

Review

miRNAs: Small but deadly

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microRNAs (miRNAs) are unique class of global gene regulators identified both in plants and animals. They can reduce protein levels of their target genes with a minor impact on the target genes mRNA. Levels of some miRNAs are found altered in cancers, so we might expect these regulatory molecules to be involved in the development of different carcinomas. The differential expression of certain miRNAs in various tumors might become a powerful tool to aid in the diagnosis and treatment of cancers. The precise biological roles of most miRNAs are still poorly understood and therefore, this review is an attempt to highlight the role and connections of human miRNA with different aspects of cancers.

Key words: microRNA, cancer, tumour, metastasis, upregulation, miRNA.

INTRODUCTION

Several hundred genes in human genome encodes small actively functional RNA molecules (Meng et al., 2007) ranging in length from 21 to 23 nucleotides known as microRNAs (miRNAs) (Dong et al., 2010). These miRNAs hybridize to the 3' untranslated region of target mRNAs causing repression at post-transcriptional level. Genes encoding these small molecules are found either inserted in introns as polycistronic clusters or in isolated regions of the genome (Jerome et al., 2007). They are conserved and important class of regulators functioning in diverse physiological and pathological phenomenon (Bagga and Pasquinelli, 2006) including apoptosis, proliferation, migration (Meng et al., 2007), hematopoietic cell differentiation (Chen et al., 2004) and brain development. They remain stable in tissues, serum, plasma and other body fluids protected from the action of endogenous RNase (Lodes et al., 2009). More than 500 miRNAs have been identified in human genome regulating up to 30% of the protein coding genes (Rajewsky, 2006).

Cancer is a complex and dynamic disease which involves a number of genetic alterations both in structure and function of genes. Traditional studies were focused on protein-coding genes and were considering them as the main players regulating cancers (Stahlhut and Slack, 2006). Role of miRNAs as regulators of cancer related signaling pathways has been fully explored since the past

few years (Jackson and Standart, 2007) and are considered one among the most important post-transcriptional gene regulators in both plants and animals (Pillai et al., 2007). Factors activating or deactivating miRNAs in tumorigenesis were considered to cooperate with abnormalities of protein coding genes (Calin and Croce, 2006). Depending upon the target mRNA, they can function either as oncogenes or tumor suppressors (Wiseman et al., 2005). Molecular characterization of 13q14 deletion in human chronic lymphocytic leukemia (CLL) suggested the involvement of miRNAs (mir-15a and mir-16-1) in human cancers for the first time. After this initial discovery, *mir-16-1* and *mir-15a* were found involved and down regulated in most of the CLL (Calin et al., 2002), miR143 and miR145 in colon carcinomas (Michael et al., 2003) and Let-7 in human lung carcinomas (Takamizawa et al., 2004). Some of the miRNAs are also found up regulated in human cancers like miR155 in Burkitt's lymphoma and some others as well (Metzler et al., 2004; Eis et al., 2005). The vital role of miRNAs in human cancer is further supported by the findings of majority of miRNAs genes located at chromosomal regions that are genetically altered in carcinogenesis (Calin et al., 2004).

Cancer is the ultimate consequence of disordered gene expression. microRNAs are suggested to play key role in cancer development, however, their precise biological roles are still poorly understood. It is therefore, suggested that the relevance of these small regulators in different types of human carcinomas may be presently underestimated. This review will highlight the role and

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connections of miRNAs with different aspects of cancers.

miRNA IN HUMAN COLON CANCER/COLORECTAL CANCER

Colon cancer is globally ranked second by causing cancer related deaths (Jemal et al., 2008; Ramana et al., 2010) with an estimated 1 million new cases and half a million deaths each year (Parkin et al., 2005). Development of colon cancer is a stepwise process causing anomalies in various genetic and epigenetic processes. Tumorigenesis are mostly caused by chromosomal instability and damaged DNA repair system, most commonly in MLH1, MSH2, MSH6 and PMS2 (Baudhuin et al., 2005). Modern research has documented the involvement of miRNA in different types of cancers including colon cancer (Bandres et al., 2007; Zhang et al., 2007). To explore the link of miRNA, colon cancer needs further detailed studies (Michael et al., 2003) because ambiguities exist about the exact nature of aberrant miRNA expression pattern (Lu et al., 2005; Volinia et al., 2006). Colon cancer is caused either by overexpression, silencing or switching off of specific miRNA involved in its pathogenesis (Rossi et al., 2009).

Many oncogenic and tumor suppressor pathways involved in colon cancer are regulated by miRNA. Majority of key signaling proteins of colon cancer pathways, like members of the Wnt/ β -catenin and phosphatidylinositol-3-kinase (PI-3-K) pathways, KRAS, p53, etc are regulated and affected by miRNA (Fearon and Vogelstein, 1990).

In human, cancers mutations have been recorded in the expression of large number of miRNAs including miR-1, miR-31, miR-133a, miR-135b, miR-182, miR-183, miR-96 and miR-592. Increased differential methylation has been studied in genomic regions having differentially expressed micro RNAs in colon cancer (Sarver et al., 2009). miR-145, miR-20a and miR-92 are the three most dysregulated miRNAs in colon cancers, among which miR-145 showed reduced expression, while miR-20a and miR-92 were up-regulated (Schepeler et al., 2008). miR-200a, miR-200b and miR-200c are also observed up-regulated in all colon cancers. The putative targets analysis of these miRNAs showed *MLH1* and *MSH2* as two candidate genes which are negatively regulated by them. Modern research has also reported differential expression of let7-family in colon cancers (Cummins et al., 2006; Akao et al., 2006). miR-145 identified as a specific miRNA negatively regulated in colon cancers (Michael et al., 2003; Cummins et al., 2006) with putative targets having oncogenic functions are *FOS*, *MYCN*, *YES* and *FLI*, cell cycle promoters such as cyclins D2 and L1 and MAPK transduction proteins such as *MAP3K3* and *MAPK4K4* (Iorio et al., 2005).

CHES1 protein which belongs to the forkhead family of transcription factors repressing apoptotic genes was

identified as a potential target for miR-96 and miR-182 (Bandrés et al., 2006). Over-expression of miR-21 has a central role in colon cancers (Schetter et al., 2008; Slaby et al., 2007) and regulates cell cycle and checkpoint activation through Cdc25A (Wang et al., 2009). miR-143 plays its function by targeting DNMT3A, which is involved in the methylation of DNA (Fabbri et al., 2007; Ng et al., 2009).

miRNAs IN THYROID AND PAPILLARY THYROID CANCERS

Significant differences in miRNA expression have been observed in various thyroid tumors. A correlation between somatic mutations and miRNA expression was found to exist in papillary carcinomas and it was also found that a set of miRNAs including miR-146b, miR-155, miR-187, miR-197, miR-221, miR-222 and miR-224 over-expressed differentially in them (Nikiforova et al., 2008). Papillary thyroid cancers (PTC) accounting for about 80% of all thyroid cancers are genetically characterized by mutations in the RET/PTC-RAS-BRAF signaling pathway (Melillo et al., 2005). BRAF and RET/PTC genes mutations during rearrangements are most common in PTC tumors (Fusco et al., 2005) and a number of miRNAs are also transcriptionally upregulated in these tumors. Five miRNAs, miR-146, miR-221 and miR-222 have been unequivocally distinguished between normal thyroid and PTC. Enhanced regulation of miR-146, miR-221 and miR-222 cause a dramatic loss of KIT transcript and related protein due to single nucleotide mutations in recognition sequences of KIT for these miRNAs. It is therefore, suggested that upregulation of numerous miRNAs and KIT down regulation are involved in the pathogenesis of PTC (Iliopoulos et al., 2005).

Overexpression of several micro RNAs were the initial evidences of their involvement in papillary thyroid cancers (He et al., 2005). miRNAs which are up-regulated in papillary thyroid cancers included miR-146, miR-221, miR-155, miR-34 and miR-181. These miRNAs donor show equal expression in this type of tumors but are found up-regulated in tumors with *BRAF* mutation (Pallante et al., 2006). It has also suggested that altered miRNAs might be early factors with the major role in the tumorigenesis of papillary thyroid cancers. There is also a genetic association between PTC and a SNP (rs2910164) in the precursor of miR-146a. Individuals which are heterozygous for the said SNP had an increased risk of acquiring PTC (Jazdzewski et al., 2008).

miR-181b has been confirmed to be overexpressed in all types of follicular cell-derived thyroid tumors and also in thyroid hyperplastic nodules. Several other miRNAs were also found up-regulated in PCs including miR-187, which is the most up-regulated in tumors with *RET/PTC* rearrangement and *RAS* mutations. It showed significantly reduced levels in tumors harboring *BRAF* mutation

(Nikiforova et al., 2008). miR-197 and miR-346 also showed significant overexpression in FCs (Weber et al., 2006).

miRNA IN BREAST CANCER

Breast cancer is a heterogeneous disease with multi step progression and constituting progressive changes in the genetics of normal tissues, resulting in invasive and metastatic carcinoma (Ramaswamy et al., 2009). Studies have revealed a fine balance of miRNAs causing stimulation and inhibition of metastasis (Iorio et al., 2005) and their involvement in breast cancer invasion (Ma et al., 2007).

In breast cancer tissues, 29 miRNAs have been identified with significant deregulation in their expression level (Iorio et al., 2005). Nine miRNAs, hsa-miR-21, hsa-miR-365, hsa-miR-181b, hsa-let-7f, hsa-miR-155, hsa-miR-29b, hsa-miR-181d, hsa-miR-98 and hsa-miR-29c were found with more than two fold enhanced level of expression, while seven miRNAs, hsa-miR-497, hsa-miR-31, hsa-miR-355, hsa-miR-320, rno-mir-140, hsa-miR-127 and hsa-miR-30a-3p were down-regulated more than two fold in breast cancer (Yang et al., 2008). Enhanced expression of miR-10b promotes metastasis and invasion, whereas its down regulation causes most breast cancers. Tumors with miR-10b over expression were highly vascularized and showed invasive behavior (Ma et al., 2007). miR-373 and miR-520c stimulate cancer cell invasion and migration which were previously considered associated with testicular cancers (Huang et al., 2008). Reduced cell growth has also been proved experimentally due to miR-21 inhibition in MCF-7 breast cancer cells (Frankel et al., 2008).

ROLE OF miRNAs IN GASTRIC CANCER

Gastric cancer is the second most common malignancy responsible for approximately 10% of tumor related deaths (Parkin et al., 2005). The molecular pathology of gastric cancer show abnormal levels of cell-cycle regulators. Cyclin-dependent kinases (Cdks) along with their modulators cause transitions between phases of cell-cycle. Cdks can be controlled by Cdk inhibitors (CKIs) that bind to Cdks. In mammalian cells, two families of Cdk inhibitors are responsible for regulating different Cdks. Members of the Ink4 family (p15^{Ink4b}, p16^{Ink4a}, p18^{Ink4c} and p19^{Ink4d}) bind to Cdk4 and Cdk6/cyclin D complexes, thereby inhibiting progression through the G1 restriction point. The Cip/Kip family proteins block the progression through all stages of G1/S, thereby functioning as a 'brake of cell cycle' (Besson et al., 2008). It has been speculated that the miRNAs can also play related biological functions. miRNAs in two clusters (miR-106b~93~25 and miR-222~221) suppress the Cip/Kip family

members of Cdk inhibitors (p57^{Kip2}, p21^{Cip1} and p27^{Kip1}). miR-25 targets p57 through the 3'-UTR. Furthermore, miR-106b and miR-93 control p21, while miR-222 and miR-221 regulate both p27 and p57. Ectopic expression of these miRNAs results in activation of Cdk2 and facilitation of G1/S phase transition. Consistent with these results, both clusters are abnormally upregulated in gastric cancer tissues compared with the corresponding normal tissues (Kim et al., 2009).

Helicobacter pylori infection was thought to be the most important factor for gastric carcinogenesis. It has been shown that expression of miR-21 was upregulated in the patients who got *H. pylori* infection, implying that the pathogen infection induces carcinogenesis in the stomach probably due to abnormal expression of some oncomiRs such as miR-21. Aberrant expression of miR-21 can alter multiple biological processes of human gastric cancer cells such as proliferation, apoptosis, migration and invasion through regulating RECK and other critical target genes (Akao et al., 2006).

miRNAs IN LUNGS CANCER

Lung cancer is one of the main causes of cancer related deaths for men and women world wide (Bommer et al., 2007). There are two main histological groups of LC that is, small cell lung cancer accounting for 15% and non small cell lung cancer accounting for 85%. The later one is further subdivided into adenocarcinomas, squamous cell, large cell and bronchoalveolar carcinomas (Wang et al., 2009).

Gene expression is regulated by miRNAs which are found deregulated in different cancers including lung cancer (Ma et al., 2007). Hypermethylation is responsible for the silencing of tumor suppressor genes, such as CDKN2A, CDH13 (Ulivi et al., 2006), FHIT, WWOX (Fabbri et al., 2005), CDH1 and RASSF1A (Suzuki et al., 2004). Methylation of the epigenome is controlled by DNA methyltransferases (DNMTs). Three catalytically active DNMTs (Dnmt1, Dnmt3A and Dnmt3B) have been identified in mammals (Jeltsch, 2002). It has been demonstrated that expression of miR-29a, miR-29b and miR-29c is down-regulated in NSCLCs (Volinia et al., 2006), while the mRNA levels of DNMT1 and DNMT3B have been found to be elevated by 58% in 102 NSCLCs (Kim et al., 2006). According to the available results, the expression of miR-29c in lung cancer tissues is inversely correlated to the expression of DNMT3A and DNMT 3B (Fabbri et al., 2007).

It has been reported that 28 miRNAs including miR-145, miR-181c, miR-30 family, miR-140, miR-143, miR-125, miR-126, miR-101, miR-95, miR-9 and miR-125a were down-regulated and 15 miRNAs including miR-210 were up-regulated in lung cancers (Son et al., 2009). In uterine leiomyomas, racial differences were reported in miRNA expression levels. It was found that a substantial

number of miRNAs significantly regulated differentially between white and black women (Wang et al., 2006). miRNAs miR-376b, miR-144, miR-520d-5p, miR-520e, miR-520f, miR-23a, miR-296-5p, miR-133a-2, miR-99b, miR-497, miR-425 and miR-338-3p expressions are reduced, while miR-371-3p is up-regulated in the earlier mentioned case. It has also been shown that in NSCLCs, miR-99b was deregulated which was negatively correlated with the expression of FGFR3 which has been reported to be frequently over expressed in NSCLC cell lines. It is therefore suggested that through up-regulation of FGFR3, miR-99b may possibly be involved in lung tumorigenesis (Son et al., 2009).

miRNAs IN PROSTATE CANCER

The most frequently diagnosed cancer among American males is the prostate cancer (PCa) (Jemal et al., 2008). Till date, the pathogenesis of PCa have been explained by several mechanisms (Nelson et al., 2003) and it was found that the consequences of PCa development are genetic and epigenetic alterations that lead to invasive carcinoma (Li, 2007). In prostate cancer, microRNAs are significantly altered which suggests their role as key regulators in the process (Coppola et al., 2010). Researchers have investigated dysregulation of miRNAs in PCa and are trying to establish a connection with the emergence and development of the disease through miRNA expression profiles (Gandellini et al., 2010). It is now clear that miRNAs express differentially in androgen-dependent and androgen-independent PCa cells (Lin et al., 2008). They are also found to play a role in the transition of PCa to the androgen-independent stage (Shi et al., 2007). It is therefore essential to have closer validation for the role of miRNAs and their differential expression patterns in both androgen-dependent and androgen-independent PCa cells (Sikand et al., 2009).

Level of miR-23a and 23b decreased significantly in human prostate cancer (Porkka et al., 2007). It was also found that in PC3 PCa cells this decrease is due to the transcriptional repression by c-Myc (Fujita et al., 2008) which leads to upregulation of glutamine catabolism. Expression of miR-101 gets decreased due to the loss of one or both of the loci encoding it (Varambally et al., 2008). Genomic losses of miR-101 have been found in approximately 37.5% of the localized PCa and 66.7% of the metastatic cases. miR-101 is the inhibitor of a polycomb group member which is an enhancer of zeste homolog-2 (EZH2). It suggests that genomic loss of miR-101 leads to over expression of EZH2 and abnormal regulation of various epigenetic pathways, resulting in progression of the disease. miR-449a is a tumor suppressor (Noonan et al., 2009) and is down regulated in PCa tissues. When miR-449a was introduced into PC-3 PCa cells, they were observed with apoptosis, cell-cycle arrest and a senescent-like phenotype. HDAC1

frequently over express in PCa but is directly targeted by miR-449a. (Weichert et al., 2008).

miRNAs IN OVARIAN CANCERS

Since the last thirty years, ovarian cancer is still a leading cause of mortality. Epithelial ovarian cancer (EOC), a type of ovarian cancer, (Corney and Alexander, 2009) is the sixth world most common cancer in women causing almost 125,000 deaths/year (Cannistra, 2004). Its high rate of mortality is mainly due to late diagnosis (Feeley and Wells, 2001). Aberrant expression or mutation of miRNAs in cancers have proved their importance as a class of oncogenes or tumor suppressors (Johnson et al., 2005) and therefore, implicated in ovarian cancer research (Iorio et al., 2007). It is now proved from the research that different miRNAs are involved in different stages of ovarian cancers.

When compared with normal ovaries, serous and endometrioid histotypes commonly shared down regulation of miR-9. Expression profiling of human immortalized ovarian surface epithelial and EOC have showed up-regulation of miR-214, miR-199a and miR-200a, while down-regulation of miR-100. It is also been observed that alterations of miR-214, miR-199a and miR-200a are associated with advanced stage ovarian tumors. It means that up-regulation of miR-214, miR-199a and miR-200a participate in progression rather than initiation of ovarian tumor (Yang et al., 2008). It as also been shown that miRNAs are histotype specific. miR-21, miR-182 and miR-205 have over expressed, miR-144, miR-222 and miR-302a have reduced expression in endometrioid tumors (Iorio et al., 2007). miRNA-21, miRNA-29a, miRNA-92, miRNA-93 and miRNA-126 were over expressed while miRNA-127, miRNA-155 and miRNA-99b expression were observed reduced in the serum of individuals with ovarian cancers (Resnick, 2009). miR-200a, miR-200b, miR-200c, miR-20a, miR-21, miR-23a, miR-23b, miR-27a, miR-141, miR-16 and miR-93 have over expressed, while miR-214, miR-26a, miR-29a, let-7b, miR-100, miR-10b, miR-125a, miR-125b, miR-143, miR-145, miR-199a-AS and miR-99a have found to be down regulated in serous ovarian cancers (Nam et al., 2008).

FUTURE PERSPECTIVES AND THERAPEUTIC POTENTIALS

Soon after the discovery of miRNAs, it became clear that they play important roles in many cellular processes. Modern research in the miRNA biology have provided us with improved understanding towards microRNA biogenesis, regulation, function and their association with molecular pathogenesis of diverse and complex diseases like cancer, cardiac disorders, viral infections and

metabolic disorders. Though, more than 530 miRNAs have been identified in human, much remains to be understood about their precise cellular function and role in the development of diseases. Scientists try to explore the mystery of miRNA biology and its potential as therapeutic agents. High-throughput target analysis combining genomics and proteomics might help delineating the spectrum of targets that are regulated by miRNAs. microRNA based cancer therapy is considered as an alternative for targeting gene networks regulated by a single miRNA. Recently, it has been suggested that specific microRNAs can play a role in drug resistance in various cancer cell lines. Altered regulation of specific microRNAs can provide information about resistance and sensitivity of tumors to different treatments.

New knowledge about the functional roles of oncomiRs is revolutionizing cancer biology and will open up new horizons in biomedical research. Further studies are therefore necessary to verify the mechanistic details in biological functions of microRNAs. A considerable investment in microRNA research is required to use miRNAs as novel biomarkers and new therapeutic targets and tools can be formulated. Rapid expansion in this field suggests their impact on the management of cancer patients in the near future. As an emerging technology, miRomics has a great potential to be utilized in drug development. In simple words, development in microRNA field, these small players could be worthless tools for different areas of basic and applied research and also for therapeutic intervention.

REFERENCES

- Akao Y, Nakagawa Y, Naoe T (2006). let-7 microRNA functions as a potential growth suppressor in human colon cancer cells. *Biol. Pharm. Bull.*, 29(5): 903-6.
- Bagga S, Pasquinelli AE (2006). Identification and analysis of microRNAs. *Gen. Eng. Principles Methods*, 27: 1-20.
- Bandres E, Agirre X, Ramirez N, Zarate R, Garcia-Foncillas J (2007). MicroRNAs as cancer players: potential clinical and biological effects. *DNA Cell Biol.* 26: 273-82.
- Bandrés E, Cubedo E, Agirre X, Malumbres R, Zárate R, Ramirez N, Abajo A, Navarro A, Moreno I, Monzó M, García-Foncillas J (2006). Identification by Real-time PCR of 13 mature microRNAs differentially expressed in colorectal cancer and non-tumoral tissues. *Mol. Cancer*, 5: pp. 29.
- Baudhuin LM, Burgart LJ, Leontovich O, Thibodeau SN (2005). Use of microsatellite instability and immunohistochemistry testing for the identification of individuals at risk for Lynch Syndrome. *Fam. Cancer*. 4(3): 255-265.
- Besson A, Dowdy SF, Roberts JM (2008). CDK inhibitors: cell cycle regulators and beyond. *Dev. Cell.* 14: 159-169.
- Bommer GT, Gerin I, Feng Y, Kaczorowski AJ, Kuick R, Love RE, Zhai Y, Giordano TJ, Qin ZS, Moore BB, MacDougald OA, Cho KR, Fearon ER (2007). Pp. 53-Mediated Activation of miRNA34 Candidate Tumor-Suppressor Genes. *Curr. Biol.* 17(15): 1298-1307.
- Calin GA, Croce CM (2006). MicroRNA-cancer connection: the beginning of a new tale. *Cancer Res.* 66: 7390-7394.
- 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 (2002). Frequent deletions and down-regulation of micro-RNA genes miR15 and miR16 at 13q14 in chronic lymphocytic leukemia. *Proc Natl. Acad. Sci. USA.* 99: 15524-15529.
- Calin GA, Sevignani C, Dumitru CD, Hyslop T, Noch E, Yendamuri S, Shimizu M, Rattan S, Bullrich F, Negrini M, Croce CM (2004). Human microRNA genes are frequently located at fragile sites and genomic regions involved in cancers. *Proc. Natl Acad. Sci. USA.* 101: 2999-3004.
- Cannistra SA (2004). Cancer of the ovary. *N. Engl. J. Med.* 351: 2519-2529.
- Chen CZ, Li L, Lodish HF, Bartel DP (2004). MicroRNAs modulate hematopoietic lineage differentiation. *Science*, 303: 83-86.
- Coppola V, Maria RD, Bonci D (2010). MicroRNAs and prostate cancer. *Endocr. Relat. Cancer*, 17: F1-F17.
- Corney DC, Nikitin AY (2009). MicroRNA and ovarian cancer. *Histol. Histopathol.* 2008 September; 23(9): 1161-1169.
- Cummins JM, He Y, Leary RJ, Pagliarini R, Diaz LAJ, Sjoblom T, Barad O, Bentwich Z, Szafranska AE, Labourier E, Raymond CK, Roberts BS, Juhl H, Kinzler KW, Vogelstein B, Velculescu VE (2006). The colorectal microRNAome. *Proc. Natl. Acad. Sci. USA.* 103(10): 3687-3692.
- Dong Q, Meng P, Wang T, Qin W, Weijun Q, Fuli W, Jianlin Y, Zhinan C, Angang Y, He W (2010). MicroRNA Let-7a Inhibits Proliferation of Human Prostate Cancer Cells In Vitro and In Vivo by Targeting E2F2 and CCND2. *PLoS. ONE*, 5(4): e10147.
- Eis PS, Tam W, Sun L, Chadburn A, Li Z, Gomez MF, Lund E, Dahlberg JE (2005). Accumulation of miR-155 and BIC RNA in human B cell lymphomas. *Proc. Natl. Acad. Sci. USA.* 102: 3627-3632.
- Fabbri M, Iliopoulos D, Trapasso F, Aqeilan RI, Amelia C, Zanesi N, Yendamuri S, Han SY, Amadori D, Huebner K, Croce CM (2005). WWOX gene restoration prevents lung cancer growth *in vitro* and *in vivo*. *Proc. Natl. Acad. Sci. USA.* 102: 15611-15616.
- Fabbri M, Ramiro G, Amelia C, Zhongfa L, Nicola Z, Elisa C, Shujun L, Hansjuerg A, Stefan C, Cecilia FC, Stefano V, Gulnur G, Carl DM, Kenneth KC, Guido M, George AC, Kay H, Carlo MC (2007). MicroRNA-29 family reverts aberrant methylation in lung cancer by targeting DNA methyltransferases 3A and 3B. *Proc. Natl. Acad. Sci. USA.* 104(40): 15805-15810.
- Fearon ER, Vogelstein B (1990). A genetic model for colorectal tumorigenesis. *Cell*, 61: 759-767.
- Feeley KM, Wells M (2001). Precursor lesions of ovarian epithelial malignancy. *Histopathol.* 38: 87-95.
- Frankel LB, Christoffersen NR, Jacobsen A, Lindow M, Krogh A, Lund AH (2008). Programmed Cell Death 4 (PDCD4) Is an Important Functional Target of the MicroRNA miR-21 in Breast Cancer Cells. *J. Biol. Chem.* 283: 1026-1033.
- Fujita Y, Kojima K, Hamada N, Ohhashi R, Akao Y, Nozawa Y, Deguchi T, Ito M (2008). Effects of miR-34a on cell growth and chemoresistance in prostate cancer PC3 cells. *Biochem. Biophys. Res. Commun.* 377:114-119.
- Fusco A, Viglietto G, Santoro MA (2005). New mechanism of BRAF activation in human thyroid papillary carcinomas. *J. Clin. Invest.* 115(1): 20-23.
- Gandellini P, Folini M, Zaffaroni N (2010). Emerging Role of microRNAs in Prostate Cancer: Implications for Personalized Medicine. *Discov. Med.* 9(46): 212-218.
- He H, Jazdzewski K, Li W, Liyanarachchi S, Nagy R, Volinia S, Calin GA, Liu CG, Franssila K, Suster S, Kloos RT, Croce CM, de la Chapelle A (2005) The role of microRNA genes in papillary thyroid carcinoma. *Proc. Natl. Acad. Sci. USA.* 102: 19075-19080.
- Huang Q, Gumireddy K, Schrier M, Le CS, Nagel R, Nair S, Egan DA, Li A, Huang G, Klein-Szanto AJ, Gimotty PA, Katsaros D, Coukos G, Zhang L, Pure Agami ER (2008). The microRNAs miR-373 and miR-520c promote tumor invasion and metastasis. *Nat. Cell Biol.* 10: 202-210.
- Iliopoulos D, Guler G, Han SY, Johnston D, Druck T, McCorkell KA, Palazzo J, McCue PA, Baffa R, Huebner K (2005). Fragile genes as biomarkers: epigenetic control of WWOX and FHIT in lung, breast and bladder cancer. *Oncogene*, 24(9): 1625-1633.
- Iorio MV, Ferracin M, Liu CG, Veronese A, Spizzo R, Sabbioni S, Magri E, Pedriali M, Fabbri M, Campiglio M, Menard S, Palazzo JP, Rosenberg A, Musiani P, Volinia S, Nenci I, Calin GA, Querzoli P, Negrini M, Croce CM (2005). MicroRNA gene expression deregulation in human breast cancer. *Cancer Res.* 65: 7065-7070.
- Iorio MV, Visone R, Di LG, Donati V, Petrocca F, Casalini P, Taccioli C,

- Volinia S, Liu CG, Alder H, Calin GA, Menard S, Croce CM (2007). MicroRNA Signatures in Human Ovarian Cancer. *Cancer Res.* 67(18): 8699-8707.
- Jackson RJ, Standart N (2007). How do MicroRNAs Regulate Gene Expression? *Sci. STKE.* 367: re1.
- Jazdzewski K, Murray EL, Franssila K, Jarzab B, Schoenberg DR, de la Chapelle A (2008). Common SNP in pre-miR-146a decreases mature miR expression and predisposes to papillary thyroid carcinoma. *Proc Natl Acad Sci USA.* 105(20): 7269-7274.
- Krystian K, Elizabeth L, Murray EL, Franssila K, Jarzab B, Daniel R, Schoenberg DR, de la Chapelle A (2008). Common SNP in pre-miR-146a decreases mature miR expression and predisposes to papillary thyroid carcinoma. *Proc. Natl. Acad. Sci. USA.* 105: 7269-7274.
- Jeltsch A (2002). Beyond Watson and Crick: DNA Methylation and Molecular Enzymology of DNA Methyltransferases. *Chembiochem.* 3: 274-293.
- Jemal A, Siegel R, Ward E, Hao Y, Xu J, Murray T, Thun MJ (2008). Cancer statistics, 2008. *CA Cancer J Clin.* 58: 71-96.
- Jerome T, Laurie P, Louis B, Pierre C (2007). Enjoy the Silence: The Story of let-7 MicroRNA and Cancer. *Curr. Genomics,* 8(4): 229-233.
- Johnson SM, Grosshans H, Shingara J, Byrom M, Jarvis R, Cheng A, Labourier E, Reinert KL, Brown D, Slack FJ (2005). RAS is regulated by the let-7 microRNA family. *Cell,* 120: 635-47.
- Kim H, Kwon YM, Kim JS, Han J, Shim YM, Park J, Kim DH (2006). Elevated mRNA levels of DNA methyltransferase-1 as an independent prognostic factor in primary non small cell lung cancer. *Cancer,* 107(5): 1042-1049.
- Kim YK, Jieun Yu, Tae SH, Park SY, Namkoong B, Kim DH, Hur K, Yoo MW, Lee HJ, Yang HK, Kim VN (2009). Functional links between clustered microRNAs: suppression of cell-cycle inhibitors by microRNA clusters in gastric cancer. *Nucleic Acids Res.* 37(5): 1672-1681.
- Li LC (2007). Epigenetics of prostate cancer. *Front. Biosci.* 12: 3377-3397.
- Lin SL, Chiang A, Chang D, Ying SY (2008). Loss of mir-146a function in hormone-refractory prostate cancer. *RNA,* 14: 417-424.
- Lodes MJ, Caraballo M, Suci D, Munro S, Kumar A, Anderson B (2009). Detection of Cancer with Serum miRNAs on an Oligonucleotide Microarray. *PLoS ONE,* 4(7): e6229.
- Lu J, Getz G, Miska EA, Saavedra EA, Lamb J, Peck D, Cordero AS, Ebert BL, Mak RH, Ferrando AA, Downing JR, Jacks T, Horvitz HR, Golub TR (2005). MicroRNA expression profiles classify human cancers. *Nature,* 435: 834-8.
- Ma L, Feldstein JT, Weinberg RA (2007). Tumour invasion and metastasis initiated by microRNA-10b in breast cancer. *Nature,* 449: 682-688.
- Ma Y, Fiering S, Black C, Liu X, Yuan Z, Memoli VA, Robbins DJ, Bentley HA, Tsongalis GJ, Demidenko E, Freemantle SJ, Dmitrovsky E (2007). Transgenic cyclin E triggers dysplasia and multiple pulmonary adenocarcinomas. *Proc Natl. Acad. Sci. USA.* 104(10): 4089-4094.
- Melillo RM, Castellone MD, Guarino V, De Falco V, Cirafici AM, Salvatore G, Caiazzo F, Basolo F, Giannini R, Kruhoffer M, Orntoft T, Fusco A, Santoro M (2005). The RET/PTC-RAS-BRAF linear signaling cascade mediates the motile and mitogenic phenotype of thyroid cancer cells. *J. Clin. Invest.* 115: 1068-1081.
- Meng F, Henson R, Wehbe JH, Ghoshal K, Jacob ST, Patel T (2007). MicroRNA-21 regulates expression of the PTEN tumor suppressor gene in human hepatocellular cancer. *Gastroenterol.* 133: 647-658.
- Metzler M, Wilda M, Busch K, Viehmann S, Borkhardt A (2004). High expression of precursor microRNA-155/BIC RNA in children with Burkitt lymphoma. *Genes Chromosomes Cancer,* 39: 167-9.
- Michael MZ, O' Connor SM, van Holst Pellekaan NG, Young GP, James RJ (2003). Reduced accumulation of specific microRNAs in colorectal neoplasia. *Mol. Cancer Res.* 1: 882-91.
- Michael MZ, SM OC, van Holst Pellekaan NG, Young GP, James RJ (2003). Reduced accumulation of specific microRNAs in colorectal neoplasia. *Mol. Cancer Res.* 1: 882-91.
- Nam EJ, Yoon H, Kim SW, Kim H, Kim YT, Kim JH, Kim JW, Kim S (2008). MicroRNA Expression Profiles in Serous Ovarian Carcinoma. *Clin. Cancer Res.* 14(9): 2690-2695.
- Nelson WG, De-Marzo AM, Isaacs WB (2003). Prostate cancer. *N. Engl. J. Med.* 349: 366-381.
- Ng EK, Tsang WP, Ng SS, Jin HC, Yu J, Li JJ, Rocken C, Ebert MP, Kwok TT, Sung JJ (2009) MicroRNA-143 targets DNA methyltransferases 3A in colorectal cancer. *Br J. Cancer* 101: 699-706.
- Nikiforova MN, Tseng GC, Steward D, Diorio D, Nikiforov YE (2008). MicroRNA expression profiling of thyroid tumors: biological significance and diagnostic utility. *J. Clin. Endocrinol. Metab.* 93:1600-1608.
- Noonan EJ, Place RF, Pookot D, Basak S, Whitson M, Hirata H, Giardina C, Dahiya R (2009). miR-449a targets HDAC-1 and induces growth arrest in prostate cancer. *Oncogene,* 28: 1714-1724.
- Pallante P, Visone R, Ferracin M, Ferraro A, Berlingieri MT, Troncone G, Chiappetta G, Liu CG, Santoro M, Negrini M, Croce CM, Fusco A (2006) MicroRNA deregulation in human thyroid papillary carcinomas. *Endocr Relat. Cancer,* 13: 497-508.
- Parkin DM, Bray F, Ferlay J, Pisani P (2005). Global cancer statistics, 2002. *CA: Cancer J. Clinicians,* 55: 74-108.
- Pillai RS, Bhattacharyya SN, Filipowicz W (2007). Repression of protein synthesis by miRNAs: How many mechanisms? *Trends Cell Biol.* 17: 118-126.
- Porkka KP, Pfeiffer MJ, Waltering KK, Vessella RL, Tammela TL, Visakorpi T (2007). MicroRNA expression profiling in prostate cancer. *Cancer Res.* 67: 6130-5.
- Rajewsky N (2006). microRNA target predictions in animals *Nat. genet.* 38(Suppl 1): S8-S13.
- Ramana KV, Tammali R, Srivastava SK (2010). Inhibition of Aldose Reductase Prevents Growth Factor-Induced G₁-S Phase Transition through the AKT/Phosphoinositide 3-Kinase/E2F-1 Pathway in Human Colon Cancer Cells. *Mol. Cancer Ther.* 9: p. 813.
- Ramaswamy B, Majumder S, Roy S, Ghoshal K, Kutay H, Datta J, Younes M, Shapiro CL, Motiwal T, Jacob ST (2009). Estrogen-Mediated Suppression of the Gene Encoding Protein Tyrosine Phosphatase PTPRO in Human Breast Cancer: Mechanism and Role in Tamoxifen Sensitivity. *Mol. Endocrinol.* 23(2):176-187.
- Resnick K (2009). The detection of differentially expressed MicroRNAs from the serum of ovarian cancer patients using a novel real-time PCR platform. *Gynecol. Oncol.* 112: 55-59.
- Rossi S, Kopetz S, Davuluri R, Hamilton SR, Calin GA (2009). MicroRNAs, ultraconserved genes and colorectal cancers. *Int. J. Biochem. Cell Biol.* 42(8): 1291-1297.
- Sarver AL, French AJ, Borralho PM, Thayaniithy V, Oberg AL, Silverstein KA, Morlan BW, Riska SM, Boardman LA, Cunningham JM, Subramanian S, Wang L, Smyrk TC, Rodrigues CM, Thibodeau SN, Steer CJ (2009). Human colon cancer profiles show differential microRNA expression depending on mismatch repair status and are characteristic of undifferentiated proliferative states. *BMC Cancer,* 9: p. 401.
- Schepeler T, Reinert JT, Ostenfeld MS, Christensen LL, Silahtaroglu AN, Dyrskjot L, Wiuf C, Sorensen FJ, Kruhoffer M, Laurberg S, Kauppinen S, Orntoft TF, Andersen CL (2008). Diagnostic and prognostic microRNAs in stage II colon cancer. *Cancer Res.* 68: 6416-6424.
- Schetter AJ, Leung SY, Sohn JJ, Zanetti KA, Bowman ED, Yanaihara N, Yuen ST, Chan TL, Kwong DL, Au GK, Liu CG, Calin GA, Croce CM, Harris CC (2008). MicroRNA expression profiles associated with prognosis and therapeutic outcome in colon adenocarcinoma. *JAMA,* 299: 425-436.
- Shi XB, Xue L, Yang J, Ma AH, Zhao J, Xu M, Tepper Evans CP, Kung HJ, Vere White RW (2007). An androgen-regulated miRNA suppresses Bak1 expression and induces androgen-independent growth of prostate cancer cells. *Proc Natl Acad Sci USA.* 104:19983-19988.
- Sikand K, Stephen DS, Girish CS (2009). Intrinsic expression of host genes and intronic miRNAs in prostate carcinoma cells. *Cancer Cell Int.* 9: p. 21.
- Slaby O, Svoboda M, Fabian P, Smerdova T, Knoflickova D, Bednarikova M, Nenutil R, Vyzula R. (2007). Altered expression of miR-21, miR-31, miR-143 and miR-145 is related to clinicopathologic features of colorectal cancer. *Oncology,* 72: 397-402.
- Son JW, Kim YJ, Cho HM, Lee SY, Jang JS, Choi JE, Lee JU, Kang MG, Lee YM, Kwon SJ, Choi E, Na MJ, Park JY (2009). MicroRNA

- Expression Profiles in Korean Non-Small Cell Lung Cancer. *Tuberc. Respir. Dis.* 67: 413-421.
- Stahlhut CE, Slack FJ (2006). The role of microRNAs in cancer. *Yale J. Biol. Med.* 79(3-4):131-140.
- Suzuki M, Shigematsu H, Shames DS, Sunaga N, Takahashi T, Shivapurkar N, Iizasa T, Frenkel EP, Minna JD, Fujisawa T, Gazdar AF (2004). RNA interference-mediated knockdown of DNA methyltransferase 1 leads to promoter demethylation and gene re-expression in human lung and breast cancer cells. *Cancer Res.* 64: 3137-3143.
- Takamizawa J, Konishi H, Yanagisawa K, Tomida S, Osada H, Endoh H, Harano T, Yatabe Y, Nagino M, Nimura Y, Mitsudomi T, Takahashi T (2004). Reduced expression of the let-7 microRNAs in human lung cancers in association with shortened postoperative survival. *Cancer Res.* 64: 3753-6.
- Ulivi P, Zoli W, Calistri D, Fabbri F, Tesei A, Rosetti M, Mengozzi M, Amadori D (2006). p16INK4A and CDH13 hypermethylation in tumor and serum of non-small cell lung cancer patients. *J. Cell. Phys.* 206: 611-615.
- Varambally S, Cao Q, Mani RS, Shankar S, Wang XS, Ateeq B, Laxman B, Cao X, Jing X, Ramnarayanan K, Brenner JC, Yu J, Kim JH, Han B, Tan P, Kumar-Sinha C, Lonigro RJ, Palanisamy N, Maher CA, Chinnaiyan AM (2008). Genomic loss of microRNA-101 leads to overexpression of histone methyltransferase EZH2 in cancer. *Science*, 322: 1695-9.
- Volinia S, Calin GA, Liu CG, Ambs S, Cimmino A, Petrocca F, Visone R, Iorio M, Roldo C, Ferracin M, Prueitt RL, Yanaihara N, Lanza G, Scarpa A, Vecchione A, Negrini M, Harris CC, Croce CM (2006). A microRNA expression signature of human solid tumors defines cancer gene targets. *Proc. Natl. Acad. Sci. USA*, 103: 2257-2261.
- Wang QZ, Xu W, Habib N, Xu R (2009). Potential Uses of MicroRNA in Lung Cancer Diagnosis, Prognosis, and Therapy. *Curr. Cancer Drug Targets*, 9: 572-594.
- Wang T, Zhang X, Obijuru L, Laser J, Aris V, Lee P, Mittal K, Soteropoulos P, Wei JJ, Zhang X, Obijuru L, Laser J, Aris V, Lee P, Mittal K, Soteropoulos P, Wei JJ, Obijuru L, Laser J, Aris V, Lee P, Mittal K, Soteropoulos P, Wei JJ, Laser J, Aris V, Lee P, Mittal K, Soteropoulos P, Wei JJ, Aris V, Lee P, Mittal K, Soteropoulos P, Wei JJ, Lee P, Mittal K, Soteropoulos P, Wei JJ, Mittal K, Soteropoulos P, Wei JJ, Soteropoulos P, Wei JJ, Wei JJ (2006). A micro-RNA signature associated with race, tumor size, and target gene activity in human uterine leiomyomas. *Genes Chrom. Cancer*, 46: 336-347.
- Weber F, Teresi RE, Broelsch CE, Frilling A, Eng C (2006). A limited set of human MicroRNA is deregulated in follicular thyroid carcinoma. *J. Clin. Endocrinol. Metab.* 91: 3584-3591.
- Weichert W, Roske A, Gekeler V, Beckers T, Stephan C, Jung K, Fritzsche FR, Niesporek S, Denkert C, Dietel M, Kristiansen G (2008). Histone deacetylases 1, 2 and 3 are highly expressed in prostate cancer and HDAC2 expression is associated with shorter PSA relapse time after radical prostatectomy. *Br. J. Cancer.* 98: 604-10.
- Wiseman SM, Makretsov N, Nielsen TO, Gilks B, Yorida E, Cheang M, Turbin D, Gelmon K, Huntsman DG (2005). Coexpression of the type 1 growth factor receptor family members HER-1, HER-2, and HER-3 has a synergistic negative prognostic effect on breast carcinoma survival. *Cancer*, 103:1770-1777.
- Yang H, Kong W, He L, Zhao JJ, O'Donnell JD, Wang J, Wenham RM, Coppola D, Kruk PA, Nicosia SV, Cheng JQ (2008). MicroRNA Expression Profiling in Human Ovarian Cancer: miR-214 Induces Cell Survival and Cisplatin Resistance by Targeting PTEN. *Cancer Res.* 68(2): 425-433.
- Zhang B, Pan X, Cobb GP, Anderson TA (2007). microRNAs as oncogenes and tumor suppressors. *Dev. Biol.* 302: 1-12.