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Review

MicroRNAs and genomic instability

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Abstract

A new species of non-coding RNA, microRNAs (miRNAs) has been identified that may regulate the expression of as many as one third to one half of all protein encoding genes. MicroRNAs are found throughout mammalian genomes, but an association between the location of these miRNAs and regions of genomic instability (or fragile sites) in humans has been suggested [1]. In this review we discuss the possible role of altered miRNA expression on human cancer and conduct an analysis correlating the physical location of murine miRNAs with sites of genetic alteration in mouse models of cancer.
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Keywords: microRNAs; Genomic instability; Mouse genome; Integration sites

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1. Introduction

Regions of genomic instability can reflect alterations that result in loss, gain, or altered gene expression depending on the nature of the underlying genetic lesion and the function of the gene(s) affected. A critical marker of genomic instability is the presence of chromosomal translocations, and the analysis of breakpoint regions surrounding such genomic rearrangements has lead to discovery of some of the most important genes in cell biology. For example, the original experiments of Nowell and Hungerford [2] connected the Philadelphia chromosome (Ph′), generated by a T(9:22) translocation with chronic myelogenous leukemia (CML) and subsequent molecular analysis of the breakpoints showed that this results in the formation of the BCR-ABL fusion oncogene [3]. Similar cytogenetic studies in Burkitt's Lymphoma identified T(8:14) as a major chromosomal translocation event [4,5]. Follow up molecular studies showed that as a result of this translocation the proto-oncogene *c-MYC* is placed adjacent to the immunoglogulin heavy chain gene leading to cell transformation [6]. In contrast to the pattern of chromosomal translocation with oncogenes, it was the loss of heterozygosity (LOH) at the 13q14 locus as reported by Cavenee and colleagues in 1983 that provided the first molecular evidence of a tumor suppressor locus [7] and identification of the retinoblastoma (RB) gene. More recently, Calin and coworkers have reported that subsets of the newly identified species of non-coding RNAs (ncRNAs) called microRNAs (miRNAs), are clustered in regions of genomic instability or fragile sites

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[1,8]. With at least several hundred miRNAs found throughout the genome and estimates that miRNAs can regulate the expression of at least 30% of protein encoding genes [9–11] not only the location but also the function of these miRNAs could play a significant role in the characterization of normal and tumor cells.

2. MicroRNA biogenesis and function

MicroRNAs are first transcribed in the nucleus as primary transcripts some of which can be very large and include polycistronic transcripts encoding multiple miRNAs. Primary miRNA transcripts are transcribed by RNA polymerase II using either independent promoters or, as some are found in the introns of protein-encoded genes, they may use the promoter of the proximal coding gene [12–14]. A critical feature of microRNA biogenesis is the formation of a precursor miRNA hairpin structure of approximately 65–80 nucleotides (nts) that is processed from the primary miRNA transcript through the action of a protein complex containing at least two proteins, a RNase III endonuclease Drosha and a newly defined protein DGCR8 (Pasha) [15–19]. Export of the precursor miRNA to the cytoplasm is mediated by Exportin 5 [20,21] where the hairpin is processed by a second RNase III endonuclease, Dicer, resulting in the formation of a small double stranded RNA structure (~22 nts) that is asymmetrically unwound based on the relative thermodynamics of the two ends of the molecule [22–24]. The single stranded mature miRNA strand is finally loaded into the multiprotein complex termed RISC (RNA induced silencing complex) which contains Argonaute 2 (AGO2), a protein also critical for small interfering RNA (siRNA) mediated RNA interference [25,26].

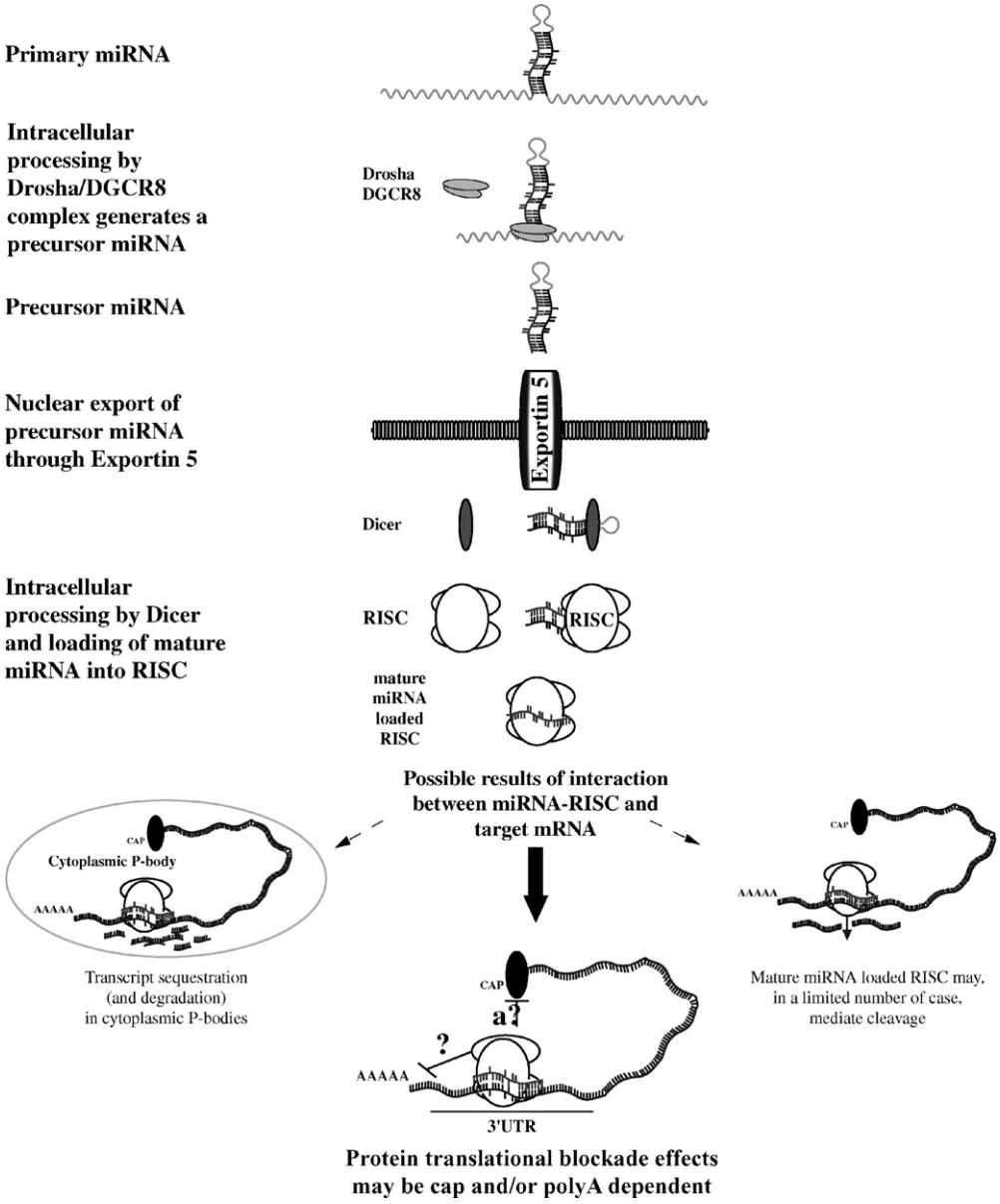

Fig. 1. A representation of the biogenesis of microRNAs and possible mechanisms by which they mediate gene silencing.

The miRNA-loaded RISC is thought to interact with binding sites within the 3′UTR of transcripts as a result of sequence complementarity between the first 5–7 nts of the mature miRNA (5′-3′) and the target transcript [27,28]. This so called 'seed sequence' [29–31] has been used to attempt the bioinformatic matching of miRNAs and gene targets, however, as the sequence nucleotide constraints for other parts of the miRNA-target interaction are much weaker and are much less well defined this has proven to be difficult [10,32]. The role of miRNAs in the regulation of gene expression is primarily thought to be through repression of translation, however, there is conflicting evidence as to whether miRNA activity is mediated through effects on the initiation of translation and/or termination [33–35]. In addition, miRNA mediated changes in gene expression may also involve RNA cleavage, RNA sequestration and/or degradation of the transcript to P-bodies [36–40] (Fig. 1). Due to the limited requirement for sequence complementarity between a miRNA and a target transcript, any one miRNA has the potential to regulate the expression of many different genes. There is also supporting evidence for the multiple binding of miRNAs (whether the same or different) to unique sites within a single transcript and that the cumulative effect of these binding events may be required for maximal repression of translation [41].

Several hundred miRNAs have been identified from a wide range of species [42,43], with conserved homology in both sequence make-up and target recognition. The first studies examining the function of miRNAs were conducted in model organisms such as *Caenorhabditis elegans* and *Drosophila*. These studies showed the critical role that some miRNAs play in development and differentiation, for example the first identified miRNAs lin-4 and let-7 [44,45]. More recent studies have shown a similar role for miRNAs in regulating genes involved in mammalian development and differentiation as well as hematopoiesis and metabolism [40,46–51].

3. MicroRNAs and cancer

One of the first reports on an association between miRNAs and human cancer involved the miRNAs hsa-miR-15a and hsa-miR-16-1 which were found to be down regulated or deleted in 70% of tumor cells from patients with B-cell chronic lymphocytic leukemia (B-CLL). As these miRNAs map to a region of minimal deletion (30 kb) that is associated with LOH (13q14) in B-CLL, a role for these particular miRNAs in oncogenesis has been proposed [8,52]. Interestingly, a predicted target of both hsa-miR-15a and hsa-miR-16-1 is *BCL2* [53], which is over-expressed in B-CLL. Transgene expression of hsa-miR-15a or hsa-miR-16 led to a decrease in the levels of BCL2 protein and the induction of apoptosis in leukaemic cell lines, suggesting that these miRNAs are involved in regulating *BCL2* and that their loss in B-CLL may be contributing to the ability of tumor cells to avoid apoptosis [53]. An example of the potential for a miRNA to drive a cancer phenotype is hsa-miR-155. Located on human chr. 21q21 within the *BIC* gene, a transcribed region originally identified as the common integration target of virally induced chicken B cell lymphomas, hsa-miR-155 has been shown to be altered in expression in primary Burkitt's lymphoma, Hodgkin's lymphoma, B-cell lymphoma and in lung cancer [54–58]. Transgenic expression of hsa-miR-155 under control of the Eμ-myc enhancer generates a polyclonal expansion of pre-B cells from leukaemic to a high-grade lymphoma in mice [59].

Studies examining the specific role for miRNAs in regulating genes that may be involved in cancer associated process have only just begun in mammalian cells, but some initial studies suggest that hsa-miR-21 found on chr. 17 and over-expressed in glioblastoma may have an anti-apoptotic function [60]. Also, one of the hsa-let-7 family members that is also part of a cluster on human chr. 9q22, has been described as having a role in modulating *RAS* expression [61] and two miRNAs found on human chr. 13q31, hsa-miR-17-5p and hsa-miR-20a, may negatively regulate *E2F1* expression [62]. Tissue specific expression of some miRNAs is well documented, and thus, in addition to specific association between miRNAs and a cancer phenotype resulting from genomic re-arrangement, expression profiling has also been used to show distinctive patterns of miRNA expression that can be used to discriminate tumor and normal cells. A variety of platforms are now available for profiling miRNA expression and include array-based oligonucleotide [63–65] and bead-based technology platforms [66]. A number of broad ranging studies have been conducted that show miRNA expression profiles are potentially valuable for cancer diagnosis and prognosis [66–69].

4. MicroRNA clusters in the human genome

Many clusters of miRNAs have been identified within the human genome. Some clusters reflect the processing of a number of miRNAs from a single large polycistronic transcript such that presumably all of the miRNAs are under the same promoter and in the same transcriptional orientation. Other clusters of miRNA genes may simply reflect close physical location but independent transcriptional regulation (either same or opposite transcriptional orientation). In terms of distance, clustering on longer chromosomes may have greater significance than clustering on shorter chromosomes and distances of less than 1 Mb between miRNAs seem significant in view of the large sizes of typically spliced genes. Predictions for clustering of human miRNAs have been made in at least one study [70] using algorithms with the criteria that 3000 nts is the maximal allowable distance for clustered miRNAs between genes and any miRNAs that fall within the same non-coding element (3′ UTR or intron) of a gene are considered clustered. With these criteria, 22 intergenic clusters (17 pairs, 4 triplets, 1 group of 5) and 9 intragenic clusters (5 pairs, 3 triplets, 1 group of 6) were identified from a total of 76 human miRNAs considered in the study. This suggests that 37.2% of miRNAs are clustered in humans assuming a minimum of a pair to form a cluster. The largest miRNA cluster identified to date is hsa-miR-127 (also known as hsa-miR-134) that resides on an imprinted region of human chr. 14q32 and is comprised of greater than 50 members [67]. The fact that they are all apparently oriented in the same transcriptional direction and they exhibit similar tissue specific expression suggests they are processed from a single large primary transcript. Therefore, the hsa-miR-127 cluster represents an extremely large polycistronic

transcript possibly regulated by differential methylation in a region 200 kb upstream of the miRNA cluster. This region of chr. 14q32.1 is a frequent target of translocations and inversions in T cell leukemias, resulting in the placement of TCL1 loci in close proximity to regulatory elements associated with T cell receptor loci [71]. It is likely that the expression of one or more miRNA from within the hsa-miR-127 cluster is significantly altered by chromosomal translocations/inversions that take place nearby.

Another relatively well characterized polycistronic cluster of miRNAs has been identified on a region of human chr. 13 and is frequently amplified in patients with diffuse large B cell lymphoma, follicular lymphoma, mantle cell lymphoma and primary cutaneous B-cell lymphoma. Although no known protein-encoding gene has been identified in this region, a large transcript originally designated as c13orf5 has been found that spans this amplicon (chr 13q31). Recently it has been shown that c13orf15 corresponds to a polycistronic cluster of seven miRNAs (hsa-miR-17-5p, hsa-miR-17-3p, hsa-miR-18, hsa-miR-19a, hsa-miR-20, hsa-miR-19b-1 and hsa-miR-92-1) termed the hsa-miR-17-92 cluster. The expression of at least five of these miRNAs is increased in lymphomas [72]. Construction of a transgene containing portions of hsa-miR17-19b-1 under the influence of an Eμ-myc enhancer will rapidly induce lymphomas in mice, suggesting that one or more of the miRNAs (independently or together) within this cluster are oncogenic [72]. Two miRNAs within this cluster, hsa-miR17-5p and hsa-miR-20a have been shown to bind to, and translationally downregulate *E2F1* that is thought to regulate the *MYC* oncogene through a feedback mechanism [62].

To investigate the possible involvement of miRNAs in deregulating key genes involved in cancer, Croce and colleagues compared the chromosomal localization of 186 miRNAs to human fragile sites and genomically unstable regions [1]. The genomically unstable regions were identified from a literature search using amplification (37 examples), deletion (154 examples) or translocation (45 examples) as key phrases. From these databases, a random effect Poisson regression model defined the number of miRNAs per 1 Mb as a defined exposure length. From this analysis, they were able to associate 48.3% (90/186) of the miRNAs studied with 36 clusters (of 2–3 miRNAs per cluster). They also found 35 of 186 miRNAs (19%) within or near fragile sites, a rate essentially nine times higher than that found at non-fragile sites. Approximately, 52.5% (98/186) of the miRNAs are found at or near regions of cancer associated genomic regions. This percentage reflects the miRNAs that were previously discovered as a result of cloning or electronic searches. For example, on chr. 7q32, the hsa-miR-29a cluster is within 1 Mb of a region of minimal deletion in prostate cancer and on chr. 19p13, LOH is associated with lung, pancreatic and gynecological cancer. Close to this region of 19p13 (0.5–4.0 Mb) is the large hsa-miR-7-3 cluster, which is composed of 47 members. The chr. Xq25 region is associated with LOH in advanced ovarian cancer and closely aligned with this region is the hsa-miR-19b-2 cluster. On chr. 9q22, a cluster of 6 miRNAs including hsa-let-7a-1 are found within a 1.46 Mb region that overlaps with a region of deletion found in urothelial cancer and is close to the Patched homolog (*PTCH*) and Fanconi's anemia complementation group C (*FANCC*) loci. A minimal LOH region of 17p13 (close to *TP53*) in both hepatocellular carcinoma and lung cancer is found near (1.9 Mb) the hsa-miR-22 cluster. Also in lung cancer, a region of homozygous deletion on chr. 21q11 is closely (2.8 Mb) aligned with a cluster that includes hsa-let-7c. Finally, the 13q32 region that harbors the hsa-miR-17-92 cluster, also exhibits LOH in Follicular lymphoma and B-CLL. In addition to deletion and amplification, a number of chromosomal breakpoints are found in close proximity to miRNAs including hsa-miR-142 which is 50 bp from the T(8:17) translocation in a leukemia and hsa-miR-180 which is 1 kb from a T(4:22) breakpoint and the *MN1* gene in meningioma. The hsa-miR-34a cluster is also very closely aligned with the breakpoint for the T(3:11) associated with B Cell leukemia. Not to diminish the importance of each miRNA, there are many additional instances of very close alignment (<1 Mb) of single miRNAs with chromosomal deletion, amplification and translocation events [1].

5. Mouse miRNAs and genomic location

The relationship between the location of miRNAs at or near sites of genomic instability has been examined mainly using human data, however, mouse models of cancer are an abundant source of chromosomal breakpoint information primarily from the use of murine retroviruses (e.g. Moloney Murine leukemia virus, Mo-MuLV) and the diseases caused by integration of these constructs. We thus compared the position of the annotated mouse miRNAs reported within the Sanger miRNA registry (http://microrna.sanger.ac.uk/) with genomically unstable regions or fragile sites, in particular retroviral integration sites found in the Mouse Retrovirus Tagged Cancer Gene Database (http://rtcgd.ncifcrf.gov); this database contains primarily information from the laboratories of Copeland and Jenkins (NCI), Berns (Netherlands Cancer Inst.) and Lenz (Albert Einstein College of Med) Release RTCGD mm7 dated 6/1/2006 [73]. The locations of the mouse miRNAs and sites of instability were positioned according to the UCSC Genome bioinformatics website (http://genome.ucsc.edu). We positioned 340 miRNAs from the current Sanger miRNA Registry release (Release 8.1) with the stringent criteria that a cluster should consist of more than two members positioned within 1 Mb (Fig. 2). Among 22 clusters of miRNAs identified by this approach, the largest included the mmu-miR-127 cluster with greater than 50 members on mouse chr. 12. Other significant clusters include the mmu-miR-29a cluster on chr. 6, the mmu-miR-23a cluster on chr. 8, the mmu-miR-17 cluster on chr. 14 and the mmu-miR-19b cluster on chr. X. If we relax the stringency to define a cluster as two or more members, we find an increase in clusters of miRNAs to 161/340 (47%) of the all the miRNAs with 51 clusters. Using the same criteria to identify clusters of retroviral integration sites as we originally used (3 or more hits within a 1 Mb region) for the miRNA cluster analysis, we find 18 clusters of retroviral integration (Fig. 2).

Overlaying retroviral integration sites with miRNA positions with the criteria that proximity should be within 1 Mb, we were then able to identify a number of cases were miRNAs reside close to integration sites (Table 1). For example, mmu-miR-9-1

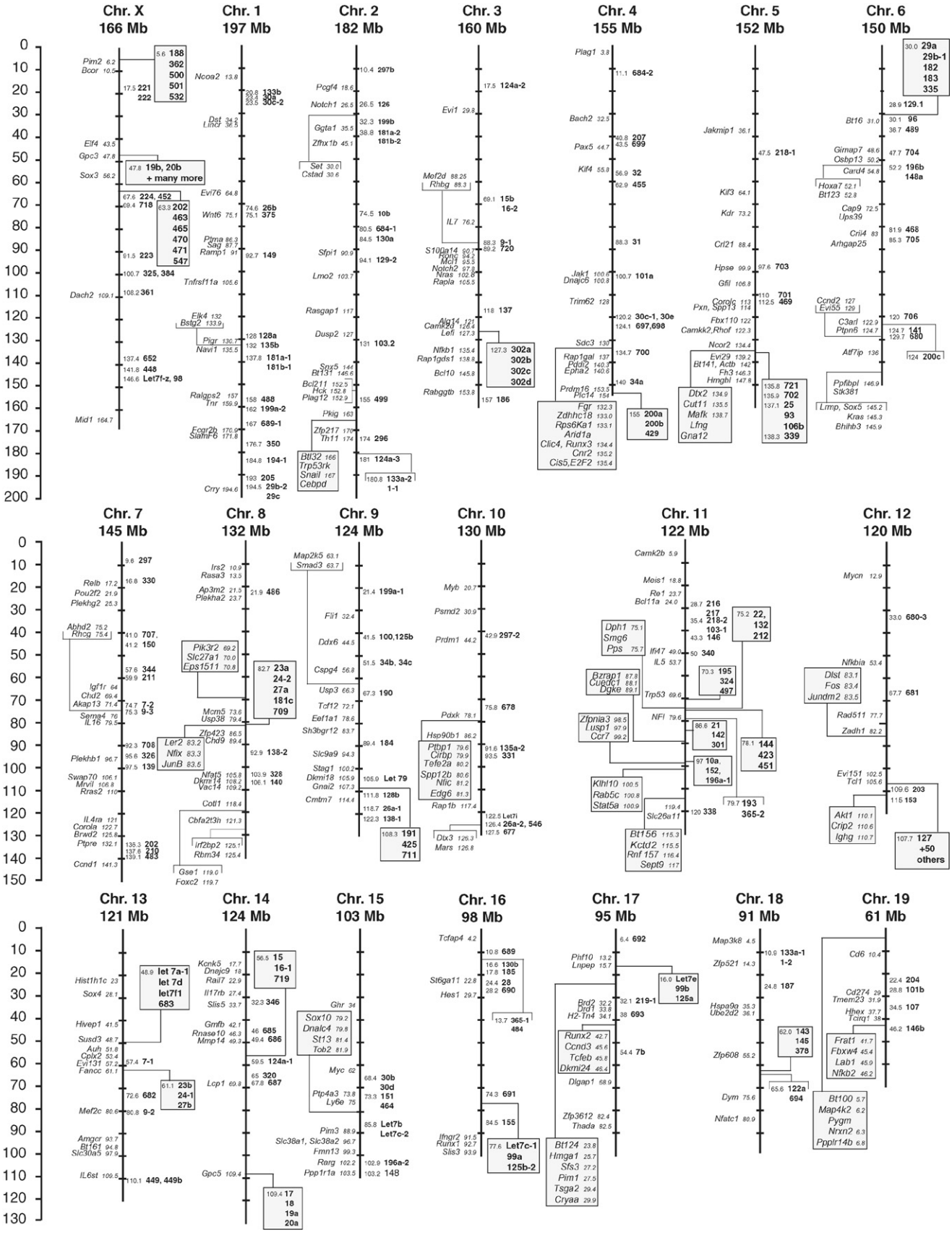

Fig. 2. Alignment of mouse microRNAs and gene markers associated with retroviral integration sites in mouse models of cancer. Gene names are shown on the left of each chromosome and the microRNAs on the right are marked by their approximate position in mega-bases (Mb). Clusters of genes associated with retroviral integration sites and clusters of miRNAs are shown within shaded boxes.

Table 1
Co-localization of murine miRNAs with retroviral integration sites associated with mouse models cancer

Chromosme	Integration or miRNA	Interval	Location
mm Chr. X	miR-19b-2		47834235
	miR-20b		47834365
	miR-92-2		47834090
	miR-106a		47834755
		1.7 kb	
	Gpc3		47836516
mm Chr. 2	*Notch 1*		26467392
		131 kb	
	miR-126		26598668
	miR-219-2		29852942
		780 kb	
	Cstad		30633080
	miR-199b		32325771
mm Chr. 3	*Mef2d*		88255552
	Rhbg		88324971
		4.4 kb	
	miR-9-1		88329399
	miR-720		89230410
		1.4 Mb	
	s100a14		90671152
	miR-302b		127383066
	miR-302c		127383201
	miR-302		127383334
	miR-302d		127383462
	miR-367		127383571
		3.3 Mb	
	Lef1		130773531
mm Chr. 4	*Jak1*		100671077
		52 kb	
	miR-101a		100723384
		160 kb	
	Dnajc6		100883753
	Zdhhc18		133049018
	Arid1a		133134330
	Rps6ka1		133190996
	Clic4		134584521
	Runx3		134558250
		236 kb	
	miR-700		134794992
		456 kb	
	Cnr2		135251385
mm Chr. 5	*Dtx2*		134929336
		417 kb	
	miR-721		135346996
	Cut11		135597145
		365 kb	
	miR-702		135962343
	miR-25		137110842
	miR-93		137111044
	miR-106b		137111258
	miR-339		138341760
		405 kb	
	Mafk		138747493
	Lfng		139581391
	Evi29		139673271
	Gna12		139741986
mm Chr. 6	miR-182		30098064
	miR-96		30101592
	miR-183		30101814
	miR-335		30673445
	miR-29a		30992959
	miR-29b-1		30993322
		18.6 kb	
	Btl6		31011947
	miR-706		120059529
		1.8 Mb	
	C3ar1		122911526
	miR-200c		124743325
	miR-141		124742917
		13.1 kb	
	Ptpn6		124756054
	Ccnd2		127175929
mm. Chr. 7	miR-7-2		74751473
		184 kb	
	Abhd2		75189552
	miR-9-3		75373555
		71.8 kb	
	Rhcg		75445389
	Sema4b		76009995
mm Chr. 8	miR-709		82761257
	miR-181c		82668721
	miR-23a		82790902
	miR-24-2		82791199
	miR-27a		82791056
		451 kb	
	Ier2		83242093
	Nfix		83358339
	JunB		83571404
mm Chr. 10	*Pttglip*		77261529
	Pdxk		78136122
	Ptbp1		79634267
	Cirbp		79931154
	Tcfe2a		80224065
	Sppl2b		80647250
	Scamp4		80390922
	Nfic		81200522
	Edg6		81293157
	miR-26-a2		126493008
	miR-546		126495918
		135 kb	
	Dtx3		126631155
	Mars		126812486
mm Chr. 11	*Smg6*		75153770
		101 kb	
	miR212		75254962
	miR-22		75545290
	miR-132		75255256
		816 bp	
	Dph1		75256072
	Pps		75735230
	miR-423		77159639
	miR-144		78154580
	miR-451		78154745
		1.4 Mb	
	Nf1		79616690
		176 kb	
	miR-193		79793544
	miR-21		86653975
		635 kb	
	miR-301		87182912
		8.6 kb	
	Bzrap1		87818078
	miR-142		87826772
mm Chr. 12	*Tcl1*		105613300
		1.5 Mb	

Table 1 (*Continued*)

Chromosme	Integration or miRNA	Interval	Location
	miR-127		107112874
	miR-134		107254159
	miR-136		107115355
	miR-154		107258453
	and many others		
	miR-203		109655825
		542 kb	
	AKT-1		110198167
	Crip2		110677230
	Ighg		110764249
mm Chr. 13	*Fancc*		61116288
		6.4 kb	
	miR-23b		61122749
	miR-27b		61122977
	miR-24-1		61123473
mm Chr. 14	*Gpc5*		109439038
		10.1 kb	
	miR-17		109449155
	miR-18		109449335
	miR-19a		109449484
	miR-20a		109449641
	Mmp14		49292580
		190 kb	
	miR-686		49483117
	miR-208		49815646
mm Chr. 15	*Rarg*		102221839
		737 kb	
	miR-196a2		102959506
	miR-148b		103273790
		230 kb	
	Ppp1r1a		103503968

is found just 4.4 kb proximal of *Rhbg*; retroviral integration into the 3′ region of the *Rhbg* gene on mouse chr. 3 is associated with brain tumors in mice [74]. About 52 kb distal to *Jak1* on chr. 4 is mmu-miR-101a; integration into the 5′ UTR region of *Jak1* results in myeloid tumors [75,76]. On mouse chr. 6, mmu-miR-29a and mmu-miR-29b-1 reside only 18.6 kb proximal to the *Btl6* integration site in an inverted in orientation. Marked by a gene of unknown function, Mo-MuLV integrations on the 3' side of *Bt16* generate lymphomas [77] or brain tumors [74] depending on the mouse model. On the distal end of chr. 6 is another cluster of integration sites, one of which is close to *Ptpn6* located 13.1 kb distal to the mmu-miR-141 locus. Disruption of the coding region of the 5′ region of *Ptpn6* results in either B cell tumors [76] or histiosarcomas [77].

Starting at 816 bp distal to mmu-miR-132 on mouse chr. 11 reside the genes *Dph1*, *Cis8,* and *Pps*. Integration into a region spanning 4–23 kb 5′ of *Dph1*, which would be predicted to interrupt mmu-miR-132 results in T cell lymphomas [77,78]. Located a little further distal on chr. 11 and located 176 kb downstream of *Nf1* (neurofibromatosis 1) is mmu-miR-193 which can be found in a cluster with four other miRNAs. Retroviral integration into *Nf1* principally results in myeloid tumors [75,76,79,80]. The mmu-miRNA-142 is also found further distal on chr. 11 and is located 8.6 kb on the 3′ UTR region (between 4 and 28.9 kb) of *Bzrap1*. Interruption of this region results in a series of T cell [78], myeloid [76] or and B cell tumors [81] depending on the model used.

On chr. 13, interruption of the *Fancc* gene in the 3′ region or in intron 15 results in brain tumors [74]. A small cluster of miRNAs including the mmu-miR-23b is located 6.4 kb distal to the *Fancc* gene and therefore, is likely to be disrupted by these retroviral integration events. Two members of the Glypican family, *Gpc3* (on chr. X) and *Gpc5* (on chr. 14) are found in close proximity to miRNA clusters. *Gpc5* is located 10.1 kb proximal to the mmu-miR-17 cluster; disruption in the 5′ region of *Gpc5* results in T cell lymphomas [78,82]. Integration into a region 5′ of *Gpc3* also generates T cell lymphomas [78,82]. The mmu-miR-19b-2 cluster resides between 1.7 kb and 2.2 kb 5′ of *Gpc3*. There are also situations where a retroviral integration site is located very close to a single miRNA. Examples of this are the integration site near *Nfkb2* that is only 33 kb from mmu-miR-146 on chr. 19 and mmu-miR-375, found 84 kb distal to the *Wnt6* integration site on mouse chr. 1.

6. Conclusion

In this analysis, we have focused on clusters of mouse miRNAs found in close proximity to known sites of retroviral integration and/or genomic instability assuming such clusters may exhibit altered expression as a consequence of genomic rearrangement. Given the potential for individual miRNAs to regulate multiple gene targets, a change in the expression of a single miRNA, let alone the aberrant expression of a miRNA cluster, could have significant consequences. Moreover, although not discussed in detail here, the conservation of miRNA positions relative to certain genes of interest (e.g. FANCC) exhibits good correlation between species (mouse to human) suggesting conserved co-regulatory processes. As such, the study of murine miRNAs in proximity to regions of known genomic instability in highly tractable mouse models of cancer should reveal critical roles for these miRNAs in a variety of cancer-related processes with many findings potentially relevant to human disease.

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