MANSOURA JOURNAL OF BIOLOGY
Official Journal of Faculty of Science, Mansoura University, Egypt
E-mail: scimag@mans.edu.eg ISSN: 2974-492X

The role of some miRNAs in bladder cancer chemoresistance to cisplatin

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Received:15/8/2021
Accepted:15 /9/2021

Abstract: For many years, the standard treatment for metastatic bladder cancer was cisplatin. The main cause of treatment failure is the resistance to cisplatin. Resistance to cisplatin remains the main obstacle to prevent clinical treatment and cause of patients' poor prognosis. In this study, we aimed to evaluate the role of microRNAs (miRNAs) and their effect on genes that can be targeted by the three miRNAs and their role in chemoresistance to differentiate between responding and non responding patients. Samples were collected from muscle invasive bladder cancer (MIBC) patients who received 4 cycles of cisplatin after cystectomy, the expression of miR-21, miR-143, miR-155, and their target genes (VEGF-C, AKT, and DMTF-1) were assessed in tumor bladder tissue sample. The results of this study suggested miR-21up regulation, decrease miR-143 and increase miR-155 expression and their target genes; increase VEGF-C, up regulation of AKT and down regulation DMTF-1; may play a vital role in chemoresistance and can be a cause of disease recurrence

keywords: chemoresistance, miRNAs, MIBCs

1.Introduction

Bladder cancer (BC) is the world's 9th most common type of cancer and the world's 7th most widely spread in men [1]. The majority of bladder cancers are urothelial carcinomas. It could be classified into non muscle invasive bladder cancer (NMIBC) and MIBC or metastatic disease. Approximately 50 % of NMIBCs are low grade whereas most MIBC or metastatic tumors are high grade [2, 3].

Radical cystectomy is considered the gold-standard therapy for MIBC patients as well as for NMIBC patients who failed with intravesical treatment [3, 4]. Moreover; among all medications that are commonly used for cancer in chemotherapy, cisplatin has shown anticancer activity in several tumors. Cisplatin is one of the most powerful medications for solid cancers. In 1978 it was the first platinum compound approved by the food and drug administration (FDA) for cancer treatment [5].

Resistance to cisplatin is considered one of the most obstacles that can prevent cancer treatment. Previous studies supposed three main theories explain resistance to cisplatin. These theories are modifications in drug cellular accumulation, intracellular detoxification and DNA damage repair. Recent studies showed that miRNAs could play a major role in the metabolism of drugs. One of the common methods of chemotherapy resistance is caused by multidrug resistance mutation (MDR1) gene activation [6].

miRNAs are unregulated small non-coding RNA molecules. miRNAs are unique in their nature in that one miRNA can control multiple protein-coding RNAs[7]. Latest studies proved that miR-21, miR-143 and miR-155 play a role in BC prognosis. It was found that high expression of miR-21 has been associated with a low overall survival rate, the high expression of miR-143 has been associated with high progression-free survival and high expression of miR-155 has been correlated with poor progression-free survival [8, 9].

Zhang et al. (2015) suggested that miR-21, mammary serine protease inhibitor (maspin) and vascular endothelial growth factor C (VEGF-C) could play a significant role in developing BC [10]. Since VEGF-C is a particular lymphangiogenic factor [10-12].

miR-143 has been seen to decrease the translation level of the AKT [13]. Increase of AKT expression in cells causes the hyperexpression of the antiapoptotic B-cell lymphoma 2 (BCL-2) [14]. miR-155 acts as an onco-miRNA in BC. It has been confirmed that miR-155 was directly repressed by cyclin-D-binding Myb-like transcription factor 1(DMTF1). DMTF1is consider tumor suppressor gene which induces the arrest of the cell cycle and prevents the proliferation of cells in BC [15].

In the current study, we investigate the functional role of three miRNAs that may play a vital role in the resistance to cisplatin, these miRNAs are miR-21, miR-143 and miR-155 and their target genes (VEGF-C, AKT and DMTF-1).

Material and methods

All patients were MIBC, they were admitted to Urology and Nephrology center (Mansoura University, Egypt) from 2011 to 2018 and received at least 4 cycles of cisplatin, while those who underwent palliative cystectomy, had histopathological variants or received adjuvant radiotherapy were excluded.

MicroRNA assay for miR-21, 155 &143

miRNA was extracted using a miRneasy Mini Kit. cDNA was synthesized from 1 µg of total miRNA, 4 µl 5x miScript HiFlex Buffer, 2 µl 10x miScript Nucleics were mixed, 2 µl nuclease free water, 2 µl miScript Reverse Transcriptase was mixed to 10 µl of miRNA sample total volume will be 20 µl cDNA. Amplification and detection were performed using real time PCR.

Gene expression assay (AKT, DMTF1 & VEGF-C)

RNA was extracted using Trizol, then reverse transcript. The reaction was performed for in a total volume of 25µl containing 12.5 µl of syber green, primers (forward and reverse)1.25 µl for each primer and 7.5 µl nuclease free water, master mix was mixed and 2.5 µl of cDNA was added. The cycling parameters were as follows: initial denaturation at 95℃ for 10 minutes, followed by 40 cycles of denaturation 95℃ for 15 seconds, annealing for 1 minutes, extension at 72℃ for 1 minutes. Data analysis was carried out using real time PCR(step one plus), using the equation 2-ΔΔct.Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was analysed as an endogenous RNAreference gene and gene expression was normalized to the expression of GAPDH. The primers used for gene amplification is shown in table 1.

Table 1: The primers used for gene amplification

Gene	Primer sequence
VEGFC	F:5-CACGAGCTACCTCAGCAAGA -3 R:3-GCTGCCTGACACTGTGGTA-5
AKT	F: 5- ACCTTTTGCGGCACACCTGA-3 R:5- CAGGCGACCGCACATCATCT-3
DMTF-1	F:5GTCTGAACCGGCCTTTGTTTG -3 R: 5–GCCCAGTCATTGCCATGCT-3

Statical analysis

The statistical analysis was performed using version 21.0 of the SPSS software. The associations between data were evaluated using spearman's rank correlation test. The results were expressed as the minimum and maximum, *P* value<0.001 was considered to indicate a statistically significant difference.

Results

One hundred and four patients were included in the study, 90 males (86.5%) and 14 females (13.5%). Mean ages were 60.32 ± 7.97 years old. Median follow-up was 11 (3-64) months.

The correlation between miR-21 and VEGF-C in bladder cancer

The expression of miR-21 and VEGF-C showed a strong positive correlation (r = 0.850) for both responding and non-responding patients (*P* < 0.001); miR-21 and VEGF-C showing high expression in non responding compared to responding patients as presented in figure (1) (*P< 0.001)*.

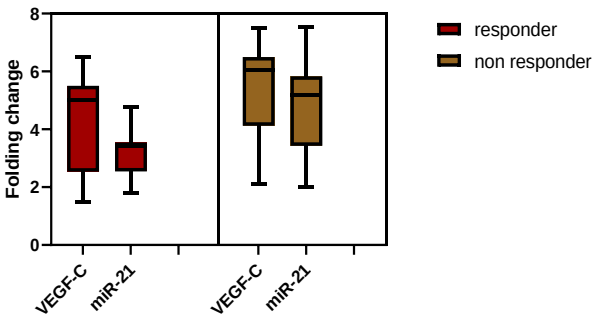

Figure (1) illustrates the correlation between miR-21 and VEGF-C.

The correlation between miR-143 and AKT in bladder cancer

In Figure (2) the expression of miR-143 and AKT showed a strong negative correlation (r = -0.727) for both responding and non-responding patients ($P < 0.001$); miR-143 was down regulated in non responding compared to responding patients, but AKT gene expression was increased in non-respondig more than responding patients as presented.

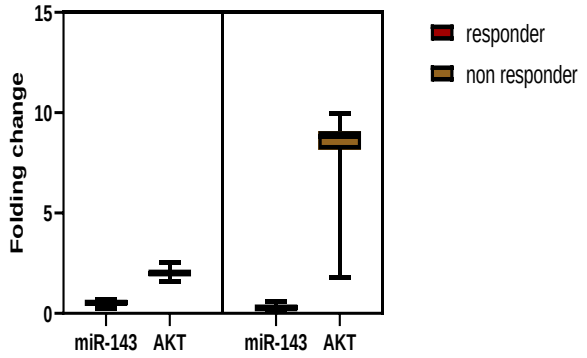

Figure (2) illustrates the correlation between miR-143 and AKT.

The correlation between miR-155 and DMTF-1

The expression of miR-155 and DMTF-1 showed a strong negative correlation (r = -0.651) for both responding and non-responding patients ($P < 0.001$), non-responding patients showing a significant increase in miR-155 and down regulation in DMTF-1 expression compared to responding patients as presented in Figure (3).

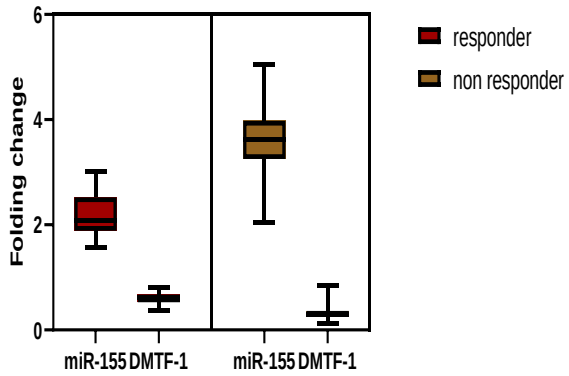

Figure (3) illustrates the correlation between miR-155 and DMTF-1.

Discussion

In tumor cells resistance, many mechanisms participate in the progression of drug resistance. Recent studies showed that alternation of many miRNAs expression plays a vital role in genes regulating including cell apoptosis, autophagy, the proliferation of the cell, epithelial-mesenchymal transition (EMT) and regulation of drug efflux [16].

Previous studies demonstrated the oncogenic role of miR-21 due to its up-regulation in many types of cancers including BC by increasing cell proliferation, promoting tumor cell growth, decreasing apoptosis and inducing chemoresistance [17].

Zhang et al. (2015) proved the correlation between miR-21 and VEGF-C as a target in BC; the up-regulation of miR-21causes reduction of maspin which leads to VEGF-C up-regulation. According to this mechanism lymphangiogenic and metastatic increase; as the VEGF-C is a lymphangiogenic factor [10, 18]. In our study, the folding change in non-responding patient's tumor tissues showed a significant increase in both miR-21and VEGF-C expression compared to the responding patients these results were confirmed by the results of Tao et al(2011).

miRNA-143 is known as a tumor suppressor and was found to reduce cancer cells in many types including BC, colon, stomach, ovarian, esophageal, osteosarcoma, B-cell leukemia and liposarcoma [19, 20]. This decrease is correlated with a greater incidence of these cancers, and the miRNA-143 expression in organs can help prevent cancer [21-23]. In a recent study, miRNA-143 has been seen to decrease the translation level of the AKT [13], which play a vital role as an antiapoptotic gene by activation BCL2 in cancer cell after cisplatin if its level up-regulated [14]. According to that our results were in agreement with Zheng et al (2017); in non-responding patients, the tumor tissues showed a significant decrease in miR-143 but a significant increase was observed in AKT expression compared to the responding patients.

High expression levels of miR-155 can cause genomic instability [24] as it considers as onco-miRNA for various types of cancers [25-27]. miR-155 acts as an onco-miRNA in bladder cancer and encourages cellular activity growth and proliferation in *vivo* as well as in *vitro* by targeting DMTF-1which consider tumor suppressor gene. The tumor suppressor

DMTF1 induces the arrest of the cell cycle and prevents the proliferation of cells in bladder cancer [15]. Depending on these studies, our results were in agreement with Peng et al (2015); in non-responding patients, the tumor tissues showed a significant increase in miR-155 but a significant decrease was observed in DMTF-1 expression compared to the responding patients.

In conclusion, the vital role of miR-21, miR-143 and miR-155 and their target genes can not be ignored and may be used as alternatives for predicting the resistance to cisplatin in the future. But many studies are needed to confirm miRNAs role before they can be used in the routine clinical regime.

4. References

1. Antoni, S., et al., (2017) Bladder cancer incidence and mortality: a global overview and recent trends.. **71**(1): p. 96-108.
2. Moch, H., et al., (2016) The 2016 WHO Classification of Tumours of the Urinary System and Male Genital Organs-Part A: Renal, Penile, and Testicular Tumours. Eur Urol,. **70**(1): p. 93-105.
3. Saginala, K., et al., (2020) Epidemiology of Bladder Cancer.. **8**(1): p. 15-27.
4. Witjes, J.A., et al., (2017) Updated 2016 EAU guidelines on muscle-invasive and metastatic bladder cancer.. **71**(3): p. 462-475.
5. Kelland, L., (2007) The resurgence of platinum-based cancer chemotherapy. Nature Reviews Cancer,. **7(8):** p. 573-584.
6. Chen, S.H. and J.Y. Chang, (2019) New Insights into Mechanisms of Cisplatin Resistance: From Tumor Cell to Microenvironment. *Int J Mol Sci*,. **20**(17): p. 4136-4157.
7. Enokida, H., et al., (2016) The role of microRNAs in bladder cancer. icu,. **57**(Suppl 1): p. S60-S76.
8. Pignot, G., et al., (2013) microRNA expression profile in a large series of bladder tumors: Identification of a 3-miRNA signature associated with aggressiveness of muscle-invasive bladder cancer.. **132**(11): p. 2479-2491.
9. Xie, Y., et al., (2017) MicroRNAs with prognostic significance in bladder cancer: a systematic review and meta-analysis. Scientific Reports,. **7**(1): p. 5619-5631.
10. Zhang, H.H., et al., (2015) Expression and clinical significance of microRNA-21, maspin and vascular endothelial growth factor-C in bladder cancer. Oncol Lett,. **10**(4): p. 2610-2616.
11. Su, J.L., et al., (2007) The role of the VEGF-C/VEGFR-3 axis in cancer progression. *British Journal of Cancer*,. **96**(4): p. 541-545.
12. Zhang, Y., et al., (2013) Serum vascular endothelial growth factor‐C levels: A possible diagnostic marker for lymph node metastasis in patients with primary non‐small cell lung cancer. Oncol Lett,. **6**(2): p. 545-549.
13. Noguchi, S., et al., (2011) MicroRNA-143 functions as a tumor suppressor in human bladder cancer T24 cells. Cancer Lett,. **307**(2): p. 211-220.
14. Zheng, H.C., (2017) The molecular mechanisms of chemoresistance in cancers. Oncotarget,. **8**(**35**): p. 59950-59964.
15. Peng, Y., et al., (2015) MicroRNA-155 promotes bladder cancer growth by repressing the tumor suppressor DMTF1. Oncotarget,. **6**(18): p. 16043-16058.
16. Pratama, M.Y., et al., (2019). The role of microRNA in the resistance to treatment of hepatocellular carcinoma. Ann Transl Med, **7(20**): p. 577-593.
17. Tao, J., et al., (2011). microRNA-21 modulates cell proliferation and sensitivity to doxorubicin in bladder cancer cells. **25**(6): p. 1721-1729.
18. Ping, Q., et al., (2021) LncRNA DANCR regulates lymphatic metastasis of bladder cancer via the miR-335/VEGF-C axis.. **10**(4): p. 1743-1753.
19. Akao, Y., Y. Nakagawa, and T. Naoe, (2006) MicroRNAs 143 and 145 are possible common onco-microRNAs in human cancers. Oncol Rep,. **16** (4): p. 845-850.
20. Nakagawa, Y., et al., (2007) Characterized mechanism of alpha-mangostin-induced cell death: caspase-independent apoptosis with release of endonuclease-G from mitochondria and increased miR-143 expression in human colorectal cancer

DLD-1 cells. Bioorg Med Chem,. **15**(16): p. 5620-5628.
21. Gao, J.S., et al., (2011) The Evi1, microRNA-143, K-Ras axis in colon cancer. FEBS Lett,. **585**(4): p. 693-699.
22. Lin, T., et al., (2009). MicroRNA-143 as a tumor suppressor for bladder cancer. *J Urol*, **181**(3): p. 1372-1380.
23. Chen, J. and M.-B.J.T.G.C. Wang, (2012) The roles of miRNA-143 in colon cancer and therapeutic implications., 2012. **1**(2): p. 169-174.
24. Tili, E., et al., (2011) Mutator activity induced by microRNA-155 (miR-155) links inflammation and cancer.. **108**(12): p. 4908-4913.
25. Li, C.-L., et al., (2012) microRNA-155 is downregulated in gastric cancer cells and involved in cell metastasis. Oncol Rep,. **27**(6): p. 1960-1966.
26. Qin, W., et al., (2013) MicroRNA-155 is a novel suppressor of ovarian cancer-initiating cells that targets CLDN1. FEBS Letters,. **587**(9): p. 1434-1439.
27. Spadaccino, F., et al., (2021) The Ambivalent Role of miRNAs in Carcinogenesis: Involvement in Renal Cell Carcinoma and Their Clinical Applications.. **14**(4): p. 322-340.