A novel long noncoding RNA (IncRNA), Braveheart (Bvht), has been defined as a critical regulator of commitment of the embryonic stem cells (ESCs) to cardiovascular lineages. Bvht activates a cardiovascular gene network and functions upstream of mesoderm posterior 1 (MESP1), a master regulator of a common multipotent cardiovascular progenitor. Bvht mediates epigenetic regulation of commitment to a cardiac lineage by interacting with suppressor of zeste12 (SUZ12), a component of polycomb repressive complex 2 (PRC2). Bvht represents the first IncRNA that defines cardiac cell fate and lineage specificity, linking IncRNAs to cardiac development and disease.

One surprise of the human genome project was that only ≈20,000 to 25,000 protein-coding genes exist in our species.1 In fact, even the simple roundworm C. elegans has similar number of protein-coding genes. Astonishingly, only <2% of the sequences in human genome are used for coding proteins.2 What are these noncoding sequences, which make up >98% of our genome for? Thanks to the innovations in sequencing technologies and new computational methods for transcriptome assembly. It is now recognized that the majority of the genome is actively transcribed to produce thousands of non-coding transcripts in many cell types and tissues.3,5 A subset of these noncoding transcripts are classified as IncRNAs, transcribed RNA molecules >200 nucleotides in length that do not encode proteins.5–6 Many of the IncRNAs are large intergenic noncoding RNAs (lncRNAs). The functions of IncRNAs have recently been investigated in diverse biological processes, such as stem cell pluripotency, immune responses, and cell-cycle regulation.7–9 IncRNAs have been reported to promote developmental transitions and function as key regulators of cell fate commitment.10 Moreover, IncRNAs were reported to interact with ESC gene regulatory networks, leading to the maintenance of ESCs and lineage-specific differentiation by regulating key transcription factors and pluripotency markers.3–7 One of the best studied examples of IncRNAs is X-inactive-specific transcript, which is involved in the process of genomic imprinting and X chromosome inactivation.14,15 X-inactive–specific transcript is transcribed from the inactive X chromosome and plays a key role in epigenetic gene silencing, primarily by recruiting PRC2 to the promoters/enhancers of target genes. Mechanistically, IncRNAs were shown to function either in cis or in trans to regulate gene expression locally or genome wide.16,17 However, little is known about the expression and functional significance of IncRNAs in the heart, in particular, during cardiac lineage commitment.

In the January 31, 2013 issue of Cell, Klattenhoff et al17 identified a novel IncRNA, Bvht, in mouse heart and demonstrated that Bvht is a key regulator of cardiac lineage commitment and cardiac gene expression. Bvht was initially discovered from database mining aimed at identifying cardiac-expressed IncRNAs. Among 47 candidate IncRNAs identified, Bvht was highly expressed in the heart. Because most IncRNA genes share similar gene structure with protein-coding genes, such as promoter histone modifications, exons, introns, and polyadenylation sites,18 it is essential to first prove the non-coding status of a given IncRNA. Bvht encodes a transcript of ≈590 nucleotides that contain 2 short open reading frames with coding potential for peptides of 48 and 74 amino acids. Two lines of evidence indicate that these open reading frames do not produce detectable proteins. First, the authors found that ectopic transfection with a Bvht expression construct did not yield detectable protein product. Second, they showed that the putative open reading frames of the Bvht transcript associated poorly with ribosomes, indicating they are not actively translated. Together, these results strongly suggest that Bvht is a true IncRNA. To further bolster this point, the authors might assess the effect of Bvht frame-shift mutations on putative functions of the IncRNA.

To study the biological function of Bvht, the investigators turned to in vitro differentiation of mouse ESCs. They knocked down Bvht by stably expressing short hairpin RNAs in ESCs. They found that Bvht was not required for global ESC differentiation nor was it required for ESC self-renewal. Instead, Bvht played a key role in cardiac commitment, promoting cardiac cell fate from nascent mesoderm. Similarly, the investigators showed that Bvht is necessary for the maintenance of cardiac fate in neonatal ventricular cardiomyocytes.

The authors found that depletion of Bvht reduced formation of contracting cardiomyocytes during ESC differentiation. Bvht-depleted ESCs failed to activate the key cardiac transcription factors that govern cardiogenesis. To connect Bvht with the regulatory pathways for cardiomyocyte
specification, the investigators performed unbiased genome-wide RNA-seq in Bvht-depleted ESCs. They found >548 genes were dysregulated when compared with that of control ESCs. Many of these altered genes are involved in transcriptional control of cardiac gene expression. In particular, MESP1, a key transcription factor for cardiac differentiation, was significantly downregulated in Bvht-depleted ESCs. MESP1 marks a multipotent cardiovascular progenitor, although MESP1 is not totally cardiovascular-specific.\(^9\)\(^{–}\)\(^{22}\) MESP1 expression, together with ETS2, was sufficient to transdifferentiate dermal fibroblasts into cardiac progenitors.\(^{23}\) Klattenhoff et al\(^{17}\) showed that Bvht regulates a core network of genes, including Mespl, to drive cardiac differentiation. More specifically, they provided evidence that Bvht functions upstream of Mespl as a permissive factor for cardiac commitment during ESC differentiation. These findings place Bvht on the top of the cardiac lineage commitment regulatory pyramid and suggest that it is among the earliest cardiac genes activated during development. Therefore, the discovery of Bvht represents a significant breakthrough that, for the first time, connects a lncRNA to cardiac specification. Together with recent reports demonstrating that dozens of lncRNAs block key lineage commitment programs within ESCs and function in crucial ESC regulatory pathways,\(^{12}\) it is evident that lncRNAs have emerged as a novel class of key regulators for cell fate determination.

To define the molecular mechanisms by which Bvht works and more specifically to determine whether Bvht acts in cis or in trans, the investigators first examined the expression of Bvht neighboring genes in Bvht-depleted ESCs. They provided convincing evidence to suggest that Bvht did not alter the expression of its neighboring genes in cis. However, the authors observed that the expression of neighboring microRNA-143/145 genes was dramatically repressed in Bvht-depleted ESCs. This decrease was attributed to the loss of differentiating cardiomyocytes, rather than being directly related to Bvht depletion. Having ruled out cis-regulatory mechanisms, the investigators next tested whether Bvht functioned in trans. Given the previously reported interaction of lncRNAs with epigenetic regulators, such as PRC2,\(^{24}\) the authors looked for Bvht–PRC2 interaction. Indeed, they found that Bvht directly interacts with PRC2 through SUZ12 during cardiomyocyte differentiation. Furthermore, H3K27me3 levels paralleled SUZ12 enrichment, suggesting epigenetic regulation by Bvht.

lncRNAs are emerging as an exciting area of investigation. However, there are more questions than answers about how they work. For example, how is the specificity of Bvht in epigenetic regulation during cardiovascular lineage commitment determined? How does Bvht–PRC2 interaction promote Mespl expression? Does Bvht epigenetically regulate the expression of a broad spectrum of targets or only a limited list of cardiac transcriptional factors? Recent studies showed that lncRNAs, such as RepA and Hotair, are required for the recruitment of PRC2 complex to a specific genomic locus and further execute its function in epigenetic regulation.\(^{25}\)\(^{–}\)\(^{26}\) It will be interesting to know whether Bvht will act in a similar manner. If so, to which chromatin locus does Bvht recruit PRC2?

Furthermore, Bvht-regulated cardiac transcription factor genes presented in this study are located in multiple genomic loci, indicating that different molecular mechanisms may exist underlying Bvht-mediated epigenetic regulation.

Genome-wide studies indicated that human lncRNAs are less conserved than protein-coding genes, and an estimated 30% of human lncRNAs are primate specific.\(^{16}\) Klattenhoff et al\(^{17}\) reported that Bvht seemed to exist as a mouse-specific lncRNA. Direct sequence alignment did not identify mouse Bvht homologs in other species. Moreover, by applying RNA-Seq approach, the authors nicely documented the expression of Bvht in mouse ESCs and heart samples. However, the orthologous human and rat genomic regions were not actively transcribed. Lack of an apparent human Bvht homolog raises a question about how well the lessons we have learned from mouse Bvht will translate to human cardiovascular disease. Perhaps, an undiscovered functional Bvht homolog, transcribed from a different genomic locus, exists in the human genome.

In their study, the authors showed that Bvht transcript is expressed in several adult mouse tissues, such as brain, colon, heart, kidney, liver, muscle, spleen, and testes, with highest expression detected in the heart. It will be important to further characterize the spatiotemporal expression of Bvht in development and disease. Most importantly, it will be essential to define the in vivo function of Bvht in mice, using genetic loss-of-function approaches. Although Bvht was shown to be an important cardiac lncRNA, additional cardiac-expressed lncRNAs, in particular selectively expressed in cardiomyocytes, remain to be identified and studied. Cardiac transcription factors and miRNAs reprogram cardiac fibroblasts into cardiomyocytes in vitro and in vivo,\(^{27}\)\(^{–}\)\(^{30}\) raising the tantalizing possibility that reprogramming strategies may be used to enhance the limited native regenerative capacity of adult mammalian hearts.\(^{31}\)\(^{32}\) It will be interesting to study whether this lncRNA might also participate in cardiac regeneration or be used to stimulate cellular reprogramming to directly reprogram cardiac fibroblasts into cardiomyocytes. Clearly, the discovery of Bvht will significantly impact cardiovascular research field and link lncRNAs to human cardiovascular disease.

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References


Build A Braveheart: The Missing Linc (RNA)
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