Multimodal imaging for the detection and characterization of liver lesions in a mouse model of neuroendocrine tumor

Imagerie multimodale pour la détection et la caractérisation de lésions hépatiques dans un modèle de tumeur neuroendocrine chez la souris

O. Beuf, C. Lartizien, L. Milot, L. Baboï, C. Roche, J.-B. Langlois, J.-Y. Scoazec, F. Pilleul

Abstract

Background. — The aim of this study was to compare in vivo magnetic resonance imaging (MRI) and ex vivo autoradiography with histopathological results for the detection and characterization of liver lesions in an experimental model of human neuroendocrine tumors.

Material and methods. — Intestinal STC-1 endocrine tumor cells were injected into 30 nude mice to achieve hepatic dissemination. Seven to 30 days after injection, T2-weighted in vivo images covering the entire liver were acquired with a 7-T system. Autoradiographs were also obtained in 28 mice after injection of fluorodeoxyglucose (18F-FDG). The autoradiographic liver samples were then stained with an antichromogranin antibody before histological analysis. Tumor size and the hepatic tumor fraction were measured using the three imaging modalities.

Results. — Metastatic tumors visualized on the histological liver sections ranged in size from 50 μm (day 7) to 3 mm (day 30). The hepatic tumor fraction increased with time, reaching 30% of the hepatic surface area on day 30. Visual analysis revealed variable tumor distribution and type (solid and/or cystic). On MRI, lesions were identified from day 12 (about 100 mm in diameter) and the hepatic tumor fraction was up to 48% at day 30. The smallest lesions (350 μm in diameter) were also detected at day 12 on the autoradiographs. There was good correlation between tumor fractions determined from autoradiographic and histological data.

© 2008 Published by Elsevier Masson SAS.
Detection and characterization of liver lesions in a mouse model

Introduction

Neuroendocrine tumors are rare [1], with autopsy series reporting a prevalence of 1/1000 for pancreatic tumors and intestinal carcinoid tumors [2], but they are also a serious therapeutic challenge in terms of controlling secretion and reducing tumor volume. Except for curative surgery, a straightforward method for localized tumors [3], the various possible treatments are complex, requiring highly specialized management. As underlined by several authors [4—7], prolonged patient survival will require the development of new compounds, specifically designed for each type of tumor. Certain simple histoprognostic factors, such as tumor size, proliferation index or signs of local invasion, allow a statistically significant, but often unsatisfactory, prediction of malignancy risk. The only relevant criterion of malignancy is the presence of metastatic dissemination, generally to the lymph nodes or liver. Hepatic metastases arising from an endocrine tumor are relatively common and have a considerable effect on therapeutic and surveillance strategies. Despite the clinical importance of this problem, little is known of the natural history of hepatic metastases and of the factors that determine tumor growth and progression.

Clinical studies have generally used morphological and molecular imaging to better understand the diversity and evolution of these tumors [8—14]. For detecting metastases, particularly from endocrine tumors, the best sensitivity is probably provided by thin-slice T2-weighted fat-saturation magnetic resonance imaging (MRI) [15]. Experimental work with small animals has enabled the development of oncological models closely mimicking human pathology that can be helpful in better targeting new compounds or therapeutic indications. Starting with an experimental model of hepatic dissemination of human enteropancreatic endocrine tumors developed in the nude mouse [1], the purpose of the present work was to develop and validate variously adapted imaging protocols. More precisely, the goal was to identify the characteristic T2-weighed MRI and nuclear-isotope-labeled imaging features of this model of neuroendocrine tumors by evaluating the appropriateness and complementary efficacy of these two imaging techniques.

Material and methods

Animal model

The animal model was based on a murine cell line, STC-1, derived from an intestinal endocrine tumor, developed in a double-transgenic mouse and expressing the rat insulin promoter-linked to the SV40-T antigen and the polyomavirus T antigen. This cell line in animals in vivo can induce tumors presenting morphological and functional properties similar to those observed in human gastrointestinal endocrine carcinomas. Tumors derived from STC-1 cells contain cells with an endocrine morphology that express characteristic markers such as chromogranin A. They can synthesize and secrete several digestive hormones such as cholecystokinin and somatostatin. These tumors are highly vascularized by small-caliber vessels with discontinuous walls. Cystic remodeling is frequent, resulting in plasma extravasation into the interstitial fluid. In this experimental model of hepatic dissemination, STC-1 cells are injected into the spleen to trigger their implantation and development within the liver. Using this method, hepatic implantation of STC-1 cells is nearly 100% [16].
**Experimental setup**

A standard exploration protocol was planned for a series of nude mice given an injection of tumor cells. Two animals were to be explored every two or three days, from seven to 30 days after the injection. MRI and autoradiography were to be performed on the same day for each animal at each stage. Practical implementation of this protocol was however difficult. MRI was nevertheless achieved for 30 animals and autoradiographs obtained for 28, with acquisitions by both methods for five animals at the same stage.

**MRI**

MRI acquisitions were made on 30 mice from day 7 to day 29 with a BioSpec-7T system (Bruker, Ettlingen, Germany) using a 32-mm inner-diameter emission/reception volume coil (Rapid Biomedical, Würzburg, Germany). A T2-weighted contrast sequence with fat saturation synchronized to respiration was acquired for each mouse. Respiration movements were captured with a pressure-sensitive sensor (DCXL01DN, Honeywell, Freeport, IL, USA) connected via tubing to a skin patch. The pressure sensor was interfaced (power and pressure measurement) using a synchronization system (ECG Trigger Unit HSB-T, Rapid Biomedical) with all the functional properties required — specifically, amplification and filtration of the input signals and regulation of levels, delays and triggering windows.

Synchronization with respiration was achieved by differentiating the respiratory period $T_{resp}$ (in the second range) from the repetition time ($TR$; greater than 3 s) to obtain the desired T2 contrast. This is particularly important for high magnetic fields (4.7 T or higher) due to the longer T1 longitudinal relaxation time observed with higher-intensity static magnetic fields.

A turbo spin-echo ($TR/TE = 3000/30\text{ ms}$) sequence with fat saturation was used. Geometric parameters were: a series of 12,700-μm thick sections; 30-mm field of view; and $256 \times 256$ pixel matrix. Voxel size was therefore $117 \times 117 \times 700\ \mu m^3$.

Parameters evaluated were: tumor volume (manual delineation of the zones considered to present point and/or diffuse high-intensity signals); liver volume (manual delineation of the liver contours); hepatic tumor fraction (tumor volume/hepatic volume); size of the lesions (minimum and maximum diameters); and the mean signal-to-noise ratio for the liver.

![Image 1](image1.png)

**Figure 1**  Representative images showing the evolution of liver lesions on days 7 (a), 12 (b), 21 (c) and 23 (d) after injection of tumor cells into the spleen.

*Images représentatives de l'évolution du foie des souris obtenues 7 (a), 12 (b), 21 (c) et 23 (d) jours après injection de cellules tumurales dans la rate.*
Detection and characterization of liver lesions in a mouse model

**Autoradiography**

Isotope imaging was obtained using a microimager (BioSpace, Paris, France). Although this material was not designed for in vivo exploration of animals, this study enabled us to establish a baseline for histological correlation. This was done by injecting 200–500 μCi $^{18}$F-labeled fluorodeoxyglucose ($^{18}$F-FDG) into 28 mice, which were then sacrificed 30 min later. A cryotom was then used to obtain 25-μm-thick sections of liver, cut in the plane of the largest diameter. Every tenth slice (250 μm) was selected to sample the entire liver. Lesions observed in the autoradiographs were counted manually using Image J software. Parameters noted were: tumor size (minimum and maximum surface and diameters, extrapolated assuming a circular surface area); tumor fraction (tumor surface/section surface); rate of FDG uptake in tumor (number of events detected in circled lesions/total number of events in section); and tumor contrast (mean uptake in lesion/mean uptake in section).

**Histology**

The Anipath system was used for histological analysis of the autoradiograph sections. After autoradiography, slices were fixed and stained with antichromogranin-A antibodies before digitization with an optical microscope (Nikon E400 Eclipse), equipped with a motorized platform and a DDC camera. This protocol enabled direct comparison between the autoradiographic and histological sections. A morphometric measurement similar to that used for the autoradiographs was then applied to the histological slides to obtain quantitative data on tumor size and fraction. Semiautomatic quantification was inoperable due to the texture visible on certain slices (cryotom striations), presence of vascular formations that could be mistaken for lesions and variable stain uptake.

**Results**

**In vivo MRI**

Hepatic lesions were detected starting from day 12. The lesions were always seen as a high-intensity signal on the T2 images. The smallest detectable lesions, measuring about 100 μm, could only be detected when in clusters. The largest lesions, which appeared as well-delineated nodules, were noted from day 23 to day 29 and measured up to 5 mm (Fig. 1(a–d)).

The hepatic tumor fraction increased linearly with time \( [\text{coefficient of correlation } r^2 = 0.89 \text{ from day } 12 \text{ (0.9%) to day } 29 \text{ (48%)}] \) (Fig. 2), but not ubiquitously. Tumors were predominantly found in the subcapsular regions, particularly in the dome. Because of insufficient variations in signal intensity, particularly in the event of cyst formation, tissue characterization was purely qualitative. Global signal analysis was insufficient for satisfactory tissue characterization as the mean hepatic signal did not increase significantly with the number of lesions.

**Ex vivo analysis of autoradiographic images**

Autoradiographic images from 25 mice, presenting lesions visible on histology were analyzed (three mice excluded). The smallest diameter measured was 350 μm for one mouse on day 12 and the largest was 2.5 mm for one mouse on day 28. The evolution of mean contrast with tumor progression is illustrated in Fig. 3. The standard deviations designate the range of contrasts measured per slice. The standard deviation was relatively stable, irrespective of the stage. Sections from two mice (day 17 and day 24), presented histologically visible lesions, but no uptake on autoradiography. $^{18}$F-FDG uptake increased with tumor progression (Fig. 4). As the contrast (Fig. 3) was constant over time, these results reflect an increase in metastatic volume. The wide data spread for a given day reflects the high interindividual variability of metastatic progression observed in the histological analysis and FDG uptake. The correlation between tumor fraction measured on histological sections and on the autoradiographs is shown in Fig. 5 and presents two regions of interest. Considering the data collected from all 25 mice, the linear correlation between these two measurements is weak \( (r^2 = 0.2) \). But given that FDG uptake was not observed in two hepatic sections from two mice with visible histological lesions and that uptake was partial in the sections from three others (in histopathological regions exhibiting...
FDG uptake), the analysis was repeated excluding these five animals and revealed a much stronger correlation ($r^2 = 0.63$). According to the qualitative results, FDG uptake was a somewhat constant feature of liver metastases, although the time curve (Fig. 4) and visual analysis of the autoradiographs revealed considerable variability. An autoradiograph obtained on day 20 and its corresponding histological section are presented in Fig. 6(a). Comparison of these two images shows similar FDG uptake within pathological (circled areas) and healthy tissues, except for one zone of very high uptake and three zones of low uptake. A similar comparison in another mouse at the same stage (day 20) is shown in Fig. 6b. The distribution of the histological lesions was similar for these two mice whereas the $^{18}$F-FDG uptakes were different.

**Histological analysis**

The autoradiographic slides from 28 mice were used for the histology study. The slides from three mice (sacrificed on day 14 and day 26) were excluded from the analysis because no lesions could be found. The results of the morphometric analysis are presented in Fig. 7(a—b). These curves illustrate the progressive metastatic dissemination of endocrine tumors in liver as observed with this model. A good fit can be seen after a power adjustment using $r^2 = 0.62$ for tumor fraction and 0.74 for mean surface area. The morphometric analysis of tumor size shows that tumors first became visible on day 7 and that the diameters started from 50—180 μm and increased up to 3 mm at the last stage studied.

Visual analysis revealed different characteristic features of spatial distribution and histological type. Two spatial dis-
Fig. 7 Time course of hepatic tumor fraction (a) and the average surface area of the lesions (b) quantified by histology. 
Évolution temporelle de la fraction tumorale (a) et de la surface moyenne des lésions (b) quantifiées en histologie.

Fig. 8 Two stained slides from mice taken on day 17 (a) and day 20 (b) highlighting the differences in metastatic dissemination. 
Deux lames colorées de souris à j17 (a) et j20 (b) soulignant les différences de dissémination métastatique.

Detections and characterization of liver lesions in a mouse model

Figure 7

Figure 8

[Image: two-stained-slides.png]

[Image: diagram.png]

Tributions are shown in Fig. 8. The first corresponds to a predominance of small-sized tumors in the peripheral areas of the liver with zones of coalescence. In the second type, the distribution was more homogeneous, with large differentiated tumors found throughout the hepatic tissue. There was also evidence of two types of lesions. At day 17, most of the tumors were non-cystic whereas, at day 20, the cystic formations appeared as either large vesicles (macrocysts) or small discrete cysts (microcysts) (Fig. 8). It was also noted that the metastatic dispersion was heterogeneous, as can be seen on comparing slides from two close stages (day 17 and day 20).

Discussion

Neuroendocrine tumors are slow-growing tumors, often discovered after the development of hepatic metastasis, with clinical manifestations generally related to tumor secretion [17,18]. The primary tumor, often composed of amine precursor uptake and decarboxylation (APUD) cells that dominate the clinical expression, generally arises in the pancreas. The rate of malignancy varies from 10 to 100%, depending on the type of secretion. Proposed therapeutic strategies are highly complex and of variable efficacy. Somatostatin and interferon can successfully control symptoms [19–21], but have disappointing antitumor effects [22,23]. Various chemotherapy protocols have been proposed, but have had a limited impact on survival [24,25].

Clinical studies have examined the contributions of morphological imaging (ultrasound, computed tomography, MRI) and diverse nuclear-medicine methods using various scintigraphy labels (MIBG, $^{111}$In-pentetreotide, somatostatin receptors) and $^{18}$F-FDG positron emission tomography (PET) [8—14]. Yet, therapeutic trials remain problematic because rigorous methodology must be applied, despite the difficulties of its practical implementation. Thus, the approval process for new compounds is long and arduous. Respecting these phases of experimentation enables study of drug toxicity, efficacy and pharmacokinetic properties and comparisons with the efficacy of the gold-standard treatment. In this framework, small-animal experimentation has been developed to create oncological models similar to human
disease to better target new compounds or new therapeutic indications. The athymic immunodepressed nude mouse is an attractive experimental model as it is unable to produce an immune response, thus allowing xenografts, particularly tumor grafts, to be readily accepted. Thus, the nude mouse is an ideal model for therapeutic experimentation using human tumors [26–28], including neuroendocrine tumors [29–33].

In vivo imaging of these small-animal models has only recently become possible. The development of imaging platforms specially designed for small animals has enabled in vivo longitudinal monitoring of tumor evolution and thus image-based therapeutic modifications.

In the present study, we used an animal model simulating a specific clinical situation to investigate the potential role of non-invasive imaging in the natural history of hepatic metastases of gastrointestinal endocrine tumors [34–43]. The histological study showed that the first lesions visualized were small intravascular aggregates suggestive of sinusoidal hepatic metastases followed by true invasion of the hepatic tissue [44,45].

With MRI, it now takes around 30 min to completely explore the hepatic parenchyma. These explorations are perfectly safe for the animal and without complications. In our model, lesions that were identifiable on histology on day 7 became visible on the MR images on day 12. The time lag is due to the different spatial resolutions of the two methods. Our MRI acquisitions were performed with a slice thickness of about 700 μm whereas the lesions observed on day 7 measured in the order of 100 μm. Despite these differences in the imaging methods, which also prevented perfect juxtaposition in the paired analyses, our results demonstrated that for metastatic tumor progression, there is a good correlation between histology (Fig. 7) and MRI (Fig. 2). More hepatic lesions were detected at the more advanced stages. The number and size of the tumors progressed, with a trend towards cystic formation in the more advanced stages. These features were easily analyzed on the MR images irrespective of stage or tissue characterization. Thus, by varying the echo times of multiple spin-echo sequences, it will be possible to characterize the lesions as micro- or macrocystic as with histology. Quantified data could therefore be obtained using mapping procedures based on the transversal T2 relaxation time. To eliminate heartbeat-related motion artifacts, observed solely in the dome of the liver, a double-acquisition synchronization procedure that applied both the respiration and cardiac signals would be a possible improvement.

Our study did not include a longitudinal follow-up of the inoculated mice. This type of follow-up, which can be done with MRI, would reduce intermouse variability and allow for more robust statistical analyses with a relatively small number of animals per group.

The autoradiographs illustrate the usefulness of FDG as a marker in this animal model. Uptake was global through the liver and was satisfactorily correlated with tumor fraction, as measured by the surface-area fraction of metastatic invasion. We did, however, note considerable variation in FDG uptake, which was even absent in some animals. In a clinical setting, several teams have observed this type of variable FDG uptake in patients with highly differentiated slow-growing endocrine tumors [46,47]. STC-1 cells are well differentiated with a moderate proliferation index of less than 5% with proliferating cell nuclear antigen (PCNA) and positive reactivity with somatostatin, chromogranin A and synaptophysin. It would therefore be logical to expect weak sensitivity in humans. The percentage observed in the present study was higher than expected, given that two of the 35 mice studied had no FDG uptake and three others presented partial FDG uptake. This aspect requires more detailed histopathological analysis to look for characteristic features that might explain these differences in uptake. Such a study could be conducted with animal models. In addition, it would be interesting to test other more specific markers, Yttrium-86-labeled octreotid, a positron emitter, is a somatostatin analog that might be useful as most endocrine tumors express receptors for this peptide. The same molecule labeled with yttrium-90 is used in metabolic radiotherapy protocols for endocrine tumors. For PET using yttrium-86-labeled octreotid, there is the problem of background noise because, as it disintegrates, this isotope emits 300% singlet gamma photons, which compromise image quality [48].

That ex vivo autoradiography study also demonstrated that tumor size varied from 350 μm to 2.5 mm, while the instrument’s spatial resolution was in the order of 250 μm. Furthermore, we measured a mean contrast of 1.5. The development of a micro-PET system with resolution in the order of 2 mm for more advanced stages (less than 20 days) is a realistic possibility that would enable detection of tumors close to the system’s resolution level. Such a system however would have to be validated experimentally because of the relatively low mean contrast measured by FDG on autoradiography. Nevertheless, we hope to obtain more striking contrast with more specific markers.

**Conclusion**

This multimodal imaging study using the nude mouse model of hepatic metastatic dissemination of neuroendocrine tumors provides encouraging results that reinforce the idea that imaging can play an important role in explorative strategies in projects involving small animals. It demonstrated that hepatic lesions from neuroendocrine tumors can be explored in the nude mouse for both the detection and surveillance required for the evaluation of new therapeutic modalities.

**Acknowledgement**

This research was funded by the CNRS-CEA’s ‘imaging of small animals’ program. The Animage platform was used for acquisitions. FDG was furnished by Cermep.

**References**

Detection and characterization of liver lesions in a mouse model


[40] Kondo Y, Aari S, Mori A, Furutani M, Chiba T, Imamura M. Enhancement of angiogenesis, tumor growth, and metasta-


