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The Relationship Between Chimeric RNAs and Gene Fusions; Potential Implications of Reciprocity in Cancer

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Abstract

Gene fusions have long been considered hallmarks of cancer. Efforts into characterization of their prevalence, cause, and function have provided significant progress toward improvements in diagnosis, prognosis assessment, and treatment of numerous cancers. More recently, detection of intergenically spliced chimeric RNAs in cancer have spurred efforts to characterize these transcripts, anticipating similar successes in translation to the clinic. Discovery of chimeric RNAs in normal cells, especially those which precede canonical translocations such as *PAX3-FOXO1* and *JAZF1-JJAZ1*, suggest that these physiologically-regulated transcripts may function as proto-oncogenic factors, in that their dysregulation can lead to cancer progression. These findings have given rise to conjecture regarding chimeric RNA-guided rearrangements giving rise to fusion genes, termed The Cart Before The Horse Hypothesis. Here, we provide context for the relationship between gene fusions, chimeric RNAs, and cancer, assemble evidence in support of the Cart Before The Horse Hypothesis, and discuss potential advantages of targeting chimeric RNAs in cancer treatment.

1.1. What are Gene Fusions?

Gene fusions are hybrid genes which possess DNA sequence from two different parental genes. These can be produced by various genomic rearrangements including, but not limited to, chromosomal translocations, inversions, tandem duplications, and interstitial deletions (Edwards, 2010), and can give rise to a variety of downstream outcomes. The fusion gene may encode for a fusion protein possessing a combination of domains from two distinct parental genes, as observed in numerous recurrent gene fusion events in cancer (Edwards, 2010; Jang et al., 2019; Maher et al., 2009; Mitelman, F., Johansson, B., & Mertens, 2012; Panigrahi et al., 2018; Tomczak et al., 2015). This junction may also alter the reading frame

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or introduce a stop codon, as observed in the *FOS-MBNL1* gene fusion (van Ijzendoorn et al., 2015).

However, not all fusion genes create a novel fusion protein. Instead, rearrangement can result in the exchange of gene regulatory regions, placing the expression of one gene under the regulatory regimen of another. This is evident in fusions such as the *TMPRSS2-ETS* group of recurrent fusions in prostate cancer (Demichelis and Rubin, 2007), *IGH-MYC* in Burkitt Lymphoma (Johnston and Carroll, 1992), and the *RET-PTC* group of fusions in papillary thyroid carcinoma (Nikiforov and Nikiforov, 2002). This exchange can result in aberrant expression of a wild-type protein, which can have significant downstream consequences, especially when the protein is involved in a signaling pathway, and functions as a transcription factor (i.e. *ETS* and *c-MYC*) or kinase (i.e. *RET*).

While fusion genes are noted for their pronounced role in cancer, it is important to note that these events are not exclusive to oncogenesis. Structural variation accounts for an estimated 3.5–6.8% of expression quantitative trait loci (eQTL) with significantly stronger effects than single nucleotide polymorphism (SNP) derived eQTLs (Chiang et al., 2017). Formation of fusion genes in healthy human populations is generally rare (Abel et al., 2018; Forabosco et al., 2009), but is a noted contributor to evolution of multi-domain proteins across larger evolutionary time scales (Durrens et al., 2008; Leonard and Richards, 2012; Pasek et al., 2006). Few polymorphic fusion genes found in healthy human populations have been studied in detail, with the exception of *TFG-ADGRG7*, a fusion gene produced by tandem duplication (Chase et al., 2010). Another recent study found *TFG-ADGRG7*, as well as *JAK3-INSL3* and *KANSL1-ARL17A/B*, to be preferentially detected in tumor samples in comparison to matched controls, suggesting that although these are polymorphic, germline fusion genes which are insufficient to drive oncogenesis, they may confer risk for tumor formation (López-Nieva et al., 2019).

1.2. What are Chimeric RNAs?

In contrast to fusion genes, which are defined by hybrid DNA sequence, chimeric RNAs are hybrid RNA transcripts comprised of nucleotide sequence from different parental genes. Traditionally, chimeric RNAs are known to be produced by transcription of a gene fusion. However, by this definition, “chimeric RNA” refers to any hybrid transcript, reliant on gene annotation rather than mechanism of generation. Li et al. provides additional rationale for the use of this definition (Li et al., 2018).

In addition to fusion gene transcripts, chimeric RNAs are also produced by intergenic splicing. Most commonly, this occurs via cis-splicing of adjacent genes (cis-SAGe), wherein splicing of a readthrough transcript yields a hybrid mRNA (Akiva et al., 2006; Barresi et al., 2019; Greger et al., 2014; Qin et al., 2016, 2015; Tang et al., 2017; Zhang et al., 2012). Cis-SAGe appears to be the predominant means for chimeric RNA production in non-cancer cells (Singh et al., 2020), although several examples including *SLC45A3-ELK4* (Qin et al., 2017; Rickman et al., 2009; Zhang et al., 2012), *D2HGDH-GAL3ST2* (Qin et al., 2016), and *FAM179B-PRPF39* (Qin et al., 2015) are enriched in cancer samples.

Other types of chimeric RNAs have also been detected, including chimeric RNAs with parental genes on different chromosomes (Babiceanu et al., 2016; Li et al., 2008; Yuan et al., 2013; C. Zhu et al., 2019), separated by linear distance on the same chromosome (Wu et al., 2018; D. Zhu et al., 2019), on opposite strands of the same chromosome within the same locus (Dorn et al., 2001; Gingeras, 2009; Li et al., 2009; McManus et al., 2010), and some are produced through trans-splicing of sense and antisense transcripts from the same parental gene (Balamurali et al., 2019). Specific mechanisms for chimeric trans-splicing are not yet proven, but several possibilities have been suggested, such as *in-vivo* template switching at regions of homology (Kandel and Nudler, 2002), premature transcription termination and trans-splicing with nearby transcripts (Kowarz et al., 2011), and the RNA-poise model, in which nascent RNA can interact with nearby transcripts or DNA (Yan et al., 2019).

Perhaps in compliment to their diverse mechanisms of generation, chimeric RNAs are also diverse in functionality (Fig. 1). First, chimeric transcripts can encode fusion proteins, and depending on the junction, may alter the reading frame of the downstream transcript (Elfman and Li, 2018). Chimeric RNAs may function as noncoding RNAs (Qin et al., 2017), and it has been suggested that they may act as competing endogenous RNA (ceRNA) sponges due to sequence similarity (Elfman and Li, 2018). Additionally, these transcripts can link 5' regulatory elements to the 3' gene transcript and conversely, 3' regulatory regions to the 5' transcript (Xie et al., 2019). Due to their ubiquity and diversity in potential function, chimeric RNAs have been suggested as a means to expand the functional genome (Gingeras, 2009; Greger et al., 2014).

2. Implications in Cancer

2.1. Gene Fusions in Cancer

An estimated 20% of all neoplasms include a gene fusion event, including over 1000 identified unique fusion partners (Mertens and Tayebwa, 2014; Mitelman et al., 2007). Fusion genes are often specific to particular cancers, and as such, have been utilized as biomarkers and therapeutic targets to improve patient outcomes. Some fusions, such as *BCR-ABL1*, are used as diagnostic markers (Tefferi et al., 2009), and others, such as *PAX3-FOXO1* and *PAX7-FOXO1* have been suggested to stratify subtypes of similar cancers, and predict prognostic outcomes (Kubo et al., 2015; Missiaglia et al., 2012).

Similarly to chimeric RNAs, fusion genes can introduce new functionality or aberrant expression patterns to normal cells. Classic examples include *BCR-ABL1*, prominent in chronic myelogenous leukemia, *PAX3-FOXO1* in alveolar rhabdomyosarcoma (ARMS), and the *TMPRSS2-ETS* group of rearrangements in prostate cancer. *BCR-ABL1* produces a fusion protein which combines additional regulatory binding domains contained within BCR to the ABL1 tyrosine kinase and interferes with normal ABL1 self-inhibition (Ren, 2005). The *PAX3-FOXO1* fusion produces a novel hybrid transcription factor, combining DNA binding domains from PAX3 to the transactivation domain of FOXO1 (Linardic, 2008). As a result, the *PAX3-FOXO1* transcription factor shares binding sites with parental *PAX3* in addition to sites unique to the fusion protein (Cao et al., 2010; Gryder et al., n.d.), and escapes micro RNA (miRNA) regulation normally targeted to the 3' regulatory

region of *PAX3* (Xie et al., 2019). Finally, the *TMPRSS2-ETS* group of fusion genes in prostate cancers, including *TMPRSS2-ERG* and *TMPRSS2-ETV1*, exhibit a similar pattern of upregulation of an ETS family transcription factor through introduction of the androgen-responsive *TMPRSS2* promoter to a functional ETS transcription factor, promoting cell growth (Demichelis and Rubin, 2007; Tomlins et al., 2008, 2005).

Identification of several fusion genes as drivers in cancers has led to successes in targeted therapy. For example, imatinib was developed as a targeted inhibitor for the *BCR-ABL1* kinase, and patient prognosis has dramatically increased (Hantschel et al., 2008; Huettner et al., 2000; Ren, 2005). Similarly, ALK inhibitors have improved prognoses in *EML4-ALK* non-small cell lung cancers (Shaw et al., 2011; Soda et al., 2007), and other groups have begun to develop inhibitors targeting *ERG* to impair *ERG*-mediated transcription (Wang et al., 2017).

2.2. Intergenically Spliced Chimeric RNAs In Cancer

Numerous chimeric RNAs generated by intergenic splicing have also been shown to associate with cancer. These include examples found in solid tumors such as prostate and breast cancers, as well as soft tissue tumors such as chronic lymphocytic leukemia and alveolar rhabdomyosarcoma (Qin et al., 2016; Rickman et al., 2009; Varley et al., 2014; Velusamy et al., 2013; Zhang et al., 2012). One well-studied example is the *SLC45A3-ELK4* chimeric RNA, which has been proposed as a potential biomarker for prostate cancer, correlating with disease detection as well as disease progression (Babiceanu et al., 2016; Kumar-Sinha et al., 2012; Rickman et al., 2009). It has been found to be present in the absence of DNA rearrangement (Kumar-Sinha et al., 2012; Rickman et al., 2009; Zhang et al., 2012), and researchers have successfully amplified its read-through pre-mRNA transcript, suggesting that *SLC45A3-ELK4* is a product of cis-SAGE (Zhang et al., 2012). Further, depletion of the chimeric transcript via RNAi impacts prostate cancer cell proliferation, and arrests cells in G1 (Zhang et al., 2012), and has been shown to function as a long noncoding chimeric RNA despite coding for wild-type ELK4 (Qin et al., 2017). Interestingly, panels of chimeric RNAs have also been used to suggest the cell of origin for ARMS (Xie et al., 2016).

Other more recent studies have attempted to characterize the landscape of chimeric transcripts in particular cancers (Wu et al., 2018; D. Zhu et al., 2019). These studies have leveraged existing data from large sequencing cohorts to provide a reference for numerous chimeric RNAs preferentially expressed in cancerous tissues when compared to matched normal samples. Each provides experimental validation for a subset of these predictions, and highlights biomarker candidates such as *CHFR-GOLGA3* in bladder urothelial carcinoma (D. Zhu et al., 2019) and *LHX-NDUFA8* in cervical cancer (Wu et al., 2018). One advantage of each of these studies is the assessment of candidate chimeric RNAs across a panel of human tissues and cell lines, providing a useful, but not comprehensive indication of biomarker tissue specificity. Other similar studies have predicted chimeric RNAs and/or gene fusions in breast cancer and T-cell lymphoblastic lymphoma (López-Nieva et al., 2019; Varley et al., 2014).

However, we advise caution in making sweeping conclusions based solely on this type of data. Numerous chimeric RNAs have also been found in normal tissues, including some which overlap with annotated “cancer-specific” chimeric RNAs (Babiceanu et al., 2016; Tang et al., 2017). While these findings certainly do not invalidate correlations with oncogenic phenotypes, they do raise concern about disease and/or tissue exclusivity, which may limit its potential as a biomarker. Of note, our group has also recently assessed the landscape of chimeric RNAs in the GTEx cohort, providing a baseline for chimeric RNA expression in normal tissues (Singh et al., 2020).

3. Chimeric RNAs Preceding Gene Fusions

3.1. The Curious Cases of JAZF1-JJAZ1 and PAX3-FOXO1

Gene fusions and their associated chimeric transcripts are often considered indicators for particular cancer types; however, there have been several important exceptions. *AML1-ETO*, *BCR-ABL*, *MLL-MF4*, *TEL-AML1*, *PML-RAR α* , and *NPM-ALK* gene fusion transcripts, characteristic of various lymphomas and leukemias, have been detected in non-neoplastic cells (Janz et al., 2003), indicating that the presence of the transcript may not always be sufficient for diagnosis. More significantly, *JAZF1-JJAZ1* and *PAX3-FOXO1* transcripts have been detected in non-neoplastic cell populations shown to be lacking the corresponding DNA rearrangement (Li et al., 2008; Yuan et al., 2013).

The *JAZF1-JJAZ1* transcript was first discovered in normal endometrial stromal cells in 2008, wherein Li et al. provided evidence to support physiologically-regulated trans-splicing of precursor *JAZF1* and *JJAZ1* mRNAs, as well as its translation into a fusion protein product (Li et al., 2008). Expression of the *JJAZ1-JAZF1* fusion protein is linked to an antiapoptotic phenotype as well as increased proliferation in the absence of normal *JJAZ1* expression (Li et al., 2007). More recent studies have shown that *JJAZ1-JAZF1* destabilizes the polycomb repressive complex 2 (PRC2), decreasing global levels of H3K27, and increasing chromatin accessibility, thus allowing for activation of normally repressed genes (Ma et al., 2017). Notably, *JAZF1* has also been suggested to have a role as a tumor suppressor (Koontz et al., 2001; Sandberg, 2007); transcriptional silencing of this locus or DNA rearrangement could feasibly contribute to this phenotype.

The *PAX3-FOXO1* gene fusion is a characteristic gene fusion in the alveolar subtype of rhabdomyosarcoma. As previously stated, it serves as a key prognostic indicator for cases with particularly poor outcomes (Kubo et al., 2015; Missiaglia et al., 2012), and its fusion protein juxtaposes the DNA-binding domain of PAX3 to the transactivation domain of FOXO1 (Linardic, 2008), generating a novel chimeric transcription factor. *PAX3-FOXO1* binding sites have been shown to overlap with known *PAX3* binding sites, and include regions associated with genes overexpressed in ARMS such as *MYCN* (Williamson et al., 2005), *IGF1R* (Shiple et al., 2011), and *FGFR4* (Cao et al., 2010; Taylor VI et al., 2009). Recent studies have also suggested that the chimeric transcription factor is capable of forming *de novo* super enhancers (Gryder et al., n.d.), and may escape miR-495 downregulation which normally targets PAX3 (Xie et al., 2019). Despite its known role in ARMS progression, *PAX3-FOXO1* has also been established as a key regulatory factor in normal myogenesis. The chimeric transcript was first detected in normal differentiating

mesenchymal stem cells in 2013, wherein Yuan et al. demonstrated transient expression of *PAX3-FOXO1* throughout myogenesis (Yuan et al., 2013). It has been shown to regulate genes involved in myogenesis, myogenic signaling, and mesodermal development (Khan et al., 1998), both promoting myogenic induction and inhibiting differentiation into mature muscle (Graf Finckenstein et al., 2008; Khan et al., 1999), and its dysregulation interferes with proper myogenic differentiation (Graf Finckenstein et al., 2008; Xie et al., 2019).

In each of these cases, a chimeric RNA created by intergenic splicing precedes a gene fusion of known consequence in cancer. Further, fusion protein produced by either chimeric RNA does not provide any new functionality to the cell. Instead, these discoveries argue that dysregulation of naturally-occurring chimeric RNAs can be an important contributor to tumorigenesis, provided the constitutive expression resulting from the permanence of gene fusion. This idea is supported by recent detection of *EML4-ALK* transcripts in a lung cancer biopsy sample which tested negative for DNA rearrangement by *ALK* break-apart fluorescence *in situ* hybridization (Yan et al., 2019), suggesting that expression of the chimeric RNA, alone, may predispose cells to neoplasm. This relationship has spurred the “Cart Before the Horse” hypothesis, wherein a chimeric transcript can give rise to gene fusion between the loci of its two parental genes (Rowley and Blumenthal, 2008). Originally proposed by Rowley and Blumenthal, this hypothesis argues for an exception to the central dogma of biology: that genetic information may flow from RNA to DNA. In these two particular cases, transiently expressed chimeric RNA *JJAZ1-JAZF1* and *PAX3-FOXO1* would facilitate the formation of constitutive gene fusions directly contributing to tumorigenesis in the cell of origin.

3.2. The Cart Before the Horse Hypothesis

Since originally proposed in 2008, significant progress has been made in fields relevant to The Cart Before The Horse Hypothesis. Within this section, we collect and present evidence in support of this hypothesis. Despite this data, however, we note that this premise remains unproven, lacking complete validation *in vivo*.

While at first glance, the Cart Before the Horse hypothesis may seem unlikely, there is considerable basis for its plausibility. First, similar phenomena have already been observed in lower eukaryotes. In ciliates, RNA has been observed to serve as a template for reassembly of ciliate genomes, and artificial RNA templates provided to these organisms are used to guide specified outcomes at the DNA level (Nowacki et al., 2008). Additionally, RNA has been shown capable of serving as a template for DNA repair in yeast (Shen et al., 2011; Storici et al., 2007), including nascent RNA as a template for repair of its own locus (Keskin et al., 2016). Similar observations hold true in human and other eukaryotic cells as well, supporting break repair of a green fluorescent protein gene (Shen et al., 2011).

For chimeric RNA-mediated DNA rearrangement to occur, translocating loci and a corresponding chimeric RNA must be in close proximity. Additionally, DSBs must occur at these loci, and DNA repair must be influenced to repair the DSB incorrectly, presumably by the chimeric RNA (Fig. 2).

Chromosomal rearrangements result from the erroneous repair of free DNA ends, and thus, are dependent upon the presence of DSBs (Gandhi et al., 2010; Lehman et al., 2017). Consequently, factors which increase the risk for DSBs also increase the risk for chromosomal rearrangement (Anderson et al., 2006; Burrow et al., 2009; Dillon et al., 2013; Lehman et al., 2017; Thys et al., 2015). This is represented in the sensitivity of fragile sites to replication stress, the abundance of cytogenetic lesions in fragile sites following aphidicolin dosage (Glover et al., 1984), and the overrepresentation of fragile sites in reported cancerous translocation events (Arlt et al., 2006; Burrow et al., 2009). Especially within these regions, DSBs tend to occur disproportionately within breakpoint cluster regions (BCRs), with increasing frequency in response to external stressors (Canela et al., 2017). For example, DSBs associated with topoisomerase II (TopII) poisons such as Etoposide (ETO) are correlated with TopII cleavage sites and loop anchor domains (Canela et al., 2017; Ezoë, 2012; Lehman et al., 2017). Appropriately, these overlap with common translocation breakpoints in therapy-related cancers following treatment with ETO (Canela et al., 2017; Ezoë, 2012).

The association of BCRs with loop anchors provides at least partial context for proximity of translocating loci. Recent work has indicated that these regions are hotspots for recombination and genome instability in cancer (Kaiser and Semple, 2018), and more specifically, that mixed lineage leukemia fusion partners in acute myeloid leukemia are enriched within these regions and genes which intersect the loop anchor domain are more likely to exhibit DSBs and undergo rearrangement (Gothe et al., 2019).

These loop anchors also serve to approximate linearly distant regions of DNA sequence into topologically-associating domains (TADs). TADs are grouped into active and inactive compartments, wherein the former tend to harbor actively transcribing genes, and the latter tend to harbor silenced genes (Lieberman-Aiden et al., 2009; Rao et al., 2014). Recent studies have suggested that these clusters are phase-separated condensates, and have shown that related factors are also present in these condensates, such as CDK9 and Mediator in transcription-associated clusters (Cisse et al., 2013; Ghamari et al., 2013). In fact, mediator has been shown to form *in vitro* phase-separated condensates in vitro with several transcription factors such as OCT4 and GCN4, suggesting a likely mechanism for their formation *in vivo* (Ann Boija et al., 2018).

These groupings can coordinate expression of related genes, such as ribosomal genes (Mcstay and Grummt, 2008), or olfactory receptor genes (Lomvardas et al., 2006; Monahan et al., 2019). Patterns of TAD groupings within active and inactive compartments have been used to explain cell-type specific expression patterns (Dixon et al., 2015; He et al., 2018), and cell type-specific contacts may help to explain the tissue-specific nature of translocation partners in cancer (Engreitz et al., 2012; Mani et al., 2009; Roukos et al., 2013). As such, these clusters serve as hubs for both intrachromosomal and interchromosomal contacts, the latter of which has been termed non-homologous chromosomal contacts (NHCCs). Further, proximity of recurrent translocating loci in numerous have been demonstrated to be in close proximity in the origin cell type, including *CCDC6-RET* in human thyroid (Nikiforova et al., 2000), *PAX3-FOXO1* in murine myoblasts (Lagutina et al., 2015), *BCR-ABL1* and *PML-RAR α* in hematopoietic precursors (Neves et al., 1999), *MYC-IGH* in lymphoblastoid

cells (Engreitz et al., 2012), and *TMPRSS2-ERG* in LNCaP cells (Mani et al., 2009). These data demonstrate that proximity of loci contribute to their capability to undergo rearrangement, that known translocating loci are often in close proximity, and that cell-type specific NHCCs between these loci may contribute to cell-type specificity of particular translocations.

While there is considerable data that places translocating DNA regions in proximity, there is comparably less experimental evidence demonstrating the presence of chimeric RNA at these hubs. A recent study mapped RNA-DNA interactions genome-wide, finding that distal RNA-DNA interactions overlapped with parental loci of predicted chimeric RNAs as well as known recurrent fusion genes in cancer (Yan et al., 2019). They summarized their findings with the production of an “RNA-poise” model, in which RNA transcribed from one gene could interact with a nearby nascent RNA or another nearby gene locus to form the trans-spliced chimeric RNA. This model requires that two parental gene loci are in proximity for the production of the chimeric RNA, which places the chimeric RNA at the site of recombination. In support of this conclusion, other proposed models for chimeric RNA generation such as *in-vivo* template switching at regions of homology (Kandel and Nudler, 2002) or premature transcription termination and subsequent trans-splicing with nearby transcripts (Kowarz et al., 2011) also require proximity of the chimeric transcript to its parental genes. The latter study also suggested that DNA damage at the parental loci would enrich for chimeric RNA generation as well as rearrangement.

Further, we have observed that most cis-spliced chimeric RNAs seem to be generated by splicing of the nearest splice donor and splice acceptor sites within a readthrough transcript (2–2 rule) (Chwalenia et al., 2017), and several other studies have found trans-spliced chimeric RNAs between neighboring genes on opposite strands of the same chromosome (Dorn et al., 2001; Gingeras, 2009; Li et al., 2009; McManus et al., 2010) or sense-antisense chimeric RNAs generated from the same gene locus (Balamurali et al., 2019). While these observations suggest importance of proximity, they cannot demonstrate capability of chimeric RNAs to influence DNA rearrangement. Previously, we have suggested the “true-true, unrelated” hypothesis to cover the possibility that the same factors which enrich for chromosomal translocation also enrich for chimeric RNA generation, but these two outcomes do not interact (Jividen and Li, 2014).

However, other recent findings add more credibility to the theory of chimeric RNA-mediated DNA rearrangement. First, RNA has demonstrated capability to be used as a template for recombination in lower organisms (Nowacki et al., 2008) as well as in humans. Nascent RNA can be used as a template for homologous recombination (HR) for the locus from which it was transcribed (Keskin et al., 2016), and alternately, as a bridge template for NHEJ (Kowarz et al., 2011). Implication of nascent RNA in these processes is particularly interesting, as this suggests that nascent chimeric RNA could serve as a chimeric template for errant recombination. Further, numerous studies have implicated the HR-related protein RAD52 in promotion of RNA-DNA annealing complexes via inverse strand exchange between single-stranded RNA (ssRNA) and double-stranded DNA (dsDNA) molecules, providing an avenue for this interaction to occur *in-vivo* (Keskin et al., 2014; Mazina et al., 2017; McDevitt et al., 2018). Further, RAD52 associates with RNA

polymerase II and is recruited to DSBs in concert with transcription (Yasuhara et al., 2018), providing additional support for connectivity between transcription and RNA-mediated repair of actively transcribed regions. Strikingly, McDevitt et al demonstrated two in vitro methodologies for RAD52/RPA-mediated RNA-dependent DSB repair, utilizing both NHEJ and HR pathways, indicating that RNA involvement in DNA repair may be possible via either major pathway (McDevitt et al., 2018).

Perhaps most importantly, several groups have been able to enrich for targeted translocations via introduction of chimeric templates. Torres-Ruiz et al. were able to recreate the *EWSR-FLII* fusion gene in human stem cells by creating targeted breaks at parental gene loci, and increased efficiency of the translocation via supplementary transfection of single-stranded DNA oligonucleotides with chimeric sequence spanning the translocation junction (Torres-Ruiz et al., 2017). Gupta et al. demonstrated recreation of the stereotypic *TMPRSS2-ERG* and *TMPRSS2-ETVI* fusions via introduction of antisense chimeric RNA templates, and provided evidence for DNA-RNA stem-like complex formation based on sequence-specific interactions, and RNase H-modulated efficiency (Gupta et al., 2018).

4. Chimeric RNAs as targets for treatment

Chimeric RNAs are increasingly being utilized as diagnostic markers for cancer, especially in cases of gene fusion-driven oncogenesis (Maher et al., 2009; Rufflé et al., 2017). As discussed, these roles continue to expand through efforts such as large-scale efforts to characterize the chimeric transcriptome (chimerome) in various cancers, factors involved in chimeric RNA production (Chwalenia et al., 2019), and to utilize of the chimerome to predict cell of origin in complicated pathologies such as ARMS (Xie and Li, 2017).

In the case of gene fusion-driven cancers, these diagnostics are useful, but reparative action is severely limited in their efficacy by the detection window occurring post-rearrangement. Existing preventative treatments for cancer such as surgery, vaccination, and medication offer promise in high-risk groups, but come with limitations (Cuzick, 2017). Other potential remedies have been identified through association of variables such as exposure or diet and incidence in case-control studies and epidemiological cohorts. While these studies offer reasonable leads for follow-up, some are later contradicted (Devassy et al., 2015; Negri et al., 1991). Generally, these studies offer meaningful benefit, but do not identify a clear oncogenic driver to target.

Chimeric RNAs found to promote oncogenesis – especially those exclusive to cancer – present a unique opportunity for both diagnosis and treatment of a root issue in these cancers. Currently, the discovery of *PAX3-FOXO1* and *JJAZ1-JAZF1* in normal tissues limits their viability as biomarkers, as positive detection does not clearly indicate an oncogenic event. However, profiling the expression of these chimeras in normal cellular processes can provide a baseline for identification of aberrant expression. If the Cart Before The Horse Hypothesis proves to be true, these chimeras offer an opportunity to identify individuals as risk for rearrangement and provide intervention before the oncogenic event.

An often-overlooked feature of chimeric RNAs is our ability to specifically modulate their activity. Numerous studies have demonstrated the ability to knock-down chimeric RNA expression without affecting parental gene expression through RNA interference (RNAi) (Qin et al., 2020, 2016; Zhang et al., 2012), and development of Cas13-based systems seem to offer another approach with considerably fewer off-target effects when compared to both RNAi and Cas9-based treatments. Many chimeric RNAs do not follow the expression patterns of their parental genes (Singh et al., 2020) and possess unique junction sequences that can be specifically targeted. These chimeric RNAs present a unique opportunity for identification and observation of at-risk patients as well as direct treatment of a phenotypic driver. Given the recent approval of the first RNAi-based treatment (Setten et al., 2019), we believe that the chimerome may provide valuable targets for preventative cancer treatment.

5. Concluding Remarks

The discovery of the Philadelphia chromosome spurred decades of research into the role of gene fusions in cancer, providing evidence of recurrent, somatic rearrangements as drivers for oncogenesis. Expansion of this field has populated databases of recurrent gene fusion events in cancer with a notable variety in pathologies. Even studies which map genome-wide structural variation are especially mindful to flag rearrangements which could produce gene fusions. On the other hand, discovery of intergenically-spliced chimeric RNAs has initiated its own subfield of study, comprising both omics-based and candidate-approach studies. Notably, chimeric RNAs also have a variety of outcomes and have been proposed as a means to expand the functional genome.

These two fields have been closely intertwined, and as our knowledge of each has grown, it seems that their relationship may be more complicated than originally thought. We have also found that this relationship harmonizes with findings in related fields such as chromatin organization, transcription-associated stress, and even treatment-associated secondary cancer development. Together, these data scribe a compelling story regarding complicated dynamics within transcription factories giving rise to rearrangements. While unproven, momentum is building within this intersection, including an independent proposition of similar hypotheses without accounting for the addition of chimeric RNAs (Osborne, 2014).

These findings, paired with recent advances in RNAi treatment, provide exciting new potential avenues for basic research into nuclear dynamics and DNA repair, as well as translational research into cancer development with potential implications in preventative cancer treatment.

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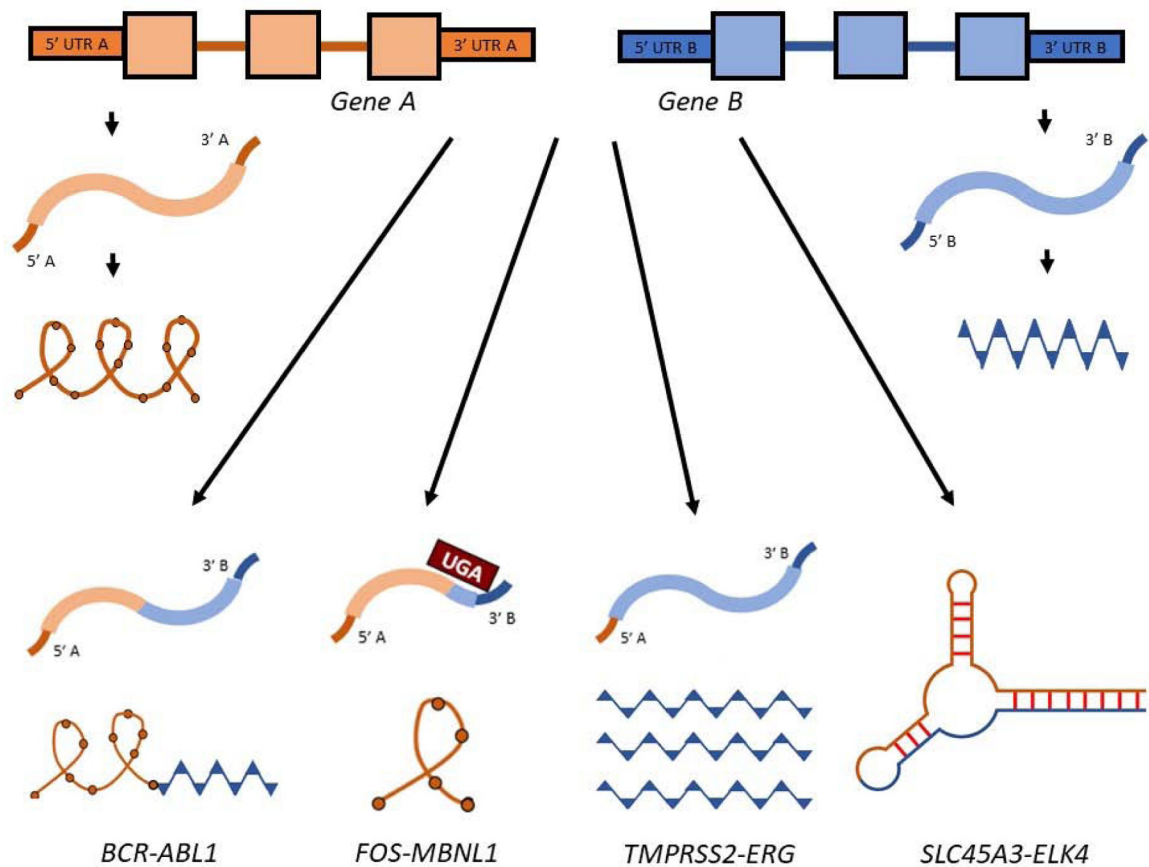


Fig. 1: Diversity in potential outcomes of chimeric RNA.

DNA is illustrated as boxes (exons) connected by lines (introns). RNA transcribed from these loci are represented by curved lines, and resultant protein is visualized as a curved line with dots (*Gene A*) or as a diagonal line with triangles (*Gene B*). Potential outcomes of chimeric RNAs shown include translation into wild-type protein; generation of a fusion protein (*BCR-ABL1*), frameshift in the protein coding sequence, resulting in a truncated protein (*FOS-MBNL1*); dysregulation of a wild-type protein (*TMPRSS2-ERG*); or generation of a chimeric noncoding RNA (*SLC45A3-ELK4*).

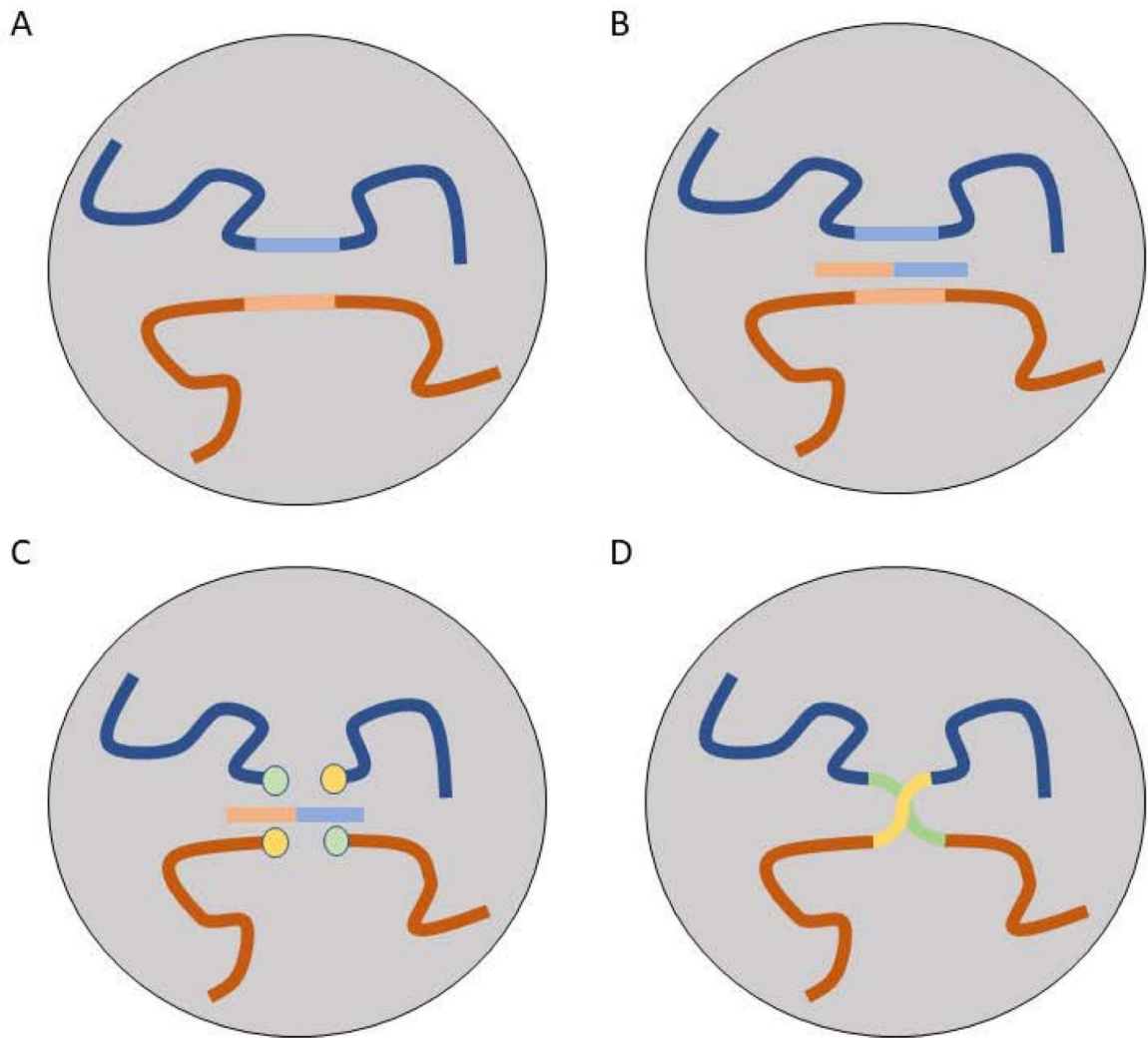


Fig. 2: Requirements for chimeric RNA mediated translocation.

A) DNA loci must be in close proximity; B) in close proximity to the chimeric RNA; (c) exhibit DSBs at each loci; and (d) exhibit errant repair of free ends, resulting in chromosomal rearrangement.