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**MiRNA in bladder carcinogenesis: A review**

Dip N *et al*. MiRNAs involved in bladder carcinogenesis

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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, 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.

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**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. 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](#_ENREF_1)] with considerable economic impact and high costs for health systems[[2](#_ENREF_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](#_ENREF_3),[4](#_ENREF_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](#_ENREF_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](#_ENREF_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](#_ENREF_6)]. 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 gene (FGFR3). 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](#_ENREF_8),[9](#_ENREF_9)], regulating several biological processes, including proliferation, differentiation, migration and apoptosis[[10](#_ENREF_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](#_ENREF_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](#_ENREF_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](#_ENREF_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](#_ENREF_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 PTEN are associated with poor prognosis and high genetic instability[[3](#_ENREF_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](#_ENREF_14)].

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](http://www.ncbi.nlm.nih.gov/gene)). RB1 inactivation is linked to UC, more specifically to invasive and aggressive disease[[17](#_ENREF_17),[18](#_ENREF_18)].

Phosphatase and tensin homolog gene (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](#_ENREF_4),[19](#_ENREF_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](#_ENREF_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](#_ENREF_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](#_ENREF_22),[23](#_ENREF_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](#_ENREF_24)]. 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](#_ENREF_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](#_ENREF_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](http://www.mirnabodymap.org))[[33](#_ENREF_33)] involved in multiple processes of development and cell differentiation, apoptosis, homeostasis and metabolic pathways[[34-36](#_ENREF_34)]. In oncologic research, miRNAs work tumor suppressors or oncogenic (oncomiR), showing specific profiles that could characterize different types of cancer[[36](#_ENREF_36),[37](#_ENREF_37)]. Albeit there are studies exploring miRNA expression profile in bladder UC, data are still scarce and biological field so vast[[38-41](#_ENREF_38)].

***MiRNA in bladder UC***

**MiR-100:** Micro RNA 100 is a protective miRNA in human cells[[42](#_ENREF_42)] , acting in a context-dependent manner[[43](#_ENREF_43)]. Under-expression profiles has been found in non-invasive bladder UC[[44](#_ENREF_44),[45](#_ENREF_45)], ovarian carcinoma[[46](#_ENREF_46)], oral cavity carcinoma[[47](#_ENREF_47)], osteosarcoma[[48](#_ENREF_48)], vulvar carcinoma[[49](#_ENREF_49)], lymphoblastic leukaemia[[50](#_ENREF_50)], gastric cancer[[51](#_ENREF_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](#_ENREF_44)]. In physiological conditions, miR-100 exerts negative control over FGFR3 decreasing their post-transcriptional expression levels (Figure 3). As Blick *et al*[[52](#_ENREF_52)], we also suggest that there might be an alternative pathway triggering UC non-invasive carcinogenesis not associated with FGFR3 activating point mutations[[53](#_ENREF_53)]. Under-expression of miR-100 could be responsible by lack of negative control and FGFR3 over-expression, promoting non-invasive UC 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](#_ENREF_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](#_ENREF_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](#_ENREF_55),[56](#_ENREF_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 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](http://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](#_ENREF_57),[58](#_ENREF_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, Orom *et al*[[59](#_ENREF_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](#_ENREF_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](#_ENREF_45)]. Moreover, miR-10a expression profile was associated with disease-free and cancer-specific survivals between groups[[45](#_ENREF_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](#_ENREF_39),[45](#_ENREF_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](#_ENREF_60)], thus triggering more aggressive disease and poor responses to treatments[[61](#_ENREF_61),[62](#_ENREF_62)].

p53 is considered the most important gene involved in invasive UC carcinogenesis[[14](#_ENREF_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](#_ENREF_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](#_ENREF_4),[19](#_ENREF_19),[63](#_ENREF_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](#_ENREF_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](#_ENREF_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](#_ENREF_64),[65](#_ENREF_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](#_ENREF_66)].

In bladder UC, evidences has been suggested aberrant methylation in chromosome 1q32.2 where is located the miR-205 gene[[69](#_ENREF_69)]. Neely *et al*[[39](#_ENREF_39)], in 2008, established that miR-21:miR-205 ratio is progressively increased according tumor progression. Interesting data are shown by Brabletz and Brabletz, demonstrating that ZEB/miR-200 feedback loop is crucial to define cell status[[70](#_ENREF_70)]. 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](#_ENREF_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](#_ENREF_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](#_ENREF_71),[72](#_ENREF_72)]. RAS is the second most important oncogene in low-grade non-invasive tumorigenesis[[73](#_ENREF_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](#_ENREF_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](#_ENREF_3)]. Moreover, p53 can enhance expression levels of miR-let7 promoting a cumulative protective effect against carcinogenesis[[74](#_ENREF_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](#_ENREF_40),[75-78](#_ENREF_75)]. 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](#_ENREF_40),[79-81](#_ENREF_79)]. 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](#_ENREF_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](#_ENREF_45),[82-85](#_ENREF_82)]. 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](#_ENREF_19)], but RAS may also be involved in high-grade tumors[[4](#_ENREF_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](#_ENREF_86)]. Noguchi *et al*[[87](#_ENREF_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](#_ENREF_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](#_ENREF_89)].The first report of miR-145 under-expression was performed by Michael *et al*[[94](#_ENREF_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](#_ENREF_95)] who showed that miR-145 under-expression was associated with malignant tumors. Sachdeva *et al*[[96](#_ENREF_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](#_ENREF_96)]. Another target of miR-145 is c-MYC gene, an oncogene implicated in carcinogenic process of high-grade invasive UC[[3](#_ENREF_3),[91](#_ENREF_91)]. Furthermore, miR-145 is induced by p53 activity, being directly related to invasive UC tumorigenesis. In 2009, Sachdeva *et al*[[91](#_ENREF_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 (p53RE). Higher levels of miR-145 inhibit c-MYC activity, allowing normal function of p21 and cell cycle arrest[[91](#_ENREF_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](#_ENREF_97)]. Following the same idea, Spizzo *et al*[[98](#_ENREF_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](#_ENREF_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](#_ENREF_100)].

**MiR-199a:** Low levels of miR-199a have already found in ovarian and endometrial cancer, testicular tumors, hepatocarcinoma (HCC) and osteosarcoma[[101-105](#_ENREF_101)] 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](#_ENREF_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](#_ENREF_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](#_ENREF_107)], 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](#_ENREF_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.

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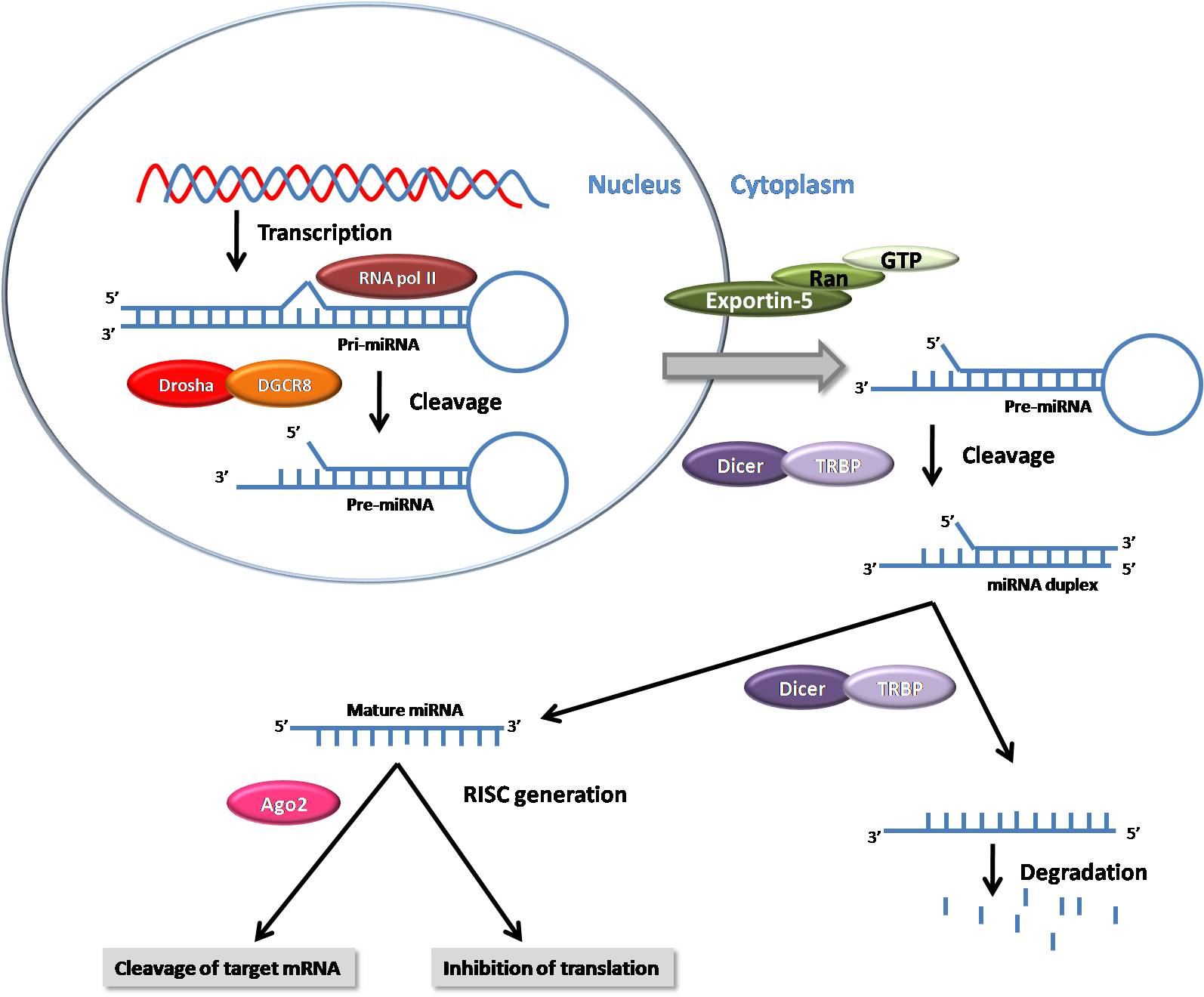
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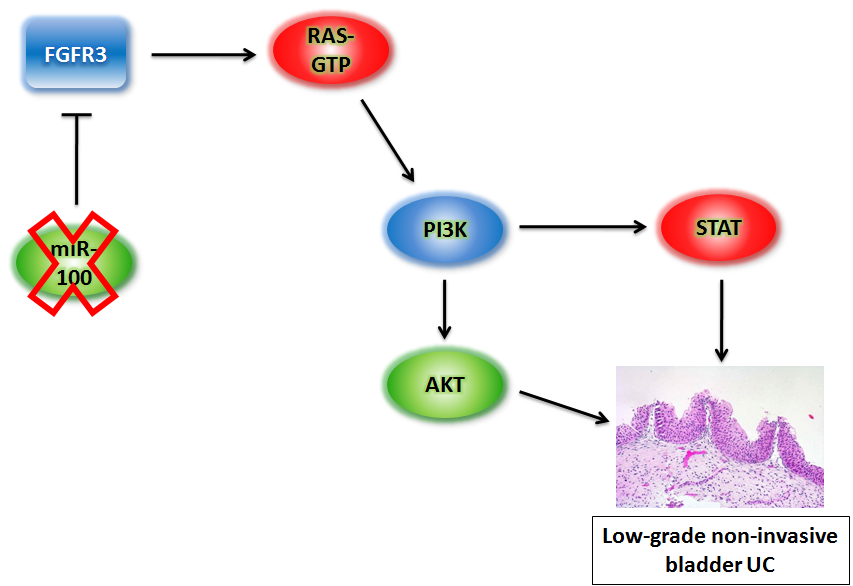
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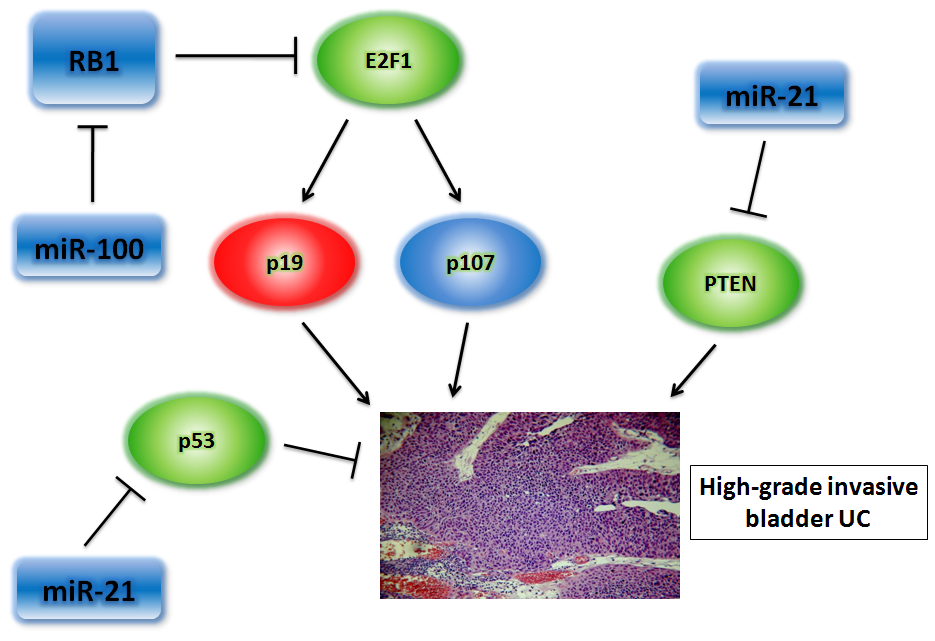
**Figure 1 Genetic carcinogenic pathways of bladder urothelial carcinomas incorporating miRNAs.**



**Figure 2 MiRNA biogenesis in human cells.** After transcription by RNA polymerase II, the Pri-miRNA (primary miRNA precursor) 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 RISC (RNA-Induced Silencing Complex). RISC drivers mature miRNA to the target 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 fibroblastic growth factor receptor 3 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 gene; UC: Urothelial carcinoma.



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