CHAPTER 2

Liver surgery enhances implantation of circulating tumour cells, but does not stimulate growth of tumour cells

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Submitted

ABSTRACT

Surgical resection remains the most effective therapy for metastatic colorectal cancer confined to the liver, although the recurrence rate is high. This is thought to be attributable to residual microscopic disease but also the liver surgery itself. The mechanism how liver surgery affects tumour recurrence is not yet known.

In this animal study the effect of partial hepatectomy (phX) on the development of tumour noduli in the lungs was evaluated. CC531 rat colon carcinoma cells were inoculated iv 24 hours before, during or 24 hours after surgery. Rat serum was obtained at different time points after phX and added to *in vitro* CC531 cell cultures. Finally, phX was compared to an ileum resection (ilX)

PhX leads to increased tumour noduli in the lungs, compared to sham operation ($p=0.002$), but only when performed directly before the injection of tumour cells and not when performed 24 hours before or after the inoculation. Comparable results were obtained for ilX. No growth stimulation of tumour cells after incubation with rat serum, obtained at different time points after phX, could be detected *in vitro*.

In conclusion, not only phX, but surgery in general promotes distant tumour recurrence exerting the effect during the early phase of tumour cell adhesion and not during tumour outgrowth.

INTRODUCTION

After potentially curative resection of colorectal carcinoma, more than 40 % of patients develop hepatic metastases[1]. Surgery is still the most effective therapy for resectable disease, offering potential for cure[2,3]. However, in most patients recurrence of tumour will develop after hepatic metastasectomy, most commonly within the remnant liver or in the lungs. Approximately 75 % of these patients develop tumour recurrence within the first two years after liver resection[4]. It has been the clinical impression that surgery itself influences the development of distant metastases.

Surgery involves tissue trauma and consequently induces wound regeneration and inflammation. Therefore, a plethora of growth factors and inflammatory mediators as reactive oxygen species and pro-inflammatory cytokines are released during and shortly after surgery. It is hypothesized that these factors may influence tumour recurrence. Indeed, several studies demonstrated enhancement of loco-regional or distant tumour recurrence caused by surgical trauma[5-8].

As the liver has the unique ability to regenerate to its original size in a relatively short period of time after resection, it has been the clinical impression for a long time that microscopic residual disease, undetected at the time of operation, might profit from the resection and the following regeneration. The release of several growth factors and cytokines during liver surgery as well as during liver regeneration has been proposed to be responsible for tumour recurrences. Experimental studies, including those from our laboratory, indicate that partial hepatectomy (phX) has a stimulating effect on tumour recurrence in the remnant liver[9-14].

At the time of surgery, circulating tumour cells are often found in patients with gastro-intestinal cancer[15-17] . Although the amount of circulating tumour cells is enhanced during resection, it will not entirely explain the high recurrence rate found after intentionally curative liver surgery. Implantation of circulating tumour cells appears to be highly inefficient and most circulating tumour cells are rapidly destroyed[18,19]. Probably surgery increases tumour recurrence by enhancing the implantation of circulating tumour cells.

Furthermore, postoperative immunosuppression, might facilitate not only dissemination of tumor cells and outgrowth of minimal residual disease / (micro) metastases but also allows more circulating tumour cells to survive and therefore may influence the recurrence rate.

In the present study, we evaluated the effect of liver surgery and another gastrointestinal resection on circulating tumor cells. We developed an *in vivo* rat model in which the effect of a hemihepatectomy (phX) on tumour recurrence in the lung was evaluated. This procedure was compared to an ileum resection (ilX). A rat colon carcinoma cell line (CC531) was used because this cell line is only weakly immunogenic and therefore the effect of a decreased immune system was minimized. In both *in vivo* and *in vitro* studies we examined the mechanisms by which surgery could modulate pulmonary development.

MATERIALS AND METHODS

Animals

Male rats of the inbred WAG/Rij strain were obtained from Harlan-CPB (Austerlitz, The Netherlands). The rats were 12-16 weeks old and weighted approximately 250 g. The animals were bred under specific pathogen-free conditions, kept under standard laboratory conditions and were given standard laboratory diet and water *ad libitum*. The experimental study was carried out in accordance with the Dutch Animal Experimentation Act and approved by the Committee on Animal Research of the Erasmus University Rotterdam.

Tumour

CC-531s is a moderately differentiated, weakly immunogenic colonic adenocarcinoma induced in the WAG/Rij rat by 1,2 dimethylhydrazine. A cell line was established from this carcinoma and maintained by serial passage after trypsinization in culture medium[20]. For continuous culture RPMI 1640 medium (Gibco, Paisly, UK) supplemented with 5% fetal calf serum, L-glutamin (200 mmol/l), penicillin (5000 U/ml) and streptomycin (5000 U/ml) was used. Before use, cells were trypsinized (10 min. at 37°C), centrifuged (5 min at 700*g*), resuspended in RPMI 1640 and counted. Viability was measured with trypan-blue exclusion (0.3% in a 0.9% NaCl-solution). Viability always exceeded 90%.

In vivo experiments

Operative Procedures

In the experiments the following procedures were performed: control group (anaesthesia but no surgical procedure) sham hepatectomy, 70% partial hepatectomy (phX) and ileum resection (ilX).

In all animals, except in the control group, a laparotomy was performed under general anaesthesia using ether. The period of anaesthesia was the same for all groups. After shaving and cleansing of the abdomen with 70% alcohol the abdominal cavity was opened using a midline incision. The animals were randomly allocated to the control, sham or phX or ileum resection group. In both the phX and the sham group the supra- and infrahepatic fibrous attachments were cut through and the liver was exteriorised. Sham hepatectomy only consisted of gentle manipulation of the liver. PhX, which included resection of the left lateral and median

liver lobes (70% of total liver volume), was performed according to the method of Higgins and Anderson[21]. Briefly, the circulation in the left lateral and median liver lobes was interrupted by ligation of the hilar vessels with a 2/0 silk tie. Subsequently the ligated lobes were resected and the laparotomy wound was closed in one layer (2-0 NC-Silk, B. Braun Melsungen AG). In the ileum resection group a segment of approximately 8 cm of small bowel was extracted from the abdominal cavity. Subsequently approximately 4 cm of terminal ileum was resected, followed directly by an end-to-end anastomosis using a running suture (7-0 Perma-hand Seide, Ethicon). All animals were intravenously (iv.) injected via the penile vein with 3.5 x 10^5 CC-531s tumour cells in 0.5 ml RPMI 1640. The specific times of injection are described later. After 40 days the animals were sacrificed. After removal the lungs were rinsed in Phosphate Buffer Saline and immersed in Bouin fixation solution to enhance visibility of the tumour nodules.

Scoring of Lung Tumour

To determine the amount of tumour present in the lungs we used a modification of the scoring system used by Singh *et al.*[22,23]. The modification was made to be able to score the size of the tumour nodules in addition to the number of the nodules. We divided the number of tumour nodules semi-quantitatively in seven groups scoring from a minimum score of zero to a maximum of six (Table 1).

Table 1. Lung tumour scoring system

Score	Number and aspect of tumour nodules in lung
0	No tumour nodules visible on lung surface
1	< 20 tumour nodules in total on surface of both lobes
2	≥ 20 tumour nodules in total on surface of both lobes
3	Disseminated tumour nodules with < 5 elevated nodules
4	Disseminated tumour nodules with 5 – 10 elevated nodules
5	Disseminated tumour nodules with > 10 elevated nodules
6	As 5, with hypertrophy of lung

Experimental design

In the first experiment the effect of phX on the development of tumour nodules in the lungs was investigated. After performing a phX or sham operation, as described previously, tumour cells were injected iv. in the penile vein, directly after closing the abdomen. A group in which anaesthesia was given and tumour cells were injected iv. (but no surgical procedure was performed) served as the control group. All groups consisted of 10 WAG/Rij rats.

In the second experiment the timing of phX and sham operation in relation to the injection of tumour cells was studied. Therefore we performed phX and sham operation in three different groups: the operation was performed **24 hours before** the injection of tumour cells, **directly before** the inoculation of tumour cells or **24 hours after** the inoculation of tumour cells. All groups consisted of 10 rats.
In the third experiment the effect of phX on tumour take in the lungs was compared to the effect of an ilX. A sham hepatectomy group and a control group, in which no surgical procedure was performed, were added. In all groups tumour cells were iv. injected directly after closing of the abdomen, again all groups consisted of 10 rats.

Reverse Transcriptase PCR experiments

Growth factor and growth factor receptor expression profiles were analysed by RT-PCR. Oligonucleotides were divised to specifically amplify rat sequences. The primers were based on rat cDNA sequences present in the Genbank database using Primer Express software (Perkin-Elmer) (Table 2). The specificity of the oligonucleotides was checked by RT-PCR procedure using RNA from various rat tissues such as spleen, liver, lung and lymph nodes. Total RNA was isolated with RNAzol^{TM} B (Campro Scientific B.V., Veenendaal, The Netherlands) according to recommendations by the manufacturer. The procedure is essentially an improved version of the RNA isolation method described by Chomczynski and Sacchi[24]. One μg of total RNA was used as a template in a reverse transcriptase reaction containing 400 U M-MLV-Reverse Transcriptase, 40 U Rnasin, 0.5 mM dNTP's (Promega, Madison, WI) and 20 ng random primers (Gibco-BRL, Gaitherburg, MD) using the M-MLV reaction buffer supplied by Promega. Part of the reaction mixture was subjected to PCR amplification using the primer sets described above. The amplified DNA fragments were cloned into the pCR2.1^{TM} cloning vector (Introgen, Carlsbad, CA) and sequenced to verify the nature of the amplified product. The presence of mRNAs encoding various growth factors and growth factor receptors was determined in total RNA preparations of CC531 colon carcinoma cells and liver and lung tissue using an identical RT-PCR protocol.

In vitro experiments

Serum Withdrawal
For our serum experiments blood was obtained by cardiac puncture from animals that underwent phX at different time intervals (0, 2, 4, 8, 12, 24, 48 and 72 hours) after surgery. Control serum was obtained from non-operated rats. The blood of each individual animal was collected in a 8.5 ml SST^{TM} gel and clot activator test tube (Becton Dickinson, Woerden, The

Table 2. Growth factor and growth factor receptor primers used for RT-PCR

	Sequence
BFGF	5'-GCG GCT TCT TCC TGC GCA TC-3' (forward) 5'-CTG CCC AGT TCG TTT CAG TGC C-3' (reverse)
bFGF-R	5'-TGT AAG GTG TAC AGC GAT CCG CAG-3' (forward) 5'-GCC ACC TGA TAG GCA CAG GAT AC-3' (reverse)
EGF	5'-AAA GAG GTG GCA TCG TTG GA-3' (forward) 5'-CGC CAG CAA ATC CTT TCA AA-3' (reverse)
EGF-R	5'-CAG GGC CCA GAG AGA GTG ACT-3' (forward) 5'-GGA CAC ACG AGC CGT GAT CT-3' (reverse)
TGFα	5'-TGT GTC AGG CTC TGG AGA ACA-3' (forward) 5'-GAT CTG CAT GCT CAC AGC GA-3' (reverse)
HGF	5'-AAA GGC CAA GGA GAA GGT TAC AG-3' (forward) 5'-TCG GAT GTT TGG ATC AGT GGT A-3' (reverse)
HGF-R	5'-CCG ACA TTC AGT CCG AGG TT-3' (forward) 5'-GCC TCA CCG ATA TTG AAT GCA-3' (reverse)
IGF1	5'-GCT ATG GCT CCA GCA TTC G-3' (forward) 5'-AGA TCA CAG CTC CGG AAG CA-3' (reverse)
IGF1-R	5'-GAG CCT GGG AGA CCT CTT CC-3' (forward) 5'-GTG CAC CCT TGG AGC ATC TGG-3' (reverse)
TGFβ	5'-TCG ACA TGG AGC TGG TGA AA (forward) 5'-CGA GCC TTA GTT TGG ACA GGA (reverse)
TGFβ-R1	5'-CTG CCA TAA CCG CAC TGT CA (forward) 5'-CCT TTG CCG ATG CTT TCT TG(reverse)
TGFβ-R2	5'-ACA AGG CCA AGC TGA AGC A (forward) 5'-CGC CGT GAT CAG CCA GTA CT(reverse)
TGFβ-R3	5'-CAC CCT GTT GGA TCC TTC GT (forward) 5'-TCG CCT GAC TCC AAG TCT TCA (reverse)

Netherlands) and allowed to clot for 60 min at room temperature. Serum was separated from the cellular fraction by centrifugation for 20 min at 2500 rpm and the samples kept frozen at -80°C until being used.

Experimental design

CC531s cells (2 x 10^4) were plated in 24-well plates (Costar, Badhoevedorp, The Netherlands) in different rat serum (RS) or foetal calf serum (FCS) concentration (2.5%, 1% and 0.5%) or the combination of RS (2.5%, 1% and 0.5%) and 1% FCS in quadruplicate. The plates were placed in an incubator with a humidified atmosphere of 95% air / 5% CO_2 at 37°C. After 72 hours, the plates were collected by washing away superfluous non-adherent cells and kept at -20°C for DNA analysis (described below). The optimal serum concentration proved to be 0.5% RS without addition of FCS (data not shown). Therefore, this concentration is used in the following experiment.

In this experiment, CC531 cells were plated in 24-well plates, using $2x10^4$ cells in 995 μl RPMI. Of each experimental condition (defined by the different time intervals after phX) 5 μl RS was added per well, to a final serum concentration in the well of 0.5%. The amount of medium was the same in all wells. Cells for each experimental serum were plated in quadruplicate. Plates were prepared for DNA analysis as described above. All experiments were performed twice.

DNA-assay

The DNA content of the tumour cells was determined using the bisbenzimide fluorescent dye (Roche Diagnostics) as described previously by Hofland *et al*.[25]. In short, after washing of the plates and storing at -20°C the cells were extracted from the plates with ammonia solution (1 mmol/L) – Triton x 100 (0.2% v/v) by sonification during 5 seconds at amplitude 15 (Soniprep 150; MSE). Thereafter assay buffer (100 mmol/L NaCl, 10 mmol/L EDTA, 10 mmol/L Tris; pH 7.0) was added. The remaining solution was centrifuged at 2000*g* during 5 min and 100 μl aliquots of the supernatant was mixed with 2 ml Hoechst dye H33258 (100 μg/L). Fluorescence was measured after 10 min with excitation and emission wavelengths set at 350 and 455 nm respectively. The fluorescence of experimental samples was referenced to a standard curve of calf thymus DNA (type II, no D-3636; Sigma, Zwijndrecht, The Netherlands).

Statistical analysis

All data are expressed as means ± SEM. Statistical analysis for all *in vivo* experiments was performed using the non-parametric Kruskal-Wallis analysis of variance, to determine overall differences, followed by the non-parametric Mann-Whitney U test, to compare differences between groups. For the *in vitro* serum experiments the Oneway ANOVA was performed with the Dunnett t (2 sided) test as a post hoc test. Statistical significance was accepted at the $p < 0.05$ level.

RESULTS

PhX and tumour recurrence

In the first experiment the effect of phX on the development of tumour take in the lungs was compared with sham hepatectomy and a control group. The tumour cells were injected in the penile vein directly after closing the abdomen. In the phX group the mean tumour score was statistically significantly higher than in the sham hepatectomy and control group (4.7 ± 0.4 *vs* 3.0 ± 0.0 *vs* 2.4 ± 0.2; p=0.002 and p=0.001; figure 1).

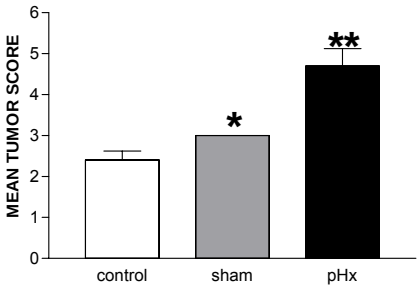

Figure 1. The effect of partial hepatectomy (phX) on remote tumour take in the lungs, compared to sham hepatectomy (sham) and control group (no surgical procedure). In all animals tumour cells were injected iv. in the penile vein. In the phX and sham groups the tumour cells were injected directly after closing the abdomen. In the phX group the mean tumour score was statistically significant higher than in the sham hepatectomy (p=0.002) and control group (p=0.001). Data are shown as mean ± SEM.

Growth factor and growth factor receptor expression profile

To find out whether these results could be explained by a growth stimulating effect of growth promoting factors released during liver regeneration after partial hepatectomy, RT-PCR was performed. In this way, the ability of CC531s colon carcinoma cells to express the main growth factor receptors (bFGF-R, EGF-R, HGF-R, IGF1-R, TGFβ-R1, TGFβ-R2 and TGFβ-R3) involved in liver regeneration was investigated. Furthermore lung and liver tissue was examined by RT-PCR for the expression of serum growth factors and associated receptors.
The integrity of total RNA isolated from CC531s, liver and lung tissue was checked on agarose gel. Amplified fragments of the expected size were found.

The results show that CC531s cells express a mRNA pattern of growth factor receptors that would allow growth stimulation of these cells by growth factors produced by the regenerating liver (Table 3). Messenger RNA for all growth factors and their associated receptors, were present in the liver. Also in the lung, apart from bFGF-R, all investigated growth factors and their receptors could be detected.

Table 3. Expression profile of growth factors and growth factor receptors.

	Liver	Lung	CC531 cells
BFGF	+	+	+
BFGF-R	+	-	-
EGF	+	+	-
EGF-R	+	+	+
TGFα	+	+	+
HGF	+	+	-
HGF-R	+	+	+
IGF	+	+	-
IGF-R	+	+	+
TGFβ	+	+	-
TGFβ-R1	+	+	+
TGFβ-R2	+	+	+
TGFβ-R3	+	+	+

Growth stimulation of CC531 after incubation with rat serum

CC531 colon carcinoma cells were cultured for 72 hours in RPMI medium at a concentration of 0.5% rat serum obtained from 0, 2, 4, 8, 12, 24, 48 and 72 hours after phx. The total amount of DNA was compared to cells cultured in medium with serum from non-operated rats. At none of the time-points a statistically significant difference was observed (figure 2).

Timing of phX in relation to tumour cell inoculation

Since no stimulation of growth was observed, a tumour cell adhesion promoting effect was suspected. Therefore, the timing of phX and sham operation in relation to the inoculation of tumour cells was investigated in the second *in vivo* experiment. The mean tumour score in the group in which the phX was performed directly before the inoculation of tumour cells (day 0) was significantly higher than in the day –1 group ($p=0.014$) and in the day +1 group ($p=0.011$). When the day 0 group was compared to the sham groups, the mean tumour score in the day 0

group was again significantly higher (vs sham day –1 p=0.005; *vs* sham day 0 p=0.008; vs sham day +1 p=0.031). These results are summarized in figure 3 and table 4.

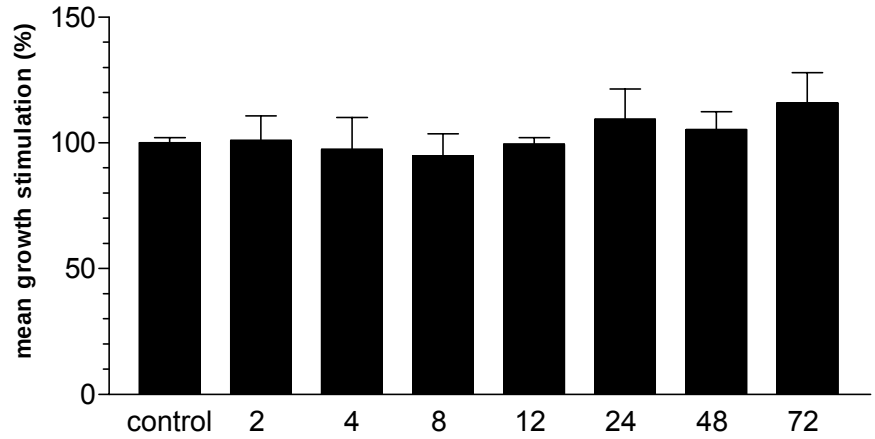

Figure 2. Growth stimulation of CC531s colon carcinoma cells after incubation with rat serum. CC531s cells were cultured for 72 hours in RPMI medium at a concentration of 0.5% rat serum, obtained at 0, 2, 4, 8, 12, 24, 48 and 72 hours after phX. The total amount of DNA was compaired to cells cultured in medium with serum from non-operated rats (control). At none of the time-points a statistically significant difference was observed (p=n.s.). Data are shown as mean ± SEM.

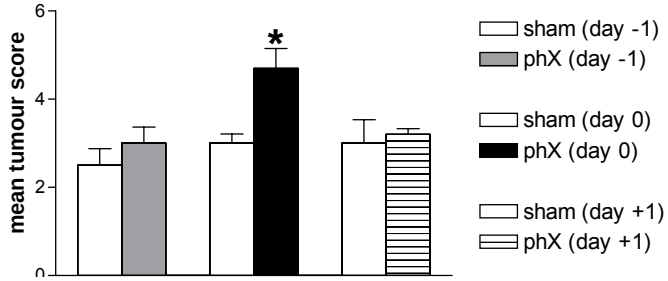

Figure 3. The effect of timing the partial hepatectomy (phX) in relation to the injection of tumour cells on remote tumour take in the lungs. Partial hepatectomy and sham hepatectomy was performed 24 hours before (day –1), directly before (day 0) or 24 hours after (day +1) injection of tumour cells in the penile vein. Compared to all other groups only the group in which phX directly preceded the injection of tumour cells (phX (day 0) scored statistically significant higher (* p≤0.031). Data are shown as mean ± SEM.

Table 4.

Group	Mean tumour score	SEM	p value *vs* phX (day 0)
sham (day –1)	2.5	0.4	0.005
phX (day –1)	3.0	0.4	0.014
sham (day 0)	3.0	0.2	0.008
phX (day 0)	4.7	0.4	
sham (day +1)	3.0	0.5	0.031
phX (day +1)	3.2	0.1	0.011

Influence of major surgery in general on tumour recurrence

The question remained whether partial hepatectomy is a special entity in enhancing tumour recurrence. To examine the influence of major surgery in general on tumour recurrence the effect of phX on tumour take in the lungs was compared with ilX. The mean tumour score was increased in both the phX and the ilX group as compared to the control group, in which no surgical procedure was performed (phX: 4.9 ± 0.6, p=0.004; ilX**: 4.9 ± 0.4, p=0.001; sham: 2.8 ± 0.1; figure 4).

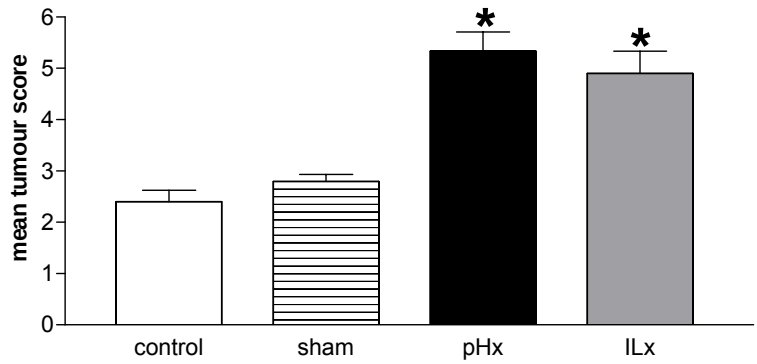

Figure 4. The effect of partial hepatectomy (phX) on remote tumour take in the lungs, compared to ileum resection (ilX). The sham hepatectomy group (sham) and control group (no surgical procedure) served as controls. In all animals tumour cells were injected iv. in the penile vein. In the phX, ilX and sham group the tumour cells were injected directly after closing the abdomen. In both the phX and the ilX group the mean tumour score was statistically significant higher than in the sham hepatectomy (*vs* phX p<0.001; *vs* ilX p=0.001) and control group (*vs* phX p<0.001; *vs* ilX p=0.001). Data are shown as mean ± SEM.

DISCUSSION

Experimental and clinical research has been done to elucidate the effect of surgical trauma on tumour recurrence. As early as 1958 Lewis and Cole described an increase of lung metastases after the surgical amputation of a tumour bearing hind limb in the mouse[26]. Possible explanations for the effect of major surgical trauma have been focussed on the release of growth-promoting factors after major surgical trauma[27]. The relation between surgical trauma and tumour take became further apparent by studies on metastases in surgical wounds[28] and on port site metastases after laparoscopic surgery[29]. Previous studies demonstrated that there is a significant correlation between the amount of surgical trauma and the degree of local tumour take in the rat[6,30,31]. Not only was it found that surgical trauma enhances the development of local tumour take, but to distant sites as well[32-34].

A direct parallel can be drawn with the influence of phX and subsequent liver regeneration on tumour take, as liver regeneration is often considered a specific form of wound healing[12]. The fact that the liver has the unique ability to regenerate after surgical resection has made research concerning the effect of phX on tumour recurrence a special entity. Various studies reported a stimulating effect of liver regeneration on microscopic residual tumour present in the remnant liver, both clinically and experimentally[11,35,36]. The aim of the present study was to elucidate the effect phX might have on distant metastases and to see whether this effect concerned tumour growth only. In the first *in vivo* experiment we clearly demonstrated that performing a 70% phX leads to a statistically significant increase of tumour nodules in the lung compared to sham hepatectomy and control group (Figure 1). Several possible explanations can be postulated for this observation. PhX has been reported to lead to immunosuppression and consequently to enhanced growth of immunogenic tumour. However, the CC531 cells used are only weakly immunogenic. Another explanation can be that growth factors, produced during liver regeneration, may influence the growth of distant metastases in the lungs, especially when there is expression of growth factor receptors on these tumour cells. A third explanation might be that these growth factors and / or cytokines modulate the expression of adhesion molecules on tumour cells or on host tissue. TNFα, for instance, plays an important role in hepatic regeneration by inducing hepatic DNA synthesis and cell proliferation[37]. On the other hand, TNFα is also noted for the enhancing effect on the expression of several adhesion molecules[38,39].

In order to verify whether CC531s tumour cells could directly be influenced by growth factors, produced during liver regeneration, we determined the growth factor and growth factor receptor expression profile of the tumour cells and the target tissues (Table 4). As messenger RNA for the EGF-receptor (being the receptor for both EGF and TGFα), the HGF receptor, the IGF1-receptor and all the TGFβ-receptors (type 1,2 and 3) were present on the tumour cells, direct stimulation of these cells by corresponding growth factors can be

expected, although the presence of messenger RNA for these receptors does not necessarily implicate the presence of biologically functional receptors. Thus, theoretically the growth of our tumour cells can be stimulated by several growth factors released during liver regeneration, such as EGF and HGF which have been shown to increase in serum of rats after phX[40,41]. However, in our present *in vitro* experiments with serum obtained from rats at different time intervals after phX we could not detect any stimulation on tumour cell growth.

Our *in vivo* experiments provide evidence that phX exerts its effect in the early phase of tumour take. When performing phX a day before or after the injection of tumour cells, no difference in number of tumour nodules compared to sham hepatectomy was seen (Figure 2). Only phX performed directly before the injection of tumour led to enhanced tumour recurrence in the lungs, which suggests an increase in tumour cell adhesion in the lungs instead of stimulation of tumour cell growth.

Our finding that not only phX, but also ilX leads to enhanced tumour nodules in the lungs suggests that surgery in general might have a stimulating effect on tumour cell adherence. Even a sham hepatectomy led to enhanced tumour nodules compared to the control group in which no operation was performed.

Therefore, we hypothesise that the enhanced tumour take seen in our experiments after surgery may be the consequence of an upregulation of adhesion molecules on the surface of circulating tumour cells, the host tissue (*i.e.* the lung) or both. During trauma, like in surgery, circulating mononuclear phagocytic cells are activated with the production of pro-inflammatory cytokines, such as IL-1, IL-6 and TNFα. IL-1β and TNFα can induce the expression of adhesion molecules, such as ICAM-1, VCAM-1 and LFA-1 on the endothelial surface[36-39,42-44]. Both the tumour cells used and the lung tissue were shown to possess receptors for several cytokines known to be involved in adhesion molecule expression modulation (data not published). Tumour cells may use the following abundance of adhesion molecules on host tissue to facilitate their penetration through and anchorage to host tissue. Indeed, in an experimental mouse melanoma model it was found that TNF-α and VCAM-1 (vascular cellular adhesion molecule) were involved in surgical stress enhanced metastasis[45].

In summary, we have shown that phX, but also ilX, leads to enhanced remote tumour recurrence in the lung. This phenomenon seems not to be mediated through growth factors produced after phX and their effect on growth of tumour cells. As the enhanced tumour recurrence is only seen when the surgical intervention coincides with the injection of tumour cells we plan to direct our future research towards a better understanding of the adhesive interactions between tumour cells and host tissue. The presence of disseminated tumour cells in patients undergoing liver resection for colorectal cancer in more than 50% of patients underlines the importance of studies to obtain better insight in the mechanism of enhanced adhesion of circulating tumour cells after surgical procedures. This may lead to the

development of strategies to prevent this common pathway of tumour recurrence after surgery.

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