Toward a microRNA Signature of Endometrial Cancer

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Abstract

A simple meta-analysis of the eight surveys of microRNA (miRNA) expression in endometrial cancers reveals a panel of sixteen miRNAs that are significantly over-expressed (n = 15) or under-expressed (n = 1) in at least three surveys. Examination of these miRNAs indicates that they target mRNAs involved in a number of basic cellular processes including the crucial epithelial to mesenchymal transition (EMT) and hypoxia response. The central role played by these miRNAs is reinforced by the demonstration that they are all members of some of the most ancient of all animal miRNA families. This suggests that they may be part of a core set of miRNAs dysregulated as part of the carcinogenic cellular reprogramming process.

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Introduction

MicroRNAs (miRNAs) are 22nt long non-coding RNAs that, since their initial discovery nearly two decades ago, have been found to be ubiquitously involved in cellular processes throughout the plant and animal kingdoms[1]. MiRNAs act to regulate gene expression at both the transcriptional and post-transcriptional level thereby influencing hundreds of normal cell functions[1]. Further, dysregulation of miRNAs has been shown to play a role in dozens of human diseases, including cancers[2].

We recently reported on an extensive survey of dysregulation of miRNA expression in endometrial endometrioid adenocarcinomas and endometrial serous adenocarcinomas[3] and have followed that with a just completed survey of miRNA dysregulation in uterine carcinosarcomas[4]. Our two surveys bring to eight the total number of extant studies of miRNA dysregulation in endometrial cancers[3-10]. These surveys were carried out on a wide range of sample sizes, utilized different methodologies, and included a spectrum of endometrial cancer types, yet the compilation of results presented here reveals a core set of sixteen miRNAs whose expression was found to be significantly dysregulated in three or more of the studies. Examination of these miRNAs reveals that they affect

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central cellular processes and are, as a group, all members of evolutionarily old miRNA families. Thus, these commonly dysregulated miRNAs represent the first true candidates forming a core panel that, with further study, comprise a miRNA signature of endometrial cancer and, perhaps, other cancers as well.

Table 1. Characteristics of the eight extant miRNA surveys

Survey (Ref)	Samples[1]	Method[2]	miRNAs[3]
Boren et al. (5)	20BE, 37EEA, 4hyperplasia	Hybridization array	335
Chung et al. (6)	38EEA, 26BE	miR-specific qPCR	157
Wu et al. (7)	10EEC and NAT	Hybridization array	nd
Hiroki et al. (8)	21ESA, 7BE	Hybridization array	470
Ratner et al. (9)	5BE, 11EEA, 6ESA, 6UCS	TLDA (A-set Only)	384
Cohn et al. (10)	141EEA, number of controls not given	Hybridization array	326
Devor et al. (3)	4EEA, 4ESA, 4BE	TLDA (A-set and B-set)	667
Devor et al. (4)	8UCS, 5BE	TLDA (A-set and B-set)	667

[1]BE, benign endometrium; EEA, endometrial endometrioid adenocarcinoma; ESA, endometrial serous adenocarcinoma; UCS, uterine carcinosarcoma; NAT, normal adjacent tissue

[2]Hybridization arrays are from various sources: Invitrogen NCode (Boren et al.), Microarrays Inc. (Wu et al.), Agilent (Hiroki et al.), Ohio State University Comprehensive Cancer Center miRNA chip v.3 (Cohn et al.) ; miR-specific qPCR primer-probe sets from Life Technologies; TLDA, Life Technologies qPCR-based Taq-Man Low Density Array (A-set = 384 miRNAs, B-set = 283 miRNAs)

[3]Wu et al. do not provide the total number of miRNAs surveyed.

Extant miRNA Surveys of Endometrial Cancers

To date, there have been eight surveys of miRNA expression in endometrial cancers of which seven have been published and the eighth is in preparation[3-10]. The general characteristics of these miRNA surveys are presented in Table 1. As noted, the sample sizes and representation of both benign endometrium controls and the endometrial tumors types vary widely from study to study. Further, these studies surveyed a varied and only partially overlapping minority of the so far known total of 1,424 human miRNAs (miRBase Release 17, April 2011). Whatever the variation in sample size, tumor composition, and miRNA coverage may be, however, the techniques for determining miRNA expression levels are limited. In fact, there are but two methods available.

One, the hybridization array, involves fluorescently labeling DNA fragments generated by reverse-transcription (RT) and hybridizing them to grids spotted with synthetic oligonucleotides composed of reverse complement (anti-sense) miRNA sequences. The fluorescence intensity of each position in the array is then measured and analyzed relative to known standards. The alternative technology, also based upon reverse transcription of cellular RNA, involves quantitative PCR (qPCR) of RT-generated cDNA fragments using primers and fluorescently labeled probes (TaqMan assays) specific to individual miRNAs. These assays are also normalized to endogenous RNA standards for subsequent expression analyses.

Taken together, the eight miRNA surveys overlap by, perhaps, only a few dozen miRNAs and yet a panel of sixteen miRNAs are found to be significantly dysregulated in endometrial cancers relative to benign endometrium in at least three of the eight surveys (Table 2). Moreover, half of these sixteen are found to be significantly dysregulated in endometrial cancers relative to benign endometrium in at least four of the eight surveys and two, *miR-210* and *miR-205*, are found in six of eight and seven of eight surveys respectively.

Discussion

MiRNAs function to both preserve the overall homeostatic regulation of cellular processes and participate in normal cellular state transitions. Several miRNAs can target the same mRNA message and each miRNA can have multiple targets. This establishes networks of gene regulation involving both feed-back and feed-forward loops. Thus, when dysregulated, miRNAs can potentially be extremely disruptive. In cancers, miRNAs have been seen to be both significantly over-expressed and significantly under-expressed relative to benign tissues and to function as tumor suppressors or oncogenes depending upon the tumor type and tissue of origin[11,12]. However, it is reasonable to expect that any particular miRNA will function consistently within the same cancer type. The sixteen miRNAs presented here are not only consistently either over- or under-expressed but can be expected to be carrying out the same pathogenic role in endometrial cancers regardless of histologic type. Proof of this principle is the presence of the entire *miR-200* family of miRNAs in Table 2. This family, which emerged in eumetazoan genomes more than 500 million years ago[13], is composed of five miRNAs in two polycistrons, *miR-200c* and *miR-141* on chromosome 12 and *miR-200a*, *miR-200b* and *miR-429* on chromosome 1. Members of this family are well known to be crucially involved in epithelial to mesenchymal transition (EMT) in uterine and other tissues[14] and *miR-200c* has been shown to regulate E-cadherin through the transcription factors ZEB1 and ZEB2[15]. Coordinately regulated with the *miR-200* family, *miR-205*, the most consistently over-expressed miRNA in these studies, also plays a key role in EMT through targeting ZEB1 and SIP1[16]. A miRNA study of EMT in uterine carcinosarcomas (UCSs) determined that expression of *miR-200* family members are significantly different in the epithelial component of UCSs versus

the mesenchymal component with the former many-fold higher than the latter[17]. Dysregulation of miRNAs involved in EMT in endometrial cancers is consistent with the central role of EMT in both development and cancer[18].

Another miRNA from Table 2 prominently represented in UCS EMT is *miR-155*, seen to be nearly 80-fold more highly expressed in the mesenchymal component than in the epithelial component[17]. This miRNA is linked to inflammation and has repeatedly been observed to be over-expressed in both hematologic cancers and solid tumors[19].

Table 2. microRNAs significantly under- or over-expressed in endometrial cancers compared with benign endometrium in three or more of the eight extant surveys of miRNA expression in endometrial cancers.

microRNA	Number of Studies	References
Under –expressed:		
miR-133	4 of 8	[3, 4, 8, 9]
Over-expressed:		
miR-205	7 of 8	[3, 4, 5, 6, 7, 8, 9, 10]
miR-210	6 of 8	[3, 4, 5, 6, 7, 8]
miR-200a	5 of 8	[3, 6, 7, 8, 9]
miR-200c	5 of 8	[3, 6, 7, 8, 10]
miR-107	4 of 8	[3, 4, 5, 6]
miR-203	4 of 8	[6, 7, 8, 10]
miR-182	4 of 8	[6, 7, 8, 9]
miR-10a	3 of 8	[6, 7, 8]
miR-31	3 of 8	[3, 7, 8]
miR-106a	3 of 8	[5, 6, 8]
miR-141	3 of 8	[3, 6, 7]
miR-155	3 of 8	[3, 6, 7]
miR-183	3 of 8	[3, 6, 8]
miR-200b	3 of 8	[3, 7, 8]
miR-429	3 of 8	[3, 7, 8]

Observed to be significantly over-expressed in six of the eight miRNA surveys, *miR-210* offers a very straight-forward example as well. Numerous studies of the relationship between hypoxia and miRNA expression have shown that *miR-210* is the most hypoxia-responsive miRNA, intimately linked to the hypoxia-inducible factor Hif1α[20]. Moreover, numerous *miR-210* mRNA targets involved in cell cycle (PLK1, E2F3) and DNA repair (BLM), among others, have been experimentally validated. Similarly straight-forward is the one miRNA in Table 2 that displays significant under-

expression, *miR-133*. Another ancient miRNA[13], *miR-133* is actually three miRNAs in the human genome; *miR-133a-1* on chromosome 18, *miR-133a-2* on chromosome 20, and *miR-133b* on chromosome 6. The 22nt mature miRNA of *miR-133a-1* and *miR-133a-2* is identical and *miR-133b* differs only in position 22 (~~G~~A). Moreover, *miR-133a-1*, *miR-133a-2*, *miR-133b* are polycistronic with *miR-1-2*/*miR-1-1*/*miR-206* respectively, which themselves share nearly identical mature sequences, and this relationship was established as long ago as 6.7 x 10^8 years ago with the emergence of the bilateria. In fact, all of the miRNAs found in Table 2 are members of ancient miRNA lineages with most appearing well before the emergence of the Chordates 5 x 10^8 years ago (Table 3). This is not surprising, since cancer is a dramatic re-programming of basic cellular functions[11] it stands to reason that deeply rooted, highly conserved miRNAs involved in regulating basic cellular functions would be preferentially affected.

Table 3. Evolutionary emergence of the miRNAs in Table 2. Taxon assignments are based upon detection of individual miRNAs in representative species reported in Sempere et al.[13].

Taxon	Emergence (Years)[1]	microRNA
Eumetazoa	700,000,000	*miR-10*
Triploblastica	680,000,000	*miR-31*
Nephrozoa	670,000,000	*miR-1, miR-133, miR-210*
Deuterostomia	535,000,000	*miR-141, miR-183, miR-200*
		miR-429
Chordata	500,000,000	*miR-107*
Osteichthyes	410,000,000	*miR-106, miR-155, miR-182*
		miR-203, miR-205

[1]Average of estimates based upon both paleontologic and molecular evidence provided primarily in Peterson et al.[21].

The miRNA dysregulation signature of human endometrial cancer presented here, because it is deeply rooted in animal evolution, is likely part of a generic dysregulation signature in many human cancers. Most of these miRNAs are seen to be significantly dysregulated in other cancers as well including leukemia, ovary, lung, prostate, colorectal, brain, and pancreas[22]. It is certainly possible that these observations are statistical artifact since

miRNAs with low miRBase archive numbers[23] are those that were first identified and have been included in various arrays for several years. On the other hand, these miRNAs continue to be represented even as the size and complexity of miRNA arrays has increased.

If there is a functional over-representation of the more ancient miRNAs in human cancers, adding to and overlaying this background are many miRNAs that appeared following the emergence of the land animals[13]. The human miRNAome retains the ancient miRNAs but also has hundreds of new miRNAs that are only found in mammals and more only found in primates or in hominids[13]. When seeking to derive a true miRNA signature of human endometrial cancers it is reasonable to expect that endometrial-specific miRNA expression patterns will be layered over the ancient, generic "cancer pattern" presented here. The task now is to identify these histology-specific miRNAs, add them to the core miRNAs, and use them to develop diagnostic and prognostic assays as well as identify their mRNA and pathway targets.

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