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# A hypothesis on paradoxical privileged portal vein metastasis of hepatocellular carcinoma. Can organ evolution shed light on patterns of human pathology, and *vice versa*?☆

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ABSTRACT

Unlike other carcinomas, hepatocellular carcinoma (HCC) metastasizes to distant organs relatively rarely. In contrast, it routinely metastasizes to liver vasculature/liver, affecting portal veins 3–10 times more often than hepatic veins. This portal metastatic predominance is traditionally rationalized within the model of a reverse portal flow, due to accompanying liver cirrhosis. However, this intuitive model is not coherent with facts: 1) reverse portal flow occurs in fewer than 10% of cirrhotic patients, while portal metastasis occurs in 30–100% of HCC cases, and 2) portal vein prevalence of HCC metastasis is also characteristic of HCC in non-cirrhotic livers. Therefore, we must assume that the route for HCC metastatic dissemination is the same as for other carcinomas: systemic dissemination via the draining vessel, i.e., via the hepatic vein. In this light, portal prevalence versus hepatic vein of HCC metastasis appears as a puzzling pattern, particularly in cases when portal HCC metastases have appeared as the sole manifestation of HCC. Considering that other GI carcinomas (colorectal, pancreatic, gastric and small bowel) invariably disseminate via portal vein, but very rarely form portal metastasis, portal prevalence of HCC metastasis appears as a paradox. However, nature does not contradict itself; it is rather our wrong assumptions that create paradoxes. The 'portal paradox' becomes a logical event within the hypothesis that the formation of the unique portal venous system preceded the appearance of liver in evolution of chordates. The analysis suggests that the appearance of the portal venous system, supplying hormones and growth factors of pancreatic family, which includes insulin, glucagon, somatostatin, and pancreatic polypeptide (HGFPF) to midgut diverticulum in the early evolution of chordates (in an Amphioxus-like ancestral animal), promoted differentiation of enterocytes into hepatocytes and their further evolution to the liver of vertebrates. These promotional-dependent interactions are conserved in the vertebrate lineage. I hypothesize that selective homing and proliferation of malignant hepatocytes (i.e., HCC cells) in the portal vein environment are due to a uniquely high concentration of HGFPF in portal blood. HGFPF are also necessary for liver function and renewal and are

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significantly extracted by hepatocytes from passing blood, creating a concentration gradient of HGFPF between the portal blood and hepatic vein outflow, making post-liver vasculature and remote organs less favorable spaces for HCC growth. It also suggested that the portal vein environment (i.e., HGFPF) promotes the differentiation of more aggressive HCC clones from already-seeded portal metastases, explaining the worse outcome of HCC with the portal metastatic pattern. The analysis also offers new hypothesis on the phylogenetic origin of the hepatic diverticulum of cephalochordates, with certain implications for the modeling of the chordate phylogeny.

In contrast to the engineer, evolution does not produce innovations from scratch. It works on what already exists, either transforming a system to give it a new functions or combining several systems to produce a more complex one. – François Jacob.

## Introduction

Since the title includes the word 'paradox', I would like to begin with the meaning of 'paradox'. There are several similar definitions of the phenomenon, including one from the Merriam Webster dictionary: "One (such as a person, situation, or action) having seemingly contradictory qualities or phases." [1]. However, the Merriam-Webster dictionary highlights the most important feature of a paradox in the etymology of the word:

"The ancient Greeks were well aware that a paradox can take us outside our usual way of thinking. They combined the prefix *para-* ("beyond" or "outside of") with the verb *dokein* ("to think"), forming *paradoxos*, an adjective meaning "contrary to expectation" [1]." In science the "expectation" unavoidably means that we analyze facts from the point of a hypothesis (model) that we have created to explain facts.

Medicine, like other areas of human activity, is full of paradoxes. While solving a medical paradox is usually a noticeable and triumphal event, there is one subtle crucial step beforehand, without which nothing could happen. It is the recognition of a paradox: because a paradox can be either not noticed or ignored. The most famous episode of ignoring and recognizing a medical paradox in happened in Austria in the middle of the 19th century.

There were two almost identical obstetric clinics in Vienna. However, there "was the remarkable difference in puerperal fever mortality between the two neighboring clinics. In 1846, for instance, in the 1st Obstetric Clinic … out of 4010 laboring patients, 459 had died of puerperal fever, a total of 11.4 per cent, while during the same period, in the 2nd Obstetric Clinic, out of 3754 laboring patients only 105 had died, i.e. 2.7 per cent. Over a stretch of five years (1841–1846), the 1st clinic had witnessed 1300 more victims of puerperal fever than the 2nd clinic" [2]. Doctors of both clinics knew about this difference in puerperal fever mortality rates, and so did the laboring patients who were mortally scared of being admitted to the 1st Obstetric Clinic. While all doctors knew about this difference in mortality between the two clinics, they perceived it as a natural inevitable event and did not view it as a violation of logic, i.e., as a paradox. Hence, they did not have a reason to ask the question: why are the rates of mortality different?

However, these figures appeared as a paradox in the eyes of Ignaz Semmelweis, who thought that mortality rates should be similar in both clinics. A Hungarian physician, Ignaz Philipp Semmelweis was the only one who recognized the paradox, and for years he relentlessly searched for an explanation, applying and testing numerous hypotheses. Dr. Semmelweis finally found that the only difference between two clinics was that the same medical personnel taking care of laboring women also routinely performed postmortem examinations of deceased patients, a practice in the first obstetric clinic but not in the second. Doctors and assistants barely washed their hands between these activities. The paradox became a logical event: the doctors' hands carried the "cadaverous matter" to laboring women, and they had to be properly cleaned before examinations. Indeed, washing hands with chlorinated lime solutions reduced mortality rates almost to zero. The concept of antiseptics was created, 20 years prior to the Louis Pasteur discovery [2].

I have included this episode not just for its historical medical significance. Someone could think that in modern medical practice/science it should not be difficult to recognize an event that contradicts common sense, whatever the paradox is. Although recognition of a paradox, i.e., to be aware of disagreement between anticipated results (hypothesis) and reality (facts), is a natural function of human cognition, to act on it or not is our choice: a paradox simply could not be noticed, which can significantly impact scientific progress. Anthony Aguirre, a physicist from University of California, writes in the essay 'The paradox':

"Paradoxes arise when one or more convincing truths contradict either each other, clash with other convincing truths, or violate unshakeable intuitions. … Nature appears to contradict itself with the utmost rarity, and so a paradox can be opportunity for us to lay bare our cherished assumptions, and discover which of them we must let go. But a good paradox can take us farther, to reveal that the not just the assumptions but the very modes of thinking we employed in creating the paradox must be replaced." [3].

I would like to elaborate the above notions in regard to another medical paradox: the privileged portal vein metastasis of hepatocellular carcinoma.

## Puzzling patterns of HCC metastasis

In general, carcinomas are characterized by metastatic spread to distant organs, which accounts for 90% of cancer-associated deaths [4]. It is agreed that cancer cells escape from the primary tumor into the blood circulation via draining vasculature, i.e. veins (lymphatic drainage to sentinel lymph nodes is not discussed in this analysis). By this route, cancer cells are carried by blood flow through the heart to the capillaries of the lungs, where metastases often seed [5]. Any cells that managed to pass through lung capillaries enter the systemic arterial circulation and then disseminate to distant organs of the body, forming metastases [6]. However, intravascular carcinomas' metastases are very rare [7,8].

HCC is one of the most common carcinomas, constituting the second-third leading cause of cancer-related mortality [9,10] and is on the rise Western countries [11–13] and worldwide [14–17].

However, unlike other carcinomas, HCC metastases to distant organs are relatively rare, even in advanced cases [18], while liver intravascular and parenchymal metastases (the latter likely evolving from the former [19]) occur very frequently [10,20,21].

The traditional explanatory model on preferential liver vascular invasion by HCC is a "local model", which assumes local intrahepatic dissemination via HCC cell detachment from a primary tumor and movement into liver vasculature [22–28]. However, under assumptions of the local intrahepatic dissemination model, metastasizing along with liver blood outflow into hepatic veins appears be more probable than metastasizing against blood inflow into portal veins, whether HCC cells float with blood within the liver or migrate on the endothelial surface. Nonetheless, portal vein metastases occur 3–10 times more frequently than hepatic veins metastases [13,26–33]

More recently, Sakon and co-authors suggested that HCC cells are

always disseminate into systemic circulation via the hepatic vein [34–37], proposing the route of HCC dissemination to be the same as for other carcinomas. This model assumes that the freshly detached, and therefore more preserved, HCC cells first pass hepatic veins, which logically should be the most metastasis-targeted compartment. Portal veins should be affected by HCC metastases less frequently because HCC cells appear in portal veins after passing heart, lung and visceral capillary nets, a cell moving associated with hypoxia, nutrient deprivation, and shear stress [38–40].

Nevertheless, the facts show the opposite distribution: portal vein metastases occur 3–10 times more frequently than hepatic veins metastases [13,26–33] We should conclude that both models (local intrahepatic spread and systemic hematogenous dissemination) are not able to explain preferential portal metastasis, which remains paradoxical.

The unusual prevalence of HCC metastases in portal vein versus hepatic vein was first noted by James Ewing in 1922 [41]. Ewing writes "An adenocarcinoma of the liver regularly appears as a circumscribed growth distending the large branches of the portal vein in this organ" (page 58). Furthermore, Ewing writes, "By this route the tumor grows into the larger veins which may be occluded by tumor masses, although their walls are intact and no point of penetration may be found" (page 687) [41]. In modern times, this puzzling prevalence of the portal pattern of HCC metastasis was re-emphasized by Albacete and co-authors in 1967 [42].

This unproportioned portal metastatic prevalence also occurs is also a feature of early HCC stages, when the primary tumor is small [13,43]. Additionally, HCC invades the main portal vein (trunk and first/second-order branches of portal veins) 3–6 times more often than the third-order and more distal branches of the portal vein [13,44–46]. All studies universally emphasize that HCC cases with portal metastasis have much worse prognosis than those without portal metastasis, although nature of the portal prevalence of HCC metastasis and worse outcome remain puzzling.

## Can a hepatofugal (retrograde or reverse) portal flow solve the paradox?

The intuitive explanation for this prevalence of portal versus hepatic vein metastasis is retrograde (hepatofugal or reverse) portal blood flow, which carries metastatic HCC cells upstream to the liver [47] against normal portal blood flow pattern. This condition is known to occur in liver cirrhosis [48], which often accompanies HCC (e.g. [49]).

However, assumption of hepatofugal flow at early [29] or later HCC stages [47] cannot resolve the paradox for the following reasons: 1) reverse portal flow occurs in less than 10% of cirrhotic patients [48], while portal metastasis occurs in 40–100% of HCC cases, and 2) portal vein prevalence of HCC metastasis is also characteristic of HCC in non-cirrhotic patients [50–54], with some studies reporting equal presence of portal HCC metastasis in cirrhotic and non-cirrhotic liver (numerous reports, for example see [55]).

The HCC portal vein metastatic pattern is depicted in Fig. 1.

Apart from the above incoherencies, all models on HCC liver vascular metastasis (local, systemic, and hepatofugal models) fail to explain baffling observations where intraportal HCC tumors have appeared as the sole HCC manifestations, without primary tumors in the liver.

## HCC metastases of the portal trunk or main portal branches as solo manifestation of HCC, with no detectable primary HCC tumor in liver parenchyma

There are eight exhaustive clinical reports describing HCC presenting ***only*** as a HCC thrombosis of a portal trunk or main portal branches with ***no*** detectable primary HCC tumor in liver parenchyma (fifteen cases total) [56–62]. It is important to emphasize again that HCC tumors in the liver parenchyma were not detected, but there were HCC metastases in the main portal veins. In four patients, a failure to detect the primary HCC was suggested to be due to heterogeneity of the malignancy [61], but yet 11 cases remain confirmed as HCC presenting only as tumors of main portal veins [56–60,62]. Notably, such HCC manifestations have never been described to occur in hepatic veins. This perplexing HCC manifestation is depicted in Fig. 2:

These findings demand the obvious question: Where did these HCC metastatic cells come from? And the more important question: Why are these HCC cells homing and grow in portal veins? To answer the first question, we can suggest that there are undetectable small HCC clusters in liver. It can be further hypothesized that these very small undetectable HCC clusters shed HCC cells, which exit the liver with the hepatic vein blood flow. Then HCC cells appear in circulation, passing the heart, lung and abdominal visceral capillary nets, and they then appear in main portal veins, colonize them and proliferate. However, a more puzzling fact ought to be explained: Why do these HCC cells not form metastases in hepatic veins, lung, and abdominal viscera, however, after a long and damaging journey, home and colonize portal veins, creating such an unexpected single manifestation of HCC? It is not surprising that the answer to this question is difficult to find; what is really astonishing is that we have rarely asked this question. However, this question is imperative because this solo intraportal HCC presentation accentuates the perplexing prevalence of portal vein metastasis versus hepatic vein metastasis in patients with established primary liver HCC. The 'Portal HCC paradox' also needs to be put in spotlight because it became clear that HCC with metastasis in portal veins at any level of portal vein system (Vp1–Vp4 [63]), is associated with poor prognosis and disease recurrence under different treatment strategies [64–78], even though the nature of this association remains obscure.

HCC privileged portal metastasis also appeared paradoxical considering that other gastrointestinal carcinomas always spread metastatic cells via the portal vein but rarely form metastases inside the portal vein itself.

## Other gastrointestinal carcinomas (colorectal, pancreatic, stomach, and small bowel) always spread cancer cells via the portal vein to liver parenchyma but very rarely form metastases in portal veins

This phenomenon of preferential portal metastasis is the distinctive feature of HCC. Portal metastases occur in 30–70% of HCC cases (and virtually in 100% of HCC with disease progression), while other carcinomas of abdominal viscera (colorectal, pancreatic, small bowel, and stomach) always spread cancer cells via the portal vein to the liver but very rarely form metastases in the portal veins themselves [79–81].

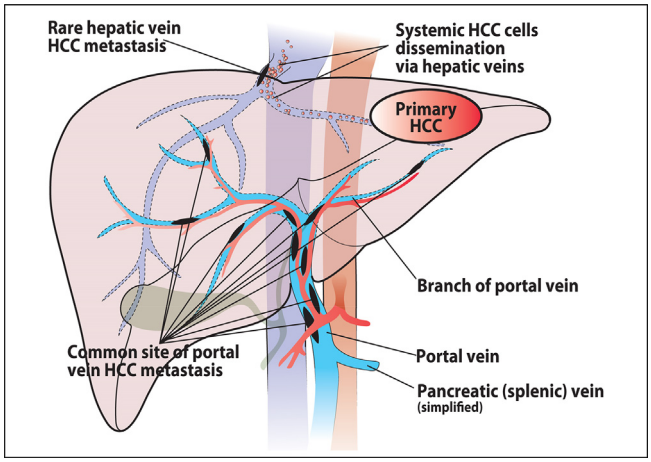


**Fig. 1.** Schematic depiction of HCC metastasis to main portal veins, while HCC cells disseminate systemically via hepatic veins.

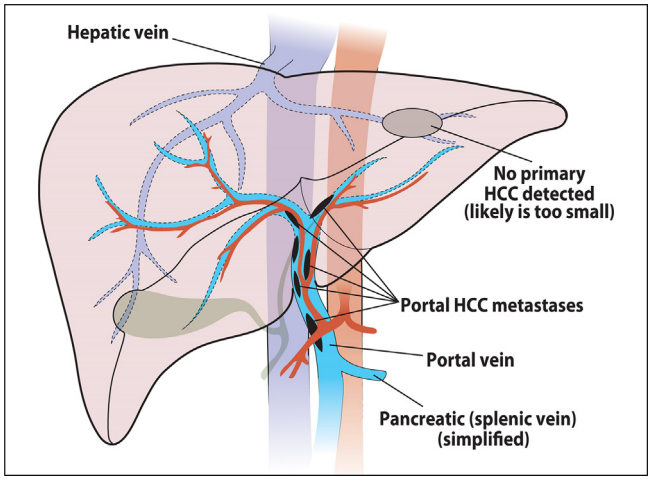


**Fig. 2.** Schematic depiction of the HCC metastases to main portal veins as solo manifestation of the disease, without detectable primary HCC in liver parenchyma.

This disproportion in portal vein metastasizing between HCC versus other abdominal visceral carcinomas is especially noticeable when it compared to colorectal cancer. Malignant colon carcinoma cells are always detected in the portal lumen [82], which also must be true for carcinomas of all organs that drain by portal vein tributaries. Nevertheless, according to the 1997 Annual of the pathological autopsy cases in Japan, the incidence of portal vein metastasis in colorectal cancer and in gastric cancer, was reported to be 0.6% and 1.2% retrospectively [83]. The frequency of portal vein metastasis in HCC versus that of other carcinomas of other abdominal organs is depicted in Fig. 3:

Therefore, we must conclude that this mystifying pattern of preferential portal vein homing/metastasizing pertains only to hepatocyte-derived malignant cells (i.e. HCC cells).

These clinical facts regarding the exceptional ability of malignant hepatocytes to colonize and proliferate inside portal veins are resonated with experimental data on non-malignant hepatocyte homing in intact intrahepatic portal veins in regenerating rat liver under special conditions (repeated FK-506 and CCl4 treatment).

## Experimental observations on hepatocytes colonizing intact intrahepatic portal veins of rat livers

Hepatocytes, that morphologically appeared normal, were discovered inside portal venous branches in the livers of rats co-treated with FK-506 (Tacrolimus) (0.2 mg/kg, three times per week) and carbon tetrachloride ($CCl_4$) twice per week (3 and 8 weeks of combined treatment, portal trunk was not harvested)*. No varices were noted, although at 8 weeks some animals developed moderate ascites. Intraportal hepatocytes appeared as compact cell clusters partially or completely occluding portal lumens. Intraportal hepatocytes often appeared only as 1–2 cell layers attached to the portal endothelium, with portal lumen potent. Mitotic figures were rare, and morphologically, the hepatocytes appeared nonmalignant. Occasionally, erythrocytes and mononuclear cells were trapped between hepatocytes. No collagen fibers or alpha smooth muscle actin-positive cells were found between portal hepatocytes, although the liver parenchyma was fibrotic and contained numerous alpha smooth muscle actin-positive hepatic stellate cells (Fig. 4):

In order to corroborate whether intraportal hepatocytes were result of a parenchymal ingrowth into portal vein lumens, paraffin blocks, containing intraportal hepatocytes, were cut into serial sections. Subsequent examination showed that the portal vein walls were intact, and has not demonstrated hepatocytes' ingrown into portal lumen, although such parenchymal extensions were occasionally present in remodeled rodent livers after repeated $CCl_4$ treatments (Fig. 5a, arrows). Artificial intrusion (replacement) of parenchymal fragments into portal lumen, which may occurs during harvesting or paraffin sectioning, was also ruled out because of the multiplicity of intraportal hepatocyte findings, none of which displayed hepatic plate-like architecture, typical for replacement artifacts (Fig. 5b).

Since the above causes (natural and artificial) were ruled out, the only logical explanation appeared to be that in regenerating/remodeling liver, hepatocytes detached from hepatic plates into sinusoids (similar to dropout of altered hepatocytes [84,85]), exit the liver via the hepatic vein, and appeared in systemic circulation (similar to systemic hematogenous dissemination of HCC cells). After passing pulmonary

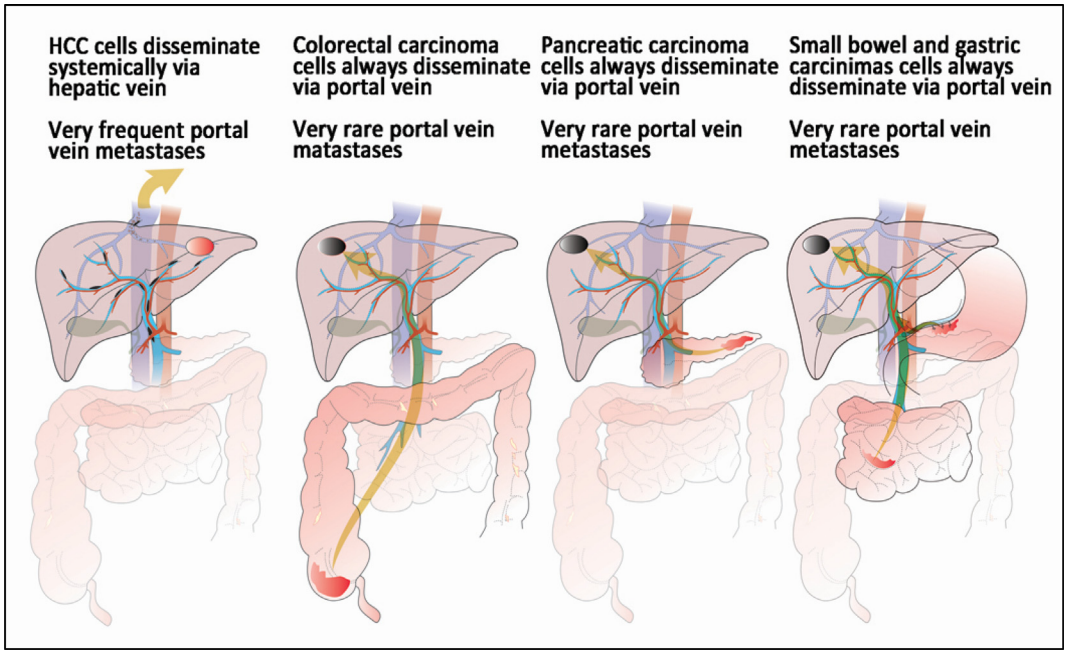


**Fig. 3.** Schematic depiction of the very frequent HCC metastasis to portal veins, while a portal metastatic pattern is very rare in other visceral carcinomas, which nonetheless disseminate via the portal vein.

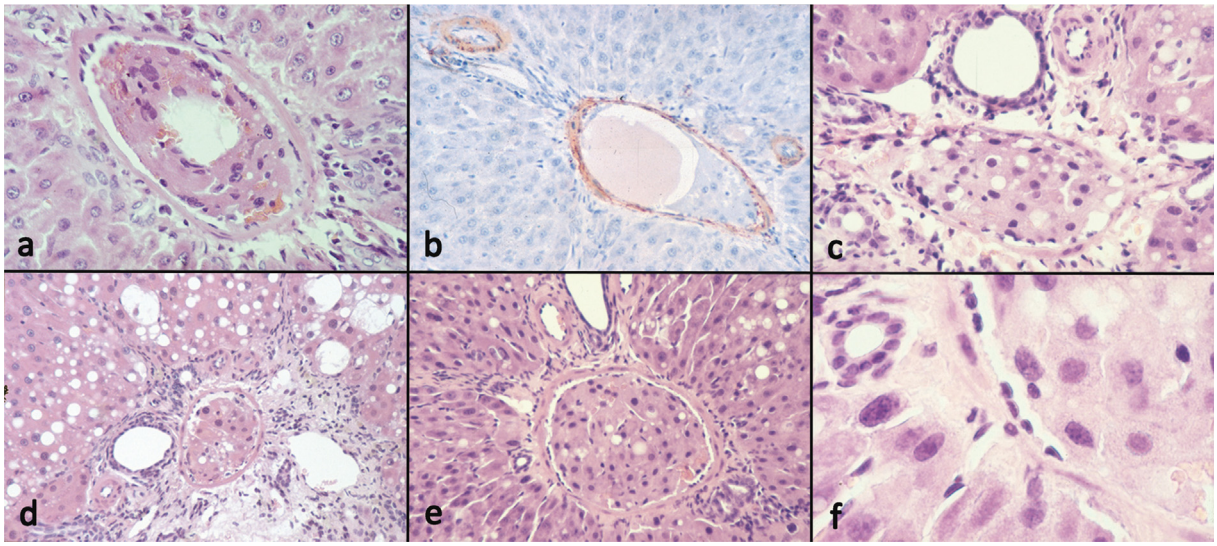


**Fig. 4.** Sections of rat livers from animals co-treated with FK-506 and $CCl_4$. a – 3 weeks of combined treatment, hepatocytes attached to the portal endothelium with portal lumen potent, H&E stain; b – 3 weeks of combined treatment, hepatocytes attached to the portal endothelium with portal lumen potent, immunostain for alpha smooth muscle actin, x400; c – 3 weeks of combined treatment, hepatocytes filled portal vein lumen, H&E stain, x400; d – 3 weeks of combined treatment, hepatocytes filled portal vein lumens, H&E stain; d – 8 weeks of combined treatment, hepatocytes filled portal vein lumens, H&E; e – 8 weeks of combined treatment, viable hepatocytes completely occupy portal lumens, H&E; f – the same field magnified. a-e – x400, f – x1000. *All animal experiments were conducted in accordance with the NIH guidelines for the Care and Use of Laboratory Animals and approved by the University of Pittsburgh IACUC.

and abdomen visceral capillary nets, hepatocytes entering portal veins, attach to the portal endothelium and colonize the lumen of portal veins [86,87]. This intraportal hepatocyte homing and survival was suggested to be due to the hepatotrophic effect of FK-506 [88,89].

A striking feature of these intraportal hepatocyte clusters of rat livers was the morphological resemblance to HCC portal vein metastasis in the clinic, Fig. 6:

The above observations suggested that both normal and malignant hepatocytes possess the unique ability of attaching to the portal vein endothelium and proliferate inside portal veins. The nature of this "portal affinity" of hepatocyte-derived cells remains unclear, prompting a search for explanatory hypotheses.

## Analysis of possible models on preferential portal metastasis of HCC

In spite that portal HCC metastasis constitute a great clinical problem, there is only one publication (to my knowledge) that puts the spotlight on this paradoxical prevalence of portal HCC metastasis. This analysis was contributed by Dr. Byung Ihn Choi. In his editorial, Dr. Choi (a prominent abdominal radiologist from Seoul National University) directly asked 'What accounts for such discrepancy in involvement of the portal vein and hepatic vein?' and outlined four potential explanatory models [90].

The first model suggests that HCC cell portal metastases occur at early stages of tumor growth when tumor replaces adenomatous nodules, when are still supplied by the portal vein. This pathogenesis was based to on the work of Nakashima and Kojiro [91].

The second suggests that HCC arterialization occurs together with arterioportal shunting during early HCC stages, and HCC cells moved from hepatic artery branches into portal vein system [92–94].

The third model is based on a hypothesis that portal branches serve as draining vessels in HCC [29].

The fourth model assumed the initial equal HCC metastases distribution in portal and hepatic veins, suggesting that small metastases of hepatic vein may be washed away from the hepatic vein at early stages of HCC, while systemically circulating HCC cells are stacked in the peripheral portal branches [91].

However, the first and second models s are not coherent with reported observations on sinusoidal/venous drainage from early HCC

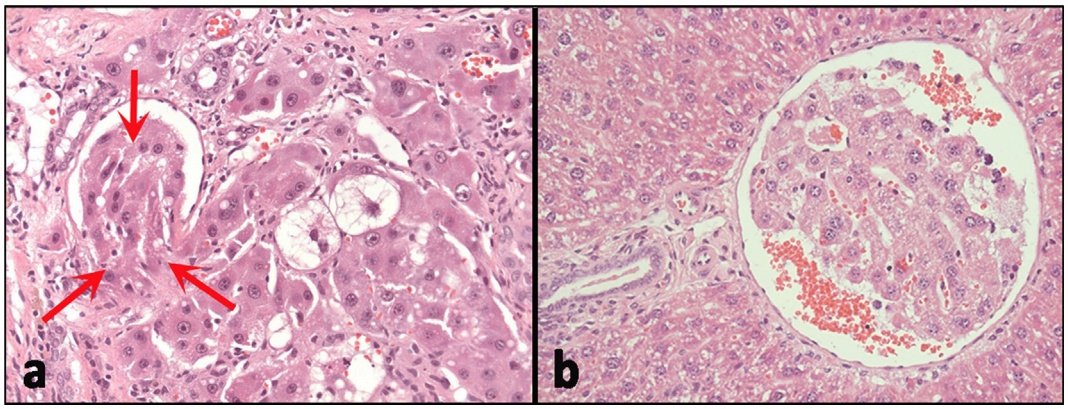


**Fig. 5.** a - an example of a direct parenchymal extension (ingrowth) into portal vein, rat liver, $CCl_4$ + Phenobarbital 8 wks, H&E, x400; b - an example of artificial parenchymal replacement (during harvesting or sectioning). Parenchymal fragment has no morphologic connection to the portal endothelium, retained hepatic plate architecture, sinusoids and small vasculature, naïve mouse liver, H&E, x400.

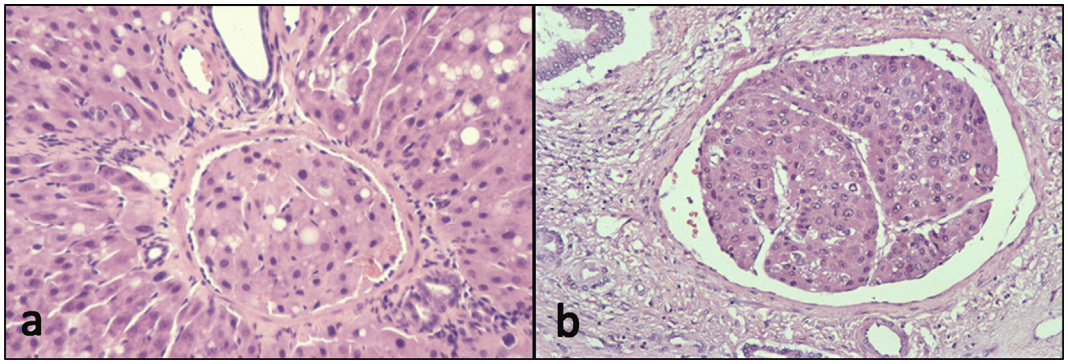


**Fig. 6.** a - Intraportal hepatocytes in rat liver, $CCl_4$ + FK-506 for 8 wks, the same as shown in Fig. 5; b - intraportal HCC metastasis in liver explant from a patient who received liver transplantation for HCC, personal observation [67], H&E, x400.

nodules [95,96], and also contradict to the fact that later HCC stages a more frequently associated with portal metastasis, e.g. [97].

In regard to portal vascularization of HCC, all reports, e.g. [98–100] showed exclusive arterial vascularization of HCC, which is the foundation for the intra-arterial treatment of liver neoplasms [39,101–103]. The presence of a portal supply in HCC is extremely rare and reported to occur only after repeated transcatheter arterial chemoembolization [104–107]. Experimental observations, also showed the exclusive arterial blood supply of liver tumors [108–111].

The fourth model outlined by Dr. Choi is not discussed in this analysis for a reason that the suggested “washed away hepatic vein metastasis”, which never manifested as such, cannot constitute a metastasis by pathology definition.

Out of all pathogenetic models on preferential portal HCC metastasis discussed by Dr. Choi, most interesting is the third model suggesting that with primary HCC growth portal veins take function as tumor draining vessels [29]. This pathogenesis of portal HCC metastasis received support from clinical observation: Matsumata and co-authors showed a lack of intrahepatic recurrence of HCC after temporary portal venous embolization, prior to liver resection, with starch microspheres [112]. Although this study [112] and a similar later report [113] focused on mechanical disturbance of the liver HCC during hepatectomy and HCC cells dislodging via the portal vein, they certainly indicated the anatomical opportunity of portal draining from HCC tumors. The ability of small HCC to spread intrahepatically via the portal vein after surgery was also noted by others [114,115].

Similar dissemination pathway of HCC to portal veins acting as efferent draining tumor vessels was detailed in publications by Toyosaka *et al.* in three leading medical journals in 1996 [26,116,117].

The model of HCC portal metastasis due to portal veins serving as a HCC draining vasculature was studied by a research group from the Kanazawa University, Japan (one article in collaboration with the Shinshu University, Japan) [95,118–121]. Using combination of radiology and histopathology analyses, this research group showed that with HCC nodule progression and formation of dense tumor fibrous capsule, drainage of blood from HCC converted: initially from hepatic veins to hepatic sinusoids, and later from hepatic sinusoids to portal veins [95,118–121].

This shift of the HCC blood draining route from hepatic to portal veins recently demonstrated by a study of Fukutomi *et al*, [19]. Fukutomi *et al*, combined preoperative 3-dimentional CT of HCC with histopathology mapping of primary HCC, metastases and liver vascular anatomy from resected specimen (anatomical resection). This technique enabled Fukutomi *et al.* to create a 3-dimentional mapping of HCC vascular invasion [19]. The resultant 3-D mappings showed HCC cells extensively formed metastases in third-order potent portal branches, supplying hepatic parenchyma in vicinity of HCC. The number of metastases abruptly declined with an increased distance from the tumor of just 5 mm [19]. Therefore, the work of Fukutomi *et al.* corroborated the hypothesis suggested by Toyosaka *et al.* [26,116], and the research group from the Kanazawa University [95,118–121].

While the frequent HCC metastasis to the main trunk and the first/second-order branches of portal veins [13,44–46,64–66,68–74], cannot be explained by the Fukutomi *et al.* model [19],the later observations raise very important questions and comments:

1) Why do HCC cells so extensively home and proliferate in the third-order potent feeding portal branches, while first passing the third-order portal branches serving as HCC draining vessels without attachment/colonization?
2) Available information suggests that HCC cells are detached from primary tumors as single cells whose diameter is about 15–20 μm. Since the diameter of the third-order portal veins is at least 1–2 mm and the blood flow rate is 10–30 ml/min (inferred from [122–124]), it is anticipated that HCC cells should be carried further into hepatic parenchyma via sinusoids, where the narrowed space and slow blood flow provide more favorable conditions for HCC cells attachment and colonization. Therefore, why do HCC cells do not colonize third-order draining portal veins they are passing, but exclusively colonize the third-order feeding portal branches?
3) According to this model [19,26,29,95,116,118–121], cancer cells from encapsulated liver HCC are dislodged into draining portal branches and further pushed into the third-order feeding portal branches due to collapse of alternative draining passages from tumors and constant high arterial pressure. However, other non-HCC liver tumors are also similarly encapsulated and fed exclusively by hepatic artery, allowing intra-arterial treatments of liver neoplasms, e.g. [125]. Therefore, why non-HCC liver secondary liver tumors, which are similarly encapsulated and vascularized, do not metastasize into the third-order afferent (feeding) portal branches?

All of the above facts indicate presence of special interactions between hepatocyte-derived cells (i.e. HCC cells and normal hepatocytes) and the portal vein conduit. The notion of a ‘special interaction’ arrived from known mechanistic on HCC metastasis to the portal trunk or main portal branches: HCC cells disseminate systemically from primary tumor and can appear in main portal veins only as single cells, as they have to pass through two capillary nets, therefore stacking of HCC cells aggregates is very unlikely. Nevertheless, upon appearance in the portal trunk, the HCC cells are capable of attaching to portal endothelium and growing into metastases, in spite of high blood velocity in main portal veins. [124]. Again, hepatofugal portal blood flow cannot serve as an explanation for the significant prevalence of HCC portal vein metastasis versus hepatic veins for the reasons outlined earlier.

## Stephen Paget’s ‘seed and soil' hypothesis of cancer metastasis

The general explanation for the HCC privilege metastasis to portal

veins can be deduced from the 'seed and soil' hypothesis suggested by Stephen Paget in 1889. [126]. Paget postulated that the site-specific location of metastasis development "was a consequence of the provision of a fertile environment (the soil) in which compatible tumor cells (the seed) could proliferate" [126] (for review on 'seed and soil' hypothesis see [127–129]).

For many years the Paget hypothesis was overshadowed by the belief that metastatic dissemination is purely governed by vascular anatomy and mechanical factors [127], a concept introduced by James Ewing In 1922 [41]. However, in the last decades, Paget's 'seed and soil' theory was confirmed and complemented by numerous facts proving its great foresight with specific details: expression of specific molecules on both 'seed and soil', specifics of metastatic environment, etc. There are thousands of publications on these subjects that need not be cited here.

The Paget hypothesis inevitably assumes the presence of specific interactions/affinity between 'seed and soil'. Since we accept the systemic HCC dissemination [34–36,130] and the 'seed and soil' notion, the explanatory hypothesis for preferential portal HCC metastasis should include the following events/sequence:

1) HCC cells dislodged from primary tumors as single cells and disseminate from the liver via hepatic/caval veins to circulation;
2) HCC cells have to pass through pulmonary and visceral capillary nets without attaching/metastasis, and drained from the visceral capillary net into portal vein tributaries and further to portal vein.
3) During short presence in portal vein HCC cells attain ability to attach to the endothelial surface of the main portal veins;
4) Attached to the portal vein endothelium, HCC cells are capable to survive and colonizing it, then proliferate, forming portal metastases.

This 'specific interactions/affinity between' model of HCC portal metastasis is the only one which is coherent with the systemic HCC dissemination pathway [34–36,131]. This model also explains different observations: 1) HCC portal metastases as the solo presentation oh HCC [56–60] and 2) notorious preferential portal metastasis with established primary HCC (see Dr. Choi editorial [90]). This model also unites clinical facts with experimental observations on intraportal hepatocytes [86,87].

However, available knowledge do not suggest any specific characteristics of the portal vein endothelium, which may constitute an essence of the 'soil' or 'fertile environment', and portal privilege HCC metastasis still appears as a paradox.

## Preferential portal metastasis of HCC is a paradox that we failed to recognize

For the last decades, we have continuously encountered more frequent HCC metastases in portal veins, which are against blood flow to liver, rather than along with hepatic blood outflow in hepatic veins, but we did not perceive it as a paradox and did not question it. We suggested that HCC cells detached from primary tumors as single sells and washed away from the liver into circulation, and then became stacked in the main portal veins. However, HCC systemic dissemination assumed that before arriving at the portal veins, the same HCC cells moved through two capillary nets (pulmonary and visceral) without metastasizing, and we did not see this as a paradox nor question this pathway. We assumed that freshly dislodged, and more preserved HCC cells passed through hepatic veins with less frequent metastasizing than that in portal veins. We also observed that after a damaging journey associated with hypoxia, nutrient deprivation, and shear stress [38–40]), HCC cells metastasized to portal veins 3–10 time more frequently than they metastasize to hepatic veins, and yet we did not perceive this as a paradox. For twenty five years, we collected cases of HCCs presenting only as HCC metastases in major portal veins with no detectable primary HCC tumor in liver and yet did not see this as a paradox. We are well aware that all other non-HCC gastrointestinal carcinomas always disseminate exclusively via the portal vein to liver yet showing significantly less frequent metastasis to portal veins than HCC, and still do not see this as a paradox. But it is a paradox, which inevitably means that all our models on HCC privileged portal metastasis, creating the paradox, must be replaced [3] and new hypotheses should be advanced and tested.

## A novel hypothesis on paradoxical privileged portal metastasis of hepatocellular carcinoma

Because the Paget 'seed and soil' hypothesis about site-specific locations of metastasis [126] and the systemic mode of HCC dissemination [34–36,130] are so far the only non-contradictory models [127–129,132], any new hypothesis on HCC metastatic mode has to incorporated both models. In the case of privileged portal metastasis of HCC we must equate another constituent of portal conduit—the portal blood—to the 'soil' or 'fertile environment'. Similarly we have to equate hepatocyte-derived cells, exposed to the portal blood, to 'compatible seeds' that could attach and proliferate. We have to accept the above notions because they unite all observations and should be incorporated in new hypothesis.

Intravenous metastasizing in very unusual for other carcinomas Mendoza, 2003 #7161;Choi, 2010 #7160}. Yet malignant hepatocytes, (i.e. HCC cells) routinely home, colonize and grow inside portal vein lumen, with occur with HCC progression literally in all cases, while frequency of portal metastasis of other carcinomas which metastasize via portal vein is 50–100 times less [83]. Such persistent morphogenesis should be perceived as a selected morphological trait or phenotype. To investigate a morphological trait or phenotype, it is known to be beneficial to analyze the phenomenon from point of view of morphologic changes in evolution. Obviously, all specific tissue characters, i.e., morphogeneses leading to a particular design of tissues and organs, are results of natural selection. Therefore, in this light, homing and proliferation of hepatocyte-derived cells into the lumen of portal veins should be perceived as the selected biological trait. Since this analysis fully agrees with the notion "Nothing in Biology Makes Sense Except in the Light of Evolution" [133], phylogenetic interpretation of the portal blood as the 'soil' and hepatocyte-derived cells as 'seeds' is worth deliberation and testing.

The above notions require analysis of data on phylogenetic formation and origin of liver and visceral portal venous system in vertebrates. Since the fossil record is mainly limited to naturally mineralized body parts (skeleton and teeth), which is not the case of visceral organs; the inquiry should be based on analysis of comparative morphology of related species.

The following arguments appeal to the acquisition of: 1) the visceral portal system; 2) the tissues expressing hormones and growth factors of pancreatic family (HGFPF); 3) and the liver in the different classes of vertebrates, which are known to be reflective of the phylogenetic sequence in the vertebrate subphylum.

## The phylogenetic overview (based on comparative data) of the portal/liver system and hormones and growth factors of pancreatic family (HGFPF) – producing tissues in the vertebrate subphylum

### *Conserved portal/liver system design in evolution of the vertebrate lineage*

Morphological changes in evolution suggest that any organ of animals must descend with modifications (small or great) from a homologous organ present in their common ancestor [134–138]. This is the essence of the Darwinian concept of Descent with Modifications, also called a Homology Principle.

An appeal to homology in biology writings is commonly complemented by specification of what particular 'kind of homology' is

discussed. The terms 'homology' and 'homologous' here and further are used only in a sense of a historical concept of homology [139,140]: "Homology, as classically defined, refers to a historical continuity in which morphological features in related species are similar in pattern or form because they evolved from a corresponding structure in a common ancestor." [141]. While citing the above statement, I believe that the application of the Homology Concept in conjunction with 'Descent with Modifications' notion does not give room for any other interpretations than in classical Darwinian logic. As Minelli and Fasco write "This is the reason why, when Darwin (1859) used homology to support his theory of descent with modification, he did not beg the question [140].

Darwin writes: "… in order to discover the early transitional grades through which the organ has passed, we should have to look to very ancient ancestral forms…". The above notion was applied to elucidate phylogenetic transitions in vertebrate lungs and hearts [142,143–149], and different hypotheses on the pre-vertebrate – vertebrate phylogenetic transition were outlined to suggest a homologous precursor of descendant forms [150,151]. Hence, the same inevitable question should be asked in regard to the vertebrate portal/liver system: What is the homologous phylogenetic precursor of the Cyclostomata portal/liver system, which already appears in this group of basal vertebrates as an elaborate organ with a unique vascularization pattern? This question must be asked for the sake of homology and because alternatively we would be forced to embrace the old rejected notion that organs in evolution "… may be developed suddenly instead of gradually." [152] and repudiate the Homology Principle together with Darwin theory.

Indeed, vertebrates' visceral organs (e.g. lung and heart [142–147,149]) demonstrate a variety of morphological transitional modifications between animal groups that carry features of major forms in vertebrate evolution. These groups usually are named after acquired characters, i.e. Agnathans, Gnathostomes, Tetrapods, and Amniotes, or after a name of a class, i.e. cyclostomata, fishes, amphibians, reptiles, mammals. This grouping is called the 'accepted phylogenetic sequence': cyclostomata → fishes → amphibians → reptiles → mammals; this sequence is based on fossil record and comparative morphology, and confirmed by molecular data as well [145,153–155], this (Note: of course, the groups of living representatives are not a 'phylogenetic sequence', and living animals themselves cannot be 'ancestors', but these groups/representatives conserved traits, i.e. morphologic features) of their retrospective phylogenetic ancestors. The conservation of ancestral traits allows extrapolation of comparative data to phylogeny, which is a common tool to reconstruct phylogeny.

The heart, for example, shows a transition from three consecutive chambers in cyclostomata [144], to four consecutive chambers in chondrichthyans and bony fishes [145], and to a double circulation in lungfishes [146]. Then it transitions to amphibians' left and right atrial chambers [145,147], further to reptiles' three-chambered hearts with two atria and one common ventricle, and then to mammalian' hearts with four chambers and parallel double circulation circuits [145,149]. This example shows a significance of intermediate form in phyletic reconstruction [156].

However, unlike other visceral organs, e.g. lung and heart [142–147,149], the design of the portal/liver system has been highly conserved in evolution of the vertebrate lineage [157]. In animal groups representing the same accepted phylogenetic sequence (cyclostomata → fishes → amphibians → reptiles → mammals), the only variations in the portal/liver system are those in hagfishes and some Teleosts, in which the portal vein receives blood from the viscera and a caudal part of the body [158,159]. Therefore, already in the most basal vertebrate Cyclostomata, the visceral post-capillary venous blood is collected into a single portal vein and directed to the already-formed liver, where it breaks into a capillary net again, forming hepatic sinusoids, which again are collected into a single hepatic vein, forming *rete mirabile,* a unique feature of the vertebrate liver.

Liver architecture as well shares the same fundamental plan in all vertebrates, from basal to the highest subclasses. In all vertebrates the liver appears as a continuous mass of cells which are channeled by the network of sinusoids [160].

Therefore, the portal/liver system in all vertebrates shows identical developmental, topological, and morphological characteristics [160–162]. This conservation strongly assumed that the common ancestor of vertebrates (including Cyclostomata) acquired a visceral portal system and a liver. This complex organ derives from two embryonic sources: endoderm and mesenchyme: and acquires a unique blood supply pattern – it is mainly vascularized not by artery but venous blood drained from peritoneal viscera.

However all evidences indicate that complex organs do not appear from nowhere, but rather undergo through intermediate transitional forms in phylogeny. Morphologic changes in evolution imply that any organ must descend with modifications (small or great) from a homologous organ of their common ancestor [134–138]. Therefore, the anatomical stability of the elaborated portal/liver system with unique vascularization in all classes of vertebrates (from basal to advanced) necessitated a search for a homologous precursor.

Hence, presence of the elaborated portal/liver system with unique vascularization in cyclostomes necessitates a question: from which homologous phylogenetic precursor the Cyclostomata portal/liver system had arrived?

## Arguments in favor of the origin of the vertebrate liver from the Amphioxus midgut diverticulum

### *Morphologic evidences*

The question "What is the homologous phylogenetic precursor of the Cyclostomata portal/liver system?" has always been asked by scientific community. All prominent experts suggested identity role of a phylogenetic homologous precursor of the vertebrate portal/liver system to a mystifying organ of Cephalochordate (Lancelet or Amphioxus) — the midgut (or hepatic) diverticulum [163–174], which appears as an evolutionary novelty in this subphylum.

All cephalochordates possess a sizable organ called a midgut diverticulum [167,170]; other terms are also common, e.g., hepatic or digestive caecum [175] or hepatic diverticulum [166], etc. This organ includes part of the midgut intestine, forming a sac and significantly extending from the midgut region in the cranial-ventral-dextral direction. The midgut diverticulum is single out by its unique blood supply.

The exceptional feature of the Amphioxus midgut diverticulum is that it is vascularized not by an arterial vessel, as the rest of body parts, but by the subintestinal vein. In Amphioxus, venous draining post-capillary vessels of the caudal intestine is collected into an unpaired subintestine vein, which breaks into a capillary network and brings venous blood to the diverticulum. Then the diverticulum's capillaries are again collected into a single vein—*vena Cardinales posterior* (analog of *vena Hepatica* or *Cava* in vertebrates) [166,168].

The unique vascularization of the Amphioxus midgut diverticulum was noted and described by many scientists. The most detailed study of Amphioxus vascular anatomy was performed by Hans Rähr [176]. However, the important vascularization patterns of the Amphioxus midgut diverticulum and caudal intestine can be demonstrated by a simplified schematic (Fig. 7):

This a vascularization pattern, i.e., post-capillary intestinal venous blood again forming a capillary net between two veins (*rete mirabile)* and supplying a derivative of intestine (i.e., portal/liver system), is the characteristics of both Cephalochordates and vertebrates. Based on this peculiar anatomy all prominent experts (a long time ago and now) share the opinion that this unique Amphioxus intestinal vein/diverticulum arrangement is a homologous precursor to the portal vein/liver system in vertebrates, although Amphioxus does not possess a liver, [163–174].

Charles Weichert in 'Elements of Chordate Anatomy' directly associates the portal-intestinal anatomical arrangement of Amphioxus

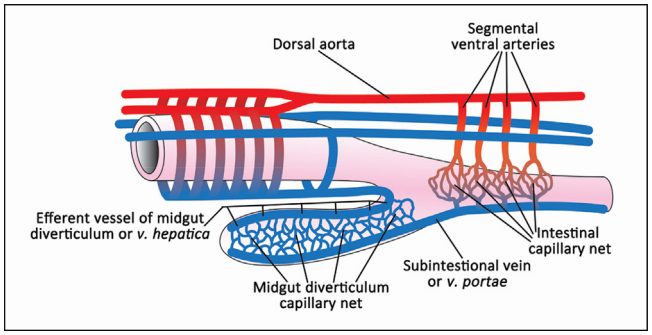


**Fig. 7.** Vascularization of Amphioxus midgut (hepatic) diverticulum. In Amphioxus, venous blood drained from the postcapillary network of the caudal intestine collects into an unpaired subintestine vein, which again breaks into a capillary network that carries blood to the diverticulum. The capillaries in the diverticulum then collect into a single vein, forming *rete mirabile*, a hallmark of the vertebrate liver.

with the acquisition of a liver by vertebrates:

> "Although no true liver is found in amphioxus, the presence of such a structure in higher chordates is foreshadowed in *Amphioxus* by a hollow, forward-projecting, ventral *hepatic caecum* which comes off the intestine just posterior to the branchial region (Fig. 2). The lining of this pouch is ciliated, and it may have some digestive function. A system of veins coming from the intestine breaks up into capillaries on the hepatic caecum, thus presaging the appearance of the hepatic portal vein of higher form" [177].

However, there is a long standing counterargument that the vascularization of a derivative of intestine by intestinal venous blood does not alone constitute sufficient explanation for the differentiation of the midgut diverticulum of *Cephalochordata* into a liver of vertebrate, e.g., [178]. In this case, other facts that supporting this homology could reinforce such type of reasoning, which is commonly used in scientific analyses [179,180] and termed by G.H. Harman as "Inference to the Best Explanation" [181]. Although, at first glance, the above task appears unsophisticated, or what is called 'common sense' it constitutes a valid and very important scientific tool:

> "…it is a method used in judging of the common events of life, and has often been used by the greatest natural philosophers." (Darwin, On the Origin of Species, 1872, p.545 [182]).

## Additional facts favoring the origin of the vertebrate liver from the Amphioxus midgut diverticulum.

### *Enterocytes of Amphioxus diverticulum expresses vertebrate liver-specific proteins*

Other facts supporting the hypothesis consist an expression of a number of vertebrate liver-specific genes in the Amphioxus hepatic diverticulum, e.g., glutathione-S-transferase, plasminogen-like protein, antithrombin, and cytochrome P450 [175,183–186]. These liver-specific gene expressions support the homology hypothesis above. The fact that Amphioxus' diverticulum is the sole tissue expressing vitellogenin [187,188], also reinforces the homology of the midgut diverticulum to vertebrate liver" [184–186,189,190], because oocytes in vertebrates never express vitellogenin themselves; this synthesis occurs mainly in the liver, and then vitellogenin is concentrated in oocytes [191]).

## Transition the expression of hormones and growth factors of pancreatic family (GHFPF, which includes insulin, glucagon, somatostatin, and pancreatic polypeptide) from neural cells to the endodermal derivatives and simultaneous acquirement of the liver in the chordate lineage

Another support for the hypothesis can be inferred from the shift of gene expression axis of GHFPF in chordate lineage. The evolution of the Cephalochordata midgut diverticulum into the liver in the vertebrate lineage could be inferred from the data on comparative morphology of GHFPF-producing tissue.

In non-chordate triploblastic animals, e.g. arthropods and nematodes, insulin is manly produced by neuronal cells [192–197]). However, in the invertebrate chordate, Amphioxus, the cells expressing insulin-like growth factors, (or HGFPF) are mainly enterocytes of caudal intestine and hepatic diverticulum [197–199]. Cyclostomes are the first Chordates (and hence the first vertebrates) that acquired a compact HGFPF-producing organ – Islet of Langerhans, in conjunction with portal circulation [200–202], and simultaneously acquired the liver.

A comparative-phylogenetic overview showing the shift of GFHPF expression in bilateral (triploblastic) animals is summarized by R. Scott Heller in "The Comparative Anatomy of Islets" [197], Fig. 8:

This schematic emphasizes the transition of HGFPF expression from neural cells of arthropods to the enterocytes of invertebrate chordates, which coincides with acquisition of the hepatic diverticulum by cephalochordates, followed by the transition of HGFPF expression from intestinal epithelium to Islets of Langerhans, which coincides with acquisition of the liver by vertebrates.

It is the opinion of this analysis that this peculiar vascular design of the Amphioxus diverticulum allows hormone-producing cells of the diverticulum to sense the level of ingested nutrients in 'portal' blood, facilitating regulation of hormones expression. Traditionally, this important physiologic mechanism (humoral regulation) was suggested first to occur in Cyclostomes [203].

Expression of a molecule which shares identity to both insulin and insulin-like growth factor, IGF (based on IGF RNA) was reported in Amphioxus [204]. Lecroisey and co-authors showed that insulin-like peptide (i.e., IGF) is highly expressed in endoderm and paraxial mesoderm during Amphioxus development and mainly expressed in the gut of both the developing embryo and adult Amphioxus [205]. Since downregulation of the IGF-1 receptor occurs after hepatic linage commitment during hepatocyte differentiation from embryonic stem cells, a role for IGF-1R in hepatocyte differentiation was suggested [206].

It was reported that in ascidians insulin and IGFs mRNAs are expressed in cortical cells of the neural ganglion (similar to non-chordate invertebrates [192,196,197]), suggesting ancient divergence of insulin and IGFs more than 600 million years ago [207]. Based on this data, McRory and Sherwood proposed the phyletic scenario of chordates, which places cephalochordates as a sister group to vertebrates [207], this scenario is supported by other works, e.g. [208].

**Hypothesis.** The hepatic diverticulum of an Amphioxus-like ancestor chordate is a homologous phylogenetic precursor of the vertebrate liver. Phylogenetic transformation the diverticulum enterocytes into hepatocyte lineage and the formation of the liver in the phylogeny of the chordate are portal blood-dependent events.

The hypothesis suggests that in early chordate evolution between Early [209,210] – Middle Cambrian [211] and upper Cambrian – lower Ordovician eras [212,213], portal venous blood drained from the intestine began to carry HGFPF to the midgut diverticulum of an Amphioxus-like ancestor via pre-existing portal circulation. The transition of the brain-gut expression axis in regards to HGFPF from neural to intestinal epithelial cells is well documented in the evolution of protochordates and chordates based on comparative data [196,198,199,214–218]. The hypothesis suggests that: 1) the transition of the expression axis of PHGFs from neuronal cells to intestinal

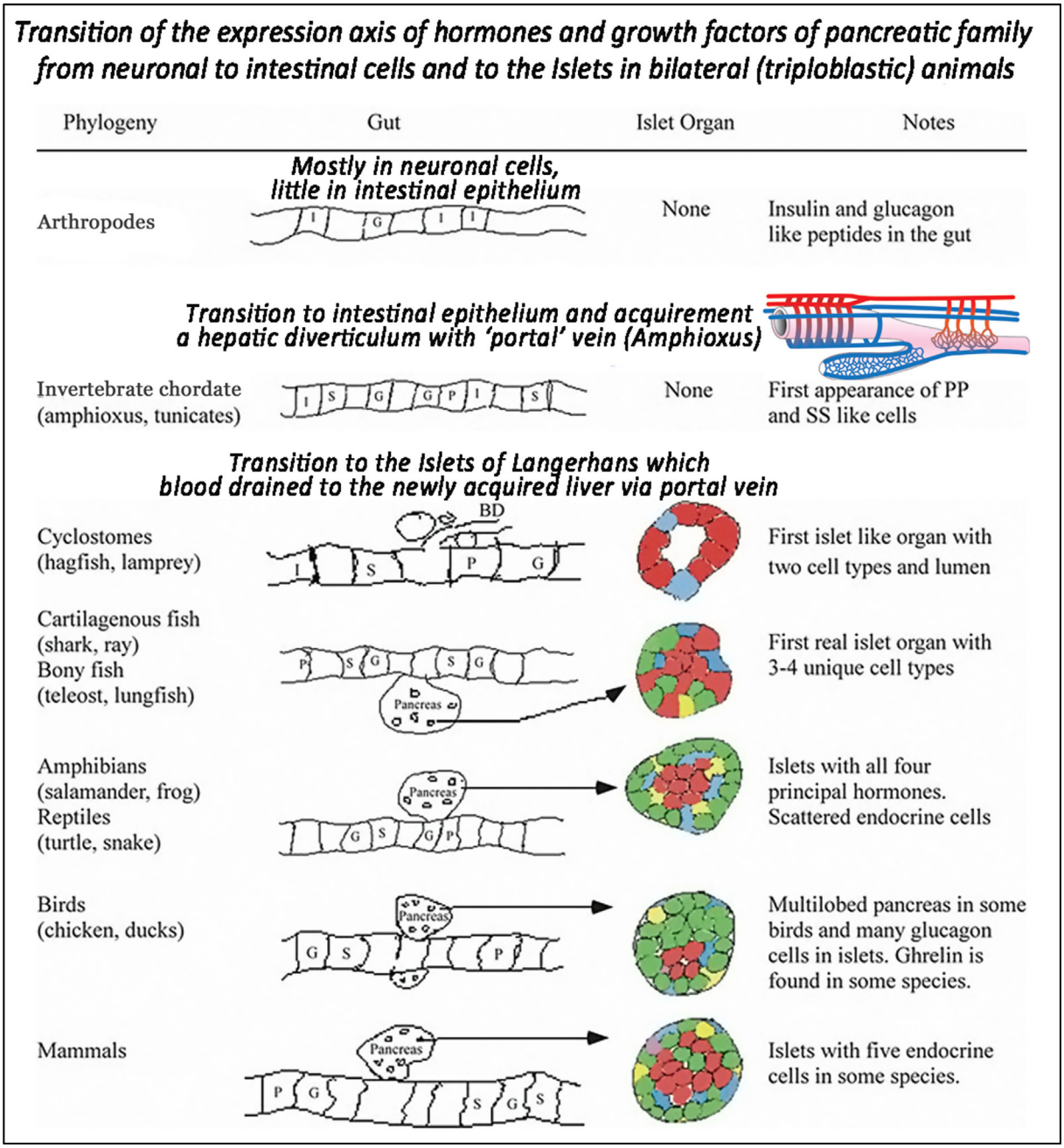


**Fig. 8.** Transition of the expression axis of hormones and growth factors of pancreatic family and acquisition of a portal/liver system in evolution of chordate, based on comparative morphology. Family member cell types that remain in the gut are represented by single letters: I, insulin (red); G, glucagon peptides (Green); SS, somatostatin peptides (blue); P, pancreatic polypeptide (PP) family peptides (yellow). The cyclostomes are the first organisms in which islet-like clusters have migrated out of the gut tube into a separate cluster (islet) surrounding the common bile duct. Ghrelin is shown in purple. Abbreviation: BD, bile duct. Reproduced with permission, from [185]. The author's additions consisted schematic of Amphioxus vasculature and comments in Italic.

epithelial cells and 2) acquisition of the portal system, which brings these growth factors back to the epithelial cells of the diverticulum of an Amphioxus-like ancestor, promoted differentiation of the midgut diverticulum enterocytes of ancestral *Cephalochordata* into hepatocytes and further into the liver of vertebrates.

From the above logic, it follows that differentiation of midgut enterocytes into hepatocytes and formation of a liver in the phylogeny of the invertebrate chordate/vertebrate lineage is driven by HGFPF of portal blood. It also follows that hepatocyte differentiation, function, and self-renewal also must depend on hormones and growth factors of portal blood.

The analysis suggests that HGFPF, probably together with other growth factors expressed by enterocytes of caudal intestine [219–224], were carried to the intestinal diverticulum by the portal vein in an Amphioxus-like ancestral chordate. The HGFPF acted on diverticulum enterocytes, promoting their differentiation into cells of hepatocyte lineage, which triggered the formation of liver in vertebrate phylogeny. It is well-documented that certain components of vertebrate portal blood (insulin, glucagon, somatostatin, pancreatic polypeptide, and augmenter of liver regeneration) exert strong morphogenic signals for hepatocyte differentiation and growth [225–233]. It was also shown that insulin receptor substrate-2 is crucial for liver development and hepatocyte survival [234,235].Under this hypothesis, this unique vascular arrangement of the Amphioxus caudal intestine and midgut

diverticulum provides the earliest phylogenetic example of an endocrine regulation between different compartments of the GI tract.

**What is the relationship between the above facts and the hypothesis regarding privileged HCC metastasis into portal vein?**

*In vitro* and *in vivo* developmental studies showed that HGFPF play an inductive role in the early formation of liver [206,231,234,236,237]. However, the same HGFPF are necessary for function and renewal of normal liver and are significantly extracted by liver cells from passing blood (as much as 70% during the first pass only [219,237–239]), which creates a significant concentration gradient of these factors between the portal blood and liver outflow/distant organs [240]. It is also known that this hepatic extraction of HGFPF becomes reduced only in very advanced stages of liver diseases [241].

The same growth factors are important for the survival and growth of hepatocyte-derived malignant cells, i.e., for HCC cells. Numerous studies have demonstrated crucial dependence of HCC survival, growth, and metastasis on insulin and insulin-like growth factors [242–250], and in particular pointing to a promoting role of insulin in the metastatic potential of human HCC cell lines [251], while treatment with an inhibitor of insulin receptor resulted in suppressed proliferation and increased apoptosis of HCC cells [252].

However, as shown by numerous studies, these growth factors are significantly extracted by parenchymal hepatocytes during blood passage though liver [219,237–239], creating factors' gradient and low concentrations of these growth factors in hepatic blood outflow. This simple mechanistic approach allows us to apply the Paget "seed and soil' hypothesis [126] to privileged portal vein HCC metastasis, perceiving HGFPF of portal blood as a cause of intraportal HCC cell attachment and growth.

The same mechanistic approach explains why HCC does not form metastases in small portal tributaries upstream of the portal vein: Considering the anatomy of the venous drainage of the pancreas, important growth factors appeared in the blood after the splenic veins united in the superior mesenteric vein to form the portal vein [253,254], as depicted in Fig. 9:

**The puzzle of "lower than anticipated" HCC pulmonary metastasis**

Although it is commonly stated that pulmonary metastasis is the most common type of extrahepatic of HCC metastasis, e.g., [255], the high frequency of metastasis to lung (39% of patients) occurs only in patients with advanced intrahepatic HCC stages (stage IVA [256]). In patients with resectable HCC, the frequency of pulmonary metastasis is much lower, in the range of 6–13% [54,255,257]. This percentage is paradoxically low since the HCC cells must continually appear in lung capillaries from the beginning of the disease, because of systemic dissemination. This paradox also can be explained only within the Paget 'seed and soil' hypothesis, in light that the growth factors (PHGFs) vital for HCC cells are significantly extracted by liver cells from portal blood, making post-liver vascular apace a less favorable compartment for HCC cells attachment, survival and growth. A logical question is: Can the diminished HGFPF extraction by liver cells, and thereby high levels of HGFPF in lung, affect the frequency of HCC pulmonary metastasis? Yes, it can: it was shown in a study on HCC with hepatofugal portal flow and esophageal varices that all cases had intravariceal HCC metastases (13/13), and 12 of 13 cases had lung metastases [258]).

**Why is the portal metastatic pattern of HCC vein strongly associated with the disease recurrence?**

It is well known that HCC metastasis to the portal vein is a significant risk factor for the disease recurrence and poor prognosis [64–70,76–78].

However, within the Paget 'seed and soil' hypothesis [126] and in light of growth-promoting properties of the portal blood, the portal vein metastasis changes its role from a risk factor to the cause of disease recurrence. Considering that the portal vein conduit acts as the 'soil' environment for HCC metastasis, it should be further logically assumed that the portal vein environment would promote the selection of more aggressive HCC clones from already seeded portal metastatic HCC cells.

Indeed, it was demonstrated, based on $^{18}$F-Fluorodeoxyglucose uptake in HCC patients, that portal HCC metastases are highly metabolic as compared to primary HCC [259,260], which indicated metabolic reprogramming and potentiated HCC cells increased aggressiveness [261].

**Comments on phylogenic reconstruction models used to infer origin of vertebrate liver**

This analysis has been initiated by experimental finding of normal hepatocyte homing in portal veins and by a study on clinical significance of HCC privileged portal vein metastasis, in my work dated by 1992–1996 [67,86]. Most of the above ideas were summarized and discusses in 1999 in Inverness, Scotland, at the conference 'Hepatic and Splanchnic Circulation in Health and Disease' in the presentation 'Formation of the unique portal venous system precedes the appearance of liver in the evolution of chordates: significance in hepatocellular carcinoma and hepatocyte transplantation'[262], and at the Fifth Congress of the International Liver Transplantation Society, Pittsburgh, 1999.

Obviously, my hypothesis is founded on Darwin concept 'Descent with modification', or Homology Principle, and incorporated the traditional evolutionary scenario on phyletic relations among the three extant groups of chordates, which considered cephalochordate (Amphioxus or Lancelet) as the close living relatives of vertebrates. This phylogenetic scheme, was persuaded by many authors [153,263–269]. At the time I formulated the above conjecture, the phylogenetic schemes above, placing the cephalochordates (lancelets) as a sister group to the vertebrates, was accepted by scientific community (e.g. [264]).

Therefore, one can ask a reasonable question: why I have not

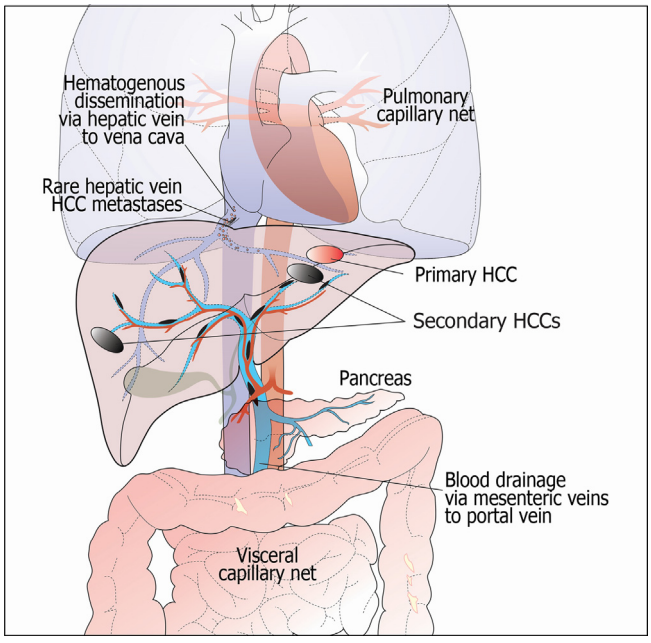


**Fig. 9.** The portal veins and hepatic sinusoidal compartments constitute 'the soil' for HCC metastases. Given the anatomy of the venous drainage of the pancreas, important growth factors appear in the blood after the splenic veins unite in the superior mesenteric vein to form the portal vein.

published this analysis at the time when it was aligned with mainstream opinion, but want to share my hypothesis now, when the phyletic relations in the phylum Chordata were reconsidered and tunicates, but not cephalochordates, designated as the closest living relatives of vertebrates. [270,271]. The answer is simple: until recently I was not able to extend the Descent with Modification concept to all logical consequences of my hypothesis. The reason for such failure was the following.

In attempt to understand the unique affinity of malignant hepatocytes (i.e. HCC cells) to portal vein conduit, but not that of other carcinomas cells that always disseminate via portal vein (and likewise prevalence HCC portal metastasis versus that to hepatic vein), I examine the problem in light of co-evolution of liver and portal system in vertebrate. Complex structures, like organs and organs' systems, do not occur in phylogeny from nowhere. Organs must have a phylogenetic precursor, meaning that organs must descent (with modifications great or small) from homologous organs of a common ancestor. Therefore, invoking the Homology Principle and a comparative-phylogenetic data I have joined other scientists [163-174,272] in the opinion that the Amphioxus' hepatic diverticulum with a unique 'portal' blood supply constitutes the phylogenetic precursor of the vertebrate liver-portal system [272,177]. However, the same logic must be applied in regard to the Amphioxus hepatic diverticulum-'portal' vein arrangement: what could be a phylogenetic precursor of such complex organ?

This question is an obligatory for two reasons. First, the Amphioxus' diverticulum-'portal' vein arrangement is a complex anatomical structure, and in theory such organs must descent from a phylogenetic precursor, unlike *de novo* acquisition of new cell types due to acquirement of new cellular functions [273]. Therefore, such putative homologous precursor must be hypothetically conceivable, regardless whether real evidences of such homologous precursors exist or not (either as fossils or comparative morphology evidences).

Inescapably, I have asked this question and with frustration realized that I am not able to hypothesize any structure for this role. There is nothing that could be imagined as a phylogenetic precursor of the Amphioxus' hepatic diverticulum-'portal' vein system. Therefore my model of liver evolution has created a paradox, meaning that the hypothesis has internal flaw and should be discarded. Therefore, in 2000 I concluded that my hypothesis does not merit publication and abandon the analysis.

However, in 2014 I came across information that offered a fresh look to the problem.

I have received an access to the original magistrate thesis of Alexander Kovalevsky [274] on development of *Amphioxus Lanceolatus*, published in Russian (somewhat old), which was also printed as a monograph in 1865. A shortened version of the work was re-published as a research article in German in 1867 [275] and much later published in Russian as part of 'The Selected Manuscripts of Kovalevsky' [276]. By reading, side to side, the earliest 1865 [274] and later editions (both German and Russian) [275,276], I found that the 1865 publication contains one fragment that was excluded from later publications. This fragment reads:

> "Developing diverticulum stretches from the gut. Some considered Amphioxus' diverticulum as the organ homologous to liver. Indeed, all cells of the diverticulum are filled with a yellow-green substance; interestingly, even before formation of the diverticulum, its function was performed by a straight part of the gut; the color of intestinal wall in this location is completely green, and food particles usually circulate in this area longer due to strong ciliary activity." [274] (page 31), (*VMS translation*).

Available publications on Amphioxus do not provide additional information about the hepatic diverticulum development, e.g. [277,278]. Since I studied only adult specimens of Amphioxus, I asked Prof. Linda Z. Holland, a prolific expert on the Lancelet, to share personal observations on Amphioxus development, in particular on the development of hepatic diverticulum. Prof. Holland replied:

> "The diverticulum forms at the very end of metamorphosis as an outgrowth of the gut. The more well fed the animals, the larger the diverticulum. Food moves into the diverticulum, which seems to store the food. Before the diverticulum forms, if the animals do not have food for a period of hours, the gut empties, they stop eating and never start again. After the diverticulum forms, if the animals do not have food for a day, the main gut empties, but the diverticulum remains full of food and if food is provided the animals will eat it and do fine." (L.Z. Holland, personal communication, with permission) [279].

Putting together observations of A. Kovalevsky and L. Holland it could be concluded that from the beginning of development the diverticulum of Amphioxus performs functions as a digestive organ and as **the food storage.** Kovalevsky' notes also suggested that the straight part of the gut, from which give the origin to diverticulum, is already enriched with yolk particles, even before the diverticulum development. However, these obvious suggestions contradict basic knowledge on the Amphioxus ovum: Amphioxus has a microlecithal or alecithal oocyte, which is traditionally considered as 'primary alecithal' (in contrast to 'secondary alecithal' oocytes of marsupials and placental mammals, and some other viviparous vertebrate, e.g. [280]). Nonetheless, developing diverticulum possesses structural and functional features that disappear in adult forms. What hypothesis can accommodate the above? There is only one biological concept that can unite the above observations and suggestions: it is the Von Baer's laws of development, also known as the Theory of Recapitulation. In spite of numerous attacks on this notion, I share the opinion that recapitulation phenomenon (or in Berrill words "phylogenetic reconstruction" [281]) is a fact which stands as a central theme in evolutionary biology and, if properly understood, cannot be eliminated or neglected [282,283]. I decided to elaborate further this hypothesis, perceiving the above phenomenon as "An actual use of recapitulated structure." (Ernst Mayr, 1994) [283].

But a recapitulation of what?

The above notions on recapitulation, together with common wisdom that such complex organ as the Amphioxus hepatic diverticulum with a unique portal blood supply must descent from a homologous organ of preceding ancestor, can offer the only one hypothesis: the Amphioxus' hepatic diverticulum descended with from the yolk sac of a phylogenetically preceding animal.

Before dealing with loud objections and counterarguments I want to introduce my readers a test on anatomical similarities between the Amphioxus diverticulum and the yolk sac.

To execute this test, I invite my readers to perform an imaginary transposition of the Amphioxus diverticulum. Imagine that a midgut diverticulum, surrounded by skin with feeding and draining vessels, is being stretched and protruded down from the Lancelet ventral site, together with skin and vasculature (Fig. 10):

## The hypothesis: *Argumenta pro et contra*

The main value of this hypothesis is that it suggests a real organ—a yolk sac—as a phylogenetic precursor of the Amphioxus' diverticulum. It is important to highlight that a yolk sac consists a yolk and the yolk-containing tissues – endoderm, mesenchyme, and ectoderm. These tissues are always present in embryos of all bilateral (triploblastic) animals. Not least point is that there is no other organ/structure that could be morphologically hypothesized as a homologous phylogenetic precursor of the Amphioxus' diverticulum.

The only theoretical constrain of the model is that the hypothesize must suggest a phylogenetic precursor of Amphioxus which ought to be advance enough to have amount of yolk sufficient for formation of yolk sac, i.e. telolecithal oocyte, similar to that of other marine triploblastic animals, e.g. [284,285].

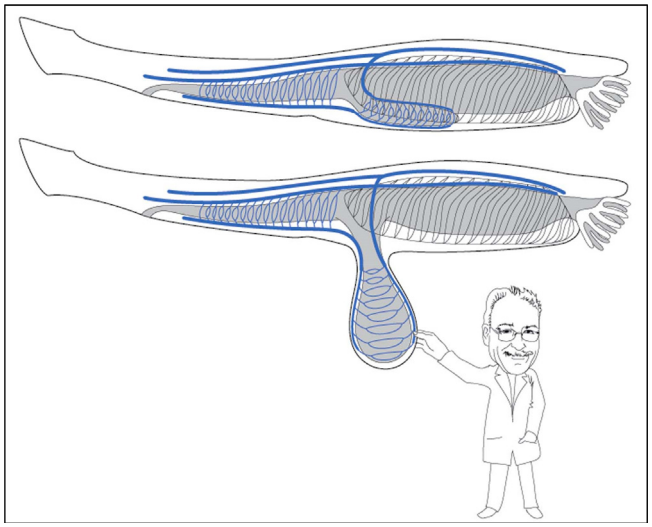

**Fig. 10.** Imaginary transposition of a midgut diverticulum, surrounded by skin with feeding and draining vessels, such that it became stretched and protruded down from Lancelet. Morphologically, this midgut diverticulum, with its unique vascular architecture, is homologous to a yolk sac (highly schematic).

It was a strange moment when I realized that I do not have to hypothesize that; this model was already suggested by prominent evolutionist Alfred Sherwood Romer (Fig. 11):

Therefore, my hypothesis only adds a yolk sac to the advanced chordate of the Romer model (Fig. 12):

## Additional arguments supporting origin of the cephalochordate hepatic diverticulum from a yolk sac

Facts from studies of early development of the digestive system in cyclostomes and fishes provided additional support for this hypothesis.

In a morphological study on the early development of lamprey's digestive and intestinal blood systems (15 days, about 5 mm long), E.W. Baxter (1957) writes:

> "In these larvae the blood can be seen traversing the lateral walls of the gut near the anterior end of the yolk mass and by this route a steady trickle of blood reaches the now mid-ventral sub-intestinal vein. In this vessel the blood passes forwards to the liver, which has now reached the stage of a hollow sac, and from anastomosing vessels in its walls the hepatic blood is returned to the heart."[286].

Please note that in lamprey larva, the only vessel feeding the yolk sac is an unpaired subintestine vein. The fact that the lamprey has two hollow sacs (liver and yolk sac) with similar vascularization patterns is puzzling, but its deliberation is beyond the scope of this communication. It could be only speculated that the yolk sac was duplicated (i.e. gene duplication) in an ancestor with one taking on the new function as the digestive/secretory organ, while the other maintaining food storage.

The crucial fact is that the lamprey's yolk sac has a vascularization pattern similar to that of the Amphioxus' diverticulum.

Another relevant note was written by the famous evolutionary scholar Harland W. Mossman in a manuscript published in the Biological Reviews of the Cambridge Philosophical Society:

> "…the blood supply of the yolk sac of teleost fishes comes from somatic veins, such as the caudal and cardinals, instead of from vitelline arteries branching off from the aorta as in amniotes." [287].

Although later studies showed that arterial supply to the yolk sac also exists in teleost fishes, e.g., [288] the early participation of the subintestinal and the posterior cardinal veins in yolk sac vascularization [287,288], favors homology between the yolk sac and Amphioxus' hepatic diverticulum.

A study on anatomical interactions between the yolk sac and intestine during early fish development was conducted by O.I. Schmalhausen (1991). In descriptions on prelarval development of Russian Sturgeon [285], which belongs to a phyletically ancient fish group [289], Olga I. Schmalhausen writes:

> "At the stage of hatching, the digestive system consists of the alimentary canal and rudiments of the digestive glands, liver, and dorsal pancreas. The alimentary canal is divided into two parts, a widened anterior (yolk sac) part and a narrow posterior part." [285].

Although this description of a Sturgeon yolk sac is short, it shows the same anatomical relation of yolk sac to intestine, as it appears in Amphioxus between the hepatic diverticulum and the caudal intestine.

Within this assumption, the main objection is a deviation from the usual phylogenetic trend in ovum size: from bigger oocyte (telolecithal – presence of yolk sac in Romer' advanced chordate) to smaller oocyte (microlecithal oocyte – no yolk sac in ancestral cephalochordate).

However, acquirement of a novel specialized digestive organ (and more effective alternative nutrition) in larval stage can result in selection of oocytes with reduced amount of yolk. The transition of the feeding pattern in larval forms from lecithotrophy to planktotrophy, or to that of facultative feeding and other intermediate forms, is known under variations of nutrient availability, and is possible in both directions [290–292]. And we know for a fact that reduced amount of yolk in 'secondary alecithal' oocytes of marsupials and placental mammals, and

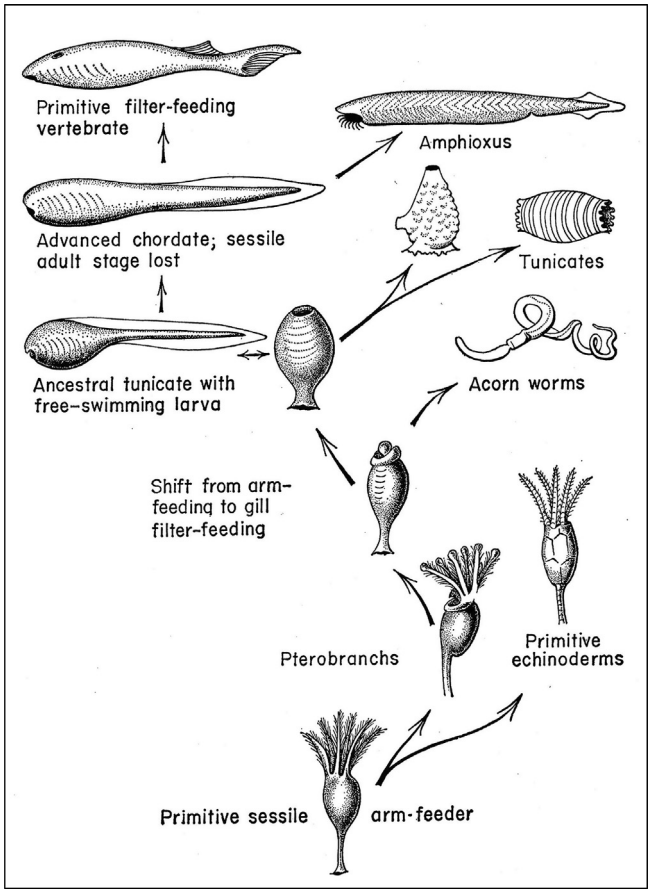


**Fig. 11.** Romer's diagram on the probable course of chordate evolution. Note that Romer emphasizes "advanced motile chordate" as common ancestor of both cephalochordate and vertebrate. From [267], with permission.

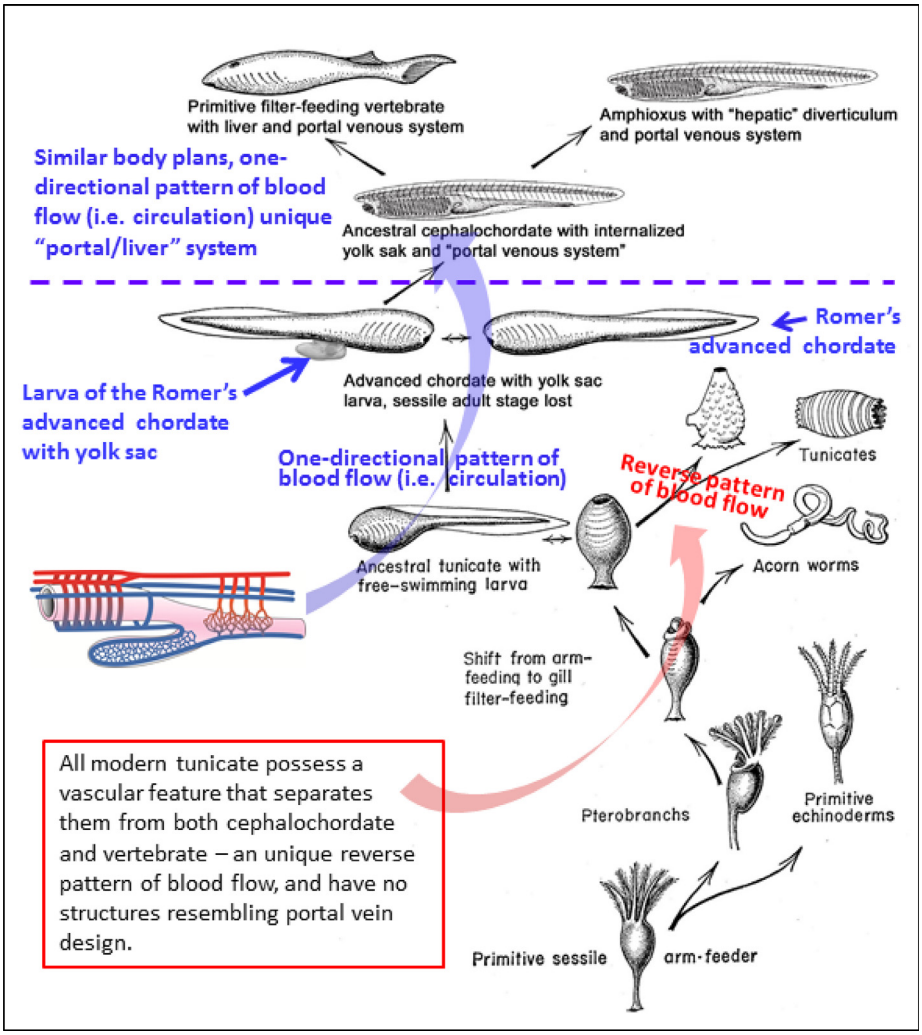


**Fig. 12.** Schematic representation of new hypothesis.

some other viviparous vertebrate, e.g. [280] is due to acquirement of an alternative source of nutrition.

Again, this analysis is a theoretical exercise aiming to suggest: 1) a hypothesis that eliminates a paradox of prevalence of HCC portal metastasis; 2) a homologous phylogenetic precursor of the vertebrate liver; and 3) a homologous phylogenetic precursor of the Amphioxus' hepatic diverticulum. To facilitate this goal, arguments are borrowed from a range of different studies concerning different representatives or are hypothetical suggestions based on logic. Since we do not have facts suggesting a putative precursor of Amphioxus' diverticulum, aligning the above arguments from different fields/subjects may help to test the hypothesis for its internal coherence.

Therefore, I hypothesize that Amphioxus evolved from an advanced motile chordate ancestor with a yolk sac, and during this transition (or cephalochordate phylogeny itself), the yolk sac ceased to function in food storage, became internalized, and acquired functions of a digestive organ. It is worth noting that the internalization/somatization of the yolk sac is a normal morphogenesis process in the development of many living fishes [285,293]. Therefore, it is most likely that the chordate lineage acquired a liver after the portal system had been acquired.

If we accept the above notion that the formation of liver followed acquirement of portal system in phylogeny of chordate, it is reasonable to contemplate causal relationships between two events.

My hypothesis is congruent with the model of chordate evolution, advanced by Alfred Sherwood Romer, who is a renowned for his contributions to the study of vertebrate evolution. In his work, Professor Romer advocated the hypothesis that chordate phylogenesis began with primitive sessile (attached) "visceral" "arm-feeding" animals, which evolved into sessile gill filter-feeding animals. Romer's hypothesis suggests further evolution with selection of ancestral tunicates, whose free-swimming larva evolved into a motile, advanced chordate. Romer suggested that the motile, advanced chordate is an ancestor of both a basal vertebrate and Amphioxus [153,266,267,294]. This assumption considered to be plausible by modern scholars, e.g. [295].

Obviously, the above model contradicts to the recently proposed rearrangements of phyletic relations in the phylum Chordata, based on analysis of molecular data [270,271]. Traditional [264,266,296–300] or 'standard' [137] perception of phylogenetic relations between Chordate subphyla (based on both morphologic and molecular data) suggested Cephalochordata as most close preceding subphylum to Vertebrata. On the contrary, recent analysis of large set molecular data suggested Tunicata as a sister taxon to Vertebrata [270,271]. It also should be mentioned, that the view of Tunicata as a sister taxon to Vertebrata was introduced in 1995 in classical morphologic analysis on phylogeny of low chordates by O.M. Ivanova-Kazas [301] (p 14).

In regard to the Homology concept, there is a concern that the recent trend in phylogenetic reconstructions disregards morphologic evidences [302–307]. I share the above general concerns [302–307], and would like to highlight particular differences and similarities in morphology of vascular system among Chordate subphyla.

Among chordates, the representatives of Tunicata phylum showed the greatest diversity of body plan [308]. However, in spite of such

diversity, all tunicate without exception, possess a unique feature that separated them from both Cephalochordata and Vertebrata – a unique reverse pattern of blood flow [309–311]. O. F. Kampmeier writes:

> "The circulation of blood in tunicates presents a phenomenon that is without parallel in the animal kingdom. The heart reverses its pulsations periodically; in other words, the waves of contraction pass along it from end to end first in one direction for a certain number of beats (from 60 to 100) and then, after a slight pause, in opposite direction (Kampmeier, 1969, p.163)" [312].

Obviously, this unique trait – the reverse pattern of blood flow (not-circulating) – must evolve in ancestral tunicates prior to their diversification. Another indirect evidence that this pattern was acquired early in tunicate phylogeny, is the fact that tunicate heart has reverse direction in the earliest developmental stages (3 days after attachment) [313]. And, as we know, none of vertebrate shares this trait, which creates a morphology gap in phyletic relations between Tunicata and Vertebrata. On the other hand, Cephalochordata and Vertebrata share the same body plan, and design of vascular system, including such unique feature as the portal/liver vascular pattern, which makes a morphology bridge for phyletic relations between Cephalochordata and Vertebrata. Such strong homology and dissimilarity argue in favor of the traditional schema on phylogenetic relations between Chordate subphyla, where Cephalochordata suggested as the most close subphylum to Vertebrata [137,264,266,296–300].

The alternative model suggests complete loss of body plan and the general vascular design, including the portal/liver pattern and one-directional blood flow, during transition from pre-Cephalochordata to pre-Tunicata, and acquirement of the reverse pattern of blood circulation in phylogeny of Tunicata. A positioning of Tunicata as a sister taxon to Vertebrata also inevitably suggests loss and acquirement of the same traits but in an opposite sequence: loss of the reversal of blood flow and re-acquirement of the one-directional vascular circulatory design, including the portal/liver vascular pattern, which makes such modeling less parsimonious. Of course, it could be disputed that existed Cephalochordata represent a relatively recent offshoot of ascidians stem [314] but Amphioxus' hepatic diverticulum argues against it.

It could be argued that, the pattern such as of one-directional blood flow in ascidians, blood circulation and diverticulum-'portal' system in cephalochordate are apomorphic traits and should not be used an argument for phyletic reconstruction. However, I do not see how apomorphic traits, such as the unique pattern of blood flow in ascidians, could bring the later closer to vertebrates. On the contrary, I believe that presence of this apomorphic trait in tunicates could serve as support of Romer hypothesis and my model, in which tunicates diverged before appearance of the motile advanced chordate (suggested precursor of both vertebrates and Amphioxus). Additionally, these facts favor the parsimonious model that requires the fewest evolutionary events [315]

## Concluding remarks

My hypothesis only adds a yolk sac to the advanced chordate of the Romer model. The Amphioxus phylogeny from an advanced chordate was initially suggested by A.S. Romer, in his hypothesis on transition from "visceral" to "somatic" animals in evolution of chordate [153,266,267,294]. In my model, the yolk sac of the advanced chordate predecessor is suggested to be the homologous precursor of the Amphioxus hepatic diverticulum.

This analysis is based on idea that all organs of living animals must descend, with modifications, great or small, from homologous organs of a common ancestor. My inquiry into origin of Vertebrate liver and Amphioxus hepatic diverticulum was thought and revised for twenty five years, but only recently it gained traction due to discovery of Alexander Kovalevsky and Linda Holland observations. Therefore, I suggest that within the Homology concept and according to Inference to the Best Explanation principal [179–181], the only organ that could be hypothesized as the homologous precursor for Amphioxus' diverticulum is the yolk sac of a preceding advanced motile chordate ancestor. I also hypothesize that during transition from the presumable advanced chordate to Amphioxus (or during cephalochordate phylogeny itself), the yolk sac ceased to function in food storage, became internalized, and acquired functions of a digestive organ.

I also suggest that within the Homology concept and in congruence with morphologic evidences, the traditional [137,264,266,296–300] perception of phylogenetic relations between Chordate subphyla is a parsimonious model.

Similarly, in the light of Homology concept, the only organ that could be hypothesized as the homologous precursor for Amphioxus' diverticulum is the yolk sac of a preceding advanced motile chordate ancestor. I also hypothesize that during transition from the presumable advanced chordate to Amphioxus (or during cephalochordate phylogeny itself), the yolk sac ceased to function in food storage, became internalized, and acquired functions of a digestive organ, establishing homologous phylogenetic precursor of vertebrate liver.

I also believe that consistency of my hypothesis with accepted model of Islets of Langerhans phylogeny [197–202], as well as congruence with well-thought model on probable course of chordate evolution outlined by the profound evolutionary scholar Alfred Sherwood Romer, is supportive. In this avenue, I find the following citation as endorsing the Romer model and my small contributions to it:

> "Some observations lately made by M. Kowalevsky [22], since confirmed by Prof. Kuppfer, will form a discovery of extraordinary interest, if still further extended, as I hear from M. Kowalevsky in Naples he has now effected. The discovery is that the larvae of Ascidians are related to the Vertebrata, in their manner of development, in the relative position of the nervous system, and in possessing a structure closely like the *chorda dorsalis* of vertebrate animals. It thus appears, if we may rely on embryology, which has always proved the safest guide in classification, that we have at last gained a clue to the source whence the Vertebrata have been derived. We should thus be justified in believing that at an extremely remote period a group of animals existed, resembling in many respects the larvæ of our present Ascidians, which diverged into two great branches—the one retrograding in development and producing the present class of Ascidians, the other rising to the crown and summit of the animal kingdom by giving birth to the Vertebrata." Charles Darwin. The Descent of Man and Selection in Relation to Sex (pp. 205–206) [316].

My analysis, based on Homology Principle, is aligned with traditional perception of phylogenetic relations between Chordate subphyla [137,264,266,296–300], which perceives cephalochordates as the closest living relatives of vertebrates. In contrast, new results from analysis of large set molecular data suggested a dramatic rearrangement in relations between Chordate subphyla [270,271]. Employing the same Homology principle on molecular level Delsuc and co-authors provide evidence that tunicates, and not cephalochordates, represent the closest living relatives of vertebrates. How could we reconcile the above?

I believe that we shall overcome the contradiction (for now) by employing the famous notion "Homology cannot be proven; it is always inferred." [317]. As Alessandro Minelli put it: "This circumstance shows how much our trees are still dependent on a body of evidence that is obviously growing, and thus changing, rapidly, so that we must be cautious before accepting this or that grouping as definitive." Alessandro Minelli [318]

Finally, I strongly believe that homology of morphologic patterns (normal or pathologic) form a great body of evidence for reconstruction of both: phyletic relations and pathogeneses of diseases. As a prominent pathologists noted: "… the power of the visual microscopic image is probably greater than the power of a gene expression microarray reporting on the activity of thousands of genes."[319].

## Conflict of interest statement

I declare that there is no conflict of interest.

## Acknowledgement

This analysis dedicated to memory my father, my teacher in morphology and embryology, my scientific mentor and colleague – Mikhail Ya. Subbotin. I want to thank Michael V. Subotin for translations from German and Chinese.

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