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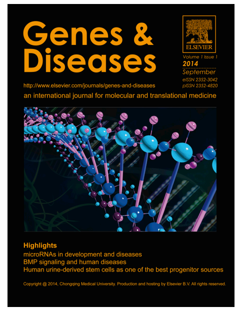


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# Gene fusions and chimeric RNAs, and their implications in cancer

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## Abstract

**Gene fusions are appreciated as ideal cancer biomarkers and therapeutic targets. Chimeric RNAs are traditionally thought to be products of gene fusions, and thus, also cancer-specific. Recent research has demonstrated that chimeric RNAs can be generated by intergenic splicing in the absence of gene fusion, and such chimeric RNAs are also found in normal physiology. These new findings challenge the traditional theory of chimeric RNAs exclusivity to cancer, and complicates use of chimeric RNAs in cancer detection. Here, we provide an overview of gene fusions and chimeric RNAs, and emphasize their differences. We note that gene fusions are able to generate chimeric RNAs in accordance with the central dogma of biology, and that chimeric RNAs may also be able to influence the generation of the gene fusions per the “horse before the cart” hypothesis. We further expand upon the “horse before the cart” hypothesis, summarizing current evidence in support of the theory and exploring its potential impact on the field.**

**Key words: gene fusion; chimeric RNA: chromosomal rearrangement; intergenic splicing; trans-splicing; cis-splicing between adjacent genes**

## Introduction

Gene fusions are hybrid genes resulting from the fusion of two previously separate genes. Gene fusions are formed by chromosomal rearrangement including translocation, inversion, deletion or tandem duplication. Interestingly, gene fusions are characteristic cytogenetic signatures of many cancer types, and have been successfully used as diagnostic tools. Often, gene fusions give rise to gene fusion transcripts and chimeric protein products, which have been used as targets for treatment. The well known examples are Gleevec (Imantinib) targeting *BCR-ABL1* gene fusion and crizotinib targeting *EML4-ALK* gene fusion[1,2].

While the term “gene fusion” refers to DNA-level fusion events, “chimeric RNA” refers to any transcript composed of exons from different parental genes, including gene fusion transcripts. Contrary to popular belief, transcription of gene fusions is not the only means of chimeric RNA generation. Chimeric RNAs can also arise from trans-splicing of two separate precursor mRNAs and alternative splicing of a readthrough transcript, otherwise known as cis-splicing of adjacent genes (cis-SAGe)[3].

Interestingly, several studies indicate the presence of chimeric RNAs which exactly mimic common gene fusion transcripts in the absence of the corresponding gene fusion. Such observations have spurred “the cart before the horse” hypothesis, in which chimeric RNA can be generated first by trans-splicing and then guide genome rearrangement to form the corresponding gene fusion[4].

Within this manuscript, we discuss known generation mechanisms for gene fusions as well as their relevance to cancer. Further, we present existing evidence in support of “the cart before the horse hypothesis” and discuss the implications and significance of these findings on our understanding of oncogenesis.

**Mechanisms of Chimeric RNAs Generation**

Transcription of gene fusions is a classic, well-studied mechanism of generating chimeric RNA. Rearrangements such as translocation, inversion, deletion, and tandem duplication have all been shown to produce gene fusions then transcribe to corresponding chimeric RNAs. The first renowned gene fusion, *BCR-ABL*, was discovered in human chronic myelogenous leukemia (CML). It is the result of translocation between the q arms of chromosomes 9 and 22 t(9;22)[5], and its chimeric transcript encodes for a fusion protein which is an altered, constitutively active ABL1 kinase. Another gene fusion, *DNAJB1-PRKACA* is the result of chromosome segmental deletion. It is now recognized as a biomarker of fibrolamellar hepatocellular carcinoma (FL-HCC), and introducing this deletion via CRISPR/CAS9 to adult mouse liver can successfully generate the *Dnajb1–Prkaca* gene fusion and effectively induce tumors resembling fibrolamellar hepatocellular carcinoma[6,7]. Additionally, the *FGFR3-TACC3* gene fusion in human glioblastoma arises via tandem duplication and insertion [8]. The fusion transcript encoding a protein, which has constitutive kinase activity and induces mitotic and chromosome separation defects then triggers aneuploidy[8]. Subsequent studies reported this gene fusion in many other cancers, indicating that *FGFR3-TACC3* is a recurring gene fusion in cancer[9]. *FGFR3-TACC3* is a potent oncogene that promotes the phosphorylation of PIN4, inducing mitochondrial respiration and promoting tumor growth[10].

Another mechanism for chimeric RNA generation is cis-SAGe, in which two neighboring genes are transcribed into one precursor RNA by transcriptional readthrough, followed by RNA splicing between exons from the two neighboring genes[11]. The cis-SAGe product *SLC45A3-ELK4* was discovered by two independent groups[12,13] and has the potential to be a biomarker in prostate cancer[11,14]. Knockdown of *SLC45A3-ELK4* in cancer cells causes reduction in cell proliferation[15]. Another example of a cis-SAGe chimeric RNA is *DUS4L-BCAP29*, which was discovered in gastric and prostate cancer and plays a tumor-promoting role in gastric cancer[16,17]. However, our group found that *DUS4L-BCAP29 also* exists in a variety of normal tissues, and its growth promoting effect is not only found in cancer but also in normal physiology[18].

A third category of chimeric RNAs arise from trans-splicing, in which exons from different RNA transcripts are spliced together[19]. While there is limited direct evidence of chimeric RNAs arising from two separate precursor mRNAs, many chimeric RNAs have been detected with concurrent evidence of no corresponding genomic rearrangement and no readthrough transcription between its parental genes. The best example of a trans-spliced chimeric RNA is *JAZF1-JJAZ1 (SUZ12)*, as described by Li et al. in 2008. RNA trans-splicing assay was used to prove that the mechanism of this chimeric RNA in normal tissue is trans-splicing between precursor messenger RNAs for *JAZF1* and *JJAZ1*[20]. Other examples of trans-spliced chimeras are *TMEM79-SMG5* and *CYCLIN D1-TROP2*. While there is no direct evidence of trans-splicing for either transcript, these chimeric RNAs have been found in cells without evidence of corresponding genomic rearrangement. Further, the orientation of the parental genes is not conducive to readthrough transcription. The *TMEM79* gene and *SMG5* are located on opposite strands in opposing orientations within the 1q22 locus, whereas *CYCLIN D1* and *TROP2* are located on entirely different chromosomes. Interestingly, *TMEM79-SMG5* is highly differentially expressed in human prostate cancer samples[21], and *CYCLIN D1-TROP2* is able to transform naïve, primary cells in vitro and induce aggressive tumor growth in vivo in cooperation with activated RAS[22].

**Detection of gene fusions and chimeric RNAs**

The first gene fusion was discovered by chromosome banding techniques. Additional techniques that can be used to detect gene fusions include Fluorescence In Situ Hybridization (FISH), Southern blotting, Comparative Genome Hybridization (CGH), PCR, and whole genome sequencing, which are based on chromosomal rearrangement. For the gene fusions that result in the formation of chimeric RNAs, RNA based assays can be used as surrogates. However, chimeric RNAs generated by trans-splicing or cis-SAGe cannot be detected by the DNA based assays, as they are produced in the absence of chromosomal rearrangement. Technologies used to detecting these chimeric RNAs are RT-PCR, Northern blotting, RNAse protection assay, and RNA sequencing. The development of microarray technology and Next-generation sequencing has lead to the discovery of a large number of gene fusions and chimeric RNAs. Thousands of chimeric RNAs and gene fusions are now deposited into several databases including Mitelman[23], FusionGDB[24], ChimerDB[25], FusionCancer[26] and ChiTaRs[27].

**The Cart: chimeric RNA which precede gene fusion**

Remarkably, several chimeric RNAs found in healthy cells exactly mimic oncogenic fusion transcripts in the absence of the corresponding rearrangement. Trans-spliced *JAZF1-JJAZ1* is a premier example of this phenomenon. The *JAZF1-JJAZ1* gene fusion is generated by the t(7;17)(p15;q21) translocation and is found in approximately 50% of human endometrial stromal sarcomas (ESSs)[28,29]. The translocation unites the first 3 exons of *JAZF1* to the last 15 exons of *JJAZ1*, and gives rise to a chimeric transcript, which is translated into chimeric protein. Forced overexpression of *JAZF1-JJAZ1* in HEK 293 cells confers resistance to apoptosis and promotes cell proliferation when cooperating with suppression of the unrearranged *JJAZ1* allele[29]. Li et al. detected the identical *JAZF1-JJAZ1* chimeric RNA and protein in normal endometrial stromal cells and proved absence of translocation between chromosome 7 and chromosome 17 in normal endometrial stromal cells[20]. This study provided new insight into the relationship between chimeric RNA and gene fusion: the chimeric RNA normally generated by trans-splicing in developing tissue could potentially lead to genome rearrangement, thus generating the corresponding gene fusion through an unknown mechanism[4].

Another “cart” is chimeric *PAX3-FOXO1*. The *PAX3-FOXO1* gene fusion arises from a t(2;13)(q35;q14) translocation exclusively expressed in alveolar rhabdomyosarcoma (ARMS). This rearrangement joins the DNA binding domain of *PAX3* to the transactivation domain of *FOXO1*, creating a new transcription factor[30]. Yuan *et al.* found that the chimeric *PAX3-FOXO1* transcript identical to the gene fusion transcript in ARMS was transiently expressed in pluripotent cells differentiating into skeletal muscle without the t(2;13) (q35;q14) translocation. Forced overexpression of *PAX3-FOXO1* led to continuous expression of *MYOD* and *MYOG* which were also overexpressed in rhabdomyosarcoma cells[31], whereas silencing the fusion led to failure of MYOD and MYOG expression. These findings support the idea that transiently expressed *PAX3-FOXO1* by trans-splicing plays an important role in myogenesis. The generation of the *PAX3-FOXO1* gene fusion results in constitutive expression of the chimeric transcript and resulting chimeric transcription factor, which establishes super enhancers directly to drive itself, MYOD1, and MYCN, and indirectly to drive MYOG, thus arresting cells in a premature myogenesis stage[32]. Of note, *PAX3-FOXO1* expression is not enough to cause transformation alone[33,34], but promotes tumorigenesis in conjunction with inactivation of *CDKN2A* and overexpression of *TERT* and *MYCN*[35].

These examples each support the claim that chimeric RNAs serving a developmental or cell/tissue specific role in normal cells without chromosomal rearrangement may potentially mediate gene fusion in the same-lineage cancer cells.

**RNA-mediated genome rearrangement**

RNA-mediating genome rearrangement is not a new concept in biology. In fact, RNA-induced genome rearrangement is a common feature of ciliates due to nuclear dimorphism[36,37]. In the ciliate *Oxytricha*, scientists have shown that maternal RNA templates can guide DNA assembly, and disruption of these specific RNAs disables the corresponding gene assembly. Further, injection of synthetic RNA templates into *Oxytricia* can mediate targeted genome rearrangement[38].

RNA-mediated genetic change is not exclusively to ciliates. Shen *et al.* used RNA-containing oligos as templates to repair a Double-Strand Break (DSB) in human cells and introduce base changes in genomic DNA.[39], demonstrating that RNA sequences can have a direct role in DNA genetic modification and remodeling.

A recently published study offers some direct evidence that chimeric RNAs may facilitate gene fusion. Sachin *et al.* showed that forced expression of a chimeric RNA can lead to genome rearrangement, resulting in generation of the corresponding gene fusion in mammalian cells[40]. The authors used the *TMPRSS2-ERG* and *TMPRSS2-ETS* fusions, common to prostate cancer, as their models for the study. These gene fusions are particularly interesting, as their parental genes are separated by considerable genomic distance, but move into close three-dimensional proximity in response to androgen stimulation. The *TMPRSS2-ERG* fusion was induced only in samples treated with dihydrotestosterone (DHT, a metabolite of testosterone) and expressing chimeric RNA templates spanning the canonical junction site. The authors proposed a potential mechanism in which the chimeric RNA template forms an imperfect stem with the sense TMPRSS2 and ERG or EVT1 genomic sequence, forming a three-way junction in a sequence-specific manner that brings the canonical breakpoints for the fusion into proximity. Surprisingly, the fusion was preferentially induced when expressing the antisense *TMPRSS2-ERG* template, potentially indicating that active transcription can prevent this junction from forming on the antisense DNA strand. In support of this hypothesis, RNA polymerase-II inhibition via α-amanitin successfully allowed for gene fusion generation by sense chimeric RNAs [36] (Figure 1a). Whether these structures can form from endogenous chimeric RNA awaits further investigation.

Another study into RNA-DNA interactions proposed the RNA-Poise model as an explanation for the cart-before-the-horse phenomenon. This model emphasizes the importance of three-dimensional proximity in generating chimeric transcripts without rearrangement. In the first subtype of the model, or the RNA Targeting model, the transcripts of gene 1 preinstall onto gene 2's genomic sequence, thus allowing for the spatial proximity of transcripts of the two genes, and trans-splicing between transcripts. The second subtype of the RNA-Poise model, the RNA Confinement model, dictates that spatial proximity brings the nascent transcripts of gene 1 close to the genomic sequence of gene 2. In both cases, proximity of the genomic regions increases the chance of translocation (Figure 1b). Additionally, this study also observed a singular lung cancer sample expressing the *EML4-ALK* chimeric RNA without harboring the corresponding *EML4-ALK* gene fusion. This is especially important, as the authors have potentially captured both the "before" and "after" states within the same study[41].

**Chimeric RNAs and gene fusions as cancer diagnostic biomarkers and treatment targets**

The tumor-specificity of gene fusions makes them ideal biomarkers for cancer. BCR-*ABL1,* has been widely utilized as a biomarker and prognosis factor in acute lymphoblastic leukemia (ALL) patients[42], and its specific inhibitor Gleevec (Imantinib) is used as an effective cancer drug targeting the *BCR-ABL* gene fusion for CML and ALL patients[1]. The *TMPRSS2-ERG* gene fusion is regarded as an early event in prostate cancer and is positively correlated with Gleason score, which is used to help evaluate the prognosis of men with prostate cancer[43]. A series of peptides inhibiting *ERG*-mediated transcription have recently been identified by Wang et al and can reduce cell invasion, proliferation, and tumor growth[44]. *ROS1* rearrangement and *EML4-ALK* account for 4% of non–small-cell lung cancers (NSCLC) carcinogenesis and is effectively targeted by crizotinib[2,45,46]. The *EVT6-NTRK3* fusion is found in 92% of human secretory breast carcinomas, and thus it is defined as a diagnostic biomarker[47]. The *PAX-FOXO1* fusion is found in 80% of aRMS patients[48,49], and has been recognized as a superior biomarker of poor event-free survival[50,51].

However, even though plenty of chimeric RNAs have been discovered in cancer and reported as biomarkers, not all are exclusively found alongside gene fusions, and not all are truly exclusive to cancer. Examples provided earlier in this manuscript such as *JJAZ1-JAZF1* and *PAX3-FOXO1* argue against exclusivity of these transcripts in cancer, and even several leukemia related fusion transcripts can be detected in healthy individuals[52-54]. Further, many chimeric RNAs have been found in normal samples, 13 of which have been found to overlap with existing annotations of supposed cancer-specific chimeras[55]. Taken together, gene fusions and chimeric RNAs have major impacts on cancer diagnosis and treatment; however, detection of either is not necessarily indicative of cancer. Thus, it is important to thoroughly validate the candidate marker before projecting its use to translational applications.

**Conclusions and future perspectives**

In this review, we provide an overview of similarities and differences between gene fusions and chimeric RNAs. We establish known origins of gene fusions as well as chimeric RNAs, and note that each may be able to influence the generation of the other. Gene fusions and the generation of their downstream chimeric products are generally well-studied; however, the inverse “cart before the horse” hypothesis is gaining traction as a possible means for existing chimeric RNAs to influence DNA-level changes.

Both gene fusions and chimeric RNAs have strong associations with cancer. They have been successfully utilized to improve patient diagnosis, treatment, and prognosis, and their value in these avenues cannot be understated. As gene fusion databases expand with the development of RNA sequencing technology, we urge additional caution in validation of these markers, as much evidence has arisen to indicate that patterns of fusion events are not sweeping indications of cancer.

Improvement in sequencing technologies has accelerated the pace at which study into chimeric RNAs has progressed. We expect that with the advent of new sequencing technologies such as low-cost whole genome sequencing and full-length sequencing, this pattern will continue. Ongoing research into this field has the potential to elucidate new mechanisms for oncogenesis which could have significant translational consequences.

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**REFERENCES**

1. Druker BJ. Current treatment approaches for chronic myelogenous leukemia. *Cancer J.* 2001;7:S14-S18.
2. Shaw AT, Ou SH, Bang YJ, et al. Crizotinib in ROS1-rearranged non-small-cell lung cancer. *N Engl J Med.* 2014;371(21):1963-1971.
3. Zhang Y, Gong M, Yuan H, Park HG, Frierson HF, Li H. Chimeric transcript generated by cis-splicing of adjacent genes regulates prostate cancer cell proliferation. *Cancer Discov.* 2012;2(7):598-607.
4. Rowley JD, Blumenthal T. Medicine - The cart before the horse. *Science.* 2008;321(5894):1302-1304.
5. Nowell PC. The minute chromosome (Phl) in chronic granulocytic leukemia. *Blut.* 1962;8:65-66.
6. Engelholm LH, Riaz A, Serra D, et al. CRISPR/Cas9 Engineering of Adult Mouse Liver Demonstrates That the Dnajb1-Prkaca Gene Fusion Is Sufficient to Induce Tumors Resembling Fibrolamellar Hepatocellular Carcinoma. *Gastroenterology.* 2017;153(6):1662-+.
7. Kastenhuber ER, Lalazar G, Houlihan SL, et al. DNAJB1-PRKACA fusion kinase interacts with beta-catenin and the liver regenerative response to drive fibrolamellar hepatocellular carcinoma. *P Natl Acad Sci USA.* 2017;114(50):13076-13084.
8. Singh D, Chan JM, Zoppoli P, et al. Transforming fusions of FGFR and TACC genes in human glioblastoma. *Science.* 2012;337(6099):1231-1235.
9. Costa R, Carneiro BA, Taxter T, et al. FGFR3-TACC3 fusion in solid tumors: mini review. *Oncotarget.* 2016;7(34):55924-55938.
10. Frattini V, Pagnotta SM, Tala, et al. A metabolic function of FGFR3-TACC3 gene fusions in cancer. *Nature.* 2018;553(7687):222-227.
11. Zhang YM, Gong M, Yuan HL, Park HG, Frierson HF, Li H. Chimeric Transcript Generated by cis-Splicing of Adjacent Genes Regulates Prostate Cancer Cell Proliferation. *Cancer Discov.* 2012;2(7):598-607.
12. Maher CA, Kumar-Sinha C, Cao X, et al. Transcriptome sequencing to detect gene fusions in cancer. *Nature.* 2009;458(7234):97-101.
13. Rickman DS, Pflueger D, Moss B, et al. SLC45A3-ELK4 Is a Novel and Frequent Erythroblast Transformation-Specific Fusion Transcript in Prostate Cancer. *Cancer Res.* 2009;69(7):2734-2738.
14. Kumar-Sinha C, Kalyana-Sundaram S, Chinnaiyan AM. SLC45A3-ELK4 Chimera in Prostate Cancer: Spotlight on cis-Splicing. *Cancer Discov.* 2012;2(7):582-585.
15. Qin FJ, Zhang YM, Liu J, Li H. SLC45A3-ELK4 functions as a long non-coding chimeric RNA. *Cancer Lett.*

2017;404:53-61.

16. Nacu S, Yuan WL, Kan ZY, et al. Deep RNA sequencing analysis of readthrough gene fusions in human prostate adenocarcinoma and reference samples. *Bmc Med Genomics.* 2011;4.
17. Kim HP, Cho GA, Han SW, et al. Novel fusion transcripts in human gastric cancer revealed by transcriptome analysis. *Oncogene.* 2014;33(47):5434-5441.
18. Tang Y, Qin FJ, Liu AQ, Li H. Recurrent fusion RNA DUS4L-BCAP29 in non-cancer human tissues and cells. *Oncotarget.* 2017;8(19):31415-31423.
19. Agabian N. Trans splicing of nuclear pre-mRNAs. *Cell.* 1990;61(7):1157-1160.
20. Li H, Wang JL, Mor G, Sklar J. A neoplastic gene fusion mimics trans-splicing of RNAs in normal human cells. *Science.* 2008;321(5894):1357-1361.
21. Kannan K, Wang LG, Wang JH, Ittmann MM, Li W, Yen LS. Recurrent chimeric RNAs enriched in human prostate cancer identified by deep sequencing. *P Natl Acad Sci USA.* 2011;108(22):9172-9177.
22. Guerra E, Trerotola M, Dell'Arciprete R, et al. A bicistronic CYCLIN D1-TROP2 mRNA chimera demonstrates a novel oncogenic mechanism in human cancer. *Cancer Res.* 2008;68(19):8113-8121.
23. Mitelman F. Recurrent chromosome aberrations in cancer. *Mutat Res.* 2000;462(2-3):247-253.
24. Kim P, Zhou X. FusionGDB: fusion gene annotation DataBase. *Nucleic Acids Res.* 2019;47(D1):D994-D1004.
25. Lee M, Lee K, Yu N, et al. ChimerDB 3.0: an enhanced database for fusion genes from cancer transcriptome and literature data mining. *Nucleic Acids Res.* 2017;45(D1):D784-D789.
26. Wang Y, Wu N, Liu J, Wu Z, Dong D. FusionCancer: a database of cancer fusion genes derived from RNA-seq data. *Diagn Pathol.* 2015;10:131.
27. Gorohovski A, Tagore S, Palande V, Malka A, Raviv-Shay D, Frenkel-Morgenstern M. ChiTaRS-3.1-the enhanced chimeric transcripts and RNA-seq database matched with protein-protein interactions. *Nucleic Acids Res.* 2017;45(D1):D790-D795.
28. Koontz JI, Soreng AL, Nucci M, et al. Frequent fusion of the JAZF1 and JJAZ1 genes in endometrial stromal tumors. *P Natl Acad Sci USA.* 2001;98(11):6348-6353.
29. Li H, Ma XY, Wang JL, Koontz J, Nucci M, Sklar J. Effects of rearrangement and allelic exclusion of JJAZ1/SUZ12 on cell proliferation and survival. *P Natl Acad Sci USA.* 2007;104(50):20001-20006.
30. Linardic CM. PAX3-FOXO1 fusion gene in rhabdomyosarcoma. *Cancer Lett.* 2008;270(1):10-18.
31. Yuan H, Qin F, Movassagh M, et al. A chimeric RNA characteristic of rhabdomyosarcoma in normal myogenesis process. *Cancer Discov.* 2013;3(12):1394-1403.
32. Gryder BE, Yohe ME, Chou HC, et al. PAX3-FOXO1 Establishes Myogenic Super Enhancers and Confers BET Bromodomain Vulnerability. *Cancer Discov.* 2017;7(8):884-899.
33. Keller C, Capecchi MR. New genetic tactics to model alveolar rhabdomyosarcoma in the mouse. *Cancer Res.* 2005;65(17):7530-7532.
34. Scheidler S, Fredericks WJ, Rauscher FJ, Barr FG, Vogt PK. The hybrid PAX3-FKHR fusion protein of alveolar rhabdomyosarcoma transforms fibroblasts in culture. *P Natl Acad Sci USA.* 1996;93(18):9805-9809.
35. Pandey PR, Chatterjee B, Olanich ME, et al. PAX3-FOXO1 is essential for tumour initiation and maintenance but not recurrence in a human myoblast model of rhabdomyosarcoma. *Journal of Pathology.* 2017;241(5):626-637.
36. Mochizuki K, Fine NA, Fujisawa T, Gorovsky MA. Analysis of a piwi-related gene implicates small RNAs in genome rearrangement in tetrahymena. *Cell.* 2002;110(6):689-699.

37. Yao MC, Fuller P, Xi XH. Programmed DNA deletion as an RNA-guided system of genome defense. *Science.* 2003;300(5625):1581-1584.
38. Nowacki M, Vijayan V, Zhou Y, Schotanus K, Doak TG, Landweber LF. RNA-mediated epigenetic programming of a genome-rearrangement pathway. *Nature.* 2008;451(7175):153-U154.
39. Shen Y, Nandi P, Taylor MB, et al. RNA-driven genetic changes in bacteria and in human cells. *Mutat Res-Fund Mol M.* 2011;717(1-2):91-98.
40. Gupta SK, Luo L, Yen L. RNA-mediated gene fusion in mammalian cells. *Proc Natl Acad Sci U S A.* 2018;115(52):E12295-E12304.
41. Yan ZM, Huang NM, Wu WX, et al. Genome-wide colocalization of RNA-DNA interactions and fusion RNA pairs. *P Natl Acad Sci USA.* 2019;116(8):3328-3337.
42. Nashed AL, Rao KW, Gulley ML. Clinical applications of BCR-ABL molecular testing in acute leukemia. *J Mol Diagn.* 2003;5(2):63-72.
43. Adamo P, Ladomery MR. The oncogene ERG: a key factor in prostate cancer. *Oncogene.* 2016;35(4):403-414.
44. Wang XJ, Qiao YY, Asangani IA, et al. Development of Peptidomimetic Inhibitors of the ERG Gene Fusion Product in Prostate Cancer (vol 31, pg 532, 2017). *Cancer Cell.* 2017;31(6):844-847.
45. Soda M, Choi YL, Enomoto M, et al. Identification of the transforming EML4-ALK fusion gene in non-small-cell lung cancer. *Nature.* 2007;448(7153):561-U563.
46. Shaw AT, Yeap BY, Solomon BJ, et al. Effect of crizotinib on overall survival in patients with advanced non-small-cell lung cancer harbouring ALK gene rearrangement: a retrospective analysis. *Lancet Oncol.* 2011;12(11):1004-1012.
47. Tognon C, Knezevich SR, Huntsman D, et al. Expression of the ETV6-NTRK3 gene fusion as a primary event in human secretory breast carcinoma. *Cancer Cell.* 2002;2(5):367-376.
48. Rudzinski ER, Teot LA, Anderson JR, et al. Dense pattern of embryonal rhabdomyosarcoma, a lesion easily confused with alveolar rhabdomyosarcoma: a report from the Soft Tissue Sarcoma Committee of the Children's Oncology Group. *Am J Clin Pathol.* 2013;140(1):82-90.
49. Arnold MA, Anderson JR, Gastier-Foster JM, et al. Histology, Fusion Status, and Outcome in Alveolar Rhabdomyosarcoma With Low-Risk Clinical Features: A Report From the Children's Oncology Group. *Pediatr Blood Cancer.* 2016;63(4):634-639.
50. Williamson D, Missiaglia E, de Reynies A, et al. Fusion Gene-Negative Alveolar Rhabdomyosarcoma Is Clinically and Molecularly Indistinguishable From Embryonal Rhabdomyosarcoma. *J Clin Oncol.* 2010;28(13):2151-2158.
51. Skapek SX, Anderson J, Barr FG, et al. PAX-FOXO1 fusion status drives unfavorable outcome for children with rhabdomyosarcoma: A children's oncology group report. *Pediatric Blood & Cancer.* 2013;60(9):1411-1417.
52. Ismail SI, Naffa RG, Yousef AMF, Ghanim MT. Incidence of bcr-abl fusion transcripts in healthy individuals. *Mol Med Rep.* 2014;9(4):1271-1276.
53. Uckun FM, Herman-Hatten K, Crotty ML, et al. Clinical significance of MLL-AF4 fusion transcript expression in the absence of a cytogenetically detectable t(4;11)(q21;q23) chromosomal translocation. *Blood.* 1998;92(3):810-821.
54. Boquett JA, Alves JR, de Oliveira CE. Analysis of BCR/ABL transcripts in healthy individuals. *Genet Mol Res.*

2013;12(4):4967-4971.

55. Babiceanu M, Qin FJ, Xie ZQ, et al. Recurrent chimeric fusion RNAs in non-cancer tissues and cells. *Nucleic Acids Res.* 2016;44(6):2859-2872.

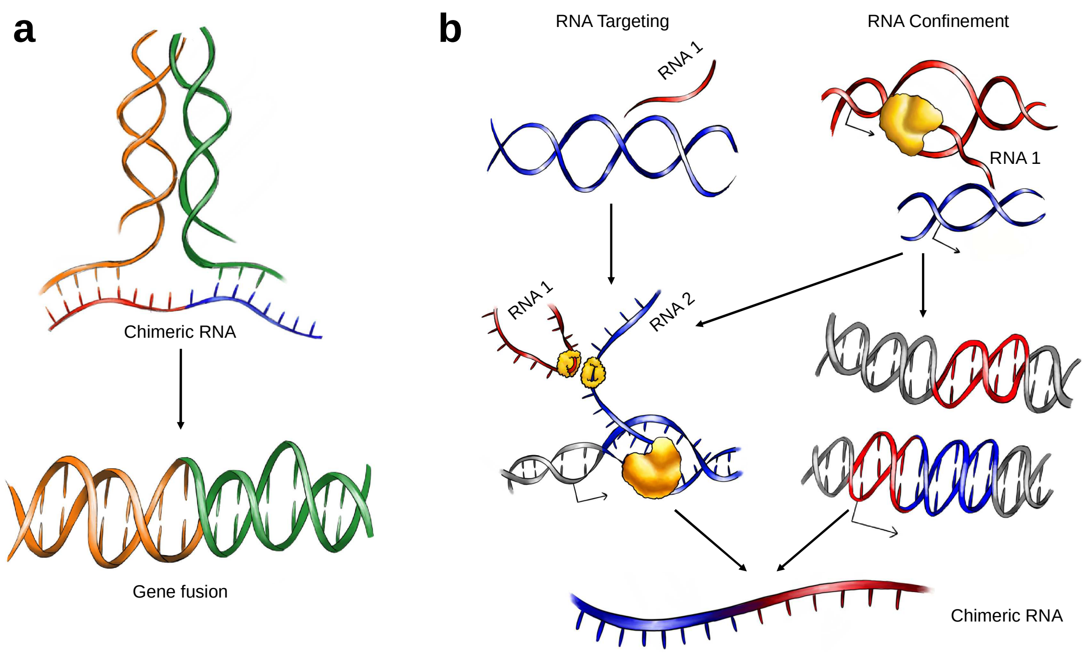


**Figure 1. Schematic diagram of mechanisms of chimeric RNA mediated gene fusion**
**a**, chimeric RNA invades chromosomal DNA of 2 genes and make a transient RNA/DNA hybrid, DNA break/repair mechanisms finally generated the corresponding gene fusion. **b**, RNA-poise model. Transcript of one gene (RNA 1) preinstalled on gene 2's genomic region, allowing for the possibility of trans-splicing (RNA Targeting). Spatial proximity of 2 genes could bring the 2 transcripts near each other, thus allowing for the possibility of trans-splicing (RNA Confinement). The RNA Confinement model also could enhance the possibility of genome rearrangement thus generate fusion gene.