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Effect of vascular endothelial growth factor on functional recovery after hepatectomy in lean and obese mice

Claudio A. Redaelli[1], David Semela[2], Francine E. Carrick[3], Monika Ledermann[2], Daniel Candinas[1], Bernhard Sauter[3], Jean-François Dufour[2,*]

[1]*Department of Visceral and Transplantation Surgery, Inselspital, University of Bern, Bern, Switzerland*
[2]*Department of Clinical Pharmacology, University of Bern, Bern, Switzerland*
[3]*Division of Gastroenterology and Center for Gene Therapy and Molecular Medicine, Mount Sinai Medical Center, New York, NY, USA*

***Background/Aims*: Liver regeneration is dependent upon coordinated proliferation of hepatocytes and endothelial cells. Vascular endothelial growth factor (VEGF) promotes angiogenesis. Hepatic steatosis delays regeneration and increases liver resection morbidity. We hypothesized that VEGF overexpression stimulates hepatic regeneration.**

***Methods*: Recombinant adenovirus expressing human VEGF165 or adenovirus control-vector (*Lac* Z) were administered before 2/3 hepatectomy in lean and ob/ob mice. Galactose elimination capacity, a quantitative liver function test, was repeatedly measured before and after hepatectomy. Expression of VEGF receptors (flt1, flk1), endoglin and hypoxia inducible factor-1α (HIF-1α) was assessed by quantitative RT-PCR and for endoglin also by immunohistochemistry.**

***Results*: After 2/3 hepatectomy, *VEGF* gene transfer increased galactose elimination capacity in lean and ob/ob mice. HIF-1α, endoglin and VEGF receptor mRNA increased during regeneration in lean but not in obese mice. Staining of endothelial cells by endoglin immunohistochemistry returned to baseline reactivity in lean mice by day 6 and remained decreased in ob/ob mice. VEGF treatment decreased HIF-1α and increased flk1 response in lean mice.**

***Conclusions*: Hepatic resection elicits an angiogenic response in the remnant liver, which is impaired in case of steatosis. Adenovirus-mediated transfer of VEGF hastens functional hepatic recovery in lean, and more importantly also, in obese mice after partial hepatectomy.**

***Keywords*: Angiogenesis; Breath test; Fatty liver; Regeneration; Steatosis**

1. Introduction

Liver resection is a life saving intervention for patients with primary or secondary malignant hepatic lesions. Progress in surgical techniques and anesthesiology has made it possible to perform extended non-anatomical resection [1]. Such interventions carry the risk of postoperative liver failure, which is only transient if the liver regenerates, but exposes the patient to potentially fatal complications. More than 20% of the patients scheduled for resection have a fatty liver [2], a condition associated with impaired liver regeneration [3] and increased postsurgical morbidity and mortality [4,5]. Moreover, organ shortage leads to more living donor liver transplantation, where both the recipient and the donor are challenged with a postoperative period during which the liver must regenerate.

Liver regeneration requires the formation of new blood vessels. The initial wave of hepatocyte proliferation is followed by endothelial cell proliferation [6] and penetration of avascular hepatocellular islands leading to the formation of new sinusoids [7]. Vascular endothelial growth factor (VEGF), which is under the transcriptional regulation of hypoxia inducible factor-1α (HIF-1α),

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* Corresponding author. Tel.: +41-31-632-3191; fax: +41-31-632-4997.
E-mail address: jf.dufour@ikp.unibe.ch (J.-F. Dufour).

Abbreviations: VEGF, vascular endothelial growth factor; HIF, hypoxia inducible factor.

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stimulates endothelial cell proliferation and migration via two receptors, flt1 and flk1. Hepatocellular production of VEGF peaks 48–72 h after hepatectomy [8]. This is accompanied by an increase in the expression of flt1 on sinusoidal endothelial cells and also on hepatocytes [9,10]. Recent studies have shown that inhibition of angiogenesis with angiostatin impairs liver regeneration [11].

From the clinical perspective, however, it is more relevant to investigate whether VEGF can stimulate liver regeneration. We tested the hypothesis that VEGF may influence liver regeneration after partial hepatectomy in lean mice as well as in obese mice with fatty liver. To avoid the difficulties linked to protein therapy, which requires frequent parenteral administrations, we chose to use adenovirus-mediated gene transfer directly to the liver, ensuring an adequate growth factor concentration in the target organ for several days [12]. Functional recovery was assessed by the galactose breath test. Galactose breath test is a quantitative liver test, which measures the capacity to metabolize galactose [13] and correlates with the functional hepatocellular mass [14].

2. Material and methods

2.1. Animals and treatments

Experiments were performed in male C57/BL6 mice (Harlan, Horst Netherlands), weighing 20–30 g, and in ob/ob male mice (Jackon Laboratories) with the same background as the lean mice and weighing 40–50 g. Animals were fed a normal laboratory diet and allowed free access to food and water. Surgical procedures were performed under aseptic conditions between 10:00 and 13:00 to avoid circadian variations. Animals received humane care in accordance with the regulations for laboratory animals and the experiments were approved by local authorities.

2.2. Surgical procedures

A two-thirds hepatectomy was performed by an experienced liver surgeon (CR) according to the method of Higgins and Anderson [15]. The left lateral and median lobes were ligated and resected. The abdominal cavity was closed by a suture. Animals were anaesthetized at different time points after surgery with pentobarbital sodium (50 mg/kg i.p.) and the livers harvested. The regeneration ratio was determined as the percentage of the liver weight at the time of hepatectomy which was harvested 3 or 6 days after a 66% hepatectomy.

2.3. Adenoviruses

Adenoviruses containing the human *VEGF165* cDNA (ADV·hVEGF) and *LacZ* cDNA (ADV·LacZ) were prepared as described [12]. Adenoviral particles (5×10^9) were administered by tail vein injection 2 days prior to surgery.

2.4. LacZ expression assay

Liver cryostat sections (12 μm) were stained overnight with 1 mg/ml X-Gal solution containing 30 mmol/l K_3FeCN_6, 30 mmol/l K_4FeCN_6, and 2 mmol/l $MgCl_2$, 0.01% Na-deoxycholate, 0.02% NP-40 in PBS.

2.5. Galactose breath test

After intraperitoneal administration of 20 mg [1-^{14}C]galactose for lean mice and 40 mg for ob/ob mice, $^{14}CO_2$ excretion in breath was measured every 10 min for 2 h [14]. The galactose elimination capacity was measured 2 days prior to the surgery and at specified times thereafter. Values were normalized to preoperative measurements.

2.6. Real-time quantitative PCR analysis

Total RNA was extracted from liver samples as described [16]. RNA was treated by DNase digestion (RQ1 RNAse free DNase, Promega) and reverse-transcribed with MMLV reverse transcriptase (GibcoBRL) with a random hexanucleotide mix (Roche) and kept at −20 °C. Specific forward and reverse primers as well as the fluorogenic probes were designed according to Perkin–Elmer guidelines (Primer Express Software) and were tested for optimal concentration and linear amplification before beginning quantitative measurements.

The primers and probes were:

Human VEGF165: AGTCCAACATCACCATGCAGATTA and GCATTCACATTTGTTGTGCTGTAG with the probe CCTCACCAAGGCCAGCACATAGGAGA
Mouse flt1: AAGCGGTTCACCTGGACTGA and CCTTGCTTTTACTCGCTATTCTCA with the probe CAAGCCCAAGGCCTCCATGAAGATAGAC
Mouse flk1: GTGGTCTTTCGGTGTGTTGCT and TCTCCTACAAAATTCTTCATCAATCTTG with the probe TTCCTTAGGTGCCTCCCCATACCCTG
Mouse HIF-1α: TCAGAGGAAGCGAAAAATGGA and CAGTCACCTGGTTGCTGCAA with the probe CCCTTTTTCAAGCAGCAGGAATTGGAA
Mouse endoglin: GCATCCAACACCATCGAACTAG and GGACTGTGACCTAGAGGTCAAT with the probe AGACACGCTCACCTGTACGAAGGCCTG

Quantitative assessment of mRNA expression was performed using 18S ribosomal RNA as an internal standard (Applied Biosystems). Δ(Ct) represents the difference in number of PCR cycles to reach the same fluorescent intensity. The lower the Δ(Ct), the more abundant is the mRNA, the relationship being exponential.

2.7. Immunohistochemistry for endoglin

The monoclonal antibody MJ7/18 against endoglin (diluted 1:100) developed by Eugene C. Butcher and Mark Jutila was obtained from the Developmental Studies Hybridoma Bank developed under the auspices of the NICHD and maintained by the University of Iowa, Department of Biological Sciences. A biotin-conjugated goat anti-rat Ig specific polyclonal was diluted 1:400 and used as secondary antibody (BD Biosciences) followed by incubation with a streptavidin–biotin-complex/alkaline (DAKO). Staining was developed with red fuchsin chromogen (DAKO). Sections were counterstained with hematoxylin and examined with an Olympus BH2 photomicroscope (Olympus Optical Co.). Negative controls were stained without the primary antibody. The immunoreactivity was quantified into four grades: 0, none (same as negative control); +, mild; ++, moderate; and +++, abundant. All of the counting was done in a blind fashion; the samples were examined without knowledge of the animal's identity.

2.8. Experimental design

C57/BL6 lean mice were randomly assigned to receive one of the following three treatments 48 h before surgery: ADV·hVEGF (5×10^9 viral particles), ADV·LacZ (5×10^9 viral particles) or sterile 0.9% saline by tail vein injection. Ob/ob mice received either ADV·hVEGF (5×10^9 viral particles) or ADV·LacZ (5×10^9 viral particles) 48 h before surgery. The galactose breath test was performed before the adenovirus administration or saline injection, 2 days after injection just before the surgery and on days 1, 2, 3 and 6 after surgery. Series of experiments with determination of the breath test on different days and harvesting either 3 days or 6 days after the surgery were conducted.

2.9. Statistics

Data were analyzed with the Statistica Software (StatSoft) and the results expressed as mean ± SE of the mean. Nonparametric statistical tests (Wilcoxon's matched pairs test, Mann–Whitney *U*-test) were applied. A $P \leq 0.05$ was considered statistically significant.

3. Results

3.1. Transgene expression in vivo

Approximately 15% of the hepatocytes were transduced with *LacZ* adenovirus 2 days after tail vein injection of 5×10^9 viral particles, as demonstrated by the β-galactosidase expression assay on sections of the resected liver at the time of surgery (Fig. 1). Importantly, the low adenovirus dose used (20–100 times below the maximal tolerable dose for mice) caused in both groups only a mild transient liver inflammation (data not shown). Transduction of *VEGF* was assessed by real-time quantitative PCR with primers and a probe specific for the human sequence. Readily detectable signals were found in all animals injected with ADV·hVEGF. In lean C57/BL6 mice VEGF mRNA was detected at 20.1 ± 1.3 cycles in the resected liver during partial hepatectomy ($n = 12$); at 16.8 ± 1.8 in the harvested liver 3 days after surgery ($n = 6$); and at 19.2 ± 1.4 in the harvested liver 6 days after the surgery ($n = 6$). In the ob/ob mice it was detected at 21.2 ± 0.6 cycles in the resected liver ($n = 5$) and at 17.1 ± 0.3 in the harvested liver 6 days after surgery ($n = 5$). No signal was detectable in non-VEGF-injected animals (40 cycles).

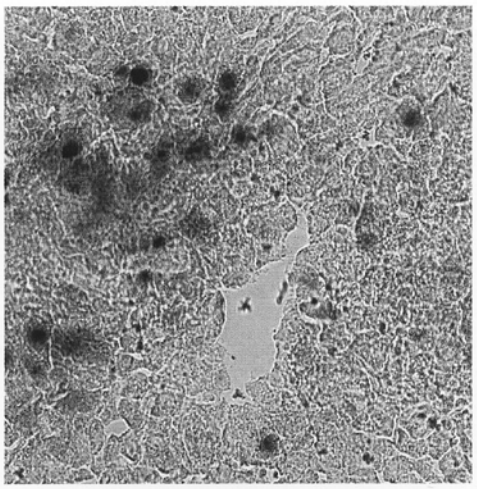

Fig. 1. Hepatic expression of β-galactosidase 2 days after tail vein injection of 5×10^9 adenovirus particles encoding for *LacZ*. About 15% of the hepatocytes display a blue coloration indicating expression of the enzyme β-galactosidase.

3.2. Effects of ADV·hVEGF gene transfer on galactose breath test

ADV·hVEGF treatment hastened the recovery of liver function as measured by the galactose breath test. Liver function was consistently higher in the mice treated with *VEGF* adenovirus compared to treatment with ADV·LacZ in lean as well as in the obese mice (Fig. 2A and B). In lean mice the difference was significant on day 1: 0.61 ± 0.07 ($n = 5$) vs 0.27 ± 0.09 ($n = 6$), Mann–Whitney *U*-test, $P < 0.03$ and on day 3: 0.87 ± 0.06 ($n = 11$) vs 0.66 ± 0.05 ($n = 5$), Mann–Whitney *U*-test, $P < 0.02$. In lean animals the galactose breath test returned to base line at day 6, in ob/ob mice it did not and remained at 80% of its basal value. In ob/ob mice the difference was significant on day 1: 0.49 ± 0.04 ($n = 5$) vs 0.29 ± 0.08 ($n = 6$), Mann–Whitney *U*-test, $P < 0.03$ and on day 3: 0.88 ± 0.07 ($n = 8$) vs 0.69 ± 0.03 ($n = 6$), Mann–Whitney *U*-test, $P < 0.04$. Galactose breath test values were not significantly different between the saline-treated mice and the ADV·LacZ-treated mice (data not shown). Breath tests determined before the administration of adenoviruses and 2 days later just before the surgery were not different indicating that adenovirus treatment itself did not affect this quantitative liver function test (data not shown).

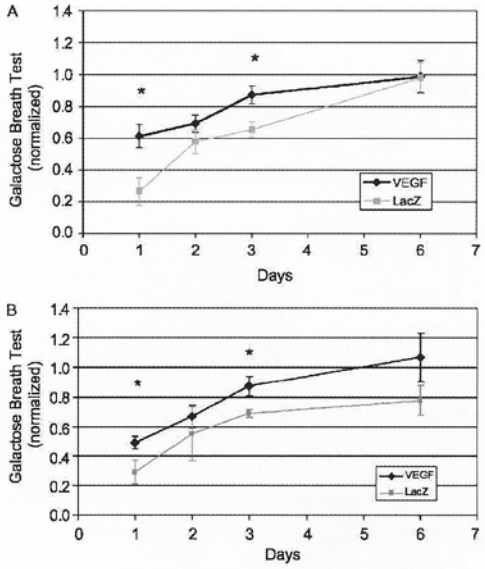

Fig. 2. Effect of VEGF on galactose breath test after 2/3 hepatectomy (day 0) normalized to preoperative values. (A) In lean C57/BL6 VEGF improved the galactose breath test. In comparison to the LacZ-control groups VEGF significantly improved functional recovery at day 1 and 3 (Mann–Whitney *U*-test, $P < 0.05$, *). Each point is the mean of the results of 5–12 animals. (B) Effects of VEGF on galactose breath test after 2/3 hepatectomy in ob/ob mice. In comparison to its LacZ-transduced controls, VEGF significantly improved functional recovery on day 1 and 3 (Mann–Whitney *U*-test, $P < 0.05$, *). Each point is the mean of the results of 5–8 animals.

3.3. Effects of ADV·hVEGF gene transfer on liver mass

Three days after surgery, liver weight restitution was 60% in the LacZ-control groups which is comparable with the galactose breath (66%). The regeneration ratio was slightly higher in the VEGF-treated animals −68%—on day 3 when the galactose breath test reached already 87% of its basal value suggesting a functional compensation by the regenerating liver treated with VEGF. The liver weight restitution was similar in the 3 groups of lean mice on day 6 (Fig. 3A and B). The regeneration ratio was significantly lower in ob/ob mice than in the lean mice reaching 79.4 ± 4.9% ($n = 6$) vs 91.6 ± 4.6% ($n = 5$) on day 6 (Mann–Whitney U-test $P < 0.05$). Transduction of VEGF had no effect on this parameter (Fig. 3C).

3.4. Effects of ADV·hVEGF gene transfer on HIF-1α, flk1, flt1 and endoglin mRNA abundance

The hepatic mRNA expression of four different angiogenic parameters was assessed. HIF-1α, flt1, flk1 and endoglin mRNAs increased progressively after partial hepatectomy in the saline control group of lean mice. Three days after partial hepatectomy HIF-1α mRNA was 2.51 ± 0.7 fold more abundant than at time of surgery, flt1 mRNA 1.31 ± 0.26, flk1 1.44 ± 0.52 and endoglin mRNA 1.50 ± 0.52 fold. Six days after the surgery these changes were larger and reached statistical significance (Table 1). At baseline, the hepatic abundance of the mRNA of HIF-1α, flk1 and endoglin in ob/ob mice was similar to that in the liver of lean C57/BL6. In contrast, flt1 mRNA was about 150 times less abundant (Table 2).

VEGF treatment affected the mRNA expression of HIF-1α and of flk1 in lean mice. The response of HIF-1α mRNA was reduced by VEGF when compared to the adenovirus control group; there was significantly less HIF-1α mRNA (0.63 ± 0.13) in VEGF-treated mice on day 3 than in LacZ-treated mice (1.38 ± 0.23; Mann–Whitney U-test $P < 0.02$) (Fig. 4A). Moreover, VEGF treatment resulted in a significant increase in flk1 mRNA 3 days after 2/3 hepatectomy (1.06 ± 0.15 vs 0.61 ± 0.10, Mann–Whitney U-test $P = 0.05$) (Fig. 4B). Flt1 and endoglin mRNA increased in mice injected with the control adenovirus in a similar manner than in the saline controls. Neither the expression of flt1 nor the expression endoglin were significantly affected by VEGF treatment (data not shown).

The evolution of these parameters after hepatectomy was different in ob/ob mice to the one observed in the lean mice. Neither HIF-1α mRNA, nor flt1 mRNA were increased 6 days after 2/3 hepatectomy (Fig. 5A and C); flk1 mRNA and endoglin mRNA were even decreased (Fig. 5B–D). Transduction of VEGF had no significant effect (Fig. 5A–D).

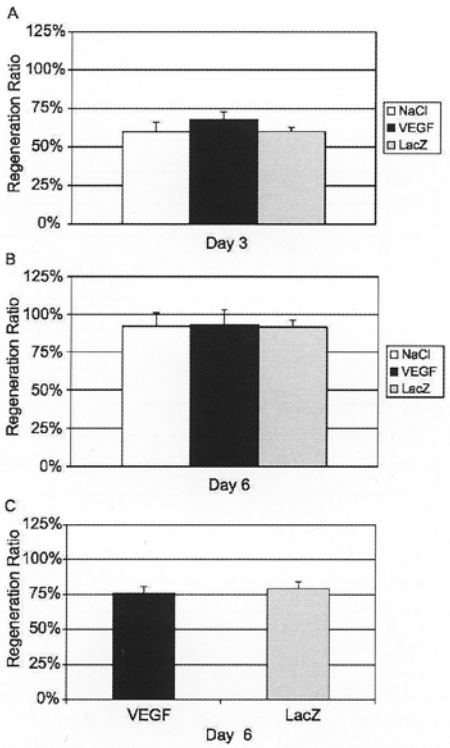

Fig. 3. Effects of VEGF on liver weight expressed as regeneration ratio in lean (A,B) and ob/ob mice (C). No significant difference was observed between the three groups of animals either 3 days after surgery (A), or 6 days after surgery (B). Effects of transduction of *VEGF* on hepatic regeneration in ob/ob mice in terms of liver weight (C). The regeneration ratio was not different in the *VEGF* treated animals and in the *LacZ*-treated group.

Table 1
Determination of hepatic mRNA abundance of HIF-1α, flt1, flk1 and endoglin by real-time quantitative PCR 6 days after 2/3 hepatectomy in C57/BL6 lean mice treated with saline (controls)

	Δ(Ct) day 0	Δ(Ct) day 6	P^a	Increase
HIF-1α	16.87 ± 1.31	15.22 ± 1.36	<0.05	3.25 ± 0.48
Flk1	17.25 ± 0.32	16.44 ± 0.09	<0.05	1.97 ± 0.56
Flt1	19.62 ± 0.12	18.80 ± 0.15	<0.05	1.79 ± 0.16
Endoglin	17.39 ± 0.08	16.38 ± 0.11	<0.05	2.06 ± 0.20

[a] Wilcoxon's matched pairs test.

Table 2
Determination of hepatic mRNA abundance of HIF-1α, flt1, flk1 and endoglin by real-time quantitative PCR in the resected liver at time of 2/3 hepatectomy in lean and ob/ob mice which received the control LacZ-adenovirus

	Δ(Ct) lean C57/BL6	Δ(Ct) ob/ob mice	P^a
HIF-1α	17.45 ± 0.41	16.86 ± 0.21	n.s.
Flk1	17.34 ± 0.26	16.99 ± 0.29	n.s.
Flt1	20.16 ± 0.32	27.52 ± 0.20	<0.0002
Endoglin	16.83 ± 0.16	16.26 ± 0.10	n.s.

[a] Mann–Whitney U-test.

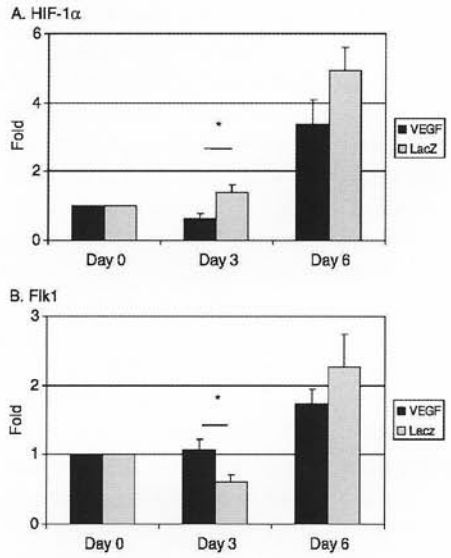

Fig. 4. Evolution of HIF-1α, and flk1 mRNAs after partial hepatectomy in lean C57/BL6 mice determined by real-time quantitative PCR. (A) Transduction with VEGF resulted in a decrease in HIF-1α mRNA when compared to LacZ adenovirus control on both time points, this effect reached statistical significance on day 3 (Mann–Whitney *U*-test $P < 0.02$, *). (B) Hepatic Flk1 mRNA was significantly more abundant 3 days after partial hepatectomy in the VEGF-treated mice than in the mice injected with control adenovirus (Mann–Whitney *U*-test $P = 0.05$, *).

3.5. Effects of ADV·hVEGF gene transfer on expression of endoglin

Endoglin (CD105) is a co-receptor for transforming growth factor-β and is specifically expressed on endothelial cells. Immunohistochemistry for endoglin revealed a strong staining lining the blood vessels (Fig. 6A and B). This staining was not affected by VEGF administration either in lean or in obese mice. Following partial hepatectomy staining for endoglin decreased of intensity

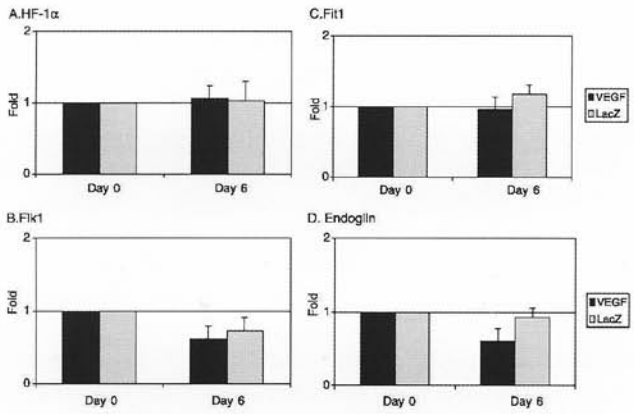

Fig. 5. Real-time quantitative PCR determination of hepatic mRNA levels for HIF-1α (A), flk1 (B), flt1 (C) and endoglin (D) in ob/ob mice after partial hepatectomy. In contrast to lean C57/BL6 mice, no significant changes were observed 6 days after partial hepatectomy compared to the expression at the time of surgery.

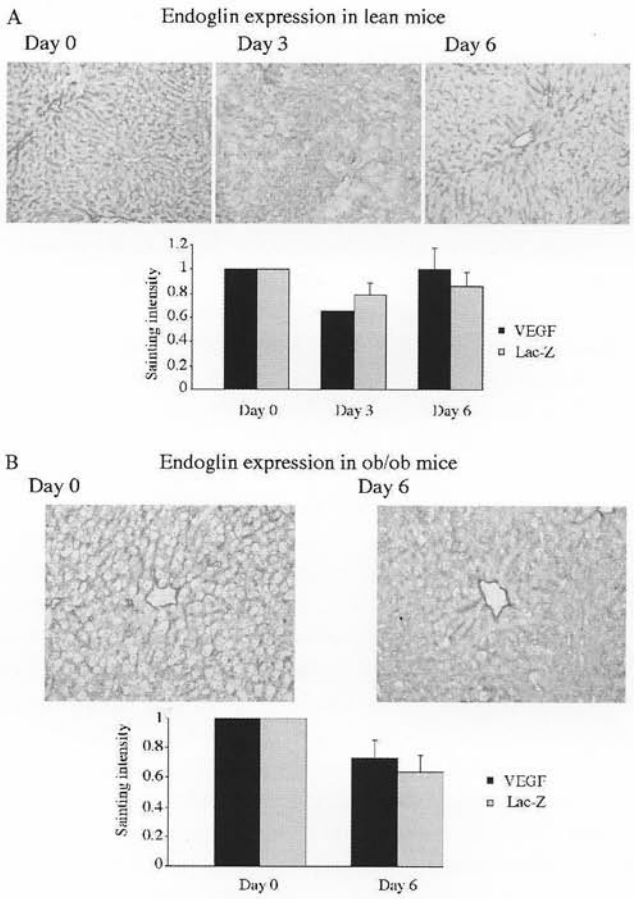

Fig. 6. Immunohistochemistry for endoglin to mark the endothelial cells (original magnification 40 ×). (A) Endoglin expression in a lean animal which received ADV·hVEGF. In comparison to the expression at time of partial hepatectomy on the resected liver, there was a decrease of the staining intensity 3 days after partial hepatectomy which returned to baseline on day 6. The bar graph shows that semi-quantitatively there was no difference between VEGF-treated ($n = 5$) and LacZ-treated control mice ($n = 5$). (B) Endoglin expression at time of partial hepatectomy on the resected liver and 6 days later in an ob/ob mouse which received ADV·hVEGF. There was a decrease of the intensity of the staining 6 days after the surgery. The bar graph shows that semi-quantitatively there was no difference between VEGF-treated ($n = 5$) and LacZ control ob/ob mice ($n = 6$). The steatosis was not affected by VEGF administration 2 days before the surgery, but it was reduced after partial hepatectomy.

3 days after surgery and returned to baseline 6 days after surgery in lean mice (Fig. 6A). In ob/ob mice intensity for endoglin staining remained decreased 6 days after partial hepatectomy. In these animals the steatosis regressed during regeneration and was not affected by VEGF-treatment.

4. Discussion

The results suggest that hepatic transduction of VEGF with adenoviruses promotes functional recovery after partial hepatectomy in lean and obese mice and that, in contrast to

normal liver, angiogenic parameters are not upregulated in fatty liver during regeneration.

Adenoviruses infect hepatocytes and are used to express a protein of interest in the liver for several days. This is advantageous in that it offers a cheaper and simpler alternative to repetitive parenteral administration of a recombinant protein. The disadvantage of this strategy however, is the inflammation due to hepatocellular infection. For this reason we studied control animals that received the same quantity of virus as the VEGF-treated animals. At the low adenoviral dose used we did not observe any toxicity due to adenovirus administration, and histological examination of the liver indicated only a mild inflammation. Human VEGF165 has been shown to be bioactive in the mouse [12] and this confers the advantage of selective determination of the transduced VEGF by PCR. Dose limiting systemic toxicity of VEGF [17] allows administration of only a relatively small amount of recombinant ADV·hVEGF (5×10^9 viral particles/mouse). This dose resulted in approximately 15% transduction of hepatocytes which was sufficient to induce sustained transgene expression in the liver over the experimental period.

To quantify non-invasively the functional hepatic recovery we determined the galactose elimination capacity which measures the function of the cytosolic galactokinase [13]. After partial hepatectomy this quantitative liver function test returns to basal values within weeks in humans [18,19] and within days in rats [20].

VEGF gene transfer resulted in a significant acceleration of functional hepatic regeneration in lean mice which was not paralleled by an increase in liver mass, even if the regeneration ratio was slightly higher in the VEGF group 3 days after the surgery. This emphasizes the importance of investigating the hepatic function. The VEGF effect would have been underestimated if only the regeneration ratio had been studied. Assy et al. reported after injection of 200 ng of VEGF a significant increase in the regenerated liver mass 2 days after 2/3 hepatectomy in rat, but they did not study functional recovery [21]. It is possible that with higher doses we would have observed an effect on the liver mass, but this would have resulted in larger hepatocellular infection and, consequently, hepatic inflammation. Ferrara and coworkers showed that VEGF induces the release of hepatocyte growth factor by sinusoidal endothelial cells [22]. The beneficial effect of VEGF on functional liver recovery could be due to a stimulatory effect on the formation of new blood vessels and/or to an effect on the hepatocytes themselves which express VEGF receptors after partial hepatectomy [10]. VEGF is not only a growth factor stimulating endothelial cell proliferation, but also a strong endothelial cell survival factor under apoptosis-inducing conditions [23]. In the present experimental conditions VEGF may prevent liver cell apoptosis rather than induce proliferation, thus possibly explaining why the regeneration ratio was less affected than the galactose breath test and why the effect of VEGF was already present 1 day after partial hepatectomy.

To explore the molecular effects of VEGF overexpression after hepatectomy, we examined hepatic mRNA of several molecules associated with angiogenesis (HIF-1α, endoglin, flt1 and flk1). Our data confirm other studies which found that flt1 and flk1 expression increases during liver regeneration [6,10]. Furthermore, 3 days after hepatectomy VEGF treatment led to significantly higher flk1 mRNA levels compared to controls. We also show that hepatic HIF-1α mRNA and endoglin mRNA become more abundant during liver regeneration. HIF-1α is a transcription factor, whose proteasomal degradation is inhibited by low-oxygen stress [24]. Interestingly we found that its mRNA expression is also regulated; after partial hepatectomy HIF-1α mRNA was increased, an observation in line with recent microarray data [25]. Moreover, HIF-1α mRNA expression was significantly inhibited in the VEGF group suggesting a negative feedback regulation. This feedback downregulation of HIF-1α mRNA in presence of VEGF may explain why the expression of endoglin was not higher in the animals treated with VEGF, since endoglin is under the transcriptional control of HIF-1α [26]. Endoglin (CD105, transforming growth factor-β receptor-3) is an integral membrane glycoprotein mainly expressed on endothelial cells [27]. The gene for endoglin is mutated in patients with hereditary hemorrhagic telangiectasia, a disease with development of vascular malformations in the gastrointestinal tract, including the liver [28]. Endoglin contributes to angiogenesis by preventing apoptosis in hypoxic endothelial cells [29]. We found that livers of lean mice contain more endoglin mRNA during regeneration than at baseline. Endothelial cell labeling by immunostaining for endoglin decreased 3 days after partial hepatectomy and increased thereafter; this reflects the delayed proliferation of the endothelial cells in comparison to the predominant hepatocytes as shown by Sato et al. [6]. This pattern was not influenced by VEGF administration.

Clinically steatosis is an important risk factor for morbidity after liver resection, which has been associated with impaired regeneration [3]. Indeed, the regeneration ratio was lower in ob/ob mice than in the lean animals. Moreover, in contrast to lean mice, there was no increase in the different angiogenic parameters after partial hepatectomy and immunohistochemistry for endoglin as an endothelial marker remained decreased suggesting that the angiogenic response is impaired in this model. This could be due to the steatosis itself or to the leptin deficiency characterizing ob/ob mice since leptin is an angiogenic factor [30]. Clavien and coworkers found that interleukin 6 was unable to stimulate the defective regeneration after partial hepatectomy in leptin receptor-deficient obese rats [3]. Others studying liver regeneration in leptin-independent models of steatosis found no impairment in liver regeneration [31,32]. Taking into account the possible

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