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Intrinsic and extrinsic modifiers of the regulative capacity of the developing liver

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ABSTRACT

Zebrafish *wnt2bb* mutants initially fail to form a liver, but surprisingly the liver eventually forms in a majority of these embryos which then develop into fertile adults. This unexpected result raised the possibility that identifying the mechanisms of liver formation in *wnt2bb* mutants could provide insights into the poorly understood yet general principle of regulative development, a process by which some cells can change fate in order to compensate for a deficiency. Here, we identify two factors that underlie the regulative capacity of endodermal tissues: an intrinsic factor, Sox32, a transcription factor of the SoxF subfamily, and an extrinsic factor, Fgf10a. *sox32* is expressed in the extrahepatic duct primordium which is not affected in *wnt2bb* mutants. Blocking Sox32 function prevented liver formation in most *wnt2bb* mutants. *fgf10a*, which is expressed in the mesenchyme surrounding non-hepatic endodermal cells, negatively impacts the regulative capacity of endodermal tissues. In Wnt/β-catenin signaling deficient embryos, in which the liver completely fails to form, the repression of Fgf10a function allowed liver formation. Altogether, these studies reveal that there is more than one way to form a liver, and provide molecular insights into the phenomenon of tissue plasticity.

1. Introduction

Regulative development refers to the process by which cells can change fate in order to compensate for a developmental defect (Gilbert, 2006; Lawrence and Levine, 2006). For example, in Hans Driesch's classic experiments with sea urchin embryos in 1892, destruction of one cell at the two-cell stage did not interfere with normal development (Sander, 1992). Regulation can also be observed at later developmental stages: for example, removal of the node and anterior primitive streak in early chick embryos did not interfere with normal development either (Psychoyos and Stern, 1996). Importantly, not all embryos display regulative development (Gilbert, 2006), highlighting that it is a specific evolutionary adaptation. Gaining insight into the cellular and molecular mechanisms underlying regulative development is likely to inform us about tissue homeostasis and regeneration.

Zebrafish *wnt2bb* mutants, as well as embryos with defective Bmp signaling, initially fail to form a liver (Ober et al., 2006; Shin et al., 2007). However, in both cases a liver

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eventually forms, and *wnt2bb* mutant embryos in fact develop into healthy and fertile adults, illustrating the remarkable regulative capacity of the foregut endoderm and its derivatives to form a liver. It has recently been reported that *wnt2*, another Wnt ligand gene which, like *wnt2bb*, is expressed in the lateral plate mesoderm compensates for the loss of Wnt2bb in liver formation (Poulain and Ober, 2011). *wnt2* knockdown abolishes liver formation in most *wnt2bb* mutants, but not in wild-type (Poulain and Ober, 2011). In addition, we have recently reported that the additional blocking of the Wnt/β-catenin signaling pathway completely abolishes liver formation in *wnt2bb* mutants (Shin et al., 2011). However, it has not been addressed whether liver formation in *wnt2bb* mutants is different than in wild-type, or whether it is the same but with a delayed onset.

In this study, we have investigated liver formation in *wnt2bb* mutants and discovered that it is significantly different from the wild-type process. Sox32 appears to be required for liver formation in *wnt2bb* mutants, but not in wild-type. In addition, repression of Fgf10a signaling allowed hepatic *sox17* expression in *wnt2bb* mutants, but not in wild-type, and could enhance liver formation in *wnt2bb* mutants but not in wild-type. These findings illustrate the regulative capacity of endodermal tissues to form a liver, and more importantly shed light into the poorly understood regulative capacity of the vertebrate embryo.

2. Results

2.1. Liver and extrahepatic duct formation in wnt2bb mutants

Liver specification is initially impaired in *wnt2bb* mutants, but surprisingly the liver eventually forms (Ober et al., 2006). We sought to understand the mechanisms that regulate liver formation in these animals. First, we systematically examined liver formation in *wnt2bb* mutants. Using the *Tg(fabp10:dsRed,ela3l:EGFP)*^{gz12} line that expresses dsRed in hepatocytes and GFP in the acinar cells of the pancreas (Korzh et al., 2008), we observed that the liver eventually formed in most *wnt2bb*^{s403} mutants (80–100%;see Table S1). At 4 days post-fertilization (dpf), the liver size in the mutant larvae was variable and much smaller than wild-type, whereas the pancreas was of wild-type size (Fig. 1A–C). We next examined in detail the expression of two hepatoblast markers, *hhex* and *prox1*, in *wnt2bb* mutants during liver development. *hhex* expression in the liver-forming region was weakly reduced in *wnt2bb* mutants compared to wild-type at 30 and 36 h ours post-fertilization (hpf) (Fig. 1E–H, arrows), whereas *prox1* expression was more severely affected (Fig. 1M–P, arrows). Interestingly, at later stages in wild-type embryos, *hhex*, but not *prox1*, is expressed in the extrahepatic duct (Fig. 1I and K, arrows), and this expression appeared unaffected in *wnt2bb* mutants (Fig. 1J and L, arrows).

We have recently reported that the additional blocking of the Wnt/β-catenin signaling pathway completely blocks liver formation in *wnt2bb* mutants (Shin et al., 2011) using the *Tg(hsp70l:dkk1-GFP)*^{w32} line that expresses Dkk1, an inhibitor of the Wnt/β-catenin signaling pathway, upon heat-shock (Stoick-Cooper et al., 2007). In Wnt/β-catenin signaling deficient embryos (*Tg(hsp70l:dkk1-GFP);wnt2bb*$^{-/-}$ embryos heat-shocked at 18 hpf) compared with *wnt2bb* mutants, both *hhex* and *prox1* expression were profoundly downregulated at 42 hpf (Fig. 2C and F, arrows). Furthermore, when assessed with the pan-endodermal marker *foxa3* at 56 hpf, a liver bud was absent in Wnt/β-catenin signaling deficient embryos (Fig. 2I, arrow), but present in *wnt2bb* mutants (Fig. 2H, arrow). Whole-mount immunostaining with anti-Prox1 and 2F11 antibody, which labels the extrahepatic and extrapancreatic ducts, additionally revealed that the extrahepatic duct as well as the liver bud was absent in Wnt/β-catenin signaling deficient embryos (Fig. 2L). Altogether, these data show that in the absence of Wnt2bb function, the extrahepatic duct forms normally and the liver eventually recovers, whereas a more severe block in Wnt/β-catenin signaling blocks the formation of the extrahepatic duct as well as the liver.

2.2. sox17 and sox32 are expressed in the forming liver

In order to investigate molecular mechanisms of liver formation in *wnt2bb* mutants, we sought to identify transcription factor genes expressed in their late forming liver bud. We previously reported that *sox17*, a transcription factor gene of the SoxF subfamily expressed in all endodermal cells during gastrulation (Alexander and Stainier, 1999), was re-expressed in a ventrally located group of cells on the left side of 48 hpf embryos (Alexander and Stainier, 1999), but we did not investigate the precise identity of these cells. In resolving this issue, we observed that *sox17* expression is off by the 6-somite stage through at least 25 hpf (Fig. 3A). By 36 hpf, it is re-expressed in a small ventral region of the liver close to the extrahepatic duct (Fig. 3G), and by 60 hpf, it is expressed in the gallbladder (Fig. 3I, arrowhead). This expression is transient and is gone by 5 dpf (data not shown). Since *sox32* is also expressed in all endodermal cells during gastrulation and regulates *sox17* expression in these cells (Kikuchi et al., 2001; Sakaguchi et al., 2001), we also examined *sox32* expression. *sox17* and *sox32* (*sox17/32*) expression patterns in the liver appeared very similar to each other (Fig. 3A–F).

We next investigated hepatic *sox17/32* expression in *wnt2bb* mutants and Wnt/β-catenin signaling deficient embryos. *sox17/32* expression was present in *wnt2bb* mutants, but not in Wnt/β-catenin signaling deficient embryos (Fig. 3N and R). Interestingly, most of the late forming liver tissue in *wnt2bb* mutants expressed *sox17* (Fig. 3J) and *sox32* (data not shown). This correlation between hepatic *sox17/32* expression and liver formation prompted us to investigate the role of these genes in this process.

2.3. Sox32 positively regulates liver formation in wnt2bb mutants

Since *sox32* is necessary and sufficient for endoderm formation during gastrulation (Dickmeis et al., 2001; Kikuchi et al., 2001; Sakaguchi et al., 2001), it was critical to be able to manipulate Sox32 levels, or activity, after gastrulation in order to investigate its function in later events including liver formation. To block *sox17/32* function after gastrulation, we generated a line that expresses a dominant-negative Sox32 upon heat-shock, *Tg(hs:dnsox32)*^{s921} (Fig. 4A). The injection of