



## Review Article

# Ductular Reaction in Total and Partial Biliary Obstruction in Experimental Settings



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## Abstract

In this paper, the features of ductular reaction (DR) and remodeling of the biliary tract in experimental models are discussed in total and selective biliary occlusion. It has been shown that the intensity of DR, as well as the shape, number, and topography of ductular profiles following common bile duct occlusion (CBDO) are closely related to the duration of the biliary obstruction. In addition, the formation of new ductular profiles can occur by the widening of existing bile ducts/ductules as a result of cholangiocyte proliferation, hepatocyte transdifferentiation, and/or activation and differentiation of stem/progenitor cells. It has been concluded that DR induced by CBDO consists of the components of all types of DRs, including I, II (A and B), and III, thus increasing the interest in further studies of this model. In the DR following CBDO, the consequent “preproliferative” and “proliferative” phases developed in parallel with cells differentiation and transdifferentiation (the “para-proliferative” phase) should be distinguished. The dynamics of these phases are important to consider for further detailed classification of DRs. During selective biliary obstruction, the full range of DR characteristics for CBDO has not been determined (mainly the events of biliary proliferation and fibrosis are noted). However, the great compensatory potential of the biliary bed has been confirmed, as reflected by the formation of new collaterals between congested and noncongested bile ducts.

## Introduction

Ductular reactions (DRs) occur in many clinical and experimental acute and chronic liver pathologies.<sup>1</sup> The term “ductular reaction” was introduced in 1957 by H. Popper and his co-authors.

They defined a DR as the aggregation of inflammatory cells in the liver with organized and/or disorganized proliferation of the biliary epithelium.<sup>2</sup>

According to modern understanding, DRs are a histopathological phenomenon, which implies an increased number of ductular profiles (DPs) lined by cells of the ductal phenotype on the histological images of the liver.<sup>3,4</sup> Besides, the proliferation of clusters of cells with the ductular phenotype, or even individual cells, is also considered as a DR.<sup>1</sup>

The interest of researchers in this topic is due to the fact that

DR is considered as a phenomenon closely related to carcinogenesis and regeneration.<sup>1,5</sup> DRs are widely studied both in the clinic and in experimental models, especially in rodents with mass liver injury, hepatocellular carcinoma, liver resection, or common bile duct occlusion (CBDO).<sup>6–10</sup>

According to the features and duration of the liver pathology, four types of DRs have been identified: I, IIA, IIB, and III (Table 1).<sup>11</sup>

Taking into the account the postulate that the presence of at least a mild component of bile congestion is necessary for the development of DRs,<sup>12</sup> the current review attempts to summarize the data from both the literature and our own studies on DRs in CBDO models, when the DR is solely caused by bile congestion and is not related to other damaging factors such as the diet, toxins, drugs, etc.

We believe that the features and characteristics of the DRs observed in rats following CBDO, a model that fairly adequately mimics biliary atresia or biliary obstruction by gallstones or tumors, provide an additional foundation for understanding the DRs in various models and pathologies of liver and bile duct diseases. The DR that occurs in acute biliary obstruction (in humans) and CBDO (in rodents) is classified as a type-I DR, otherwise called a “typical” DR.<sup>11</sup> The formation of new DPs in a typical DR is considered to be based on the proliferation of existing ducts, based on which the biliary bed is remodeled to adapt to the new environ-

**Keywords:** Ductular reactions; Common bile duct occlusion; Selective biliary obstruction; Biliary remodeling; Biliary collaterals; Hepatocyte transdifferentiation.

**Abbreviations:** CBDO, common bile duct occlusion; CK, cytokeratin; DP, ductular profile; DR, ductular reaction; miRNA, microRNA; SBO, selective biliary obstruction;  $\alpha$ -SMA, alpha-smooth muscle actin; 3D, three-dimensional.

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**Table 1. Different types of ductular reactions (according to Desmet, 2011)<sup>11</sup>**

Type of ductular reaction	Disease/condition or animal models	Location	Cellular mechanism
Type I (typical)	Occurs during acute biliary obstruction (in humans), CBDO (in rodents)	In the portal areas and periportal zones of the liver lobule	Proliferation of cholangiocytes
Type IIA (atypical)	Occurs during chronic cholestatic and inflammatory diseases, postnecrotic regeneration, malignant liver disease	In the periportal zones of the liver lobule	Activation of progenitor cells that exist or result from hepatocyte dedifferentiation
Type IIB (atypical)		In the central zones of the liver lobule in the areas of hypoxia	
Type III (atypical)	Occurs after damage to the liver parenchyma in rodents	Intralobular	Proliferation of progenitor cells (oval cells) from the canals of Hering

CBDO, common bile duct occlusion.

ment. DRs involve not only an increase in the number of DPs but also the infiltration of inflammatory cells (mainly neutrophils) and the proliferation of vascular and fibrotic elements, which together create a typical picture.<sup>13–15</sup>

Sharp hypertrophy of the cholangiocytes and their nuclei lining the small-caliber periportal ducts can be confirmed as early as 6 h after CBDO. Sometimes, the size of the nucleus of cholangiocytes exceeds the size of the nucleus of adjacent hepatocytes. This phenomenon, which confirms the high reactivity of these cells and continues to be observed during 24–48 h after CBDO, should also be considered as one of the manifestations of DRs.<sup>16</sup>

Under the conditions of CBDO, the reasons for the development of DPs, except for stationary bile duct/ductule proliferation, can be associated with peribiliary glands (the existence of which has been shown in mammals with a gallbladder but continues to be in doubt in mammals without a gallbladder), as well as the periportal plexus described in rats by Murakami and co-authors (which is considered as one of the alternatives to peribiliary mucous glands).<sup>17–19</sup> DPs can also be given by the “wonderful biliary plexus” and “vaginal ductuli” located in the thickness of the portal plate of the liver. Some researchers also equate this plexus with peribiliary glands, which originate from the bile ducts that are located in the large portal tracts.<sup>20,21</sup> All of the abovementioned structures associated with the biliary network, especially in CBDO, can lead to the observation of DPs on histological slices.

### Biliary architectonics in relation with DRs

During the last two decades, many important research studies focusing on the cellular and molecular mechanisms of liver and biliary pathologies have been performed.<sup>1,11,22,23</sup> The obtained results provide new insights into the micro-architectonics of the biliary tree,<sup>24–27</sup> greatly contributing to our current understanding of biliary disorders as well as the pathological mechanisms and translational significances of the DR in various liver diseases. In general, bile ducts exhibit unique plasticity. They can dynamically remodel and adapt to different pathological conditions, which is important for maintaining liver homeostasis.<sup>5,28</sup> This opinion has been confirmed by the development of collaterals between the intrahepatic bile ducts;<sup>19,29</sup> in addition, targeted tropism of the bile duct proliferates toward the damaged locus (*e.g.*, the locus of the duodenal ulcer penetration<sup>30</sup>), which actually means making new “tunnels” in the liver. However, the mechanism that defines the intensity and direction of biliary tract branching remains unclear.

The trigger of type-I DRs is considered to be increased pressure in the bile ducts.<sup>31–33</sup>

The cells of the epithelial phenotype taking part in the DR differ by form and size. Their sizes vary from 6 µm (which is the diameter of the smallest cholangiocytes of the Hering’s canals) to 40 µm (which is the normal diameter of hepatocytes). This finding, which has been provided for type-II and type-III DRs, is absolutely acceptable for DRs accompanying CBDO as well and has been confirmed by the results of our research.

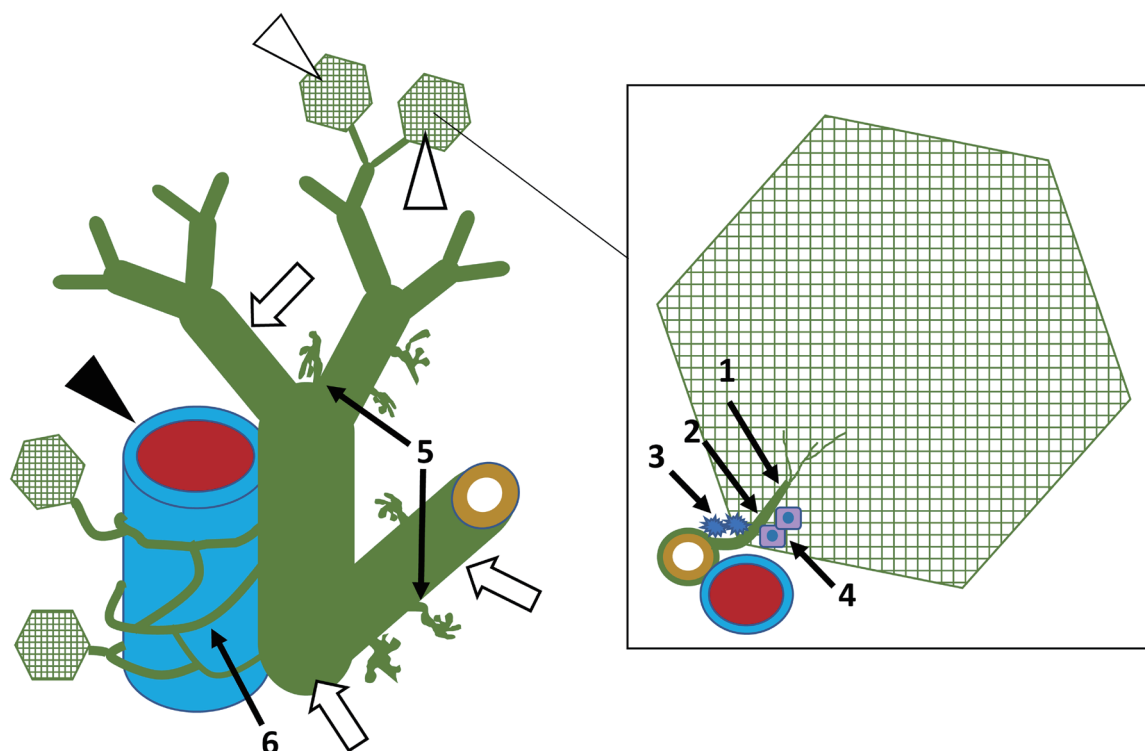
Nowadays, many researchers support the idea that in all types of DRs, the smallest caliber ducts of the biliary tree and the canals of Hering, which contain reactive cholangiocytes (so-called “small cholangiocytes” and progenitor/stem cells), play an important role. This re-actualized the importance of detailed examination of the morphology of the biliary system using modern technologies (including three-dimensional (3D) imaging).<sup>26,27,34–36</sup> Notably these ductules in rodents present a unique cytokeratin (CK)19<sup>+</sup>/CK7<sup>−</sup> immunophenotype, the kind of which is not found in the human liver. All ductules in the human liver are CK19<sup>+</sup>/CK7<sup>+</sup>.<sup>27</sup>

The presence of four progenitor/stem cell niches in the liver has been identified: ductules of Hering, intralobular bile ducts, periductal so-called “null” mononuclear cells, and peribiliary hepatocytes.<sup>37</sup> Additionally, the peribiliary mucosal glands are the fifth niche of progenitor/stem cells, which are dedicated to repair the damaged ductal epithelium.<sup>38,39</sup> Kordzaia *et al.* have reported the existence of one more niche observed in nongallbladder rodents. This is the periportal biliary plexus,<sup>19</sup> the diameter of the ductules of which is similar to the diameter of Hering’s canals and extra-portal lobular ducts,<sup>18</sup> and the sizes of their epithelial cells are also equal to the sizes of cholangiocytes of Hering’s ductules (Fig. 1).

### Cell proliferation and transdifferentiation induced by biliary obstruction

In rats, the peak of cholangiocellular proliferation in large ducts is observed on days 2–3 following CBDO, and in small ducts on day 5,<sup>8</sup> after which it gradually decreases up to day 28 from CBDO. In addition, the number of smooth muscle actin-positive, myofibroblast-like cells continuously increases, indicating that there is a negative correlation between the proliferative activity of biliary and myofibroblast-like cells.<sup>40</sup>

It should be noted that different opinions exist regarding the proliferation of biliary cells in CBDO. Various studies have shown that cholangiocytes divide, regardless of the size of the ducts



**Fig. 1. The niches of progenitor/stem cells in the liver.** 1, the ductules of Herring; 2, the intralobular bile ducts; 3, the intraportal so-called “null cells,” mononuclear cells; 4, the peribiliary hepatocytes; 5, the peribiliary mucosal glands; 6, the periportal biliary plexus; white arrows, bile ducts; black arrowhead, a branch of the portal vein; white arrowhead, hepatic lobule.

that they line. Two subpopulations (fractions) of cholangiocytes in rodents: small cholangiocytes ( $<8\text{ }\mu\text{m}$ ), which line so-called small ducts, and large cholangiocytes ( $>14\text{ }\mu\text{m}$  lining so-called large ducts) were distinguished; the proliferative activity of both subpopulations can be assessed by measuring  $[3\text{H}]$  thymidine incorporation and H3 histone gene expression. They conclude that under CBDO conditions, large cholangiocytes proliferate, while small cholangiocytes do not; they participate in the modulation of ductal bile secretion through  $\text{Ca}^{2+}$ - and inositol 1,4,5-trisphosphate-dependent (but not cAMP-dependent) mechanisms, in a different way from the pathway confirmed for secretin.<sup>41</sup> However, other studies have shown that in another models of ductal hyperplasia (e.g., partial hepatectomy or acute  $\text{CCl}_4$  poisoning), small cholangiocytes proliferate actively.<sup>42,43</sup> These findings indicate that small cholangiocytes have a proliferative potential that might not be realized under CBDO conditions. In mice after CBDO, the proliferation of biliary epitheliocytes begins in the large ducts, followed by proliferation in the small ducts, indicating the presence of a regenerative wave-from extrahepatic to intrahepatic ducts, until a kind of equilibrium level is reached in both types of ducts.<sup>8</sup> Also, other studies have shown that mitoses of large duct-lining epitheliocytes follow activation of the protein kinase A/Src/MEK/ERK1/2 pathway,<sup>44</sup> while small ducts undergo differentiation into large ducts via inositol trisphosphate/ $\text{Ca}^{2+}$ /calmodulin signaling,<sup>45</sup> which is accompanied by the restoration of the biliary epithelium, probably through the activation of stem cell niches (it is believed that a subset of small cholangiocytes are progenitor cells in the bile ducts, which are more resistant to damage than large cholangiocytes<sup>46,47</sup>).

DRs developed during CBDO are closely related not only to

the proliferation of cholangiocytes but also to hepatocytes. CBDO causes acute hepatocellular injury in mice on the second and third days following biliary obstruction, as evidenced by biliary infarcts and increased blood alanine aminotransferase levels. At the same time, the enhanced release of proliferative mediators has been confirmed, followed by the clear peak of hepatocellular proliferation on day 5.<sup>8,48</sup> The peak of cholangiocellular proliferation in large ducts is observed on days 2–3 following CBDO, and in small ducts on day 5,<sup>8</sup> after which it gradually decreases up to day 28 from CBDO. These data as well as the data provided by Kamimoto *et al.*,<sup>49</sup> contradict the findings claiming that the mitoses of epitheliocytes of small bile ducts do not occur in CBDO.<sup>41,50</sup>

Proliferation of hepatocytes in relation to DRs in CBDO is interesting due to the following circumstances: at the early stage of cholestasis, with the development of foci of the biliary infarction and the appearance of xanthomatous and pseudoxanthomatous cells—the so-called cholestatic rosettes. They are formed as a result of transformation of the liver parenchymal cell plates into tubular structures. They appear in the form of glandular acini, which include four or more hepatocytes located around the central lumen, that might be empty or filled with eosinophilic or bilirubin-containing material of variable density. Cholestatic rosettes of the liver are better visualized after labeling with a CK7 marker. They can also be detected during fluorescence microscopy of hematoxylin and eosin-stained preparations, when cells of the size and shape of hepatocytes, participating in the formation of the DPs, exhibit fluorescence characteristic of cholangiocytes. It is likely that these hepatocytes represent the population that underwent ductal transdifferentiation. Kaneko *et al.* have reported that hepatocytes adjacent to or near ducts should be subjected to biliary transdif-

ferentiation in the first zone of the acinus (periportal region) as well as in the hepatocytes that form the cholestatic rosettes of the liver cells.<sup>28,51–53</sup> Thus, during CBDO, the increase in the number of cells of the cholangiocyte phenotype of DRs may represent not only the proliferation of existing cholangiocytes but also the result of biliary transdifferentiation of hepatocytes.<sup>28</sup>

The presence of CK7<sup>+</sup> hepatocytes is considered as a morphological sign of chronic cholestasis.<sup>51,54</sup> They are mainly located in the first zone of the acinus, either periportal or paraseptal, and are considered to be an early sign of biliary metaplasia (while the detection of CK7<sup>+</sup> hepatocytes in the third zone is associated with ischemia of various genesis).<sup>16,51,55–57</sup>

The number of CK7<sup>+</sup> hepatocytes increases with the duration of cholestatic disease, while a small number of hepatocytes additionally show immunoreactivity for CK19. Thus, during ductal metaplasia, CK7<sup>+</sup> and then only CK19<sup>+</sup> hepatocytes appear, which is the reversed sequence of events observed during normal embryonic development. However, we found CK19<sup>+</sup> hepatocytes during the first week after CBDO (unpublished materials).

The degree of hepatocyte transdifferentiation during CBDO is thought to be lower than the degree of the same process with the 3,5-diethoxycarbonyl-1,4-dihydrocollidine diet.<sup>58</sup> In addition, it should be noted that the ductular transdifferentiation of hepatocytes is reversible, depending on the microenvironment,<sup>59</sup> which is important for more accurate understanding of the pathogenesis of chronic liver diseases and for the development of new therapeutic interventions.

Desmet has proposed the concept that most liver DRs are presented in a “ductal plate” configuration.<sup>11</sup> The ductal plate is the primitive form of intrahepatic bile ducts at the stage of embryonic development of the liver, which is manifested as a perforated cylinder that is formed by a double layer of biliary-type cells, the lumen of which has a circular or slit-like shape.<sup>11</sup> The “mini-ductal plates” found in DRs consist of a double layer of biliary-type epithelial cells and a small central blood vessel (usually a modified sinusoid or venule) surrounded by a small amount of mesenchyme originating from the space of Disse. An almost virtual luminal fissure exists between the two layers of epitheliocytes, often with multiple areas of dilatation. This configuration almost exactly corresponds to the double-layered embryonic ductular plate construction during the early remodeling stage. If cholestasis is persistent, this remodeling occurs rapidly, which in turn results in a prolonged need for the cholehepatic cycle of bile acids.<sup>10</sup> Given the larger resorptive cholangiocytic surface in mini-ductal plates, this cycle is more substantial. During an intense DR, the patterning of the ductal plates may occur simultaneously around many “incoming sinusoids,” forming a network at the periphery of the lobe.<sup>11</sup> The results provided by Fabris *et al.*<sup>60</sup> also confirm that the atypical DR in chronic liver diseases recapitulates a program similar to that involved in the early stages of biliary ontogenesis, when neural cell adhesion molecule-positive ductular cells arise from hepatoblasts that undergo a phenotypic switch. These greatly expanded the understanding of the essence of DRs. However, Desmet believes that all types of DRs might be characterized by the form of the mini-ductal plate patterns, excluding the type-I DRs. However, we also observed similar patterns in the type-I DRs in CBDO rats. This finding may be due to the fact that Desmet was investigating clinical materials (liver biopsies of patients), while we were investigating rat livers with bile congestion. Taking this into account, we suppose that the concept provided by Desmet is more comprehensive than he assumed himself.

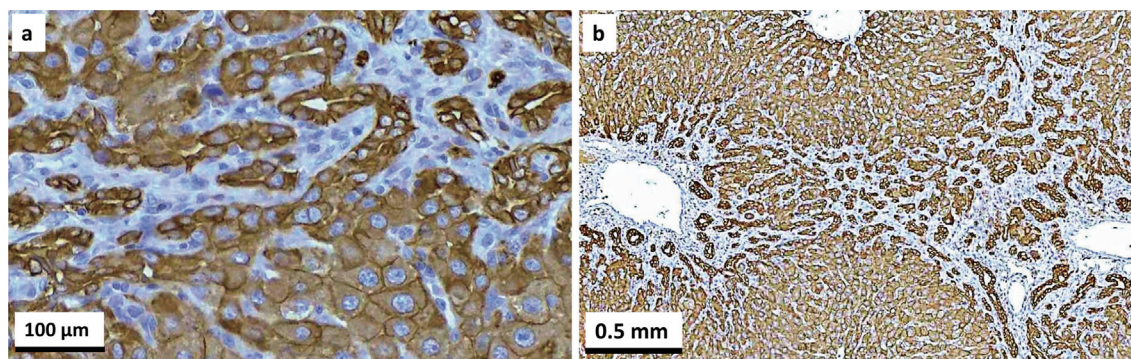
Studying CBDO-associated DRs, it is critical to determine

not only whether the newly identified DRs are derived from the existing bile ducts and their cholangiocytes or whether they are structures formed by stem cells or transdifferentiated and/or dedifferentiated hepatocytes,<sup>61–64</sup> but also to what extent these newly formed DRs are related to the existing bile duct and to each other.<sup>65</sup> Given that the bile ducts represent a potential stem/progenitor cell niche in the liver,<sup>62,66</sup> it is plausible to hypothesize that during the CBDO, intralobular bile ductules may contribute to the migration of progenitor cells into the lobular parenchyma. These cells, in turn, can facilitate the local delivery of new cells and support the formation of new canaliculo-ductal connections if needed, similar to what occurs in a choline-deficient, ethionine-supplemented liver injury model. In this model, it has been demonstrated that DRs can generate asymmetric hepatocytes that interconnect DR-biliary cells and resident hepatocytes, thus providing a continuous hepatobiliary pathway.<sup>10,67</sup> The number of these hepatocytes is apparently insignificant for parenchymal regeneration but important for observing the intralobular drainage of bile. This view is supported by a study by Pradhan-Sundd and co-authors<sup>68</sup> based on quantitative intravital liver microscopy. However, according to a number of authors, bile duct obstruction does not cause the spread of DRs in the parenchyma and is characterized by the formation of a denser network of interlobular ducts around the portal vein.<sup>69</sup> The intralobular arrangement of DR components has been described by us on the CBDO model, which gives us a reason to support the concept of maintaining the hepatobiliary continuum with DRs.<sup>23</sup> In addition, this mechanism can be implemented through the involvement of the morphogenetic pathways that are involved in the prenatal development of the bile ducts.<sup>70–73</sup>

During cholestatic disease, DRs are accompanied by the development of periductal fibrosis; moreover, DRs are a type of driver for the development of portal-portal septal fibrosis (biliary-type fibrosis).<sup>11,74</sup> Besides, the functional significance of this characteristic histological picture in cholestatic liver disease is still not completely clear.

Wedge-shaped periportal expansion of the DR, accompanied by an inflammatory infiltrate, creates an irregular portal-parenchymal interface with the formation of foci of gradual biliary necrosis. This process is further “complicated” by the development of portal-central connective tissue “bridges”, which are accompanied by nodal regeneration of the parenchyma and the formation of biliary cirrhosis; nevertheless, the detailed explanation of the mechanism requires additional research.<sup>11,75–77</sup> Also, it is very important to know that the DR is reversible after restoring the bile flow: expanded ductules are thought to disappear by apoptosis.<sup>78</sup> This issue is related to the problem of fibrosis reversal and may represent one of its key explanations.

MicroRNAs (miRNAs), which are endogenous noncoding RNAs that regulate gene expression by affecting specific mRNAs and at the same time are highly stable to chemical and enzymatic degradation, play an important role in the development of pathological processes associated with cholestasis. They modulate proliferation, apoptosis, fibrosis, and cancer. Studies have shown that the expression level of specific miRNAs changes in the blood serum, peripheral blood mononuclear cells, and the liver tissue itself of patients with chronic liver disease (primary biliary cirrhosis).<sup>79</sup> miRNAs play a critical role in hepatic stem cell activation; for example, miR-183-5p is increased in bile duct ligation-induced liver fibrotic tissue, which in turn activates LX-2 cells (a hematopoietic stem cell line),<sup>80</sup> and miRNA-29a also inhibits bromodomain-containing protein 4, leading to suppression of hematopoietic stem cell activation and resulting in reduced liver fibrosis.<sup>81</sup> In addition,



**Fig. 2. DR induced at 1 week after CBDO in rats.** (a) DR in the configuration of the ductal plates. (b) Ductular profiles accompanying porta-portal connective tissue bridges. Light microscopy images after immunohistochemistry with the CK8 antibody. CBDO, common bile duct occlusion; DR, ductular reaction.

miRNAs play an important role in maintaining bile acid homeostasis.<sup>79</sup> In turn, increased levels of bile acids lead to progressive liver damage, fibrosis, and end-stage liver disease.<sup>82,83</sup> The potential of miRNAs as therapeutic targets is increasingly being established. The discovery of each participant involved in the development of pathological processes is crucial for the introduction of new strategies for the treatment of cholestasis.<sup>82</sup>

Dezső and co-authors have presented a model obtained by further refinement of the widely known architecture of intraparenchymal bile ducts in the normal human liver,<sup>27,84</sup> according to which the canals of Hering with accompanying blood vessels are located in rudimentary interlobular septal zones. Studies based on Masson's trichrome-stained and CK19-labeled slides have confirmed that fibrotic septa that develop in conditions of liver pathology appear to follow the "pathways of Hering's ductules".<sup>85</sup> This is classically demonstrated in the rat liver under CBDO conditions (Fig. 2).

### Phases of DRs

It is known that the number, length, and shape of detected Hering's canals depend on the liver tissue samples (normal or pathological tissue) and on the immunohistochemical markers used (e.g., CK7, CK19, or epithelial cell adhesion molecule). Without immunostaining, in a normal human liver, 0.4 ducts (range: 0–4) are revealed on average within one interlobar portal tract,<sup>86</sup> while after the use of CK7, this number increases to 2.5–5.<sup>87</sup> It is logical that the dilatation of biliary structures caused by bile congestion and increased biliary pressure should enable "visualization of the invisible ducts". Thus, the sharp increase in the number of DPs described by us during the first 24 h of CBDO in rats reflects the widening and exposure of those "hidden" ducts, which together with the abovementioned sharp hypertrophy of ductal-lining cells (cholangiocytes and progenitor cells) and their nuclei might be considered as the so-called "preproliferative phase" of DR.<sup>16</sup> From day 2 of the CBDO, it is followed by a "proliferative phase" that is characterized by the proliferation of cholangiocytes, hepatocytes, and progenitor cells (this process can be quantified using CK markers and morphometry<sup>88</sup>) as well as the proliferation of other components of the DR: inflammatory cells as well as vascular and fibrous elements. It is believed that the transdifferentiation of periportal hepatocytes towards biliary epitheliocytes is involved in the latter process. However, the biliary transdifferentiation of hepatocytes has been described as early as 12 h after CBDO.<sup>89</sup>

All of the above should be taken into account when listing the characteristics of the acute and chronic phases of cholestasis.

Before CBDO, the interlobular bile ducts are presented as a sparse mesh. The proliferation rate of cholangiocytes lining this mesh is less than 0.5%. During the first three days, in the acute phase of CBDO, a strong proliferative reaction of cholangiocytes develops, which is reflected by the marked corrugation (folding) of the surface of the bile duct lumen, which, in turn, increases the luminal surface of the biliary bed. At 3 days after CBDO, the bile ducts begin to bifurcate and trifurcate. Branching continues systematically until day 14 from CBDO. From the day 7 of cholestasis, the proliferation rate decreases but still exceeds the control rate. During the same period, an increase in the length of the ducts is also observed. This is accompanied by a reduction in the frequency of epithelial folds (wrinkles). At the same time, the height of the remaining folds increases. The increase in the length of the bile ducts can be explained as the result of "relaxation." This indicates that the transitive increase of the inner relief folds of the ducts acts as a temporary buffer, which "delays" the extension of the ducts alongside the maximum rate of cholangiocyte proliferation. From day 7 following CBDO, the length of the ducts increases. From days 7 to 14, the probability of the formation of biliary collaterals increases to a maximum. The frequency of Hering's canals (canalicular-ductal connections) is moderately reduced. This completes the acute phase of cholestasis.<sup>26</sup>

The chronic phase of CBDO (from day 14 to day 28 and possibly longer) is characterized by a decrease in cell proliferation, accompanied by a further increase in the duct length, volume, and luminal surface area. The frequency of development of collaterals and the probability of formation of a loop are maintained at the same maximum level, which is observed on day 14 after CBDO.<sup>26,33</sup>

### Spatial architecture of the biliary tree and topography of the DRs

During CBDO-induced DRs, we documented the emergence (development or exposure) of an increased number of DPs in the portal field (adjacent to pre-existing bile ducts and/or intramurally-in the thickness of the duct wall), around the portal vein, periportal (at the border plate and between the portal tract), inside the lobules (at different depths, including in the second and third zones of the acinus), in the septa connecting the portal tracts and adjacent areas, in the "hilar plate," as well as in the thickness of the porta-caval fibrous connections and in the adventitia of the hepatic veins. The reason and possibility of detecting ductules in the last two locations have been shown by us in a special review.<sup>90</sup> From the point of view of participation in DRs, the attention of researchers has

been particularly focused on the periportal ducts, because this biliary segment is known for its impressive proliferative activity and high potential for adaptive remodeling.

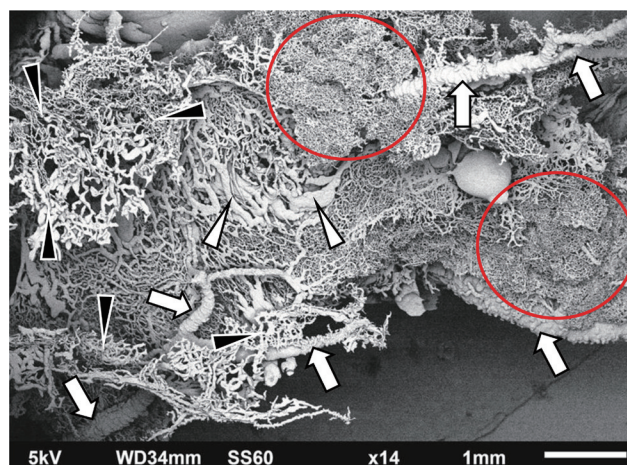
Studies using confocal microscopy for 3D reconstruction have shown a significant abundance of small-caliber biliary structures in mice.<sup>4,28</sup> A study of the bile ducts in the dynamics of CBDO (including 28 days) by the method of 3D confocal image reconstruction and analysis has demonstrated that the common and large-caliber bile ducts of the liver are predominantly widening.<sup>91</sup> In addition, the proliferation of cholangiocytes lining these ducts initially causes corrugation of their inner surface (bed), resulting in an approximately five-fold increase in the surface area. Similar events were observed by us during scanning electron microscopy of large-caliber bile ducts of dogs.<sup>19</sup> If a similar corrugation of the epithelium develops in fine ducts as well, then this process must be based on the activation of cholangiocyte mitoses, which is contradicted by a number of studies<sup>41,45</sup> claiming that the cholangiocytes lining the fine-caliber ducts do not involve the mitotic cycle but undergo remodeling based on the activation of stem cell niches.<sup>46,47</sup> Thus, the reaction of interlobular ducts to bile congestion is significantly different from the reaction of large-caliber bile ducts as well as from the process of new duct formation during embryogenesis.<sup>92,93</sup> The diameter of the interlobular bile ducts does not increase, despite the fact that there is an increase in hydrostatic pressure in the biliary bed.<sup>8</sup> Studies have shown that the diameter of the interlobular ducts remains unchanged ( $\sim 10\ \mu\text{m}$ ) during the period of 28 days of CBDO, indicating that CBDO-induced cholestasis does not cause dilation of the interlobular bile ducts or their branches.<sup>26</sup> Instead, they are extended and branched. Furthermore, regardless of extension and branching, the interlobular bile ducts remain within a 10–15- $\mu\text{m}$  radius of the portal vein branch. Thus, the intralobular ductal response seen in CBDO must be the result of the proliferation of already existing Hering ducts within the lobe and not the intralobular expansion of proliferating ducts from the portal field. But, at the same time, it has been shown that during CBDO, the number of Hering's ducts does not increase; on the contrary, it decreases (normally 5 for every 100  $\mu\text{m}$ , and 1 for every 100  $\mu\text{m}$  on day 28 after CBDO), which confirms the reduction of the canalicular-ductular connections.

All of the above studies confirm that many aspects of DRs in the setting of CBDO still remain unexplored.

The result of the study conducted by the 3D reconstruction method shows that the total length of the bile ducts increases mainly due to their branching, which occurs during the first 14 days after common bile duct ligation. Interestingly, there is no increase in the biliary frequency between days 14 and 28, despite continued significant proliferation of biliary epithelial cells and an increase in the total ductal length. The average length of the biliary branches increases steadily after 28 days of the CBDO. It has been confirmed that the peak of the formation of biliary offspring occurs on days 6–7.<sup>26</sup> In addition, the increase in the number of bi- and trifurcations of the bile ducts is especially noticeable on day 3 after CBDO. Subsequently, a decrease in the rate of this process is noted. As a result, the architecture of the interlobular bile ducts shifts from a sparse network with branches every 100  $\mu\text{m}$ , on average, to a relatively complex network with branches every 20  $\mu\text{m}$ .<sup>26,40,45,94</sup>

These data differ from those reported by Slott *et al.*, according to whom the biliary obstruction causes the elongation and less branching of the ducts. But if we consider the research methods used, the data of Vartak and co-authors should be considered more reliable.<sup>26</sup>

It has been hypothesized that this “adaptive remodeling” and accordingly increased surface area in contact with the bile serve



**Fig. 3. Scanning electron microscopy of corrosion casts of the biliary pathway of rats at 2 weeks after CBDO.** Inside the red circles, the casts of networks of intralobular bile canaliculi; white arrows, casts of bile ducts; white arrowheads, casts of lymphatic vessels and prelymphatic pathways; black arrowheads, corrosion cast of ductular plates and proliferated bile ducts/ductules inside of the portal tracts. CBDO, common bile duct occlusion.

various purposes: reducing intrabiliary pressure by increasing the volume of the biliary bed and/or providing a means of bypassing dysfunctional biliary tracts.<sup>36</sup>

Scanning electron microscopy of biliary corrosion casts can play an important role in the research of bile duct system remodeling and DRs. Although a lot of research on vascular corrosion casts has been performed, the details of the biliary tree, including small-caliber bile ducts, have been described in only a few experimental studies using this method.<sup>28,93,95,96</sup>

There are even fewer articles that refer to 3D visualization and quantification of biliary structures in obstructive cholestasis.<sup>4,33,97</sup> From this point of view, the results of our study obtained by scanning electron microscopy of corrosion casts of the biliary tree in CBDO have triggered additional interest: it has been shown that the proliferation of large- and medium-sized bile ducts is accompanied by the appearance and/or deepening of blind pocket-like bulges of the bile ducts, which are clearly visible on corrosive preparations in the form of hemispherical or bud-like growths. If we compare the normal biliary casts to the biliary casts created on days 4 and 6 of CBDO, it is clear that under the condition of bile congestion, the number of biliary casts as well as the size of their bulges exceed the similar characteristics of normal biliary casts (Fig. 3).

In addition, we critically re-analyzed the scanograms of our corrosion specimens in the CBDO model and made sure that the periportal abundant network of the casts is not only a combination of connective tissue channels and lymphatic vessels that are connected with them, but part of this network is also represented by casts of the “biliary” component of the DR (some of these prints represent the impression of a “slit-shaped” lumen of a flattened mini-ductal plate). The studied corrosion casts confirm the proliferation of the bile ducts, the formation of anastomoses between the proliferated ducts, and, as a result, the formation of a “wonderful network” of bile ducts/ductules. Besides, some ducts are connected to the network of bile capillaries, while others are not.

On the other hand, at later stages of biliary congestion, the absence of biliary ductal casts in some areas where the presence of a multiple DP is evident on the histological slides indicates that not all newly formed DPs are connected to the biliary bed.<sup>16</sup> The fact

that in the intralobular labyrinth of DPs, some ducts are widened, while others are not, or moreover, cannot be identified practically, supports the assumption of a heterogeneous connection of the “neo-ductules” constituting the DR to the existing biliary bed.

The described features emphasize the importance of adaptive remodeling of interlobular bile ducts in mitigating the effects of cholestasis. Furthermore, this remodeling may be specific to the interlobular ducts and qualitatively different from the events occurring in the larger bile ducts, which is in full agreement with the concept of the heterogeneity of bile duct epitheliocytes.<sup>19,50,98</sup>

Interestingly, the mechanisms of small-caliber bile duct remodeling have been described predominantly in mice.<sup>4,26,28,97</sup> Also, since 3D imaging of the mouse biliary tree is a fairly new research approach, especially in cholestatic liver diseases, more experimental studies are needed to refine 3D imaging methods.<sup>36</sup>

### Ductular reaction following partial (selective) biliary obstruction

Based on the above, it is important to determine the type of remodeling that occurs during selective biliary obstruction (SBO), when the draining bile ducts of one or more lobes of the animal are ligated. It has been shown that obstruction of the bile ducts draining 75% of the liver tissue in dogs and monkeys (even 95% in some dogs) can be accomplished without the development of jaundice.<sup>99</sup> Also, it is widely believed that SBO in rats is difficult to achieve.<sup>100</sup> With the exception of the technical difficulties associated with the small size of the bile ducts and the close proximity of the arterial branches, it was discovered that the formation of biliary collaterals provides rapid elimination of obstructive cholestasis, as confirmed by microcholangiography and magnetic resonance studies.<sup>29,101</sup>

Additionally, the results of a study by *Tannuri et al.*<sup>101</sup> indicate that in the conditions of SBO, ductal proliferation and collagen formation occur in both occluded and nonoccluded lobes. The pressure in congested bile ducts and chemical irritation of the epithelium are considered to be the initiators of cholangiocyte proliferation. After two days, the existing collaterals widen enough to bypass the obstruction and restore bile drainage from the congested lobes. Subsequently, within one week after SBO, a network of collaterals develops in the liver hilar area, which is formed by the interconnection of excrescences (“outgrowths”) of congested and free bile ducts<sup>29</sup> not only as a result of the twisting of already existing branches with developed anastomoses.

The number of bile ducts increases dramatically at one week after occlusion of the lobular duct. In addition, it decreases relatively between two and three weeks, and then increases again between four and eight weeks. A re-increase in biliary profiles after the fourth week indicates maintenance of biliary proliferative stimuli.<sup>101</sup>

An increase in collagen and portal areas is observed in both congested and noncongested lobes at two weeks after SBO. After three weeks, the deposition of collagen in the congested lobe is further increased, but in the noncongested lobe, its content is reduced compared with the amount present after two weeks. Immunohistochemical analysis has shown that both congested and noncongested lobes have increased expression of alpha-smooth muscle actin ( $\alpha$ -SMA), which is a protein associated with liver fibrogenesis.  $\alpha$ -SMA and desmin are structural proteins of intermediate filaments located in the cytoskeleton of smooth muscle cells as well as in other cell types, and they are responsible for the increased synthesis of type-I collagen. Another important profibrogenic cytokine involved in this process is transforming growth factor beta 1,<sup>102</sup> which regulates type-I collagen gene expression at the

transcriptional level in Ito cells.<sup>103</sup> The gene expression of  $\alpha$ -SMA is increased in the congested lobe at one week after SBO. After eight weeks, its expression is increased in the intact lobe as well. Considering that  $\alpha$ -SMA in the liver is present in myofibroblasts, which arise from the activation and transdifferentiation of hepatic stellate cells or portal fibroblasts,<sup>104</sup> it might be concluded that SBO causes an increase in the myofibroblast population in both the cholestatic and noncholestatic liver parenchyma and that this is associated with collagen deposition and portal fibrosis. Based on this study, the researchers conclude that the ligation of the duct responsible for the biliary drainage from the liver lobe contributes to the development of changes driven by paracrine and/or endocrine mechanisms, both in congested and noncongested lobes.<sup>101</sup>

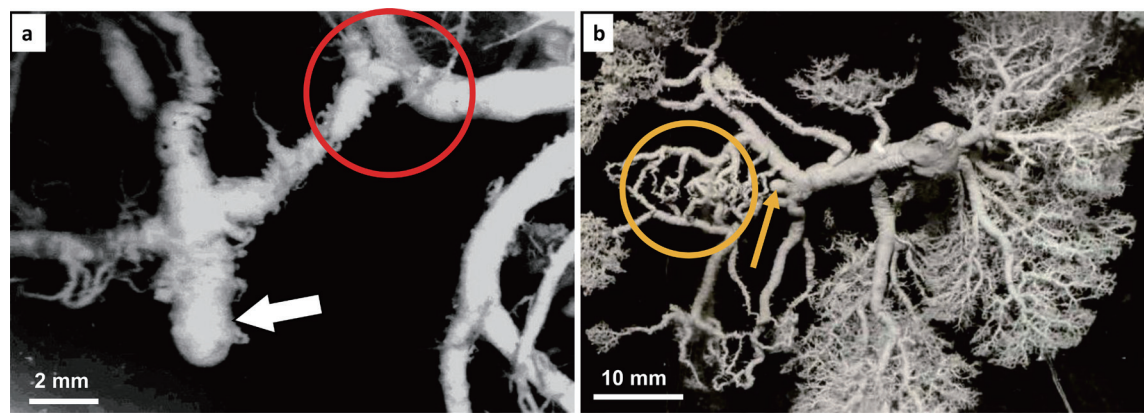
Gastrointestinal hormones, bile acids, angiogenic factors, neurotransmitters, and steroid hormones are mediators of the cholangiocytes’ proliferative response to cholestasis.<sup>105</sup> It is possible that some of them (or even one of them) may also act on (or influence) the surrounding noncholestatic parenchyma and induce a similar proliferative response. However, revealing these mechanisms requires additional research.

It has been confirmed that in both experimental and in clinical conditions, SBO causes atrophy of the congested lobe and compensatory hypertrophy of the contralateral lobe. The experimental research has shown that the bile secretion capacity of the nonobstructive lobes is enhanced, thereby compensating the dysfunction of the obstructive lobes.<sup>106</sup> Interestingly, this phenomenon of the atrophy-hypertrophy complex also has been described in selective obstruction of the portal vein.<sup>107</sup> The selective occlusion of the main branches of the portal vein or bile duct causes atrophy of the corresponding part of the liver, whereas the remaining liver undergoes compensatory hypertrophy until the original mass of the liver is restored.<sup>108,109</sup> The characteristics of hepatic lobular atrophy and compensatory hypertrophy associated with selective cholestasis should be identified in order to develop both diagnostic and therapeutic approaches. From this point of view, attention should be paid to such a phenomenon as the finding of ducts in the “contour” between atrophied and nonatrophied lobes.<sup>110</sup> The detection of DPs in the contour of the advanced liver lobes should be considered as an original form of DR that develops under the conditions of SBO.

Bile drainage from the congested lobes through the ducts of the adjacent lobes is facilitated by three factors. The first factor is the connection between the networks of bile canaliculi of the congested and noncongested lobes in the area where the parenchyma of the lobes passes into each other. If we take into account that in rats, the network of bile capillaries of all adjacent lobes is connected to each other,<sup>18</sup> it becomes clear that under the conditions of occlusion of any lobular duct, bile drainage from the bile canaliculi of the occluded lobe will be carried out through the duct of the adjacent (nonoccluded) lobe. The second factor is the presence of connections between congested and noncongested ducts—by means of the structures related with the above ducts and located in the hilar plate (Laennec’s capsule). The third factor is the possibility of developing anastomoses between congested and noncongested bile ducts. During the study of corrosion casts of the biliary bed (Fig. 4a), we observed a similar anastomosis in the model of SBO in dogs.<sup>19</sup> It should be noted that a similar phenomenon was described by Professor Shamshe Kevanishvili in 1988 after occlusion of the lobular branch of the portal vein (Fig. 4b).

### Conclusion

Based on all of the abovementioned studies, it can be concluded



**Fig. 4. Corrosion casts of biliary (a) and portal vein (b) branches in dogs.** White arrow, the remnant of ligated segmental bile duct; inside the red circle, the anastomosis developed among the intrahepatic branches of the ligated and nonligated segmental ducts; yellow arrow, the remnant of the occluded segmental branch of the portal vein; inside the yellow circle, the anastomoses developed inside of the left segment without portal circulation and connecting the portal branches of the neighboring segments with each other adjusted from two opposite sides.

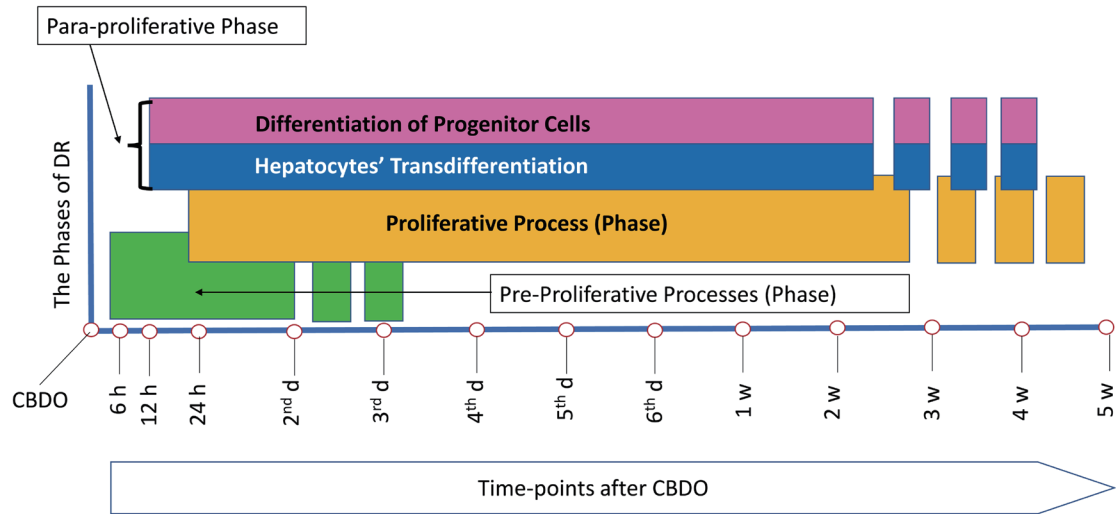
**Table 2. Features of ductular reactions in CBDO rodents**

Type of ductular reaction	Animal model	Location	Cellular mechanism
Type I (typical)	CBDO in rodents	In the portal areas (adjacent to pre-existing bile ducts and/or intramurally (in the thickness of the duct wall), around the portal vein, periportal, inside the lobules, in the septa connecting the portal tracts, in the “hilar plate,” in the thickness of the porta-caval fibrous connections, and in the adventitia of the hepatic veins	Dilation of existing bile ductules (preproliferative phase), proliferation of the cholangiocytes (proliferative phase), biliary transdifferentiation of hepatocytes, activation and differentiation of progenitor/stem cells (paraproliferative phase)

CBDO, common bile duct occlusion.

that the intensity (the number of DPs) and the topography of DRs in the CBDO rat model are closely related to the duration of biliary obstruction. The formation of new DPs can occur through the dilation of existing bile ducts/ducts, the proliferation of cholangiocytes, the transdifferentiation of hepatocytes, and the activation of stem/progenitor cells. Thus, the CBDO-induced DRs consist of all types of DRs, including I, II (A and B), and III, which increases

the interest in further studies of this model. In the DR following CBDO, the consequent “preproliferative” and “proliferative” phases in parallel with cells differentiation and transdifferentiation (the “paraproliferative” phase) should be distinguished (Table 2, Fig. 5). The dynamics of these phases are important to consider for further detailed classification of DRs. The study of both the bile ducts and the intralobular biliary pathway by means of direct 3D



**Fig. 5. The phases of the ductular reaction induced by CBDO in rats.** CBDO, common bile duct occlusion.

visualization (without the need for reconstruction), such as scanning electron microscopy of corrosion casts, has the potential to reveal new features of the biliary bed structure.

Thus, the current review highlights the less-known features of the forms, topography, and different phases of the DRs that develop in complete occlusion of the common bile duct. Besides, it shows the reasons why the DRs are minimal or not manifested under conditions of partial biliary obstruction.

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## Conflict of interest

The authors have no conflict of interests related to this publication.

## Author contributions

Review concept and design (DK and SG), illustration preparation (IB and DK), data collection and interpretation (SG, LG, LS, KT, IB, and DK), and drafting and revision of the manuscript (SG, KT, LS, and DK). All authors have made a significant contribution to this paper and have approved the final text.

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