University of Groningen

Experimental and clinical studies on the effect of liver regeneration on tumor growth

Jong, Koert Pieter de

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2000

Citation for published version (APA):
Jong, K. P. D. (2000). *Experimental and clinical studies on the effect of liver regeneration on tumor growth*. s.n.

Summary, discussion, and conclusions

Colorectal cancer is the third commonest cancer in men, and the second commonest in women. Resection is the standard therapy, and results in a five-year survival rate of around 50%. Liver metastases occur in about 40 -70% of patients during the course of their disease, but in only 10 - 15% liver metastases are initially limited to the liver. In these patients, partial liver resection offers the only chance for cure. Unfortunately, only 25% of the patients after liver resection will be definitely cured.

The liver is a unique organ, in that, after removal or destruction of liver cell mass, it is able to restore liver tissue by a process, which is called regeneration. This restorative capacity is regulated by various growth factors and cytokines generated in the liver itself, but also by distant organs and tissues. Tumor cells, although behaving more autonomous than non-transformed cells, are also influenced by growth factors. The common role of growth factors in liver regeneration and tumor growth, led to the hypothesis that changes in serum growth factor levels, induced by partial hepatectomy, might also influence tumor growth of micrometastases. This hypothesis is tested in this thesis, using an experimental animal model and by analyzing serum growth factor levels and growth factor(receptor) expression on human colorectal liver metastases.

Section I. Introduction

Chapter 1 gives a general introduction to the topic and formulates the questions to be answered in the ensuing chapters. In **chapter 2**, a review is presented on the various mechanisms which can potentially play a role in the interaction between liver regeneration and tumor growth.

Section II. Experimental studies

The aim of the experimental studies, performed in rats, is to investigate whether increased tumor growth after partial liver resection is restricted to the liver or also occurs outside the liver, and whether this is related to changes in serum composition. The experiments described in **Chapter 3** demonstrate that, 14 days after removal of 70% of the (non-tumor bearing) liver, tumors in the remnant liver had a tumor weight which was twice as high as that in sham operated rats. Tumors placed beneath the renal capsule, did not show this difference in weight, suggesting that tumor growth stimulation is restricted to tumors in the liver and not elsewhere. In order to differentiate between liver related changes and changes in serum composition as an explanation for the differences in tumor growth, tumor cells were cultured in the presence of portal and systemic serum, which was obtained at days one and 14 after partial hepatectomy or sham operation. These experiments did not show a difference in proliferation (measured by thymidine incorporation) between tumor cells cultured in hepatectomy serum and sham serum. If, however, hepatocytes were added to these cultures, it was found that tumor cells had a higher proliferation rate than tumor cells cultured separately. It appeared that this increased tumor cell proliferation only occurred if the ratio tumor cells:hepatocytes was 1:1 or 1:10, but not in a ratio of 10:1. These findings suggest that increased tumor growth after hepatectomy is more likely to be explained by direct (paracrine) stimulation of tumor cells by hepatocytes than by changes in serum composition at days 1 and 14 after partial hepatectomy. It might also be, however, that changes in growth factor serum concentration after partial hepatectomy, are time dependent, and that they occur at other time

147

intervals than at days one and 14 after partial hepatectomy. Therefore, in the experiments described in **chapter 4**, tumor cells were cultured in portal and systemic serum, obtained from rats at days one, three and 14 day after 70% partial hepatectomy or sham operation. Serum obtained at day three after operation, added to tumor cell cultures, revealed that proliferation (measured by thymidine incorporation into DNA) was 25 - 40% higher in tumor cells cultured in portal hepatectomy serum than in sham operated portal serum. Addition of systemic hepatectomy serum obtained at day 14 resulted in a 30% higher proliferation rate in tumor cells as compared to (day 14) systemic serum of sham operated rats. A possible explanation for the fact that the experiments described in chapter 4, using day 14 systemic hepatectomy serum, resulted in a higher tumor cell proliferation, whereas the same serum experiments in chapter 3 did not, could be the difference in technique of measuring cell proliferation. Also, changes in epidermal growth factor receptor (EGFr) expression on tumor cells cultured in hepatectomy and sham serum, from portal and systemic origin obtained at day 3, were analyzed as a possible explanation for differences in tumor growth. Other investigators showed that growth factors can influence EGFr expression on cells, and changes in growth factor serum concentration could result in an altered EGFr expression. We used immunocytochemistry, northern blot analysis and ligand binding assay to determine EGFr expression and it appeared that the increased proliferation of tumor cells was not mediated by upregulation of EGFr by the various types of sera used.

In order to study cell proliferation in humans, it is necessary to use techniques that do not need the administration of potentially toxic materials, like BrdU or [^{3}H]thymidine. A naturally occurring antigen, expressed in proliferating cells, is proliferating cell nuclear antigen (PCNA). Immunostaining of this antigen makes it possible to quantify cell proliferation in humans. Also, analysis of S-phase labeled nuclei, using flow cytometry, is a method used for proliferation assays. These techniques, however, need to be validated in the setting of liver regeneration in the presence of (micro)metastases. It was, for instance, shown that PCNA expression can be upregulated, without concomitant increase in thymidine labeling, in the presence of growth factors produced by tumors. In **chapter 5** flow cytometry and PCNA immunostaining are studied in rats with liver metastases, comparing liver cell proliferation in partially hepatectomized and sham operated rats at days one, two, three, four and 10 after operation. It was found that both PCNA expression and flow cytometric analysis of S-phase nuclei, nicely correlated with BrdU immunostaining, and that these techniques are well suited for the study of liver cell proliferation in tumor bearing rats. The validation of these techniques, makes it possible to apply those in human beings.

Section III. Clinical studies

The aim of these studies is to test the hypothesis that partial hepatectomy, and the ensuing regeneration response, is associated with an increase in growth factor serum levels, which could potentially influence growth rate of occult micrometastases in man. To this end, the serum growth factor response in patients after partial hepatectomy was compared to that in patients after laparotomy or cryosurgery, in whom a regeneration response is not foreseen. Furthermore, the expression of growth factors and their receptors in colorectal liver metastases was analyzed in relation to tumor characteristics and prognosis after partial hepatectomy.

148

One of the growth factors important for liver regeneration and tumor growth is transforming growth factor α (TGFα). **Chapter 6** describes the expression of this growth factor and its receptor (EGFr) in colorectal liver metastases and corresponding primary colorectal carcinomas in relation to prognosis. Expression of the tumor suppressor gene p53 and proliferation rate were also studied. It was found that higher TGFα expression in liver metastases was associated with unfavorable prognostic characteristics of the primary tumor. Expression of p53 and the proliferation marker Ki67 were not associated with clinicopathological characteristics of the primary tumor or the metastasis. Using multivariate analysis, it appeared that p53 expression, absence of EGFr expression and a lower TGFα expression in the liver metastasis than in the primary tumor, was associated with a better prognosis after partial hepatectomy than in patients with absence of p53 expression, EGFr expression and a higher TGFα expression in the liver metastasis than in the primary tumor.

Other growth factors/cytokines for which a role in liver regeneration has been described are interleukin-6 (IL-6) and hepatocyte growth factor (HGF). Experiments in rats suggested that IL-6 initiates HGF production and thereby induce liver regeneration. The effect of administration of recombinant human IL-6, in cancer patients participating in a phase I-II study, on HGF serum levels was the topic of the study in **chapter 7**. HGF levels were measured on days 1, 2, 3, 8 and 15 after intravenous IL-6 infusion (day 1) and subcutaneous IL-6 administration for the subsequent 6 days. It was found that at days three and eight, median HGF levels were increased to 124% and 157% respectively, as compared to serum levels at day 1. At day 8, the increase in HGF levels were IL-6 dose dependent. It can be concluded that solely administered IL-6 induced an upregulation of HGF production.

The fact that in vitro experiments revealed that serum from partially hepatectomized rats stimulated tumor cell growth, suggest that changes in serum composition after hepatectomy occur. Therefore, the aim of the study presented in **chapter 8** was to compare the serum response of various growth factors after laparotomy or partial hepatectomy in patients with colorectal liver metastases. We choose to analyze changes in serum growth factor concentration of those growth factors which play a dominant role in liver regeneration, namely epidermal growth factor (EGF), HGF, IL-6 and insulin-like-growth factor-I (IGF-I). Preoperative levels of the growth factors were comparable in both groups. The response, measured from day 0 until the 10th postoperative day, was not different in both groups except for IGF-I. This growth factor, demonstrated a lower response in hepatectomized patients than in laparotomized patients. In general, these findings suggest that the systemic response of growth factors is already initiated by laparotomy, and that partial liver resection has no additional effect on this response. The lower IGF-I response in hepatectomized patients, could be related to tumor growth, because IGF-I is known to induce apoptosis. This finding might be of importance because the phenomenon of increased tumor growth after partial liver resection, found in rats, might thus be related to a decrease in tumor apoptotic rate instead of an increase in tumor proliferation rate. In the same chapter the results are presented of a comparison between growth factor serum levels in portal and systemic serum. Several growth factors are produced by the liver, whereas others are known to be extracted by the liver. Also, some growth factors are produced by organs and tissues which have their venous drainage only via the portal vein. These facts initiated the comparative study of growth factor levels in portal and systemic serum. It appeared that, except for insulin, the serum levels of the studied growth factors were comparable in both serum types. Insulin levels were higher in portal than

149

in systemic serum. Furthermore, the serum growth factor response was compared in patients after partial hepatectomy in whom blood loss was low (< 1200 mL) with that in patients in whom blood loss was high (>2500 mL). This comparison revealed that the amount of blood loss did not influence serum growth factor response. Additionally, a comparison in growth factor levels was made in patients after right lobectomy versus those after left lobectomy. EGF response was higher in patients after right lobectomy than after left lobectomy. Possibly this difference can be explained by a difference in EGF receptor status between the right and left lobe of the liver. IGF-I serum levels were lower in right lobectomy than in left lobectomy patients, and the most likely explanation is that after right lobectomy a larger amount of IGF-I synthetic capacity has been removed than after left lobectomy.

Another treatment modality for (irresectable) colorectal liver metastases is destruction of metastases by cryosurgery. Because cryosurgery is not associated with removal of liver tissue, a regeneration response is not occurring. Because of this it is expected that changes in serum growth factor levels associated with regeneration, do not occur after cryosurgery. On the other hand, in comparison to patients treated by partial liver resection, the extent of injury in cryo surgically treated patients is expected to be higher, because necrotic tumor is left in situ and initiates an ongoing inflammatory response. In order to compare the extent of injury and the growth factor response in these two patient groups, acute phase proteins, HGF, and IL-6 serum response is described in **chapter 9**. This study revealed that in cryosurgically treated patients the HGF and IL-6 response is comparable to that in patients after partial hepatectomy. The acute phase response was higher in patients who underwent cryosurgery than in patients after liver resection.

Conclusions

-1- **Increased tumor growth after partial liver resection is well established in experimental animals.** It is not restricted solely to the in vivo situation, but also in vitro experiments revealed that tumor cell growth can be stimulated by factors in serum obtained from partially hepatectomized rats.

-2- **The influence of serum on in vitro tumor cell proliferation, is in the experimental animal model dependent on the time lapse after partial liver resection.** Differences in tumor cell proliferation were found only in cultures with serum obtained at day 3 and day 14 after operation, but not in cultures with day 1 serum.

-3- **EGFr expression on cultured CC531 tumor cells is not influenced by the type of serum added to the culture medium, and is therefore not an explanation for the differences in tumor cell proliferation in the studied animal model.** In fact, no EGFr expression was found on the used CC531 cells.

-4- **PCNA expression and flow cytometric analysis of the number of S-phase nuclei appear to be reliable methods for quantifying liver cell proliferation in livers containing metastases.** These two techniques, can replace standard techniques for quantifying cell proliferation, which are potentially dangerous to use in human research.

-5- **In human colorectal liver metastases, EGFr expression, absence of p53 expression and higher TGFα expression than in the primary tumor are associated with an unfavorable prognosis.** Both primary tumor characteristics and survival after partial liver resection are associated with TGFα expression, which is an important liver regeneration factor.

150

-6- **Recombinant human IL-6 induces a dose dependent HGF serum response.** In cancer patients, participating in a phase I-II trial, solely administered IL-6 resulted in an increase of HGF serum levels. This finding confirms that the relation between IL-6 and HGF, which was proven in animal models, also exists in the human situation.

-7- **Only the acute phase and IGF-I serum response are different in partially hepatectomized patients than in patients after laparotomy.** The search for growth factors, responsible for liver regeneration and the enhancement of tumor growth in humans, was not (yet) successful. Of the growth factors studied in this thesis only IGF-I serum levels were lower in hepatectomized patients as compared to laparotomized patients. Also, the acute phase protein serum levels in hepatectomized patients were lower than in laparotomized or cryosurgically treated patients.

-8- **HGF behaves like an acute phase protein.** The very close resemblance of HGF serum levels and acute phase proteins after laparotomy and cryosurgery suggests that HGF is more behaving like an acute phase protein than as a liver regeneration factor.

-9- **The comparison of growth factor levels in portal versus systemic serum only revealed a difference in insulin levels.** As a possible explanation for differences in growth rate of tumors in the liver and elsewhere, growth factor levels were analyzed in portal and systemic serum. No differences for EGF, HGF, IL-6 and IGF-I in both sera were found. Only insulin levels were higher in portal than in systemic serum.

-10- **EGF and IGF-I response is different after right lobectomy as compared with left lobectomy.** The finding that right lobectomy is associated with a higher EGF and a lower IGF-I response, requires additional studies, but can possibly be explained by differences in the right and left liver lobe regarding (EGF) receptor status and (IGF-I) synthetic capacity.

Samenvatting, discussie en conclusies

De incidentie van het colorectale carcinoom staat bij mannen (na long en prostaat carcinoom) op de derde, en bij vrouwen (na het mammacarcinoom) op de tweede plaats. Chirurgische verwijdering is de standaard therapie en leidt tot een 5-jaars overleving van ongeveer 50%. Levermetastasen komen bij 40 tot 70% van de patiënten met een colorectaal carcinoom voor. Bij 10-15% van de patiënten is de lever aanvankelijk het enige aangedane orgaan en zou partiële leverresectie tot genezing kunnen leiden. Slechts 25% van de aldus behandelde patiënten bereikt een definitieve genezing.

De lever bezit het unieke vermogen zich te herstellen wanneer er sprake is geweest van weefselverlies of beschadiging. Dit herstel treedt op door de uitgroei van resterend normaal leverweefsel. Dit proces wordt regeneratie genoemd. Verschillende groeifactoren en cytokines, die zowel door de lever zelf als door andere organen en weefsels worden geproduceerd, reguleren dit proces. Ook tumorcellen, hoewel deze zich in het algemeen meer autonoom gedragen dan niet-getransformeerde cellen, zijn afhankelijk van groeifactoren. De gemeenschappelijke rol die groeifactoren spelen bij leverregeneratie en tumorgroei, leidde tot de volgende hypothese: veranderingen in serumgroeifactor concentraties, optredend tijdens leverregeneratie na partiële leverresectie, kunnen van invloed zijn op de groei van niet detecteerbare micrometastasen. Deze hypothese wordt in dit proefschrift op twee manieren getoetst. In een dierexperimenteel model en door bestudering van serum groeifactor concentraties en groeifactor(receptor) expressie bij patiënten met colorectale levermetastasen.

151