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Use of bioluminescence imaging and quantitative RT-PCR to monitor tumor progression and treatment response in orthotopic AML mouse models: Application to the targeted cytotoxic agent F14512

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After xenotransplantation of human cells into mice, it is necessary to detect those cells as early as possible and at a low level to monitor their engraftment, dissemination and growth in immunodeficient mice. To this purpose, we developed two methods based on bioluminescence imaging and quantitative RT-PCR. HL-60 and KG-1 AML cell lines were constitutively modified to express the firefly luciferase (HL60-Luc and KG1-Luc) allowing thus in vivo detection by non-invasive bioluminescence imaging. We also developed a PCR method for the specific detection of human and mouse glyceraldehyde-3-phosphate-desydrogenase (GAPDH) mRNA. HL60-Luc and KG1-Luc cells were implanted intravenously or in the tibia bone marrow of irradiated NOD-SCID mice. Leukemic cell proliferation, monitored by optical imaging, was correlated to the quantitation of human leukemic cells in mouse blood and bone marrow by quantitative RT-PCR and FACS. In an effort to mimic the human disease, we injected approximately 10^6 AML cells (from patient LAM09–012) in irradiated NOD-SCID mice and allowed them to establish as xenografts. Circulating leukemic cells were detected in peripheral blood of these living mice by quantitative RT-PCR after 8 weeks. This technology was also used to assess the antileukemic activity of F14512, a potent spermine–drug conjugate exploiting the polyamine transport system for tumor cell delivery, against these models of primary AML. Multiple i.v. administrations of F14512 at 0.32 mg/kg, induced an extensive reduction of the number of leukemic cells in mouse blood and bone marrow (97−99%), assessed by quantitative RT-PCR and confirmed by FACS analysis and histology. In order to determine if key properties of leukemic stem cells such as self-renewal are targeted by F14512, a secondary transplantation of LAM09–012 was performed following in vivo treatment and was monitored by quantitative RT-PCR. While the LAM09–012 AML cells harvested from the drug-vehicle treated mice have maintained homing and repopulation to the bone marrow of secondary recipient mice, the repopulation capacity of LAM09–012 AML cells harvested from F14512-treated mice was abolished. No re-growth was observed after 25 weeks post secondary transplantation. In conclusion, the quantitative RT-PCR and bioluminescence imaging methods presented here provide a sensitive and reliable detection and quantitation of low numbers of human cells in immunodeficient mice. In addition, these methods permit a non-invasive monitoring of drug effects in vivo, reducing thus the extensive use of mice for conventional pharmacological studies. Furthermore, these results also demonstrated that F14512 exhibits a marked preclinical antileukemic activity in patient-derived AML models and support its on going phase I clinical trials.

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Bioluminescence imaging for monitoring tumor growth of GI-tract tumors

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Introduction: Bioluminescence imaging (BLI) is an attractive non-invasive technique for monitoring and characterizing tumor growth. For this purpose different stable cell cancer cell lines (CRC, pancreatic, NET) expressing the luciferase gene (Renilla or firefly) were established and characterized *in vitro* and *in vivo*. By administrating the substrates luciferin or colelenterazine, growth of different tumor models (ectopic and orthotopic) were monitored.
Material and Methods: Several human gastrointestinal tumor cell lines (colorectal, pancreatic, neuroendocrine), stably expressing luciferase or renilla constructs were validated in vitro and in vivo. For the in vitro characterization proliferation assays and kinetic studies were performed. Furthermore different cell clones were tested for their in vivo growth characteristics. For this purpose the cells were inoculated either subcutaneously (5×10^6 cells) into the flanks of nude mice or orthotopically (1×10^6) into the head of the pancreas (PaCa) or into the wall of the colon ascendens (CRC model). Shortly before in vivo BLI, the substrates were either injected i.p. (Luciferin) or iv (Coelenterazine).
Results: Several gastrointestinal cell lines were successfully stably transfected with either luciferase (luc) or renilla (Rluc) constructs. In vitro analysis of the established cell lines showed high bioluminescence signals in comparison to purchased luc-expressing cell lines. In vitro, Rluc expressing cell lines showed a 10 fold higher bioluminescence signal (10.000–80.000 phtotons/sec) compared to luc expressing cells (1000–6000 photons/sec). In addition, kinetic studies in Rluc-cells showed a fast decrease of the signal within the first 10 min after adding the substrate, whereas Luc-cells showed a stable expression over 60 min. In vivo tumor growth of s.c. growing tumors was measured by BLI and calliper measurements which showed similar growth characteristics. Contrary to the in vitro results, in vivo, Luc-expressing cells showed higher BL signal compared to Rluc-cell lines. For the first time we could also measure the tumor growth of our orthotopically growing tumors by BLI which showed an increase of signal over time.
Conclusion: Our findings show hat BLI improves monitoring of tumor growth of subcutaneous growing xenografts and especially offer non invasive monitoring tumor growth of orthotopically growing tumors of the gastrointestinal tract by using fewer animals. BLI is a powerful tool for longitudinal monitoring of tumor load in orthotopic models with almost the same simplicity as ectopic tumors. The establishment of these gastrointestinal bioluminescent xenograft models are powerful tools for ongoing studies concerning the detection of metastases and for the application in pre-clinical therapy interventions.

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A collection of patient-derived pancreatic adenocarcinoma xenografts: pharmacological and molecular characterization

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There is a high medical need to identify new treatments for patients with pancreatic cancer, since in many cases only palliative treatment is possible. The standard 1st line chemotherapy in inoperable, locally advanced (stage II and III) and metastatic (stage IV) adenocarcinoma of the pancreas is Gemcitabine as a single agent with a median survival of about 6 months. In addition, Gemcitabine is indicated as adjuvant chemotherapy after surgery. Although the anti-metabolite 5-Flourouracil (5-FU) and the EGF-R inhibitor Tarceva (Erlotinib) have been approved for 2nd line treatment, new and more efficient drugs are urgently needed. In the present study, more than 60 samples of pancreatic carcinomas were transplanted subcutaneously (s.c.) into NMRI nude mice directly after tumor resection. In most cases, tumor material from chemonaive patients with defined histology and staging was used for implantation. Up to now, 20 tumor models were passaged in nude mice and characterized comprehensively. Except for less stroma, histology of the established xenograft was comparable to that of the primary tumor. Chemosensitivity *in vivo* was evaluated by treatment of tumor bearing nude mice with 5-FU (100 or 75 mg/kg, q7dx3, i.p.), Gemcitabine (240 mg/kg, q7dx3, i.v.) and Erlotinib (25 and 50 mg/kg, qdx21, p.o.). In general, tumor growth was not inhibited with best T/C values >50% highlighting the general chemoresistance of pancreatic cancer. Only in three models (PAXF 1872, PAXF 1998, PAXF 2011), a high sensitivity towards Gemcitabine was evident with best T/C values of 8%, 3.8% and 0%, respectively. Concerning Erlotinib, best T/C values ranged from 89.6% (PAXF 1876) to 38.5% (PAXF 1982) with no correlation to EGFR expression status. A more broad chemosensitivity profile was established with the *ex-vivo* clonogenic assay. Interestingly, several tumors responded strongly to treatment with mTOR Inhibitors ($IC_{50} \leqslant 10$ nM). Based on this data, in vivo treatment experiments with RAD001 (Everolimus) were performed with best T/C values around 50% for most tumors as tested and transient regressions in a few experiments. In summary, a unique collection of patient-derived pancreatic xenograft models of high clinical relevance has been established. These models are available for translational research studies including in-vivo efficacy testing of new investigational drugs. According to our data, mTOR Inhibitors might be useful in selected patients.

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Establishment and characterization of human luminal breast cancer xenografts

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Background: Luminal breast cancers (BC) are characterized by a persisting long term risk of relapse. Preclinical models of estrogen-dependent human BC are requested for a better understanding of estrogen