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1 α ,25(OH) $_2$ D $_3$ differentially regulates miRNA expression in human bladder cancer cells

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Abstract

Bladder cancer is the fourth most commonly diagnosed cancer in men and eighth leading cause of cancer-related death in the US. Epidemiological and experimental studies strongly suggest a role for 1 α ,25(OH) $_2$ D $_3$ in cancer prevention and treatment. The antitumor activities of 1 α ,25(OH) $_2$ D $_3$ are mediated by the induction of cell cycle arrest, apoptosis, differentiation and the inhibition of angiogenesis and metastasis. MiRNAs play important regulatory roles in cancer development and progression. However, the role of 1 α ,25(OH) $_2$ D $_3$ in the regulation of miRNA expression and the potential impact in bladder cancer has not been investigated. Therefore, we studied 1 α , 25(OH) $_2$ D $_3$ -regulated miRNA expression profiles in human bladder cancer cell line 253J and the highly tumorigenic and metastatic derivative line 253J-BV by miRNA qPCR panels. 253 J and 253J-BV cells express endogenous vitamin D receptor (VDR) which can be further induced by 1 α , 25(OH) $_2$ D $_3$. VDR target gene 24-hydroxylase was induced by 1 α ,25(OH) $_2$ D $_3$ in both cell lines, indicating functional 1 α ,25(OH) $_2$ D $_3$ signaling. The miRNA qPCR panel assay results showed that 253J and 253J-BV cells have distinct miRNA expression profiles. Further, 1 α ,25(OH) $_2$ D $_3$ differentially regulated miRNA expression profiles in 253J and 253 J-BV cells in a dynamic manner. Pathway analysis of the miRNA target genes revealed distinct patterns of contribution to the molecular functions and biological processes in the two cell lines. In conclusion, 1 α ,

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25(OH)₂D₃ differentially regulates the expression of miRNAs, which may contribute to distinct biological functions, in human bladder 253J and 253J-BV cells.

Keywords

1 α ; 25(OH)₂D₃; bladder cancer; miRNA; vitamin D

1. Introduction

Bladder cancer is the second most frequent malignancy of the genitourinary tract, the fourth most common cancer diagnosed and eighth leading cause of cancer death in men. In 2014, there are estimated 74,690 new cases of bladder cancer diagnosed in the US and 15,580 deaths related to bladder cancer (1). Over 90 % of bladder cancer is the transitional cell carcinoma (TCC), 5 % is squamous cell carcinoma and less than 2 % is adenocarcinoma (2, 3). Approximately 25–30 % of the patients will be diagnosed as having muscle invasive or metastatic bladder cancer (2, 3). Additionally, 50–70 % of patients who are initially diagnosed with a superficial cancer will recur and 10–20 % will develop into an invasive tumor (2, 3). Metastasis is the major cause of bladder cancer-related mortality. Combination treatment with gemcitabine and cisplatin is the current standard chemotherapy regimen for locally advanced and metastatic bladder cancer (4, 5). However, limited response rate and drug resistance remain major clinical problems. Therefore, new and effective approaches to treatment of bladder cancer are urgently needed.

Although the major function of vitamin D is to regulate calcium homeostasis and bone mineralization, it plays an important role in many other physiological activities (6). 1 α , 25-dihydroxyvitamin D₃ (1 α ,25(OH)₂D₃) is the most active vitamin D metabolite. Epidemiological and experimental studies support a role of 1 α ,25(OH)₂D₃ in cancer prevention and treatment (7, 8). Low levels of serum 25(OH)₂D₃ have been associated with increased risk of bladder cancer in male smokers (9). A large study assessed the risk of bladder cancer in association with plasma 25(OH)₂D₃ levels in 1125 patients with bladder cancer and 1028 control individuals with matched age, sex, ethnic origin and region (10). Low levels of plasma 25(OH)₂D₃ was found to be associated with risk of bladder cancer in a dose dependent manner, especially for more aggressive invasive tumors. Notably, vitamin D deficient patients had the highest risk for developing low-FGFR3-expressing muscle-invasive bladder cancer (10). 1 α ,25(OH)₂D₃ exerts growth inhibitory effect in various cancers through the induction of apoptosis, cell cycle arrest and differentiation of cancer cells and inhibition of angiogenesis (11). In bladder cancer cells RT112, J-82, MGH-U3 and MGH-U4, 1 α ,25(OH)₂D₃ suppresses cell growth and induces the expression of p21 and p27 (10). FGFR3 expression is also induced by 1 α ,25(OH)₂D₃ in bladder cancer cells (10). 1 α , 25(OH)₂D₃ enhances the therapeutic effect of Bacillus Calmette-Guerin (BCG) by promoting BCG-induced secretion of interleukin-8 (IL-8), an important prognostic marker for BCG treatment, by bladder cancer cells T24 and TCC-SUP (12).

MicroRNAs (miRNAs) are endogenous non-coding RNAs of 18–25 nucleotides that cause post-transcriptional gene silencing (13). miRNAs have been shown to contribute to the regulation of cancer-related processes such as cell growth, apoptosis, angiogenesis, and

metastasis. The expression of miRNAs correlates with many cancer types (13). Emerging data support the involvement of miRNAs in the progression of bladder cancer. Low and high grade bladder cancer can be distinguished by specific miRNA expression (14). miRNAs are dysregulated in bladder cancer and show promise to be used as diagnostic and prognostic markers or therapeutic targets (15). miRNA expression has been reported to be regulated by $1\alpha,25(\text{OH})_2\text{D}_3$ (16–18). Nevertheless, studies on the regulation of miRNAs by $1\alpha,25(\text{OH})_2\text{D}_3$ are limited and the impact of $1\alpha,25(\text{OH})_2\text{D}_3$ on bladder cancer remains unknown.

In the current study, we investigate the regulation of miRNA expression in bladder cancer cells 253J and 253J-BV by $1\alpha,25(\text{OH})_2\text{D}_3$. Furthermore, The potential molecular functions and biological processes affected by $1\alpha,25(\text{OH})_2\text{D}_3$ was analyzed. The results of this study shed new light on our understanding of $1\alpha,25(\text{OH})_2\text{D}_3$ -mediated regulation of miRNAs in bladder cancer.

2. Materials and methods

2.1. Materials

$1\alpha,25(\text{OH})_2\text{D}_3$ (Hoffmann-LaRoche, Nutley, NJ) was reconstituted in 100% ethanol (EtOH) and stored, protected from light, under nitrogen gas at -80°C . Anti-vitamin D receptor (VDR) (D-6) was purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Anti-24-hydroxylase (CYP24) was a gift from Cytochroma (Ontario, Canada). Anti-actin (CP-01) was from Calbiochem (San Diego, CA).

2.2. Cell culture

Human bladder cancer cells 253J were obtained from ATCC (Manassas, VA). 253 J-BV cells were generously provided by Dr. Ashish Kamat (MD Anderson Cancer Center) (19, 20). 253J cells were maintained in RPMI 1640 supplemented with 10% fetal bovine serum (FBS), penicillin and streptomycin. 253J-BV cells were maintained in modified Eagle's MEM supplemented with 10% FBS, vitamins, sodium pyruvate, L-glutamine, penicillin, streptomycin, and nonessential amino acids.

2.3. Immunoblot analysis

Human bladder cancer cells 253J and 253J-BV were treated with vehicle control ethanol (EtOH) or 10 – 500 nM $1\alpha,25(\text{OH})_2\text{D}_3$ for 48 h, harvested and lysates prepared as previously described (21). Immunoblot analysis was performed as described (21).

2.4. miRNA qPCR panels

Human bladder cancer cells 253J and 253J-BV were treated with EtOH or 500 nM $1\alpha,25(\text{OH})_2\text{D}_3$ for 24 or 48 h. MiRNAs were isolated with mirVana miRNA isolation kit following the manufacturer's instructions (Life Technologies, Grand Island, NY). cDNA was synthesized with 40 ng RNA using Universal cDNA synthesis kit II (Exiqon, Woburn, MA) following the manufacturer's protocol. The miRNA expression profile was assessed on human miRNA miRNome PCR panels including 740 human miRNAs using miRCURY LNA Universal PCR starter kit obtained from Exiqon. This miRNome PCR paneling was

chosen because these human miRNAs are the most highly expressed, may present the most biological relevance, and are most likely to be differentially regulated in disease conditions. All the PCR assays were carried out on an ABI 7900HT Fast Real-Time PCR system (Applied Biosystems – Life Technologies, Grand Island, NY). The RT-PCR data were normalized using miR-103 as the reference microRNA. Unsupervised hierarchical clustering algorithm based on the average linkage and Pearson correlation metric was performed based on the normalized expression profiles. MiRNAs regulated by $1\alpha,25(\text{OH})_2\text{D}_3$ in 253J and 253J-BV cells were identified as either up- or down-regulated for > 2.0 fold. The Venn diagrams were generated using the R programming language.

2.5. miRNA function prediction by pathway analysis

To explore the potential functions of the miRNAs differentially regulated by $1\alpha,25(\text{OH})_2\text{D}_3$ in 253J and 253J-BV cells and pathways involved, the predicted gene targets of miRNAs were analyzed using the Protein Analysis Through Evolutionary Relationships Classification System (PANTHER, <http://www.pantherdb.org>), as part of Gene Ontology Reference Genome Project and maintained by the Thomas Lab at the University of Southern California. The PANTHER system categorizes the functions of genes based on their evolutionary relationships as well as experimental evidence. The potential target genes of regulated miRNAs were analyzed for the biological processes and molecular functions and presented as pie charts. Each slice of the pie chart represents the significance of the specific process or function among the entire list of biological processes or molecular functions. The sum of $-\log_2$ of the P value is 1. The P values were calculated through the PANTHER pathway analysis using the overrepresentation test.

3. Results

3.1. Vitamin D receptor is expressed and inducible in human bladder cancer cells

To study the potential role of $1\alpha,25(\text{OH})_2\text{D}_3$ in the regulation of miRNAs, we first examined integrity of vitamin D signaling in the human bladder cancer cells. We assessed the expression and induction of the VDR, which mediates most of the activities of $1\alpha,25(\text{OH})_2\text{D}_3$ and is also transcriptionally regulated by itself. Two human bladder cancer cell lines 253J and 253J-BV were selected in this study. 253J cells are low tumorigenic and non-metastatic. 253J-BV cell line is a highly tumorigenic and metastatic line derived from 253J through serial passages of orthotopic 253J tumors in the bladder (19). Therefore, it is a valuable model system to study the contribution of miRNA in tumorigenesis and metastasis. 253J and 253J-BV cells were treated with vehicle control EtOH or 10 – 500 nM of $1\alpha,25(\text{OH})_2\text{D}_3$ for 48 h and VDR protein expression was evaluated by immunoblot analysis. Both 253J and 253J-BV cells expressed endogenous VDR, although VDR protein level was lower in 253J-BV compared with 253 J cells (Fig. 1). $1\alpha,25(\text{OH})_2\text{D}_3$ induced VDR expression in a dose dependent manner in both cell lines (Fig. 1). Further, we examined the expression of another VDR transcriptional target CYP24. $1\alpha,25(\text{OH})_2\text{D}_3$ induced CYP24 protein expression in both 253J and 253J-BV cells as shown by immunoblot analysis (Fig. 1). These results indicate that 253J and 253J-BV cells have functional $1\alpha,25(\text{OH})_2\text{D}_3$ signaling.

3.2. $1\alpha,25(\text{OH})_2\text{D}_3$ differentially regulates miRNA expression in 253J and 253J-BV cells

To investigate the impact of $1\alpha,25(\text{OH})_2\text{D}_3$ on the expression of miRNAs in bladder cancer cells, 253J and 253J-BV cells were treated with 500 nM $1\alpha,25(\text{OH})_2\text{D}_3$ for 24 or 48 h and the expression of a panel of 740 human miRNAs were examined by qRT-PCR panel assays. Unsupervised clustering analysis revealed that 253 J and 253 J-BV cells have distinct miRNA expression profiles as shown in the control treated cells (Fig. 2A). Within each cell line, $1\alpha,25(\text{OH})_2\text{D}_3$ markedly regulated miRNA expression as compared to EtOH control, and the regulation showed dynamic changes at 24 and 48 h (Fig. 2A). Further analysis of the miRNA PCR panel data revealed that a total of 94 and 105 miRNAs were significantly regulated by $1\alpha,25(\text{OH})_2\text{D}_3$ for > 2.0 fold at either time point in 253J and 253J-BV cells, respectively (Fig. 2B–C). There were 3 and 16 miRNAs that were regulated at both 24 and 48 h in 253J and 253J-BV cells, respectively (Fig. 2B–C). The 3 miRNAs with expression changes at both time points in 253J cells are miR-17, let-7a, and miR-1201. The 16 miRNAs changed in 253J-BV cells are miR-10a, miR-22, miR-29a, miR-30d, miR-96, miR-125b-1, miR-126, miR-130a, miR-147, miR-147b, miR-193b, miR-335, miR-421, miR-454, miR-542-5p, and miR-1237. Other differentially regulated miRNAs at 24 and 48 h in 253J and 253J-BV cells are listed in Supplementary Data (Suppl. Table 1–2). These results show that $1\alpha,25(\text{OH})_2\text{D}_3$ differentially regulates the miRNA expression in 253 J and 253 J-BV cells.

3.3. $1\alpha,25(\text{OH})_2\text{D}_3$ -regulated miRNAs contribute to molecular functions and biological processes

We next analyzed the functions of the gene targets of miRNAs regulated by $1\alpha,25(\text{OH})_2\text{D}_3$ for their contributing molecular functions and biological processes using the PANTHER program. Functional clustering of miRNA target genes revealed that $1\alpha,25(\text{OH})_2\text{D}_3$ differentially regulated the molecular functions in 253J and 253J-BV cells (Fig. 3A). Likewise, the biological processes involved in 253J and 253J-BV cells show different patterns (Fig. 3B). The top 3 regulated biological processes were protein metabolism and modification (16%), protein phosphorylation (11%) and MAPKKK cascade (10%) in 253J cells and stress response (21%), amino acid biosynthesis (15%) and sulfur metabolism (11%) in 253 J-BV cells (Fig. 3B). The P values for the PANTHER analysis of the 253J and 253J-BV cells are presented in Supplementary Table 3. Interestingly, the most regulated biological process in 253J-BV cells is stress response (21%), which presents 2% of the significantly regulated processes in 253J cells. The amino acid biosynthesis process also shows marked difference in the two cell lines: 15% in 253 J-BV vs. 3% in 253 J cells. These differences may be caused by the adapted phenotypes in 253J-BV cells during the generation of this metastatic line and may contribute to differential response to $1\alpha,25(\text{OH})_2\text{D}_3$ treatment. These results indicate that $1\alpha,25(\text{OH})_2\text{D}_3$ has differential effects on the biological functions in human bladder cancer cells with different tumorigenic and metastatic potentials.

Discussion

The antitumor activity of $1\alpha,25(\text{OH})_2\text{D}_3$ has been supported by epidemiological evidence and preclinical studies in a number of cancer types including colorectal, breast, prostate,

ovarian and bladder cancers. We previously demonstrated that $1\alpha,25(\text{OH})_2\text{D}_3$ potentiates the anti-tumor activity of gemcitabine and cisplatin, the first line treatment for advanced bladder cancer, in vitro and in vivo in bladder cancer model systems (22). $1\alpha,25(\text{OH})_2\text{D}_3$ has inhibitory roles in cancer cell proliferation, angiogenesis and tumor metastasis (23–26). However, the underlying mechanisms for these anticancer effects remain to be fully understood.

In the current study, we selected human bladder cancer cells 253J and 253J-BV. 253J-BV cells are derived from 253J cells and are highly tumorigenic and metastatic. Both cell lines express endogenous VDR and are responsive to $1\alpha,25(\text{OH})_2\text{D}_3$ treatment. $1\alpha,25(\text{OH})_2\text{D}_3$ induces the expression of VDR target genes including VDR and CYP24.

miRNAs are important gene regulators that contribute to many processes in tumor development and progression. Multiple studies have analyzed the miRNA expression profiles in bladder cancer and altered expression was found in human bladder cancer tissue samples compared with normal bladder tissue (27–29). Distinct miRNA expression signatures may separate the low grade and high grade non-muscle invasive and invasive bladder cancers (14). The dysregulated miRNAs contribute to the regulation of cell cycle progression, proliferation, apoptosis, metastasis as well as drug resistance. miRNAs have also been studied for their potential as diagnostic or prognostic markers in bladder cancer using tissue samples or urine samples. The expression of miR-129, miR-133b and miR-518c* were found to be up-regulated with the progression of cancer and have prognostic potential for bladder cancer (30). Another study indicates that miR-143, miR-222, miR-452 expression in urine may have diagnostic value for bladder cancer (31).

miRNAs have been shown to be regulated by $1\alpha,25(\text{OH})_2\text{D}_3$ in various cancer types including prostate, colon, lung, ovarian and breast cancers, melanoma and leukemia (32). $1\alpha,25(\text{OH})_2\text{D}_3$ induces miR-98 which contributes to the anti-proliferative effect of $1\alpha,25(\text{OH})_2\text{D}_3$ in prostate cancer cells (16). $1\alpha,25(\text{OH})_2\text{D}_3$ induces miR-498 and results in the suppression of human telomerase reverse transcriptase in ovarian cancer cells (17). $1\alpha,25(\text{OH})_2\text{D}_3$ -induced miR-22 is involved in the anti-proliferative and anti-migratory activities of $1\alpha,25(\text{OH})_2\text{D}_3$ in colon cancer cells (18). In non-malignant prostate epithelial cells RWPE-1, $1\alpha,25(\text{OH})_2\text{D}_3$ induces VDR binding to MCM7 gene which promotes the production of miR-106b and results in the inhibition of p21 expression (33). This regulation is preceded by individual histone modification at three VDR binding sites on the p21 coding gene CDKN1A (33). On the other hand, $1\alpha,25(\text{OH})_2\text{D}_3$ signaling has also been shown to be regulated by miRNAs. The expression of VDR mRNA is suppressed by miR-125b (34) and miR-27b and miR-298 (35). miR-125b also inhibits the expression of CYP24, the metabolizing enzyme for $1\alpha,25(\text{OH})_2\text{D}_3$ (36). Nevertheless, the regulation of miRNAs by $1\alpha,25(\text{OH})_2\text{D}_3$ in bladder cancer has not been reported.

In this study, we show that human bladder cancer cell lines 253 J and 253 J-BV, which have different tumorigenic and metastatic capacities, present very different miRNA expression profiles which can be separated by unsupervised clustering. Since 253 J cells are low tumorigenic and non-metastatic while 253 J-BV cells are high tumorigenic and metastatic, this observation indicates that miRNAs may play a role in the tumorigenesis and metastasis.

Several miRNAs that are regulated by $1\alpha,25(\text{OH})_2\text{D}_3$ in our study in 253J and 253J-BV cells have been reported previously to be dysregulated in bladder cancer. For instance, miR-96 is up-regulated in bladder cancer tissue assessed by deep sequencing (29) and also detected in urine samples of bladder cancer patients (37). A potential tumor suppressor miR-125b-1 has been shown to be down-regulated in bladder cancer (15). In addition, the evaluation of urine miRNAs identified the ratio of miR-126: miR-152 as a potential marker for bladder cancer detection (38). Further, $1\alpha,25(\text{OH})_2\text{D}_3$ differentially modulates the expression of miRNAs in 253J and 253J-BV cells in a dynamic manner. PANTHER pathway analysis revealed that the miRNA target genes are involved in different patterns of molecular functions and biological processes in the two cell lines. The most regulated biological process by $1\alpha,25(\text{OH})_2\text{D}_3$ in 253 J-BV cells is stress response (21%), which only counts for 2% of the significantly regulated processes in 253 J cells. Stress response genes contribute directly to metastasis. These genes regulate inflammation, wound healing and angiogenesis which ultimately lead to metastasis (39). Stress in the tumor microenvironment such as hypoxia encourages metastasis (40). Increased hypoxia-inducible factor-1 (HIF-1) in hypoxia condition promotes tumor invasiveness and metastasis (40). Besides local stress, systemic stress can also promote metastasis. In an orthotopic breast cancer mouse model, chronic stress accelerates metastasis through the induction of sympathetic nervous system β -adrenergic signaling which leads to increased infiltration of macrophages in the primary tumor (41). Therefore, $1\alpha,25(\text{OH})_2\text{D}_3$ may affect metastasis through the regulation of stress response genes in 253J-BV cells. These analyses also indicate that $1\alpha,25(\text{OH})_2\text{D}_3$ -regulated miRNAs contribute distinctively in biological functions in different bladder cancer cells.

miRNAs may function as oncogenes or tumor suppressors depending on their target genes. Therefore, it will be beneficial to integrate the miRNA and mRNA expression profiles to further identify important molecular signatures and targets regulated by $1\alpha,25(\text{OH})_2\text{D}_3$ in bladder cancer. The Cancer Genome Atlas Research Network has comprehensively analyzed the molecular characteristics of bladder cancer in 131 high-grade muscle-invasive bladder cancer (42). Distinct subsets of bladder cancer can be identified by the integrated analysis of mRNA, miRNA and protein data (42), which further emphasizes on the importance of the network of mRNAs, miRNAs and proteins.

In conclusion, we show that human bladder cancer cells 253J and 253 J-BV have functional vitamin D signaling and distinct miRNA expression profiles. $1\alpha,25(\text{OH})_2\text{D}_3$ differentially regulates miRNA expression in these two cell lines. Pathway analysis of miRNA target genes revealed that distinct patterns of molecular and biological processes were modulated by $1\alpha,25(\text{OH})_2\text{D}_3$ in 253J and 253J-BV cells. Although further mechanistic investigation is needed, our data indicate that $1\alpha,25(\text{OH})_2\text{D}_3$ -regulated miRNAs may play a role in the tumorigenesis and metastasis in bladder cancer.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

CYP24	24-hydroxylase
ETOH	ethanol
FBS	fetal bovine serum
miRNA	microRNA
PANTHER	protein analysis through evolutionary relationships classification system
TCC	transitional cell carcinoma
VDR	vitamin D receptor

References

1. Siegel R, Ma J, Zou Z, Jemal A. Cancer statistics, 2014. *CA Cancer J Clin.* 2014; 64:9–29. [PubMed: 24399786]
2. Chou R, Dana T. Screening adults for bladder cancer: a review of the evidence for the U.S. preventive services task force. *Annals of internal medicine.* 2010; 153:461–8. [PubMed: 20921545]
3. Kaufman DS, Shipley WU, Feldman AS. Bladder cancer. *Lancet.* 2009; 374:239–49. [PubMed: 19520422]
4. Sternberg CN, Donat SM, Bellmunt J, Millikan RE, Stadler W, De Mulder P, et al. Chemotherapy for bladder cancer: treatment guidelines for neoadjuvant chemotherapy, bladder preservation, adjuvant chemotherapy, and metastatic cancer. *Urology.* 2007; 69:62–79. [PubMed: 17280909]
5. Barocas DA, Clark PE. Bladder cancer. *Current opinion in oncology.* 2008; 20:307–14. [PubMed: 18391631]
6. Brown AJ, Dusso A, Slatopolsky E. Vitamin D. *Am J Physiol.* 1999; 277:F157–75. [PubMed: 10444570]
7. Garland CF, Garland FC, Gorham ED, Lipkin M, Newmark H, Mohr SB, et al. The role of vitamin D in cancer prevention. *Am J Public Health.* 2006; 96:252–61. [PubMed: 16380576]
8. Giovannucci E, Liu Y, Rimm EB, Hollis BW, Fuchs CS, Stampfer MJ, et al. Prospective study of predictors of vitamin D status and cancer incidence and mortality in men. *Journal of the National Cancer Institute.* 2006; 98:451–9. [PubMed: 16595781]
9. Mondul AM, Weinstein SJ, Mannisto S, Snyder K, Horst RL, Virtamo J, et al. Serum vitamin D and risk of bladder cancer. *Cancer Res.* 2010; 70:9218–23. [PubMed: 20978193]
10. Amaral AF, Mendez-Pertuz M, Munoz A, Silverman DT, Allory Y, Kogevinas M, et al. Plasma 25-Hydroxyvitamin D3 and Bladder Cancer Risk According to Tumor Stage and FGFR3 Status: A Mechanism-Based Epidemiological Study. *Journal of the National Cancer Institute.* 2012
11. Deeb KK, Trump DL, Johnson CS. Vitamin D signalling pathways in cancer: potential for anticancer therapeutics. *Nature reviews Cancer.* 2007; 7:684–700.
12. Hsu JW, Yin PN, Wood R, Messing J, Messing E, Lee YF. 1 alpha, 25-dihydroxylvitamin D3 promotes Bacillus Calmette-Guerin immunotherapy of bladder cancer. *Oncotarget.* 2013; 4:2397–406. [PubMed: 24353168]
13. Esquela-Kerscher A, Slack FJ. Oncomirs – microRNAs with a role in cancer. *Nature reviews Cancer.* 2006; 6:259–69.

14. Catto JW, Miah S, Owen HC, Bryant H, Myers K, Dudzic E, et al. Distinct microRNA alterations characterize high- and low-grade bladder cancer. *Cancer Res.* 2009; 69:8472–81. [PubMed: 19843843]
15. Yoshino H, Seki N, Itesako T, Chiyomaru T, Nakagawa M, Enokida H. Aberrant expression of microRNAs in bladder cancer. *Nature reviews Urology.* 2013; 10:396–404.
16. Ting HJ, Messing J, Yasmin-Karim S, Lee YF. Identification of microRNA-98 as a Therapeutic Target Inhibiting Prostate Cancer Growth and a Biomarker Induced by Vitamin D. *J Biol Chem.* 2013; 288:1–9. [PubMed: 23188821]
17. Kasiappan R, Shen Z, Tse AK, Jinwal U, Tang J, Lungchukiet P, et al. 1,25-Dihydroxyvitamin D3 Suppresses Telomerase Expression and Human Cancer Growth through MicroRNA-498. *J Biol Chem.* 2012; 287:41297–309. [PubMed: 23055531]
18. Alvarez-Diaz S, Valle N, Ferrer-Mayorga G, Lombardia L, Herrera M, Dominguez O, et al. MicroRNA-22 is induced by vitamin D and contributes to its antiproliferative, antimigratory and gene regulatory effects in colon cancer cells. *Human molecular genetics.* 2012; 21:2157–65. [PubMed: 22328083]
19. Dinney CP, Fishbeck R, Singh RK, Eve B, Pathak S, Brown N, et al. Isolation and characterization of metastatic variants from human transitional cell carcinoma passaged by orthotopic implantation in athymic nude mice. *The Journal of urology.* 1995; 154:1532–8. [PubMed: 7658585]
20. Kamat AM, Karashima T, Davis DW, Lashinger L, Bar-Eli M, Millikan R, et al. The proteasome inhibitor bortezomib synergizes with gemcitabine to block the growth of human 253JB-V bladder tumors in vivo. *Mol Cancer Ther.* 2004; 3:279–90. [PubMed: 15026548]
21. Ma Y, Yu WD, Kong RX, Trump DL, Johnson CS. Role of Nongenomic Activation of Phosphatidylinositol 3-Kinase/Akt and Mitogen-Activated Protein Kinase/Extracellular Signal-Regulated Kinase Kinase/Extracellular Signal-Regulated Kinase 1/2 Pathways in 1,25D3-Mediated Apoptosis in Squamous Cell Carcinoma Cells. *Cancer Res.* 2006; 66:8131–8. [PubMed: 16912191]
22. Ma Y, Yu WD, Trump DL, Johnson CS. 1,25D(3) Enhances antitumor activity of gemcitabine and cisplatin in human bladder cancer models. *Cancer.* 2010; 116:3294–303. [PubMed: 20564622]
23. McGuire TF, Trump DL, Johnson CS. Vitamin D(3)-induced apoptosis of murine squamous cell carcinoma cells. Selective induction of caspase-dependent MEK cleavage and up-regulation of MEKK-1. *J Biol Chem.* 2001; 276:26365–73. [PubMed: 11331275]
24. Mantell DJ, Owens PE, Bundred NJ, Mawer EB, Canfield AE. 1 alpha,25-dihydroxyvitamin D(3) inhibits angiogenesis in vitro and in vivo. *Circ Res.* 2000; 87:214–20. [PubMed: 10926872]
25. Bao BY, Yao J, Lee YF. 1alpha, 25-dihydroxyvitamin D3 suppresses interleukin-8-mediated prostate cancer cell angiogenesis. *Carcinogenesis.* 2006; 27:1883–93. [PubMed: 16624828]
26. Yudoh K, Matsuno H, Kimura T. 1alpha,25-dihydroxyvitamin D3 inhibits in vitro invasiveness through the extracellular matrix and in vivo pulmonary metastasis of B16 mouse melanoma. *J Lab Clin Med.* 1999; 133:120–8. [PubMed: 9989763]
27. Gottardo F, Liu CG, Ferracin M, Calin GA, Fassin M, Bassi P, et al. Micro-RNA profiling in kidney and bladder cancers. *Urologic oncology.* 2007; 25:387–92. [PubMed: 17826655]
28. Lin T, Dong W, Huang J, Pan Q, Fan X, Zhang C, et al. MicroRNA-143 as a tumor suppressor for bladder cancer. *The Journal of urology.* 2009; 181:1372–80. [PubMed: 19157460]
29. Han Y, Chen J, Zhao X, Liang C, Wang Y, Sun L, et al. MicroRNA expression signatures of bladder cancer revealed by deep sequencing. *PLoS One.* 2011; 6:e18286. [PubMed: 21464941]
30. Dyrskjot L, Ostensfeld MS, Bramsen JB, Silahtaroglu AN, Lamy P, Ramanathan R, et al. Genomic profiling of microRNAs in bladder cancer: miR-129 is associated with poor outcome and promotes cell death in vitro. *Cancer Res.* 2009; 69:4851–60. [PubMed: 19487295]
31. Puerta-Gil P, Garcia-Baquero R, Jia AY, Ocana S, Alvarez-Mugica M, Alvarez-Ossorio JL, et al. miR-143, miR-222, and miR-452 are useful as tumor stratification and noninvasive diagnostic biomarkers for bladder cancer. *Am J Pathol.* 2012; 180:1808–15. [PubMed: 22426337]
32. Ma Y, Trump DL, Johnson CS. Vitamin D and miRNAs in Cancer. *Current gene therapy.* 2014; 14:269–75. [PubMed: 25039615]

33. Thorne JL, Maguire O, Doig CL, Battaglia S, Fehr L, Sucheston LE, et al. Epigenetic control of a VDR-governed feed-forward loop that regulates p21(waf1/cip1) expression and function in non-malignant prostate cells. *Nucleic acids research*. 2011; 39:2045–56. [PubMed: 21088000]
34. Mohri T, Nakajima M, Takagi S, Komagata S, Yokoi T. MicroRNA regulates human vitamin D receptor. *Int J Cancer*. 2009; 125:1328–33. [PubMed: 19437538]
35. Pan YZ, Gao W, Yu AM. MicroRNAs regulate CYP3A4 expression via direct and indirect targeting. *Drug metabolism and disposition: the biological fate of chemicals*. 2009; 37:2112–7. [PubMed: 19581388]
36. Komagata S, Nakajima M, Takagi S, Mohri T, Taniya T, Yokoi T. Human CYP24 catalyzing the inactivation of calcitriol is post-transcriptionally regulated by miR-125b. *Mol Pharmacol*. 2009; 76:702–9. [PubMed: 19570947]
37. Yamada Y, Enokida H, Kojima S, Kawakami K, Chiyomaru T, Tatarano S, et al. MiR-96 and miR-183 detection in urine serve as potential tumor markers of urothelial carcinoma: correlation with stage and grade, and comparison with urinary cytology. *Cancer science*. 2011; 102:522–9. [PubMed: 21166959]
38. Hanke M, Hoefig K, Merz H, Feller AC, Kausch I, Jocham D, et al. A robust methodology to study urine microRNA as tumor marker: microRNA-126 and microRNA-182 are related to urinary bladder cancer. *Urologic oncology*. 2010; 28:655–61. [PubMed: 19375957]
39. Weber GF, Ashkar S. Stress response genes: the genes that make cancer metastasize. *J Mol Med (Berl)*. 2000; 78:404–8. [PubMed: 11097109]
40. Cui H, Grosso S, Schelter F, Mari B, Kruger A. On the Pro-Metastatic Stress Response to Cancer Therapies: Evidence for a Positive Co-Operation between TIMP-1, HIF-1 α , and miR-210. *Frontiers in pharmacology*. 2012; 3:134. [PubMed: 22807917]
41. Sloan EK, Priceman SJ, Cox BF, Yu S, Pimentel MA, Tangkanangnukul V, et al. The sympathetic nervous system induces a metastatic switch in primary breast cancer. *Cancer Res*. 2010; 70:7042–52. [PubMed: 20823155]
42. Comprehensive molecular characterization of urothelial bladder carcinoma. *Nature*. 2014; 507:315–22. [PubMed: 24476821]

Highlights

- Human bladder cancer cells have functional vitamin D signaling
- Distinct miRNA expression pattern observed in bladder cancer cells
- $1\alpha,25(\text{OH})_2\text{D}_3$ differentially regulate miRNA expression in bladder cancer cells

Fig. 1.

VDR expression in human bladder cancer cells. Human bladder cancer cells 253J and 253J-BV were treated with control ETOH or 10 – 500 nM $1\alpha,25(\text{OH})_2\text{D}_3$ for 48 h. VDR and CYP24 expression was examined by immunoblot analysis. Actin was the loading control. Results are representative of three independent experiments.

Fig. 2.

1 α ,25(OH) $_2$ D $_3$ differentially regulates miRNA expression in 253 J and 253J-BV cells. Cells were treated with EtOH or 1 α ,25(OH) $_2$ D $_3$ for 24 or 48 h. miRNA PCR panels were performed with isolated RNA. A. The heatmap analysis of the unsupervised clustering of miRNA expression in 253J and 253J-BV cells was presented. E, EtOH; D, 1 α ,25(OH) $_2$ D $_3$. B–C, Differentially expressed miRNAs with > 2.0 fold changes in 253J (B) and 253J-BV (C) cells are summarized in Venn diagrams. The miRNAs regulated at both 24 and 48 h (3 in 253J cells and 16 in 253J-BV cells) are listed.

Fig. 3. $1\alpha,25(\text{OH})_2\text{D}_3$ -regulated miRNAs contribute to molecular functions and biological processes. The gene targets of $1\alpha,25(\text{OH})_2\text{D}_3$ -regulated miRNAs were analyzed for their contributing molecular functions and biological processes using the PANTHER program. The top molecular functions (A) and biological processes (B) involved in 253J and 253J-BV cells were presented as the $-\log_2$ of the P value in pie charts. Each slice of the pie chart represents the significance of the specific pathway. The sum of $-\log_2$ of the P-value is 1. The P-values were calculated through the PANTHER pathway analysis.