Current Topics in Pharmacology
Vol. 23, 2019

Mini-Review

# Role of serotonin in liver regeneration

**Kota Naito, Hajime Moteki, Mitsutoshi Kimura* and Masahiko Ogihara**
Department of Clinical Pharmacology, Faculty of Pharmaceutical Sciences, Josai University,
1-1 Keyakidai, Sakado City, Saitama 350-0290, Japan.

## ABSTRACT

One of the characteristic functions of the liver is hepatic regeneration. In an experimental animal (e.g., rats), when liver tissue is affected by an agent (e.g., viral infection, chemical toxin), or removed surgically (up to 70%), the remnant liver spontaneously undergoes cell division to regenerate the organ. Such proliferative reactions are ceased when the whole organ is restored. Several methods to elucidate this complex phenomenon became available in the 20th century, and various studies have been performed in many laboratories. As a result, many factors that promote hepatocyte proliferation, as well as some growth inhibitors, were confirmed. Among these, serotonin (5-HT: 5-hydroxytryptamine) was recently identified as a promising hepatocellular growth-promoting factor. In this review, we attempt to integrate the intracellular signal transduction pathway for 5-HT-induced hepatocyte proliferation in primary cultures of adult rat hepatocytes and discuss the physiological significance of 5-HT in liver regeneration *in vivo*.

**KEYWORDS:** serotonin (5-HT), signal transduction, 5-$HT_{2B}$ receptor, proliferation (cultured hepatocytes), autocrine secretion, transforming growth factor-α, EGF/TGF-α receptor tyrosine kinase, 70-kDa ribosomal protein S6 kinase (p70S6K).

## 1. Introduction

The liver plays a major role in metabolism with more than 500 known metabolic functions. Another human and animal hepatic function of great interest is the organ's ability to regenerate from the remnant liver through spontaneous cell division after hepatic tissue injury caused by viral infection, chemical toxins or surgical resection up to about 70%. Once the original volume is restored, this proliferative reaction ceases spontaneously. This characteristic is unique to the liver and is not observed in other organs. This phenomenon is called liver regeneration [1, 2]. For instance, in rats subjected to 70% hepatectomy, the 30% remnant liver begins cell proliferation spontaneously, with the original liver mass restored after about 10 days. Research into the mechanisms of liver regeneration took a great leap forward in the first half of the twentieth century after Higgins and Anderson published a model of 70% partial hepatectomy [3].

This section examines the phenomenon of liver regeneration at the cellular level. Mature hepatocytes, under physiological conditions, exit the cell cycle, cease to proliferate, and enter the so-called quiescent phase ($G_0$). To begin proliferation, cells must enter the $G_1$ phase (competent) and undergo transition sequentially through each phase of the cell cycle. In the S phase, large amounts of DNA that make up the chromosomes are replicated accurately to exactly double the genome. In the M phase, the nucleus undergoes mitosis (increase in the number of nuclei), followed by division of the cytoplasm to form two daughter cells. The entry of hepatic parenchymal cells from the quiescent phase into the cell cycle reportedly involves various factors associated with local inflammation, such as neurotransmitters, hormones, growth factors, cytokines, and nutrients [1, 2]. Despite many studies on the mechanism of liver regeneration, the overall picture of the very complex mechanism remains unknown.

*Corresponding author: mkimura@josai.ac.jp

## 2. Experimental systems for studying liver regeneration

Studies have revealed that liver regeneration after partial hepatectomy involves growth factors such as epidermal growth factor (EGF), hepatocyte growth factor (HGF), and transforming growth factor (TGF)-α, as well as cytokines including tumor necrosis factor (TNF)-α and interleukin (IL)-6 [1, 2]. In addition to these factors, hormones and autacoids, among other factors, are also involved in the promotion and inhibition of liver regeneration, indicating that liver regeneration is achieved through complex multifactorial interactions. In detailed investigations of the mechanism involving specific growth factors, however, an *in vivo* model makes analyses difficult due to complex interactions among many factors. Thus, scientists have been developing *in vitro* models, in parallel with *in vivo* models, to study detailed mechanisms of each factor. The primary culture, under proper conditions, of hepatic parenchymal cells isolated by the *in situ* collagenase perfusion method developed by Seglen *et al*. has provided hepatocytes with high levels of metabolic activities equivalent to those in *in vivo* systems and the ability to retain responses to various growth factors and cytokines for a relatively long period of time [4, 5]. Using such an experimental system has allowed elucidation of the mechanisms employed by numerous growth factors and cytokines to promote the proliferation of hepatic parenchymal cells.

Our laboratory has used biochemical, molecular biological, and pharmacological approaches to investigate the mechanisms of hepatocyte growth-promoting factors and growth-inhibitory factors involved in liver regeneration. Specifically, we have studied mediators of the regulatory systems (signal transduction systems) involved in the homeostasis of the body, including the metabolic (nutrients), autonomic nervous, endocrine, and immune systems, on whether and how they promote the proliferation of hepatic parenchymal cells. The present paper provides a brief overview of the activities and mechanisms of action of such factors and information on the intracellular signal-transduction mechanism of serotonin (5-hydroxytryptamine, 5-HT), which has been found in recent years to be a hepatocyte growth-promoting factor. We would also like to discuss the physiological significance and the action mechanisms of serotonin in *in vivo* liver regeneration by comparing the *in vitro* study results we obtained with several relevant literature papers.

## 3. Factors affecting hepatocyte proliferation

Table 1 categorizes hepatocyte growth-promoting and inhibitory factors. Factors found to promote hepatocyte proliferation independently include EGF, HGF, platelet-derived growth factor, insulin-like growth factor (IGF)-I, IGF-II, TGF-α, and insulin [6-12]. Moreover, factors found to have no independent activity to promote (or inhibit) hepatocyte proliferation but show a modifying effect when used in combination with one of the above factors include α- and β-adrenoceptor agonists and glucagon. Moreover, numerous factors such as TNF-α, IL-1 β, branched-chain amino acid, vitamin A, vitamin C, prostaglandin $E_2$ ($PGE_2$), and prostacyclin ($PGI_2$) were also found to promote hepatocyte proliferation mediated by an autocrine factor [12-18]. Meanwhile, very few factors inhibit (stop) the hepatocyte growth-promotion response, including TGF-$\beta_1$, which exhibits a powerful inhibitory effect on hepatocyte proliferation [19].

**Table 1.** Mitogenic growth factors and inhibitory factors in liver regeneration.

| Growth factor | Bioactive substances | References |
|---|---|---|
| 1. Primary mitogen | EGF, HGF, PDGF, TGF-α, insulin, TNF-α, IGF-I, GF-II | [6-12] |
| 2. Co-mitogen | Adrenaline, noradrenaline, α-,β-adrenoceptor agonists, glucagon | [6-11] |
| 3. Indirect mitogen | TNF-α, BCAA, $PGE_2$, $PGI_2$, IL-1β, vitamin A, C | [12-18] |
| 4. Inhibitory factor | TGF-$\beta_1$, glucocorticoids | [19-21] |

EGF: epidermal growth factor, HGF: hepatocyte growth factor, PDGF: platelet-derived growth factor, TGF: transforming growth factor, TNF: tumor necrosis factor, IGF: insulin-like growth factor, BCAA: branched chain amino acid, PG: prostaglandin, IL: interleukin.

In addition, glucocorticoids have been also found to exhibit a powerful inhibitory effect on the hepatocyte growth factor-induced hepatocyte proliferation at relatively low concentrations [20, 21].

The results we obtained from the studies using the *in vitro* experimental model of primary cultured hepatic parenchymal cells collectively showed the following. In the *in vivo* response to promote (initiate) the proliferation of hepatic parenchymal cells after hepatectomy, under certain conditions, various growth-promoting factors express cell growth-promoting effects duplicatively in conjunction with mediators in the sympathetic nervous and endocrine systems to promote cell proliferation. Moreover, mediators of the inflammatory and immune systems and nutrients (amino acids and vitamins) may also promote the proliferation of hepatic parenchymal cells. Nevertheless, it remains unknown as to when such factors exert their effects during the natural course of *in vivo* liver regeneration and the details of their synergism with other factors. Meanwhile, although very few factors have been found to inhibit (stop) the hepatocyte growth-promotion response, their activities are powerful.

## 4. The role of serotonin in liver regeneration

In recent years, progress has been made in the research of the deep involvement of 5-HT, an autacoid, in promoting liver regeneration [22, 23]. The 5-HT in peripheral tissues is mostly present in the intestinal tract, followed by the platelets. The physiological actions of 5-HT are wide ranging, including regulating intestinal motility and inducing emesis and blood-clotting reactions. After hepatectomy, platelets quickly gather at the wound of the incision, and platelet aggregation begins. Meanwhile, activated platelets secrete 5-HT, and platelet aggregation increases. Furthermore, hepatectomy induces the expression of 5-$HT_{2A}$ and 5-$HT_{2B}$ receptors in the liver and inhibits 5-HT synthesis, thereby delaying liver regeneration, thus indicating that 5-HT is an important factor in liver regeneration and has the potential to become a "liver regeneration-promoting agent" [23]. Nevertheless, the mechanism of how 5-HT promotes the proliferation of hepatocytes remains unknown.

## 5. Serotonin receptor subtypes

The genes that code for serotonin 5-HT receptor subtypes have been cloned, allowing physiological and biochemical studies to be conducted. Fourteen 5-HT receptor subtypes have been discovered. Except for 5-$HT_3$, a receptor with internal ion channel, the other 5-HT receptor subtypes are known to be the G protein-coupled type that promotes the metabolic turnover of phosphatidylinositol (PI) or increases the cAMP production for intracellular signal transduction [24, 25].

## 6. Serotonin receptor subtypes involved in promoting hepatocyte proliferation and intracellular signal-transduction mechanisms

We have investigated the effects of 5-HT and 5-$HT_2$ receptor subtype agonists on the proliferation of hepatic parenchymal cells using the experimental system of primary cultured hepatic parenchymal cells. The selective agonist for serotonin 5-$HT_{2A}$ or 5-$HT_{2C}$ receptor (TCB-2 or CP809101, respectively) exhibited no effect on promoting hepatocyte proliferation. On the other hand, BW723C86, a selective agonist for 5-HT and serotonin 5-$HT_{2B}$ receptors, has been shown to promote both DNA synthesis and cell proliferation independently in culture-time- and dose-dependent manners. In addition, a pharmacological study of a specific signal transduction factor that inhibits the activity of 5-HT in promoting the proliferation of hepatic parenchyma cells showed the involvement of signaling factors such as phospholipase C (PLC), receptor tyrosine kinase (RTK), phosphoinositide 3-kinase (PI3K), mitogen-activated protein kinase (MAPK) kinase, and mammalian target of rapamycin (mTOR) in the activity of 5-HT in promoting the proliferation of hepatic parenchymal cells [26].

In light of such results, we measured the phosphorylation activities of hepatic parenchymal cellular RTK, MAPK, and ribosomal p70 S6 kinase (p70S6K) by the Western blot technique to further understand the intracellular signal-transduction mechanism in the 5-HT-induced promotion of cell proliferation. The results showed that the pathways of 5-$HT_{2B}$ receptor/PLC/$Ca^{2+}$ and EGF/TGF-α RTK/PI3K/extracellular signal-regulated kinase (ERK) 2/mTOR/p70S6K are deeply involved in the 5-HT-induced promotion of cell proliferation. Nevertheless, the data did not shed light on the relationship between the two signal transduction systems. Meanwhile, given that somatostatin, which inhibits granule secretion, inhibited the 5-HT-induced phosphorylation activities of RTK, MAPK, and

p70S6K (with the activity peaking at 10, 20 to 30, and 30 minutes after the addition of 5-HT, respectively), 5-HT probably causes hepatic parenchymal cells to secrete an autocrine factor [27, 28]. Candidates for such an autocrine factor were thought to include IGF-I and TGF-α.

We then investigated the involvement of autocrine factor secretion in the activity of 5-HT to promote the proliferation of hepatic parenchymal cells. The results confirmed that stimulation by 5-HT rapidly increases the TGF-α concentration in culture medium (5 to 10 minutes after addition of 5-HT). Meanwhile, the concomitant addition of an anti-TGF-α monoclonal antibody (mAb to TGF-α) completely inhibited the activity of 5-HT in promoting the proliferation of hepatic parenchyma cells. Moreover, a study using a selective PLC inhibitor showed that the 5-HT-induced autocrine secretion of TGF-α is mediated through the $5\text{-HT}_{2B}$ receptor/PLC/$Ca^{2+}$ pathway [27], (Fig. 1). TGF-α reportedly exhibits its effect in the relatively early phase of *in vivo* liver regeneration [29].

The results presented above collectively indicate that stimulation by 5-HT on the $5\text{-HT}_{2B}$ receptor subtype initiates the 5-HT-induced promotion of the proliferation of hepatic parenchymal cells. Thereafter, downstream PLC and the PI metabolism are activated, and intracellular $Ca^{2+}$ levels increase. The increased intracellular $Ca^{2+}$ levels trigger the secretion of TGF-α stored within the hepatic parenchymal cells to extracellular spaces. The secreted TGF-α binds with the EGF/TGF-α receptor on the hepatic parenchymal cell membrane, resulting in phosphorylation of RTK, and such a proliferative signal is transduced sequentially over time through PI3K, ERK2, mTOR, and p70S6K, resulting ultimately in cell proliferation (Fig. 2). While 5-HT is an indirect mitogen of hepatocytes that promotes the proliferation of hepatic parenchymal cells *via* a complex intracellular signaling pathway, it is of great interest that TNF-α and IL-1β, which are cytokines, and $PGE_2$ and $PGI_2$, which are also autacoids, promote the proliferation of hepatocytes *via* an intracellular signal-transduction mechanism similar to that for 5-HT [12, 13, 17, 18].

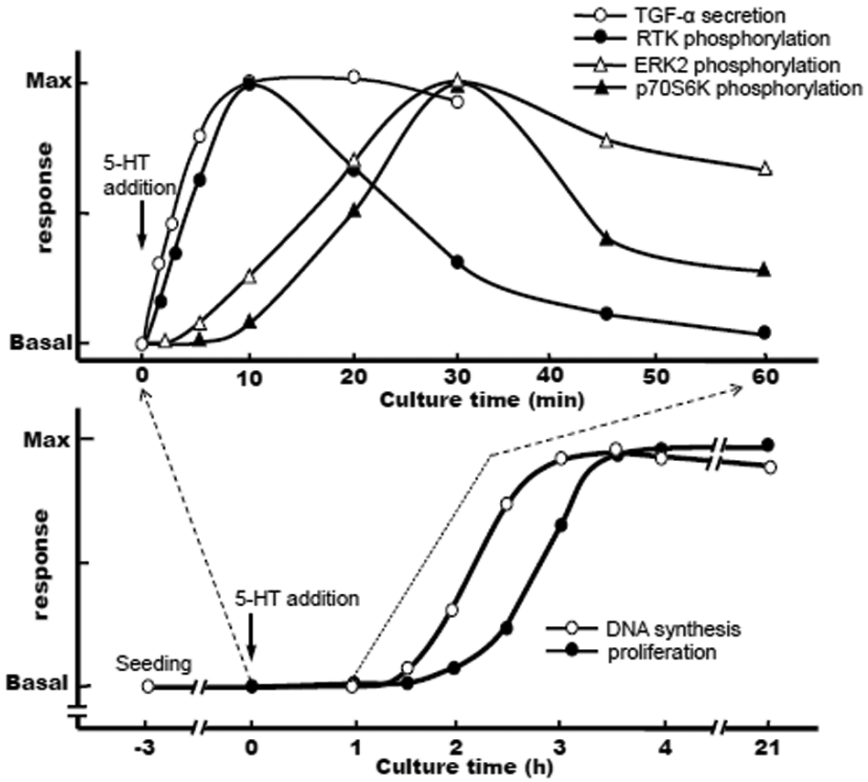


**Fig. 1.** Time course of activation of signal transducing elements and proliferation of hepatocytes induced by serotonin.

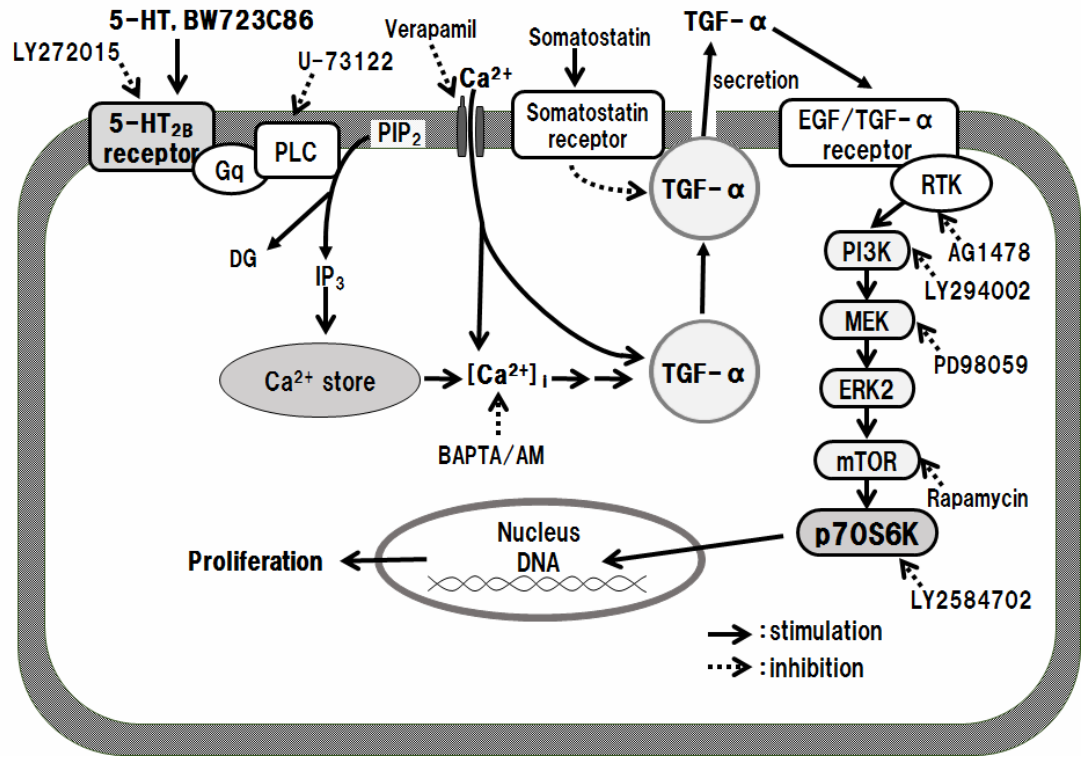


**Fig. 2.** Mechanism of 5-HT-induced DNA synthesis and proliferation in hepatocytes.
PLC: phospholipase C, $PIP_2$: phosphatidylinositol 4,5-bisphosphate, DG: diacylglycerol, $IP_3$: inositol 1,4,5-triphosphate, RTK: receptor tyrosine kinase, PI3K: phosphoinositide 3-kinase, MEK: mitogen-activated protein extracellular kinase, ERK2: extracellular signal-regulated kinase 2, mTOR: mammalian target of rapamycin, p70S6K: ribosomal p70 S6 kinase (Reproduced with permission from Naito, K., Kurihara, K., Moteki, H., Kimura, M., Natsume, H. and Ogihara, M. 2019, Biol. Pharm. Bull., 42, 631-637; Copyright (2019) The Pharmaceutical Society of Japan.).

## 7. Physiological significance and clinical applications of 5-HT in liver regeneration

Studies in recent years have reported that 5-HT exhibits a cell-growth promoting activity on various cells. The present research is the first of such studies to show that the mechanism of 5-HT in promoting the proliferation of hepatic parenchymal cells is mediated by the $5\text{-}HT_{2B}$ receptor. In *in vivo* models, activated platelets that collect at the site of a wound or inflammation at the early stage release 5-HT, which stimulates the $5\text{-}HT_{2B}$ receptor, causes the autocrine secretion of TGF-α by hepatic parenchymal cells, and is considered a growth factor that plays a very important role in achieving liver regeneration. Thus, the findings obtained in the present research contribute to the development of a novel 5-HT-based "liver regeneration-promoting agent" and are also useful in gaining an overall understanding of the phenomenon of liver regeneration. Particularly, given the deep involvement of the $5\text{-}HT_{2B}$ receptor in the activity of 5-HT in promoting proliferation of hepatic parenchymal cells, needs to develop both drugs that are highly selective for the $5\text{-}HT_{2B}$ receptor and efficient systems that deliver drugs to hepatic parenchymal cells are expected. Furthermore, given that the level of $5\text{-}HT_{2B}$ receptor expression is reportedly elevated in 30% of patients with liver cancer [30], the present research is expected to make contributions to the investigation of carcinogenic mechanisms and development of anticancer drugs.

## CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

## REFERENCES

1. Fausto, N., Campbell, J. S. and Riehle, K. J. 2006, Hepatology, 43, 45-53.

2. Michalopoulos, G. K. and DeFrances, M. 1997, Science, 276, 60-66.
3. Higgins, G. M. and Andeson, R. M. 1931, Arch. Pathol., 12, 186-202.
4. Seglen, P. O. 1975, Methods Cell Biol., 13, 29-83.
5. Ichihara, A., Nakamura, T., Noda, C. and Tanaka, K. 1986, A. Guillouzo and C. Guguen-Guillouzo (Eds.), Isolated and Cultured Hepatocytes, John Libbey Eurotext, London/ Paris, 187-208.
6. Kimura, M. and Ogihara, M. 1997, Eur. J. Pharmacol., 324, 267-276.
7. Kimura, M. and Ogihara, M. 1997, J. Phamacol. Exp. Ther., 282, 1146-1154.
8. Kimura, M. and Ogihara, M. 1998, Jpn. J. Pharmacol., 76, 165-174.
9. Kimura, M. and Ogihara, M. 1998, Eur. J. Pharmacol., 354, 271-281.
10. Kimura, M. and Ogihara, M. 1999, J. Pharmacol. Exp. Ther., 291, 171-180.
11. Kimura, M. and Ogihara, M. 1998, Eur. J. Pharmacol., 327, 87-95.
12. Okamoto, H., Kimura, M., Watanabe, N. and Ogihara, M. 2009, Eur. J. Pharmacol., 604, 12-19.
13. Kimura, M., Moteki, H. and Ogihara, M. 2014, Eur, J. Pharmacol., 745, 223-233.
14. Kimura, M. and Ogihara, M. 2005, Eur. J. Pharmacol., 510, 167-180.
15. Kimura, M., Watanabe, M., Ishibashi, N., Yanagida, S. and Ogihara, M. 2010, Eur. J. Pharmacol., 643, 267-273.
16. Moteki, H., Shimamura, Y., Kimura, M. and Ogihara, M. 2012, Eur. J. Pharmacol., 683, 276-284.
17. Kimura, M., Osumi, S. and Ogihara, M. 2001, Endocrinology, 142, 4428-4440.
18. Kimura, M., Okamoto, H., Natsume, H. and Ogihara, M. 2009, J. Pharmacol. Sci., 109, 618-629.
19. Kimura, M. and Ogihara, M. 1999, Eur. J. Pharmacol., 386, 271-277.
20. Kimura, M., Moteki, H. and Ogihara, M. 2011, J. Pharmacol. Sci., 115, 390-398.
21. Kimura, M., Moteki, H. and Ogihara, M. 2011, Biol. Pharm. Bull., 34, 682-687.
22. Lesurtel, M., Graf, R., Aleil, B., Walther, D. J., Tian, Y., Jochum, W., Gachet, C., Bader, M. and Clavien, P. A. 2006, Science, 312, 104-107.
23. Lesurtel, M. and Clavien P.-A. 2012, Clin. Res. Hepatol. Gastroenterol., 36, 319-322.
24. Schmuck, K., Ullmer, C., Engels, P. and Lübbert, H. 1994, FEBS letters, 342, 85-90.
25. Porter, R. H. P., Benwell, K. R., Lamb, H., Malcolm, C. S., Allen, N. H., Revell, D. F., Adams, D. R. and Sheardown, M. J. 1999, Br. J. Pharmacol.,128, 13-20.
26. Naito, K., Tanaka, C., Mitsuhashi, M., Moteki, H., Kimura, M., Natsume, H. and Ogihara, M. 2016, Biol. Pharm. Bull., 39,121-129.
27. Naito, K., Kurihara, K., Moteki, H., Kimura, M., Natsume, H. and Ogihara, M. 2019, Biol. Pharm. Bull., 42, 631-637.
28. Naito, K., Moteki, H., Kimura, M., Natsume, H. and Ogihara, M. 2016, Biol. Pharm. Bull., 39, 570-577.
29. Mead, J. E. and Fausto, N. 1989, Proc. Natl. Acad. Sci. USA, 86, 1558-1562.
30. Soll, C., Jang, J. H., Riener, M. O., Moritz, W., Wild, P. J. and Clavien, P. A. 2010, Hepatology, 51, 1244-1254.