cells could play a role in cancer progression. When analysing the expression of BE in 30 human melanoma biopsies obtained from patients, we found a correlation between beta endorphin expression and stage of the malignancy (p < 0, 05). We analysed the potential role of BE in preventing immune response against tumor cells and performed a mice model of cancer progression by subcutaneous injection of melanoma B16 cells to both mu opioid receptor deficient mice (MOR -/-) and their WT counterparts. A profound decrease in tumor growth was observed in MOR -/- mice compared to WT animals (median volume 0,2 cm3 versus 0,8 cm3 at day 15 post injection; p < 0,01). This was paralleled by a significant higher infiltration of CD4+, CD8+, NK and dendritic cells at tumor site of MOR -/- mice determined by flow cytometry and immunohistochemistry. Adoptive transfer experiment with PKH-26-labeled MOR -/- leukocytes in combination with PKH-67-labeled WT leukocytes demonstrated that the higher presence of immune cells was not du to a higher recruitment of cells at tumor sites, but rather to proliferation and activation of leukocytes. NK cell activation was indeed increased by the use of BE-blocking antibody.

These findings demonstrate that endgenous beta endorphin secretion by melanoma cells plays a role in tumor growth and immune escape. Blocking beta endorphin therefore appear to be a promising new attractive therapeutic strategy to limit cancer progression.

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The monocarboxylate transporter 1 (MCT1) and Hypoxia-induced MCT4 are key targets promoting tumor cell survival

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In the hypoxic tumor microenvironment, cancer cells switch their glucose metabolism to glycolysis for their energy demands. This is known to generate a large amount of lactic acid that must be rapidly exported to maintain a permissive intracellular pH, essential in sustaining glycolysis, protein synthesis and cell survival. Cells have evolved several transport systems to extrude lactic acid. A large family of H+-linked MonoCarboxylate Transporters, represented by the ubiquitously expressed MCT1, co-transport H+/lactate- in both directions. Interestingly, MCT4, a member of this family widely expressed in tumor cells, is up-regulated in hypoxia by the transcription factor HIF. In addition, the functional expression of MCT1 and MCT4 in the plasma membrane is finely regulated by specific interaction with the glycoprotein chaperone CD147/Basigin.

Why do tumor cells co-express two lactate transporters? What are their respective functions? Are they both essential for tumor metabolism?

Firstly, we show in Ras-transformed fibroblasts, which express only MCT1, that blockade of this transporter (siRNA or AstraZeneca inhibitor) severely restricts cell growth and survival in vitro in hypoxia and in vivo in xenografted tumors in nude mice. Secondly, we show that ectopic expression of MCT4 in these Ras-transformed fibroblasts bypasses the MCT1 blockade. MCT4 expression restored ATP levels and growth in vitro in hypoxia and in vivo, induced tumors to escape the block in MCT1. Thirdly, we demonstrate that silencing CD147 or MCT4 in several human tumor cell lines grown in hypoxia (breast, prostate, colon, head & neck, melanoma) induces rapid cell death when MCT1 is co-inactivated.

We therefore conclude that MCT1 and MCT4 are two key steps in hypoxic tumor metabolism and as such represent new targets for anticancer therapy.

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Hypoxia-induced BNIP3 reduces proliferation of colon carcinoma cells through downregulation of ERK1/2

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The development of solid tumors is often accompanied by the appearance of hypoxic regions. Tumor cells rapidly respond to this new micro-environment by activating the Hypoxia-induced transcription factor HIF, an acknowledged strong promoter of tumor growth. HIF activates a complex gene expression program ensuring cell survival by inducing glycolysis, angiogenesis, autophagy, migration and invasion but also by controlling proliferation. Amongst these adaptive processes the control of tumor cell proliferation by hypoxia remains unclear and largely uncharacterized at the molecular level. We present here new evidence confirming the negative impact of hypoxia (1% O2) on cell proliferation. We also provide proof that the hypoxia-induced HIF-1a gene known as Bcl2/adenovirus E1B 19kDa-interacting protein 3 (BNIP3) does not induce cell death but mediates the decrease in tumor cell proliferation induced by hypoxia. We demonstrate, in both non-tumoral fibroblast (CCL39) and colon carcinoma (BE) cell lines, that BNIP3 triggers a decrease in the level of phosphorylated ERK leading to a substantial increase in the accumulation of the CDK inhibitor p27 and a delay in S-phase entry. We show that inhibition of ERK activity by treatment with the MEK inhibitor U0126 abolished the hypoxic and HIF-1-induced expression of BNIP3 suggesting the existence of a negative feedback loop regulating BNIP3 expression and cell proliferation in tumors. We propose that hypoxia-induced BNIP3 participates in the general HIF-induced adaptive mechanism leading to tumor cell survival through the attenuation of the rate of cell proliferation but also through the activation of autophagy (see Mazure et al. abstract).

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Caveolin-1, a novel Id1 binding partner, and its role on Id1-induced behavioral change in prostate cancer cells

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Id-1 (Inhibitor of Differentiation/DNA binding-1) is reported to promote cell proliferation, invasion and survival in many types of human cancer cells through multiple signaling pathways. However, how Id1 interacts with these pathways and the immediate downstream effectors of the Id1 protein are not known. In this study, we identified a novel Id-1 interacting protein, caveolin-1 (Cav-1), a cell membrane protein and a positive regulator of cell survival and metastasis in prostate cancer. Using immunoprecipitation method, we found that the helix-loop-helix domain of the Id-1 protein was essential for the physical interaction between Id-1 and Cav-1. We also demonstrated in prostate cancer cells that the physical interaction between Id-1 and Cav-1 played a key role in the Id-1-induced epithelial-mesenchymal transition and cell migration as well as resistance to taxol-induced apoptosis. Our results also revealed that the Id-1-induced Akt activation through promoting the binding activity between Cav-1 and protein phosphatase 2A was responsible for the synergistic effect between these two proteins. Our study demonstrates a novel Id-1 binding partner and suggests a molecular mechanism that mediates the function of Id-1 in promoting prostate cancer cell invasion and survival through activation of the Akt pathway [(HKU7478/03M) to XHW and YCW (HKU7490.03M, 7470/04M, NSFC/RGC N_HKU738/03, HKU Foundation Seed Fund, 03)].

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SEMA3F Semaphorin is involved in tumor angiogenesis

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Class-3 semaphorins (SEMA3s) and VEGF are secreted proteins that bind neuropilin receptors suggesting antagonism, although recent studies argue against binding competition. In addition, SEMA3s regulate integrin activation in the vascular system, as well as in tumor cells.

SEMA3F was identified as a tumor suppressor in lung cancer by chromosome mapping of heterozygous deletions. We and others subsequently demonstrated that SEMA3F blocks in vivo tumorigenesis using different animal models. This was associated with inhibition of integrin-mediated attachment and reduced angiogenesis.

We studied intracellular signaling changes due to overexpression of SEMA3F in H157 lung cancer cells and found lower activity of ILK, ERK1/2, AKT and STAT3. Importantly, we observed downregulation of HIF-1α protein, along with VEGF transcription reduction. In a mouse subcutaneous tumorigenesis model, SEMA3F overexpression had a growth-inhibitory effect that was coupled with reduced vascular support. To further our investigations, we adapted the chick chorioallantoic membrane (CAM) assay to test the angiogenic capacity of lung cancer cells transfected with SEMA3F or VEGF165. Our results confirm the anti-angiogenic activity of SEMA3F but also suggest that VEGF repression at the level of transcription is only partially responsible for the inhibition of angiogenesis. Moreover, VEGF stable transfection in SEMA3F-expressing lung cancer cells did not reverse the inhibitory action of SEMA3F on phospho-ERK1/2.

These data suggest that VEGF antagonism by SEMA3F might not explain all of its anti-tumorigenic properties, and thereby support the development of SEMA3F as a therapeutic agent in cancer.

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The effect of covalent linkage and the number of Arg residues on the in vitro cytostatic effect and cellular uptake of daunomycin-conjugates on HepG2 and HL-60 tumour cells

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Daunomycin (Dau) is an anthracycline derivative widely used in cancer chemotherapy. Dau inhibits the growth and division of the cells mainly by

interaction with DNA. Although it is one of the most efficient chemotherapeutical agents, it has serious side effects: nausea, vomiting, hair spilling, cardiotoxic effect, etc. These could be limited by conjugation of Dau to polypeptide carriers (1). Oligoarginine is a de novo designed cell penetrating peptide, capable to translocate covalently attached cargo (2). For the comparative analysis of the effect of covalent linkage and of the length of the peptide chain on antitumor and cellular uptake properties we have prepared three groups of new Dau-oligoarginine conjugates containing different number of Arg residues. In these compounds we have insererted oxime-, hydrazone- or squaric acid linkage between Dau and oligoraginine. New conjugates were characterized by mass spectrometry and RP-HPLC. The antitumor activity of the conjugates was evaluated in vitro on HL-60 human leukemia and HepG2 human hepatoma cells by MTT assay. Cellular uptake properties under different conditions (e.g. concentration) was studied by flow cytometry on two cell lines. We found that Dau-conjugates were more effective and uptake was also higher on HL-60 cells. The type of the bond in the conjugates as well as the number of Arg residues influenced markedly both cytostatic effect and cellular uptake.

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Experimental/Molecular therapeutics, pharmacogenomics 1

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Dietary flavonoid fisetin induces a forced exit from mitosis by targeting the spindle assembly checkpoint

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The spindle assembly checkpoint (SAC) regulates the fidelity of cell division by ensuring that chromosome segregation is delayed until all sister kinetochore pairs have achieved stable bipolar attachments with spindle microtubules. Interference with the SAC is a promising strategy for treatment of cancer as premature SAC inactivation causes chromosome mis-segregation leading to massive aneuploidy and subsequent cell death. To discover small molecules with anti-SAC activity, we performed a high-throughput screen (HTS) for compounds that cause a forced exit from mitotic arrest induced by the microtubule destabilizing drug nocodazole. In most human cell lines with a robust SAC nocodazole treatment leads to a cell cycle arrest at mitosis during which the mitotic cells round up and become loosely attached to the substrate. Our screening strategy was based on the different cell-to-substrate attachment properties of round loosely attached mitotic and well-adhered flat interphase HeLa cells. From a library consisting of 2000 biologically active and structurally diverse compounds we identified the flavonoid fisetin (3,3',4',7-tetrahydroxy-flavone) as a strong SAC inhibitor. Time lapse microscopy of H2B-GFP expressing HeLa cells confirmed that fisetin induces escape from nocodazole arrest in a proteasome-dependent manner. We also showed that fisetin can overcome taxol (a microtubule stabilizing drug) and monastrol (an Eg5 inhibitor) induced mitotic arrests. Also non-drug treated mitotic cells underwent premature mitotic exit accompanied by cytokinetic defects upon fisetin treatment. Next we investigated how fisetin interferes with SAC signaling by studying kinetochore accumulation of key SAC proteins in the presence of the drug. We showed that fisetin causes a significant reduction in kinetochore affinity of BubR1 and Bub1 proteins, and delocalization of Aurora B kinase from the inner centromere to the chromosome arms. Furthermore, fisetin inhibited Aurora B and Cdk1 kinase activities as indicated by reduced phosphorylation of CenpA, Cdc27, and nucleolin-1, known substrates of the two kinases. We speculate that inhibition of SAC by fisetin is mediated through interference with Cdk1 and/or Aurora B function. In conclusion, utilizing our novel HTS and subsequent biochemical assays we have identified the flavonoid fisetin as a potential SAC inhibitor, which provides a mechanism of action to explain the drugs' previously reported anti-carcinogenic activity.

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Tissue distribution and pharmacokinetics of an ATWLPPR-conjugated chlorine-type photosensitizer targeting neuropilin-1 in glioma-bearing nude mice

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Destruction of the neovasculature is essential for efficient tumor eradication by photodynamic therapy (PDT). The PDT anti-vascular effect can be promoted by developing addressed photosensitizers localized preferentially to the tumor vascular compartment. A new photosensitizer conjugated to an heptapeptide [H-Ala-Thr-Trp-Leu-Pro-Pro-Arg-OH (ATWLPPR)] targeting neuropilin-1, a Vascular Endothelial Growth Factor (VEGF) co-receptor, has been synthesized. It was administered intravenously for an easier access to endothelial cells lining the vasculature in human malignant glioma-bearing nude mice. Plasma pharmacokinetic parameters were derived from plasma concentration–time data using a non-compartmental analysis and validated a relatively rapid elimination from the blood compartment with an elimination rate constant of 0.062 h^{-1} and a biological half-life of 11.0 h. The photosensitizer was mainly concentrated in organs such as liver, spleen and kidneys, which are rich in reticuloendothelial cells. In these organs, the elimination profiles of the photosensitizer were comparable, with half-lives as short as 12.2, 15.1 and 19.7 h, respectively. The peptidic moiety of the conjugated photosensitizer was degraded to various rates depending on the organ considered, most of the degradation process occurred in organs of the reticuloendothelial system. A metabolic product resulting from the enzymatic cleavage of the peptide bond between Ala and Thr was detected in plasma at all the examined time points from 2 h post-injection. The conjugated photosensitizer accumulated rapidly and at high levels in the tumor, with 2.3% of injected dose per gram of tumor tissue at 1 h after injection. Taking into account the aspecific uptake of the degradation product, the tumor levels of total photoactivable compounds might exhibit an interesting photodynamic activity. On the contrary, levels of total photoactivable compounds remained low in the skin. This study provides essential information for the choice of the time interval not to exceed to activate the photosensitizer.

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Targeting of neuropilin-1 to improve the anti-vascular effect of photodynamic therapy in xenograft human malignant glioma

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The principle of photodynamic therapy (PDT) is based on the combined action of a photosensitizer (PS) localized in the tumor, light and oxygen. After light irradiation of the PS and in the presence of molecular oxygen, photo-oxydation reactions will lead to the production of reactive oxygen species, inducing a localized eradication of the tumor. However, PDT effects are mediated not only through direct killing of tumor cells but also through indirect effects, involving both initiation of an immune response and destruction of the neovasculature (anti-vascular effect).

The strategy developed in the laboratory aims to favour this anti-vascular effect by targeting tumor neovasculature. This approach was considered by coupling a PS (chlorin) to the heptapeptide ATWLPPR, targeting neuropilin-1 (NRP-1), a $VEGF_{165}$ (Vascular Endothelial Growth Factor, isoform 165) co-receptor. We previously confirmed molecular and cellular affinity for the conjugated PS and its in vitro photocytotoxicity (Tirand et al., J. Control Release, 2006). In vivo, we demonstrated that only the conjugated PS allowed a selective accumulation in endothelial cells lining tumor vessels (Thomas et al., Photochem. Photobiol. Sci., 2008). Metabolic profile and optimization of treatment conditions were performed in nude mice xenografted ectopically with U87 human malignant glioma cells (Tirand et al., Drug Metab. Dispos., 2007). The aim of this study was to validate and