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ORIGINAL ARTICLE

Bilobar colorectal liver metastases: a new model for preclinical studies

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Summary

This study aimed to develop a new model of colorectal liver metastases (LM) in the rat. Both single macroscopic and multiple bilobar microscopic LM were investigated, as this closely resembled the human situation, before right hepatectomy was performed for 'single' right LM. The single macroscopic LM was elicited by direct injection of DHD/K12 colorectal cancer cells under the capsule of the median liver lobe in immunocompetent BDIX rats. The bilobar micrometastases were elicited by intraportal injection of DHD/K12 cells. A preliminary protocol was conducted to assess the dose of cells required to inject in to the portal vein, using 10^6, 2×10^6 and 3×10^6 DHD/K12 cells ($n = 15$ rats). The resultant protocol for the experimental model used intraportal injection of 10^6 DHD/K12 cells and direct injections of 0.5×10^6, 10^6 and 1.5×10^6 DHD/K12 cells ($n = 15$ rats). For both protocols, BDIX rats were sacrificed at day 30 after injection. The preliminary protocol showed that intraportal injection of 10^6 DHD/K12 cells was associated with bilobar micrometastases of 0.8 mm mean diameter at day 30. The main protocol assessed that direct injection of 0.5×10^6 under the liver median lobe capsule and intraportal injection of 10^6 DHD/K12 cells were associated at day 30 with a single macroscopic metastasis confined to a liver lobe and bilobar micrometastases, without peritoneal carcinomatosis or lung metastasis. Thus we have developed a new experimental model of bilobar colorectal LM including both macro- and microscopic colorectal LMs, which mimics the human situation and which will be useful in preclinical studies.

Keywords
BDIX rats, colorectal cancer, DHDK12 cells, experimental model, liver metastasis

Approximately 60% of the million patients diagnosed with colorectal cancer worldwide every year will develop liver metastases (LM) during the course of their disease. Recently, surgical treatment with perioperative oxaliplatin-based chemotherapy has been established as the standard therapy for resectable LM, with an associated 5-year survival approaching 50% (Nordlinger *et al.* 2008). Despite these encouraging results, more than 30% of the patients will develop intrahepatic recurrence within the three years following complete surgical LM resection (D'Angelica *et al.* 2011). This high rate of intrahepatic recurrence might be explained, at least in part, by the presence of hepatic micrometastases, undetected during preoperative radiological staging using computed tomography (CT). Yokoyama *et al.* (2002) Yokoyama *et al.* studied such micrometastases by immunohistochemical detection and suggested that their presence in pathologic specimens after LM resection indicates widespread hepatic involvement and thus predicts an increased risk of intrahepatic recurrence.

For optimization of diagnostic tools and oncological management of such micrometastases *in vivo* experimental models are needed. To be relevant, such experimental model should meet several requirements (de Jong *et al.* 2009): (i) be easy to perform and be reproducible, (ii) obtained experimental LM should be histologically accurate, as close as possible to the histological features of genuine LM, (iii) animal hosts should have a normal immune system, as immunity is a key feature in responses to cancer (Banchereau & Steinman 1998), and finally, (iv) the experimental model should resemble human clinical settings closely.

In the past, we have used the association of syngenic immunocompetent BDIX rats and the DHD/K12 cell line extensively to produce several experimental models of LM (Panis *et al.* 1990, 1992, 1996; Caruso *et al.* 1993; Maggiori *et al.* 2011). The DHD/K12 cell line is an established transplantable carcinoma cell line originating from a 1,2-dimethylhydralazine-induced colon adenocarcinoma in the BDIX rat (Martin *et al.* 1983). Using this association, LM can be obtained by intraportal injection (Panis *et al.* 1992) or by direct injection under the liver capsule (Caruso *et al.* 1993), inducing in both cases poorly differentiated adenorcarcinoma consisting mainly of tumour cells organized in lobules or thick cell cords, with comparable histological features to genuine LM (Panis *et al.* 1990, 1992; Caruso *et al.* 1993). These previous models have been used to evaluate the impact of liver regeneration after portal vein occlusion (Maggiori *et al.* 2011) and after hepatectomy on immunosuppressive therapy (Panis *et al.* 1992), and to evaluate the benefit of HSV1-TK and interleukin-12 gene therapies (Caruso *et al.* 1993; Alves *et al.* 2004). More recently, we developed a model of micrometastases to assess the possibilities of early detection of such micrometastases by perfusion CT scan. However, to our knowledge, no study focused on closely mimicking the clinical setting of bilobar LM including both macroscopic metastases (i.e. potentially detectable on CT and leading to surgery) and non-detectable micrometastases (Figure 1). We consider that this new experimental model could be particularly relevant in preclinical studies concerning firstly, the development of new imaging technics for detection of 'undetectable' LM (i.e. elastoMRI, perfusion CT scan, etc.); and secondly, to evaluate the potential benefit of new drugs in a situation, which is close what is observed in humans: a patient with unilobar macroscopic liver metastase, and possible 'undetectable' controlateral LM: (i) before hepatectomy, in neodjuvant setting, (ii) after partial hepatectomy to prevent recurrence within the remnant liver.

Thus, the aim of this study was to develop and describe an *in vivo* relevant experimental model of bilobar LM including both macroscopic colorectal liver metastasis and micrometastases using immunocompetent BDIX rats and DHD/K12 cell line.

Materials and methods

Animals

The experiments were performed on male BDIX rats weighing 180–220 g (Charles River, France). The animals were maintained in a temperature-controlled environment with 12-h light–dark cycle, free access to standard chow pellets and tap water *ad libitum*. All procedures were performed in compliance with institutional animal care guidelines.

Ethical Approval

This study has been approved by our ethical commitee with the protocol number: 2011-14/773-0057

Cell line

The DHD/K12 cells were cultured in Dulbecco's modified Eagle medium (GIBCO, Invitrogen, Carlsbad, CA, USA) and 1:1 Ham's F10 medium (GIBCO, Invitrogen) containing 10% of foetal calf serum (GIBCO, Invitrogen). All media were supplemented with 4 mM of L-Glutamine (GIBCO, Invitrogen), 100 UI/ml of penicillin (GIBCO, Invitrogen) and 100 μg/ml of streptomycin (GIBCO, Invitrogen). Culture in 150-cm² Falcon flasks (TPP, Trasadingen, Switzerland) at 37°C and 5 per cent CO2. When cells reached 80% confluence the medium was discarded and the cells were washed three times with Dulbecco's phosphate-buffered saline (D-PBS, GIBCO, Invitrogen) before incubation with 4 ml of trypsin-EDTA (Trypsin 0.05% and EDTA 0.2 g/l, GIBCO, Invitrogen) at 37 °C for 5 min. Cells were then harvested, centrifuged, counted and re-suspended in D-PBS for introduction in to the experimental animals.

Surgical procedures

The rats were anaesthetized with intraperitoneal injection of pentobarbital (0.001 ml/g). During anaesthesia, the rats were allowed to breathe spontaneously. All operative procedures were performed through a midline laparotomy closed with a single running suture. After the surgical procedure, the rats were maintained for 30 min under a heating lamp.

Experimental protocols

Preliminary protocol 1: Bilobar microscopic metastases. Bilobar microscopic metastases were induced by injection of DHD/K12 cells suspended in 0.2 ml of D-PBS into the main portal trunk. At day 0, 15 rats were randomly assigned in three groups: Group A ($n = 5$): injection of 1×10^6 DHD/K12 cells; Group B ($n = 5$): injection of 2×10^6 DHD/K12 cells; and Group C ($n = 5$): injection of 3×10^6 DHD/K12 cells. All injections were performed using 30 gauge needles. Haemostasis was obtained by portal vein plugging with a cotton stem. All rats were sacrificed and necropsied at day 30.

This preliminary protocol was designed to assess the ideal dose of intraportal DHD/K12 cells injection to obtain bilobar micrometastases with a mean diameter of <1 mm (Figure 2).

Main protocol: established macroscopic metastasis and bilobar micrometastases. Macroscopic metastasis was induced by direct injection of DHD/K12 cells suspended in 0.1 ml of D-PBS under the liver capsule of the median lobe. At day 0, 15 rats were randomly assigned in three groups: Group 1 ($n = 5$): injection of 0.5×10^6 DHD/K12 cells; Group 2 ($n = 5$): injection of 1×10^6 DHD/K12 cells; and Group 3 ($n = 5$): injection of 1.5×10^6 DHD/K12 cells. Bilobar microscopic metastases were induced in all rats by intraportal injection of 1×10^6 DHD/K12 cells suspended in 0.2 ml of D-PBS into the main portal trunk. All rats were sacrificed and necropsied at day 30.

This second protocol was conducted to assess the ideal doses of DHD/K12 direct and intraportal injection to obtain

bilobar metastases with a mean diameter of <1 mm and a single macroscopic metastasis confined to a liver lobe (Figure 2).

Control group. A control group was composed of 3 rats in which sham injections were performed using 0.2 ml of D-PBS and 0.1 ml D-PBS, in the main portal trunk and under the liver capsule of the median liver lobe respectively. Rats from the control group were sacrificed and necropsied at day 30.

Pathological evaluation

Necropsy and liver harvesting was performed immediately after sacrifice. Abdominal cavity and lungs were systematically checked for peritoneal carcinomatosis and extra-hepatic metastasis. After harvesting, livers were split into right (including superior and inferior right lobes, and anterior and posterior caudate lobes) and left (including left lateral and median lobes) liver. The liver tissue was fixed in 10% formaldehyde, embedded, sectioned, stained with haematoxylin–eosin–saffran and analysed with computer-assisted optical microscopy. Metastases were identified and counted. Metastases diameters were measured on their maximal cut surface using the Histolab software (Microvision Instruments, Evry, France). Additionally, in the main protocol, liver inflammation was assessed. All histological analyses were performed in a blinded fashion with respect to the experimental groups.

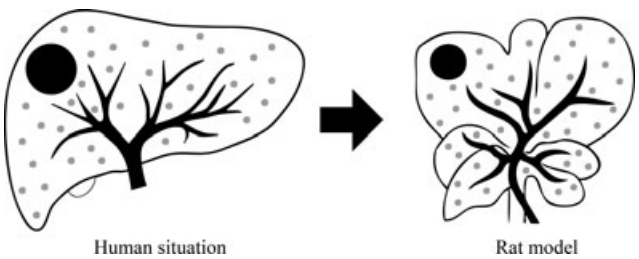

Figure 1 Experimental model (right) and corresponding clinical situation (left): single macroscopic liver metastasis (black colour) and multiple diffuse micrometastases (grey colour).

Statistical analysis

Continuous data are expressed as mean ± standard deviation. Proportions are expressed as number of cases (percentage of cases). Statistical analysis was performed using the Statistical Package for the Social Sciences software (SPSS, version 17.0, Chicago, IL, USA).

Results

Preliminary protocol

Group A: intraportal injection of 1 × 10^6 DHD/K12. The mean weight of the rats, at day 0, was 201 ± 13 g (range, 187–215 g). At day 30 no rats had either peritoneal carcinomatosis or extra-hepatic metastases. Pathological examination showed bilobar micrometastases in all cases. Micrometastases were well-defined rounded nodules composed of poorly differentiated non-secreting carcinoma cells arranged in a glandular pattern, showing hyperchromatic nuclei and atypia (Figure 3). Mean total number of micrometastases was 12 ± 2

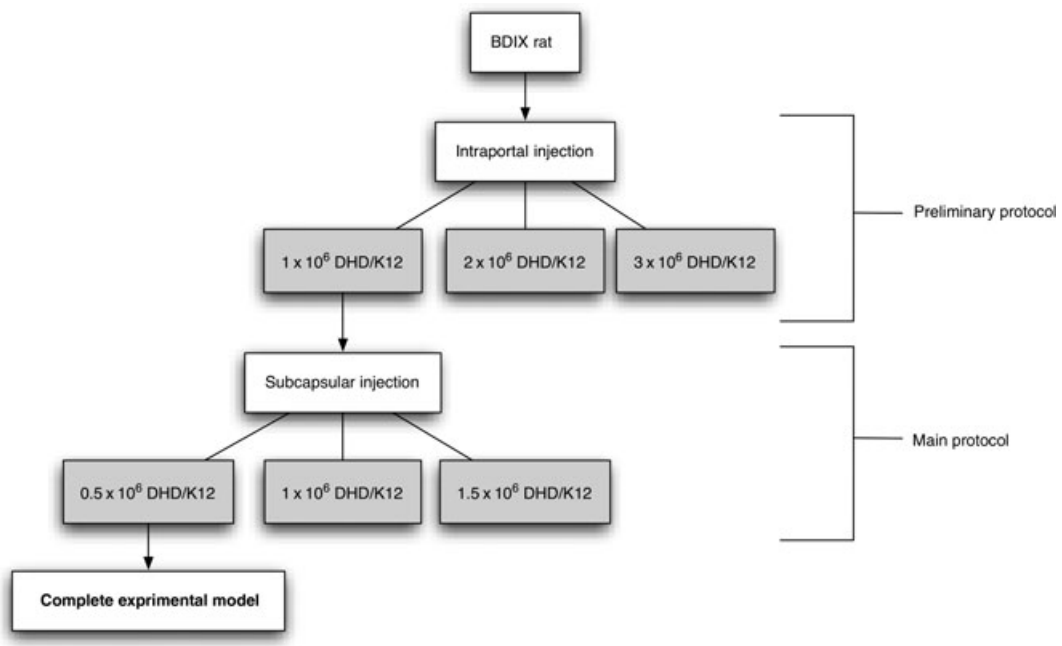

Figure 2 Experimental workflow.

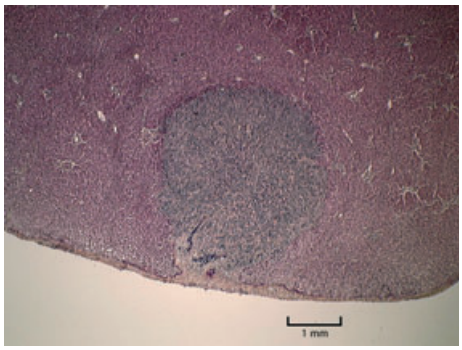

Figure 3 Optical microscopy of micrometastasis obtained 30 days after intraportal injection of 1×10^6 DHD/K12 cells (stain: haematoxylin–eosin–saffran).

(range, 9–14), including 3 ± 1 (range, 1–4) micrometastases in the right liver and 9 ± 1 (range, 7–10) micrometastases in the left liver (Table 1). The mean diameter of the micrometastases was 0.8 ± 0.4 mm (range, 0.2–1.8 mm) (Figure 4).

Group B: intraportal injection of 2×10^6 DHD/K12. The mean weight of the rats at day 0 was 196 ± 10 g (range, 185–209 g). At day 30 necropsy revealed peritoneal carcinomatosis in 2/5 (40%) but no rats had extra-hepatic metastases. Histopathology showed bilobar micrometastases in all cases, with the same features as Group A. The mean total number of micrometastases was 17 ± 2 (range, 15–20), including 5 ± 1 (range, 4–6) micrometastases in the right liver and 12 ± 1 (range, 11–14) micrometastases of the left liver (Table 1). The mean diameter of the micrometastases was 1.2 ± 0.7 mm (range, 0.2–3.4 mm) (Figure 4).

Group C: intraportal injection of 3×10^6 DHD/K12. The mean weight of the rats at day 0 was 205 ± 15 g (range, 180–220 g). At day 30 necropsy revealed peritoneal carcinomatosis in 4/5 (80%) but no rats had extra-hepatic metastases. Histopathology showed bilobar micrometastases in all cases, with the same features as Group A. The Mean total number of micrometastases was 22 ± 2 (range, 21–25), including 7 ± 1 (range, 6–9) metastases in the right liver and 15 ± 1 metastases (range, 14–16) of the left liver (Table 1). The mean diameter of the micrometastases was 1.8 ± 1.0 mm (range, 0.1–5 mm) (Figure 4).

Main protocol

Group 1: intraportal injection of 1×10^6 DHD/K12 and direct injection of 0.5×10^6 DHD/K12. The mean weight of the rats at day 0 was 201 ± 17 g (range, 185–220 g). At day 30 neither peritoneal carcinomatosis nor extra-hepatic metastases were found. Pathological examination of the liver showed a macroscopic metastasis (obtained by direct injection), composed of poorly differentiated cells non-secreting carcinoma cells arranged in a glandular pattern with focal areas of necrosis in all cases. This metastasis was single and located in the median liver lobe, at the site of direct injection, in all cases (Figure 5a and 5b). Mean macroscopic metastasis diameter was 7.9 ± 1.0 mm (range, 6.4–9.3 mm) (Figure 4). Bilobar micrometastases were observed in all cases and were composed of tumours with the pathologic features as preliminary protocols. The mean total number of micrometastases was 11 ± 1 (range, 9–12), including 3 ± 1 micrometastases (range, 3–5) in the right liver and 7 ± 1 (range, 6–9) micrometastases in the left liver (Table 1). The mean diameter of the micrometastases was 0.8 ± 0.5 mm (range, 0.1–2.0 mm).

Group 2: intraportal injection of 1×10^6 DHD/K12 and direct injection of 1×10^6 DHD/K12. The mean weight of the rats at day 0 was 204 ± 17 g (range, 182–221 g). At day 30 neither peritoneal carcinomatosis nor extra-hepatic metastases were present. Pathological examination showed a macroscopic metastasis (obtained by direct injection) in all cases. This metastasis was single and located in the median liver lobe, at the site of direct injection, in all cases. The mean macroscopic metastasis diameter was 10.1 ± 0.6 mm (range, 9.5–11.0 mm) (Figure 4). Bilobar micrometastases were observed in all cases and were composed of tumours with the same pathologic features as seen in the preliminary protocols. The mean total number of micrometastases was 12 ± 3 (range, 9–15), including 4 ± 0 micrometastases (range, 4–5) in the right liver and 8 ± 3 (range, 5–11) micrometastases

Table 1 Number of micrometastases and macrometastases observed 30 days after injection of DHD/K12 cell line in BDIX rats

Protocol	Metastases	Mean total number	Mean number in the right liver	Mean number in the left liver
Intraportal injection of 1×10^6 DHD/K12	Micrometastases	12 ± 2 (9–14)	3 ± 1 (1–4)	9 ± 1 (7–10)
	Macrometastases	0	0	0
Intraportal injection of 2×10^6 DHD/K12	Micrometastases	17 ± 2 (15–20)	5 ± 1 (4–6)	12 ± 1 (11–14)
	Macrometastases	0	0	0
Intraportal injection of 3×10^6 DHD/K12	Micrometastases	22 ± 2 (21–25)	7 ± 1 (6–9)	15 ± 1 (14–16)
	Macrometastases	0	0	0
Intraportal injection of 1×10^6 and direct injection of 0.5×10^6 DHD/K12	Micrometastases	11 ± 1 (9–12)	3 ± 1 (3–5)	7 ± 1 (6–9)
	Macrometastases	1	0	1
Intraportal injection of 1×10^6 and direct injection of 0.5×10^6 DHD/K12	Micrometastases	12 ± 3 (9–15)	4 ± 0 (4–5)	8 ± 3 (5–11)
	Macrometastases	1	0	1
Intraportal injection of 1×10^6 and direct injection of 0.5×10^6 DHD/K12	Micrometastases	10 ± 1 (10–12)	4 ± 1 (3–5)	6 ± 1 (5–7)
	Macrometastases	1	0	1

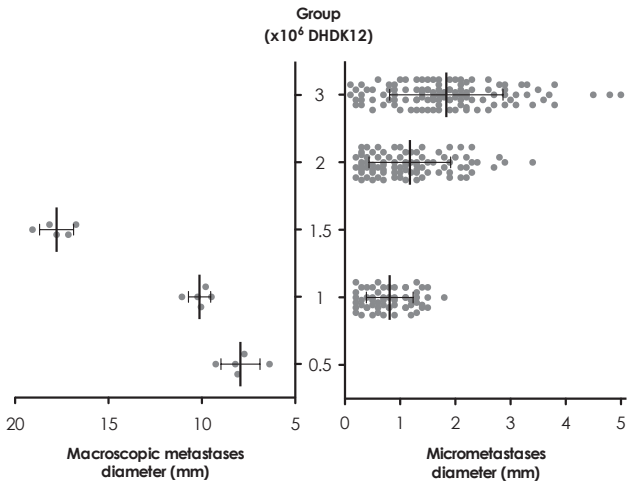

Figure 4 Micrometastases diameter obtained 30 days after intraportal injection of 1×10^6 DHD/K12 cells, 2×10^6 DHD/K12 cells or 3×10^6 DHD/K12 cells and macrometastases diameter obtained 30 days after subcapsular injection of 0.5×10^6 DHD/K12 cells, 1×10^6 DHD/K12 cells or 1.5×10^6 DHD/K12 cells under the liver capsule of the median lobe.

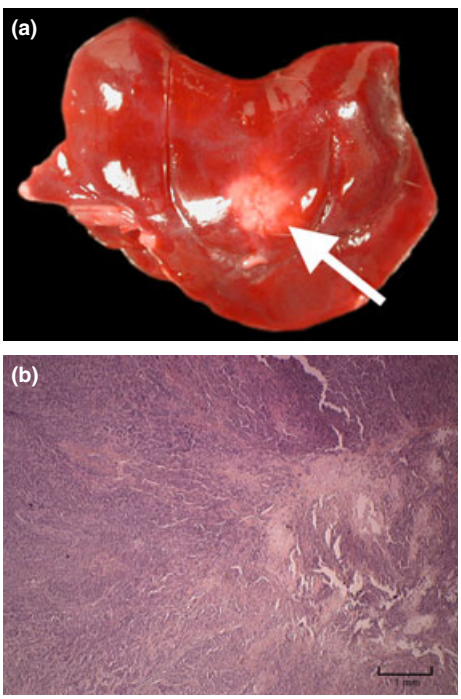

Figure 5 Macrometastasis obtained 30 days after subcapsular injection of 0.5×10^6 DHD/K12 cells under the liver capsule of the median lobe. (a): Full liver specimen; (b): optical microscopy of macrometastasis (stain: haematoxylin–eosin–saffran).

in the left liver (Table 1). The mean diameter of the micrometastases was 0.9 ± 0.5 mm (range, 0.1–2.1 mm).

Group 3: intraportal injection of 1×10^6 DHD/K12 and direct injection of 1.5×10^6 DHD/K12. The mean weight of the rats at day 0 was 195 ± 13 g (range, 185–215 g). At day 30 2/5 rats (40%) had peritoneal carcinomatosis. No rats had extra-hepatic metastases. Pathological liver examination showed a macroscopic metastasis (obtained by direct injection) in all cases. This metastasis was single and located in the median liver lobe, at the site of direct injection, in all cases. The mean macroscopic metastasis diameter was 17.7 ± 0.8 mm (range, 17.1–19.1 mm) (Figure 4) with tumoural extension to adjacent organs in 2/5 cases (40%): pancreas ($n = 1$) or abdominal wall ($n = 1$). Bilobar micrometastases were observed in all cases and were composed of tumours with the same pathologic features as those seen in the preliminary protocols. The mean total number of micrometastases was 10 ± 1 (range, 10–12), including 4 ± 1 micrometastases (range, 3–5) in the right liver and 6 ± 1 (range, 5–7) micrometastases in the left liver (Table 1). The mean diameter of the micrometastases was 0.8 ± 0.4 mm3 (range, 0.1–1.7 mm3).

Inflammation. At the interface between the liver metastases and the liver parenchyma very few inflammatory foci were identified. The presence and discontinuous distribution of the inflammatory foci were similar whatever the diameter of the liver metastases. These foci were composed chiefly of lymphocytes. No inflammatory foci were identified inside the liver metastases and in the healthy liver parenchyma.

Control Group

The mean weight of the rats, at day 0, was 205 ± 7 g (range, 197–210 g). At day 30 neither surgical exploration nor pathological examination showed pathologic features.

Discussion

This study aimed to develop and describe a new and relevant experimental model of colorectal LM including both single macroscopic LM and multiple bilobar micrometastases using a combination of immunocompetent BDIX rats and the DHD/K12 colon cancer cell line. Bilobar micrometastases were obtained by intraportal injection of DHD/K12 cells, and macroscopic metastasis was obtained by direct injection of DHD/K12 cells under the liver capsule.

First, the preliminary protocol was conducted to assess the ideal dose of DHD/K12 cells to inject in the main portal trunk to obtain bilobar metastasis with a mean diameter of less than 1 mm and with a low risk of peritoneal carcinomatosis and lung metastases. This preliminary protocol assessed that an intraportal injection of 1×10^6 DHD/K12 suspended in 0.2 ml of D-PBS was associated, at day 30, with bilobar micrometastases of a 0.8 mm mean diameter without peritoneal carcinomatosis or lung metastasis. Second, the main protocol was conducted to develop the complete experimental model including a single macroscopic metastasis confined to a liver lobe along with bilobar

micrometastases and without peritoneal carcinomatosis or lung metastasis. This protocol assessed that a direct injection of 0.5×10^6 DHD/K12 suspended in 0.1 ml of D-PBS under the liver median lobe capsule and an intraportal injection of 1×10^6 DHD/K12 suspended in 0.2 ml of D-PBS were associated at day 30 with a single macroscopic metastasis confined to the median lobe and bilobar micrometastases without peritoneal carcinomatosis or lung metastasis.

In vivo experimental models play a key role in preclinical testing of emerging therapies and in the better comprehension of cancer development and progression. Ideally, animal models should mimic the human condition in terms of biological, histological and morphological features (Kobaek-Larsen *et al.* 2000; de Jong *et al.* 2009).

Rodents, and especially rats, are the animals used most frequently for colorectal cancer experimental models. Two pathways for obtaining experimental LM are available: xenografts of human colorectal cancer cells in immunodeficient mice or allografts in immunocompetent animals, such as the association used in the present study. Several studies have described LM using nude mice (Rashidi *et al.* 2000; Cai *et al.* 2005; de Jong *et al.* 2009). However, the use of an immunodeficient host has the major disadvantage that it does not not reflect the normal immune system present in the majority of patients with colorectal cancer. This leads to a, major bias in cancer development studies. Furthermore, immunodeficient animals show a less stable health status (de Jong *et al.* 2009) and do not allow studies concerning immunotherapy-based cancer treatments (Calman 1975; Kobaek-Larsen *et al.* 2000). As immunity is a cornerstone in both the response to cancer and in carcinogenesis (Banchereau & Steinman 1998), the ideal experimental model host should have a normal immune system, and resemble the normal human immune system as closely as possible. Several studies have compared human and rodent carcinogenesis, suggesting that both species have very similar pathways leading to similar cancer development patterns (Rangarajan & Weinberg 2003; Anisimov *et al.* 2005).

Several associations of cancer cell lines and immunocompetent rodent hosts have been described (Robertson *et al.* 2008; de Jong *et al.* 2009). The DHD/K12 cell line used in the present study is a chemically induced colon cancer from BDIX rats, initially described by Martin *et al.* (Martin *et al.* 1983). Therefore, the DHD/K12 cell line and BDIX rat association is biologically compatible and might be an accurate *in vivo* model of LM. Furthermore, this association leads to a high output of LM. Indeed, Robertson *et al.* (2008) studied different combinations of hosts and cell lines, using DHD/K12 and HT29 human colorectal cancer cells injected in BDIX and Sprague-Dawley rats. In their study, the DHD/K12-BDIX combination was shown to have the highest metastatic rate.

Different methods of inoculation of cancer cells are available. LM can be obtained by orthotopic (i.e. in the colon) injection of syngeneic tumour cells (Goldrosen 1980; Bresalier *et al.* 1987; Garcia-Olmo *et al.* 2003), injection into the spleen (Lafreniere & Rosenberg 1986; Casillas *et al.* 1997), direct injection into the liver (Caruso *et al.* 1993; Maggiori *et al.* 2011), or by intraportal injection (Robertson *et al.* 2008; Maggiori *et al.* 2011). However, only models using direct injection into the liver or intraportal injection represent a satisfactory experimental cancer model (de Jong *et al.* 2009). The LM incidence obtained after orthotopic injection varies considerably between the different studies, ranging from 100% (Goldrosen 1980) to 0% (Garcia-Olmo *et al.* 2003), suggesting a lack of reproducibility. Intrasplenic injection of the cancer cell line seems to offer reproducible rates of LM with a 100% reported LM incidence rate (Lafreniere & Rosenberg 1986; Casillas *et al.* 1997) but leads to the problem of spleen management. A tumoural spleen might be a continuous source of cancer cells in the portal system, leading to possible bias of experiment and extra-hepatic metastases, such as the 20% pancreatic tumour rat observed by Lafreniere and colleagues (Lafreniere & Rosenberg 1986). Conversely, a splenectomy might affect immunity response. In the present study, both micro- and macroscopic LM were obtained in 100% of the cases, with neither peritoneal carcinomatosis nor extra-hepatic metastasis.

We have reported previously on studies using several different experimental LM models, where LM were elicited using several different methods. (Panis *et al.* 1990, 1992, 1996; Caruso *et al.* 1993; Alves *et al.* 2004). In the present study, LM were obtained by both intraportal and subcapsular injection of DHD/K12 cells. The intraportal injection of cancer cells mimics the generally accepted hypothesis of haematogenous spread of human colorectal cancer cells through the portal system (de Jong *et al.* 2009), allowing reliable study of micrometastases and early development stages of LM. Furthermore, this inoculation method allows a diffuse distribution of the LM in all the liver lobes, as confirmed in the present study. However, this method of implantation, used as an experimental model of LM, has some limitations. First, human tumours consist of a heterogeneous population whereas cell lines are homogenous. Second human metastasis occurs after spreading from an original tumour, during which several transformations occur that facilitate the metastatic event.

Conversely the subcapsular injection method does not resemble the haematogenous spread, but has the advantage of allowing for a precise localization of the LM, which is crucial if the aim is to assess tumoural response to treatment by imaging techniques or necropsy. In the present study, 100% of the macroscopic LM developed in the median liver lobe, at the precise site of injection.

This experimental model was developed to mimic as closely as possible the clinical situation of a patient with a detectable single macroscopic metastasis and multiple undetectable micrometastases. Such experimental model might be of great interest for preclinical studies focusing on curative and preventive management of LM. On a neoadjuvant setting, this model might be use to study: (i) radiologic detection of liver micrometastases to optimize complete surgical

resection and potentially improve postoperative disease-free survival, (ii) chemotherapy response of both established macroscopic and microscopic LM at an early development stage, allowing a better selection of resectable LM and (iii) impact on tumour growth of preoperative induced liver regeneration by portal vein ligation or embolization. On a postoperative setting, this model might allow preclinical studies focusing on: (i) early detection of intrahepatic LM recurrence and (ii) impact of liver regeneration induced by partial hepatectomy on tumour growth (of possible 'micrometastases') in the remnant liver.

In conclusion, the present study proposes an *in vivo* relevant experimental model of bilobar LM including both macro- and microscopic colorectal liver metastasis using immunocompetent BDIX rats and DHD/K12 cell line. Both macro- and microscopic LM obtained are highly controllable, predictable and reproducible, without extra-hepatic metastastic involvement. The utility of this experimental model lies in the precise mimicking of the clinical situation of a patient with a large, detectable LM and with synchronous undetectable micrometastases. It can be used to study new diagnostic tools (such as innovative imaging procedures), new therapies and novel combination of approaches. Recently, we have already used this model to assess impact of preoperative portal vein ligation and embolization on LM growth (Maggiori *et al.* 2011).

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Conflict of Interest

There were no conflicts of interest.

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