MINI-REVIEW

Experimental Investigation of Opisthorchiasis-associated Cholangiocarcinoma Induction in the Syrian Hamster - Pointers for Control of the Human Disease

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Abstract

Appropriate animal models for specific diseases in man can facilitate elucidation of mechanisms underlying tumour development and allow potential interventions and therapeutic regimens to be tested in vivo before consideration for use in the human situation. In the North-east of Thailand exceptionally high levels of cholangiocellular carcinomas (CCCs) are encountered, related to infestation with *Opisthorchis viverrini* liver flukes. The Syrian hamster can also be infected with metacercariae of the fluke and heavy loads of parasites cause the development of cirrhotic livers. While the presence of flukes alone does not give rise to neoplasms, large yields of cholangiofibrotic lesions and CCCs can be readily induced with additional carcinogenic insult. While removal of the parasite with the antihelminthic drug Praziquantel can protect against carcinogenesis, this is dependent on the timing of the drug administration and the efficacy of application to the human situation remains to be confirmed. The available information would suggest that interest needs to be concentrated on potential chemopreventive agents which could be administered to individuals at high risk. Furthermore, understanding of the genesis of CCCs and the characteristics of preneoplastic lesions, again as assessed in the animal model, might allow novel approaches to identification of early stage cases and effective surgical intervention.

Key Words: Opisthorchiasis - hepatocarcinogens - hamster liver - cholangiocellular lesions - chemoprevention

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Introduction

There is increasing general awareness that chronically elevated rates of cell division predispose to tumor development and establishment of prevention approaches aimed at reducing or normalising proliferation is therefore a high priority in the battle against neoplasia (Cohen and Ellwin, 1991; Ames et al., 1995; Moore and Tsuda, 1998). Infectious agents which can clearly impact in this way include viruses (Mayer and Ebbesen, 1994; Morris et al., 1995; Zur Hausen, 1991), bacteria and parasites (Parsonnet,1995; Pisani et al.,1996).

Liver cancer is a major problem in Thailand in terms of both hepatocellular and cholangiocellular carcinomas (HCCs and CCCs) (Deerasamee et al.,1999). There is large variation in incidences of the two types and the North-East region of the country, populated by ethnic Lao has the highest incidence of CCCs in the world at over 85/100,000 in males and over 35/100,000 in females (Deerasamee et al., 2001). With regard to this tumour type, there is abundant epidemiological evidence that liver flukes are a responsible factor (Kurathong et al., 1985) and a strong association between high intensity of past and/or present *Opisthorchis viverrini* infection has been found in the majority of cases (Itoh et al., 1994). The related liver fluke, *Clonorchis sinensis*, may be similarly responsible for the pathogenesis of CCCs in other countries of Southeast and East Asia (Kim 1984; Shin et al., 1996; Abdel-Rahim, 2001).

For studies of how parasites or other agents causing chronically elevated cell proliferation might impact on carcinogenesis, *in vivo* animal models have clear advantages and a number have already become well established (see Table 1). For example, duodenogastroesophageal reflux in the rat provides a condition closely resembling Barrett's eosophagus in man which is highly susceptible to carcinogens causing adenocarcinoma development (Chen and Yang, 2001). In the stomach, *Helicobacter pylori* bacteria are an acknowledged risk factor for neoplasia and

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Table 1. Animal Models for Specific Human Diseases Causing Chronic Proliferation Associated with Neoplasia

Organ	Tumour Type	Agent		Rodent Species
		Human	Animal	
Oesophagus	Adenocarcinoma	Bile Reflux	Bile Reflux + Carcinogen	Rat
Stomach	Adenocarcinoma	H pylori	H pylori + Carcinogen	Mongolian Gerbil
Colon	Adenocarcinoma	Colitis	DSS* + Carcinogen	Mouse
Liver	Hepatocellular Carc	HBV/HCV	WHV# + Aflatoxin	Woodchuck
	Hepatocellular Carc	Schistosomes	S haematobium +	Mouse
	Cholangiocellular Carc	Opisthorchis	O. viverrini + Carcinogen	Syrian Hamster

*Dextran Sulphate Sodium # Woodchuck Hepatitis Virus

an experimental model using the Mongolian gerbil has been developed (Shimizu et al., 1999). In the mouse colon, induction of colitis with acetic acid or dextran sulfate sodium (DSS) treatment similarly provides an environment conducive to 1,2-dimethyl-hydrazine induction of colon tumorigenesis (Hagihara, 1982; Takesui et al., 2001). As a model for HCC development in the liver, use of the woodchuck hepatitis virus with aflatoxin (Bannasch et al., 1995) has been established. For the CCC case the Syrian hamster appears to provide the most appropriate experimental animal (Thamavit et al., 1978; Lee et al., 1994).

Syrian Hamster Model

Syrian golden hamsters can be readily infected with metacercariae of *Opisthorchis viverrini*, resulting in inflammatory and fibrotic changes with increase in cell turnover (see Fig 1), but without additional carcinogen exposure no carcinomas result, suggesting that parasite infestation is itself not strongly carcinogenic, if at all (Thamavit et al., 1987; 1996). Rather, it exerts a marked promoting influence on cholangiocellular (see Fig 2) and hepatocellular tumor development in the hamster via chronic irritation and increased cell turnover (Thamavit et al., 1996). Yields of cholangiocellular lesions, including preneoplastic cholangiofibrosis and CCCs (Moore et al., 1996) are dependent on both carcinogen dose and numbers of metacercariae (Thamavit et al., 1987a). Even as low as 12 metacercariae is sufficient for DMN induction of bile duct carcinogenesis. The liver fluke infection also promotes development of diethylnitrosamine (DEN)-induced hepatocellular nodules (Thamavit et al., 1987b)

An investigation of the effect of endogenously generated carcinogen, with combined administration of 0.1% nitrite and 0.1% aminopyrine in the drinking water for eight to ten weeks, also resulted in subsequent development of both hepatocellular nodules and cholangiofibrotic lesions/ cholangiocellular carcinomas in Syrian golden hamsters. Additional prior dosing with Opisthorchis viverrini metacercariae (100/animal) induced inflammatory and proliferative changes in the livers of infected hamsters and was associated with a significant increase in yields of hepatocellular and cholangiocellular preneoplastic and neoplastic lesions (Thamavit et al., 1988a).

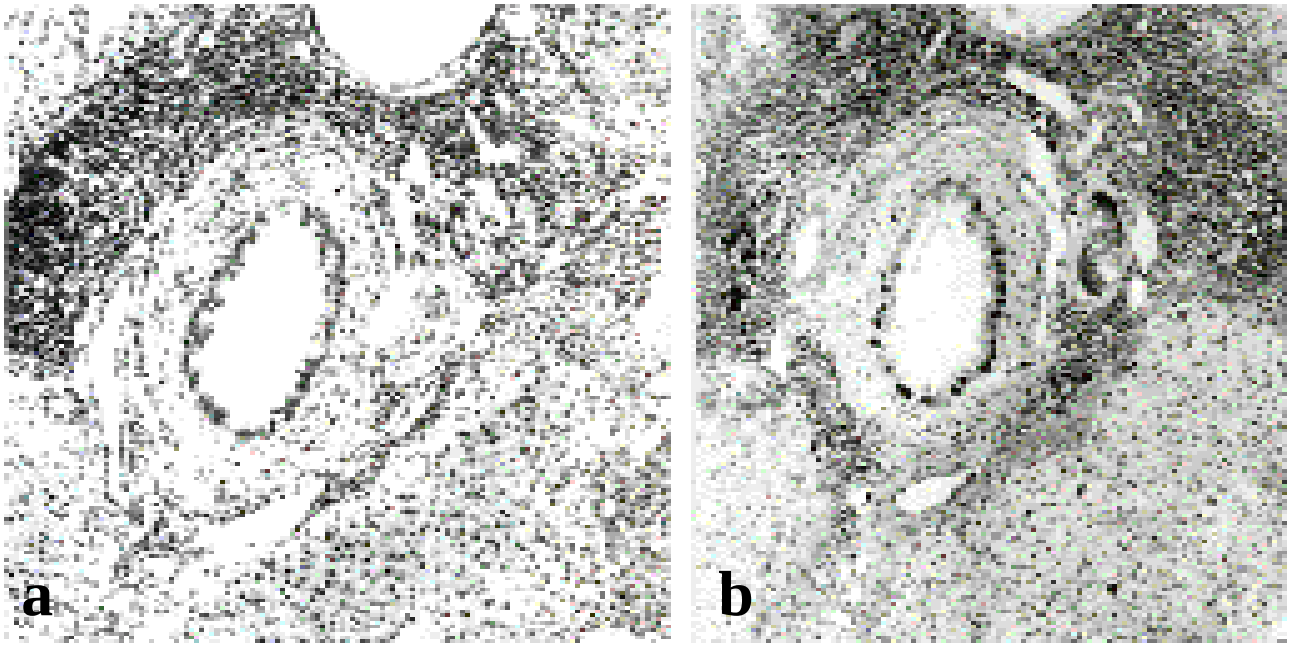

Figure 1. Inflammatory and Fibrotic Change around a Duct in the Liver of a Syrian Hamster Infected with Opisthorchis viverrini. a) H&E b) BrdU Labeling

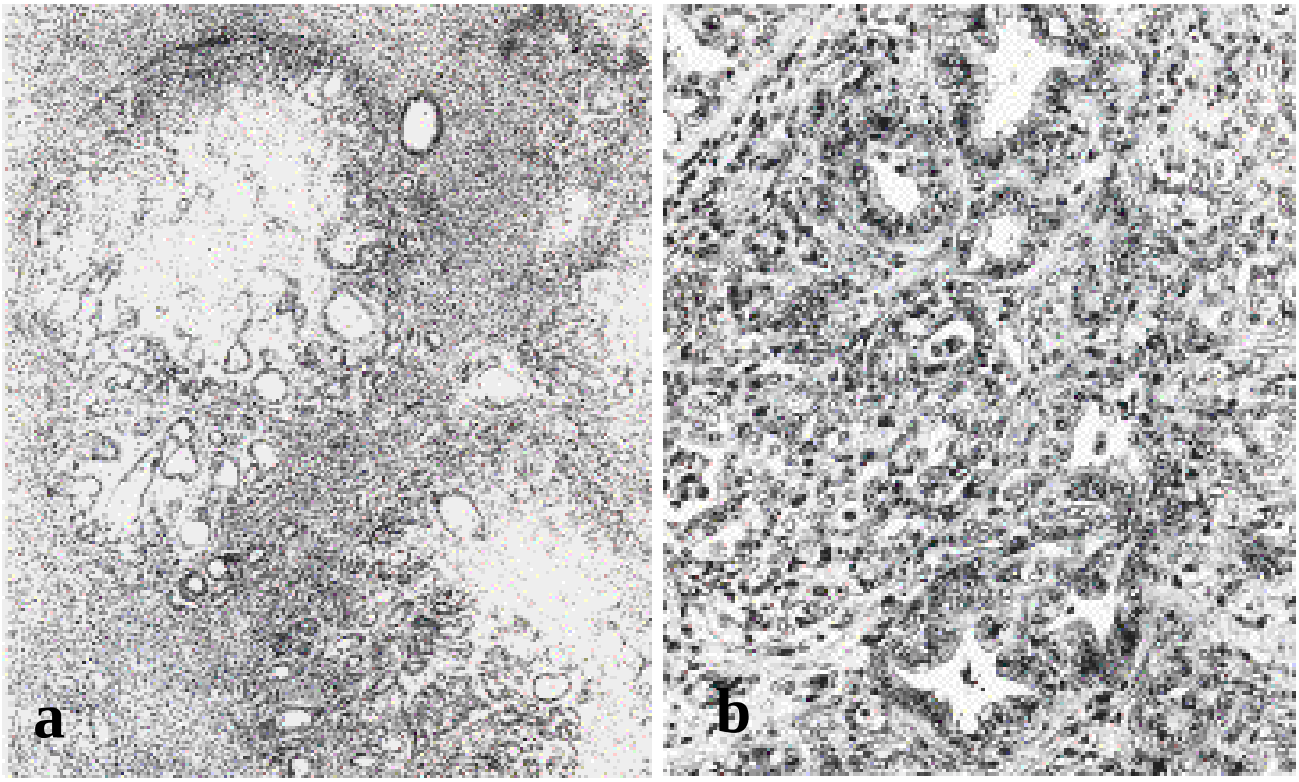

Figure 2. A Cholangiocellular Carcinoma in a Syrian Hamster a) Overview b) Detail

With dihydroxy-di-n-propyl nitrosamine (DHPN) (1000 mg/kg body weight) parasite infection brought about significant enhancement of resultant preneoplastic lesion development in Syrian hamster pancreas tissue as well as the liver (Thamavit et al., 1988b).

Infection of hamsters by the human liver fluke Opisthorchis viverrini elevates liver procollagen prolyl hydroxylase activity, reflecting increased collagen biosynthesis proportional to the intensity of infection (Hutradilok et al., 1983a; 1983b). A conclusion that the parasite is acting by epigenetic physical events, rather than as an initiating carcinogen, is supported by findings for complete ligation of the bile duct. This itself leads to a series of events, obstruction of bile flow being followed by dilatation, cyst formation, and necrosis of the bile duct epithelium and surrounding affected areas followed by regenerative proliferation. If this occurs in hamsters initiated with dimethylnitrosamine, promotion of cholangiocellular lesion development is the result (Thamavit et al., 1993b). Thus parasite-associated proliferation in target cell populations is, at least in part, responsible for the influence of OV on liver tumor development.

Modulation of Cholangiocellular Lesion Development

Experimental findings with the Syrian hamster model and eradication of parasites with the antihelminthic drug Praziquantel indicate that enhancement of DHPN-initiated bile duct carcinogenesis by opisthorchiasis is both rapid and to a large degree irreversible (Thamavit et al., 1993a). Hepatocellular lesion development in this model, on the other hand, appears to correlate more closely with the duration of parasite-associated proliferative stimulus. With Praziquantel the procollagen prolyl hydroxylase activity decreases, similar to collagen levels (Hutradilok et al., 1983a; 1983b), and cell turnover may also return to normal. Clearly there is great interest in the possibility of using Praziquantel to eradicate opisthorchiasis in the affected human polulations and thereby control CCC. While one study suggested that the antihelminthic drug might have promoting potential in the Ito model of hepatocarcinogenesis (Shirai et al., 1991), repeated exposure at levels sufficient for successful removal of parasite infestation did not itself appear to carry carcinogenic risk (Thamavit et al., 1992) and no effects were noted on nitrosamine-induced lesions in the hamster liver (Thamavit et al., 1992b). What effect the repeated use of Praziquantel has in high risk human populations in which re-infection may occur remains to be clarified.

If exposure to carcinogen can be reduced then the induction of neoplasia would be expected to decrease. In fact, vitamin E, given in the diet at 0.5 or 1%, to Syrian hamsters receiving long term combined administration of sodium nitrite and aminopyrine in the drinking water, caused inhibition of both cholangiofibrosis and cholangiocarcinoma development. The underlying mechanisms presumably involve alteration of endogenous dimethylnitrosamine formation by the vitamin, with clear implications for prevention in the human environment.(Thamavit et al., 2001). It should be noted in this context that the Thai diet

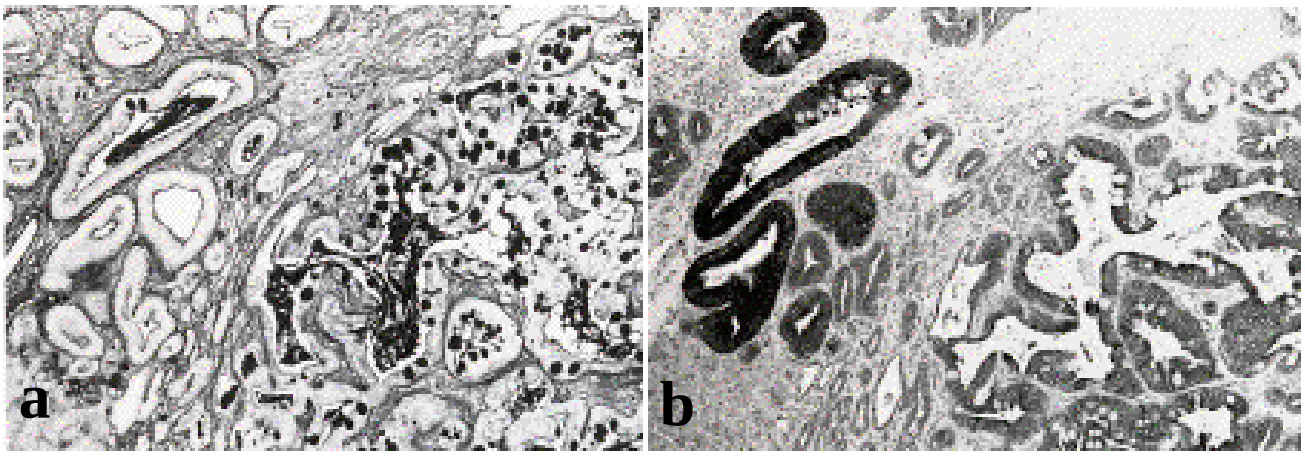

Figure 3. Semi-serial Sections of an Area of Cholangiofibrosis a) Alcian blue/PAS b) Glutathione S-transferase P

may contain nitrates and nitrites (Migasena et al., 1980)

The early preneoplastic lesions induced by carcinogens in the Syrian hamster liver and gallbladder are characterized by change in carbohydrate metabolism, with production of mucin in ductal populations, and increase in expression of enzymes like glucose-6 phosphate dehydrogenase and the glutathione S-transferase placental form (see Fig 3) (Moore et al., 1986; 1996). This was the rationale for two studies conducted with the adrenal steroid dehydroepiandrosterone (DHEA) and the antioxidant butylated hydroxyanisole (BHA). Unexpectedly, when DHEA was given concomitant with Opisthorchis infestation and/or administration of dihydroxy-di-n-propylnitrosamine (DHPN), increase in carcinogen toxicity was apparent with enhanced generation of glutathione-S-transferase P (GST-P)-positive hepatocellular foci, liver cysts and focal proliferative changes in the pancreas (Moore et al., 1988). However, when the timing of DHEA was subsequent to Praziquantel administration, after exposure to DHPN and the parasite, significant reduction in frequency of cholangiofibrosis and cholangiocellular carcinoma was achieved (Moore et al., 1998). The results indicate that although cholangiocellular lesion development may, unlike generation of hepatocellular nodules, be to a certain extent independent of the continued presence of parasite, it can be influenced by exogenous treatments.

Conclusions and Future Perspectives

The mechanisms of carcinogenesis with Opisthorchis viverrini infection have been the subject of considerable research and there is evidence that the presence of parasites induces DNA damage and mutations as a consequence of the formation of carcinogens/free radicals and of cellular proliferation of the intrahepatic bile duct epithelium (Parkin et al., 1993). The Syrian hamster model features histopathological lesions very similar to those evident in man (Pairojkul et al., 1991), although the question of size of the liver and the dimensions of the ductal system relative to the parasites means that particular attention needs to be concentrated on pathophysiology.

Abnormalities significantly associated with intensity of infection in man include gallbladder enlargement, presence of sludge, irregular gallbladder wall, liver enlargement, and enhanced portal vein echoes (Elkins et al., 1996). While gallbladder enlargement is not sex-specific, the prevalence odds of the other abnormalities are 2-3 times higher among males compared with females. Individuals recently treated with the anthelmintic praziquantel have higher odds of abnormalities compared with others with the same infection status who remain untreated. The low prevalence of gallstones suggests that any impairment of gallbladder structure and function does not frequently stimulate gallstone formation. Other studies of biliary pathology related to worm burden in humans demonstrated that only a minority of subjects with parasites demonstrate a pathology of adenomatous hyperplasia, which is believed to predispose bile ducts to subsequent development of carcinomas (Pairojkul et al., 1991). Biliary changes in nontumorous areas of hepatectomy specimens, including fibrosis (with or without adenomatous hyperplasia) which is found in most cases, and dysplasia in the fibrotic ducts, indicate a conversion event in carcinogenesis: other factors may be required to aggravate the simple proliferation lesion so that they subsequently change to carcinomas. Comparison of tumor phenotypes and expressions of ras p21 in fluke related and non-fluke-related cholangiocarcinomas suggest that some similar mechanisms might be operating, at least in the relatively late stages of multistage carcinogenesis involving the bile ducts (Pairojkul et al., 1991). In another study. significant differences in the expression of p53 protein or c-erbB-2 protein were found between the two series of patients, but proliferative activity was significantly higher in the Thai patients (Suzuki et al., 2000).

With regard to the histogenesis in the hamster, nearly all infected hamsters that tested positive for cancer also tested positive for p53 immunostaining in the epithelia of the small bile ducts. Electron micrographs of these positive p53-immunostained cells showed characteristics of early cancer so that it has been proposed that p53 is a candidate as a tumor marker (Tesana et al., 2000).

To return to the possibility of eradication of parasites, as

proposed for *Helicobacter pylori* in the stomach cancer case (Shimizu et al., 2000), it is a high priority that the effects of past administration of antihelminthic drugs be now epidemiologically assessed with a case-control approach (see Table 2). Establishing a cohort for longer term follow-up would allow the dynamics of physiological change in response to infection and parasite eradication to be elucidated. Studies have provided evidence that nitric oxide synthesis is elevated during human liver fluke infection, with increased nitrosation of proline and thioproline (thiazolidine-4-carboxylic acid) among infected men which can be specifically abolished by co-administration of ascorbic acid with proline and by elimination of parasites by praziquantel treatment (Satarug et al., 1996). Whether dietary supplementation with antioxidants should be included with Praziquantel treatment in intervention studies therefore requires attention.

Given the importance of inflammation for neoplasia in multiple organ sites and the wide potential of specific cycooxygenase inhibitors to prevent cancer development in different tissues (Wakabayashi, 2000), investigation of the effects of COX-1 and COX-2 inhibitors in the Syrian hamster model would appear warranted. Selective and nonselective COX-2 inhibitors can inhibit inflammation and development of adenocarcinoma induced by reflux in the oesophagus (Buttar et al., 2002). Based on mechanistic understanding, antioxidants, inhibitors of arachidonic acid metabolism and receptor antagonists of certain eicosanoids have been proposed as potential chemopreventive agents for testing in the animal surgical model (Chen and Yang, 2001), in which vitamin E supplementation has already demonstrated beneficial influence (Chen Xet al 2000). A series of experiments to elucidate the effects of different chemopreventive agents, alone and in combination, on CCC development in Opisthorchis-infected animals would provide a basis for optimal intervention in the human situation. Indeed, it could be argued that a preliminary intervention study with aspirin might already be worthy of attention.

For screening purposes, serum antibody responses to liver fluke antigens may be useful in the identification of infected individuals who are at high risk for liver fluke-associated CCA. (Akai et al., 1994). A tumor-associated soluble antigen defined by MAb 6E5 has been reported to be a useful marker for the detection of tumors at an early stage of development (Prempracha et al., 1994). Markers like CA 19-9 and carcinoembryonic antigen have a relatively high specificity but low sensitivity for the detection of cholangiocellular tumours in primary sclerosing cholangitis patients (Bjornsson et al., 1999) and attention has been paid to changes in serum sialic acid in serum as a tool for early detection (Wongkham et al., 2001; 2003). Glycoproteins secreted from tumors and inflammatory cells might be responsible for the increased total sialic acid in the serum in CCC patients (Wongkham et al, 2003), but there does not appear to be sufficient specificity for neoplasia (Wongkham et al, 2001). The potential for using changes in glycoproteins from cholangiocellular lesions released into the bile and thus finding their way into the faeces as an alternative approach to marker development, might warrant interest.

One approach to clinical diagnosis and staging of bile duct cancer which might also deserve more stress is positron emission tomography (PET) with [(18)F]fluoro-2-deoxy-D-glucose for (Kluge et al., 2001), although the possibility of false positive results means that this approach is inappropriate for patients with mucinous cholangiocarcinomas (Fritscher-Ravens et al., 2001). Whether PET could have promise for screening, at least for determination of those cases for which surgical intervention might be worthwhile, is another question requiring further attention in the future.

Table 2. Possible Future Research Projects

1. What is the efficacy of Praziquantel?
   - Case-control study
   - Prospective study
2. What is the influence of COX inhibitors?
   - Intervention study - Syrian hamster - COX II
   - Intervention study- human - aspirin
3. What potential has mucin as a screening tool?
   - Syrian hamster model - Glycoproteomics
     - Stimulation of mucin release?
   - Human population study
4. What potential has PET as a screening/diagnostic tool?

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