Optimized Mouse Model for the Imaging of Tumor Metastasis upon Experimental Therapy

Sergio Lavilla-Alonso¹,²,⁶, Usama Abo-Ramadan³, Juha Halavaara⁴,⁵, Sophie Escutenaire¹,², Turgut Tatlisumak³, Kalle Saksea⁶, Anna Kanerva¹,⁷, Akseli Hemminki¹,²,⁹, Sari Pesonen¹,²,⁹

¹Cancer Gene Therapy Group, Molecular Cancer Biology Program, Transplantation Laboratory, Haartman Institute and Finnish Institute of Molecular Medicine, University of Helsinki, Helsinki, Finland, ²HUSLAB, Helsinki University Central Hospital, Helsinki, Finland, ³Department of Neurology, Helsinki University Central Hospital, Helsinki, Finland, ⁴Department of Radiology, Helsinki University Central Hospital, Helsinki, Finland, ⁵Department of Radiology, Jorvi Hospital, Espoo, Finland, ⁶Department of Virology, Haartman Institute, University of Helsinki and Helsinki University Central Hospital, Helsinki, Finland, ⁷Department of Obstetrics and Gynecology, Helsinki University Central Hospital, Helsinki, Finland

Abstract

Development of new cancer treatments focuses increasingly on the relation of cancer tissue with its microenvironment. A major obstacle for the development of new anti-cancer therapies has been the lack of relevant animal models that would reproduce all the events involved in disease progression from the early-stage primary tumor until the development of mature metastatic tissue. To this end, we have developed a readily imageable mouse model of colorectal cancer featuring highly reproducible formation of spontaneous liver metastases derived from intrasplenic primary tumors. We optimized several experimental variables, and found that the correct choice of cell line and the genetic background, as well as the age of the recipient mice, were critical for establishing a useful model system. Among a panel of colorectal cancer cell lines tested, the epithelial carcinoma HT29 line was found to be the most suitable in terms of producing homogeneous tumor growth and metastases. In our hands, SCID mice at the age of 125 days or older were the most suitable in supporting consistent HT29 tumor growth after splenic implantation followed by reproducible metastasis to the liver. A magnetic resonance imaging (MRI) protocol was optimized for use with this mouse model, and demonstrated to be a powerful method for analyzing the antitumor effects of an experimental therapy. Specifically, we used this system to with success to verify by MRI monitoring the efficacy of an intrasplenic administered oncolytic adenovirus therapy in reducing visceral tumor load and development of liver metastases. In summary, we have developed a highly optimized mouse model for liver metastasis of colorectal cancer, which allows detection of the tumor load at the whole body level and enables an accurate timing of therapeutic interventions to target different stages of cancer progression and metastatic development.

Citation: Lavilla-Alonso S, Abo-Ramadan U, Halavaara J, Escutenaire S, Tatlisumak T, et al. (2011) Optimized Mouse Model for the Imaging of Tumor Metastasis upon Experimental Therapy. PLoS ONE 6(11): e26810. doi:10.1371/journal.pone.0026810

Editor: Matthew Bogyo, Stanford University, United States of America

Received May 24, 2011; Accepted October 4, 2011; Published November 3, 2011

Copyright: © 2011 Lavilla-Alonso et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Funding: Funding was provided by European Research Council, European Union grants THERADPOX and APOTHERAPY, Helsinki University Central Hospital, Sigrid Juselius Foundation, Academy of Finland, Emil Aaltonen Foundation, Finnish Cancer Society, Biocentrum Helsinki, Research Funds of the University of Helsinki, and Helsinki Biomedical Graduate School. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: sari.pesonen@helsinki.fi (SP); akseli.hemminki@helsinki.fi (AH)

¹ These authors contributed equally to this work.

Introduction

Uncontrolled progression of primary tumors is a usual cause of cancer-related deaths, especially in colorectal cancer. For this reason, in the last decades cancer research focuses, not only on the genetic causes of cancer, but on epigenetic variables and influence of tumor microenvironment. Phenomena like tumor invasiveness, progression and metastases need to be understood in order to find more efficient treatment modalities. However, a big demand exists for more realistic and reliable metastatic models that feature all phases of the metastatic process.

For basic research on metastasis and development of new anticancer agents, the selection of the optimal animal model is of great importance and is dependent on which biological questions are addressed. Subcutaneous xenograft murine models are often used since such tumors are easy to grow, and the tumor size and treatment responses can be followed non invasively. However, such models are not optimal for all purposes, as the subcutaneous tumor environment may not fully resemble relevant clinical situation such as liver metastases in the context of advanced colorectal cancer [1]. It is increasingly recognized that the tumor environment plays a key role in the behavior of the tumor and the response to therapeutics [2]. In this regard, subcutaneous tumors are problematic, as the growth environment is different from naturally occurring tumors, making their morphology and especially stromal elements markedly different [3]. Most importantly, subcutaneous tumors are poorly invasive rarely lead to metastases. This is a major disadvantage since majority of overall cancer related deaths are due to metastases. Therefore, detection, prevention, and treatment of distant metastases have become increasingly important objectives in cancer research.

Fortunately, metastasis is a rare event. Specific conditions are needed for disseminating cells to develop metastasis, and only selected cells detached from their primary site are capable of...
establishing a mature tumor in a distant ectopic location, given that the appropriate conditions are available [4]. Even when metastatic cells have reached a recipient organ, they may remain as single cells or as micrometastases until certain factors permit their development to metastases [5]. Metastasis is a multistage process including cell migration, entry into vasculature, survival in the circulation/lymphatic vessels, invasion of vessel wall, migration to target organ, implantation, and tumor formation at the metastatic site [6]. The whole process may take several years or even decades [6]. Such complexity makes it difficult to study metastatic cancers, and most in vivo models only reproduce some of the aforementioned steps.

For the purpose of research and therapeutic development, some metastatic animal models are available [7]. In many of them, cells are injected directly to the systemic circulation and the site of metastasis is mostly defined by the first capillary bed the tumor cells meet after injection and, in a lesser extent, influenced by cancer cell type [7]. For example, after tail vein injection tumors are found in the lungs, while after intracardiac injection, tumors can be found at several sites [7]. In this type of experimental models, early steps of the metastatic process are not recapitulated, which may lead to differences in tumor behavior when compared to metastases deriving from a primary tumor [8].

Several genetically engineered mouse strains producing metastatic disease bona fide are available [9]. The tumors arising in these models are, however, fully of murine origin, and may not accurately represent the properties of human tumors, nor do they allow to study certain human specific therapeutic agents, such as oncolytic adenoviruses.

Magnetic Resonance Imaging (MRI) is a powerful imaging modality for characterizing animal models of disease. Contrast-enhanced MRI has significantly improved the accuracy of the detection of several tumors, such as brain and liver tumors. Tissue-specific contrast agents such as superparamagnetic iron oxide (SPIO) have been reported to enable highly sensitive to the detection of liver tumors [10].

Based on systematic testing of different colorectal cancer cell lines and mouse backgrounds, we describe here a new colorectal cancer mouse model featuring spontaneous liver metastases originating from an intrasplenic primary tumor. In this mouse model, MRI allowed effective in vivo monitoring of tumor growth and treatment response which permitted a variety of longitudinal studies not possible with invasive methods. For example, repeated measurements of the same animal were possible thus reducing the total number of animals needed in the study. The sensitivity of the model for detecting the effects of antitumor drugs was demonstrated by using oncolytic adenovirus as an example of a novel therapeutic agent.

Results

Selection of mice strain and provider

In order to identify an optimal mouse strain to support our model, athymic NMRI nude and SCID mice were tested. For successful implantation and proper growth of intrasplenic tumors, the consistency, size, and structure of the spleen were found to be of critical importance. In general, mutations used to generate deficiencies of the immune system, such as found in nude and SCID mice, truncate normal spleen development [11] and lead to morphologically immature spleens in adult mice (Figure 1). Interestingly, important differences in spleen morphology were seen in MRI analysis between mice strains. As a curiosity, variation in the splenic structure was also seen among mice with the same genetic defect (SCID) but from a different provider.

As confirmed by morphological observation after laparotomy, NMRI nude mice had a solid, compact and relatively large spleen. In contrast, SCID mice of the same age often presented an immature spleen: the organ was fragile, small, soft, and pale.

![Figure 1. Developmental stage of the spleens of adult SCID and NMRI nude mice.](https://example.com/figure1.png)

125 to 135 days old SCID and NMRI nude mice from different providers were imaged with MRI to evaluate the developmental stage of the spleens (arrows). For SCID mice, animals purchased from Charles River (A) and Harlan (B) showed poorly developed, diffuse, and small spleens while animals from Taconic (C) had solid, compact, and relatively large spleens. NMRI nude mice from Charles River (D) and Scanbur (E) showed fully developed spleens with a similar appearance.

doi:10.1371/journal.pone.0026810.g001
comparison to wild type (NMRI) mice. This was also observed with MRI (Figure 1) where the spleens of SCID mice not only appear smaller than seen in wild type (NMRI) mice, but are squeezed between neighboring organs due to their diffuse/malleable structure. In comparison to SCID mice, nude mice under anesthesia were more susceptible to hypothermia during the imaging session and were therefore used for optimization of the MRI method. To further evaluate the spleen development of SCID mice, animals were imaged at the age of 125 days or older. All animals obtained from Taconic had mature splenic tissue at the time of imaging (Figure 1) and were therefore selected for further experiments.

After cancer cell injection (HT29 cells), only mice with mature splenic tissue showed proper tumor engraftment (Table 1). Animals with immature spleens showed no intrasplenic tumor development, and, when present, tumors were most often localized in the peritoneal cavity or in tissues adjacent to the spleen, suggesting spillage of injected. In contrast, 20 out of 20 SCID mice from Taconic presented detectable tumors 21 days after cancer cell implantation, indicating spontaneous origin from the primary splenic tumor. For NMRI nude mice, 3 out of 7 (Charles River) and 2 out of 4 (Scanbur) animals showed intrasplenic tumor development.

With regard to liver metastases originating from the primary spleen tumor, SCID mice presented hepatic tumors in 100% of the mice used. These tumors were first seen with MRI one week after injection while tumors in the liver were first observed 1 to 4 weeks after intrasplenic implantation of the cells in hepatic tissue after intrasplenic injection. Therefore, it is crucial to distinguish between these events. The development of hepatic tumors in the absence of tumors in the spleen would be assumed to be a consequence of a direct implantation of the injected cells in the liver. This was seen in mice injected intrasplenically with the cell line HT2116. On day 21 after intrasplenic cancer cell injection, 2 out of 7 mice presented hepatic tumors but no intrasplenic tumors and 1 mouse showed detectable intrasplenic tumor several days after appearance of the intrahepatic tumor. On the contrary, for all mice injected with HT29 cells, the intrahepatic liver tumors developed 7 to 21 days after the appearance of primary intrasplenic tumors, strongly supporting the metastatic origin of these tumors.

Metastatic origin of the hepatic tumors

The portal system allows easy spread of metastatic cells from splenic tumors to the liver but may also mediate direct implantation of the cells in hepatic tissue after intrasplenic injection. Therefore, it is crucial to distinguish between these events. The development of hepatic tumors in the absence of tumors in the spleen would be assumed to be a consequence of a direct implantation of the injected cells in the liver. This was seen in mice injected intrasplenically with the cell line HT2116. On day 21 after intrasplenic cancer cell injection, 2 out of 7 mice presented hepatic tumors but no intrasplenic tumors and 1 mouse showed detectable intrasplenic tumor several days after appearance of the intrahepatic tumor. On the contrary, for all mice injected with HT29 cells, the intrahepatic liver tumors developed 7 to 21 days after the appearance of primary intrasplenic tumors, strongly supporting the metastatic origin of these tumors.

MRI method optimization

Current MRI methodology in small animals is not readily applicable for testing large number of subjects in a short time, because it would increase costs and would require to keep the animals under anesthesia for too long. Therefore, our MRI method was optimized in order to achieve the following goals: 1) Fast and accurate detection of liver tumors 2) minimized imaging time in order to be able to image a large amount of animals within a reasonable time; and 3) minimal time under anesthesia to avoid associated health risks. In order to achieve two latter goals, we designed an MRI protocol with an imaging time of around 10 minutes with a requirement for the mice to be under isoflurane anesthesia for less than 30 minutes. This schedule was well tolerated and no mortality was seen even upon repeated imaging. For optimal visualization of liver tumors, we chose to assist imaging with systemic administration of Endorem (Roisys, Cedex, France), an SPIO contrast agent that accumulates in the Kupffer cells of the liver. Its predominant action is to shorten the T2 or T2* relaxation time by disturbing the local magnetic field in the liver parenchyma. The signal intensity from normal liver dramatically decreases after SPIO administration. While malignant liver foci do not contain Kupffer cells, their signal remains unaltered, and the signal difference between liver and tumor lesions increases.

In our work, liver signal was reduced to 32% and 25% after 15 and 45 minutes from contrast agent administration, respectively. At later time points, signal remained at a constant 19% compared to non-treated livers. To maximize the lesion–liver contrast, all tests were performed at least 30 minutes after contrast agent administration.

---

**Table 1.** Morphology of the spleens of 30- and 125-day-old SCID and NMRI nude mice.

| Mouse strain | Provider | Suitable for IS injection at age of 30 days | Suitable for IS injection at age of 125 days | Mice developing intrasplenic tumor (ratio) | Mice developing hepatic metastases (ratio) |
|--------------|----------|-------------------------------------------|-------------------------------------------|------------------------------------------|------------------------------------------|
| SCID         | Charles River (Sulzfeld, Germany) | No | No | NA | NA |
| SCID         | Harlan (Horst, Holland) | No | No | NA | NA |
| SCID         | Taconic (Lille Skensved, Denmark) | No | Yes | 20/20 (100%) | 20/20 (100%) |
| NMRI-nu/nu   | Charles River (Sulzfeld, Germany) | Yes | Yes | 3/7 (43%) | 3/7 (43%) |
| NMRI-nu/nu   | Scanbur (Sollentuna, Sweden) | Yes | Yes | 2/4 (50%) | 2/4 (50%) |

SCID, severe combined immunodeficiency; NA, not applicable.

doi:10.1371/journal.pone.0026810.t001
The stable decrease of liver signal from 15 minutes until late timepoints by Endorem administration optimized the quality of the images in a minimal analysis time. In addition, an imaging time of only 10 minutes reduced dramatically the possibility of animals dying of hypothermia in the MRI device. With this improved MRI method, each animal could be fully imaged only in 30 minutes. This increases the performance of the method, reduces number of mice needed and permits screening of large number of animals in short periods of time, decreasing costs.

Use of the optimized mouse model to study the effects of an experimental treatment

Animals were implanted with HT29 cells and imaged with MRI weekly for 6 weeks. As expected, mice developed both intrasplenic and liver metastases (Figure 3). As also verified before, liver metastases were detected in each case 1 to 3 weeks after intrasplenic (primary) tumor formation. At least one week before metastases were detected, primary tumors were considered suitable for an 8 μl microinjection of the therapeutic agent. Of note, this time window of at least one week makes the model useful for the evaluation of possible pro- and anti-metastatic properties as well as the intervention, in this case the viral injection.

Adequate sensitivity to detect changes in the visceral tumor load is a critical requirement for a model to be useful in the evaluation of the potency of therapeutic agents for orthotopic colorectal cancer. Oncolytic adenoviruses have been widely tested in mouse xenograft models of human cancers and shown to effectively reduce tumor masses [12]. Here, we used an oncolytic adenovirus Ad5-D24-RGD as an example of a developmental anti-cancer drug to assess model utility for quantifying treatment responses. Significant intrasplenic tumor growth reduction, detected after a latency period of two weeks (i.e. days 21 to 35 after tumor implantation), was seen following a single intratumoral administration of a moderate dose of oncolytic adenovirus (Figure 4A). In contrast to untreated mice, whose tumors grew steadily until the end of the experiment, tumors of treated animals stopped growing. Intratumoral treatment of primary spleen tumors with oncolytic adenovirus did not have a pro-metastatic effect. This was seen both as a reduced number of metastatic lesions (Figure 4B) as well as significantly smaller amount of total tumor mass in the liver (Figure 4C).

Comparison of tumors in laparotomy and MRI

For its optimal utility, the imaging method should allow fast and progressive identification of tumor development in the spleen and the liver. A general difficulty with MRI is to differ between tumors and stromal elements such as vessels, the gall bladder, hepatic lymph nodes or ligaments. Therefore it was important to compare our MRI results with laparotomy findings. As shown in figure 5, laparotomy observations of the entire organ (5A) and of a section of the organ where the tumor is engrafted (5B) give an accurate idea of the location and shape of the tumor within the organ, which corresponds to its predicted location and shape by MRI (5C). This demonstrates that MRI is a reliable method for splenic and liver tumors diagnosis in mice.

Discussion

Here we describe the development and use of an extensively optimized liver metastatic colorectal cancer mouse model, which provides several important advantages compared to other metastatic mouse models described before. Moreover, the utility of MRI for monitoring changes in tumor growth and rate of

Figure 2. Development of intrasplenic and intrahepatic tumors in SCID mice. Four human colorectal cancer cell lines (Co115, HCT116, SW620, HT29) were tested for their ability to induce growth of intrasplenic and intrahepatic tumors in SCID mice. 1x10⁶ cells were injected into the spleen and primary tumor growth (A), number of metastasis in the liver (B), and total volume of the liver tumors (C) were analyzed weekly by MRI. Each line represents an individual animal. Number of animals used for testing each cell line is presented in parentheses.

doi:10.1371/journal.pone.0026810.g002
metastasis in that model was optimized and demonstrated by using oncolytic adenovirus Ad5-D24-RGD as a model treatment agent.

Four colorectal cancer cell lines were tested and remarkable differences in primary tumor growth rates and metastasis formation were observed. Epithelial HT29 cell line showed the best reproducibility both in primary tumor and metastasis development. 100% (20/20) of animals showed growth of intrasplenic tumors within 2 weeks from cell transplantation, and liver metastases were detected in all animals 1 to 3 weeks
afterwards. This suggests a metastatic nature for the HT29 liver tumors rather than being a consequence of direct immediate dissemination from the spleen through the portal vein, as has been reported for other colorectal cancer cell lines [13]. If the liver tumors would have been a consequence of accidental direct injection into the portal vein, they would have been expected to appear concurrently with spleen tumors. In comparison to other colorectal cancer cell lines, the growth rate of HT29 tumors was relatively slow and uniform, which might be an advantage with regard to flexibility of experimental design. Also, slower growth would correspond better with the typical behavior of human tumors. All animals were killed due to progressing metastatic disease 6 to 7 weeks from the beginning of the experiment.

Spleen was used as a primary target organ for tumor cell implantation in our model. It is known that the mutations that render laboratory mice immune deficient, can result also in developmental defects in their spleens [11], and the developmental stage of the spleen at the time of tumor cell injection had a significant effect on the outcome of cell implantation. Immature spleens did not allow tumor engraftment at the injection site and consequently tumors were often found in the peritoneal cavity or in tissues adjacent to the spleen, suggesting direct extravasation from the injection site. Based on our observations, differences in the developmental stage of spleens may significantly alter the outcome of tumor cell injection, which is of critical importance for reduction of the biological variation in tumor and metastasis development. Therefore, anatomical confirmation of the proper structure of the spleens prior to cancer cell implantation (with e.g. MRI) is crucial in order to ensure uniform development of liver metastases.

Metastatic progression is one of the most severe aspects in cancer. The metastatic process not only depends on the characteristics of the primary tumor cells, but also on the tumor microenvironment, where extreme conditions such as poor oxygenation and nutrition, high interstitial pressure, presence of immune cells, extracellular matrix and isolation from the host organ by stromal barriers are often present [14–16]. Metastatic development also depends on the target tissue and the capability of its stromal cells to give rise to a metastatic tumor [15,17]. Liver is the primary target organ for metastases in colorectal cancers [6,18]. To develop clinically relevant models, different approaches have been utilized for human cancer cell engraftment in the murine liver. Direct injection of cancer cells either in the liver parenchyma [19] or in the portal vein [20] has been used to induce tumors in the liver. Neither approach allows for selection of metastatic cells from injected cells. This may partially explain the variability often seen in the number of mice developing intrahepatic tumors in this type of experimental set-up [13,19,20]. Also, implantation of a primary tumor (individual cells or solid tumors) into spleen [13] or cecum [21] for later release of disseminated cells to the portal system has been described earlier. Main disadvantage of these models is that only a portion of animals develop disseminated tumors and/or the location of primary or metastatic tumors vary increasing the number of animals needed in each experiment.

It has been shown that metastatic cells detaching from the spleen are assisted by splenocytes on their way to the liver, demonstrating the involvement of microenvironmental factors in the metastatic process also in mice [13]. As supportive evidence for the importance of metastatic selection of the cells, more uniform tumor development was achieved if the cells were implanted in the spleen (vascular access to the liver through the portal system) in comparison to direct injection of the same cell line into the portal

---

**Figure 5. Visualization of primary tumor in the spleen and liver metastasis.** Laparotomy observations of the entire organs and sections of the organs where the tumors are engrafted give an accurate idea of the location and shape of the spleen and liver tumors within the organ, which corresponds to its predicted location and shape by MRI. doi:10.1371/journal.pone.0026810.g005
Materials and Methods

Ethics Statement

All animal experiments were conducted according to the rules set by the Provincial Government of Southern Finland.

Cell lines

Human colorectal cancer cell lines Co115, HCT116, SW620, and HT29 were acquired from ATCC (American Type Culture Collection), cultured in the recommended growth media with 10% fetal bovine serum (FBS) and maintained in a humidified atmosphere at 37°C and 5% CO2.

Oncolytic adenovirus

Ad5-D24-RGD is an oncolytic adenovirus previously described [22]. Briefly, 24hp deletion (“D24”) in the constant region 2 of adenovirus E1A, renders the virus unable to bind retinoblastoma (Rb) and therefore allows virus replication only in Rb/p16 mutant tumor cells. Also, a Arg-Gly-Asp (RGD) aminoacid sequence is inserted in the HI loop of the fiber knob to enhance integrin-dependent entry into cells. Virus was propagated on A549 cells and purified on cesium chloride gradients. The particle concentration was measured at 260 nanometers (nm) and a standard TCID50 test on 293 cells was performed to determine functional units and were assessed to be 1.1x106CFU/ml and 2.8x106CFU/ml, respectively.

Mice

Pathogen-free, 10–11-week-old female SCID (severe combined immunodeficiency) mice were purchased from Taconic (Elby, Denmark), Charles River (Sulzfeld, Germany), and Harlan (Horst, Holland) and NMRI nude mice from Charles River and Scanbur (Sollentuna, Sweden). Animals were quarantined for 2 weeks.

MRI

MRI studies were performed with a 4.7 T scanner (PharmaScan, Bruker BioSpin, Ettlingen, Germany) using a 90-mm shielded gradient capable of producing a maximum gradient amplitude of 300 mT/m with an 80-μs rise time. A linear birdcage RF coil with an inner diameter of 30 mm was used. T2-weighted images were acquired using rapid acquisition with relaxation enhancement (RARE) sequence (TR / TEEff = 3767/63 ms, matrix size = 256×256, Rare Factor = 8, field-of-view = 33×33 mm2, 32 slices, slice thickness = 0.7 mm, number of averages = 8).

Spleen-to-liver murine model of metastasized colorectal cancer

The surgical procedure was similar to that previously described [23]. Briefly, 125-day-old SCID mice were anesthetized with ketamine (Ketaminol® 75 mg/kg; Intervet, Boxmeer, Netherlands) / dexamethasone (Dexdormitor® 1 mg/kg; Orion Pharm., Espoo, Finland) and the spleen was exteriorized through a left lateral flank incision. Tumors were established by intrasplenic injection of 1x106 HT29 cells suspended in 50 μl of serum-free growth media using a 27-gauge needle. The injection site on the spleen was pressed with a cotton stick wet in iodine-polividone solution (Betaine®, Leiras, Helsinki, Finland) in order to destroy extravasated cells and ensure hemostasis. The peritoneum and skin were closed in a single layer with surgical materials.

Affect their development.

for the study of metastasis and how experimental treatments can limit progression.

metastatic process. All in all, our method is a promising tool for the study of metastases in mouse as experimental model.
thread. Finally, atipamezole (Antisedan® 1 mg/kg; Orion Pharm., Espoo, Finland) was injected subcutaneously to reverse anesthesia.

For the treatment study, single injection of Ad5-D24-RGD at a dose of $2.5 \times 10^7$ VP in 10 ul volume was given into intrasplenic tumors 21 days after cancer cell implantation. Tumor growth (both in the spleen and liver) was followed weekly with MRI for 6 weeks.

Statistical analyses
Analysis was done with SPSS 15.0 software for Windows. Comparison between mock treated and oncolytic virus treated animals was carried out using t-test for independent samples. A p value less than 0.05 was considered significant.

Acknowledgments
We thank Paivi Hannukela, Sirkka-Liisa Holm, Eerika Karli and Aila Karioja-Kallio for technical assistance.

Author Contributions
Conceived and designed the experiments: SLA AH SP. Performed the experiments: SLA SE. Analyzed the data: SLA UAR JH AH SP. Contributed reagents/materials/analysis tools: TT UAR JH AK AH SP. Wrote the paper: SLA KS AH SP.

References
1. Ito K, Govindarajan A, Ito H, Feng Y (2010) Surgical treatment of hepatic colorectal metastasis: Evolving role in the setting of improving systemic therapies and ablative treatments in the 21st century. Cancer J 16: 103–110.
2. Meads MB, Gatenby RA, Dalton WS (2009) Environment-mediated drug resistance: A major contributor to minimal residual disease. Nat Rev Cancer 9: 665–674.
3. Zeckmann CM, Woenje EC, Reix G, Radzwill N, Ilg M, et al. (2007) Impact of stroma on the growth, microcirculation, and metabolism of experimental prostate tumors. Neoplasia 9: 57–67.
4. Klein CA (2009) Parallel progression of primary tumours and metastases. Nat Rev Cancer 9: 392–312.
5. Peeters CF, de Waal RM, Wobbes T, Ruers TJ (2008) Metastatic dormancy imposed by the primary tumor: Does it exist in humans? Ann Surg Oncol 15: 3108–3115.
6. Nguyen DX, Ros PD, Massague J (2009) Metastasis: From dissemination to organ-specific colonization. Nat Rev Cancer 9: 274–284.
7. Khana C, Hunter K (2005) Modeling metastasis in vivo. Carcinogenesis 26: 513–523.
8. Yamanoto N, Yang M, Jiang P, Xu M, Tsuchiya H, et al. (2003) Determination of clonality of metastasis by cell-specific color-coded fluorescent-protein imaging. Cancer Res 63: 7785–7790.
9. Hansen K, Khamma C (2004) Spontaneous and genetically engineered animal models: use in preclinical cancer drug development. Eur J Cancer 40: 858–880.
10. Koike N, Cho A, Nasu K, Seto K, Nagaya S, et al. (2009) Role of diffusion-weighted magnetic resonance imaging in the differential diagnosis of focal hepatic lesions. World J Gastroenterol 15: 5095–5102.
11. Seymour R, Sundberg JP, Hogenesch H (2006) Abnormal lymphoid organ development in immunodeficient mutant mice. Vet Pathol 43: 401–423.
12. Liu XY, Gu JF (2006) Targeting gene-virotherapy of cancer. Cell Res 16: 740.
13. Baurier M, Tsuji K, Yang M, Jiang P, Moossa AR, et al. (2006) In vivo color-coded imaging of the interaction of colon cancer cells and splenocytes in the formation of liver metastases. Cancer Res 66: 11293–11297.
14. Fukumura D, Jain RK (2007) Tumor microvasculature and microenvironment: Targets for anti-angiogenesis and normalization. Microvasc Res 74: 72–84.
15. Nakagawa H, Lyamarrachchi S, Davahari RV, Auer H, Martin EW, et al. (2004) Role of cancer-associated stromal fibroblasts in metastatic colon cancer to the liver and their expression profiles. Oncogene 23: 7366–7377.
16. Boekhorst M, Jain RK, Munn LL (2007) Active versus passive mechanisms in metastasis: Do cancer cells crawl into vessels, or are they pushed? Cancer Oncol 8: 444–448.
17. Joyce JA, Pollard JW (2009) Microenvironmental regulation of metastasis. Nat Rev Cancer 9: 239–252.
18. Chappa A, Makuch M, Lygidakis NJ, Zbar AP, Chong G, et al. (2009) The management of colorectal liver metastases: Expanding the role of hepatic resection in the age of multimodal therapy. Crit Rev Oncol Hematol 72: 65–75.
19. Satoh Y, Esche C, Gambotto A, Shurin GV, Yurkovetsky ZR, et al. (2002) Local administration of 8-12-transfected dendritic cells induces anti-tumor immune responses to colon adenocarcinoma in the liver in mice. J Exp Ther Oncol 15: 337–349.
20. Thalheimer A, Otto C, Bueter M, Illert B, Gattenhohner S, et al. (2009) Tumor cell dissemination in a human colon cancer animal model: Orthotopic implantation or intraportal injection? Eur Surg Res 42: 195–200.
21. Cespedes MV, Espina C, Garcia-Cabezas MA, Trias M, Bohula A, et al. (2007) Orthotopic microinjection of human colon cancer cells in nude mice induces tumor foci in all clinically relevant metastatic sites. Am J Pathol 170: 1077–1085.
22. Baurier M, Tsuji K, Yang M, Jiang P, Moossa AR, et al. (2006) In vivo color-coded imaging of the interaction of colon cancer cells and splenocytes in the formation of liver metastases. Cancer Res 66: 11293–11297.