Micro-RNAs as diagnostic or prognostic markers in human epithelial malignancies

Angela Hui¹, Christine How¹,², Emma Ito¹,³ and Fei-Fei Liu¹,²,³,4*

Abstract

Micro-RNAs (miRs) are important regulators of mRNA and protein expression; the ability of miR expression profilings to distinguish different cancer types and classify their sub-types has been well-described. They also represent a novel biological entity with potential value as tumour biomarkers, which can improve diagnosis, prognosis, and monitoring of treatment response for human cancers. This endeavour has been greatly facilitated by the stability of miRs in formalin-fixed paraffin-embedded (FFPE) tissues, and their detection in circulation. This review will summarize some of the key dysregulated miRs described to date in human epithelial malignancies, and their potential value as molecular bio-markers in FFPE tissues and blood samples. There remain many challenges in this domain, however, with the evolution of different platforms, the complexities of normalizing miR profiling data, and the importance of evaluating sufficiently-powered training and validation cohorts. Nonetheless, well-conducted miR profiling studies should contribute important insights into the molecular aberrations driving human cancer development and progression.

Introduction

Micro-RNAs (miRs) are important regulators of mRNA and protein expression which play important yet complex roles in human cancers [1]. Their biogenesis and biological networks are complex (Figure 1); they are first synthesized as large RNA precursors, processed in the nucleus into approximately 70 nt pre-miRs, folded into imperfect stem-loop structures, transported to the cytoplasm, whereupon they are incorporated into RISC (RNA-induced silencing complex) (reviewed in [2]). Cleavage by Argonaute-2, then Dicer, results in an approximately 22-nt mature miR duplex; the “guide” strand is retained within the RISC; the “passenger” strand is degraded. Through the seed region (nt 2 to 8), the miR can then bind to the 3′UTR of target mRNA sequences, preventing protein translation, leading to mRNA degradation. More recently, miRs have also been described to target 5′UTR, and even coding regions of transcripts [3]. The current miRDatabase (http://www.mirbase.org) has catalogued more than 1,300 human sequences. Given their ability to target mRNA with imperfect complementarity, and predicted to regulate the expression of approximately one-third of all human transcripts [4], miRs are considered to be among the largest class of gene regulators [5,6].

Multiple mechanisms can mediate miR dysregulations in human cancers, including chromosomal gains or losses [7], mutations of miR located loci [8], or epigenetic aberrations [8]. Any misstep in miR biogenesis (Figure 1) can also affect miR expression [9,10], exemplified by the down-regulation of Drosha and Dicer being associated with worse survival in ovarian, lung, and breast cancers [11]. MiRs can be either over- or under-expressed, functioning as tumour suppressors or oncogenes, depending on their downstream target genes [12]. MiR-15a and miR-16-1 are two of the first described down-regulated miRs in chronic lymphocytic leukemia [13], both target Bcl-2 [14]; thus their absence inhibits apoptosis. Alternatively, miR-21, one of the most commonly over-expressed miRs in solid malignancies, targets PTEN [15] and pro-apoptotic genes [16,17]; hence pro-survival signals dominate.

Micro-RNA as bio-markers in epithelial cancers

Biomarkers are biological indicators of disease states, utilized to define tumor subtypes, or assess efficacy of interventions [18]. Useful biomarkers can provide insights into tumorigenesis, and facilitate the
development of improved therapies. Some current biomarkers include prostate-specific antigen (PSA) [19], carcinoembryonic antigen (CEA) [20], CA125 [21], and α-fetoprotein [22,23]. More recently, the role of mRNA or miRs as cancer biomarkers have also been investigated and developed. The prototype mRNA signature is Oncotype DX, the 21-gene set utilized to predict recurrence risks for patients with breast cancer [24].

MiR expression profilings could distinguish different cancer types [12], classify sub-types of prostate or breast cancers [25], identify the tissue origin of tumors [26], and facilitate the diagnosis of colon [27], or lung cancers [28]. MiRs can also predict outcome, such as let-7a [28].
and miR-155 [29] for lung cancer, and select patients for targeted therapy (for example, breast cancer [30]). Finally, predictive miR signatures have been reported for several malignancies, such as lung [31-34], hepatocellular [35], esophageal [36], gastric [37], prostate [38], cervical [39], and colon cancers [40].

**Micro-RNAs in FFPE samples**

The ability to examine FFPE specimens, a universally standard histologic processing procedure, allows the expeditious discovery and evaluation of potential biomarkers, given their possible link to clinical databases with mature follow-up. Transcript (miRNA) profiling is technically challenging with FFPE samples due to significant RNA degradation during formalin fixation [41,42], and continued deterioration with storage over time [43]. In contrast, miRs are not significantly affected by fixation, and can be readily extracted from FFPE samples due to their small sizes (approximately 22 nt in length) and remarkable stability [44,45]. Hence, this greatly enhances the ability to evaluate miRs as cancer biomarkers, leading to a multitude of reports describing miR expressions in many epithelial malignancies, summarized as per anatomical site in Table 1.

As already mentioned, miR-21 up-regulation is the most commonly observed aberrant miR in human cancers, with oncogenic consequences [46] (Table 1). It was first reported in glioblastoma [16], but also described for epithelial cancers such as head and neck, breast, colon, lung, prostate, and others [12,44,47]; often associated with worse outcome [40]. Over-expression of miR-21 has been shown to increase cell proliferation, migration, invasion and survival [48]; in contrast, suppression of miR-21 induced apoptosis and decreased cell proliferation and invasion [49].

Mir-155 is another commonly dysregulated miR, wherein the majority of studies report its over-expression associated with tumorigenesis in lymphomas, breast, lung, colon, pancreatic cancers, and others [50]. Aside from these two miRs, there is usually minimal overlap of dysregulated miRs described among different studies, even when examining the same cancer type; the same variation as previously observed for mRNA profiling. Perhaps this might relate to multiple redundant

| Table 1 Micro-RNAs as Diagnostic or Prognostic markers in FFPE Samples |
|----------------|----------------|----------------|----------------|
| Cancer | Diagnostic miRs | Prognostic miRs | References |
| Head and Neck Squamous cell carcinoma | miR-16, -20a, -21, -106b, -142-3p, -155, -423, let-7i (up); miR-10a, -125b, -375 (down) | miR-451 (up) | [47] |
| Breast cancer | miR-21 (up); let-7a, miR-145, -205 (down) | miR-21 (up) | [70] |
| Lung cancer | miR-21, -155, -191, -196a (up); miR-125b, -221 (down) | miR-16 (up) | [72] |
| Gastrointestinal Cancers | miR-106a (up) | miR-10b, -21, -223, -338 (up); miR-30a-5p, -126, let-7a (down) | [74] |
| Gastric cancer | miR-31 (down) | miR-10b-21, -223, -338 (up); miR-30a-5p, -126, let-7a (down) | [75] |
| Pancreatic cancer | miR-452, -105, -127, -518a-2, -187, -30a-3p (up) | miR-196a-2 (up) | [76] |
| | miR-21, -155 (up) | miR-200c (down) | [77] |
| | miR-21, -221, -222, let-7a (up) | | [78] |
| Gynecological Cancers | | | |
| Cervical cancer | miR-9, -200a | miR-9, -200a | [39] |
| Ovarian cancer | miR-223 (up); miR-9 (down) | miR-223 (up); miR-9 (down) | [80] |
| | miR-200a, -200b, -429 (down) | miR-200a, -200b, -429 (down) | [81] |
| | miR-23a, -27a (up) | miR-23a, -27a (up) | [82] |
| Prostate cancer | miR-125b (up) | miR-29b (up) | [84] |
| | miR-15a, -16 (down) | | [85] |
| | miR-184 (up); miR-146a (down) | miR-124 (up); miR-146a (down) | [86] |
| | miR-203 (down) | miR-203 (down) | [87] |
| | miR-34c (down) | miR-34c (down) | [88] |
| | | miR-34c (down) | [89] |
Table 2  Micro-RNAs as Non-invasive Biomarkers in Blood Samples

| Cancer                        | Samples            | Diagnostic miRs                                                                 | Prognostic miRs                                                                 | References |
|-------------------------------|--------------------|---------------------------------------------------------------------------------|----------------------------------------------------------------------------------|------------|
| Head and Neck Squamous cell carcinoma | Plasma             | miR-184 (up)                                                                     |                                                                                  | [90]       |
|                               | Plasma             | miR-24 (up)                                                                      |                                                                                  | [91]       |
|                               | Plasma             | miR-31 (up)                                                                      |                                                                                  | [92]       |
|                               | Plasma             | miR-181 (up)                                                                      | miR-181 (up) correlated with poor survival, lymph-node metastasis, and vascular invasion | [93]       |
| Breast cancer                 | Serum              |                                                                                  | miR-155 (up) in PR+ve patients                                                   | [68]       |
|                               | Plasma             | miR-425*(up); let-7d*(down)                                                      |                                                                                  | [94]       |
|                               | Serum              | miR-10b, -34a, -155 (up)                                                         | miR-10b, -34a, -155 (up) correlated with metastasis                              | [95]       |
|                               | Serum              | miR-21, -106a, -155 (up); miR-126, -199a, -335 (down)                             |                                                                                  | [96]       |
|                               | Whole blood        | miR-195 (up)                                                                     | miR-21, -10b (up) in ER -ve patients; let-7a (down) in lymph node +ve patients   | [97]       |
|                               | Serum              |                                                                                  | miR-21 (up) correlated with visceral metastasis                                  | [98]       |
| Non-small-cell lung carcinoma | Pooled serum       | miR-25, -223 (up)                                                                |                                                                                  | [99]       |
|                               | Exosome from plasma| miR-17-3p, -21, -106a, -146, -155, -191, -192, -203, -205, -210, -212, -214 (up) | miR-486, -30d (up); miR-1, -499 (down) associated with overall survival          | [58]       |
|                               | Pooled serum       |                                                                                  | miR-10b (up) associated with lymph node metastasis                              | [100]      |
|                               | Serum              | miR-10b, -155 (up)                                                               |                                                                                  | [101]      |
|                               | Vesicles of plasma samples | let-7d, let-7f, miR-223, -383, -192, -30e-5p, -301, -572, -20b, -345 (down)      | let-7f, miR-30e-3p (up) associated with poor outcome                             | [59]       |
| Gastrointestinal Cancers      | Colorectal cancer  |                                                                                  |                                                                                  | [102]      |
|                               | Plasma             | miR-17-3p, -92 (up)                                                              |                                                                                  | [103]      |
|                               | Plasma             | miR-29a, -92a (up)                                                               |                                                                                  | [104]      |
|                               | Plasma             | miR-221 (up)                                                                     | miR-221 (up) associated with poor overall survival                              | [105]      |
| Esophageal                    | Serum              | miR-10a, -22, -100, -148b, -223, -133a, -127-3p (up)                              |                                                                                  | [106]      |
| Gastric cancer                | Serum              | miR-1, -20a, -27a, -34, -423-5p (up)                                             | miR-1, -20a, -27a, -34, -423-5p (up)                                            | [107]      |
|                               | Plasma             | miR-17-5p, -21, -106a, -106b (up); let-7a (down)                                 |                                                                                  | [108]      |
| Hepatocellular carcinoma      | Serum              | miR-500 (up)                                                                     |                                                                                  | [109]      |
|                               | Serum              | miR-1, -25, -92a, -206, -375, -let-7f (up) (HBV-associated); miR-25, -375, and let-7f (up) (HCC detection) |                                                                                  | [110]      |
|                               | Serum              | miR-21, -122, -223 (up)                                                          |                                                                                  | [111]      |
|                               | Serum              | miR-122 (up)                                                                     |                                                                                  | [112]      |
|                               | Serum              | miR-885-5p (up)                                                                  |                                                                                  | [113]      |
|                               | Serum              | miR-16 (down) combined with AFP, AFP DCP increases specificity of HCC detection  |                                                                                  | [63]       |
| Pancreatic cancer             | Plasma             | miR-21, -210, -155, -196a (up)                                                    |                                                                                  | [62]       |
|                               | Plasma             | miR-210 (up)                                                                     |                                                                                  | [60]       |
|                               | Plasma             | miR-21 (up)                                                                      |                                                                                  | [61]       |
|                               | Serum              | miR-21, -155, -196a (up)                                                         | miR-196a (up)                                                                     | [57]       |
| Ovarian cancer                | Exosome from serum | miR-21, -141, -200a, -200c, -200b, -203, -205, -214 (up) correlated with stage  | miR-21, -141, -200a, -200c, -200b, -203, -205, -214 (up) correlated with stage  | [64]       |
|                               | Serum              | miR-21, -92, -93, -126, -29a (up); miR-155, -127, -99b (down)                    |                                                                                  | [114]      |
|                               | Whole blood        | miR-30c1* (up); miR-342-3p, -181a*, -450b-5p (down)                              |                                                                                  |            |
Serum miR-21 (up) associated with resistant to docetaxel-based chemotherapy

| Table 2 Micro-RNAs as Non-invasive Biomarkers in Blood Samples (Continued) |
| Prostate cancer Serum | miR-141 (up) | miR-100, -125b, -141, -143, -296 (up) |
| Serum | miR-16, -92a, -103, -107, -197, -34b, -328, -485-3p, -486-5p, -92b, -574-3p, -636, -640, -766, -885-5p (up) |
| Serum | miR-20b, -874, -1274a, -1207-5p, -93, -106a (up); miR-223, -26b, -30c, -24 (down) |
| miR-24 (down) in metastatic cancers |
| Serum | miR-375, -141 (up) |
| miR-21 (up) |
| Serum | miR-21, -141, -221 (up) |

Micro-RNAs in blood samples

There is emerging interest in the investigation of miRs as non-invasive biomarkers in circulating blood. This was first described in B-cell lymphoma, reporting elevated levels of miR-155, -210 and -21 in patients’ sera, with miR-21 associating with relapse-free survival [53]. In epithelial cancers, Mitchell et al. first identified tumor-derived miRs in plasma samples, and suggested that variations in miR abundance reflected tumor burden [54]. MiRs have been detected as free miRs in either plasma or serum, or contained within microvesicles such as exosomes; the latter being minute, natural membrane vesicles secreted by a variety of different cell types [55]. In addition to miRs, exosomes also carry intact and functional mRNA [56], with the probable purpose of transferring information and signals throughout the body [55]. Association of epithelial cancer and exosome miRs was first illustrated in ovarian cancer, wherein tumor-derived miR profiles strongly correlated with levels of peripheral blood-derived exosomal miRs [57]. Similar observations have also been reported for lung cancer [58,59].

As shown in Table 2, the list of potential blood miR biomarkers is even more diverse than those from tissue studies (Table 1). The greatest degree of overlap was reported for miR-21, miR-196a and miR-210 from four different pancreatic cancer studies [60-63]. As observed for the tissue studies, miR-21 and miR-155 are also the two most common aberrant miRs in circulation with putative diagnostic and prognostic value (Table 2). However, down-regulation of miR-155 was reported in one serum study of ovarian cancer [64]. There is some controversy surrounding miR-155; the majority of reports suggest an oncogenic role; however, in a lung cancer study, its up-regulation predicted for worse outcome for adenocarcinomas, but improved outcome for squamous cell carcinoma patients [65]. One possible tumor suppression function for miR-155 was demonstrated in miR-155 deficient mice, which appeared to reduce oncogenic translocations generated by activation-induced cytidine deaminase (AICD) [66]. Micro-RNA expression levels in circulation can also relate to hormone receptor status in that estrogen negative breast cancer sera samples had higher levels of miR-21 and miR-10b [67]; in contrast, miR-155 was detected for progesterone receptor positive patients [68].

In summary, there are multitudes of reports describing the potential value of miRs as both diagnostic and prognostic bio-markers for human malignancies. None to date, however, have been translated into clinical practice, likely a reflection of its complex biology, and lack of validation studies using appropriately-powered sample sizes.

Challenges of Micro-RNA as bio-markers

Despite the promising data supporting the potential value of miRs as biomarkers, many challenges remain. First, robust platforms, as well as appropriate statistical and bio-computational analyses must be utilized in order to identify potential candidate miR signatures for predicting outcome. Furthermore, such candidate signatures must be validated using independent cohorts statistically powered to confirm the existence of a predictive signature. Second, the selection of the appropriate reference controls is extremely important for normalization of biological variation. Recent reports have observed that some of the commonly-utilized reference miRs, such as RNU43, RNU44 or RNU48, in fact fluctuate with the biological entity of interest [69]; hence it is critical to determine the most stable miRs for each condition under examination. Third, it is conceivable that given the “upstream” effects of miRs, and their biological complexities which we are just starting to unravel, their pattern of expression might be too subtle and variable to serve as robust predictive signatures. Nonetheless, pursuit of investigations such as prognostic signatures, or their measurements in sera/plasma are definitely warranted, particularly when using appropriately-sized population cohorts.
Conclusion
Application of the potential role of miRs as molecular bio-markers in human epithelial malignancies is widely supported by the large number of studies conducted in different cancers. There is great promise that they will aid in the early diagnosis of cancer, and the development of personalized therapies. Further research into miR biogenesis and regulation, along with functional target identifications will definitely lead to an improved understanding of the complex mechanisms underlying human cancer development and progression.

Abbreviations
AICD: activation-induced cytidine deaminase; CEA: carcinoembryonic antigen; FFPE: formalin fixed and paraffin embedded; miR: micro-RNAs; PSA: prostate-specific antigen; RISC: RNA-induced silencing complex.

Author details
1Ontario Cancer Institute (OCI)/Campbell Family Cancer Research Institute (CFCRI); University Health Network (UHN); Toronto, ON, Canada. 2Department of Medical Biophysics; University of Toronto; Toronto, ON, Canada.

Authors’ contributions
AH performed the literature review and participated in manuscript preparation. FL designed and edited the manuscript. All authors read and approved the final manuscript.

Competing interests
FF Liu is a Section Editor for BMC Cancer.

Received: 8 June 2011 Accepted: 30 November 2011
Published: 30 November 2011

References
1. Garzon R, Calin GA. Croce CM: MicroRNAs in cancer. Annu Rev Med 2009, 60:167-179.
2. Kai ZS, Pasquonelli AE: MicroRNA assassins: factors that regulate the disappearance of miRNAs. Nat Struct Mol Biol 2010, 173-10.
3. Breving K, Esquela-Kerscher A: The complexities of microRNA regulation: Mirroring around the rules. Int J Biochem Cell Biol 2009, 42:1316-1329.
4. Griffiths-Jones S, Grocock RJ, van Dongen S, Bateman A, Enright AJ: miRBase: microRNA sequences, targets and gene nomenclature. Nucleic Acids Res 2006, 34:D104-144.
5. Bentwich I, Avniel A, Karov Y, Aharonov R, Gilad S, Barad O, Barzilai A, Einat H, Einav U, Mint E, Sharon E, Spector Y, Bentwich Z: Identification of hundreds of conserved and nonconserved human microRNAs. Nat Genet 2005, 37:766-770.
6. Stefani G, Slack FJ: Small non-coding RNAs in animal development. Nat Rev Mol Cell Biol 2008, 9:219-230.
7. Calin GA, Croce CM: MicroRNA signatures in human cancers. Nat Rev Cancer 2006, 6:855-866.
8. Calin GA, Ferracin M, Cimmino A, Di Leva G, Shimizu M, Wojcik SE, Iorio MV, Vissone R, Severi N, Fabbri M, Iuliano R, Palmroth T, Pichiorri F, Roldo C, Garzon R, Sevignani C, Rassenti L, Alder H, Volinia S, Liu CG, Kipps TJ, Negrini M, Croce CM: A MicroRNA signature associated with prognosis and progression in chronic lymphocytic leukemia. N Engl J Med 2005, 353:1793-1805.
9. Wiemer EA: The role of microRNAs in cancer: no small matter. Eur J Cancer 2007, 43:1529-1544.
10. Zhang B, Pan X, Cobb GP, Anderson TA: microRNAs as oncogenes and tumor suppressors. Dev Biol 2007, 302:1-12.
11. Merritt WM, Lin YG, Han LY, Kamat AA, Spannuth WA, Schmandt R, Urbauer D, Pennacchio LA, Cheng JF, Nick AM, Deavers MT, Moudad-Zeidan A, Wang H, Mueller P, Lenburg ME, Gray JW, Mok S, Birrer MJ, Lopez-Berestein G, Coleman RL, Bar-Eli M, Sood AK: Dicer, Drosha, and outcomes in patients with ovarian cancer. N Engl J Med 2008, 359:2641-2650.
12. Volinia S, Calin GA, Liu CG, Ambs S, Cimmino A, Petrocca F, Sorice R, Iorio M, Roldo C, Ferracin M, Pue rtel RL, Yaniahara N, Lanza G, Scarpa A, Vecchione A, Negrini M, Harris CC, Croce CM: A microRNA expression signature of human solid tumors defines cancer gene targets. Proc Natl Acad Sci USA 2006, 103:2257-2261.
13. Calin GA, Dumitru CD, Shimizu M, Bichi R, Zupo S, Noch E, Aldler H, Rattan S, Keating M, Rai K, Rassenti L, Kipps T, Negrini M, Bullrich F, Croce CM: Frequent deletions and down-regulation of micro-RNA genes miR15 and miR16 at 13q14 in chronic lymphocytic leukemia. Proc Natl Acad Sci USA 2002, 99:15524-15529.
14. Cimmino A, Calin GA, Fabbri M, Iorio MV, Ferracin M, Shimizu M, Wojciech SE, Angelani RI, Zupo S, Dono M, Rassenti L, Alder H, Volinia S, Liu CG, Kipps TJ, Negrini M, Croce CM: miR-15 and miR-16 induce apoptosis by targeting BCL2. Proc Natl Acad Sci USA 2005, 102:13944-13949.
15. Meng F, Henson R, Wehbe-Janek H, Ghoshal K, Jacob ST, Patel T: Micr0RNA-21 regulates expression of the PTEN tumor suppressor gene in human hepatocellular cancer. Gastroenterology 2007, 133:647-658.
16. Chan JA, KriskoV S, Kosik KS: MicroRNA-21 is an antia apoptotic factor in human glioblastoma cells. Cancer Res 2005, 65:6029-6033.
17. Si ML, Zhu S, Wu H, Lu Z, Wu F, Mo YY: miR-16-mediated tumor growth. Oncogene 2007, 26:2799-2803.
18. Ratain MJ, Glassman RH: Biomarkers in phase I oncology trials: signal, noise, or expensive distraction? Clin Cancer Res 2007, 13:6545-6548.
19. Balk SP, Ko VJ, Bubley GJ: Biology of prostate-specific antigen. J Clin Oncol 2003, 21:383-391.
20. Thomas SN, Tong Z, Stebe KJ, Konstantopoulos K: Identification, characterization and utilization of tumor cell selectin ligands in the design of colon cancer diagnostics. Biothecology 2009, 46:207-225.
21. Osman N, O’Leary N, Mulcahy E, Barrett N, Wallis E, Hickey K, Gupta R: Correlation of serum CA125 with stage, grade and survival of patients with epithelial ovarian cancer at a single centre. J Med 2008, 101245-247.
22. Heyward WL, Lanier AP, Bender TR, McMahan BJ, Kilkenney S, Procopi RK, Kline KT, Silipreri DR, Maynard JE: Early detection of primary hepatocellular carcinoma by screening for alpha-fetoprotein in high-risk families. A case-report. Lancet 1981, 23161-162.
23. Lange PH, Vogelzang NJ, Goldman A, Kennedy BJ, Fraley EE: Marker half-life analysis as a prognostic tool in testicular cancer. J Urol 1982, 128:708-711.
24. Palc S, Shak S, Tang G, Kim C, Baker J, Cronin M, Baehner FL, Walker MG, Watson D, Park T, Hiller W, Fisher ER, Wickerham DL, Bryant J, Wolmark N: A mi gene assay to predict recurrence of tamoxifen-treated, node-negative breast cancer. N Engl J Med 2004, 351:2817-2826.
25. Mattie MD, Benz CC, Bowers J, Sensinger K, Wong L, Scott GK, Fedele V, Ginzinger D, Gets R, Haqq C: Optimized high-throughput microRNA expression profiling provides novel biomarker assessment of clinical prostate and breast cancer biologies. Mol Cancer 2006, 5:24.
26. Liu J, Getz G, Miska EA, Alvarez-Saavedra E, Lamb J, Picard D, Sweet-Cordero A, Ebert BL, Mak RH, Ferrando AA, Downing JR, Jacks T, Horvitz HR, Golub TR: MicroRNA expression profiles classify human cancers. Nature 2005, 435:834-838.
27. Michael MZ, Connor SM, van Holst Pellekaan NG, Young GP, James RJ: Reduced accumulation of specific microRNAs in colorectal neoplasia. Mol Cancer Res 2003, 1:862-869.
28. Takamizawa J, Konishi H, Yanagisawa K, Tomida S, Osada H, Endoh H, Harano T, Yatabe Y, Kawahara K, Sekido Y, Takahashi T, Hanada K, Yatabe Y, Nagino M, Nimura Y, Matsudomi T, Yamada T: Reduced expression of the let-7 microRNAs in human lung cancers in association with shortened postoperative survival. Cancer Res 2004, 64:3753-3756.
29. Hayashiha Y, Osada H, Tatematsu Y, Yamada H, Yanagisawa K, Tomida S, Yatabe Y, Kawanaka T, Sekido Y, Takahashi T: A polycistrionic microRNA cluster, miR-17-92, is overexpressed in human lung cancers and enhances cell proliferation. Cancer Res 2005, 65:9628-9632.
30. Chen JQ, Russo J: ERalpha-negative and triple negative breast cancer: molecular features and potential therapeutic approaches. Biochim Biophys Acta 2009, 1796:162-175.

31. Du L, Schagamen JJ, Imov, Girard L, Hammond SM, Minna JD, Gazdar AF, Pertemtiselli A: MicroRNA expression distinguishes SCLC from NSCLC lung tumor cells and suggests a possible pathological relationship between SCLCs and NSCLCs. Exp Clin Cancer Res 2010, 29:75.

32. Raponi M, Dossey L, Jaklose T, Wu X, Chen G, Fan H, Beer DG: MicroRNA classifiers for predicting prognosis of squamous cell lung cancer. Cancer Res 2009, 69:5776-5783.

33. Yanaihara N, Caplen N, Bowman E, Seike M, Kumamoto K, Yi M, Stephens RM, Okamoto A, Yokota J, Tanaka T, Calin GA, Liu CG, Croce CM, Harris CC: Unique microRNA molecular profiles in lung cancer diagnosis and prognosis. Cancer Cell 2006, 9:189-198.

34. Yu SL, Chen HY, Chang GC, Chen CY, Chen HW, Singh S, Cheng CL, Yu CJ, Yanaihara N, Caplen N, Bowman E, Seike M, Kumamoto K, Yi M, Stephens RM, Okamoto A, Yokota J, Tanaka T, Calin GA, Liu CG, Croce CM, Harris CC: Identification of metastasis-related microRNAs in hepatocellular carcinoma. Hepatology 2008, 47:897-907.

35. Guo Y, Chen L, Wang L, Zhou F, Shi S, Feng X, Li B, Meng X, Ma X, Luo M, Shao K, Li N, Qiu B, Michelson K, Cheng J, He J: Distinctive microRNA profiles relating to patient survival in esophageal squamous carcinoma. Cancer Res 2008, 68:36-33.

36. Li X, Zhang Y, Ding J, Wu K, Fan D: Survival prediction of gastric cancer by a seven-microRNA signature. Gut 2010, 59:579-585.

37. Tong AW, Fulgham P, Jay C, Chen P, Khalil I, Liu S, Senzer N, Bludac AH, Han J, Nemunaitis J: MicroRNA profile analysis of human prostate cancers. Cancer Gene Ther 2008, 15:206-216.

38. Zhu W, Qin W, Atasoy U, Sauter ER, Hurvitz PN, Riner J, Reddy M, Trichur R, Sweeney KJ, Newell J, Kerin MJ: Circulating microRNAs in breast cancer. Cell Transl Med 2011, 3:979-987.

39. Kimmelman AC, Bui HK, Lu W, Buchkovich KM, Cheng C, Lu Y, Argo CM, Metzger R, Cano DA, Vousden KH: MicroRNA expression distinguishes SCLC from NSCLC. Proc Natl Acad Sci USA 2008, 105:10513-10518.

40. Concucci E, Raccetti G, Rupnik M, Meldolesi J: The regulated exocytosis of enlargesosomes is mediated by a SNARE machinery that includes VAMP4. J Cell Sci 2008, 121:2983-2991.

41. Valadi H, Ekstrom K, Bossios A, Sjostrand M, Lee JJ, Lotvall JO: Exosome-mediated transfer of miRNAs and microRNAs is a novel mechanism of genetic exchange between cells. Nat Cell Biol 2004, 6:654-659.

42. Taylor DO, Gercel-Taylor C: MicroRNA signatures of tumor-derived exosomes as diagnostic biomarkers of ovarian cancer. Gynecol Oncol 2010, 110:19-21.

43. Bockisch A, Kachelriess M, Kuenen JG, Nooter K, van Houwelingen HC, van de Velde CJ: Circulating microRNAs as stable blood-based markers for cancer detection. Proc Natl Acad Sci USA 2008, 105:10513-10518.

44. Mitchel P, Parkin RK, Kroh EM, Fritz BR, Wyman SK, Pogosova-Agadjanyan EL, Peterson A, Noteboom J, O’Briant KC, Allen A, Lin DW, Urban N, Drescher CW, Knudsen BS, Shirewalt DL, Gentelman R, Vessella RL, Nelson PS, Martin DB, Tewari M: Circulating microRNAs as stable blood-based markers for cancer detection. Cancer. 2010, 110:19-21.

45. Rabinovits G, Gercel-Taylor C, Day JM, Taylor DD, Kloecker GH: Exosomal microRNA: a diagnostic marker for lung cancer. Clin Lung Cancer 2009, 10:42-46.

46. Silva J, Garcia V, Zaballos A, Provencio M, Lombardia L, Almonacid L, Garcia JM, Dominguez G, Perla C, Diaz R, Herrera M, Varela A, Bonilla F: Vesicle-related microRNAs in plasma of nonsmall cell lung cancer patients and correlation with survival. Eur Respir J 2011, 37:617-623.

47. Ali S, Almanhika K, Chen W, Philip PA, Safark KH: Differentially expressed miRNAs in the plasma may provide a molecular signature for aggressive pancreatic cancer. Am J Transl Res 2011, 3:28-47.

48. Kong X, Du Y, Wang G, Gao J, Gong Y, Li L, Zhang Z, Zhu J, Jing Q, Qin Y, Li Z: Detection of differentially expressed microRNAs in serum of pancreatic ductal adenocarcinoma patients: miR-196a could be a potential marker for poor prognosis. Dig Dis Sci 2011, 56:602-609.

49. Ho AS, Huang X, Cao H, Christman-Skeller C, Berneth K, Le QT, Koong AC: Circulating miR-210 as a Novel Hypoxia Marker in Pancreatic Cancer. Cancer Transl Oncol 2010; 3:109-113.

50. Wang J, Chen J, Chang P, LeBlanc A, Li D, Frazier ML, Killary AM, Sen S: MicroRNAs in plasma of pancreatic ductal adenocarcinoma patients as novel blood-based biomarkers of disease. Cancer Prev Res (Phila) 2009, 2:807-813.

51. Reischke K, Alder H, Hagan JP, Richardson DL, Croce CM, Cohn DE: The detection of differentially expressed microRNAs from the serum of ovarian cancer patients using a novel real-time PCR platform. Gynecol Oncol 2009, 115:53-59.

52. Donnem T, Eklo K, Berg T, Sorbye SW, Lonvik K, Al-Saad S, Al-Shibi K, Andersen S, Stenvold H, Bremsen BM, Busund LT: Prognostic impact of miR-155 in non-small cell lung cancer evaluated by in situ hybridization. J Transl Med 2011, 9:6.

53. Donnert Y, McCabe RM, Jankovic M, Gazzarian Y, Thai TH, Robbiano DI, Duglipo M, Reina San-Martín B, Heidkamp G, Schwikert GA, Eischen T, Rajewsky K, Nussenzweig MC: MicroRNA-155 suppresses activation-induced cytolytic deamidase-mediated Myc-Igh translocation. Immunity 2008, 29:630-639.

54. Heneghan HM, Miller N, Lowery AJ, Sweeney KJ, Newell J, Kerin MJ: Circulating microRNAs as novel minimally invasive biomarkers for breast cancer. Ann Surg Oncol 2010, 257:499-505.

55. Zhu W, Qin W, Atasoy U, Sauter ER, Hurvitz PN, Riner J, Reddy M, Trichur R, Sweeney KJ, Newell J, Kerin MJ: Circulating microRNAs as novel minimally invasive biomarkers for breast cancer. Ann Surg Oncol 2010, 257:499-505.
nucleolar RNAs commonly used for microRNA normalisation correlate with tumour pathology and prognosis. Br J Cancer 2010;104:1168-1177.

70. Sempere LF, Christensen M, Shahrazioglu A, Bak M, Heath CV, Schwartz G, Wells W, Kauppinen S, Cole CN. Altered MicroRNA expression confined to specific epithelial cell subpopulations in breast cancer. Cancer Res 2007, 67:1162-11620.

71. Yan LX, Huang XF, Qiao O, Huang MY, Deng L, Wu QL, Zeng YX, Shao JY. MicroRNA-21 overexpression in human breast cancer is associated with advanced clinical stage, lymph node metastasis and patient poor prognosis. RNA 2008, 14:2388-2360.

72. Lebaniou D, Benjamin H, Gilad S, Ezagouri M, Dov A, Ashkenazi N, Gefen N, Izraeli S, Rechavi G, Pass H, Nonaka D, Li J, Spector Y, Rosenfeld N, Chajut A, Cohen D, Aharonov R, Manshali M. Diagnostic assay based on hsa-miR-205 expression distinguishes squamous from nonsquamous non-small-cell lung carcinoma. J Clin Oncol 2009, 27:2030-2037.

73. Navarro A, Diaz T, Gallardo E, Vinolas N, Marrades RM, Gallardo E, Vinolas N, Marrades RM, Gel B, Campayo M, Lebanony D, Benjamin H, Gilad S, Ezagouri M, Dov A, Ashkenazi K, Gefen N, Izraeli S, Rechavi G, Pass H, Nonaka D, Li J, Spector Y, Rosenfeld N, Chajut A, Cohen D, Aharonov R, Manshali M. Diagnostic assay based on hsa-miR-205 expression distinguishes squamous from nonsquamous non-small-cell lung carcinoma. J Clin Oncol 2009, 27:2030-2037.

74. Xiao B, Guo J, Miao Y, Jiang Z, Huan R, Zhang Y, Li D, Zhong J: The miR-15a-miR-16-1 cluster controls prostate cancer by targeting multiple core cancer genes. Cancer Res 2010, 70:1168-1177.

75. Zhang C, Wang C, Chen X, Yang C, Li K, Wang J, Dai J, Hu Z, Zhou X, Zhang C, Wang C, Chen X, Yang C, Li K, Wang J, Dai J, Hu Z, Zhou X, Zhang C, Wang C, Chen X, Yang C, Li K, Wang J, Dai J, Hu Z, Zhou X. Specific epithelial cell subpopulations in breast cancer. Clin Chem Lab Med 2010, 48:411-424.

76. Saini S, Majid S, Yamamura S, Tabatabi ZL, Suh SO, Shahyani V, Chen Y, Deng G, Tanaka Y, Dahlia R. Regulatory role of miR-203 in prostate cancer progression and metastasis. Clin Cancer Res 2011, 17:5287-5298.
Circulating microRNAs in plasma of patients with gastric cancers. *Br J Cancer* 2010, 102:1174-1179.

108. Yamamoto Y, Kosaka N, Tanaka M, Kozumi F, Kanai Y, Mizutani T, Murakami Y, Kuroda M, Miyajima A, Katou T, Ochiya T: MicroRNA-500 as a potential diagnostic marker for hepatocellular carcinoma. *Biomarkers* 2009, 14:529-538.

109. Li LM, Hu ZB, Zhou ZX, Chen X, Liu FY, Zhang JF, Shen HB, Zhang CY, Zen K: Serum microRNA profiles serve as novel biomarkers for HBV infection and diagnosis of HBV-positive hepatocarcinoma. *Cancer Res* 2010, 70:9798-9807.

110. Xu J, Wu C, Che X, Wang L, Yu D, Zhang T, Huang L, Li H, Tan W, Wang C, Lin D: Circulating microRNAs, miR-21, miR-122, and miR-223, in patients with hepatocellular carcinoma or chronic hepatitis. *Mol Carcinog* 2011, 50:136-142.

111. Morita K, Taketomi A, Shirabe K, Umeda K, Kayashima H, Ninomiya M, Uchiyama H, Soejima Y, Maehara Y: Clinical significance and potential of hepatic microRNA-122 expression in hepatitis C. *Liver Int* 2011, 31:474-484.

112. Gui J, Tian Y, Wen X, Zhang W, Zhang P, Gao J, Run J, Tian L, Jia X, Gao Y: Serum microRNA characterization identifies miR-885-5p as a potential marker for detecting liver pathologies. *Clin Sci (Lond)* 2011, 120:183-193.

113. Qu KZ, Zhang K, Li H, Alfihal NH, Albstar M: Circulating MicroRNAs as Biomarkers for Hepatocellular Carcinoma. *J Clin Gastroenterol* 2011, 45:355-360.

114. Hausler SF, Keller A, Chandran PA, Ziegler K, Zipp K, Heuer S, Krockenberger M, Engel JB, Honig A, Scheffler M, Dietl J, Wischhusen J: Whole blood-derived miRNA profiles as potential new tools for ovarian cancer screening. *Br J Cancer* 2010, 103:693-700.

115. Lodes MJ, Caraballo M, Suciu D, Munro S, Kumar A, Anderson B: Detection of cancer with serum miRNAs on an oligonucleotide microarray. *PLoS One* 2009, 4:e6229.

116. Moltzahn F, Olishen AB, Baehner L, Peck A, Fong L, Stoppler H, Simko J, Hilton JF, Caroll P, Belloch R: Microfluidic-based multiplex qRT-PCR identifies diagnostic and prognostic microRNA signatures in the sera of prostate cancer patients. *Cancer Res* 2011, 71:550-560.

117. Bende JC, Johannes M, Schromm T, Falth M, Haase A, Steuber T, Beissbarth T, Kuner R, Sultmann H: Circulating miRNAs are correlated with tumor progression in prostate cancer. *Int J Cancer* 2011, 128:608-616.

118. Zhang HL, Yang LF, Zhu Y, Yao XD, Zhang SL, Dai B, Zhu YP, Shen YJ, Shi GH, Ye DW: Serum miRNA-21: elevated levels in patients with metastatic hormone-refractory prostate cancer and potential predictive factor for the efficacy of docetaxel-based chemotherapy. *Prostate* 2011, 71:326-331.

119. Yaman Agaoglu F, Kovanclari M, Ozdar Y, Darendeliler E, Holdenrieder S, Dalay N, Gezer U: Investigation of miR-21, miR-141, and miR-221 in blood circulation of patients with prostate cancer. *Tumour Biol* 2011, 32:583-588.

Pre-publication history
The pre-publication history for this paper can be accessed here:
http://www.biomedcentral.com/1471-2407/11/500/prepub
doi:10.1186/1471-2407-11-500
Cite this article as: Hui et al.: Micro-RNAs as diagnostic or prognostic markers in human epithelial malignancies. *BMC Cancer* 2011 11:500.