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REVIEW

DEAD-box RNA Helicases: The microRNA managers of breast cancer

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The roles of non-coding RNAs in cancers, microRNA (miRNA) especially, have sparked interest in the field of RNA research in recent years. The once widely accepted 'central dogma of genetics' describing the flow of cellular protein expression is now being challenged following the discovery of non-coding RNA research. miRNAs belong to the family of non-coding RNAs, in which many have been shown to be involved in cancer progression, including breast cancer. *Goh et al.* have recently summarized comprehensively, the roles played by miRNAs in the hallmarks of breast cancer progression. In this research highlight, we provide a brief summary of these miRNA-associated hallmarks in breast cancer progression and also highlight DEAD-box RNA helicases, a family of proteins known to be associated with miRNA-associated tumorigenesis. There are an increasing number of studies on DEAD-box RNA helicases in recent years, with different roles being reported in numerous cancer types. DDX20, a member of the DEAD-box RNA helicase family, was most recently revealed to be involved in breast cancer progression and metastasis. New data from our group found a possible novel miRNA-processing role of DDX20 in breast cancer. In an ongoing study, we found that miR-222 expression inversely correlates with DDX20, suggesting a possible tumor-suppressing role of miR-222 in invasive breast cancers, contrary to previous reports where miR-222 was associated with invasion in breast cancers. Our work thus provides another dimension to the complexity, where miRNAs and DEAD-box RNA helicases play in breast cancers.

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Cancer arises as a result of dynamic changes to the genome, producing oncogenes and repressing of tumor suppressors. Such non-specific insults to the genome lead to the heterogeneity of cancers, including breast cancers. Breast cancer can be classified into various molecular subtypes, hormone receptor-positive luminal subtype, HER2 receptor-enriched subtype and triple negative breast cancer subtype (TNBC) [1]. The treatment of triple negative breast cancers (TNBC) remains challenging due to the lack of therapeutic targets. Gene expression analysis from 587 TNBC cases showed TNBCs to be highly diverse, indicating a need to better understand the disease and identify specific

biomarkers for targeted therapies [2].

Over the past decades, a growing number of studies have focused on the involvement of miRNAs in various diseases, including cancers [3, 4]. miRNAs are non-coding small RNAs, which regulate gene expression post-transcriptionally by binding to the 3'-UTR of target mRNAs. miRNAs regulate a wide range of important cellular processes, including proliferation, apoptosis, immune response and metabolism [5]. In cancers, including breast cancer, miRNAs are reported to be dysregulated [6, 7], and miRNA profiling has shown that tumor samples exhibit lower global miRNA level compared with normal tissues [8]. Due to the extensive dysregulation of miRNAs in cancers, miRNA profiles can provide valuable diagnostic and prognostic information. In addition, numerous studies have reported pivotal functions of miRNAs in the hallmarks of cancers, summarized in a recent review by *Goh et al.* [5, 9, 10]. *Hanahan D et al.* described the acquired capabilities of cancers as six hallmarks, including sustaining proliferative signals, insensitivity to anti-growth signals, evasion of apoptosis, infinite replicative potential, sustained angiogenesis, and invasion and metastasis [11]. Over a decade's time, reprogrammed energy metabolism and evasion of immune system have added to the emerging cancer hallmarks [12].

Dysregulation of cell proliferation is one of the crucial characteristics of cancer. Numerous mitogenic growth signals and anti-proliferative signals contribute to the regulation of cell proliferation. Many studies have suggested that miRNAs regulate cell proliferation through various pathways, with some miRNAs promoting oncogenic signals and others inhibiting tumor suppressing signals. miR-107 and miR-206 have been found to interact with cell cycle regulators to regulate cell cycle progression and the expression of cell cycle related proteins, while miR-24 and miR-100, involved in cell survival signaling [13-16]. In breast cancers, estrogen receptor (ER) signaling is also indirectly regulated by miRNAs such as miR-17-5p [17].

In addition to regulating cell growth, miRNAs also play critical roles in apoptosis by targeting mRNAs to regulate the expression of pro and anti-apoptosis proteins. Tumor-suppressing miRNAs (tsmiRNAs), such as miR-34a and miR-195, inhibit the expression of oncogenes Bcl-2, MYC or Raf-1 etc, while oncogenic miRNAs associate with upregulation of pro-apoptotic proteins [18, 19]. However, certain miRNAs, miR-26a as an example, exert either oncogenic or tumor suppressing effects in a context-dependent manner [20, 21]. In chemo-resistant cancer cells, manipulation of miRNAs can increase their sensitivity to chemotherapeutic drugs; miR-128 and miR-26b target Bax and SLC7A11 respectively to induce apoptosis in breast cancer cells [22, 23].

Cancer cells are known to evade immune attacks through many signaling pathways, and accumulating studies have demonstrated that miRNAs are involved in the immune responses in cancers. miR-10b and miR-155 have been reported to be both oncogenic and inflammatory responsive by inhibiting major histocompatibility complex Class I polypeptide-related sequence B (MICB) or enhancing suppressor of cytokine signaling 1 (SOCS1), respectively [24, 25]. Another important link between inflammation and cancers is M2-polarized tumor-associated macrophages, which have been shown to contribute to cancer progression and epithelial-mesenchymal transition (EMT) [26]. miR-92a was found to inversely correlate to infiltrating macrophages in tumor stroma and down-regulation of miR-92a enhanced tumor migration [27].

Metastasis is the main cause of cancer related deaths. There are numerous proteins and pathways discovered to drive metastasis in cancers, and miRNAs are found to target important metastasis regulators to either promote or suppress metastasis. miR-183 cluster members, miR-182 and miR-96, target tumor suppressor RECK, a matrix metalloproteinase inhibitor, to increase invasiveness [28, 29]. Negative regulators of Wnt/ß-catenin pathway are suppressed by miR-301a and miR-374a to promote breast cancer invasion and metastasis [30, 31]. miR-200 family is known to be the negative regulators of metastasis by inhibiting EMT and tumor cell migration [32].

The dysregulated metabolism of cancer cells has been studied for many decades, with examples such as RAS and TP53 being discovered to be important modulators. However, it is until recently that an increasing number of studies associating miRNAs with cancer metabolism have been carried out. In normal breast cells, miR-203 was uncovered to inversely correlate with caveolin-1, whose loss was found to fuel the growth of tumor cells [33]. In breast cancer, miR-378* was reported to be regulated by HER2 and lead to a metabolic change in breast cancers cells via blocking estrogen-related receptor gamma (ERRγ) and GA-binding protein alpha (GABPA), resulting in decreased tricarboxylic acid cycle gene, increased lactate level and proliferation [5].

It has been shown that miRNAs are involved in all the cancer hallmarks and exhibit the potential as biomarkers for breast cancers. Moreover, a very recent study suggested profiling a cluster of miRNAs is more accurate than analyzing a single miRNA in clinical samples [34].

In the complex machinery of miRNA processing, a family

of proteins known as the DEAD-box RNA helicases, have been found to be essential. As its name suggests, DEAD-box RNA helicases function by unwinding double stranded RNA molecules, and are crucial in cellular processes involving RNA metabolism, including miRNA processing [35]. Eukaryotic DEAD-Box RNA helicases belong to the largest group of helicase superfamily, known as Superfamily 2 (SF2). They are characterized by nine conserved motifs [36]; motif I, II, VI and Q are essential for ATP binding and hydrolysis, while motifs Ia, Ib, III, IV, and V are involved in intramolecular rearrangements and RNA interaction [37]. Amongst the nine conserved motifs, motif II (or Walker B motif) is the most characteristic, where amino acids D-E-A-D (asp-glu-ala-asp) are found [38].

Several DEAD-box RNA helicases have been reported to regulate expression of potential oncogenic proteins through miRNA processing [39]. Recently, DEAD-box RNA helicase DDX1 was found to promote specifically, the maturation of miR-200 family of tumor suppressing miRNAs and in turn inhibit metastasis [12]. DEAD-box RNA helicase paralogs DDX5/DDX17 have been reported to be part of the drosha miRNA processor complex, processing both oncogenic (e.g. miR-21, miR-182 and let-7 family miRNAs) and growth suppressing miRNAs (e.g. miR16-1, miR-143, miR-145) in various cancer types [39, 40]. In addition to miRNA processing, *Etienne et al.* have also reported DDX5/DDX17 to regulate miRNA production at transcriptional level [40]. In breast cancer, DDX5 was shown to directly regulate oncomiR-182, resulting in increased expression of cofilin and profilin, which are crucial for cytoskeletal reorganization during breast cancer invasion and metastasis [41]. RNA helicase, DDX6, was found to regulate miRNA processing in cancers, where they negatively regulate miR-143/145 expression via post-transcriptional regulation. DDX6 interacts with AGO1/2, components of the RNA-induced silencing complex (RISC) and also essential proteins for Dicer-independent miRNA processing [42]. From recent reports, DEAD-box RNA helicases play a pivotal role in miRNA processing and production, and these reports are possibly only a tip of the iceberg; it would not be surprising to see more association between DEAD-box RNA helicases, miRNA processing and cancers in the near future.

Over the years, DEAD-box RNA helicases have been often associated with breast cancers, examples include: DDX3, DDX5/17, DDX6, DDX10, DDX21, DDX43 and most recently DDX20 [39, 43]. A recent study from our group has reported for the first time DDX20 (also known as DP103 or Gemin 3) to be a novel onco-protein involved in breast cancer metastasis [43]. Expression of DDX20 was not only found to be significantly higher in highly metastatic triple negative breast cancers (TNBCs) compared to other less invasive subtypes, abrogating DDX20 effects via siRNA knockdown significantly reduced invasion and migration of TNBC cell lines.

DDX20, like other DEAD-box RNA helicases in the family, is an important protein for RNA metabolism. One of the earliest reports studied the role of DDX20 in mRNA splicing. DDX20 has been reported to interact with proteins in the survival motor neurons (SMN) complex, the spinal muscular atrophy gene product, together with other small nuclear RNAs (snRNAs) and ribonucleic proteins (snRNPs), forming the spliceosomal complex during mRNA splicing [44, 45]. DDX20 has also been reported in miRNA processing, where some reports showed its association with cancers. In one of the earliest reports, *Mourelatos et al.* showed DDX20 as a miRNA biogenesis factor and a component of miRNP complex via immunoprecipitation and sucrose gradient centrifugation. In addition, DDX20 was found to be part of a complex that contains eIF2C2, a member of the Agronaute (ago) protein family [46]. A later study showed that DDX20 specifically interacts with ago2 and *let-7* programmed RISC complex, which are essential for miRNA and RNA interference (RNAi) formation [47]. Furthermore, *Mourelatos et al.* described a total of forty known and novel miRNAs to be associated with the gemin3-gemin4-eIF2C2 complex; examples include miR-21 to miR-23 and miR-24, miR-110 to miR-121 [46]. In relation to cancer, *Takata et al.* published two reports describing DDX20's role in miR-140 processing in hepatocellular carcinoma (HCC) [48, 49]. In the first study, DDX20 was found to be inversely associated with NF-κB activity in liver cancer cells, where reduced DDX20 expression significantly increased NF-κB activity. DDX20 expression is commonly found to be downregulated in HCC patients, it was later discovered that DDX20 interacted directly with miR-140 and its deficiency resulted in a defect in miR-140 processing and expression [49]. miRNA-140 is one of the critical suppressors of NF-κB activity, and its expression is often lost in HCC [50]. Further mechanistic studies found miR-140 and DDX20 deficient cells to display increased methylation in the CpG islands/shores of metallothionein (MT) promoters, mediated by DNA methyl transferase (Dmnt) [48].

Interestingly, in our ongoing study, *in silico* analysis of the 3'-UTR of DDX20 showed that DDX20 could be regulated by miR-222 in invasive breast cancers. Many studies have reported the oncogenic potential of miR-221/222. In breast cancer, miR-221/222 has also been shown to play significant roles, including conferring drug resistance and promoting EMT [44, 50]. Notably, in our study, the effects of manipulation of miR-222 on DDX20 expression revealed an inverse correlation between miR-222 and DDX20 in breast cancer cells, indicating the possible tumor suppressor role of

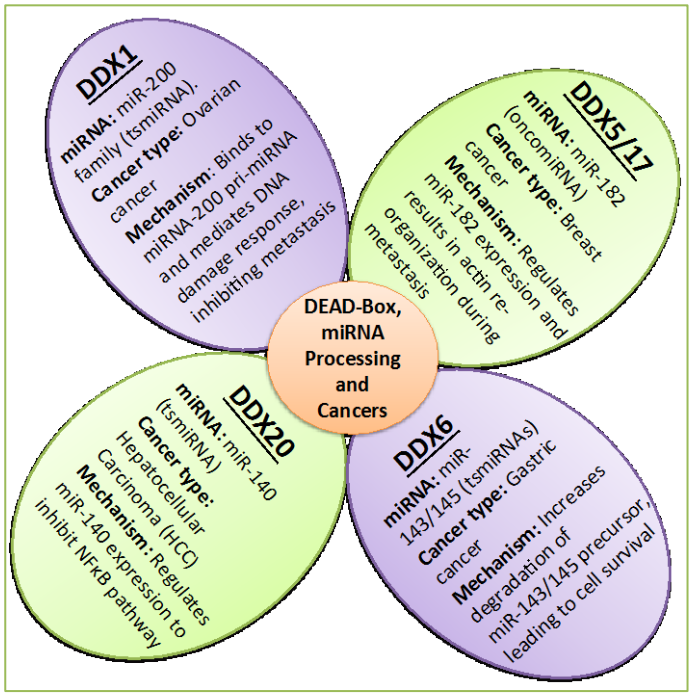

Figure 1. A summary of DEAD-Box RNA helicases' roles in miRNA processing in cancers.

miR-222. The tumor-suppressing effect of miR-221/222 was also observed in luminal invasive carcinomas, where miR-221/222 inversely correlates with Ki67 and the most high-grade tumors express low levels of miR-221/222 [51].

In conclusion, miRNAs are involved in different hallmarks of breast cancers and may be exploited for diagnostic and therapeutic purposes. DEAD-box RNA helicases have been shown to be involved in miRNA regulation in several cancer types (Figure 1), however, their roles in breast cancer remain to be explored. Our observation of miR-222 and DDX20 provided another dimension of the possible roles miRNAs and DEAD-box RNA helicases may play in breast cancers, where targeting miRNAs or DDX20 for breast cancer treatment may be a possible solution in the future.

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References

1. Perou, CM, T Sorlie, MB Eisen, M van de Rijn, SS Jeffrey, CA Rees, *et al*. Molecular portraits of human breast tumours. Nature 2000; 406: 747-52.
2. Lehmann, BD, JA Bauer, X Chen, ME Sanders, AB Chakravarthy, Y Shyr, *et al*. Identification of human triple-negative breast cancer subtypes and preclinical models for selection of targeted therapies. J Clin Invest 2011; 121: 2750-67.
3. Whiteoak, SR, R Felwick, T Sanchez-Elsner, and JR Fraser Cummings. MicroRNAs in Inflammatory Bowel Diseases: Paradoxes and Possibilities. Inflamm Bowel Dis 2015.
4. Song, JL, P Nigam, SS Tektas, andE Selva. microRNA regulation of Wnt Signaling Pathways in Development and Disease. Cell Signal 2015.
5. Goh, JN, SY Loo, A Datta, KS Siveen, WN Yap, W Cai, *et al*. microRNAs in breast cancer: regulatory roles governing the hallmarks of cancer. Biol Rev Camb Philos Soc 2015.
6. Graveel, CR, HM Calderone, JJ Westerhuis, ME Winn, and LF Sempere. Critical analysis of the potential for microRNA biomarkers in breast cancer management. Breast Cancer (Dove Med Press) 2015; 7: 59-79.
7. Kang, L, J Mao, Y Tao, B Song, W Ma, Y Lu, *et al*. MiR-34a Suppresses the Breast Cancer Stem Cell-like Characteristics by Downregulating Notch1 Pathway. Cancer Sci 2015.
8. Lu, J, G Getz, EA Miska, E Alvarez-Saavedra, J Lamb, D Peck, *et al*. MicroRNA expression profiles classify human cancers. Nature 2005; 435: 834-8.
9. Bennett, PE, L Bemis, DA Norris, and YG Shellman. miR in melanoma development: miRNAs and acquired hallmarks of cancer in melanoma. Physiol Genomics 2013; 45: 1049-59.
10. Ruan, K, X Fang, and G Ouyang. MicroRNAs: novel regulators in the hallmarks of human cancer. Cancer Lett 2009; 285: 116-26.
11. Hanahan, D and RA Weinberg. The hallmarks of cancer. Cell 2000; 100: 57-70.
12. Hanahan, D and RA Weinberg. Hallmarks of cancer: the next generation. Cell 2011; 144: 646-74.
13. Zhou, J, Y Tian, J Li, B Lu, M Sun, Y Zou, *et al*. miR-206 is down-regulated in breast cancer and inhibits cell proliferation through the up-regulation of cyclinD2. Biochem Biophys Res Commun 2013; 433: 207-12.
14. Li, XY, QF Luo, CK Wei, DF Li, J Li, and L Fang. MiRNA-107 inhibits proliferation and migration by targeting CDK8 in breast cancer. Int J Clin Exp Med 2014; 7: 32-40.
15. Gebeshuber, CA and J Martinez. miR-100 suppresses IGF2 and inhibits breast tumorigenesis by interfering with proliferation and survival signaling. Oncogene 2013; 32: 3306-10.
16. Martin, EC, S Elliott, LV Rhodes, JW Antoon, C Fewell, Y Zhu, *et al*. Preferential star strand biogenesis of pre-miR-24-2 targets PKC-alpha and suppresses cell survival in MCF-7 breast cancer cells. Mol Carcinog 2014; 53: 38-48.
17. Hossain, A, MT Kuo, and GF Saunders. Mir-17-5p regulates breast cancer cell proliferation by inhibiting translation of AIB1 mRNA. Mol Cell Biol 2006; 26: 8191-201.
18. He, M, L Gao, S Zhang, L Tao, J Wang, J Yang, *et al*. Prognostic

significance of miR-34a and its target proteins of FOXP1, p53, and BCL2 in gastric MALT lymphoma and DLBCL. Gastric Cancer 2014; 17: 431-41.

19. Yang, G, D Wu, J Zhu, O Jiang, Q Shi, J Tian, *et al*. Upregulation of miR-195 increases the sensitivity of breast cancer cells to Adriamycin treatment through inhibition of Raf-1. Oncol Rep 2013; 30: 877-89.
20. Zhang, B, XX Liu, JR He, CX Zhou, M Guo, M He, *et al*. Pathologically decreased miR-26a antagonizes apoptosis and facilitates carcinogenesis by targeting MTDH and EZH2 in breast cancer. Carcinogenesis 2011; 32: 2-9.
21. Guo, P, Q Nie, J Lan, J Ge, Y Qiu, and Q Mao. C-Myc negatively controls the tumor suppressor PTEN by upregulating miR-26a in glioblastoma multiforme cells. Biochem Biophys Res Commun 2013; 441: 186-90.
22. Ji, S, G Shao, X Lv, Y Liu, Y Fan, A Wu, *et al*. Downregulation of miRNA-128 sensitises breast cancer cell to chemodrugs by targeting Bax. Cell Biol Int 2013; 37: 653-8.
23. Liu, XX, XJ Li, B Zhang, YJ Liang, CX Zhou, DX Cao, *et al*. MicroRNA-26b is underexpressed in human breast cancer and induces cell apoptosis by targeting SLC7A11. FEBS Lett 2011; 585: 1363-7.
24. Tsukerman, P, J Enk, and O Mandelboim. Metastamir-mediated immune evasion: miR-10b downregulates the stress-induced molecule MICB, hence avoid recognition by NKG2D receptor. Oncoimmunology 2013; 2: e22245.
25. Jiang, S, HW Zhang, MH Lu, XH He, Y Li, H Gu, *et al*. MicroRNA-155 functions as an OncomiR in breast cancer by targeting the suppressor of cytokine signaling 1 gene. Cancer Res 2010; 70: 3119-27.
26. Thanee, M, W Loilome, A Techasen, N Namwat, T Boonmars, C Pairojkul, *et al*. Quantitative Changes in Tumor-Associated M2 Macrophages Characterize Cholangiocarcinoma and their Association with Metastasis. Asian Pac J Cancer Prev 2015; 16: 3043-50.
27. Nilsson, S, C Moller, K Jirstrom, A Lee, S Busch, R Lamb, *et al*. Downregulation of miR-92a is associated with aggressive breast cancer features and increased tumour macrophage infiltration. PLoS One 2012; 7: e36051.
28. Chiang, CH, MF Hou, and WC Hung. Up-regulation of miR-182 by beta-catenin in breast cancer increases tumorigenicity and invasiveness by targeting the matrix metalloproteinase inhibitor RECK. Biochim Biophys Acta 2013; 1830: 3067-76.
29. Zhang, J, X Kong, J Li, Q Luo, X Li, L Shen, *et al*. miR-96 promotes tumor proliferation and invasion by targeting RECK in breast cancer. Oncol Rep 2014; 31: 1357-63.
30. Ma, F, J Zhang, L Zhong, L Wang, Y Liu, Y Wang, *et al*. Upregulated microRNA-301a in breast cancer promotes tumor metastasis by targeting PTEN and activating Wnt/beta-catenin signaling. Gene 2014; 535: 191-7.
31. Cai, J, H Guan, L Fang, Y Yang, X Zhu, J Yuan, *et al*. MicroRNA-374a activates Wnt/beta-catenin signaling to promote breast cancer metastasis. J Clin Invest 2013; 123: 566-79.
32. Lim, YY, JA Wright, JL Attema, PA Gregory, AG Bert, E Smith, *et al*. Epigenetic modulation of the miR-200 family is associated with transition to a breast cancer stem-cell-like state. J Cell Sci 2013; 126: 2256-66.
33. Sotgia, F, UE Martinez-Outschoorn, A Howell, RG Pestell, S Pavlides, and MP Lisanti. Caveolin-1 and cancer metabolism in the tumor microenvironment: markers, models, and mechanisms. Annu Rev Pathol 2012; 7: 423-67.
34. Sakurai, M, M Masuda, Y Miki, H Hirakawa, T Suzuki, and H Sasano. Correlation of miRNA expression profiling in surgical pathology materials, with Ki-67, HER2, ER and PR in breast cancer patients. Int J Biol Markers 2015: 0.
35. Rocak, S and P Linder. DEAD-box proteins: the driving forces behind RNA metabolism. Nat Rev Mol Cell Biol 2004; 5: 232-241.
36. Umate, P, N Tuteja, and R Tuteja. Genome-wide comprehensive analysis of human helicases. Commun Integr Biol 2011; 4: 118-37.
37. Tanner, NK, O Cordin, J Banroques, M Doere, and P Linder. The Q motif: a newly identified motif in DEAD box helicases may regulate ATP binding and hydrolysis. Mol Cell 2003; 11: 127-38.
38. Schmid, SR and P Linder. D-E-A-D protein family of putative RNA helicases. Mol Microbiol 1992; 6: 283-91.
39. Fuller-Pace, FV. DEAD box RNA helicase functions in cancer. RNA Biol 2013; 10: 121-32.
40. Fukuda, T, K Yamagata, S Fujiyama, T Matsumoto, I Koshida, K Yoshimura, *et al*. DEAD-box RNA helicase subunits of the Drosha complex are required for processing of rRNA and a subset of microRNAs. Nat Cell Biol 2007; 9: 604-11.
41. Wang, D, J Huang, and Z Hu. RNA helicase DDX5 regulates microRNA expression and contributes to cytoskeletal reorganization in basal breast cancer cells. Mol Cell Proteomics 2012; 11: M111 011932.
42. Iio, A, T Takagi, K Miki, T Naoe, A Nakayama, and Y Akao. DDX6 post-transcriptionally down-regulates miR-143/145 expression through host gene NCR143/145 in cancer cells. Biochim Biophys Acta 2013; 1829: 1102-10.
43. Shin, EM, HS Hay, MH Lee, JN Goh, TZ Tan, YP Sen, *et al*. DEAD-box helicase DP103 defines metastatic potential of human breast cancers. J Clin Invest 2014; 124: 3807-24.
44. Charroux, B, L Pellizzoni, RA Perkinson, A Shevchenko, M Mann, and G Dreyfuss. Gemin3: A novel DEAD box protein that interacts with SMN, the spinal muscular atrophy gene product, and is a component of gems. J Cell Biol 1999; 147: 1181-94.
45. Shpargel, KB and AG Matera. Gemin proteins are required for efficient assembly of Sm-class ribonucleoproteins. Proc Natl Acad Sci U S A 2005; 102: 17372-7.
46. Mourelatos, Z, J Dostie, S Paushkin, A Sharma, B Charroux, L Abel, *et al*. miRNPs: a novel class of ribonucleoproteins containing numerous microRNAs. Genes Dev 2002; 16: 720-8.
47. Hutvagner, G and PD Zamore. A microRNA in a multiple-turnover RNAi enzyme complex. Science 2002; 297: 2056-60.
48. Takata, A, M Otsuka, T Yoshikawa, T Kishikawa, Y Hikiba, S Obi, *et al*. MicroRNA-140 acts as a liver tumor suppressor by controlling NF-kappaB activity by directly targeting DNA methyltransferase 1 (Dnmt1) expression. Hepatology 2013; 57: 162-70.
49. Takata, A, M Otsuka, T Yoshikawa, T Kishikawa, Y Kudo, T Goto, *et al*. A miRNA machinery component DDX20 controls

NF-kappaB via microRNA-140 function. Biochem Biophys Res Commun 2012; 420: 564-9.

50. Callegari, E, L Gramantieri, M Domenicali, L D'Abundo, S Sabbioni, and M Negrini. MicroRNAs in liver cancer: a model for investigating pathogenesis and novel therapeutic approaches. Cell Death Differ 2015; 22: 46-57.

51. Dentelli, P, M Traversa, A Rosso, G Togliatto, C Olgasi, C Marchio, *et al*. miR-221/222 control luminal breast cancer tumor progression by regulating different targets. Cell Cycle 2014; 13: 1811-26.