

microRNA expression profiling of endometrial endometrioid adenocarcinomas and serous adenocarcinomas reveals profiles containing shared, unique and differentiating groups of microRNAs

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Received April 18, 2011; Accepted May 23, 2011

DOI: 10.3892/or.2011.1372

Abstract. microRNAs (miRNAs) control a multitude of pathways in human cancers. Differential expression of miRNAs among different histological types of tumors within the same type of tissue offers insight into the mechanism of pathogenesis and may help to direct treatment to improve prognosis. We assessed expression of 667 miRNAs in endometrial endometrioid and serous adenocarcinomas using RNA extracted from benign endometrium as well as from primary endometrial tumors. Quantitative miRNA profiling of endometrial adenocarcinomas revealed four overlapping groups of significantly overexpressed and underexpressed miRNAs. The first group was composed of 20 miRNAs significantly dysregulated in both adenocarcinoma types compared with benign endometrium, two groups were composed of miRNAs significantly dysregulated in either endometrioid adenocarcinomas or in serous adenocarcinomas compared with benign endometrium, and the fourth group was composed of 17 miRNAs that significantly distinguished between endometrioid adenocarcinomas and serous adenocarcinomas themselves. Validation of the expression levels of the selected miRNAs was carried out in a second panel composed of ten endometrioid and five serous tumors. Experimentally validated mRNA targets of these dysregulated miRNAs were identified using published sources, whereas TargetScan was used to predict targets of miRNAs in the first and fourth profile groups. These validated and potential miRNA target lists were filtered using published lists of genes displaying significant overexpression or underexpression in endometrial cancers compared to benign endometrium.

Our results revealed a number of dysregulated miRNAs that are commonly found in endometrial (and other) cancers as well as several dysregulated miRNAs not previously identified in endometrial cancers. Understanding these differences may permit the development of both prognostic and diagnostic biomarkers.

Introduction

Endometrial carcinoma is the fourth most common cancer among women worldwide (1). Endometrioid adenocarcinoma accounts for three-quarters of all endometrial cancers, and serous adenocarcinoma ranks as the second most common type (2). According to the National Cancer Institute, there were 42,160 new cases of endometrial cancer in the US with nearly 8,000 deaths in 2009; the relative 5-year survival has not improved over many decades. In spite of these data, endometrial cancer is perhaps the least studied and understood cancer affecting women. In the past few years, substantial progress has been made in the arenas of clinical trials and translational research, particularly the understanding of the importance of post-transcriptional gene regulation. For example, an area of particular interest is the relative expression patterns of microRNAs (miRNAs) in cancer and how these molecules contribute to oncogenesis (3-7). By understanding how the miRNA expression profile reflects the grade and stage of disease, it may be possible to better design patient treatment regimens.

In the present study we report an analysis of the comparative expression levels of 667 human miRNAs in benign endometrial tissues, endometrial endometrioid adenocarcinomas and endometrial serous adenocarcinomas using a quantitative PCR (qPCR) array platform. Our data demonstrated that the two types of adenocarcinomas (endometrioid and serous) are generally similar with respect to miRNAs that are significantly either overexpressed or underexpressed compared to benign endometrial tissues. However, we also identified numerous miRNAs that differentiate between the two adenocarcinomas. Several of these significantly overexpressed or underexpressed miRNAs correspond to recently identified miRNA expression

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Key words: microRNA, endometrial cancer, endometrioid adenocarcinoma, serous adenocarcinoma

networks known to be reprogrammed in human cancers (8). We also identified a potential coexpression clique potentially affecting the *Wnt* signaling pathway in both forms of endometrial adenocarcinoma, signifying the importance of this pathway in the development of all endometrial cancers.

Materials and methods

Endometrial tumor and benign endometrium tissues. Endometrial tumor samples and samples of benign endometrium were obtained under informed consent from patients undergoing surgery at the University of Iowa Hospitals and Clinics. All tumor specimens were from primary sites and were snap-frozen and stored at -80°C following histological examination. Clinicopathologic characteristics of the patients from whom the tissues used in this study were obtained are presented in Table I.

RNA extraction. Prior to RNA extraction, tissues were transitioned from -80 to -20°C following perfusion in RNAlater ICE (Ambion) according to the supplier's instructions. Total RNA was extracted using the miRVana miRNA isolation kit (Ambion). RNA quality and concentrations were assessed on a NanoDrop M-1000 and an Agilent 2100 Bioanalyzer. Only RNAs of sufficient quality (RIN ≥ 7.00) were used for expression assays. RNAs were standardized to 200 ng/ μ l and these concentrations were reverified on the NanoDrop M-1000.

qPCR arrays. miRNA expression levels were assessed on Applied Biosystems TaqMan Low Density miRNA arrays (TLDA). Both the A-Set TLDA card, containing qPCR assays for 377 human miRNAs and the B-Set TLDA card, containing qPCR assays for 290 additional human miRNAs, were used for expression screening of a panel composed of four benign endometrial tissues, four endometrial endometrioid adenocarcinomas and four endometrial serous adenocarcinomas. A total of 600 ng of tissue RNA was used for each assay. Reverse transcription was carried out using the TaqMan miRNA reverse transcription kit (Applied Biosystems) and either the Human A Pool or Human B Pool Megaplex RT primers (Applied Biosystems).

All 24 TLDA cards were processed on an Applied Biosystems Model 7900 Genetic Analyzer, and the resulting data were analyzed using the Applied Biosystems StatMiner software. Expression of all 667 miRNAs in all samples was normalized against the RNU48 endogenous RNA control prior to statistical analyses. Comparisons between endometrioid adenocarcinoma and benign endometrium, serous adenocarcinoma and benign endometrium, and endometrioid adenocarcinoma and serous adenocarcinoma were carried out using the $\Delta\Delta C_t$ method where fold change was expressed as $2^{-\Delta\Delta C_t}$ (9). Statistical significance of the fold changes was assessed via two-tailed t-tests with unequal variances. We report unadjusted p-values ($p \leq 0.05$) as significant. No adjustment of p-values to account for a false discovery rate (FDR) was made as inherent FDR in miRNA studies is insufficient to warrant the type of increased stringency required by other types of arrays (10).

microRNA validation. Following analysis of the TLDA array data, we selected a set of 10 miRNAs with which to validate

Table I. Clinical/pathologic characteristics of the tissues used for this study.

ID	Tumor type	Age	Grade	Stage
NE10	Benign	58	NA	NA
NE13	Benign	48	NA	NA
NE59	Benign	48	NA	NA
NE86	Benign	63	NA	NA
EA11	Endometrioid	72	3	IB
EA30	Endometrioid	66	2	IC
EA38	Endometrioid	74	1	IB
EA44	Endometrioid	53	2	IIA
EA46	Endometrioid	54	1	IB
EA49	Endometrioid	64	2	IB
EA66	Endometrioid	82	1	IB
EA68	Endometrioid	49	2	IIB
EA69	Endometrioid	59	1	IB
EA71	Endometrioid	52	3	IIIC
EA73	Endometrioid	54	1	IB
EA83	Endometrioid	85	1	IA
EA92	Endometrioid	61	1	IC
EA96	Endometrioid	62	1	IIIC
SA12	Serous	61	3	IB
SA36	Serous	59	3	IIIC
SA39	Serous	64	3	IIIC
SA48	Serous	60	3	IVB
SA50	Serous	67	3	IIIC
SA55	Serous	69	3	IB
SA72	Serous	83	3	IIIC
SA79	Serous	87	3	IIIC
SA93	Serous	85	3	IIIC

Tissues used for the qPCR arrays are indicated in bold type. NA, not applicable. Age is in years.

the array results. miRNA-specific qPCR assays for miR-21, miR-34a, miR-129-3p, miR-135a, miR-135b, miR-137, miR-200c, miR-205, miR-210 and miR-299-3p (Applied Biosystems) were carried out on an RNA panel composed of the original four benign endometrial tissues plus 10 additional endometrial endometrioid adenocarcinomas and five additional endometrial serous adenocarcinomas. For validations, reverse transcriptions and qPCR assays were carried out using miRNA-specific RT primers and qPCR primer/probe sets (Applied Biosystems). These assays were also run on an Applied Biosystems Model 7900 Genetic Analyzer and the resulting data again were analyzed using the Applied Biosystems StatMiner software following normalization against the RNU48 endogenous RNA control.

Results

Twenty microRNAs are significantly dysregulated in both endometrial adenocarcinomas compared with benign endometrium. miRNAs displaying either significant overexpression

Table II. microRNAs significantly underexpressed or overexpressed in both endometrioid and serous adenocarcinomas compared to normal endometrium.

	Endometrioid tumors		Serous tumors	
	Fold change	p-value	Fold change	p-value
Underexpressed microRNAs				
miR-1	-83.3	0.001	-15.9	0.048
miR-133a	-200.0	0.001	-83.3	0.021
miR-504	-125.0	0.001	-16.1	0.011
miR-133b	-333.3	0.002	-377.2	0.022
miR-137	-200.0	0.002	-30.3	0.030
miR-125b-2*	-166.7	0.003	-33.3	0.024
miR-129-3p	-47.6	0.043	-37.0	0.026
Overexpressed microRNAs				
miR-15b	5.7	0.005	6.9	0.020
miR-142-5p	9.0	0.028	10.4	0.023
miR-142-3p	13.7	0.018	15.0	0.015
miR-135a	19.5	0.014	48.6	0.001
miR-519a	22.9	0.027	79.6	0.037
miR-135b	41.4	0.004	90.1	0.018
miR-200c	102.0	0.003	102.3	0.002
miR-141	186.8	0.001	127.0	0.003
miR-200a	194.4	0.001	85.6	0.004
miR-200b	221.4	0.002	104.5	0.004
miR-429	345.0	0.003	209.2	0.006
miR-888	400.8	0.001	74.4	0.032
miR-205	806.4	0.005	301.5	0.010

or underexpression in both endometrioid adenocarcinoma and serous adenocarcinoma as compared to benign endometrium are presented in Table II. A total of seven miRNAs was significantly underexpressed in both adenocarcinomas and a total of 13 miRNAs was significantly overexpressed in both adenocarcinomas. The lowest relatively expressed miRNA in both adenocarcinomas was miR-133b while the highest was miR-205, which is consistent with miRNA studies of endometrial cancers (4-7). Similar to other reports, miR-135b and all five members of the miR-200 family (miR-200a, miR-200b, miR-200c, miR-141 and miR-429) were significantly overexpressed in both types of endometrial cancers (4-6).

Endometrioid adenocarcinoma and serous adenocarcinoma each display tumor type-specific dysregulated microRNAs. We next examined how miRNA expression patterns might be used to distinguish between different types of endometrial carcinoma. miRNAs significantly underexpressed or overexpressed in either endometrioid adenocarcinoma or serous adenocarcinoma as compared to benign endometrium are shown in Table III. We identified three times as many miRNAs differentially expressed in endometrioid adenocarcinoma (n=38) than

there were in serous adenocarcinoma (n=12) in comparison to the benign endometrium. These included many of the miRNAs commonly observed in other studies such as miR-155, miR-203 and miR-210, the most frequently observed differentially expressed miRNA in endometrial cancers (3-6). For many of these miRNAs, however, the significance of overexpression varied greatly between the two types of adenocarcinomas. For example, miR-155 was significantly overexpressed in serous adenocarcinoma (p=0.049) but was just above statistical significance in endometrioid adenocarcinoma (p=0.053). Similarly, miR-370 was significantly overexpressed in serous adenocarcinoma (p=0.024) but was not statistically significant in endometrioid tumors (p=0.07); miR-210 was significantly overexpressed in endometrioid adenocarcinoma (p=0.029) but, again, fell short of statistical significance in serous tumors (p=0.10). Taken together, these data indicate that, while these individual miRNAs were overexpressed in both types of adenocarcinomas relative to the benign samples, there was a wide range of expression levels within each tumor type.

microRNA-specific expression validations by qPCR. We chose 10 miRNAs for subsequent validation using miRNA-specific qPCR in an expanded panel of patients. These miRNAs (miR-21, miR-34a, miR-129-3p, miR-135a, miR-135b, miR-137, miR-200c, miR-205, miR-210 and miR-299-3p) were selected based in part upon their expression levels as well as reported cancer-related targets. Furthermore, we specifically chose miRNAs with non-significant as well as those with significant fold change compared with benign endometrium. Of the 20 comparisons of endometrial cancer expression vs. benign endometrium, 16 (80.0%) were concordant with the array results (Fig. 1).

Algorithmically predicted and experimentally validated mRNA targets of dysregulated miRNAs present numerous potential avenues for examining endometrial carcinogenesis. Utilizing both an extensive literature search and the miRNA target prediction algorithm TargetScan, we assembled files composed of algorithmically predicted and experimentally validated mRNA targets for each of the miRNAs significantly dysregulated in both endometrioid and serous adenocarcinomas compared to benign endometrium. We screened the predicted mRNA target files for each miRNA using a filter composed of mRNAs shown to be differentially expressed in endometrial cancers compared to benign endometrium (3,4,11,12). These filtered predicted targets for each of the significantly dysregulated miRNAs are presented in Table IV along with experimentally validated targets for each.

Seventeen microRNAs discriminate between endometrioid and serous adenocarcinomas. In addition to identifying miRNAs that were significantly dysregulated in endometrial cancers compared to benign endometrium, these data also permitted identification of 17 miRNAs that significantly differentiated between endometrioid and serous tumors themselves. These miRNAs are presented in Table V. In 11 of the 17 miRNAs, the fold change for one of the two tumor types compared to benign endometrium was <2, whereas for the remaining six miRNAs there was at least a 2-fold difference in expression compared to benign endometrium in both tumor types. For most of these

Table III. microRNAs significantly underexpressed or overexpressed in either endometrioid and serous adenocarcinomas compared to normal endometrium.

	Fold change	p-value
Underexpressed microRNAs		
Endometrioid tumors		
miR-502-3p	-2.2	0.003
miR-130a	-3.2	0.005
miR-214	-9.1	0.006
miR-218	-6.7	0.010
miR-99a	-7.5	0.010
miR-410	-3.8	0.012
miR-100	-6.6	0.014
miR-199a-3p	-4.4	0.015
miR-424	-3.6	0.020
miR-199a-5p	-3.4	0.021
miR-214*	-5.4	0.024
miR-99a*	-15.4	0.029
let-7c	-7.8	0.030
miR-212	-2.8	0.031
miR-130a*	-32.3	0.035
miR-495	-3.9	0.035
miR-100*	-20.4	0.036
miR-125b	-6.8	0.042
miR-218-2*	-5.2	0.043
miR-502-5p	-3.5	0.043
miR-532-5p	-1.7	0.047
Serous tumors		
miR-370	-37.0	0.024
miR-423-5p	-2.7	0.030
Overexpressed microRNAs		
Endometrioid tumors		
let-7g*	18.1	0.002
miR-181c*	14.7	0.007
miR-516a-3p	8.1	0.014
miR-9	9.3	0.014
miR-203	14.1	0.017
miR-375	333.9	0.021
miR-652	3.5	0.021
miR-146a	43.1	0.022
miR-9*	5.8	0.024
miR-210	9.1	0.029
miR-32	5.9	0.030
miR-148a	4.2	0.036
miR-425	5.4	0.043
miR-592	20.0	0.047
miR-21	6.6	0.048
miR-7-1*	4.8	0.049
miR-107	9.2	0.049
Serous tumors		
miR-31	19.0	0.006
miR-885-5p	23.9	0.015

Table III. Continued.

	Fold change	p-value
miR-490-3p	20.4	0.016
miR-644	7.5	0.034
miR-522	65.1	0.037
miR-519d	37.5	0.041
miR-98	5.9	0.045
miR-425	5.4	0.049
miR-518e	27.3	0.049
miR-155	5.4	0.049

miRNAs it is clear which of the two tumor types is responsible for the differentiating fold change. These distinctions are important in order to better evaluate the nature of miRNA differentiation between the two tumor types.

Discussion

microRNA dysregulation in cancer is a well-established phenomenon (13,14). However, studies of miRNA expression in a wide range of human cancers have shown that the specifics of dysregulation and, by implication, the consequences vary enormously with respect not only to which miRNAs are involved but also to whether specific miRNAs are overexpressed or underexpressed and whether they act as oncogenes or tumor suppressors (15). To date, there have been five surveys of miRNA expression patterns in endometrial cancers (3-7). These studies have employed hybridization array methods and/or qPCR-based methods assessing from 157 to 470 miRNAs in samples ranging from ten to nearly one hundred patients. Cumulative with our data, there are 16 miRNAs in which significant expression dysregulation was observed in at least three studies (data not shown). One of these 16 miRNAs, miR-133b was significantly underexpressed in three of the six studies while the remaining 15 were all significantly overexpressed. miR-133b, and its nearly identical duplicate, miR-133a, target the suspected oncoprotein pyruvate kinase type M2 (PKM2) as well as several other potentially important mRNAs including the oncogenic fascin 1 (FSCN1). In addition, the TargetScan predicted mRNA target taglin 2 (TAGLN2) has previously been shown in other types of cancers to promote invasiveness of tumorigenic cells (16). The TAGLN2 mRNA is also a predicted target of miR-1 and all three of these miRNAs have recently been linked as a co-regulated clique (8). Significant co-regulated underexpression of miR-133a,b and miR-1 would, therefore, influence overexpression of several tumorigenic proteins. It is important to note in this context that another oncogenic target mRNA of miR-1, MET, is also a target of miR-133a,b.

Among the overexpressed miRNAs, miR-210 was reported in all but one of the extant surveys. Overexpression of miR-210 in these cancers is not unexpected as it is well known as the principle hypoxia-responsive miRNA (17) with mRNA targets involved in DNA repair (BLM), cell cycle (PLK1, E2F3) and metabolism (GPD1L1, LDH-B, ATP5A1) among others. As

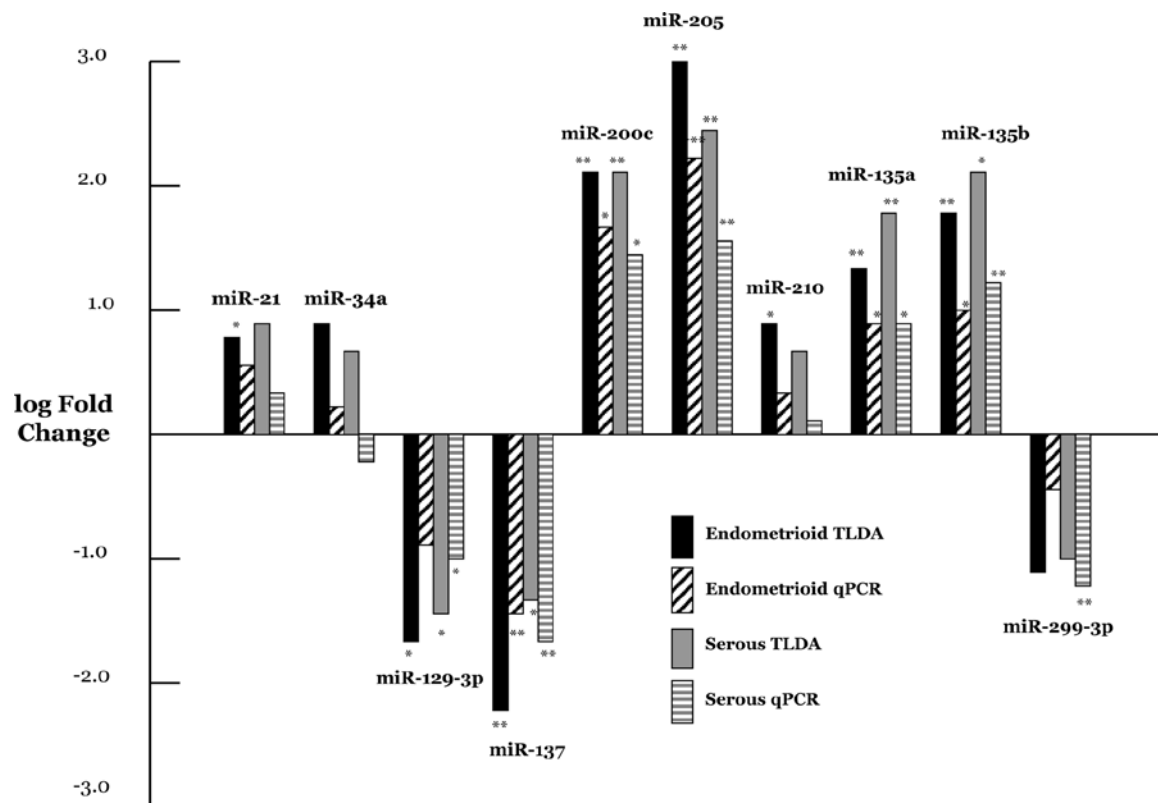


Figure 1. qPCR validations of selected miRNAs. Log fold change is shown for both the TLDA array and the miR-specific qPCR assay for endometrioid adenocarcinoma and serous adenocarcinoma. Significant values (* $p<0.05$ and ** $p<0.01$) are comparing adenocarcinoma with benign endometrium.

Table IV. mRNA targets of microRNAs significantly underexpressed or overexpressed in endometrial adenocarcinomas compared to benign endometrium.

	Target mRNAs
Underexpressed microRNAs	
miR-133a,b	TAGLN2, BCL2L2 , FSCN1, MET, MCL-1, PKM2
miR-137	MITF, STK38, ZAK, Cdc42
miR-504	OLFML2A, p53
miR-1	TRIM2, HDAC4, MET, Pim-1, FOXP1, TAGLN2
miR-125b-2*	CXCL5
miR-129-3p	SOX4 , Cdk6
Overexpressed microRNAs	
miR-205	LRP1 , HER3, ERBB3, PRKCE
miR-888	PPP1R12B, ZAK, REV3L, PGR
miR-135a,b	APC , SFRP4
miR-519a	ZNF238, CXCL12
miR-142-3p	MYLK, ZEB2, BNC2
miR-142-5p	ADAMTS5
miR-15b	BCL2L2
miR-141 ^a	ZEB2, CXCL12, TSHZ3, VEGFA
miR-429 ^b	ZEB1 , ZEB2, LEPR, PPP1R12B

The mRNAs shown in bold were experimentally validated. The remaining mRNA targets were identified as underexpressed or overexpressed in the endometrial adenocarcinomas compared to benign endometrium in Boren *et al* (3), Risinger *et al* (11), Chung *et al* (4) or Smid-Koopman *et al* (12). ^aIncludes family members miR-141, miR-200a, miR-200b. ^bIncludes family members miR-429 and miR-200c.

Table V. microRNAs found to significantly differentiate serous tumors from endometrioid tumors.

microRNA	Fold change ^a	p-value	EA/BE ^b	SA/BE ^b	mRNA target ^f
miR-218	5.9	0.001	-6.7 ^d	-1.4	ZEB2
miR-9	-9.8	0.011	9.3 ^c	-1.1	NEDD4
miR-423-5p	-4.6	0.011	1.7	-2.7 ^c	
miR-542-3p	5.8	0.018	-2.5	2.3	BLCAP, PARP12, FIGF
miR-490-3p	11.6	0.024	1.8	20.4	
miR-504	8.1	0.026	-131.6 ^e	-16.3 ^d	GPC4, OLFML2A
miR-338-3p	10.9	0.030	-1.9	5.9	
miR-9*	-13.6	0.030	-15.4 ^c	-2.3	
miR-146a	-8.9	0.031	43.1 ^c	4.9	ERBB4, ABL2
miR-130a	5.9	0.032	-3.2 ^d	1.8	IGF1, ZAK
let-7c	3.2	0.033	-7.7 ^c	-2.4	
miR-375	-232.7	0.035	333.9 ^c	1.4	PDK1
miR-675	80.2	0.037	-1.5	53.2	RB1
miR-570	27.6	0.041	-1.1	26.0	ABL2, XYLT1, IGF1R
miR-99a/100	4.8	0.041	-7.5	-1.6	PPP3CA, IGF1R
miR-518e	19.8	0.048	1.4	27.3 ^c	
miR-450a	5.4	0.050	-2.1	2.6	

^aFold change is presented as serous tumors compared to endometrioid tumors. ^bFold changes for endometrioid adenocarcinoma (EA) compared to benign endometrium (BE) and for serous adenocarcinoma (SA) compared to benign endometrium (BE) for the same miRNAs. ^cp<0.05, ^dp<0.01, ^ep<0.001. ^fExperimentally validated targets are shown in bold. TargetScan predicted targets were filtered in the same manner as those presented in Table IV.

endometrial adenocarcinomas are solid tumors in which hypoxic microenvironments abound, induction of miR-210 expression through the HIF-1 response mechanism should be an expected outcome.

Similarly represented in the miRNA expression surveys are miR-205 and miR-200a. Recent studies of miR-205 as well as the entire miR-200 family, which is composed of the two polycistrons miR-141/miR-200c and miR-429/miR-200a/miR-220b, show that all of these miRs are coordinately regulated (18) and are involved in mesenchymal transition, tumor invasiveness and metastasis (7,19,20). Expression of these miRNAs is increased in ovarian, bladder and kidney cancers as well but is decreased in prostate and esophageal cancers (7). In addition, miR-182, found to be overexpressed in four of the six surveys, is involved in both tumor invasiveness and anti-apoptosis (21). Overexpression of miR-182, along with miR-210 and miR-200c, was recently reported in ovarian cancers and was linked to tumor progression (22). In addition, miR-182 has been linked to repression of the tumor-suppressor gene FOXO1 in both endometrial (23) and breast cancers (24).

The other miRNA found to be significantly overexpressed in four of the six surveys, miR-203, has been associated with poor survival in pancreatic cancers (25); in ovarian cancers there is evidence that overexpression of this miRNA is due to hypomethylation (26). This latter finding suggests that epigenetic phenomena play an important role in miRNA dysregulation in cancers of the female reproductive tract (27,28).

We also identified a number of significantly dysregulated miRNAs whose expression status in endometrial cancers was

either not well known in general or was described for the first time in this study. For example, miR-129-3p was noted here to be significantly underexpressed in both the endometrioid and serous tumors. Whether this was due to the fact that the expression of this miRNA did not achieve statistical significance in other surveys or that it was not one of the miRNAs surveyed is unknown. However, miR-129-3p was found here to be nearly 50-fold underexpressed in the endometrioid tumors and nearly 40-fold underexpressed in the serous tumors compared to benign endometrium. A validated mRNA target of miR-129-3p in endometrial cancers is the SOX4 oncogene. Underexpression of miR-129-3p leads to overexpression of SOX4 and this relationship has been shown to be under epigenetic control through hypomethylation of the miR-129 CpG island (29).

Another important observation in our study is the significant overexpression of miR-135a,b in both endometrioid and serous tumors. This overexpression could have direct consequences for the Wnt signaling pathway, a pathway well known to be important in a variety of tumors (30,31). The adenomatous polyposis coli gene (APC) is a key component of the Wnt pathway (32-34) and its mRNA has recently been shown to be a direct target of miR-135a,b (35). Consistent with the effects of oncogenic mutations arising in various crucial components of the pathway (31), suppression of APC by miR-135a,b overexpression would have the effect of locking the pathway into a ligand-independent state of constitutive activity. The result of leaving the Wnt switch 'on' would be accumulation of β -catenin and subsequent overexpression of downstream proteins for which β -catenin is

a known transcription factor. One of these downstream targets is c-MYC, which has been shown to be involved in a number of feedback regulatory loops with a host of additional miRNAs that regulate cell progression, angiogenesis and metastasis (36). Thus, overexpression of miR-135a,b in endometrial adenocarcinomas may result in a wide range of downstream miRNA-mediated phenomena.

Another potentially important miRNA dysregulation reported here for the first time is the significant overexpression of miR-888 in both endometrioid and serous tumors compared to benign endometrium. This miRNA lies at Xq27.3 between miR-890 and the two tandem duplication pairs miR-892a,b and miR-891a,b. It has recently been shown that these miRNAs are unique to primate genomes and that they are exclusively expressed in reproductive tissues (37). Consequences of miR-888 overexpression in endometrial cancers are, as yet, unknown. However, one of the TargetScan predicted mRNA targets of miR-888 is the progesterone receptor, PR, which is lost in a substantial proportion of endometrial tumors (38). Moreover, the role of progesterone and progesterone receptors in endometrial carcinogenesis and early treatment is well established (39-41). Thus, investigation of the role of miR-888 in endometrial cancers is warranted.

A number of dysregulated miRNAs are reported here to differentiate endometrioid and serous adenocarcinomas. One of these, miR-675, is particularly notable as it is transcribed as part of the maternally imprinted non-coding RNA H19 (42,43). While its function remains unknown, H19 has been called an 'oncofetal' non-coding RNA (44) since it is primarily expressed during fetal development while it has been shown to re-emerge in a variety of cancers including colorectal (45), hepatocellular (46), testicular (47), esophageal (48), bladder (49) and breast (50). The most important observation relative to the potential role of H19 in endometrial cancer is that H19 expression is clearly associated with serous tumors of the ovary (44). This is consistent with our observation that miR-675 expression was greater than 50-fold overexpressed in the serous tumors compared with the benign endometrium but was not different from the benign endometrium in the endometrial tumors. Thus, miR-675 overexpression in serous tumors is the driver of the 80-fold expression differentiation between serous and endometrioid tumors. H19 transcription and miR-675 expression were recently shown to be significantly correlated in colorectal tumors ($r=0.86$, $p<0.001$) and cultured cells ($r=0.88$, $p<0.001$) (51). Moreover, the one experimentally validated mRNA target of miR-675, retinoblastoma RB1, is a tumor suppressor (51). As H19 and, by extension, miR-675 transcription is maternally imprinted, one mechanism to consider in overexpression in endometrial serous adenocarcinomas is epigenetic. However, it has been shown that the H19 promoter is efficiently repressed by p53 (52) and there is also evidence that H19 expression is negatively regulated by progesterone levels (53). Both p53 mutation and loss of progesterone and its receptor are well characterized phenomena in endometrial cancers so the mechanistic interplay among epigenetics, specific tumor-suppressor mutations and hormones is likely to be complex and warrants future investigation.

We presented evidence of substantial miRNA dysregulation in endometrial endometrioid adenocarcinomas and serous adenocarcinomas compared both to benign endometrium

and to each other. Some of these data, such as the consistent overexpression of miR-205, miR-210 and members of the miR-200 family, suggest that these miRNAs are excellent candidates for development as biomarkers for prognosis and possibly early diagnosis. In addition, the opportunity to offer adjuvant chemotherapy after initial hysterectomy for patients at a particularly high risk for recurrence (as identified from predictive miRNA patterns) has the potential to significantly improve survival in this disease. Expression profiles of other miRNAs, notably miR-135a,b, miR-888 and miR-675, point the way to mechanistic studies that may elucidate previously unknown aspects of endometrial carcinogenesis.

Acknowledgements

This study was supported by NIH Grant R01CA99908 to K.K.L. and in part by the Department of Obstetrics and Gynecology Research Development Fund. We thank Kristina W. Thiel for assistance in the manuscript preparation.

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