other hand, rapamycin shows its anti-angiogenesis function in several cancer therapy. In order to explore the possibility of combination therapy by rapamycin and FTY720, we designed an orthotopic liver tumor nude mice model to investigate the effect of this combination therapy by comparison of the tumor growth and metastases, as well as the related signaling pathways.
Materials 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. In the single treatment group, rapamycin was given by ip injection at 0.8 mg/kg/3 days. In the combination treatment group, rapamycin (0.5 mg/kg) and FTY720 (2 mg/kg) was administrated by ip injection every 3 days. The treatments were started at 7 days after tumor implantation. The mice were sacrificed at day20, 30 and 40 after treatment, respectively. The tumor growth, proliferation (Ki67), apoptosis (TUNEL) 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) and angiogenesis (VEGF) were compared. The effect of FTY720 and rapamycin on MHCC97H and MHCC97L was also studied in vitro functional tests.
Results
The tumor growth was significantly suppressed by both single and combination treatments by comparison of liver tumor volume. The incidence of lung metastasis was significantly lower in the treatment group at day 40 in a higher metastatic potential model (MHCC97H: 2/8 vs 7/8, $p = 0.041$; MHCC97L: 0/8 vs 4/8, $p = 0.077$). Suppression of hepatic stellate cell activation was mainly found in the combination treatment groups. The tumor invasiveness including venous invasion/tumor thrombus was mainly presented in the control groups. The tumor proliferation was significantly suppressed by both single and combination treatments. The RNA and protein expression of Rho, ROCK and VEGF was down regulated in the combination treatment group.
Conclusion
Low dose rapamycin and FTY720 combination therapy significantly inhibited liver tumor growth and lung metastases.

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Targeting bladder tumor cells in vivo and in the urine by a peptide identified using phage display

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Bladder cancer is one of the most common tumors of genitourinary tract. Selective delivery of drugs to tumor tissues is important for effective tumor therapy. Here we identified a peptide targeting bladder tumor cells using phage display. A phage library containing CX7C random peptides was screened for selective binding to cells from human bladder tumor xenograft. Selected phage clones were individually evaluated for binding to cultured bladder tumor cells and for binding to cells from fresh human tumor tissues over cells from normal bladder tissues. The displayed peptide of the most promising clone was synthesized and named as Bld-1. Fluorescein-conjugated Bld-1 peptide showed selective binding to frozen sections of human bladder tumor tissues, while little binding to normal bladder tissues was observed. In vivo tumor targeting was examined in a carcinogen-induced rat tumor model. When the fluorescent peptide was instillated into the bladder lumen, it selectively bound to tumor epithelium, while little binding was observed in normal bladder epithelium. Moreover, when the fluorescent peptide were intravenously injected into the tail vein, it homed to the bladder tumor, while not detected in normal bladder and control organs such as lung. The peptide distributed in the tumor tissues in colocalization with cykokeratin, an epithelial cell marker. Next, we examined whether the peptide can detect tumor cells in the urine. The fluorescent Bld-1 peptide bound to urinary cells collected from tumor patients, while little binding was observed in urinary cells from healthy individuals. These results indicate that the Bld-1 peptide could be useful for targeting bladder tumor cells in vivo and in the urine.

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Limited penetration of paclitaxel and doxorubicin in multicellular layers of human cancer cells

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The multicellular layer (MCL) is a 3D in vitro model which resembles solid tumor conditions in vivo. Many studies reported different pharmacodynamics of chemotherapeutic agents between monolayers and 3D culture models. Paclitaxel (PTX) and doxorubicin (DOX) are widely used in the treatment of human solid tumors. The cells grown as MCL showed decreased sensitivity to both PTX and DOX compared with monolayers indicating the relative resistance of the cells in 3D condition, which can be attributed to the limited drug penetration. In the present work we used MCL of DLD-1 human colorectal adeno-carcinoma cells (Pgp + cells) to study the penetration of PTX and DOX into multilayers of human cancer cells. PTX (50 μM) showed slow penetration over 72 hr. Lower concentration of PTX (1 μM) failed to obtain a significant accumulation up to 48 hr exposure. Under drug exposure of 24 μM·hr, the penetration/accumulation of PTX was greater with higher drug conc rather than longer exposure. Dox showed a relatively faster penetration compared to PTX, i.e. 100 μM DOX showed a full penetration within 1 hr. However, lower concentration of DOX (10 μM) also failed to show a significant accumulation after 4 hr exposure. Another P-gp substrate, Calcein-AM, showed moderate penetration through the MCL after 2 hr of exposure, and inhibition of P-gp activity (incubation at 4ºC) induced only partial enhancement of penetration. These data indicate that P-gp substrates, PTX, DOX, and calcein-AM, are not similar in their penetration through the MCL of Pgp + cancer cell layers. Ethidium homodimer-1 (EthD-1), a highly hydrophilic agent also failed to penetrate through the MCL after 2 hr similar to PTX. Overall, our data suggest that not only physicochemical property(hydrophilicity) and P-gp affinity but also other factors such as tissue binding may be important factors in MCL penetration. Extensive tissue binding of PTX and DOX may hinder the penetration within avascular regions of human solid tumors in vivo, which warrants further investigation.

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In vitro and in vivo evaluation of pegaspargase for the treatment of solid tumors and lymphomas

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Background: Pegaspargase (Oncaspar®) is a PEGylated version of *E. coli* L-asparaginase (ASNase), and is approved for use in patients with acute lymphoblastic leukemia. Previous work suggests that ASNase may be efficacious in solid tumors and lymphomas and that its activity may correlate with the cellular levels of asparagine synthetase (ASNS). We evaluated the *in vitro* and *in vivo* efficacy of pegaspargase in pancreatic, ovarian and lymphoma cells with varying expression of ASNS.
Methods: The *in vitro* cytotoxicity of pegaspargase was determined using a MTS assay and levels of ASNS were measured by a quantitative RT-PCR. Pharmacokinetics (PK) and pharmacodynamics (PD) of pegaspargase were studied in rats and therapeutic efficacy was evaluated in xenograft models of pancreatic cancer.
Results: *In vitro*, pegaspargase had potent cytotoxicity against MiaPaCa-2, PANC-1 and Panc 10.05 pancreatic cells, and OV-90 and TOV-21G ovarian cells with IC_{50} values below 1.0 IU/mL. Similar cytotoxicity was observed against B- and T-lymphoma lines including Raji, Daudi, Molt-4 and Ramos. In a low ASNS-expressing model, MiaPaCa-2, treatment with a single dose of 12.5 IU/g pegaspargase resulted in 46% tumor growth inhibition (TGI). Further, although treatment with gemcitabine alone (80 mg/kg q3d × 4) or with low dose pegaspargase (0.8 IU/kg, single dose) alone was not significantly better than controls, treatment with the combination of the two resulted in improved efficacy compared to controls ($P < 0.05$) and a TGI of 48%. In contrast, in a high ASNS-expressing pancreatic model, ASPC-1, treatment with pegaspargase at various doses was ineffective. In PK/PD studies, the C_{max} and $AUC_{0-\infty}$ of pegaspargase increased and asparagine (ASN) levels decreased in a dose-proportional manner when pegaspargase was dosed via either intramuscular (IM) or intravenous (IV) routes. The elimination half-lives by IM or IV routes were comparable. ASN levels depleted rapidly following pegasparagase treatment and recovered with low dose but not with high dose treatment.
Conclusions: Pegaspargase had potent cytotoxicity against various pancreatic, ovarian and lymphoma cells and had significant antitumor efficacy in a xenograft of pancreatic cancer. The therapeutic efficacy of pegaspargase correlates with cellular ASNS which could serve as a biomarker in clinic. Pegaspargase either as a single agent or in combination with gemcitabine should be evaluated clinically for the treatment of solid tumors and lymphomas.