A Breath of Fresh Air(n) in Molecular Cardiology

Airn Long Noncoding RNA Regulates Cardiomyocyte Resistance to Stress and Igf2bp2 Translation

Simona Greco, Fabio Martelli

Numerous human genome sequencing projects have clearly indicated that only ≈2% of the human genome is constituted by protein-coding genes and that >90% of the genome is actively transcribed. This apparent lack of efficiency of mammalian cells has been explained with the existence of classes of RNAs biochemically similar to mRNA, but functionally different because they do not act as a template for protein synthesis. LncRNAs (long noncoding RNAs) belong to one of these classes and their crucial role in both cardiac physiology and disease has been clearly demonstrated for some of them, such as CHAST (cardiac hypertrophy–associated transcript), MIAT (myocardial infarction-associated transcript), CHRF (cardiac hypertrophy–related factor), and BACE1-AS (beta-secretase 1-antisense).[1–4] The function of the vast majority of them remains, however, unknown.

Article, see p 1347

LncRNAs have been identified both in the nucleus and in the cytoplasm and can function through their sequence and structure. Indeed, by associating with DNA, RNA, and proteins, they can modify gene expression and activity, either transcriptionally or posttranscriptionally.

One of the functions of lncRNAs is to regulate genomic imprinting, that is, the monoallelic silencing of specific genes, based on the parent-of-origin of the allele. In mammals, during early gametogenesis, almost 1% of protein-coding genes are epigenetically marked so that their expression occurs in a parent of origin-specific manner.[5]

Genomic imprinting relies on *cis*-acting long-distance gene regulatory mechanisms that make use of chromatin insulators and lncRNAs.[5] There are several imprinted RNAs maternally or paternally expressed, and one of them is the paternally expressed *Airn* (antisense of Igf2r [insulin growth factor 2 receptor] nonprotein coding RNA, also known as *Air* or *IGF2RAS*) lncRNA (Figure).[6,7] *Airn* belongs to a gene cluster located on mouse chromosome 17 that includes both maternally expressed genes (*Igf2r*, *Slc22a2* [solute carrier family 22 member 2], and *Slc22a3* [solute carrier family 22 member 3]) and nonimprinted genes (*Slc22a1* [solute carrier family 22 member 1], *Mas* [proto-oncogene, G protein-coupled receptor], and *Plg* [plasminogen]). *Airn* is involved in the imprinting of the maternally expressed genes of the locus, and it is transcribed from the opposite strand of the *Igf2r* gene, overlapping its 5′ end.[8] Worth noting is that it is the *Airn* transcriptional overlap through the *Igf2r* promoter, and not its lncRNA products, that induces imprinted *Igf2r* silencing, likely via transcriptional interference.[8]

Most Airn transcripts avoid splicing, thus generating a large mature transcript located in the nucleus. However, spliced Airn isoforms also exist and are generally more stable and located in the cytoplasm[9]; moreover, *Airn* displays conservation during evolution. These facts suggest that, although Airn sequence is not important for *Igf2r* imprinting, Airn might be involved in other functions requiring a cytoplasmic localization and a defined structure.

Airn Dysregulation in Mouse Heart Failure and Its Involvement in Cardiomyocyte Apoptosis and Cell Migration

The article of Hosen et al[10] elegantly investigated the functions of both unspliced and spliced Airn isoforms in mouse heart failure tissues and in a cardiomyocyte cell line (HL-1). Indeed, a careful analysis of all different isoforms expressed should be the starting point of all studies on both coding and noncoding transcripts.

Starting from previous data showing that unspliced Airn is abundantly expressed also in the heart,[9] they found that both unspliced (Airn) and one of the spliced isoforms (Airn-001) were downregulated after 4 weeks of coronary artery occlusion in a mouse model of ischemic heart failure. This dysregulation was limited to the noninfarcted remote area, suggesting a potential involvement in cardiac remodeling. Both isoforms were highly expressed into the nucleus of HL-1 cardiomyocytes, but a fraction of Airn-001 was also detected in the cytoplasm. In keeping with previous findings from different experimental systems,[9] Airn-001 spliced isoform was more stable compared with the unspliced one, suggesting an independent function.

Prompted by these observations, Hosen et al[10] analyzed the function of Airn and Airn-001 in HL-1 cardiomyocytes. *Airn* silencing induced cell apoptosis, decreased cells resistance to chemical stress, and reduced their ability to migrate.

New *Airn* Function: the Binding of *Airn* to Igf2bp2 Regulates Protein Translation

By pull-down experiments, the authors demonstrated that both unspliced Airn and spliced Airn-001 showed preferential

The opinions expressed in this article are not necessarily those of the editors or of the American Heart Association.

From the Molecular Cardiology Laboratory, IRCCS-Policlinico San Donato, Milan, Italy.

Correspondence to Fabio Martelli, PhD, Molecular Cardiology Laboratory, IRCCS-Policlinico San Donato, Via Morandi, 30, 20097 San Donato Milanese, Milan, Italy. E-mail fabio.martelli@grupposandonato.it

(*Circ Res*. 2018;122:1321-1323.
DOI: 10.1161/CIRCRESAHA.118.312966.)

© 2018 American Heart Association, Inc.

***Circulation Research* is available at http://circres.ahajournals.org**
DOI: 10.1161/CIRCRESAHA.118.312966

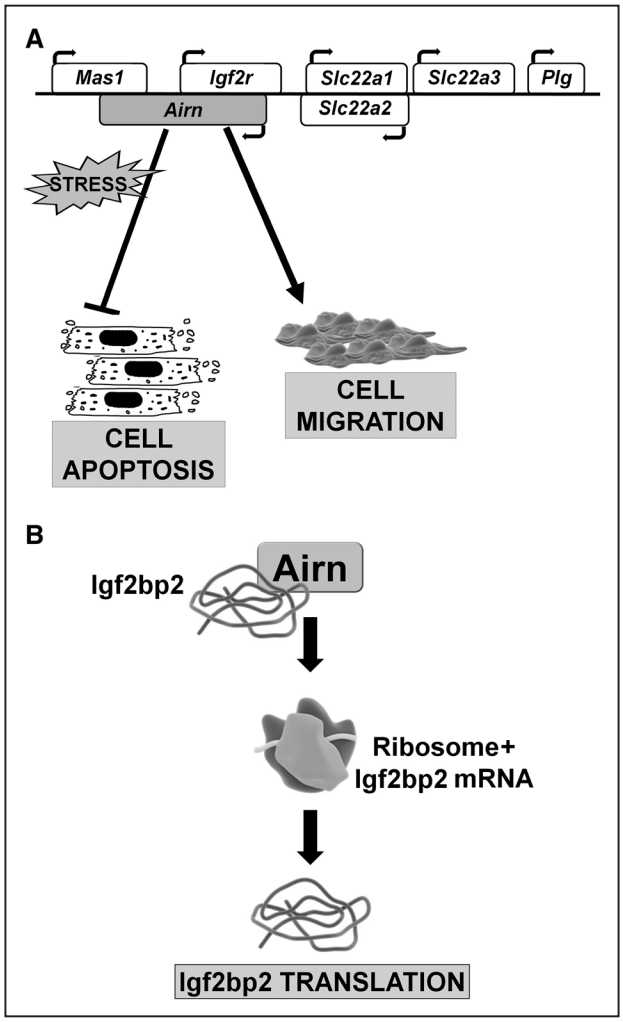

Figure. Airn (antisense of Igf2r nonprotein coding RNA) has other functions besides imprinting regulation. A, *Airn* belongs to the *Igf2r* gene cluster located on mouse chromosome 17. Airn controls cardiomyocyte cell apoptosis in response to stress *stimuli* and regulates cell migration. **B**, Airn binding of Igf2bp2 (insulin-like growth factor 2 mRNA binding protein 2) transcript controls the translation of Igf2bp2 itself and of a number of other genes.

binding to the protein Igf2bp2 (insulin-like growth factor 2 mRNA binding protein 2), which is important for glucose metabolism. Indeed, Igf2bp2 binds, along with many other mRNAs, the 5′ UTR (untranslated region) of IGF2 transcript regulating its translation. Intriguingly, human *AIRN* polymorphisms are associated with type 2 diabetes mellitus.[11] High throughput analysis showed that several mRNAs bound to Igf2bp2 protein. After *Airn* and *Igf2bp2* silencing, the levels of their mRNAs did not change, whereas the corresponding proteins decreased, indicating a new role for Airn as a protein translation regulator. Somewhat surprisingly, Airn silencing also reduced the levels of Igf2bp2 protein itself, which controls the translation efficiency of many mRNAs. The limited number of examples of lncRNAs regulating protein translation available to date (eg, AS Uchl1 [UCHL1 antisense RNA 1 (head to head)] and BC1 [brain cytoplasmic RNA 1])[12] suggests that this might be an uncommon function for lncRNAs.

AIRN's Potential Role in Human Cardiovascular Diseases

As often happens with groundbreaking studies, these findings prompt many more questions than they answer.

Most of the in vitro data of this first study were generated in HL-1 cells, a murine atrial-cardiomyocyte cell line. As all cell lines, HL-1 can proliferate indefinitely, in striking contrast to adult primary cardiomyocytes.

A crucial issue is understanding the link between Igf2bp2 translational-regulation by Airn, its importance for cell survival and motility, and the molecular mechanisms underpinning cardiac remodeling in failing hearts. Indeed, postischemic remodeling is characterized by both loss of cardiomyocytes[13] and extracellular matrix remodeling,[14] which further contribute to systolic dysfunction. Many efficacy studies in mouse models of ischemic and nonischemic heart failure as well as toxicology studies need to be performed to gain the necessary mechanistic insight. In this respect, mice displaying tissue-specific gain- and loss-of-function of *Airn* and *Igf2bp2* might provide important data.

Another critical issue is the role played by AIRN for human heart physiology and disease. AIRN has been identified in humans.[15] Hosen et al[10] showed that, as in mice, human AIRN is well expressed in the heart. The extent to which the findings of this study are applicable to humans is still unknown. To fully assess the clinical relevance of AIRN, its modulation needs to be assayed in patients affected by a variety of ischemic and nonischemic heart diseases.

In human embryonic kidney cells (HEK-293) *AIRN* silencing decreased cell resistance to apoptosis induced by oxidative stress, suggesting functional conservation. The regulation and function of AIRN should be investigated in more relevant in vitro models, such as human iPS (induced pluripotent stem cells)-derived cardiomyocytes.

Conclusions

In conclusion, data presented by Hosen et al,[10] although preliminary and requiring further mechanistic investigation, show that Airn plays an important role in cardiomyocyte regulation of translation, migration, and resistance to stress, thus implying that AIRN dysregulation in ischemic hearts might be involved in the development of cardiac dysfunction.

Sources of Funding

This study was supported by Ministero della Salute (Ricerca Corrente, RF-2011-02347907, and PE-2011-02348537).

Disclosures

None.

References

1. Viereck J, Thum T. Long noncoding RNAs in pathological cardiac remodeling. *Circ Res*. 2017;120:262–264. doi: 10.1161/CIRCRESAHA.116.310174.
2. Greco S, Zaccagnini G, Fuschi P, Voellenkle C, Carrara M, Sadeghi I, Bearzi C, Maimone B, Castelvecchio S, Stellos K, Gaetano C, Menicanti L, Martelli F. Increased BACE1-AS long noncoding RNA and β-amyloid levels in heart failure. *Cardiovasc Res*. 2017;113:453–463. doi: 10.1093/cvr/cvx013.
3. Kopp F, Mendell JT. Functional classification and experimental dissection of long noncoding RNAs. *Cell*. 2018;172:393–407. doi: 10.1016/j.cell.2018.01.011.

4. Greco S, Salgado Somoza A, Devaux Y, Martelli F. Long noncoding RNAs and cardiac disease [published online ahead of print August 30, 2017]. *Antioxid Redox Signal*. doi: 10.1089/ars.2017.7126. https://www.liebertpub.com/doi/10.1089/ars.2017.7126.
5. Kanduri C. Long noncoding RNAs: lessons from genomic imprinting. *Biochim Biophys Acta*. 2016;1859:102–111. doi: 10.1016/j.bbagrm.2015.05.006.
6. Sleutels F, Zwart R, Barlow DP. The non-coding Air RNA is required for silencing autosomal imprinted genes. *Nature*. 2002;415:810–813. doi: 10.1038/415810a.
7. Braidotti G, Baubec T, Pauler F, Seidl C, Smrzka O, Stricker S, Yotova I, Barlow DP. The Air noncoding RNA: an imprinted cis-silencing transcript. *Cold Spring Harb Symp Quant Biol*. 2004;69:55–66. doi: 10.1101/sqb.2004.69.55.
8. Latos PA, Pauler FM, Koerner MV, Şenergin HB, Hudson QJ, Stocsits RR, Allhoff W, Stricker SH, Klement RM, Warczok KE, Aumayr K, Pasierbek P, Barlow DP. Airn transcriptional overlap, but not its lncRNA products, induces imprinted Igf2r silencing. *Science*. 2012;338:1469–1472. doi: 10.1126/science.1228110.
9. Seidl CI, Stricker SH, Barlow DP. The imprinted Air ncRNA is an atypical RNAPII transcript that evades splicing and escapes nuclear export. *EMBO J*. 2006;25:3565–3575. doi: 10.1038/sj.emboj.7601245.
10. Hosen MR, Militello G, Weirick T, Ponomareva Y, Dassanayaka S, Moore JB, Döring C, Wysoczynski M, Jones SP, Dimmeler S, Uchida S. *Airn* regulates Igf2bp2 translation in cardiomyocytes. *Circ Res*. 2018;122:1347–1353. doi: 10.1161/CIRCRESAHA.117.312215.
11. Christiansen J, Kolte AM, Hansen Tv, Nielsen FC. IGF2 mRNA-binding protein 2: biological function and putative role in type 2 diabetes. *J Mol Endocrinol*. 2009;43:187–195. doi: 10.1677/JME-09-0016.
12. Yoon JH, Abdelmohsen K, Gorospe M. Posttranscriptional gene regulation by long noncoding RNA. *J Mol Biol*. 2013;425:3723–3730. doi: 10.1016/j.jmb.2012.11.024.
13. Moe GW, Marín-García J. Role of cell death in the progression of heart failure. *Heart Fail Rev*. 2016;21:157–167. doi: 10.1007/s10741-016-9532-0.
14. Frangogiannis NG. The extracellular matrix in myocardial injury, repair, and remodeling. *J Clin Invest*. 2017;127:1600–1612. doi: 10.1172/JCI87491.
15. Yotova IY, Vlatkovic IM, Pauler FM, Warczok KE, Ambros PF, Oshimura M, Theussl HC, Gessler M, Wagner EF, Barlow DP. Identification of the human homolog of the imprinted mouse Air non-coding RNA. *Genomics*. 2008;92:464–473. doi: 10.1016/j.ygeno.2008.08.004.

KEY WORDS: Editorials ■ apoptosis ■ cell line ■ heart failure ■ myocardial infarction