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REVIEW

MiRNA in bladder carcinogenesis: A review

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Abstract

Bladder cancer (BC) is the second urological malignancy in incidence, currently being one of the most neoplasms studied with profile and biology poorly defined. In the world, BC is responsible by about 386000 new cases and 150000 deaths annually with considerable economic impact and high costs for health systems. After its discovery more than 20 years, micro RNAs (miRNAs) have been recognized as molecules that work specifically in post-transcriptional control in majority of eukaryote genomes. MiRNAs are a family of small non-coding RNAs of 19-25 nucleotides in length, expressed in a wide variety of organisms, comprising plants, worms and mammals, including humans. They have a fundamental role in physiological and pathological processes in organs and tissues in a context-dependent manner. This review brings new roles of protective and oncogenic miRNAs linked to carcinogenesis of urothelial carcinoma of the bladder, and associated with behavior of disease. Many studies have demonstrated promising roles of miRNAs working as diagnostic and prognostic biomarkers or involved in target therapies, consolidating miRNAs as crucial players in human cancer. This review allowed a reflection about the true functions of miRNAs in bladder carcinogenesis. Not only by their wide capacities of action, but also by abilities in define the cell date. The future of anti-tumor target therapies will be based not in one, but in groups of miRNAs working together in several steps of carcinogenic process, being able to identify the disease, predicting behavior and effectively treat bladder cancer.

Key words: Bladder cancer; Urothelial carcinoma; MiRNA; Biomarkers

Core tip: Bladder cancer is the second urological malignancy in incidence, currently being one of the most neoplasms studied with profile and biology poorly defined. Micro RNAs (miRNAs) are a class of small non-coding RNAs that play roles in many physiological and pathological processes, including cancer. This review brings new roles of protective and oncogenic miRNAs linked to carcinogenesis of urothelial carcinoma of the bladder, and associated with behavior of disease. Most importantly, we provided a reflection about the true functions of miRNAs in bladder carcinogenesis, not only by their wide capacities of action, but also by abilities in define the cell date.

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INTRODUCTION

Bladder cancer (BC) is the second urological malignancy in incidence, currently being one of the most neoplasms studied with profile and biology poorly defined. BC development is related to environmental exposures that by genetic and epigenetic mechanisms can modify the cellular machinery and trigger the carcinogenic process. In the world, BC is responsible by about 386000 new cases and 150000 deaths annually[1] with considerable economic impact and high costs for health systems[2].

Conventional clinical and pathological parameters are used for BC histological graduation and stage and they are still the only tools now available employed to predict the prognosis of disease. However, this ability is limited and lacking data allowing a prospective analysis of risk of progression and behavior of BC.

Scientific evidences support the concept that BC is a many phases disease, and several alterations are needed until clinical presentation of BC. Thus, BC has been used as a main information source about mutational events that trigger carcinogenic pathways of solid human tumors[3,4]. Although genetic and molecular pathways are relatively well demonstrated and some biomarkers established, there are many questions to be answered about biological behavior or BC and novel methods that more specifically predict BC behavior are necessary.

The most common histological type of BC is bladder urothelial carcinoma (UC), occurring in 80%-90% of cases. UC can present in some different forms, from a small low-grade non-invasive tumor to advanced disease invading bladder wall and adjacent organs, with grade and stage established as main prognostic factors in bladder UC[4]. At diagnosis, 70% are low-grade non-invasive tumors evolving with optimal survival rates, while 10%-20% is high-grade invasive and aggressive disease with poor prognosis and increased index of mortality.

MOLECULAR BIOLOGY OF UC

A wide number of genetic events are involved in etiology, progression and treatment responses of UC[5]. The light of the molecular pathways related to UC carcinogenic process is crucial to know its etiopathogenesis and behavior. Biological variations such as both carcinogens conversion and detoxification and DNA repair can modify the expression and action of related genes in different phases of UC carcinogenesis.

Genetic events in non-invasive UC

Carcinogenic pathways that trigger low-grade non-invasive and high-grade invasive tumors are specific and mutually exclusives, and are showed in Figure 1[6-8]. The most of bladder malignancies is non-muscle invasive in initial presentation and its main tumorigenic route is mediated by fibroblastic growth factor receptor 3 (*FGFR3*) gene. Nevertheless, less common mutations in *RAS* gene have been described.

FGFR3 has 18 exons, it is located in 4p16.3 chromosomal region and belongs to tyrosine kinase growth factors receptors family, involved in functions related to embryogenesis and tissue homeostasis[8,9], regulating several biological processes, including proliferation, differentiation, migration and apoptosis[10]. Point mutations or other alterations which lead to *FGFR3* over-activity can alter cellular proliferation and trigger low-grade well differentiated UC, having little effect in cellular differentiation and apoptosis. These influences propitiate advantage to cell proliferation, but they do not change the genomic stability. *FGFR3* point mutations were described for the first time by Cappellen *et al*[11] (1999) that identified mutations in 35% of tumors. Point mutations in codons 248, 249 and 375 comprise more than 95% of *FGFR3* mutations.

Mutations in *RAS* oncogene has also been associated with UC non-invasive tumors and can be responsible by 30% of human cancers[12]. RAS works in regulation of cellular functions as proliferation, differentiation, motility and apoptosis in response to extracellular signals. In bladder UC, RAS seems to act through both mitogenic-activated protein kinase (MAPK) and AKT/STAT pathways[13]. Interestingly, *RAS* and *FGFR3* mutations do not occur at the same time, being considered mutually exclusive events indicating biological equivalence between these two types of point mutations[13].

Genetic events in invasive UC

The most of known genetic events in bladder UC is described in high-grade invasive tumors and many of them, as mutations and loss of function in the protective genes *p53*, retinoblastoma (RB1) and phosphatase and tensin homolog gene (*PTEN*) are associated with poor prognosis and high genetic instability[3].

p53 gene product is a tumor suppressor protein that is activated in response to signals of cellular stress, promoting transcriptional regulation of genes that induce cell cycle arrest, apoptosis, senescence, DNA repair and alterations in metabolism of the cell. Somatic mutations in *p53* are described in more than 50% of human tumors and germinal mutations can promote the tumor development in some hereditary syndromes. Unlike FGFR3, loss of function of p53 lead to important genomic instability associated with high-grade and stage tumors (Figure 1)[14-16].

RB1 suscetibility, a prototype of suppressor tumor gene, has been associated with UC progression and development. This phosphoprotein is a negative regulator of cell cycle and promotes chromatin stabilization allowing maintenance of its structure. RB1 mutations are strongly related to infant retinoblastoma, osteogenic sarcoma and bladder cancer (www.ncbi.nlm.nih.gov/gene). RB1 inac-

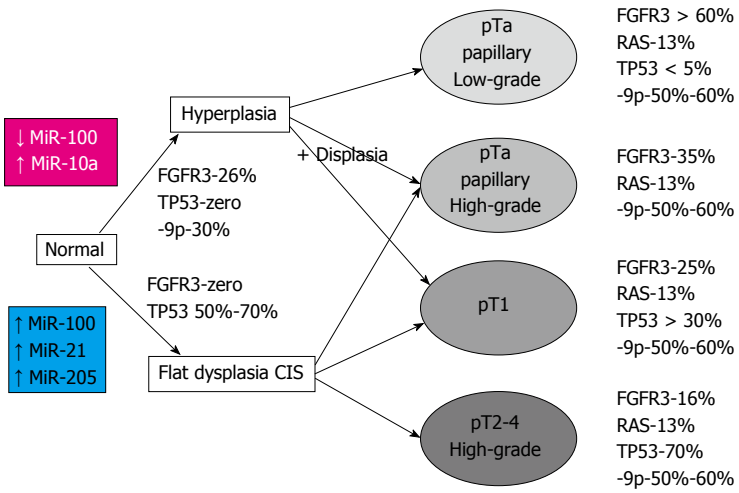

Figure 1 Genetic carcinogenic pathways of bladder urothelial carcinomas incorporating micro RNA. FGFR3: Fibroblastic growth factor receptor 3.

tivation is linked to UC, more specifically to invasive and aggressive disease[17,18].

PTEN is located in chromosome 10 (10q23) and works like a traditional tumor suppressor, acting in proliferation control, migration and cellular invasion by PI3K/AKT/mTOR pathway[4,19]. Despite its influence in non-invasive bladder UC, PTEN is more associated with invasive carcinogenic pathways. Although there are evidences that show its role in initiation and neoplastic progression, PTEN is not able to trigger them alone[20]. Recent data show that, when loss of function of PTEN is associated with *p53* mutations, invasive UC progresses more quickly, demonstrating worst prognosis and lower survival rates[21].

Associations of genetic alterations into UC high-grade invasive carcinogenic pathway seem to be the key event leading to initiation and progression of bladder urothelial carcinomas.

Epigenetic events in UC

Genetic alterations only are not able to explain cancer molecular diversity. Other mechanisms can also affect gene expression and signal pathways. Epigenetic changes, such as DNA methylation and histones deacetylation, can occur without changing DNA structure and seem contribute to malignant transformation and UC progression[22,23]. They can be promoted by external agents, including smoke, diet and carcinogens exposure.

A wide variety of important genes in several cellular processes could present DNA methylation oscillating from 1% to 98%, and appear in initial stages of disease[24-30]. Another epigenetic mechanism is the transcription repression through interaction between micro RNA (miRNA) and specific sequences in messenger RNA (mRNA), as discussed below.

MiRNA

After its discovery more than 20 years[31], miRNAs have been recognized as molecules that work specifically in post-transcriptional control in majority of eukaryote genomes. miRNAs are a family of small non-coding RNAs of 19-25 nucleotides in length, expressed in a wide variety of organisms, comprising plants, worms and mammals, including humans[32]. They have a fundamental role in physiological and pathological processes in organs and tissues in a context-dependent manner. Many miRNAs are highly conserved between species and the machinery of its biogenesis can be found in archaebacteria and eubacterias, establishing its ancestral characteristic. Currently, there are more than 2500 miRNAs with specific biogenesis (Figure 2) and related to control of more than 30% of human genes (www.mirnabodymap.org)[33] involved in multiple processes of development and cell differentiation, apoptosis, homeostasis and metabolic pathways[34-36]. In oncologic research, miRNAs work tumor suppressors or oncogenic (oncomiR), showing specific profiles that could characterize different types of cancer[36,37]. Albeit there are studies exploring miRNA expression profile in bladder UC, data are still scarce and biological field so vast[38-41].

MiRNA in bladder UC

MiR-100: MiR-100 is a protective miRNA in human cells[42], acting in a context-dependent manner[43]. Under-expression profiles has been found in non-invasive bladder UC[44,45], ovarian carcinoma[46], oral cavity carcinoma[47], osteosarcoma[48], vulvar carcinoma[49], lymphoblastic leukaemia[50], gastric cancer[51] and several other types of human cancer.

In non-invasive bladder UC, miR-100 has as main target the *FGFR3* gene, whose mutation and over-activity is related to this neoplasm[44]. In physiological conditions, miR-100 exerts negative control over FGFR3 decreasing their post-transcriptional expression levels (Figure 3). As Blick *et al*[52], we also suggest that there might be an alternative pathway triggering UC non-invasive carcinogenesis not associated with *FGFR3* activating point mutations[53]. Under-expression of miR-100 could be responsible by lack of negative control and FGFR3 over-expression, promoting non-invasive UC

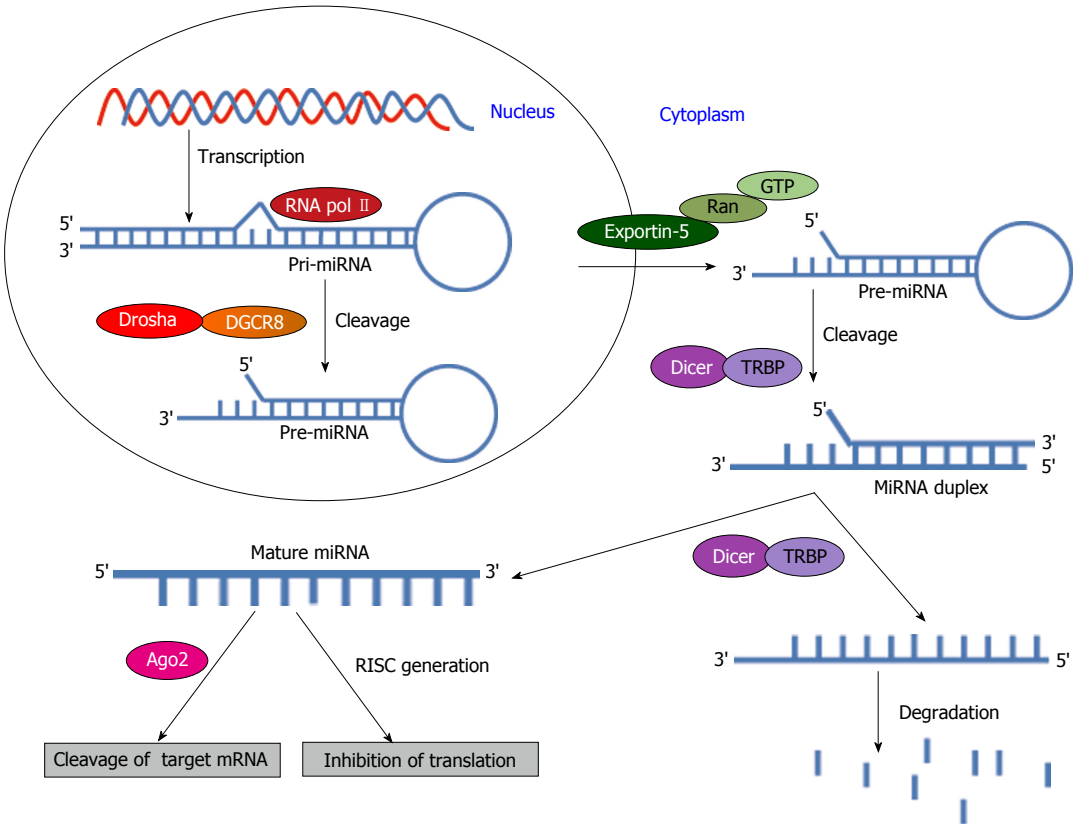

Figure 2 Micro RNA biogenesis in human cells. After transcription by RNA polymerase Ⅱ, the primary micro RNA (miRNA) precursor (Pri-miRNA) is cleaved by Drosha microprocessor complex and converted in Pre-miRNA, a 60-70 nt double-strand molecule. The Pre-miRNA is transported from nucleus to the cytoplasm by Exportin-5 and then it is cleaved by Dicer to generate the miRNA duplex. Again, Dicer enzyme acts over miRNA duplex and produces single-strand mature miRNA that, in turn, is incorporated into RNA-Induced Silencing Complex (RISC). RISC drivers mature miRNA to the target messenger RNA (mRNA), triggering mRNA cleavage (Slicer activity) or inhibition of translation by complete or incomplete complementarity, respectively.

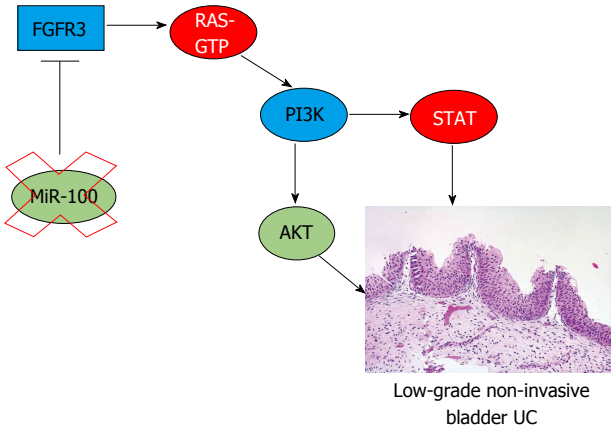

Figure 3 MiR-100 in low-grade non-invasive urothelial carcinoma carcinogenesis. Under-expression of miR-100 leads to *FGFR3* gene over-expression, stimulus to PI3K/AKT/STAT pathway and low-grade non-invasive tumor development. Adapted by Wu[19], 2009. FGFR3: Fibroblastic growth factor receptor 3; UC: Urothelial carcinoma.

carcinogenesis and low-grade tumor development (Figure 3). Catto *et al*[44] found an inverse ratio between miR-100 and *FGFR3*, where the under-expression of miR-100 led to increased gene activity before the occurrence of point mutation, suggesting that increased levels of *FGFR3* could facilitate the mutational event through increased cellular turnover or natural selection of mutant cells. Maybe, miR-100 loss of expression can be the first trigger event of disease and could occur before clinical presentation of the tumor. This fact is important because this molecular characteristic could be used for initial diagnostic and predicts disease behavior, allowing a conservative treatment due to rare chance of progression and excellent survival. We speculate that miR-100 will be used in clinical practice as a diagnostic and prognostic biomarker and employed in target therapies.

On the other hand, we showed an over-expression of miR-100 in high-grade invasive UC[45]. We suggest that miR-100 acts as a negative controller of *THAP-2* gene, directly involved in proliferation control through modulation of proteins that control cell cycle such as pRB and E2F[54]. Loss of function of RB1, p53 and PTEN is involved in carcinogenic route of invasive UC, promoting genomic instability and facilitating tumor progression. MiR-100 over-expression could trigger THAP-2 silencing and, consequently, RB1 inactivity (Figure 4). *BAZ2A* and *SMARCA5* genes are also targets of miR-100 and are associated with DNA transcription repression and chromosomal instability[55,56]. Recently we demonstrated in cell cultures of BC that miR-100 has a role over BAZ2A and SMARCA5 activity (data submitted for publication), but better investigation are needed to establish the role of

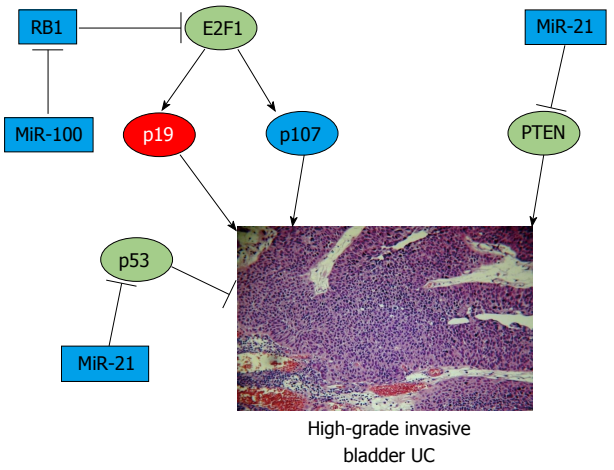

Figure 4 MiR-100 and miR-21 in high-grade invasive urothelial carcinoma carcinogenesis. High levels of miR-100 inhibit retinoblastoma (RB1) and over-expression of miR-21 suppresses p53 and phosphatase and tensin homolog gene (*PTEN*), three crucial protective genes. Inactivity of RB1 associated with loss of function of p53 and *PTEN* trigger high-grade urothelial carcinoma (UC) carcinogenesis. Adapted by Wu[19], 2009.

miR-100 in invasive UC carcinogenesis.

MiR-10a: MiR-10a comprises 23 nitrogenous bases and is located in chromosome 17q21.23, within *HOXB* gene cluster, upstream of *HOXB4* (www.mirbase.org). A number of *HOX* genes have been found to be regulated by miR-10. These genes encode mainly transcription factors which have crucial roles in embryonic development and cell differentiation. In humans, miR-10a exerts a negative control over *HOXA1* and *HOXA3* genes supporting that this miRNA can play fundamental roles in physiological activities of the cell[57,58].

MiR-10a has two different mechanisms of action over control of gene expression. The first one, extensively demonstrated by literature, is the canonical inhibition of protein product by miR-10a binding in target 3'UTR mRNA. On the other hand, Ørom *et al*[59] have described the second mechanism of action, and demonstrated a positive effect of miR-10a through its complementary interaction with mRNA 5´UTR, allowing a translational stimulus of proteins associated to the ribosomal machinery, increasing global cellular activities[59]. Although miR-10a is linked to carcinogenesis of several human tumors, it is believed that its functions are involved in physiological situations of the cell. Nowadays it is well established the crucial role of miR-10a in physiological process of cellular differentiation. Even being able to act through mechanisms described above, translational inhibition by negative control of miR-10a on its target genes highlights as the most active mechanism in the differentiation process.

Recently, we have demonstrated a miRNA expression profile in bladder urothelial carcinoma and we found that miR-10a over-expression was one of the most evident changes. MiR-10a is able to effectively separate two genetically distinct tumor groups, which are, low-grade non-invasive pTa from high-grade invasive pT2-3 UC, with over-expression in first and under-expression in the second tumor group[45]. Moreover, miR-10a expression profile was associated with disease-free and cancer-specific survivals between groups[45].

Regarding low-grade non-invasive pathway, FGFR3 over-activity triggered by down-regulation of miR-100 could be corroborated through up-regulation of miR-10a, both promoting higher cell proliferation rates. Maybe this mechanism miR-10a-mediated is constituted in a negative control of physiological inhibitor of FGFR3 through its canonical activity, increasing cellular proliferation without modify the genetic stability of the neoplastic cell. About high-grade invasive tumors, sharing close homology with miR-100, miR-10a maybe could be continuously blocking *HOX* genes, promoting poor differentiation, enhanced aggressiveness and worse tumor behavior.

MiR-21: Corroborating with findings published by Neely *et al*[39], we recently have demonstrated that miR-21 presented strong over-expression (17-fold higher) in high-grade invasive UC[39,45]. Recent evidences show that miR-21 is a truly oncogenic miRNA, presenting over-expressed in wide majority of human tumors. miR-21 can promote tumorigenesis by inducing cell proliferation and blocking of apoptotic control mechanisms[60], thus triggering more aggressive disease and poor responses to treatments[61,62].

p53 is considered the most important gene involved in invasive UC carcinogenesis[14]. p53 is responsible for control of global activities of the cell by cell cycle arrest, stimulus of apoptosis and DNA repair. Catto *et al*[44] have observed miR-21 over-expression associated with p53 inactivation, invasiveness and tumor progression. Another important protective gene related to UC carcinogenesis is PTEN, also being target of miR-21[4,19,63]. PTEN is a lipid phosphatase that inhibits PI3K/AKT pathway, blocking cell proliferation. In an actual review about genetic and molecular mechanisms involved in initiation and progression of UC, McConkey *et al*[4] have suggested that loss of function of PTEN is much more common in invasive disease related to PTEN/PI3K/AKT/mTOR characterizing a worse prognostic factor. Figure 4 shows a schematic flowchart regarding miR-21 roles in high-grade invasive bladder UC.

We showed that miR-21 under-expression was associated with better disease-free survival in non-invasive UC, consolidating oncogenic behavior for miR-21[45].

MiR-205: MiR-205 has been defined as a tumor suppressor miRNA involved in epithelial-to-mesenchymal transition (EMT), a process provided by malignancies to perform a fundamental step to tumor progression and systemic dissemination. Like miR-200 family, miR-205 acts negatively over ZEB-1 and ZEB-2 that, in turn, suppress E-cadherin, an adhesion molecule responsible for physiological conditions of bladder epithelia[64,65]. Under-expression of miR-205 leads to ZEB-1 and ZEB-2 over-

activity and sequential E-cadherin inhibition, facilitating the metastasizing process. EMT is crucial to success and maintenance of bladder carcinogenesis, being directly associated with worse behavior, high tumor aggressiveness, poor prognosis and shorter survival. The role of miR-205 is similar in several human tumor types, blocking tumor progression and dissemination. Some authors have already demonstrated a miR-205 under-expression in lung, breast and prostate cancer[66-68].

In bladder UC, evidences has been suggested aberrant methylation in chromosome 1q32.2 where is located the *miR-205* gene[69]. Neely *et al*[39], in 2008, established that miR-21:miR-205 ratio is progressively increased according tumor progression. Interesting data are shown by Brabletz *et al*[70], demonstrating that ZEB/miR-200 feedback loop is crucial to define cell status. Thus, an invasive and progressive tumor status occurs when the loop is favoring ZEB, leading tumor dissemination beyond epithelial barriers. On the other hand, a proliferative environment predominates when the loop tend to miR-200 family over-expression, allowing tumor growth. Second authors, both states are needed to carcinogenic process, a first early proliferative promoting tumor growth and a second late progressive triggering tumor dissemination[70]. A very interesting fact is that miR-200 over-expression could favor the metastasizing process but, after metastasis sites are defined, the proliferation occur again, being necessary the re-expression of miR-200. In 2012, we published data supporting this idea, where miR-205 was under-expressed in 100% of low-grade non-invasive pTa UC, according with early carcinogenic state, while there was a re-expression of miR-205 in a third of cases of high-grade invasive UC[45].

MiR-let7c: The literature consolidates the suppressor role of miR-let7c in almost all human cancers, reflecting its important suppressive action against malignant events. MiR-let7c targets RAS and c-MYC oncogenes and under-expression of this miRNA is related to neoplasm development[71,72]. RAS is the second most important oncogene in low-grade non-invasive tumorigenesis[73]. Point mutations can lead to genetic alterations that, in turn, promote a strong stimulus of AKT and STAT pathways, triggering high rates of cellular proliferation. c-MYC is a prototype of oncogene promoting RB1 inhibition in high-grade invasive UC[71]. c-MYC is able to stimulate cyclins CDK4, CDK6 and D1 leading to phosphorylation and inactivation of RB1 and increased mitogenic and proliferative processes. Otherwise, c-MYC can stimulate p53 over-activity *via* MDM2 inhibition, favoring cell cycle arrest and apoptosis[3]. Moreover, p53 can enhance expression levels of miR-let7 promoting a cumulative protective effect against carcinogenesis[74].

MiR-125b: Evidences are controversial regarding role of miR-125b in cancer. While some studies define miR-125b as a tumor suppressor, other suggest its oncogenic functions[40,75-78]. However, miR-125b seems to have a protective role in bladder UC. Four main authors demonstrate the suppressive role of miR-125b in bladder cancer, acting over transcription factors, oncogenes and metallopeptidases[40,79-81]. In agreement to these authors, we have also verified an under-expression profile for miR-125b in almost all cases of low-grade non-invasive and high-grade invasive UC, suggesting its suppressive function in bladder UC[45].

MiR-143 and miR-145: MiR-143 and miR-145 are closely located in 5q32 chromosome and share similar suppressive tumor functions, including bladder cancer[45,82-85]. Evidences demonstrate RAS as a target gene of miR-143. Mutations in RAS lead to stimulus of MAPK/AKT/STAT pathway, triggering low-grade non-invasive UC[19], but RAS may also be involved in high-grade tumors[4]. Lin *et al*[86] showed by studies in malignant tissues and cell cultures that under-expression of miR-143 is the rule in UC. In tumor tissues, miR-143 presented 13.7 fold-changes down-regulated in comparison to normal bladder tissues, while in EJ and T24 cell lines it was not identified. When transfected in cell lines, miR-143 significantly inhibited cellular proliferation[86]. Noguchi *et al*[87] also showed the suppressive role of miR-143 in bladder cancer cell lines, and replacement treatment with miR-143 and miR-145 induced synergistic inhibition of tumor by regulating PI3K/AKT/MAPK signaling pathways. Moreover, miR-143 can regulate other target genes involved in UC carcinogenesis. For example, Song *et al*[88] established an inverse correlation between miR-143 and COX-2, an oncogene associated with grade, prognosis and recurrence of bladder UC. Furthermore, the authors verified in T24 cell line that restoration of miR-143 by transfection decreased COX-2 expression and reduced proliferation and motility of tumor cells.

Many studies have validated miR-145 as an inhibitor of cell cycle and tumor growth, promoting induction of apoptosis and lower progression of disease. MiR-145 is a protective miRNA presenting under-expressed in many human tumors, such as colorectal, lung, breast, prostate and renal cancer, and in non-malignant disease such as benign prostatic hyperplasia[89-93]. The first report of miR-145 under-expression was performed by Michael *et al*[94] in a study suggesting that these alterations could be involved in the initiation of colorectal cancer. These findings were confirmed by Shi *et al*[95] who showed that miR-145 under-expression was associated with malignant tumors. Sachdeva *et al*[96] showed in breast cancer cell lines that *MUC-1* gene was associated with tumor onset, invasion and dissemination, where miR-145 was able to inhibit these factors controlling tumor development[96]. Another target of miR-145 is *c-MYC* gene, an oncogene implicated in carcinogenic process of high-grade invasive UC[3,91]. Furthermore, miR-145 is induced by p53 activity, being directly related to invasive UC tumorigenesis. In 2009, Sachdeva *et al*[91] observed that, in physiological conditions, cellular stress promoted higher levels of p53 that, in turn, increased concentrations of miR-145 through

p53 response element. Higher levels of miR-145 inhibit c-MYC activity, allowing normal function of p21 and cell cycle arrest[91]. Under-expression of miR-145 could promote fail in c-MYC control, decreasing p21 levels and stimulates cell proliferation. These complex mechanisms could lead, or at least initiate, UC carcinogenesis[97]. Following the same idea, Spizzo *et al*[98] analyzing breast cancer cell lines demonstrated the crucial suppressor role of miR-145, and its transfection inhibited growth and cell proliferation, inducing p53-mediated apoptosis. Chiyomaru *et al*[99] demonstrated an association between FSCN-1 oncogene and miR-145 under-expression in UC, suggesting that loss of expression of miR-145 and consequent FSCN-1 up-regulation may be associated with bladder tumors in all stages, promoting more aggressive and invasive tumors. Last year we published a work demonstrating that miR-145 is a well-characterized tumor suppressor miRNA in UC. We hypothesize that lack of protector role promoted by miR-145 over probable target genes PI3K/AKT, FSCN-1, MDM-2, c-MYC and MUC-1 could be involved in carcinogenic process of low-grade, non-invasive and high-grade invasive urothelial carcinomas, being an interesting candidate diagnostic biomarker[100].

MiR-199a: Low levels of miR-199a have already found in ovarian and endometrial cancer, testicular tumors, hepatocarcinoma (HCC) and osteosarcoma[101-105] and it seems to be directly involved in tumor progression and worse prognosis. Fornari *et al*[102] studying HCC suggest that miR-199a targets mTOR, demonstrating an inverse relationship between them. Similarly to HCC, PI3K/AKT/mTOR pathway is also involved in high-grade UC carcinogenesis and miR-199a under-expression could explain mTOR over-activity and, at least partially, the tumor onset and progression of disease.

In bladder UC, even though we have shown miR-199a under-expression in most of both low-grade non-invasive and high-grade invasive tumors, statistical differences were not observed[45]. On the other hand, Ratert *et al*[106] found miR-199a down-regulated in malignant bladder samples compared to healthy tissue. Also, the authors demonstrated that miR-199a was differentially expressed between non-invasive and invasive bladder cancer, underling together miR-205 and miR-141, the potential to work as biomarkers of diagnosis and prognosis in bladder UC[106].

MiR-452: There are few and controversial studies concerning miR-452 in human cancer. Some works demonstrate an under-expression of miR-452 in squamous lung tumors, gliomas, prostate and breast cancer[107-110], suggesting a protective role of miR-452 in these assessed tumors.

In bladder cancer, Veerla *et al*[41] and Puerta-Gil *et al*[111] showed that higher levels of miR-452 are the rule in UC and associated with high-grade and invasive disease, poor behavior and metastatic process of disease. In disagreement with the former two researchers, but in concordance with other several studies in human cancer, we verified a strong under-expression profile for miR-452 in both low-grade non-invasive and high-grade invasive UC, suggesting a tumor suppressor role for this miRNA[45]. Moreover, we observed increased under-expression levels according higher grade and stage, reinforcing the protective function of miR-452. Human miR-452 has approximately 220 target genes and we speculate that loss of control over genes involved in cell growth and proliferation as E2F3 and MEF2C could explain the involvement of miR-452 in bladder carcinogenesis. However, complementary mechanistic studies are needed to consolidate this hypothesis.

Final considerations

Many studies have demonstrated promising roles of miRNAs working as diagnostic and prognostic biomarkers or involved in target therapies, consolidating miRNAs as crucial players in human cancer. This review allows a reflection about the true functions of miRNAs in bladder carcinogenesis, not only by their wide capacities of action, but also by abilities in define the cell date. MiRNAs characterization in plasma and urine, representing tissue levels, are potential non-invasive methods that could assist diagnosis, treatment and evaluation of bladder cancer improving management of this prevalent disease.

Finally, the future of anti-tumor target therapies will be based not in one, but in groups of miRNAs working together in several steps of carcinogenic process, being able to identify the disease, predicting behavior and effectively treat bladder cancer.

REFERENCES

1 **Jemal A**, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. *CA Cancer J Clin* 2011; **61**: 69-90 [PMID: 21296855 DOI: 10.3322/caac.20107]

2 **Botteman MF**, Pashos CL, Redaelli A, Laskin B, Hauser R. The health economics of bladder cancer: a comprehensive review of the published literature. *Pharmacoeconomics* 2003; **21**: 1315-1330 [PMID: 14750899 DOI: 10.1007/BF03262330]

3 **Knowles MA**. Molecular pathogenesis of bladder cancer. *Int J Clin Oncol* 2008; **13**: 287-297 [PMID: 18704628 DOI: 10.1007/s10147-008-0812-0]

4 **McConkey DJ**, Lee S, Choi W, Tran M, Majewski T, Lee S, Siefker-Radtke A, Dinney C, Czerniak B. Molecular genetics of bladder cancer: Emerging mechanisms of tumor initiation and progression. *Urol Oncol* 2010; **28**: 429-440 [PMID: 20610280 DOI: 10.1016/j.urolonc.2010.04.008]

5 **Garcia del Muro X**, Condom E, Vigués F, Castellsagué X, Figueras A, Muñoz J, Solá J, Soler T, Capellà G, Germà JR. p53 and p21 Expression levels predict organ preservation and survival in invasive bladder carcinoma treated with a combined-modality approach. *Cancer* 2004; **100**: 1859-1867 [PMID: 15112266 DOI: 10.1002/cncr.20200]

6 **Bakkar AA**, Wallerand H, Radvanyi F, Lahaye JB, Pissard S, Lecerf L, Kouyoumdjian JC, Abbou CC, Pairon JC, Jaurand MC, Thiery JP, Chopin DK, de Medina SG. FGFR3 and TP53 gene mutations define two distinct pathways in urothelial cell carcinoma of the bladder. *Cancer Res* 2003; **63**: 8108-8112 [PMID: 14678961 DOI: 10.4238/vol10-1gmr923]

7 **van Rhijn BW**, van der Kwast TH, Vis AN, Kirkels WJ, Bo-

evé ER, Jöbsis AC, Zwarthoff EC. FGFR3 and P53 characterize alternative genetic pathways in the pathogenesis of urothelial cell carcinoma. *Cancer Res* 2004; **64**: 1911-1914 [PMID: 15026322 DOI: 10.1158/0008-5472.CAN-03-2421]

8 **Pandith AA**, Shah ZA, Siddiqi MA. Oncogenic role of fibroblast growth factor receptor 3 in tumorigenesis of urinary bladder cancer. *Urol Oncol* 2013; **31**: 398-406 [PMID: 20822928 DOI: 10.1016/j.urolonc.2010.07.014]

9 **Thompson LM**, Plummer S, Schalling M, Altherr MR, Gusella JF, Housman DE, Wasmuth JJ. A gene encoding a fibroblast growth factor receptor isolated from the Huntington disease gene region of human chromosome 4. *Genomics* 1991; **11**: 1133-1142 [PMID: 1664411 DOI: 10.1016/0888-7543(91)90041-C]

10 **Ornitz DM**, Xu J, Colvin JS, McEwen DG, MacArthur CA, Coulier F, Gao G, Goldfarb M. Receptor specificity of the fibroblast growth factor family. *J Biol Chem* 1996; **271**: 15292-15297 [PMID: 8663044 DOI: 10.1074/jbc.271.25.15292]

11 **Cappellen D**, De Oliveira C, Ricol D, de Medina S, Bourdin J, Sastre-Garau X, Chopin D, Thiery JP, Radvanyi F. Frequent activating mutations of FGFR3 in human bladder and cervix carcinomas. *Nat Genet* 1999; **23**: 18-20 [PMID: 10471491 DOI: 10.1038/12615]

12 **van der Weyden L**, Adams DJ. The Ras-association domain family (RASSF) members and their role in human tumourigenesis. *Biochim Biophys Acta* 2007; **1776**: 58-85 [PMID: 17692468 DOI: 10.1016/j.bbcan.2007.06.003]

13 **Jebar AH**, Hurst CD, Tomlinson DC, Johnston C, Taylor CF, Knowles MA. FGFR3 and Ras gene mutations are mutually exclusive genetic events in urothelial cell carcinoma. *Oncogene* 2005; **24**: 5218-5225 [PMID: 15897885 DOI: 10.1038/sj.onc.1208705]

14 **Esrig D**, Elmajian D, Groshen S, Freeman JA, Stein JP, Chen SC, Nichols PW, Skinner DG, Jones PA, Cote RJ. Accumulation of nuclear p53 and tumor progression in bladder cancer. *N Engl J Med* 1994; **331**: 1259-1264 [PMID: 7935683 DOI: 10.1056/NEJM199411103311903]

15 **Sarkis AS**, Dalbagni G, Cordon-Cardo C, Melamed J, Zhang ZF, Sheinfeld J, Fair WR, Herr HW, Reuter VE. Association of P53 nuclear overexpression and tumor progression in carcinoma in situ of the bladder. *J Urol* 1994; **152**: 388-392 [PMID: 8015077]

16 **Hainaut P**, Hollstein M. p53 and human cancer: the first ten thousand mutations. *Adv Cancer Res* 2000; **77**: 81-137 [PMID: 10549356 DOI: 10.1016/S0065-230X(08)60785-X]

17 **Cordon-Cardo C**, Wartinger D, Petrylak D, Dalbagni G, Fair WR, Fuks Z, Reuter VE. Altered expression of the retinoblastoma gene product: prognostic indicator in bladder cancer. *J Natl Cancer Inst* 1992; **84**: 1251-1256 [PMID: 1640484 DOI: 10.1093/jnci/84.16.1251]

18 **Gallucci M**, Guadagni F, Marzano R, Leonardo C, Merola R, Sentinelli S, Ruggeri EM, Cantiani R, Sperduti I, Lopez Fde L, Cianciulli AM. Status of the p53, p16, RB1, and HER-2 genes and chromosomes 3, 7, 9, and 17 in advanced bladder cancer: correlation with adjacent mucosa and pathological parameters. *J Clin Pathol* 2005; **58**: 367-371 [PMID: 15790699 DOI: 10.1136/jcp.2004.021154]

19 **Wu XR**. Biology of urothelial tumorigenesis: insights from genetically engineered mice. *Cancer Metastasis Rev* 2009; **28**: 281-290 [PMID: 20012171 DOI: 10.1007/s10555-009-9189-4]

20 **Tsuruta H**, Kishimoto H, Sasaki T, Horie Y, Natsui M, Shibata Y, Hamada K, Yajima N, Kawahara K, Sasaki M, Tsuchiya N, Enomoto K, Mak TW, Nakano T, Habuchi T, Suzuki A. Hyperplasia and carcinomas in Pten-deficient mice and reduced PTEN protein in human bladder cancer patients. *Cancer Res* 2006; **66**: 8389-8396 [PMID: 16951148 DOI: 10.1158/0008-5472.CAN-05-4627]

21 **Puzio-Kuter AM**, Castillo-Martin M, Kinkade CW, Wang X, Shen TH, Matos T, Shen MM, Cordon-Cardo C, Abate-Shen C. Inactivation of p53 and Pten promotes invasive bladder cancer. *Genes Dev* 2009; **23**: 675-680 [PMID: 19261747 DOI: 10.1101/gad.1772909]

22 **Dalmay T**. MicroRNAs and cancer. *J Intern Med* 2008; **263**: 366-375 [PMID: 18298485 DOI: 10.1111/j.1365-2796.2008.01926.x]

23 **Enokida H**, Nakagawa M. Epigenetics in bladder cancer. *Int J Clin Oncol* 2008; **13**: 298-307 [PMID: 18704629 DOI: 10.1007/s10147-008-0811-1]

24 **Kim YT**, Park SJ, Lee SH, Kang HJ, Hahn S, Kang CH, Sung SW, Kim JH. Prognostic implication of aberrant promoter hypermethylation of CpG islands in adenocarcinoma of the lung. *J Thorac Cardiovasc Surg* 2005; **130**: 1378 [PMID: 16256792 DOI: 10.1016/j.jtcvs.2005.06.015]

25 **Friedrich MG**, Chandrasoma S, Siegmund KD, Weisenberger DJ, Cheng JC, Toma MI, Huland H, Jones PA, Liang G. Prognostic relevance of methylation markers in patients with non-muscle invasive bladder carcinoma. *Eur J Cancer* 2005; **41**: 2769-2778 [PMID: 16242928 DOI: 10.1016/j.ejca.2005.07.019]

26 **Marsit CJ**, Houseman EA, Schned AR, Karagas MR, Kelsey KT. Promoter hypermethylation is associated with current smoking, age, gender and survival in bladder cancer. *Carcinogenesis* 2007; **28**: 1745-1751 [PMID: 17522068 DOI: 10.1093/carcin/bgm116]

27 **Yates DR**, Rehman I, Abbod MF, Meuth M, Cross SS, Linkens DA, Hamdy FC, Catto JW. Promoter hypermethylation identifies progression risk in bladder cancer. *Clin Cancer Res* 2007; **13**: 2046-2053 [PMID: 17404085 DOI: 10.1158/1078-0432.CCR-06-2476]

28 **Ellinger J**, El Kassem N, Heukamp LC, Matthews S, Cubukluoz F, Kahl P, Perabo FG, Müller SC, von Ruecker A, Bastian PJ. Hypermethylation of cell-free serum DNA indicates worse outcome in patients with bladder cancer. *J Urol* 2008; **179**: 346-352 [PMID: 18006010 DOI: 10.1016/j.juro.2007.08.091]

29 **Lodygin D**, Tarasov V, Epanchintsev A, Berking C, Knyazeva T, Körner H, Knyazev P, Diebold J, Hermeking H. Inactivation of miR-34a by aberrant CpG methylation in multiple types of cancer. *Cell Cycle* 2008; **7**: 2591-2600 [PMID: 18719384 DOI: 10.4161/cc.7.16.6533]

30 **Brait M**, Begum S, Carvalho AL, Dasgupta S, Vettore AL, Czerniak B, Caballero OL, Westra WH, Sidransky D, Hoque MO. Aberrant promoter methylation of multiple genes during pathogenesis of bladder cancer. *Cancer Epidemiol Biomarkers Prev* 2008; **17**: 2786-2794 [DOI: 10.1158/1055-9965.EPI-08-0192]

31 **Lee RC**, Feinbaum RL, Ambros V. The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. *Cell* 1993; **75**: 843-854 [PMID: 8252621 DOI: 10.1016/0092-8674(93)90529-Y]

32 **Pillai RS**. MicroRNA function: multiple mechanisms for a tiny RNA? *RNA* 2005; **11**: 1753-1761 [PMID: 16314451 DOI: 10.1261/rna.2248605]

33 **Lewis BP**, Burge CB, Bartel DP. Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. *Cell* 2005; **120**: 15-20 [PMID: 15652477 DOI: 10.1016/j.cell.2004.12.035]

34 **Ambros V**. MicroRNA pathways in flies and worms: growth, death, fat, stress, and timing. *Cell* 2003; **113**: 673-676 [PMID: 12809598 DOI: 10.1016/S0092-8674(03)00428-8]

35 **Bartel DP**. MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell* 2004; **116**: 281-297 [PMID: 14744438 DOI: 10.1016/S0092-8674(04)00045-5]

36 **Blenkiron C**, Miska EA. miRNAs in cancer: approaches, aetiology, diagnostics and therapy. *Hum Mol Genet* 2007; **16** Spec No 1: R106-R113 [PMID: 17613543 DOI: 10.1093/hmg/ddm056]

37 **Calin GA**, Croce CM. MicroRNA signatures in human cancers. *Nat Rev Cancer* 2006; **6**: 857-866 [PMID: 17060945 DOI: 10.1038/nrc1997]

38 **Gottardo F**, Liu CG, Ferracin M, Calin GA, Fassan M, Bassi P, Sevignani C, Byrne D, Negrini M, Pagano F, Gomella LG, Croce CM, Baffa R. Micro-RNA profiling in kidney and bladder cancers. *Urol Oncol* 2007; **25**: 387-392 [PMID: 17826655 DOI: 10.1016/j.urolonc.2007.01.019]

39 **Neely LA**, Rieger-Christ KM, Neto BS, Eroshkin A, Garver J, Patel S, Phung NA, McLaughlin S, Libertino JA, Whitney D, Summerhayes IC. A microRNA expression ratio defining the invasive phenotype in bladder tumors. *Urol Oncol* 2010; **28**: 39-48 [PMID: 18799331 DOI: 10.1016/j.urolonc.2008.06.006]

40 **Ichimi T**, Enokida H, Okuno Y, Kunimoto R, Chiyomaru T, Kawamoto K, Kawahara K, Toki K, Kawakami K, Nishiyama K, Tsujimoto G, Nakagawa M, Seki N. Identification of novel microRNA targets based on microRNA signatures in bladder cancer. *Int J Cancer* 2009; **125**: 345-352 [PMID: 19378336 DOI: 10.1002/ijc.24390]

41 **Veerla S**, Lindgren D, Kvist A, Frigyesi A, Staaf J, Persson H, Liedberg F, Chebil G, Gudjonsson S, Borg A, Månsson W, Rovira C, Höglund M. MiRNA expression in urothelial carcinomas: important roles of miR-10a, miR-222, miR-125b, miR-7 and miR-452 for tumor stage and metastasis, and frequent homozygous losses of miR-31. *Int J Cancer* 2009; **124**: 2236-2242 [PMID: 19127597 DOI: 10.1002/ijc.24183]

42 **Chen J**, Zheng B, Wang C, Chen Y, Du C, Zhao G, Zhou Y, Shi Y. Prognostic role of microRNA-100 in various carcinomas: evidence from six studies. *Tumour Biol* 2014; **35**: 3067-3071 [PMID: 24258109 DOI: 10.1007/s13277-013-1398-3]

43 **Leite KR**, Morais DR, Reis ST, Viana N, Moura C, Florez MG, Silva IA, Dip N, Srougi M. MicroRNA 100: a context dependent miRNA in prostate cancer. *Clinics* (Sao Paulo) 2013; **68**: 797-802 [PMID: 23778488 DOI: 10.6061/clinics/2013(06)12]

44 **Catto JW**, Miah S, Owen HC, Bryant H, Myers K, Dudziec E, Larré S, Milo M, Rehman I, Rosario DJ, Di Martino E, Knowles MA, Meuth M, Harris AL, Hamdy FC. Distinct microrna alterations characterize high- and low-grade bladder cancer. *Cancer Res* 2009; **69**: 8472-8481 [DOI: 10.1158/0008-5472.CAN-09-0744]

45 **Dip N**, Reis ST, Timoszczuk LS, Viana NI, Piantino CB, Morais DR, Moura CM, Abe DK, Silva IA, Srougi M, Dall'Oglio MF, Leite KR. Stage, grade and behavior of bladder urothelial carcinoma defined by the microRNA expression profile. *J Urol* 2012; **188**: 1951-1956 [PMID: 22999546 DOI: 10.1016/j.juro.2012.07.004]

46 **Nagaraja AK**, Creighton CJ, Yu Z, Zhu H, Gunaratne PH, Reid JG, Olokpa E, Itamochi H, Ueno NT, Hawkins SM, Anderson ML, Matzuk MM. A link between mir-100 and FRAP1/mTOR in clear cell ovarian cancer. *Mol Endocrinol* 2010; **24**: 447-463 [PMID: 20081105 DOI: 10.1210/me.2009-0295]

47 **Henson BJ**, Bhattacharjee S, O'Dee DM, Feingold E, Gollin SM. Decreased expression of miR-125b and miR-100 in oral cancer cells contributes to malignancy. *Genes Chromosomes Cancer* 2009; **48**: 569-582 [PMID: 19396866 DOI: 10.1002/gcc.20666]

48 **Huang J**, Gao K, Lin J, Wang Q. MicroRNA-100 inhibits osteosarcoma cell proliferation by targeting Cyr61. *Tumour Biol* 2014; **35**: 1095-1100 [PMID: 24317814 DOI: 10.1007/s13277-013-1146-8]

49 **de Melo Maia B**, Lavorato-Rocha AM, Rodrigues LS, Coutinho-Camillo CM, Baiocchi G, Stiepcich MM, Puga R, de A Lima L, Soares FA, Rocha RM. microRNA portraits in human vulvar carcinoma. *Cancer Prev Res* (Phila) 2013; **6**: 1231-1241 [PMID: 24048714 DOI: 10.1158/1940-6207.CAPR-13-0121]

50 **Li XJ**, Luo XQ, Han BW, Duan FT, Wei PP, Chen YQ. MicroRNA-100/99a, deregulated in acute lymphoblastic leukaemia, suppress proliferation and promote apoptosis by regulating the FKBP51 and IGF1R/mTOR signalling pathways. *Br J Cancer* 2013; **109**: 2189-2198 [PMID: 24030073 DOI: 10.1038/bjc.2013.562]

51 **Shi DB**, Xing AY, Gao C, Gao P. [Expression of microRNA-100 in human gastric cancer]. *Zhonghua Binglixue Zazhi* 2013; **42**: 15-19 [PMID: 23611267 DOI: 10.3760/cma.j.issn.0529-5807.2013.01.004]

52 **Blick C**, Ramachandran A, Wigfield S, McCormick R, Jubb A, Buffa FM, Turley H, Knowles MA, Cranston D, Catto J, Harris AL. Hypoxia regulates FGFR3 expression via HIF-1α and miR-100 and contributes to cell survival in non-muscle invasive bladder cancer. *Br J Cancer* 2013; **109**: 50-59 [PMID: 23778527 DOI: 10.1038/bjc.2013.240]

53 **Dip N**, Reis ST, Timoszczuk LS, Abe DK, Dall'Oglio M, Srougi M, Leite KRM. Under-expression of mir-100 may be a new carcinogenic pathway for low-grade pta bladder urothelial carcinomas. *J MolBiomarkDiagn* 2011; **1**: 2-6 [DOI: 10.4172/2155-9929.1000121]

54 **Bessière D**, Lacroix C, Campagne S, Ecochard V, Guillet V, Mourey L, Lopez F, Czaplicki J, Demange P, Milon A, Girard JP, Gervais V. Structure-function analysis of the THAP zinc finger of THAP1, a large C2CH DNA-binding module linked to Rb/E2F pathways. *J Biol Chem* 2008; **283**: 4352-4363 [PMID: 18073205 DOI: 10.1074/jbc.M707537200]

55 **Zhou Y**, Santoro R, Grummt I. The chromatin remodeling complex NoRC targets HDAC1 to the ribosomal gene promoter and represses RNA polymerase I transcription. *EMBO J* 2002; **21**: 4632-4640 [PMID: 12198165 DOI: 10.1093/emboj/cdf460]

56 **Bozhenok L**, Wade PA, Varga-Weisz P. WSTF-ISWI chromatin remodeling complex targets heterochromatic replication foci. *EMBO J* 2002; **21**: 2231-2241 [PMID: 11980720 DOI: 10.1093/emboj/21.9.2231]

57 **Garzon R**, Pichiorri F, Palumbo T, Iuliano R, Cimmino A, Aqeilan R, Volinia S, Bhatt D, Alder H, Marcucci G, Calin GA, Liu CG, Bloomfield CD, Andreeff M, Croce CM. MicroRNA fingerprints during human megakaryocytopoiesis. *Proc Natl Acad Sci USA* 2006; **103**: 5078-5083 [PMID: 16549775]

58 **Lund AH**. miR-10 in development and cancer. *Cell Death Differ* 2010; **17**: 209-214 [PMID: 19461655 DOI: 10.1038/cdd.2009.58]

59 **Ørom UA**, Nielsen FC, Lund AH. MicroRNA-10a binds the 5'UTR of ribosomal protein mRNAs and enhances their translation. *Mol Cell* 2008; **30**: 460-471 [PMID: 18498749 DOI: 10.1016/j.molcel.2008.05.001]

60 **Si ML**, Zhu S, Wu H, Lu Z, Wu F, Mo YY. miR-21-mediated tumor growth. *Oncogene* 2007; **26**: 2799-2803 [PMID: 17072344 DOI: 10.1038/sj.onc.1210083]

61 **Li B**, Ren S, Li X, Wang Y, Garfield D, Zhou S, Chen X, Su C, Chen M, Kuang P, Gao G, He Y, Fan L, Fei K, Zhou C, Schmit-Bindert G. MiR-21 overexpression is associated with acquired resistance of EGFR-TKI in non-small cell lung cancer. *Lung Cancer* 2014; **83**: 146-153 [PMID: 24331411 DOI: 10.1016/j.lungcan.2013.11.003]

62 **Zhang W**, Bai W, Zhang W. MiR-21 suppresses the anticancer activities of curcumin by targeting PTEN gene in human non-small cell lung cancer A549 cells. *Clin Transl Oncol* 2014; **16**: 708-713 [PMID: 24293118]

63 **Meng F**, Henson R, Wehbe-Janek H, Ghoshal K, Jacob ST, Patel T. MicroRNA-21 regulates expression of the PTEN tumor suppressor gene in human hepatocellular cancer. *Gastroenterology* 2007; **133**: 647-658 [PMID: 17681183 DOI: 10.1053/j.gastro.2007.05.022]

64 **Gregory PA**, Bert AG, Paterson EL, Barry SC, Tsykin A, Farshid G, Vadas MA, Khew-Goodall Y, Goodall GJ. The miR-200 family and miR-205 regulate epithelial to mesenchymal transition by targeting ZEB1 and SIP1. *Nat Cell Biol* 2008; **10**: 593-601 [PMID: 18376396 DOI: 10.1038/ncb1722]

65 **Tran MN**, Choi W, Wszolek MF, Navai N, Lee IL, Nitti G, Wen S, Flores ER, Siefker-Radtke A, Czerniak B, Dinney C, Barton M, McConkey DJ. The p63 protein isoform δnp63α inhibits epithelial-mesenchymal transition in human bladder cancer cells: Role of mir-205. *J BiolChem* 2013; **288**: 3275-3288 [DOI: 10.1074/jbc.M112.408104]

66 **Lebanony D**, Benjamin H, Gilad S, Ezagouri M, Dov A, Ash-

kenazi K, Gefen N, Izraeli S, Rechavi G, Pass H, Nonaka D, Li J, Spector Y, Rosenfeld N, Chajut A, Cohen D, Aharonov R, Mansukhani 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 [PMID: 19273703 DOI: 10.1200/JCO.2008.19.4134]

67 **Wu H**, Zhu S, Mo YY. Suppression of cell growth and invasion by miR-205 in breast cancer. *Cell Res* 2009; **19**: 439-448 [PMID: 19238171 DOI: 10.1038/cr.2009.18]

68 **Gandellini P**, Folini M, Longoni N, Pennati M, Binda M, Colecchia M, Salvioni R, Supino R, Moretti R, Limonta P, Valdagni R, Daidone MG, Zaffaroni N. miR-205 Exerts tumor-suppressive functions in human prostate through down-regulation of protein kinase Cepsilon. *Cancer Res* 2009; **69**: 2287-2295 [PMID: 19244118 DOI: 10.1158/0008-5472.CAN-08-2894]

69 **Wiklund ED**, Bramsen JB, Hulf T, Dyrskjøt L, Ramanathan R, Hansen TB, Villadsen SB, Gao S, Ostenfeld MS, Borre M, Peter ME, Ørntoft TF, Kjems J, Clark SJ. Coordinated epigenetic repression of the miR-200 family and miR-205 in invasive bladder cancer. *Int J Cancer* 2011; **128**: 1327-1334 [PMID: 20473948 DOI: 10.1002/ijc.25461]

70 **Brabletz S**, Brabletz T. The ZEB/miR-200 feedback loop--a motor of cellular plasticity in development and cancer? *EMBO Rep* 2010; **11**: 670-677 [PMID: 20706219 DOI: 10.1038/embor.2010.117]

71 **Reinhart BJ**, Slack FJ, Basson M, Pasquinelli AE, Bettinger JC, Rougvie AE, Horvitz HR, Ruvkun G. The 21-nucleotide let-7 RNA regulates developmental timing in Caenorhabditis elegans. *Nature* 2000; **403**: 901-906 [PMID: 10706289 DOI: 10.1038/35002607]

72 **Johnson SM**, Grosshans H, Shingara J, Byrom M, Jarvis R, Cheng A, Labourier E, Reinert KL, Brown D, Slack FJ. RAS is regulated by the let-7 microRNA family. *Cell* 2005; **120**: 635-647 [PMID: 15766527 DOI: 10.1016/j.cell.2005.01.014]

73 **Wu XR**. Urothelial tumorigenesis: a tale of divergent pathways. *Nat Rev Cancer* 2005; **5**: 713-725 [PMID: 16110317 DOI: 10.1038/nrc1697]

74 **Lee JY**, Kim HJ, Yoon NA, Lee WH, Min YJ, Ko BK, Lee BJ, Lee A, Cha HJ, Cho WJ, Park JW. Tumor suppressor p53 plays a key role in induction of both tristetraprolin and let-7 in human cancer cells. *Nucleic Acids Res* 2013; **41**: 5614-5625 [PMID: 23595149 DOI: 10.1093/nar/gkt222]

75 **Le MT**, Teh C, Shyh-Chang N, Xie H, Zhou B, Korzh V, Lodish HF, Lim B. MicroRNA-125b is a novel negative regulator of p53. *Genes Dev* 2009; **23**: 862-876 [PMID: 19293287 DOI: 10.1101/gad.1767609]

76 **Klusmann JH**, Li Z, Böhmer K, Maroz A, Koch ML, Emmrich S, Godinho FJ, Orkin SH, Reinhardt D. miR-125b-2 is a potential oncomiR on human chromosome 21 in megakaryoblastic leukemia. *Genes Dev* 2010; **24**: 478-490 [PMID: 20194440 DOI: 10.1101/gad.1856210]

77 **Rajabi H**, Jin C, Ahmad R, McClary C, Joshi MD, Kufe D. Mucin 1 oncoprotein expression is suppressed by the mir-125b oncomir. *Genes Cancer* 2010; **1**: 62-68 [PMID: 20729973 DOI: 10.1177/1947601909357933]

78 **Liang L**, Wong CM, Ying Q, Fan DN, Huang S, Ding J, Yao J, Yan M, Li J, Yao M, Ng IO, He X. MicroRNA-125b suppressesed human liver cancer cell proliferation and metastasis by directly targeting oncogene LIN28B2. *Hepatology* 2010; **52**: 1731-1740 [PMID: 20827722 DOI: 10.1002/hep.23904]

79 **Huang L**, Luo J, Cai Q, Pan Q, Zeng H, Guo Z, Dong W, Huang J, Lin T. MicroRNA-125b suppresses the development of bladder cancer by targeting E2F3. *Int J Cancer* 2011; **128**: 1758-1769 [PMID: 20549700 DOI: 10.1002/ijc.25509]

80 **Han Y**, Liu Y, Zhang H, Wang T, Diao R, Jiang Z, Gui Y, Cai Z. Hsa-miR-125b suppresses bladder cancer development by down-regulating oncogene SIRT7 and oncogenic long noncoding RNA MALAT1. *FEBS Lett* 2013; Epub ahead of print [PMID: 24512851]

81 **Wu D**, Ding J, Wang L, Pan H, Zhou Z, Zhou J, Qu P. microRNA-125b inhibits cell migration and invasion by targeting matrix metallopeptidase 13 in bladder cancer. *Oncol Lett* 2013; **5**: 829-834 [PMID: 23425975 DOI: 10.3892/ol.2013.1123]

82 **Noguchi S**, Mori T, Hoshino Y, Maruo K, Yamada N, Kitade Y, Naoe T, Akao Y. MicroRNA-143 functions as a tumor suppressor in human bladder cancer T24 cells. *Cancer Lett* 2011; **307**: 211-220 [PMID: 21550168 DOI: 10.1016/j.canlet.2011.04.005]

83 **Villadsen SB**, Bramsen JB, Ostenfeld MS, Wiklund ED, Fristrup N, Gao S, Hansen TB, Jensen TI, Borre M, Ørntoft TF, Dyrskjøt L, Kjems J. The miR-143/-145 cluster regulates plasminogen activator inhibitor-1 in bladder cancer. *Br J Cancer* 2012; **106**: 366-374 [PMID: 22108519 DOI: 10.1038/bjc.2011.520]

84 **Pignot G**, Cizeron-Clairac G, Vacher S, Susini A, Tozlu S, Vieillefond A, Zerbib M, Lidereau R, Debre B, Amsellem-Ouazana D, Bieche I. microRNA expression profile in a large series of bladder tumors: identification of a 3-miRNA signature associated with aggressiveness of muscle-invasive bladder cancer. *Int J Cancer* 2013; **132**: 2479-2491 [PMID: 23169479 DOI: 10.1002/ijc.27949]

85 **Yoshino H**, Seki N, Itesako T, Chiyomaru T, Nakagawa M, Enokida H. Aberrant expression of microRNAs in bladder cancer. *Nat Rev Urol* 2013; **10**: 396-404 [PMID: 23712207 DOI: 10.1038/nrurol.2013.113]

86 **Lin T**, Dong W, Huang J, Pan Q, Fan X, Zhang C, Huang L. MicroRNA-143 as a tumor suppressor for bladder cancer. *J Urol* 2009; **181**: 1372-1380 [PMID: 19157460 DOI: 10.1016/j.juro.2008.10.149]

87 **Noguchi S**, Yasui Y, Iwasaki J, Kumazaki M, Yamada N, Naito S, Akao Y. Replacement treatment with microRNA-143 and -145 induces synergistic inhibition of the growth of human bladder cancer cells by regulating PI3K/Akt and MAPK signaling pathways. *Cancer Lett* 2013; **328**: 353-361 [PMID: 23104321 DOI: 10.1016/j.canlet.2012.10.017]

88 **Song T**, Zhang X, Wang C, Wu Y, Dong J, Gao J, Cai W, Hong B. Expression of miR-143 reduces growth and migration of human bladder carcinoma cells by targeting cyclooxygenase-2. *Asian Pac J Cancer Prev* 2011; **12**: 929-933 [PMID: 21790228]

89 **Ozen M**, Creighton CJ, Ozdemir M, Ittmann M. Widespread deregulation of microRNA expression in human prostate cancer. *Oncogene* 2008; **27**: 1788-1793 [PMID: 17891175 DOI: 10.1038/sj.onc.1210809]

90 **Izzotti A**, Calin GA, Arrigo P, Steele VE, Croce CM, De Flora S. Downregulation of microrna expression in the lungs of rats exposed to cigarette smoke. *FASEB J* 2009; **23**: 806-812 [DOI: 10.1096/fj.08-121384]

91 **Sachdeva M**, Zhu S, Wu F, Wu H, Walia V, Kumar S, Elble R, Watabe K, Mo YY. p53 represses c-Myc through induction of the tumor suppressor miR-145. *Proc Natl Acad Sci USA* 2009; **106**: 3207-3212 [PMID: 19202062 DOI: 10.1073/pnas.0808042106]

92 **Viana NI**, Reis ST, Dip NG, Morais DR, Moura CM, Silva IA, Katz B, Srougi M, Leite KR, Antunes AA. MicroRNAs 143 and 145 may be involved in benign prostatic hyperplasia pathogenesis through regulation of target genes and proteins. *Int J Biol Markers* 2014; **29**: e246-e252 [PMID: 24474452 DOI: 10.5301/jbm.5000069]

93 **Lu R**, Ji Z, Li X, Zhai Q, Zhao C, Jiang Z, Zhang S, Nie L, Yu Z. miR-145 functions as tumor suppressor and targets two oncogenes, ANGPT2 and NEDD9, in renal cell carcinoma. *J Cancer Res Clin Oncol* 2014; **140**: 387-397 [PMID: 24384875 DOI: 10.1007/s00432-013-1577-z]

94 **Michael MZ**, O' Connor SM, van Holst Pellekaan NG, Young GP, James RJ. Reduced accumulation of specific microRNAs in colorectal neoplasia. *Mol Cancer Res* 2003; **1**: 882-891 [PMID: 14573789]

95 **Shi B**, Sepp-Lorenzino L, Prisco M, Linsley P, deAngelis T, Baserga R. Micro RNA 145 targets the insulin receptor sub-

strate-1 and inhibits the growth of colon cancer cells. *J Biol Chem* 2007; **282**: 32582-32590 [PMID: 17827156 DOI: 10.1074/jbc.M702806200]

96 **Sachdeva M**, Mo YY. MicroRNA-145 suppresses cell invasion and metastasis by directly targeting mucin 1. *Cancer Res* 2010; **70**: 378-387 [PMID: 19996288 DOI: 10.1158/0008-5472.CAN-09-2021]

97 **Sachdeva M**, Mo YY. miR-145-mediated suppression of cell growth, invasion and metastasis. *Am J Transl Res* 2010; **2**: 170-180 [PMID: 20407606]

98 **Spizzo R**, Nicoloso MS, Lupini L, Lu Y, Fogarty J, Rossi S, Zagatti B, Fabbri M, Veronese A, Liu X, Davuluri R, Croce CM, Mills G, Negrini M, Calin GA. Mir-145 participates with tp53 in a death-promoting regulatory loop and targets estrogen receptor-alpha in human breast cancer cells. *Cell Death Differ* 2010; **17**: 246-254 [DOI: 10.1038/cdd.2009.117]

99 **Chiyomaru T**, Enokida H, Tatarano S, Kawahara K, Uchida Y, Nishiyama K, Fujimura L, Kikkawa N, Seki N, Nakagawa M. miR-145 and miR-133a function as tumour suppressors and directly regulate FSCN1 expression in bladder cancer. *Br J Cancer* 2010; **102**: 883-891 [PMID: 20160723 DOI: 10.1038/sj.bjc.6605570]

100 **Dip N**, Reis ST, Srougi M, Dall'Oglio MF, Leite KR. Expression profile of microrna-145 in urothelial bladder cancer. *Int Braz J Urol* 2013; **39**: 95-101; discussion 102 [PMID: 23489501 DOI: 10.1590/S1677-5538.IBJU.2013.01.12]

101 **Chen R**, Alvero AB, Silasi DA, Kelly MG, Fest S, Visintin I, Leiser A, Schwartz PE, Rutherford T, Mor G. Regulation of IKKbeta by miR-199a affects NF-kappaB activity in ovarian cancer cells. *Oncogene* 2008; **27**: 4712-4723 [PMID: 18408758 DOI: 10.1038/onc.2008.112]

102 **Fornari F**, Milazzo M, Chieco P, Negrini M, Calin GA, Grazi GL, Pollutri D, Croce CM, Bolondi L, Gramantieri L. MiR-199a-3p regulates mTOR and c-Met to influence the doxorubicin sensitivity of human hepatocarcinoma cells. *Cancer Res* 2010; **70**: 5184-5193 [PMID: 20501828 DOI: 10.1158/0008-5472.CAN-10-0145]

103 **Cheung HH**, Davis AJ, Lee TL, Pang AL, Nagrani S, Rennert OM, Chan WY. Methylation of an intronic region regulates miR-199a in testicular tumor malignancy. *Oncogene* 2011; **30**: 3404-3415 [PMID: 21383689 DOI: 10.1038/onc.2011.60]

104 **Duan Z**, Choy E, Harmon D, Liu X, Susa M, Mankin H, Hornicek F. MicroRNA-199a-3p is downregulated in human osteosarcoma and regulates cell proliferation and migration. *Mol Cancer Ther* 2011; **10**: 1337-1345 [PMID: 21666078 DOI: 10.1158/1535-7163.MCT-11-0096]

105 **Wu D**, Huang HJ, He CN, Wang KY. MicroRNA-199a-3p regulates endometrial cancer cell proliferation by targeting mammalian target of rapamycin (mTOR). *Int J Gynecol Cancer* 2013; **23**: 1191-1197 [PMID: 23851675 DOI: 10.1097/IGC.0b013e31829ea779]

106 **Ratert N**, Meyer HA, Jung M, Lioudmer P, Mollenkopf HJ, Wagner I, Miller K, Kilic E, Erbersdobler A, Weikert S, Jung K. miRNA profiling identifies candidate mirnas for bladder cancer diagnosis and clinical outcome. *J Mol Diagn* 2013; **15**: 695-705 [PMID: 23945108 DOI: 10.1016/j.jmoldx.2013.05.008]

107 **Mascaux C**, Laes JF, Anthoine G, Haller A, Ninane V, Burny A, Sculier JP. Evolution of microRNA expression during human bronchial squamous carcinogenesis. *Eur Respir J* 2009; **33**: 352-359 [PMID: 19010987 DOI: 10.1183/09031936.00084108]

108 **Liu L**, Chen K, Wu J, Shi L, Hu B, Cheng S, Li M, Song L. Downregulation of miR-452 promotes stem-like traits and tumorigenicity of gliomas. *Clin Cancer Res* 2013; **19**: 3429-3438 [PMID: 23695168 DOI: 10.1158/1078-0432.CCR-12-3794]

109 **Liu C**, Kelnar K, Vlassov AV, Brown D, Wang J, Tang DG. Distinct microRNA expression profiles in prostate cancer stem/progenitor cells and tumor-suppressive functions of let-7. *Cancer Res* 2012; **72**: 3393-3404 [PMID: 22719071 DOI: 10.1158/0008-5472.CAN-11-3864]

110 **van Schooneveld E**, Wouters MC, Van der Auwera I, Peeters DJ, Wildiers H, Van Dam PA, Vergote I, Vermeulen PB, Dirix LY, VanLaere SJ. Expression profiling of cancerous and normal breast tissues identifies microrna that are differentially expressed in serum from patients with (metastatic) breast cancer and healthy volunteers. *Breast Cancer Res* 2012; **14**: R34 [DOI: 10.1186/bcr3127]

111 **Puerta-Gil P**, García-Baquero R, Jia AY, Ocaña S, Alvarez-Múgica M, Alvarez-Ossorio JL, Cordon-Cardo C, Cava F, Sánchez-Carbayo M. miR-143, miR-222, and miR-452 are useful as tumor stratification and noninvasive diagnostic biomarkers for bladder cancer. *Am J Pathol* 2012; **180**: 1808-1815 [PMID: 22426337 DOI: 10.1016/j.ajpath.2012.01.034]

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