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Tie-ing up angiogenesis to treat NASH

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There is increasing interest in the role of angiogenesis in fibrogenic signaling, via generation of proangiogenic mediators that perpetuate injury and promote fibrosis [reviewed in (1)]. This is a timely development in understanding the underlying mechanisms and pathways that promote progression of nonalcoholic fatty liver disease (NAFLD) to steatohepatitis (NASH) with fibrosis and hepatocellular cancer (HCC), given the global impact of these diseases (2).

A role for angiogenesis in the progression of human NAFLD/NASH was shown a decade ago in studies where increased CD34 immunostaining (a surrogate for neovascularization) was observed in liver samples from NASH patients and in particular those with F2–F4 fibrosis (3). Predicated in part by those findings in humans, other studies examined the role for angiogenic signaling in preclinical models of NASH, including via vascular endothelial growth factor (VEGF) and placental growth factor (PlGF) signaling (4). Those studies demonstrated that mice fed a methionine-choline deficient (MCD) diet exhibited increased angiogenesis with a significant increase in both angiogenic and inflammatory mediators (4). Treatment of MCD fed mice with anti-VEGF Receptor-2 antibody decreased the angiogenic response, reduced vascular architectural distortion and attenuated both inflammation and steatosis, while anti-PlGF antibody treatment of MCD fed mice was ineffective in mitigating those adverse events (4). So at least in mice fed an MCD diet, the findings suggest that hepatic steatosis and inflammation are associated with an abnormal angiogenic response that may together further promote fibrogenesis and that inhibiting VEGF signaling is potentially therapeutic.

The work by Lefere and colleagues in the current issue of *Hepatology* (5) addresses those questions by studying a key signaling pathway involved in angiogenesis, namely the Tie1/Tie2 receptor complex and angiopoietin ligands. Tie receptors (tyrosine kinases with immunoglobulin and epidermal growth factor homology domains) exist in two forms, Tie1 and Tie2, which interact and both of which are required for normal angiogenesis [reviewed in (6)]. Angiopoietins (principally ANG1 and ANG2) function by signaling through Tie receptors to regulate both normal developmental angiogenesis and, depending on the circumstances (described below) function as either context dependent agonists or antagonists of Tie2 signaling. This dual role is explained in part because Tie1 is considered an orphan receptor with no naturally identified ligand, but rather modulates ANG signaling via Tie2 (6). ANG1 signaling via Tie1/Tie2 promotes normal vascular homeostasis under basal (ie

non-inflammatory) conditions, but in the setting of inflammatory mediators the Tie1 receptor undergoes cleavage of its ectodomain and ANG2 then becomes the dominant angiogenic signal, where it functions as a Tie2 antagonist and promotes a neoangiogenic, abnormal vasculature, prone to increased leakage (7). The inflammation-associated cleavage of Tie1 thus initiates a feedforward activation loop in which ANG2 promotes abnormal vascular remodeling (8). Lefere and colleagues show that serum ANG2 levels are increased in NASH patients and in proportion to the histological grade of inflammation, steatosis and ballooning although, surprisingly, not with the degree of fibrosis (5). They further show that serum ANG2 levels correlate with CD34 immunohistochemical staining in liver sections with reactivity confined to macrophages and endothelial cells. Those findings together strongly suggest that NASH subjects display increased circulating serum levels of ANG2 which correlates with markers of neovascularization (as indicated by CD34 staining) and that hepatic vascular endothelial cells are a plausible source of ANG2.

In the next phase, the authors undertook to inhibit the antagonistic role of ANG2 signaling via Tie2, using an engineered antibody (L1–10) that blocks ANG2-Tie2 interaction. They show that MCD fed mice exhibit increased serum ANG2 levels, confirming the expected findings alluded to above. They also studied another NASH model, namely neonatal streptozotocin and 16 weeks western diet exposure (9) and confirmed that ANG2 serum levels were increased in that model also. Administration of L1–10 reduced inflammation, ballooning and fibrosis in MCD fed mice, but did not change the degree of steatosis. The authors further undertook elegant 3D reconstruction imaging of the hepatic vasculature in those various groups of MCD fed mice and demonstrated that L1–10 treatment, as either a preventive or therapeutic intervention, reduced abnormal angiogenesis with decreased vascular density, compared to placebo (5). They also showed that L1–10 treatment reduced angiogenic signaling from cultured endothelial cells following LPS stimulation, confirming their observations that L1–10 reduces neoangiogenesis primarily through cell autonomous (ie endothelial) signaling. In a final series of studies, the authors returned to the streptozotocin-western diet model of NASH and asked if L1–10 would mitigate the development of HCC. They show that steatosis and histologic inflammation was unaltered in the L1–10 treated group, although there was a trend to reduced fibrosis and reduced mRNA expression of proinflammatory cytokines. In addition, they observed a decreased tumor burden in the L1–10 treated mice with reduced expression of glypican-3 and Hsp70 as well as reduced alpha fetoprotein. Those findings suggest that L1–10 treatment attenuates tumorigenesis in experimental NASH associated HCC, although the underlying mechanisms are still to be explored (5).

These elegant and comprehensive studies provide a tantalizing insight into the complex, multicellular signaling network that modulates NALFD → NASH → HCC progression and further raises the prospect for targeted therapeutic interventions. There are distinctive differences, however, in the pathways at play with ANG2-Tie interactions as compared, say, to other angiogenic pathways that have been studied in the context of experimental NASH. Among these differences is why anti-VEGFR2 treatment in MCD fed mice led to reduced hepatic steatosis (4) while L1–10 had no effect? Presumably those anti-angiogenic interventions differ in the downstream effects on hepatic lipogenesis and/or other homeostatic pathways. Another question is the role of inflammatory mediators in the initial

process of cleaving the ectodomain of Tie1. What is the protease involved and is baseline inflammation a prerequisite for L1–10 to ameliorate further damage? Would L1–10 treatment mitigate fibrosis in other, less inflammatory preclinical models of hepatic steatosis? Among the other intriguing findings from this study is the observation that L1–10 reduced the tumor burden in a preclinical model of NASH associated HCC. Those findings are important because high circulating levels of ANG2 are a strong negative predictor of overall patient survival in HCC (10). However, in a recent study using trebananib (which sequesters ANG1 and ANG2 and blocks interaction with Tie2) in addition to standard sorafenib treatment, there was no improvement in progression-free survival in patients with advanced HCC (10).

The studies of Lefere and colleagues will hopefully propel further investigation of this important signaling pathway, which could in turn pave the way for clinical trials.

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