OAE
Open Access Publisher

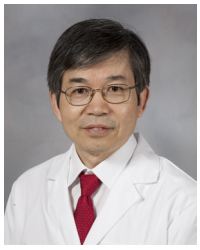
Review

Long non-coding RNAs as key regulators of cancer metastasis

Pratirodh Koirala[1], De-Hong Zou[2], Yin-Yuan Mo[1,3]

[1]*Department of Biochemistry and Cancer Institute, University of Mississippi Medical Center, Jackson, MS 39201, USA.*
[2]*Department of Breast Surgery, Zhejiang Cancer Hospital, Hangzhou 310022, China.*
[3]*Department of Pharmacology/Toxicology, University of Mississippi Medical Center, Jackson, MS 39201, USA.*

Correspondence to: Dr. Yin-Yuan Mo, Department of Pharmacology/Toxicology, University of Mississippi Medical Center, Jackson, MS 39201, USA. E-mail: ymo@umc.edu



Yin-Yuan Mo is Professor in the Department of Pharmacology/Toxicology, Cancer Institute, at University of Mississippi Medical Center. His research interests are epigenetic regulation of genes involved in tumorigenesis and chemoresistance, cancer susceptibility due to alterations of microRNA expression, and lncRNA-mediated gene expression in cancer.

ABSTRACT

The recent advances in functional genomics have discovered that a large number of long non-coding RNAs (lncRNAs) are pervasively transcribed from the human genome. Increasing evidence further indicates that lncRNAs are important for gene expression during cell differentiation and development through various mechanisms such as nuclear organization, post-transcription regulation, alternative splicing, and epigenetic regulation. Thus, aberrant expression of lncRNAs can cause abnormality in those cellular functions and lead to various pathological conditions. One of such fatal consequences is cancer metastasis which is responsible for more than 90% of cancer-related deaths. A good understanding of how lncRNAs regulate different genetic and epigenetic changes during different stages of cancer metastasis is important not only for general cancer biology but also for identification of novel biomarkers and therapeutic targets for treatment of metastatic cancer. A significant progress has been made regarding the role of lncRNAs in cancer for past several years. In this study, we first discuss general functions of lncRNAs and then highlight recent findings of how lncRNAs impact cancer metastasis, and finally we provide our perspectives on clinical implications of lncRNAs.

Key words: Cancer metastasis; epigenetics; gene regulation; long non-coding RNA

INTRODUCTION

It is well-known now that protein-coding genes account only about 2% of the human genome,[1] whereas the vast majority of the transcripts do not code for protein.[2] Although these non-coding RNAs were considered "transcriptional noise", their functions are increasingly valued for defi ning the cellular complexity of organisms. For instance, the number of protein-coding genes in humans is only a 2-fold more than that in worms such as *Caenorhabditis elegans* do,[1] implying that the protein alone is not sufficient to determine the complexity of organisms. Instead, this complexity may be achieved by efficient programming, which helps in handy expression and functioning of protein in a different context. The versatility and plasticity of non-coding RNAs help in such programming of protein function by regulating their expression and assembly in contextual cues.[3]

Non-coding RNAs include a broad category of RNA molecules. Some of them are constitutively expressed in the cells, and they may play a housekeeping role such as ribosomal RNA, transfer RNA, small nuclear RNA, and small nucleolar RNA (snoRNA). In contrast, other non-coding RNAs may be spatiotemporally expressed, and they often play a regulatory role.

Access this article online

Quick Response Code:

Website: www.jcmtjournal.com

DOI: 10.4103/2394-4722.171829

How to cite this article: Koirala P, Zou DH, Mo YY. Long non-coding RNAs as key regulators of cancer metastasis. J Cancer Metastasis Treat 2016;2:1-10.

Received: 06-08-2015; **Accepted:** 25-11-2015.

LncRNA-ATB

This lncRNA is activated by cytokine TGF-β (lncRNA-ATB) that is well-known for its role in tumor metastasis. TGF-β modulates different signaling pathways involved in EMT, migration, invasion, and metastasis.[66-68] A long time treatment of cells with TGF-β induces EMT (decreased E-cadherin and increased N-cadherin, vimentin, slug, twist1, ZEB-1 and ZEB-2). Similar treatment of hepatocellular carcinoma (HCC) cells with TGF-β activates the lncRNA-ATB in a time- and dose-dependent manner. Clinically, lncRNA-ATB level is high in HCC tumors as compared to adjacent normal tissue. Similarly, a high level of lncRNA-ATB is positively correlated with microvascular invasion and portal vein tumor thrombosis. Consistent with these observations, injection of HCC tumor cells overexpressing lncRNA-ATB into orthotropic mice promotes metastasis to different organs.[69] One of the possible mechanisms is through enhancement of EMT by interfering the action of miR-200 which can inhibit EMT by suppressing ZEB-1 and ZEB-2.[70] This 2.5 kb long lncRNA carries 6 binding sites for miR-200. Therefore, lncRNA-ATB traps miR-200 and prevents degradation of ZEB-1 and ZEB-2 by miR-200. The high level of ZEB-1 and ZEB-2 ultimately promotes EMT and invasiveness of different cells *in vitro* and *in vivo*. In addition, lncRNA-ATB enhances colonization of migrating cells by enhancing the function of IL-11-STAT3 signaling pathway. In this case, lncRNA-ATB binds to IL-11 mRNA and stabilizes it. The increased stability of IL-11 facilitates its secretion. As a ligand, IL-11 promotes phosphorylation of STAT3. This autocrine mitogenic signal helps in robust cell survival and effective colonization in distant organs.[69]

LncRNA-low expression in tumor

Low expression in tumor (LET) was originally identified in HCC cells.[71] Along with HCC, a reduced level of LET is also found in lung squamous carcinoma and colorectal cancer as compared to adjacent normal tissue. Overexpression of lncRNA-LET suppresses metastasis of HCC and colon cancer cells *in vivo*.[72] LncRNA-LET could limit HCC metastasis in both hypoxic and normoxic condition by different mechanisms. In hypoxic condition, lncRNA-LET interferes with the function of hypoxia-inducible factor-1α (HIF-1α), a transcription factor that regulates a number of genes under tumor hypoxia, and promotes angiogenesis and metastasis.[73] The high expression of lncRNA-LET suppresses HIF-1α through inhibiting NF90 which is required for accumulation of HIF-1α mRNA. However, hypoxia keeps the level of lncRNA-LET low by deacetylating its promotor. As a result, HIF-1α is increased promoting metastasis. In normoxic condition, lncRNA-LET inhibits expression of CDC42 (which is required for trans-endothelial migration) of circulating tumor cells. The low level of lncRNA-LET in HCC keeps CDC42 high and this results in profound metastasis of HCC.[72]

Colon cancer-associated transcript 1

Colon cancer-associated transcript 1 (CCAT1) was found up-regulated in colon cancer tissue, circulating blood cells of colon cancer patient and metastasis cases, indicating its role in colon cancer progression.[74] Besides, high expression of CCAT1 is also associated with primary tumor tissue, lymph node metastasis, and metastatic cases of gastric carcinoma.[75] The elevated level of CCAT1 reduces the survival of HCC patients. In both gastric cancer and HCC cell lines, overexpression of CCAT1 enhances the proliferation and migration of cells driven by c-Myc, an oncogenic transcription factor required for cell survival. On one hand, c-Myc binds to promoter of the CCAT1 and up-regulates its level in cancer cells.[75] On the other hand, CCAT1 prevents degradation of c-Myc by interaction with let-7, a known miRNA that can target c-Myc through its 3'-UTR.[76]

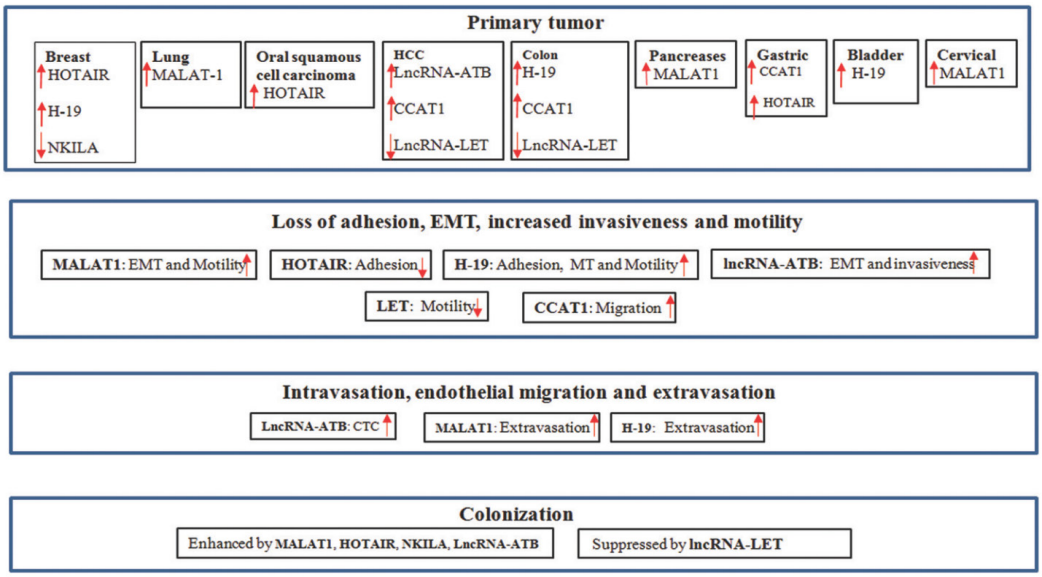


Figure 2: Long non-coding RNAs involved in different stages of cancer metastasis

DEREGULATION OF LNCRNA EXPRESSION IN CANCER

It is well-known that most of lncRNAs are transcribed through RNA polymerase II, just like protein-coding genes; they are also spliced products via canonical genomic splice site motifs, frequently ended with a poly A tail. Importantly, lncRNAs are often regulated by well-established transcription factors and are expressed in a tissue-specific manner.[77] For example, we have shown that wild-type p53 can transcriptionally induce linc-RoR and loc285194, but mutant p53 cannot.[17,24] On the other hand, c-Myc, as an oncogene, can regulate a group of lncRNAs.[78] In cancer, c-Myc is often amplified or up-regulated, which may explain why some lncRNAs are often deregulated.

CONCLUSION AND PERSPECTIVE

A tremendous progress has been made in our understanding of the genes and events involved in metastasis in recent years. Moreover, emerging evidence indicates that lncRNAs have also joined this complex regulatory network and may serve as very important regulators at different stages of metastasis (e.g. EMT, invasion, migration, and colonization) often through their expression levels [Figure 2]. However, overall, lncRNA research in this field is still at the infancy stage. Given the complex interactions of lncRNAs with DNA, RNA, and protein, a systematic approach may be needed to better understand the molecular mechanism of lncRNA-mediated metastasis. With the development of advanced technology such as CRISPR/Cas9, it is now feasible to perform knockout or knockin experiments and these research tools will no doubt speed up new discovery. In this system, nuclease Cas9 assisted by a sequence-specific guild RNA (gRNA) which is functionally similar to RNAi, cuts targeted DNA sequence.[79] Once the double strand break is made, the cell employs one of two major DNA repair mechanisms, non-homologous end joining (NHEJ), and homologous recombination (HR). Unlike HR, the NHEJ mechanism often leads to deletions or insertions, and thus it is an error-prone repair, a feature important for knockout. The HR mechanism would allow for introducing mutations or correcting a mutant sequence by knockin. Increasing evidence indicates that this technology has a potential to transform the field of cancer genetics such as the development of next-generation models of human cancer.[80]

Given the nuclear localization nature for a number of lncRNAs, genetic manipulations at the DNA level provides a better alternative to RNAi approach which mainly works through RISC complex in the cytoplasm. Our recent study indicates that a dual gRNA/Cas9 system combined with donor vector for HR can greatly improve the efficiency of obtaining complete lncRNA knockouts in various cancer cell lines.[81] As this field advances, we anticipate that more lncRNAs will be identified to be important players in cancer metastasis. More importantly, further characterization of this regulatory system will reveal many of detailed mechanisms. As a result, these studies will help develop novel strategies for cancer treatment. Furthermore, lncRNAs may serve as biomarkers for diagnosis/prognosis as supported by profiling studies of clinical specimens. Finally, given their important role in metastasis, lncRNAs may also prove to be valuable targets for cancer therapy. In particular, ribonucleoprotein complexes through lncRNAs are critical to lncRNA-mediated metastasis, drugs that block or enhance such interactions may have a bright future.

Financial support and sponsorship

This work was supported by NIH grant R01 CA154989 (YM).

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Lander ES, Linton LM, Birren B, Nusbaum C, Zody MC, Baldwin J, Devon K, Dewar K, Doyle M, FitzHugh W, Funke R, Gage D, Harris K, Heaford A, Howland J, Kann L, Lehoczky J, LeVine R, McEwan P, McKernan K, Meldrim J, Mesirov JP, Miranda C, Morris W, Naylor J, Raymond C, Rosetti M, Santos R, Sheridan A, Sougnez C, Stange-Thomann N, Stojanovic N, Subramanian A, Wyman D, Rogers J, Sulston J, Ainscough R, Beck S, Bentley D, Burton J, Clee C, Carter N, Coulson A, Deadman R, Deloukas P, Dunham A, Dunham I, Durbin R, French L, Grafham D, Gregory S, Hubbard T, Humphray S, Hunt A, Jones M, Lloyd C, McMurray A, Matthews L, Mercer S, Milne S, Mullikin JC, Mungall A, Plumb R, Ross M, Shownkeen R, Sims S, Waterston RH, Wilson RK, Hillier LW, McPherson JD, Marra MA, Mardis ER, Fulton LA, Chinwalla AT, Pepin KH, Gish WR, Chissoe SL, Wendl MC, Delehaunty KD, Miner TL, Delehaunty A, Kramer JB, Cook LL, Fulton RS, Johnson DL, Minx PJ, Clifton SW, Hawkins T, Branscomb E, Predki P, Richardson P, Wenning S, Slezak T, Doggett N, Cheng JF, Olsen A, Lucas S, Elkin C, Uberbacher E, Frazier M, Gibbs RA, Muzny DM, Scherer SE, Bouck JB, Sodergren EJ, Worley KC, Rives CM, Gorrell JH, Metzker ML, Naylor SL, Kucherlapati RS, Nelson DL, Weinstock GM, Sakaki Y, Fujiyama A, Hattori M, Yada T, Toyoda A, Itoh T, Kawagoe C, Watanabe H, Totoki Y, Taylor T, Weissenbach J, Heilig R, Saurin W, Artiguenave F, Brottier P, Bruls T, Pelletier E, Robert C, Wincker P, Smith DR, Doucette-Stamm L, Rubenfield M, Weinstock K, Lee HM, Dubois J, Rosenthal A, Platzer M, Nyakatura G, Taudien S, Rump A, Yang H, Yu J, Wang J, Huang G, Gu J, Hood L, Rowen L, Madan A, Qin S, Davis RW, Federspiel NA, Abola AP, Proctor MJ, Myers RM, Schmutz J, Dickson M, Grimwood J, Cox DR, Olson MV, Kaul R, Raymond C, Shimizu N, Kawasaki K, Minoshima S, Evans GA, Athanasiou M, Schultz R, Roe BA, Chen F, Pan H, Ramser J, Lehrach H, Reinhardt R, McCombie WR, de la Bastide M, Dedhia N, Blocker H, Hornischer K, Nordsiek G, Agarwala R, Aravind L, Bailey JA, Bateman A, Batzoglou S, Birney E, Bork P, Brown DG, Burge CB, Cerutti L, Chen HC, Church D, Clamp M, Copley RR, Doerks T, Eddy SR, Eichler EE, Furey TS, Galagan J, Gilbert JG, Harmon C, Hayashizaki Y, Haussler D, Hermjakob H, Hokamp K, Jang W, Johnson LS, Jones TA, Kasif S, Kaspryzk A, Kennedy S, Kent WJ, Kitts P, Koonin EV, Korf I, Kulp D, Lancet D, Lowe TM, McLysaght A, Mikkelsen T, Moran JV, Mulder N, Pollara VJ, Ponting CP, Schuler G, Schultz J, Slater G, Smit AF, Stupka E, Szustakowski J, Thierry-Mieg D, Thierry-Mieg

J, Wagner L, Wallis J, Wheeler R, Williams A, Wolf YI, Wolfe KH, Yang SP, Yeh RF, Collins F, Guyer MS, Peterson J, Felsenfeld A, Wetterstrand KA, Patrinos A, Morgan MJ, de Jong P, Catanese JJ, Osoegawa K, Shizuya H, Choi S, Chen YJ; International Human Genome Sequencing Consortium. Initial sequencing and analysis of the human genome. *Nature* 2001;409:860-921.

2. Bertone P, Stolc V, Royce TE, Rozowsky JS, Urban AE, Zhu X, Rinn JL, Tongprasit W, Samanta M, Weissman S, Gerstein M, Snyder M. Global identification of human transcribed sequences with genome tiling arrays. *Science* 2004;306:2242-6.
3. Mattick JS. Challenging the dogma: the hidden layer of non-protein-coding RNAs in complex organisms. *Bioessays* 2003;25:930-9.
4. Ponting CP, Oliver PL, Reik W. Evolution and functions of long noncoding RNAs. *Cell* 2009;136:629-41.
5. Carthew RW, Sontheimer EJ. Origins and mechanisms of miRNAs and siRNAs. *Cell* 2009;136:642-55.
6. Carninci P, Kasukawa T, Katayama S, Gough J, Frith MC, Maeda N, Oyama R, Ravasi T, Lenhard B, Wells C, Kodzius R, Shimokawa K, Bajic VB, Brenner SE, Batalov S, Forrest AR, Zavolan M, Davis MJ, Wilming LG, Aidinis V, Allen JE, Ambesi-Impiombato A, Apweiler R, Aturaliya RN, Bailey TL, Bansal M, Baxter L, Beisel KW, Bersano T, Bono H, Chalk AM, Chiu KP, Choudhary V, Christoffels A, Clutterbuck DR, Crowe ML, Dalla E, Dalrymple BP, de Bono B, Della Gatta G, di Bernardo D, Down T, Engstrom P, Fagiolini M, Faulkner G, Fletcher CF, Fukushima T, Furuno M, Futaki S, Gariboldi M, Georgii-Hemming P, Gingeras TR, Gojobori T, Green RE, Gustincich S, Harbers M, Hayashi Y, Hensch TK, Hirokawa N, Hill D, Huminiecki L, Iacono M, Ikeo K, Iwama A, Ishikawa T, Jakt M, Kanapin A, Katoh M, Kawasawa Y, Kelso J, Kitamura H, Kitano H, Kollias G, Krishnan SP, Kruger A, Kummerfeld SK, Kurochkin IV, Lareau LF, Lazarevic D, Lipovich L, Liu J, Liuni S, McWilliam S, Madan Babu M, Madera M, Marchionni L, Matsuda H, Matsuzawa S, Miki H, Mignone F, Miyake S, Morris K, Mottagui-Tabar S, Mulder N, Nakano N, Nakauchi H, Ng P, Nilsson R, Nishiguchi S, Nishikawa S, Nori F, Ohara O, Okazaki Y, Orlando V, Pang KC, Pavan WJ, Pavesi G, Pesole G, Petrovsky N, Piazza S, Reed J, Reid JF, Ring BZ, Ringwald M, Rost B, Ruan Y, Salzberg SL, Sandelin A, Schneider C, Schönbach C, Sekiguchi K, Semple CA, Seno S, Sessa L, Sheng Y, Shibata Y, Shimada H, Shimada K, Silva D, Sinclair B, Sperling S, Stupka E, Sugiura K, Sultana R, Takenaka Y, Taki K, Tammoja K, Tan SL, Tang S, Taylor MS, Tegner J, Teichmann SA, Ueda HR, van Nimwegen E, Verardo R, Wei CL, Yagi K, Yamanishi H, Zabarovsky E, Zhu S, Zimmer A, Hide W, Bult C, Grimmond SM, Teasdale RD, Liu ET, Brusic V, Quackenbush J, Wahlestedt C, Mattick JS, Hume DA, Kai C, Sasaki D, Tomaru Y, Fukuda S, Kanamori-Katayama M, Suzuki M, Aoki J, Arakawa T, Iida J, Imamura K, Itoh M, Kato T, Kawaji H, Kawagashira N, Kawashima T, Kojima M, Kondo S, Konno H, Nakano K, Ninomiya N, Nishio T, Okada M, Plessy C, Shibata K, Shiraki T, Suzuki S, Tagami M, Waki K, Watahiki A, Okamura-Oho Y, Suzuki H, Kawai J, Hayashizaki Y; FANTOM Consortium, RIKEN Genome Exploration Research Group and Genome Science Group (Genome Network Project Core Group). The transcriptional landscape of the mammalian genome. *Science* 2005;309:1559-63.
7. Harrow J, Frankish A, Gonzalez JM, Tapanari E, Diekhans M, Kokocinski F, Aken BL, Barrell D, Zadissa A, Searle S, Barnes I, Bignell A, Boychenko V, Hunt T, Kay M, Mukherjee G, Rajan J, Despacio-Reyes G, Saunders G, Steward C, Harte R, Lin M, Howald C, Tanzer A, Derrien T, Chrast J, Walters N, Balasubramanian S, Pei B, Tress M, Rodriguez JM, Ezkurdia I, van Baren J, Brent M, Haussler D, Kellis M, Valencia A, Reymond A, Gerstein M, Guigo R, Hubbard TJ. GENCODE: the reference human genome annotation for the ENCODE project. *Genome Res* 2012;22:1760-74.
8. Sparmann A, van Lohuizen M. Polycomb silencers control cell fate, development and cancer. *Nat Rev Cancer* 2006;6:846-56.
9. Pasmant E, Laurendeau I, Heron D, Vidaud M, Vidaud D, Bieche I. Characterization of a germ-line deletion, including the entire INK4/ARF locus, in a melanoma-neural system tumor family: identification of ANRIL, an antisense noncoding RNA whose expression coclusters with ARF. *Cancer Res* 2007;67:3963-9.
10. Yap KL, Li S, Munoz-Cabello AM, Raguz S, Zeng L, Mujtaba S, Gil J, Walsh MJ, Zhou MM. Molecular interplay of the noncoding RNA ANRIL and methylated histone H3 lysine 27 by polycomb CBX7 in transcriptional silencing of INK4a. *Mol Cell* 2010;38:662-74.
11. Plath K, Fang J, Mlynarczyk-Evans SK, Cao R, Worringer KA, Wang H, de la Cruz CC, Otte AP, Panning B, Zhang Y. Role of histone H3 lysine 27 methylation in X inactivation. *Science* 2003;300:131-5.
12. Kim K, Choi J, Heo K, Kim H, Levens D, Kohno K, Johnson EM, Brock HW, An W. Isolation and characterization of a novel H1.2 complex that acts as a repressor of p53-mediated transcription. *J Biol Chem* 2008;283:9113-26.
13. Yoon JH, Abdelmohsen K, Srikantan S, Yang X, Martindale JL, De S, Huarte M, Zhan M, Becker KG, Gorospe M. LincRNA-p21 suppresses target mRNA translation. *Mol Cell* 2012;47:648-55.
14. Derrien T, Johnson R, Bussotti G, Tanzer A, Djebali S, Tilgner H, Guernec G, Martin D, Merkel A, Knowles DG, Lagarde J, Veeravalli L, Ruan X, Ruan Y, Lassmann T, Carninci P, Brown JB, Lipovich L, Gonzalez JM, Thomas M, Davis CA, Shiekhattar R, Gingeras TR, Hubbard TJ, Notredame C, Harrow J, Guigo R. The GENCODE v7 catalog of human long noncoding RNAs: analysis of their gene structure, evolution, and expression. *Genome Res* 2012;22:1775-89.
15. Rodriguez A, Griffiths-Jones S, Ashurst JL, Bradley A. Identification of mammalian microRNA host genes and transcription units. *Genome Res* 2004;14:1902-10.
16. Cai X, Cullen BR. The imprinted H19 noncoding RNA is a primary microRNA precursor. *RNA* 2007;13:313-6.
17. Liu Q, Huang J, Zhou N, Zhang Z, Zhang A, Lu Z, Wu F, Mo YY. LncRNA loc285194 is a p53-regulated tumor suppressor. *Nucleic Acids Res* 2013;41:4976-87.
18. Zhang Z, Zhu Z, Watabe K, Zhang X, Bai C, Xu M, Wu F, Mo YY. Negative regulation of lncRNA GAS5 by miR-21. *Cell Death Differ* 2013;20:1558-68.
19. Poliseno L, Salmena L, Zhang J, Carver B, Haveman WJ, Pandolfi PP. A coding-independent function of gene and pseudogene mRNAs regulates tumour biology. *Nature* 2010;465:1033-8.
20. Schneider C, King RM, Philipson L. Genes specifically expressed at growth arrest of mammalian cells. *Cell* 1988;54:787-93.
21. Kino T, Hurt DE, Ichijo T, Nader N, Chrousos GP. Noncoding RNA gas5 is a growth arrest- and starvation-associated repressor of the glucocorticoid receptor. *Sci Signal* 2010;3:ra8.
22. Good MC, Zalatan JG, Lim WA. Scaffold proteins: hubs for controlling the flow of cellular information. *Science* 2011;332:680-6.
23. Loewer S, Cabili MN, Guttman M, Loh YH, Thomas K, Park IH, Garber M, Curran M, Onder T, Agarwal S, Manos PD, Datta S, Lander ES, Schlaeger TM, Daley GQ, Rinn JL. Large intergenic non-coding RNA-RoR modulates reprogramming of human induced pluripotent stem cells. *Nat Genet* 2010;42:1113-7.
24. Zhang A, Zhou N, Huang J, Liu Q, Fukuda K, Ma D, Lu Z, Bai C, Watabe K, Mo YY. The human long non-coding RNA-RoR is a p53 repressor in response to DNA damage. *Cell Res* 2013;23:340-50.
25. Fox AH, Lam YW, Leung AK, Lyon CE, Andersen J, Mann M, Lamond AI. Paraspeckles: a novel nuclear domain. *Curr Biol* 2002;12:13-25.
26. Emili A, Shales M, McCracken S, Xie W, Tucker PW, Kobayashi R, Blencowe BJ, Ingles CJ. Splicing and transcription-associated

proteins PSF and p54nrb/nonO bind to the RNA polymerase II CTD. *RNA* 2002;8:1102-11.
27. Auboeuf D, Dowhan DH, Li X, Larkin K, Ko L, Berget SM, O'Malley BW. CoAA, a nuclear receptor coactivator protein at the interface of transcriptional coactivation and RNA splicing. *Mol Cell Biol* 2004;24:442-53.
28. Fox AH, Bond CS, Lamond AI. P54nrb forms a heterodimer with PSP1 that localizes to paraspeckles in an RNA-dependent manner. *Mol Biol Cell* 2005;16:5304-15.
29. Clemson CM, Hutchinson JN, Sara SA, Ensminger AW, Fox AH, Chess A, Lawrence JB. An architectural role for a nuclear noncoding RNA: NEAT1 RNA is essential for the structure of paraspeckles. *Mol Cell* 2009;33:717-26.
30. Morin GB. The human telomere terminal transferase enzyme is a ribonucleoprotein that synthesizes TTAGGG repeats. *Cell* 1989;59:521-9.
31. Greider CW, Blackburn EH. A telomeric sequence in the RNA of tetrahymena telomerase required for telomere repeat synthesis. *Nature* 1989;337:331-7.
32. Ly H, Blackburn EH, Parslow TG. Comprehensive structure-function analysis of the core domain of human telomerase RNA. *Mol Cell Biol* 2003;23:6849-56.
33. Vulliamy T, Marrone A, Dokal I, Mason PJ. Association between aplastic anaemia and mutations in telomerase RNA. *Lancet* 2002;359:2168-70.
34. Chaffer CL, Weinberg RA. A perspective on cancer cell metastasis. *Science* 2011;331:1559-64.
35. Oka H, Shiozaki H, Kobayashi K, Inoue M, Tahara H, Kobayashi T, Takatsuka Y, Matsuyoshi N, Hirano S, Takeichi M, Mori T. Expression of E-cadherin cell adhesion molecules in human breast cancer tissues and its relationship to metastasis. *Cancer Res* 1993;53:1696-701.
36. Schipper JH, Frixen UH, Behrens J, Unger A, Jahnke K, Birchmeier W. E-cadherin expression in squamous cell carcinomas of head and neck: inverse correlation with tumor dedifferentiation and lymph node metastasis. *Cancer Res* 1991;51:6328-37.
37. Werb Z. ECM and cell surface proteolysis: regulating cellular ecology. *Cell* 1997;91:439-42.
38. Cheng GZ, Chan J, Wang Q, Zhang W, Sun CD, Wang LH. Twist transcriptionally up-regulates AKT2 in breast cancer cells leading to increased migration, invasion, and resistance to paclitaxel. *Cancer Res* 2007;67:1979-87.
39. Chambers AF, Groom AC, MacDonald IC. Dissemination and growth of cancer cells in metastatic sites. *Nat Rev Cancer* 2002;2:563-72.
40. Nash GF, Turner LF, Scully MF, Kakkar AK. Platelets and cancer. *Lancet Oncol* 2002;3:425-30.
41. Wang H, Fu W, Im JH, Zhou Z, Santoro SA, Iyer V, DiPersio CM, Yu QC, Quaranta V, Al-Mehdi A, Muschel RJ. Tumor cell alpha3beta1 integrin and vascular laminin-5 mediate pulmonary arrest and metastasis. *J Cell Biol* 2004;164:935-41.
42. Gupta GP, Massague J. Cancer metastasis: building a framework. *Cell* 2006;127:679-95.
43. Aguirre-Ghiso JA. Models, mechanisms and clinical evidence for cancer dormancy. *Nat Rev Cancer* 2007;7:834-46.
44. Ji P, Diederichs S, Wang W, Boing S, Metzger R, Schneider PM, Tidow N, Brandt B, Buerger H, Bulk E, Thomas M, Berdel WE, Serve H, Muller-Tidow C. MALAT-1, a novel noncoding RNA, and thymosin beta4 predict metastasis and survival in early-stage non-small cell lung cancer. *Oncogene* 2003;22:8031-41.
45. Tripathi V, Ellis JD, Shen Z, Song DY, Pan Q, Watt AT, Freier SM, Bennett CF, Sharma A, Bubulya PA, Blencowe BJ, Prasanth SG, Prasanth KV. The nuclear-retained noncoding RNA MALAT1 regulates alternative splicing by modulating SR splicing factor phosphorylation. *Mol Cell* 2010;39:925-38.
46. Gutschner T, Hammerle M, Eissmann M, Hsu J, Kim Y, Hung G, Revenko A, Arun G, Stentrup M, Gross M, Zornig M, MacLeod AR, Spector DL, Diederichs S. The noncoding RNA MALAT1 is a critical regulator of the metastasis phenotype of lung cancer cells. *Cancer Res* 2013;73:1180-9.
47. Tano K, Mizuno R, Okada T, Rakwal R, Shibato J, Masuo Y, Ijiri K, Akimitsu N. MALAT-1 enhances cell motility of lung adenocarcinoma cells by influencing the expression of motility-related genes. *FEBS Lett* 2010;584:4575-80.
48. Jiang Y, Li Y, Fang S, Jiang B, Qin C, Xie P, Zhou G, Li G. The role of MALAT1 correlates with HPV in cervical cancer. *Oncol Lett* 2014;7:2135-41.
49. Jiao F, Hu H, Han T, Yuan C, Wang L, Jin Z, Guo Z, Wang L. Long noncoding RNA MALAT-1 enhances stem cell-like phenotypes in pancreatic cancer cells. *Int J Mol Sci* 2015;16:6677-93.
50. Gupta RA, Shah N, Wang KC, Kim J, Horlings HM, Wong DJ, Tsai MC, Hung T, Argani P, Rinn JL, Wang Y, Brzoska P, Kong B, Li R, West RB, van de Vijver MJ, Sukumar S, Chang HY. Long non-coding RNA HOTAIR reprograms chromatin state to promote cancer metastasis. *Nature* 2010;464:1071-6.
51. Tsai MC, Manor O, Wan Y, Mosammaparast N, Wang JK, Lan F, Shi Y, Segal E, Chang HY. Long noncoding RNA as modular scaffold of histone modification complexes. *Science* 2010;329:689-93.
52. Wu Y, Zhang L, Zhang L, Wang Y, Li H, Ren X, Wei F, Yu W, Liu T, Wang X, Zhou X, Yu J, Hao X. Long non-coding RNA HOTAIR promotes tumor cell invasion and metastasis by recruiting EZH2 and repressing E-cadherin in oral squamous cell carcinoma. *Int J Oncol* 2015;46:2586-94.
53. Niinuma T, Suzuki H, Nojima M, Nosho K, Yamamoto H, Takamaru H, Yamamoto E, Maruyama R, Nobuoka T, Miyazaki Y, Nishida T, Bamba T, Kanda T, Ajioka Y, Taguchi T, Okahara S, Takahashi H, Nishida Y, Hosokawa M, Hasegawa T, Tokino T, Hirata K, Imai K, Toyota M, Shinomura Y. Upregulation of miR-196a and HOTAIR drive malignant character in gastrointestinal stromal tumors. *Cancer Res* 2012;72:1126-36.
54. Pachnis V, Belayew A, Tilghman SM. Locus unlinked to alpha-fetoprotein under the control of the murine raf and rif genes. *Proc Natl Acad Sci U S A* 1984;81:5523-7.
55. Brannan CI, Dees EC, Ingram RS, Tilghman SM. The product of the H19 gene may function as an RNA. *Mol Cell Biol* 1990;10:28-36.
56. Hao Y, Crenshaw T, Moulton T, Newcomb E, Tycko B. Tumour-suppressor activity of H19 RNA. *Nature* 1993;365:764-7.
57. Hibi K, Nakamura H, Hirai A, Fujikake Y, Kasai Y, Akiyama S, Ito K, Takagi H. Loss of H19 imprinting in esophageal cancer. *Cancer Res* 1996;56:480-2.
58. Ariel I, Miao HQ, Ji XR, Schneider T, Roll D, de Groot N, Hochberg A, Ayesh S. Imprinted H19 oncofetal RNA is a candidate tumour marker for hepatocellular carcinoma. *Mol Pathol* 1998;51:21-5.
59. Ayesh S, Matouk I, Schneider T, Ohana P, Laster M, Al-Sharef W, De-Groot N, Hochberg A. Possible physiological role of H19 RNA. *Mol Carcinog* 2002;35:63-74.
60. Luo M, Li Z, Wang W, Zeng Y, Liu Z, Qiu J. Long non-coding RNA H19 increases bladder cancer metastasis by associating with EZH2 and inhibiting E-cadherin expression. *Cancer Lett* 2013;333:213-21.
61. Adriaenssens E, Lottin S, Berteaux N, Hornez L, Fauquette W, Fafeur V, Peyrat JP, Le Bourhis X, Hondermarck H, Coll J, Dugimont T, Curgy JJ. Cross-talk between mesenchyme and epithelium increases H19 gene expression during scattering and morphogenesis of epithelial cells. *Exp Cell Res* 2002;275:215-29.
62. Liang WC, Fu WM, Wong CW, Wang Y, Wang WM, Hu GX, Zhang L, Xiao LJ, Wan DC, Zhang JF, Waye MM. The lncRNA H19 promotes epithelial to mesenchymal transition by functioning as miRNA sponges in colorectal cancer. *Oncotarget* 2015;6:22513-25.
63. Liu B, Sun L, Liu Q, Gong C, Yao Y, Lv X, Lin L, Yao H, Su F, Li

D, Zeng M, Song E. A cytoplasmic NF-kappaB interacting long noncoding RNA blocks IkappaB phosphorylation and suppresses breast cancer metastasis. *Cancer Cell* 2015;27:370-81.
64. Chaturvedi MM, Sung B, Yadav VR, Kannappan R, Aggarwal BB. NF-kappaB addiction and its role in cancer: 'one size does not fit all'. *Oncogene* 2011;30:1615-30.
65. Ghosh S, Karin M. Missing pieces in the NF-kappaB puzzle. *Cell* 2002;109 Suppl: S81-96.
66. Thiery JP, Acloque H, Huang RY, Nieto MA. Epithelial-mesenchymal transitions in development and disease. *Cell* 2009;139:871-90.
67. Akhurst RJ, Hata A. Targeting the TGFbeta signalling pathway in disease. *Nat Rev Drug Discov* 2012;11:790-811.
68. Pardali K, Moustakas A. Actions of TGF-beta as tumor suppressor and pro-metastatic factor in human cancer. *Biochim Biophys Acta* 2007;1775:21-62.
69. Yuan JH, Yang F, Wang F, Ma JZ, Guo YJ, Tao QF, Liu F, Pan W, Wang TT, Zhou CC, Wang SB, Wang YZ, Yang Y, Yang N, Zhou WP, Yang GS, Sun SH. A long noncoding RNA activated by TGF-beta promotes the invasion-metastasis cascade in hepatocellular carcinoma. *Cancer Cell* 2014;25:666-81.
70. Burk U, Schubert J, Wellner U, Schmalhofer O, Vincan E, Spaderna S, Brabletz T. A reciprocal repression between ZEB1 and members of the miR-200 family promotes EMT and invasion in cancer cells. *EMBO Rep* 2008;9:582-9.
71. Yang F, Zhang L, Huo XS, Yuan JH, Xu D, Yuan SX, Zhu N, Zhou WP, Yang GS, Wang YZ, Shang JL, Gao CF, Zhang FR, Wang F, Sun SH. Long noncoding RNA high expression in hepatocellular carcinoma facilitates tumor growth through enhancer of zeste homolog 2 in humans. *Hepatology* 2011;54:1679-89.
72. Yang F, Huo XS, Yuan SX, Zhang L, Zhou WP, Wang F, Sun SH. Repression of the long noncoding RNA-LET by histone deacetylase 3 contributes to hypoxia-mediated metastasis. *Mol Cell* 2013;49:1083-96.
73. Peinado H, Olmeda D, Cano A. Snail, Zeb and bHLH factors in tumour progression: an alliance against the epithelial phenotype? *Nat Rev Cancer* 2007;7:415-28.
74. Nissan A, Stojadinovic A, Mitrani-Rosenbaum S, Halle D, Grinbaum R, Roistacher M, Bochem A, Dayanc BE, Ritter G, Gomceli I, Bostanci EB, Akoglu M, Chen YT, Old LJ, Gure AO. Colon cancer associated transcript-1: a novel RNA expressed in malignant and pre-malignant human tissues. *Int J Cancer* 2012;130:1598-606.
75. Yang F, Xue X, Bi J, Zheng L, Zhi K, Gu Y, Fang G. Long noncoding RNA CCAT1, which could be activated by c-Myc, promotes the progression of gastric carcinoma. *J Cancer Res Clin Oncol* 2013;139:437-45.
76. Deng L, Yang SB, Xu FF, Zhang JH. Long noncoding RNA CCAT1 promotes hepatocellular carcinoma progression by functioning as let-7 sponge. *J Exp Clin Cancer Res* 2015;34:18.
77. Prensner JR, Chinnaiyan AM. The emergence of lncRNAs in cancer biology. *Cancer Discov* 2011;1:391-407.
78. Winkle M, van den Berg A, Tayari M, Sietzema J, Terpstra M, Kortman G, de Jong D, Visser L, Diepstra A, Kok K, Kluiver J. Long noncoding RNAs as a novel component of the Myc transcriptional network. *FASEB J* 2015;29:2338-46.
79. Jinek M, Chylinski K, Fonfara I, Hauer M, Doudna JA, Charpentier E. A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. *Science* 2012;337:816-21.
80. Sanchez-Rivera FJ, Jacks T. Applications of the CRISPR-Cas9 system in cancer biology. *Nat Rev Cancer* 2015;15:387-95.
81. Ho TT, Zhou N, Huang J, Koirala P, Xu M, Fung R, Wu F, Mo YY. Targeting non-coding RNAs with the CRISPR/Cas9 system in human cell lines. *Nucleic Acids Res* 2015;43:e17.