CLIN. EXPL. METASTASIS, 1991, VOL. 9, NO. 4, 335–349

Facilitation by partial hepatectomy of tumor growth within the rat liver following intraportal injection of syngeneic tumor cells

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(*Received 20 July 1990; accepted 8 January 1991*)

The effects of both mechanical trauma and regeneration on the growth of intraportally injected tumor in the rat liver were investigated using two-thirds partial hepatectomy (PH). Tumor grew at the excision scar when PH was performed less than 2 days before tumor injection (34/34 animals). However, when the PH was performed 4–7 days before injection, tumor developed within the regenerating lobe, but not at the scar (50/51). Injecting the same cell dose into rats with intact livers caused few tumors to develop in 12/30 animals. Intraportally injected ^{51}Cr-labelled tumor cells distributed uniformly in the liver irrespective of the time after PH. Patterns of tumor take seen at different times after PH were not due to selective trapping of the injected cells. Liver extracts showed that epidermal growth factor-like activity was unaltered by PH, while heparin-binding growth factor activity peaked at 2 days post-PH, before the incidence of tumor growth in the parenchyma increased. We observed two peaks of DNA synthesis at days 1 and 4 post-PH by pulse labeling with [^{125}I]deoxyuridine and bromodeoxyuridine. Bromodeoxyuridine immunohistochemistry showed the first peak to be confined to hepatocytes. The second peak involved non-hepatocytes and coincided with the beginning of enhanced tumor take in the regenerating lobe.

Introduction

The liver is clinically a major site of metastatic growth but the factors which determine whether a tumor cell which has arrested in the liver will develop into tumor are not as yet clear, either in the clinical situation or in experimental models.

That trauma enhances the ability of a tissue to support the growth of cancer cells was first observed by Jones and Rous [15] and has subsequently been seen in a wide variety of tissues in a number of species [1, 6, 7, 14, 25, 26]. When the syngeneic MC28 sarcoma—the cancer used in this study—was injected into rats intra-arterially, parts of tissues that were traumatized grew tumor preferentially compared to untraumatized areas of the same tissues. In this model, if the muscle in the colon had been wounded by an end-to-end anastomosis prior to tumor inoculation or if the muscle in the peritoneum had been traumatized by a laparatomy incision, the probability that a single cell trapped in wounded muscle would develop into a tumor was more than a thousand times greater than for a cell trapped in healthy muscle [22, 26]. Fisher and Fisher [6–9] reported that,

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when given intraportally, allogeneic Walker carcinoma cells grew in the liver of rats from smaller innocula if the liver had been traumatized mechanically, by administration of carbon tetrachloride or had undergone partial hepatectomy. Murphy *et al.* [23] carried out similar studies with the syngeneic rat sarcoma MC28 and found that more than 95% of radioactively labeled cells arrested in the liver when given intraportally and that the same types of trauma as had been investigated by Fisher and Fisher greatly facilitated MC28 take in the liver when a single cell suspension of tumor was given intraportally or intra-arterially via left ventricular injection.

We chose to investigate the partial hepatectomy model which offered us the opportunity to study, in the same organ, the effect of trauma on tumor growth—at the surgical excision site— and the effect of regeneration on tumor growth—in the untraumatized parenchyma of the remaining lobes. Both healing following trauma [13, 17, 19, 24] and regeneration following partial hepatectomy [4, 12, 18, 21, 27] are characterized by cell proliferation which is the result of a well-ordered but not perfectly understood cascade, which involves growth factors. Healing also involves an influx of inflammatory cells [19] while regenerating liver accepts an influx of blood; also, circulating monocytes may be involved in Kupffer cell repopulation [2, 3].

In the present paper, the growth of MC28 sarcoma cells delivered intraportally was determined at different stages of liver regeneration following partial hepatectomy (PH). The extent of the enhancement of tumor take was quantified at both the sites of trauma and regeneration. Experiments that were conducted to elucidate the mechanisms that facilitate tumor growth investigated patterns of cell trapping, cell proliferation and growth factor profiles over the time of regeneration after PH.

Materials and methods

Animals and tumor

The transplantable, syngeneic, methylcholanthrene induced MC28 sarcoma was propagated in young (250 g), adult, hooded Lister rats of either sex, by subcutaneous passage of small tumor fragments. The rats were maintained on a 12 h light/dark cycle. Within 2 weeks of implantation, the tumor grew to approximately 15 g when it was suitable both for subsequent passage and for the preparation of a cell suspension. To avoid biological drift the tumor was re-established from a frozen stock after approximately eight passages.

Tumor suspension

The excised tumor was mechanically disaggregated in Hanks' balanced salt solution (HBSS) and necrotic areas were discarded. A single cell suspension was obtained by incubating tumor at room temperature on a slowly rotating magnetic stirrer, with a protease/DNAse mixture (0·5 and 0·005 mg/ml, respectively, Sigma) for 30 min. Large, undigested fragments were allowed to settle out and the cell-rich supernatant was washed and spun for 5 min, 300 g, 3 times in HBSS. Cell counts and viability were determined by Trypan Blue dye exclusion, using a hemocytometer and the concentration of viable cells was adjusted to 10^6/ml.

Chromium labeled MC28 cells

Five million MC28 cells in 5 ml of HBSS were incubated with 100 μl of chromium-51 (Amersham, CJS1) for 30 min at 37 °C, mixing very gently. The cells were then spun at 300 g for 5 min, the supernatant was discarded appropriately and 10 ml of HBSS were added to the pellet. The washing procedure was repeated at least four times to ensure that the unbound chromium was removed from the preparation. Cell counts were determined as above and the concentration was adjusted to 5×10^6/ml.

Two-thirds partial hepatectomy

PH involved the removal of the median and left lobes under ether anesthesia, leaving behind the right and caudate lobes which constitute approximately 33% of the liver [11].

Intraportal injections

To avoid the risk of causing trauma by manipulation of the liver, a suspension of single tumor cells or radioactive chromium-labeled cells were introduced into the liver by cannulating a tributary of the superior mesenteric vein, under ether anesthesia. Fine ($0{\cdot}4 \times 12$ mm) needles were used and care was taken to prevent any back flow of blood.

Relative timing of intraportal injection to PH

Tumor cells were injected at different times after PH had been performed. The times chosen were 10 min (day 0), 1, 2, 3, 4, 5, 6, 7 and 9 days.

Post-mortem examinations (tumor incidence)

Animals were killed 14 days after cell injection and the liver was sectioned with a scalpel into approximately 2 mm sections. The presence of tumor was assessed visually at three situations: (a) at the excision scar, (b) on the surface of the right lobe and (c) within the parenchyma of the right lobe. Tumor growing on or in the liver as opposed to the scar was classified into two groups, less than five (< 5) and equal or more than five foci present ($\geqslant 5$). Representative cases were checked histologically and were invariably identified as sarcoma.

Post-mortem examinations (initial arrest of tumor cells)

To investigate the trapping of malignant cells in the liver after intraportal inoculation, 10^6 ^{51}Cr labeled MC28 cells were injected at two different times after PH: day 0 (i.e. 10 min) and day 5. The animals were killed within 10 min of injection. The livers were excised and segmented in seven parts; four for the right lobe and three for the caudate (Figure 3). Each fragment was weighed and radioactivity was measured in a gamma-counter.

IUDR/BUDR injections

To investigate stimulation of DNA synthesis after PH, 200 μCi of ^{125}IUDR (Amersham, IM355) mixed with 5 μg/g body weight of BUDR ($1{\cdot}5$ ml total volume) were injected into the tail veins of partially hepatectomized rats at different times after PH (10 min, 1, 2, 3 ... 10 days). The animals were killed between 4 and 6 h after injection. The livers were excised and representative segments were taken from each lobe for immunohistochemistry. After 5%

trichloroacetic acid precipitation, acid-insoluble material from weighed segments from the two lobes were counted in a gamma-counter.

Immunohistochemistry

Liver samples were fixed in 10% formalin for at least 48 h and after routine wax processing using the procedure according to Magaud *et al.* [20] and preferentially using 1 N hydrochloric acid at 60 °C for 20 min as a denaturing agent. The antibody was incorporated into DNA and visualized by the avidin/biotin procedure (Sigma).

HPLC/mitogenic assay

Animals. PH was performed on male rats (300–350 g). Animals were killed at 2, 5 and 21 days post-PH. All operations took place between 2 and 2.30 p.m. to minimize any possible diurnal fluctuations in growth factor concentration.

Tissue extractions. Liver samples (1 g) were homogenized in either 10 ml of acid [1 M acetic acid, 0·1% bovine serum albumin (BSA), 1·0 mM phenylmethyl sulfonylurea (PMSF), 0·2 μM aprotinin and 20 μM leupeptin protease inhibitors] or 10 ml of Tris buffer (20 mM Tris–HCl, pH 8·0, 0·14 M NaCl, 0·1% BSA). Samples were extracted for 1 h at 4 °C and centrifuged at 20,000 ***g*** for 30 min at 4 °C. Acid extracts were neutralized to pH 7·4 and sediment was precipitated by the same centrifugation regime. Supernatants were dialyzed against 25 mM Tris–HCl, pH 7·4, for 16 h.

Protein concentration was determined with the MICRO BCA method (Pierce, Life Science Laboratories, U.K.).

Before testing on the mitogenic assay, the liver extracts were further treated in one of two ways: (1) fractionation of acid liver extracts—samples were fractionated by anion exchange chromatography using a fast protein liquid chromatography system (FPLC Pharmacia, Upsala, Sweden) on a Mono Q HR5/5 column. A sample containing 25 mg of protein was loaded onto the column in 25 mM Tris–HCl, pH 7·4, and bound protein was eluted with a linear gradient of NaCl (0–0·35 M). The concentration of NaCl used throughout the gradient does not significantly alter EGF- or TGFa-stimulated mitogenesis. (2) Heparin extraction of Tris buffer extracts—heparin Sepharose gel CL6B (Pharmacia, Upsala, Sweden) was equilibrated with 25 mM Tris–HCl, pH 7·4, 0·14 M NaCl and 1% BSA. One part gel was mixed with two parts of the liver buffer extract and incubated for 2·5 h at 4 °C. The gel was then separated from the extract by low speed centrifugation and the supernatant was retained for assaying. The bound growth factors were eluted sequentially by incubation with 0·6 M and then 2·0 M NaCl in 25 mM Tris–HCl, pH 7·4, 1% BSA. Eluates were desalted on a BioRad 10DG (BioRad Labs, Richmond, CA, U.S.A.) equilibrated in Tris–HCl, pH 7·4, 0·14 M NaCl, 1% BSA. Samples were filter sterilized and assayed for growth factor induced activity.

Assay for mitogenic activity of liver extracts. DNA synthesis was determined by measuring the incorporation of ^{125}IUDR into DNA of low passage human foreskin fibroblasts (HFF). These cells were obtained from circumcision tissue samples and seeded in 96-well plates at $2{\cdot}5 \times 10^4$ cells per well in Dulbecco's modified Eagles medium (DMEM), containing 10% foetal calf serum (FCS). The cells were incubated for 4 days at 37 °C in a humidified air atmosphere (5% CO_2). On day 4, when the cells were confluent, the medium was replaced with

1% FCS in DMEM and cells were incubated for a minimum of 48 h to allow for complete depletion of growth factor activity from the medium. The extraction samples resulting from either the acid or buffer processes were applied to the wells in serum-free medium (equal volumes of phosphate-buffered saline and DMEM containing 12·5 mM HEPES, 1% BSA, 8 μg/ml insulin and 240 μg/ml transferrin). After incubation for 24 h at 37 °C, cells were labeled with 0·125 μCi ^{125}IUDR (sp. act. 1963 Ci/mmol) plus 20 μM FUDR. Cells were incubated for a further 2 h at 37 °C and were then washed and fixed three times with 5% trichloroacetic acid at 4 °C and finally washed twice with absolute alcohol.

The cells were left to dry, solubilized in 0·2 M NaOH and samples were counted on a LKB1282 gamma counter. Results were compared with a standard curve of IUDR incorporation obtained by treating cells with increasing concentrations of human EGF (Leu 21 EGF) or fibroblast growth factor (FGF bovine brain, both peptides from Boehringer, Mannhein, East Sussex, U.K.).

To distinguish growth factors which act via the EGF receptor samples were run (where indicated) in the presence of anti-receptor antibody. The antibody (purified by ammonium sulphate precipitation and DEAE ion exchange chromatography) was raised in a sheep against EGF receptor affinity purified from A431 plasma membrane vesicles. The antibody is known to inhibit EGF- and TGFa-induced mitogenesis but does not inhibit that induced by PDGF or FGF.

Results

Tumor cell injections into rats with intact livers

Rats with intact livers were intraportally injected with 10^5 MC28 cells. When they were killed, 14 days afterwards, no tumor was observed in the liver or elsewhere (15/15); when 2×10^5 MC28 cells in 0·2 ml HBSS were injected, 12/30 animals (40%) were found to have liver tumors. By 14 days after inoculation, these had grown into small foci of not more than 0·5 cm diameter and the number of foci per rat was less than 5. It is noteworthy that tumors were confined to the surface of the liver and none could be detected internally. When 10^6 cells were injected, then 10/10 rats had large numbers of foci, but again these were entirely superficial. This pattern of tumor growth is characteristic for intraportally injected MC28 cells in animals with an intact and untraumatized liver.

Tumor cell injections into partially hepatectomized animals

MC28 cells (2×10^5) in 0·2 ml were injected into different groups of animals within 10 min following PH (day 0) or at 1, 2, 3, 4, 5, 6, 7 and 9 days following PH. Each group had from 9 to 14 animals and they were killed 14 days after injection. The site of tumor growth was markedly different from that seen in rats with intact livers. If the cells were delivered soon after PH (between days 0 and 2), tumor growth was almost entirely confined to the suture line, i.e. the line of excision of the median and left lobes. If the interval between PH and the injection of the cells was greater than 2 days, large numbers of tumor foci were observed within the parenchyma of the regenerating lobes, but not at the scar.

Figure 1 shows that tumor growth in the scar reached 100% in the first 2 days after PH and then fell rapidly towards zero values as the time interval between

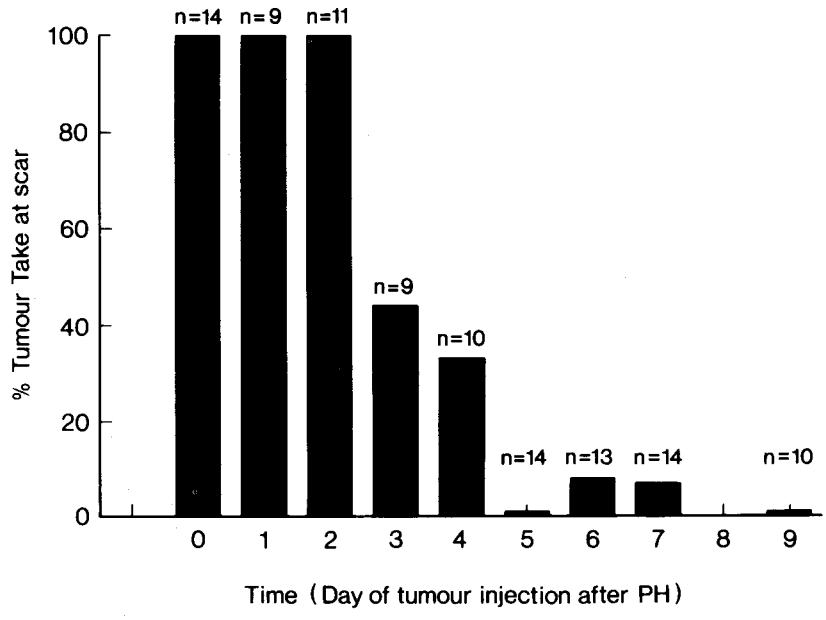

Figure 1. Tumor take associated with trauma. Partial hepatectomy was performed on day 0; the *a*-axis shows the time in days of the intraportal MC28 injection (2×10^5 cells), after PH. The *y*-axis represents the percentage of animals from each group that grew tumor at the surgical excision scar. Animals (n = number per group, accumulated from three experiments) were scored positive or negative for tumor at the scar 14 days after injection.

PH and tumor injection was extended. While tumor associated with the scar was at its maximum, a few (< 5) small tumor foci also appeared on the surface of the remaining right and caudate lobes and these were similar in incidence and location to the lesions seen when tumor was injected into rats with intact livers. Tumor growth at the scar was indistinguishable between the groups that received cells 10 min (day 0) or 2 days after PH, despite the fact that by day 2 the total weight of the liver had returned to 75% of normal (unpublished data).

Figure 2 shows that in the groups where PH was performed 3–7 days prior to cell injection, there was a striking change in the location of tumor growth. Growth in the scar decreased and tumor foci were found within the parenchyma of the regenerating lobes, both superficially and internally. The data shown corresponds to the right lobe. The caudate lobe grew to varying extents, if at all, and therefore these studies on the role of regeneration on tumor growth were confined to the right lobe which always grew extensively during regeneration. Maximum tumor incidence occurred within the right lobe when cells were injected 5 and 6 days following PH; the number of foci was variable and in a fraction of animals was very large, often exceeding 100.

By day 9, tumor incidence fell to 40–50% and the number of foci was < 5 and these were all superficial (Figure 2), i.e. tumor growth resembled that seen in rats with intact livers.

The minimum number of intraportal MC28 cells necessary to produce foci at

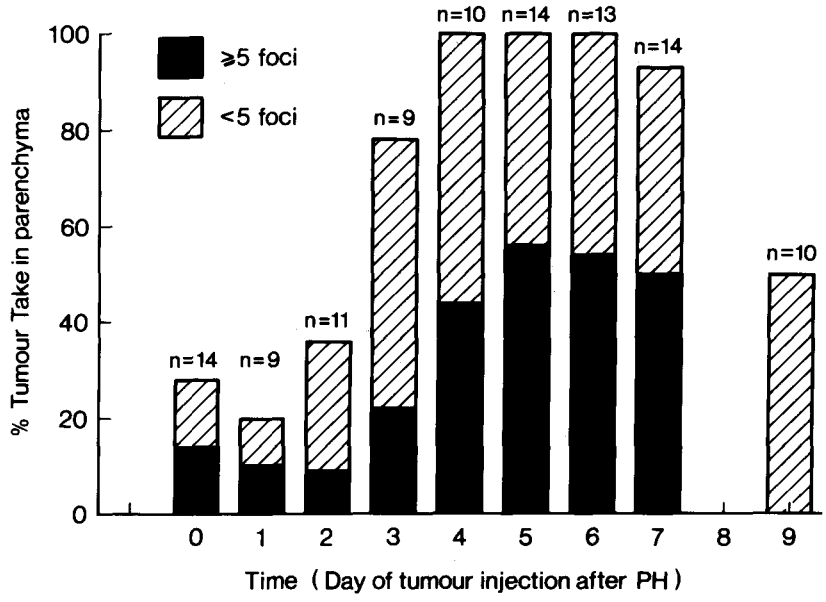

Figure 2. Tumor take associated with regeneration. Partial hepatectomy was performed on day 0; the x-axis shows the time in days of the intraportal MC28 injection (2×10^5 cells), after PH. The y-axis represents the percentage of animals from each group that had tumor within the regenerated right lobe. Animals (n = number per group, accumulated over three experiments) were scored positive (≥ 5 foci or < 5 foci), or negative for tumor within the parenchyma of the right lobe 14 days after injection.

the scar at 0–2 days after PH was 10^3, while the number of cells necessary to produce foci in the regenerating lobe at day 5 was 10^4.

Trapping of ^{51}Cr labeled MC28 cells and their distribution during regeneration

The trapping of MC28 cells within the liver following PH and regeneration was measured by innoculating with radiolabeled tumor cells. 10^6 cells in 0·2 ml of HBSS were injected intraportally in two groups of four animals at either 20 min or 5 days after PH. The animals were killed within 15 min of injection and the livers were excised and segmented (see Figure 3 insert).

Figure 3a shows that the distribution of radioactive cells within the livers of four rats at 5 days post-PH was similar for the right and caudate lobes and also for the different segments within each lobe ranging from 9·8 to 15·1 cpm/mg, with a slight drop of radioactivity at the tip. The distribution of cells in the livers of four rats at day 0 after PH (Figure 3b) was also of the same order throughout both lobes, ranging from 14–28 cpm/mg. The two experiments were conducted on different days, hence the difference in activities for the MC28 cells.

^{125}IUDR/BUDR incorporation

1·5 ml of 200 μCi IUDR and 5 μg/g body weight BUDR were injected intravenously 0–10 days after PH ($n = 25$) and the animals were killed 4–6 h later. The livers were excised and segmented for gamma-counting and samples were fixed for BUDR staining. Figure 4 shows the uptake of ^{125}IUDR in the

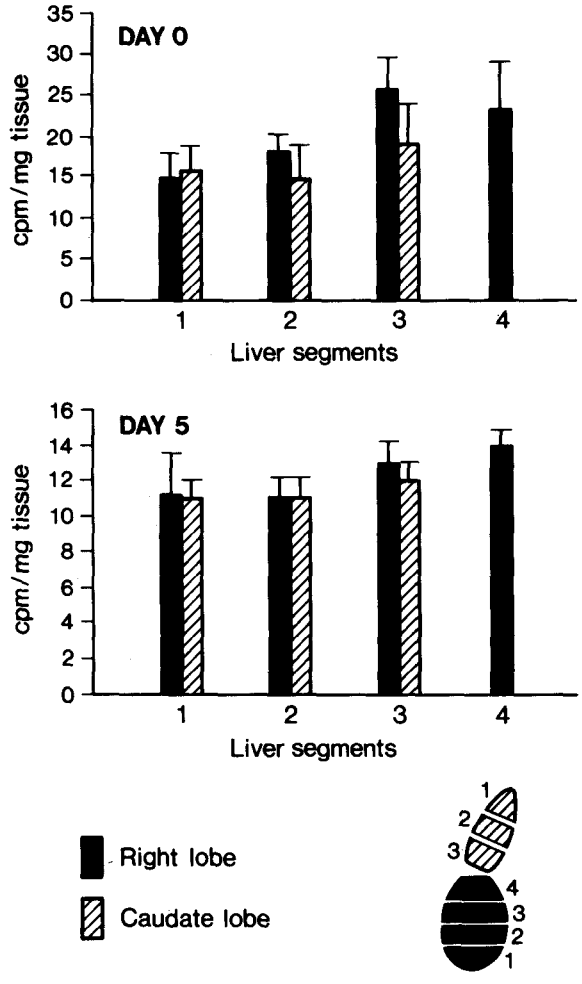

Figure 3. Uniform ^{51}Cr labeled MC28 cell distribution in the regenerating liver. 10^6 cells were injected intraportally at either 10 min after PH (for day 0, $n = 4$) or 5 days after PH (for day 5, $n = 40$). The livers were excised within 15 min of injection and segmented as shown in the insert (right lobe, 4 segments; caudate lobe, three segments). Counts plotted on the y-axis are the mean values for four animals ± SEM, corrected for weight of liver section.

regenerating right lobe at different times after PH. There are two incorporation peaks at day 1 (98 cpm/mg) and day 4 (58 cpm/mg). Regeneration did not vary within any one region of the right lobe. This corresponds to the classical pattern of IUDR or radioactive thymidine uptake seen in the regenerating rat liver [5, 10]. Incorporation of BUDR (Figure 5a) was seen only in hepatocytes on day 1 post-PH and 35% of parenchymal hepatocytes in the right lobe were stained. Figure 5b shows incorporation at day 4; most BUDR uptake was observed in elongated structural cells and littoral cells, i.e. sinusoidal endothelial cells, Kupffer cells and perhaps Ito cells (25% of non-hepatocytes in the right lobe were stained) although some hepatocytes were also stained.

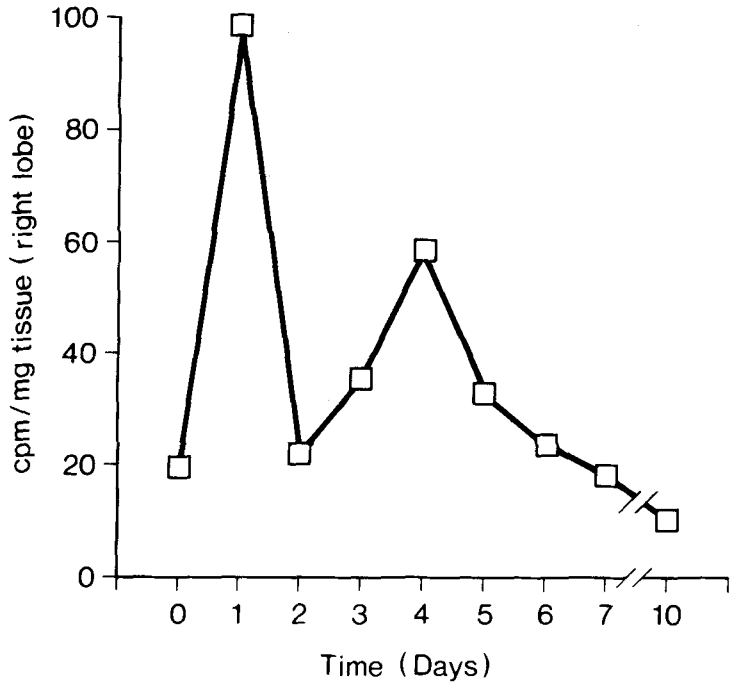

Figure 4. ^{125}IUDR incorporation in the regenerating right lobe. IUDR (200 μCi) was injected intravenously at different times after PH (x-axis: days 0–8, n = 18) and the livers were excised 4–6 h afterwards. The y-axis shows counts/mg wet weight of tissue. The largest SEM was 3·2 cpm/mg; for clarity SEMs are therefore omitted.

Isolation of growth factors from acid and buffer extracts of liver at different times after PH

We measured the actual mitogenic activity present in acid and buffer extracts from livers before and after PH, using a bioassay set up with human foreskin fibroblasts. Tris–HCl buffer extracts contained growth factors which bind strongly to heparin; acid extracts of livers contained growth factors which initiate mitogenicity by binding to the EGF-receptor (EGFR) and these were tested both in the absence and presence of an antibody directed against EGFR. This antibody abolishes mitogenicity of growth factors such as EGF and TGFa.

Figure 6 shows the mitogenic action of the different fractions from the anion exchange column which was capable of being inhibited by the antibody directed against EGFR. These curves were derived by subtracting mitogenic activity which was not inhibitable by antibody against EGFR. The peak of action of growth factors binding to EGFR was seen in fraction 7 (0·47 ng EGF equivalent) and it was in this fraction also that rat TGFa appeared (data not shown). Figure 6 shows that there was no significant change in the activity of growth factors binding to EGFR at 2, 5 and 21 days after PH from that found in normal liver. Our assay measures activity of all of the growth factors binding to EGFR.

Table 1 shows the mitogenic activity of an extract made from livers with buffer at pH 8·0 and which was bound to heparin and extracted with 2 M NaCl. The mitogenic activity is expressed as equivalent for a weight of FGF. This

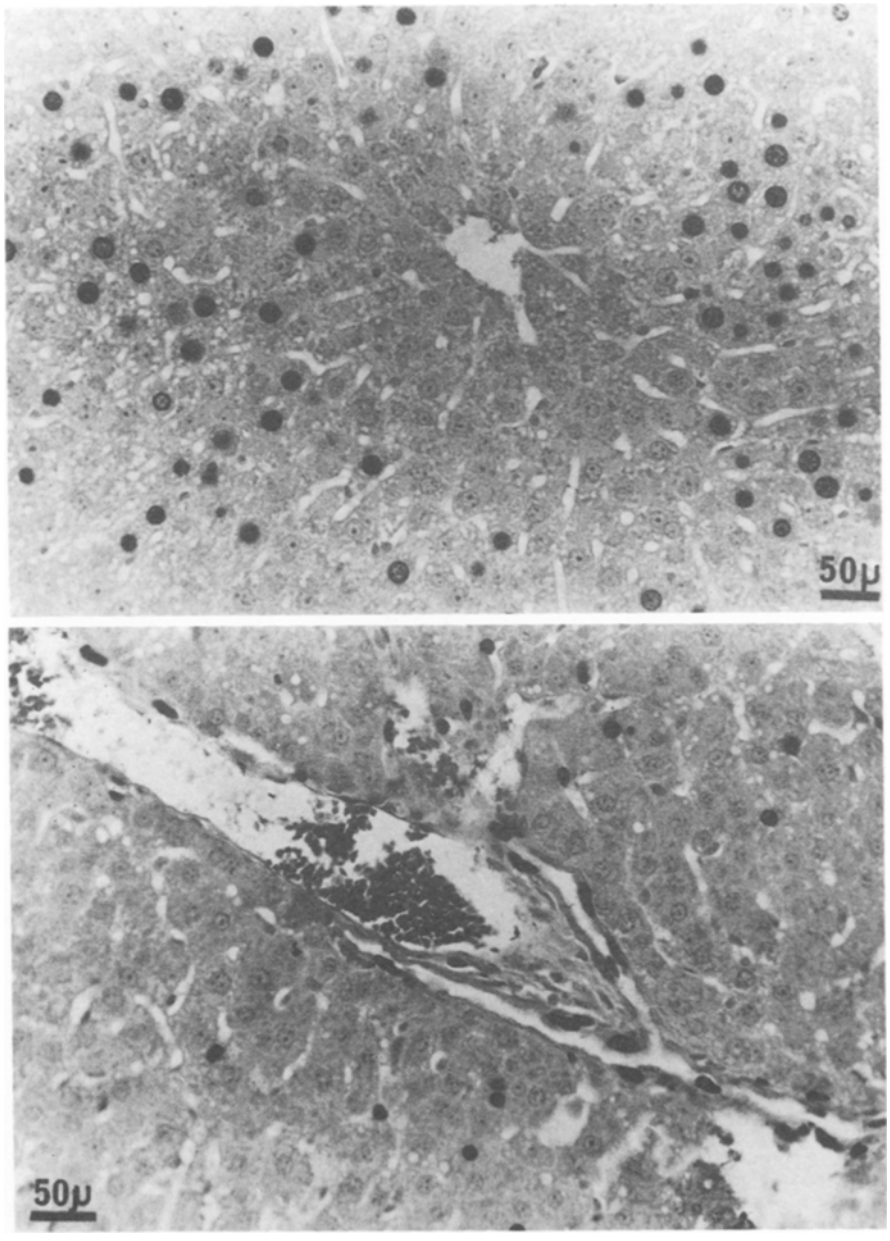

Figure 5(a) BUDR staining of regenerating liver at day 1. Peroxidase stained nuclei indicate incorporation into DNA. Only hepatocytes are so labeled. Note the zonal pattern of uptake. (b) BUDR staining of regenerating liver at day 4. Peroxidase stained nuclei indicate incorporation into DNA. Much BUDR uptake is observed in structural and littoral cells. Some hepatocytes are also labeled.

activity rose at day 2 (494, SEM = 57·6 ng FGF equivalent) after PH but reached normal values by day 5 after PH (304, SEM = 43·5 ng FGF equivalent). Our measurements detected all heparin binding FGFs and not only acidic FGF.

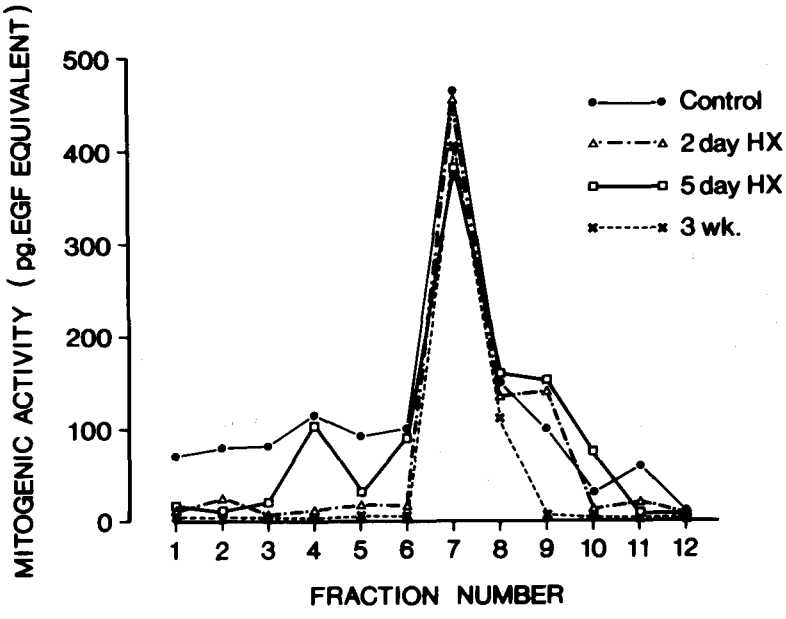

Figure 6. EGFR ligand activity of hepatectomized liver extracts. Normal livers and livers from rats 2, 5 and 21 days after PH were acid extracted and fractionated as described in Materials and Methods. The *y*-axis data represent the EGFR ligand mitogenic activity (expressed as equivalent of human EGF) in a 40 μl aliquot from post-gradient fractions taken from three pooled liver samples for each time point. Samples were assayed in duplicate.

Table 1. Heparin binding mitogenic activity of rat liver extracts after hepatectomy.

Time after partial hepatectomy (days)	0	2	5	21
ng FGF equivalent per gram of liver (wet weight)	288 ± 55·6	494 ± 57·6	304 ± 43·5	187 ± 28·8

Samples of liver were extracted in Tris–HCl buffer, pH 8·0, and passed over heparin sepharose as described in Materials and Methods. The desalted 2·0 M fraction was assayed for mitogenic activity. Data represent mean ± SEM for three assays at each time point: normal liver and days 2, 5 and 21 post-PH.

Discussion

Like Fisher and Fisher [9] and Murphy *et al.* [22, 23] we observed that intraportally injected sarcoma cells grew much better when administered after PH. Several phenomena contributed to the facilitation of tumor growth after PH.

If PH was performed immediately before or up to 2 days before injection of tumor cells, tumor growth was confined to the suture line. Thereafter at this site, tumor take fell sharply and was no longer detectable at 5 days after PH.

The probability that a cell trapped in the scar would develop into tumor appeared to be at least 200 times more likely than for a cell trapped in an untraumatized liver, since the minimum cell numbers required for tumor growth were 2×10^5 for healthy livers and 10^3 for the PH scar. This appeared to be another example of pronounced facilitation of tumor growth by trauma, but was different in some respects from that seen following trauma to muscle, e.g. at the site of a laparatomy incision [23] or an end-to-end anastomosis of gut [26]. Maximum tumor growth from blood-borne cancer cells in traumatized muscle occurred when the tumor cells were injected at 4–5 days after surgical incision. We have no explanation to offer for these differences between muscle and liver.

The second pattern of tumor take following intraportal innoculation appeared to be associated with the process of liver regeneration. Tumor growth was seen both superficially and within the parenchymal mass of the regenerating lobe and this was maximal when the PH was performed 4–6 days before the intraportal cell injection. The growth pattern at 4–7 days post-PH was markedly different from that seen after rats with intact livers were given tumor cells intraportally where only small foci appeared originally on the surface of the liver and penetrated down into the parenchyma during growth. Also, the probability that a cell trapped in the regenerating liver would develop into tumor seemed to be at least 20 times greater than for a malignant cell trapped in a non-PH liver, since the minimum cell numbers necessary for tumor growth were 2×10^5 for healthy livers and 10^4 for regenerating livers.

In an effort to clarify the processes responsible for the metastatic patterns obtained, the role of tumor cell trapping, growth factor profiles and liver cell proliferation were investigated.

The arrest of the injected tumor cells was investigated using radioactive chromium labeled MC28 tumor [23]. Cells arriving via the portal blood flow were trapped in a similar manner and numbers both in the right lobe, which regenerated extensively and in which tumor grew consistently, and in the caudate lobe, which enlarged by varying degrees and presented erratic tumor growth. This pattern of cell trapping did not change irrespective of the time of cell injection after PH. It was consistent with the tumor pattern seen in the experiments conducted 4–7 days post-PH (Figure 2), but not with the tumor seen growing around the scar in the experiments carried out up to 2 days after PH (Figure 1). The proximal liver segment included the scar and coincided with the area that developed the tumor mass in the early injection times (Figure 1). The maximum difference in trapped cell numbers was seen between the proximal and the distal segments and was 30%. The drop in the number of malignant cells towards the distal end of the right lobe cannot account for the predominance of tumor seen at the scar between 0 and 2 days after PH; a 10-fold difference in cell concentrations between the top and the bottom of the lobe would be necessary for such a change in tumor growth. Therefore, other factors apart from trapping play a role in the susceptibility of the scar area to tumor growth.

It seemed possible that the presence of growth stimulating factors within the regenerating liver might be responsible for the enhanced growth of tumor between 4 and 7 days after PH. Attention was directed at two classes of growth factors. Firstly, those which like EGF and TGFa act via the EGFG and secondly those which bind to heparin and require 2 M salt for elution from heparin, e.g.

fibroblast growth factor. Both of these classes of growth factors are potent mitogens for hepatocytes [12]; an increase in messenger RNA for TGFa was found in rat liver one day after PH [21] and an increase in messenger RNA for acid FGF was observed for 7 days following PH and was found both in parenchymal and non-parenchymal cells [16].

Our results showed no change in the mitogenicity of acid extracts from livers before and after PH. Our assay measured activity of all of the growth factors binding to EGFR and was, therefore, not directly comparable with the data for messenger RNA for TGFa, which was found to be increased on day 1 after PH by Mead and Fausto [21]. Moreover, it is quite possible that an increase in the expression of messenger RNA is not accompanied by an increase in transcription to give the protein product.

Mitogenic activity attributable to FGF-type growth factors was shown to be increased 2 days after PH and therefore preceded but did not correlate with the facilitation of tumor growth within the liver parenchyme which occurred between days 4 and 7 after PH. The finding by Kann *et al.* [16] of an increase in messenger RNA for acidic FGF, which persisted for 7 days post-PH, was not incompatible with our observations, as our measurements detected all heparin binding FGFs and not only acidic FGF; again translation of messenger RNA to protein may not paralel the increase in the amount of messenger RNA produced.

It seemed possible that the growth of the tumor cells trapped in the liver might correlate with the expansion of the endogenous cell populations. Liver cell proliferation as shown by uptake of IUDR was maximal at days 1 and 4 for the right lobe, in accordance with previous studies [4, 5], the results visualized by BUDR staining. The hepatocyte proliferation on day 1 post-PH did not appear to influence tumor take within the parenchyma, since in the first 48 h after PH the tumor grew maximally at the scar while the incidence in the parenchyma remained similar to that found in rats that had not had PH. However, DNA synthesis of non-hepatocytes which was evident at day 3 and maximum at day 4 coincided with the increased growth of tumor in the parenchyma on day 3 and the start of the maximal tumor take in the parenchyma on day 4. Therefore, the peak of DNA synthesis on day 1 occurring in hepatocytes was not synchronous with the maximal tumor incidence seen in the liver after PH, but the day 4 peak corresponding to littoral and structural cell proliferation coincided with the beginning of the maximal tumor take. There may be an association between these two phenomena but we have no evidence which would allow us to distinguish whether the facilitation of tumor cell growth is a consequence of cell division of non-parenchymal liver cells or a paralel phenomenon caused by a common factor(s). Additionally, the methodology used allowed the investigation of *in situ* proliferation but could not detect the possible extrahepatic recruitment of relevant cell populations of non-hepatocytes.

Further studies are in hand to identify possible mediators of tumor growth, since clearly they are likely to be important factors in determining the distribution and growth of malignant 'micrometastases' within the liver.

References

[1] ALEXANDER, J. W., and ALTEMEIER, W. A., 1964, Susceptibility of injured tissues to haematogenous metastases: an experimental study. *Annals of Surgery*, **159**, 933–944.

[2] BOUWENS, L., BAEKELAND, M., and WISSE, E., 1982, Importance of local proliferation in the expanding Kupffer cell population after zymosan stimulation and partial hepatectomy. *Hepatology*, **4**, 213–219.

[3] BOUWENS, L., KNOOK, D. L., and WISSE, E., 1986, Local proliferation and extrahepatic recruitment of Kupffer cells in partial-body irradiated rats. *Journal of Leukocyte Biology*, **39**, 687–697.

[4] BUCHER, N. L. R., PATEL, U., and COHEN, S., 1977, Hormonal factors and liver growth. *Advances in Enzyme Regulation*, edited by G. Weber (New York: Pergamon), pp. 205–213.

[5] BURKI, K., SCHAER, J. C., GRETILLAT, P., and SCHINDLER, R., 1975, Radioactively labeled iododeoxyuridine in the experimental liver regeneration. *Current Topics in Pathology*, **60**, 33–61.

[6] FISHER, B., and FISHER, E. R., 1959, Experimental studies of factors influencing hepatic metastases: II. Effect of partial hepatectomy. *Cancer*, **12**, 929–932.

[7] FISHER, B., and FISHER, E. R., 1959, Experimental studies of factors influencing hepatic metastases: III. Effect of surgical trauma with special reference to liver injury. *Annals of Surgery*, **150**, 731–744.

[8] FISHER, E. R., and FISHER, B., 1960, Experimental studies of factors influencing hepatic metastases: IV. Effect of cirrhosis. *Cancer*, **13**, 860–864.

[9] FISHER, E. R., and FISHER, B., 1965, Experimental study of factors influencing development of hepatic metastases from circulating tumour cells. *Acta Cytologica*, **9**, 146–158.

[10] GRISHAM, J. W., LEONG, G. F., ALBRIGHT, M. L., and EMERSON, J. D., 1962, Effect of exchange transfusion on labeling of nuclei with ^{3}H-thymidine and on mitosis in hepatocytes of normal and regenerating rat liver. *Cancer Research*, **26**, 1476–1485.

[11] HIGGINS, G. M., and ANDERSON, R. M., 1931, Experimental pathology of the liver. *Archives of Pathology*, **12**, 186–202.

[12] HOFFMAN, B., and PAUL, D., 1990, Basic fibroblast growth factor and transforming growth factor-alpha are hepatotrophic mitogens *in vitro*. *Journal of Cellular Physiology*, **142**, 149–154.

[13] HUNT, T. K., CONOLLY, W. B., ARONSON, S. B., and GOLDSTEIN, P., 1978, Anaerobic metabolism and wound healing: a hypothesis for the initiation and cessation of collagen synthesis in wounds. *American Journal of Surgery*, **135**, 328–332.

[14] ICHIHASHI, H., MABUCHI, H., SUENAGA, M., and KONDO, T., 1984, Liver regeneration and tumour growth in the rat after partial hepatectomy. *Japanese Journal of Surgery*, **14**, 510–514.

[15] JONES, F. S., and ROUS, P., 1914, On the cause of the localization of secondary tumours at point of injury. *Journal of Experimental Medicine*, **20**, 404–412.

[16] KANN, M., HUANG, J., MANSSON, P. E., YAMAMITSU, H., CARR, B., and MCKEEHAN, W. L., 1989, Heparin-binding growth factor type 1 (acidic fibroblast growth factor): A potential biphasic autocrine and paracrine regulator of hepatocyte regeneration, *Proceedings of the National Academy of Science, U.S.A.*, **86**, 7432–7436.

[17] KNIGHTON, D. R., HUNT, T. K., THAKRAL, K. K., and GOODSON, W. H., 1982, Role of platelets and fibrin in the healing sequence. An *in vitro* study of angiogenesis and collagen synthesis. *Annals of Surgery*, **196**, 379–387.

[18] LEFFERT, H. L., KOCH, K. S., MORAN, T., and RUBALCAVA, B., 1979, Hormonal control of rat liver regeneration, *Gastroenterology*, **76**, 1470–1482.

[19] LEIBOVICH, S. J., and ROSS, R., 1974, The role of the macrophage in wound repair. *American Journal of Pathology*, **78**, 71–91.

[20] MAGUAD, J. P., SARGENT, I., CLARKE, P. J., FRENCH, M., RIMOCK, R., and MASON, D. Y., 1989, Double immunocytochemical labeling of cell and tissue samples with monoclonal antibromodeoxyuridine. *Journal of Histochemistry and Cytochemistry*, **37**, 1517–1527.

[21] MEAD, J. E., and FAUSTO, N., 1988, Transforming growth factor α may be a physiological regulator of liver regeneration by means of an autocrine mechanism. *Cell Biology*, **86**, 1558–1562.

[22] MURPHY, P., ALEXANDER, P., KIRKHAM, N., FLEMING, J., and TAYLOR, I., 1986, Pattern of spread of bloodborne tumour. *British Journal of Surgery*, **73**, 829–834.
[23] MURPHY, P., ALEXANDER, P., SENIOR, P. V., FLEMING, J., KIRKHAM, N., and TAYLOR, I., 1988, Mechanism of organ selective tumour growth by bloodborne cancer cells. *British Journal of Cancer*, **57**, 19–31.
[24] RAPOLEE, D. A., MARK, D., BANDA, M. J., and WERB, Z., 1988, Wound macrophages express TGF-α and other growth factors *in vivo*: Analysis by mRNA phenotyping. *Science*, **241**, 708–712.
[25] ROBINSON, K. P., and HOPPE, E., 1962, The development of bloodborne metastases. Effect of local trauma and ischaemia. *Archives of Surgery*, **85**, 720–724.
[26] SKIPPER, D., JEFFREY, M. J., COOPER, A. J., ALEXANDER, P., and TAYLOR, I., 1989, Enchanced growth of tumour cells in healing colonic anastomoses and laparatomy wounds. *International Journal of Colorectal Disease*, **4**, 172–177.
[27] STRAIN, A. J., 1988, Transforming growth factor β and inhibition of hepatocellular proliferation. *Scandinavian Journal of Gastroenterology*, **23**(Suppl.), 37–45.