with significant increases in tumor cell and endothelial cell apoptosis ($P \leqslant 0.05$).

Table 1. Correlation of change in p-PDGFR-β activity with clinical benefit.

Clinical outcome	No. of patients	Change in p-PDGFR-β activity
CB	8	18.2% decrease (P = 0.006) (42% decrease [P = 0.008])*
PR	2	26.1% decrease (P = 0.001)
SD	6	13.9% decrease (P = 0.04)
PD (SD < 6 months)	12	9.9% increase (P = 0.06) (23% increase [P = 0.443])*

*Change in p-PDGFR-β activity in tumor-associated endothelial cells.

Conclusions: PDGFR-β phosphorylation was significantly lower in tumor biopsies from patients with GIST who experienced CB but not in biopsies from patients with PD. Sunitinib appears to exert its antiangiogenic effects by inhibiting PDGFR-β activity in tumor-associated endothelial cells, in addition to inhibiting VEGFR-2 activity. Endothelial cell PDGFR-β phosphorylation may be a sensitive marker of sunitinib biological activity.

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Antiangiogenic and anti-invasive activities of the kinase inhibitor sunitinib malate on experimental human glioblastoma in vitro and in vivo

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Background: Angiogenesis inhibitors appear to be promising therapies for highly vascularized tumors such as glioblastoma multiform (GBM). Sunitinib (SUTENT®, SU11248) is an oral multitargeted tyrosine kinase inhibitor with both antiangiogenic and antitumor activities due to selective inhibition of various receptor tyrosine kinases, including those important for angiogenesis (VEGFRs and PDGFRs).
Material and Methods: Here we evaluated the antitumor activities of sunitinib on orthotopic models of GBM *in vitro* and *in vivo*.
Results: Sunitinib potently inhibited angiogenesis which was stimulated by implantation of U87-MG and GL15 cells into organotypic brain slices at concentrations as low as 10 nM. At high dose (10 μM), sunitinib induced direct antiproliferative and proapoptotic effects on GL15 cells and decreased invasion of these cells implanted into brain slices by 49% ($P < 0.001$). Treatment was also associated with decreases in src (60%) and FAK (73%) phosphorylation. However, anti-invasive activity was not observed *in vivo* at the highest dose level utilized (80 mg/kg/day). Survival experiments involving athymic mice bearing intracerebral U87-MG GBM demonstrated that oral administration of 80 mg/kg sunitinib (5 days on, 2 days off) improved median survival by 36% ($P < 0.0001$). Sunitinib treatment caused a 74% reduction in microvessel density ($P < 0.05$), an increase in tumor necrosis, and a decrease in number of MIB-1-positive GBM cells.
Conclusions: The main finding of the present study is that sunitinib exhibited potent antiangiogenic activity which was associated with a meaningful prolongation of survival of mice bearing intracerebral GBM. This data supports the potential utility of sunitinib in the treatment of GBM.

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Adiponectin as a novel therapy for the suppression of liver cancer growth and metastasis

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Background: Recently, adipocyte-derived factor – adiponectin has been demonstrated to be able to suppress angiogenesis in addition to its anti-inflammatory function. It will have great clinical impact to explore the possibility of the application of adiponectin in liver cancer therapy, together with the underlying liver diseases, such as liver cirrhosis and NASH. In the present study, we aim to investigate the effect of adiponectin in the suppression of liver cancer growth and metastasis.
Material and Methods: The orthotopic liver tumor nude mice models with different metastatic potential were applied. 5×10^6 MHCC97H or MHCC97L cells were injected subcutaneously into the right flank of the mice. Once the subcutaneous tumor reached 1 cm in diameter, it was removed and cut into about 1–2 mm cubes which were implanted into the left liver lobe of another group of nude mice. Ad-adiponectin (1×10^8) (treatment group) or Ad-luciferase (control group) was injected via portal vein after tumor implantation. The animals were sacrificed at day 30, 40 and 50 after tumor implantation. The tumor growth and proliferation (Ki67) and local/distant metastases were compared among the groups. Hepatic stellate cell activation in the tumor tissue was detected by α-SMA staining. Cell signaling related to invasion, migration (ROCK-Rho, CAK and FAK) and angiogenesis (VEGF) were compared. The effect of adiponectin on hepatic stellate cell was also investigated by in vitro functional study.
Results: The tumor growth was significantly inhibited by adiponectin treatment at different time points accompanied with the lower incidence of lung metastasis compared to the control groups at different time points. The hepatic stellate cell activation by α-SMA staining in the liver tumors was suppressed by adiponectin treatment. The treatment group got lower incidence of Ki67 positive tumor cells. Protein expression of CAK and FAK was down-regulated in the adponectin treatment groups by immunostaining. Gene and Protein expression of Rho, ROCK and VEGF in the liver tumors was also suppressed.
Conclusion: Adiponectin treatment significantly inhibited liver tumor growth and metastasis by suppression of hepatic stellate cell activation in tumor and down-regulation of cell invasion and angiogenesis pathways.

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Targeting the chemokine receptor CXCR4 and ligand SDF-1/CXCL12 in tumor vasculature and stroma

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Background: The chemokine receptor CXCR4 is expressed at high levels in human cancers. The CXCR4 ligand, SDF-1 (stromal-derived factor 1) is a pro-angiogenic factor secreted by stromal cells. Tumor stroma consists of a variety of cell types including endothelial cells (EC), fibroblasts, progenitor cells, and pericytes. Tumor vasculature may be targeted by therapeutics directed against this pathway.
Materials and Methods: Gene expression analysis was performed on cell lines and databases generated from normal and tumor tissues. Flow cytometry assessed CXCR4 expression; ELISA quantified SDF-1 secretion. Inhibitors were tested in tube formation assays. Progenitor cells were co-injected with colon carcinoma cells to further explore the CXCR4-SDF-1 axis in vivo.
Results: RT-PCR analysis of a 61-cell line panel revealed CXCR4 mRNA expression in EC were comparable to human carcinoma cells. Gene expression analysis confirmed CXCR4 expression in healthy artery tissue, bulk bone marrow, white blood cells, and EC that were isolated from normal lung, brain, breast, and colon samples; CXCR4 was overexpressed 2-fold in EC derived from tumors of the same patients. Comparison of CXCR4 expression in tumor tissue vs. normal counterpart revealed a 2–9-fold increase in CXCR4 mRNA expression in many tumor types. Secreted SDF-1 levels in cultured media were measured by ELISA. MSC and HDF secreted the highest levels of SDF-1 compared to pericytes and EC. Flow cytometry indicated that HUVEC, HMVEC, pericytes, and fibroblasts in vitro express CXCR4 while mesenchymal stem cells (MSC) do not.
HUVEC tube formation on Matrigel was inhibited by antibodies against SDF-1 or CXCR4. Pericyte tube formation was also affected by an antibody against CXCR4 and AMD3100. Immunohistochemistry performed on tumors arising from the co-injection of MSC that secrete SDF-1 and CXCR4-positive colon cancer cells indicate that MSC contribute not only to the stroma, but associate with EC, suggesting that SDF-1 production by MSC can influence both cancer cells and developing blood vessels.
Conclusions: While CXCR4 overexpression in malignant cells is becoming more widely recognized, the tumor vasculature offers additional therapeutic targets. Secretion of SDF-1 by fibroblasts or MSC enhances angiogenesis through the recruitment of CXCR4+ EC, progenitors, and pericytes. A dual approach with antagonists against CXCR4 and/or its ligand SDF-1 against cancer cells and stroma may provide clinical benefit.

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Participation of paxillin in the inhibition by 4-hydroxycoumarin of experimental melanoma metastases

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During the metastasic process, cancerous cells change their adhesiveness and increase their motility. Paxillin is a multidomain adapter protein that interacts with integrins as well as with cytoskeletal proteins, having a crucial participation in the reorganization of the cytoskeleton needed for adhesion and migration. Therefore, changes in paxillin expression or activation correlate with the metastatic potential of cancerous cells. Previously, we