Editorial

Long non-coding RNA *lncHand2*: the key to make a Prometheus?

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Εν τούτω προσηλωθείς Προμηθεύς πολλών ετών αριθμόν εδέδετο. καθ'εκάστην δέ ημέραν αετός εφιπτάμενος αυτώ τούς λοβούς ενέμετο τών ηπάτών αυξανομένων διά νυκτός.

Chained to this (i.e., the Caucasus), Prometheus spent many years; every day, an eagle flew to him, devoured his liver, which would grow again during the night.

It is tempting to open any commentary to liver regeneration research with these immortal words written by Apollodorus (1). Two-thousand years after the classical Greek culture's intriguing sense that the liver can rebuild itself following injury, the issue remains more relevant than ever. Liver disease in its various forms is a frequent cause of death worldwide (2). Transplantation is an effective solution, but its application is hampered by the paucity of donors (3). It follows that the quest for effective and safe liver regeneration methods is a crucial part of modern experimental hepatology. In a recent study published in the *Journal of Hepatology*, Wang and co-workers moved the field a step forward in that direction, by uncovering a novel long non-coding RNA (lncRNA) that stimulate hepatocytes to proliferate and regenerate liver tissue following partial hepatectomy (PHx) (4). The lncRNA in question is *lncHand2*. The rationale for focusing on this particular lncRNA was robust overexpression in a mouse model of PHx and conservation in several species including humans. A closer look revealed that *lncHand2* is a divergent transcript to the heart and neural crest derivatives expressed 2 (*Hand2*) coding gene and its expression is restricted to the liver and heart.

To test the hypothesis that *lncHand2* is involved in liver regeneration, the authors exploit a wide range of tools, from the latest genome editing technology, to good old days' Northern blot and much of what there is in between. First, they establish that *lncHand2* is present in pericentral hepatocytes and that its expression spreads out from sites where the latter cell type normally resides. Combined with previous evidence that pericentral hepatocytes display stem cell-like properties, the data were consistent with a potential role for *lncHand2* in liver regeneration. Based on this evidence, the authors examined a range of independent loss-of-function mouse mutants, both complete and conditional. Perhaps surprisingly, *lncHand2* loss did not result in any embryonic or perinatal phenotype, indicating no involvement in liver development. As predicted though, *lncHand2* loss produced liver damage and no regeneration in the PHx assay.

To further corroborate their results, the authors employed the fumarylacetoacetate hydrolase (FAH)-deficient (*Fah*$^{-/-}$) mouse model. *Fah*$^{-/-}$ mice display fatal liver failure unless rescued by 2-(2-nitro-4-trifluoromethylbenzoyl)-1,3-cyclohexanedione (NTBC). Hepatocytes that were *lncHand2*-deficient or with rescued *lncHand2* expression, colonized *Fah*$^{-/-}$ livers and made the treatment with NTBC dispensable for survival, to the predicted extent.

Following that flurry of physiological data, the study turned to molecular mechanisms. *In cis* transcriptional regulation, a previously documented function of specific lncRNAs (5), was ruled out. Indeed, the gene which *lncHand2* is divergent to *Hand2*—is a transcription factor involved in cardiac development and moderately expressed in the liver [(6-7); www.proteinatlas.org/ENSG00000164107-HAND2/tissue]. That negative results prompted a transcription factor-focused transcriptomics analysis in *lncHand2*-deficient cells. The main finding was that *lncHand2* loss suppressed the expression of Nkx1-2. Reassuringly, *lncHand2*-loss and *Nkx1-2*-loss

hepatic phenotypes were remarkably overlapping in a PHx assay, suggesting that they might operate within the same pathway. In parallel, the authors went to a fishing expedition for *lncHand2*-interacting proteins that might participate in Nkx1-2 expression regulation. RNA pull-down followed by a simple SDS-PAGE and silver staining, yielded a clearly identifiable differential band that could be successfully analysed by mass spectrometry. This simple and smooth proteomics—at least judged so from the reader's perspective—yielded Ino80 as *lncHand2*-interacting factor. The finding was biologically plausible, since Ino80 is an ATP-dependent chromatin modifier involved in gene expression regulation (8). Functionally, the *lncHand2*-Ino80 complex was detected at the *Nkx1-2* promoter and its disruption suppressed transcription of the latter gene. Once that established, the authors asked what were the downstream targets of *Nkx1-2*. The next set of experiments was based on the rationale that liver regeneration must be driven by growth factors, in line with evidence such as that insulin-like growth factor 2 is involved (9). Signalling receptor-focused transcriptomics revealed that c-Met was downregulated in *lncHand2*-null cells. This was another well-fitting piece of the puzzle, as c-Met is a cell surface receptor known for mediating hepatocyte growth factor (HGF) signalling (10). A range of expression manipulation experiments then showed that c-Met activation lies downstream to *lncHand2* and Nkx1-2, and that it participates in liver regeneration together with the two latter factors. Interestingly, double *Nkx1-2*/*c-Met*-null mice scored more poorly in liver regeneration ability than *Nkx1-2*-null counterparts. Although the authors do not further discuss the matter, it can be concluded that other liver regeneration pathways in addition to *lncHand2*/Nkx1-2 converge on c-Met.

In summary, Wang and co-workers produced an impressive amount of convincing data. Now, the exciting and often frustrating phase begins, of translating those findings into benefits for liver disease patients. One can only expect that the full range of available and yet to be discovered small molecule, RNA interference and genome editing weapons will be fired to achieve that goal.

From a molecular viewpoint, the study produced yet another important insight into lncRNA biology. *LncHand2* is new entry in the list of epigenetic modifiers-binding RNAs (11). The field brought about a significant change in how epigenetic modifiers are viewed. In the light of evidence that factors such as DNA methyltransferase 1 bind to RNA (12) and even more avidly than it binds to DNA, the text book definition of DNA-binding factors for epigenetic modifiers warrants some conceptual revision. Along this same line, a further merit of the paper by Wang and co-workers is that the word epigenetics is mentioned not even once—excluding the references. The issue is how epigenetics is currently defined. It is true that the *lncHand2*/Ino80 complex modifies the histone repertoire at the *Nkx1-2* promoter, but should these phenomena be included in the definition of epigenetics? The two epigenetic marks known so far—DNA methylation and histone posttranslational modifications—are written, maintained and erased by a range of DNA-binding factors such as transcription factors (13,14) and RNAs (12-15). By including the latter within the definition of epigenetics, virtually any transcription regulation phenomena become epigenetic, making one wonder why any field called epigenetics should exist at all and whether "broad transcriptional regulation mechanisms" would cover pretty much everything.

Lastly, one pending issue is the potential function of *lncHand2* in cardiac biology. Loss-of-function mice indicated that *lncHand2* does not significantly impact embryonic and perinatal heart development (4). Yet, cardiac expression data suggests a possible role for *lncHand2* in the heart that mirrors its function in the liver, i.e., driving cardiac tissue regeneration. It is possible that *Hand2*, a regulator of coronary artery development (7), cooperates with *lncHand2* in an adult heart-specific pathway. These are worth exploring topics that may lead to novel strategies to repopulate regions of the heart damaged by ischemia in cardiovascular disease. If so, a modern Prometheus should not worry about heart-eating diseases, either.

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