys-$^{®}$-lys-NT(1–6) resulted in an increase in intracellular Ca^{2+} and in intracellular alkalinization of 0.1–0.15 pH-units that has not been described so far. In contrast, MIAPaCa-2 cells that lack significant NTR1 surface expression revealed a minor intracellular acidification in the presence of a normal Ca^{2+} response. Extracellular acidosis (pH = 6.8) stimulated proliferation of these cell lines, in comparison to normal or alkaline (pH = 7.8) conditions. Dense and acidic cultures revealed a higher expression of NTR1 inversely related to the expression of EGFR. Since intracellular pH-regulation under these conditions is accomplished by the Na^{+}/H^{+}-exchanger 1 (NHE1), the signal transduction pathways linking NTR to NHE1 were further investigated. The NT-induced alkalinization was abrogated in presence of the NHE1-inhibitor amiloride and linked to an increased proton flux. Application of PKC inhibitors in combination with lys-$^{®}$-lys-NT(1–6) resulted in an impaired pH response for H-7 and staurosporine in BxPC-3, and for bisindolylmaleimide I and II in PANC-1, respectively. In contrast, no inhibitory effect was observed in MIAPaCa-2 cells for those PKC inhibitors. The phosphatase inhibitor calyculin enhanced the NT-induced alkalinization, and NHE1 was found to become phosphorylated in a time- and dose-dependent manner by lys-$^{®}$-lys-NT(1–6) in BxPC-3 and PANC-1 cells.
Conclusion: NT stimulates the activity of the NHE1 in NTR1-positive pancreatic cancer cells, resulting in a proliferative response and intracellular alkalinization/extracellular acidification. This NT-induced acidic condition may contribute to the overexpression of VEGF and IL-8 in pancreatic tumors, ultimately resulting in a highly metastatic phenotype.

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Complete remission of advanced autologous intracranial gliomas by oncolytic Parvovirus H-1

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Background: Virotherapy of malignant gliomas is an alternative strategy to improve the prognosis of this rapidly fatal disease. The oncolytic and non-pathogenic Parvovirus H-1 possesses strong cytotoxic effects in glioma cells *in vitro*. In this study, we investigated the therapeutic potential of H-1 virus in a glioma model in immunocompetent rats.
Material and Methods: RG-2 rat gliomas were implanted into immunocompetent animals. MRI was performed to demonstrate tumour growth. When tumours had reached a size of >6mm in the largest diameter, animals received intratumoural stereotactic injection of H-1 virus (n = 12) or sham-operation (n = 12). Treatment effects were monitored by MRI. When animals were sacrificed, PCR, histology and immunostaining of brain tissue were performed.
Results: H-1 virus treatment of animals with large intracranial gliomas resulted in rapid tumour regression in 8 animals. 4 of the animals were sacrificed prior to complete tumour remission. Histology showed widespread destruction of the tumour tissue, but no toxic or inflammatory side effects in the surrounding brain tissue. Viral proteins could be demonstrated by immunostaining. The remaining 4 animals survived for >1 year without tumour recurrence. All control animals died within 22 days. The difference between the survival time of H-1 therapy group and control group were statistically signifcant (logrank test: $p < 0.001$).
Conclusions: Parvovirus H-1 possesses strong antitumour activity in glioma cells *in vivo*. This finding and the absence of pathogenic side effects make H-1 virus a promising candidate for oncolyticvirotherapy of malignant gliomas.

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Tumor gene expression influences the chance of cure and the type of recurrence after colectomy in a colon cancer model using mice grafts

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Background: After surgical resection of a colon cancer, 3 pathways are possible: cure, local recurrence or a metastatic process. Our hypothesis was to consider that the accumulation of genetic alterations that characterize colonic carcinogenesis must impact on different pathways after surgical tumor resection.
Model: We used the human MSI (Microsatellite Instability) colon cancer cell line HCT116, typical of non hereditary colon cancer and 15% of sporadic cancers. Three clones were used: the parental cells lines with a non functional MLH1 protein, the complemented cell line with a vector containing the HMLH1 gene (HCT-MLH1-3) and the complemented cell line with the chromosome 3 (containing the HMLH1 gene and the TGFβRII) named HCT-K3. Each tumor was grafted intra-caecaly on *node-scid* mice (n = 10). Natural history of the tumor evolution was analyzed at day 45, and evolution after surgical resection of the graft at day 8 was analyzed at day 45.
Results: The cell lines HCT-116 and HCT-K3 present more voluminous tumors than HCT-MLH1-3 ($p < 0.005$). Only the tumor from HCT-K3 presented lymph node or liver metastasis after 45 days, in 20% of cases. After resection of the HCT-1161–3 tumors, all mice were cured at 45 days (if histological and biological data are confirmed). However, surgery only cured 20% of mice with the other two tumors ($p < 0.04$). For the HCT-116 tumors, the recurrences are usually reported as peritoneal carcinomatosis without lymph node or liver metastasis. However, for the HCT-K3 tumors the recurrence after colectomy was always associated with lymph node metastasis and 20% of cases with liver metastasis.

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