The Eurasia Proceedings of Health, Environment and Life Sciences (EPHELS)
ISSN: 2791-8033

The Eurasia Proceedings of Health, Environment and Life Sciences (EPHELS), 2024

Volume 13, Pages 128-131

ICGeHeS 2024: International Conference on General Health Sciences

Determination of miRNA Expression Levels in Bladder Cancer

Mehmet Aydin Dagdeviren
Gaziantep University

Mehmet Ozaslan
Gaziantep University

Abstract:Bladder cancer is the ninth most common cancer worldwide, with the highest incidence rates observed in men in some countries in Southern and Western Europe, North America, North Africa, or Western Asia (Antoni, S. et al., 2017). Although gender differences vary greatly between countries, incidence rates are lower in men than in women. According to the data announced by the World Health Organization in 2022: In European and Asian comparisons; This rate is 21.1% and 5.6% per 100,000 people, respectively, and 22.6% in Turkey (Globocan, 2022). MicroRNAs (miRNAs) are non-protein-coding, single-stranded RNA molecules of 18-25 nucleotides in length and constitute a class of endogenous small RNAs (Celik et al., 2013). Studies have shown that microRNAs may also function as oncogenes or tumor suppressors in Bladder cancer. Although the expression levels of microRNA 22 5p and microRNA 337 have been determined in many cancers, including lung, prostate and colon cancer, no studies have been found on their expression levels in bladder cancer patients. In our study, miRNA 22 5p and miRNA 337 5p expression levels in bladder cancer patients will be calculated quantitatively using the Real-Time PCR method. In the first stage of the study, samples will be taken from the bladder using the TUR method, one from healthy tissue and the other from cancerous tissue. miRNA will be isolated from the tissue samples taken, cDNA will be synthesized from the miRNA samples and expression levels will be determined with the Real-Time PCR method using miRNA 22 5p, miRNA 337 5p specific primers and U6 primer as the reference gene. The data will be analyzed and interpreted with the SPSS package program.This study was planned to determine whether these two miRNAs can guide early diagnosis and diagnosis in bladder cancer patients and to provide clinicians with diagnosis and treatment planning.

Keywords: Bladder cancer, miRNA, Real-time PCR, Expression

Introduction

Cancer is a disease that occurs at the cellular level. Clinically, cancer; It is defined as a condition that covers nearly a hundred complex diseases that behave differently depending on the cell type from which it originates. Cancer types differ depending on the patient's age at onset, cancer's growth rate, spread, stage, and response to treatment. However, all cancer types have common features at the molecular level, and these common features group them into a single category (Klug et al., 2011).

Cancer is one of the deaths with known causes both in the world and in our country, and it is an important public health problem as it is the second cause of death after heart and circulatory system diseases. It is also a chronic disease that is increasing worldwide and causing significant material, spiritual, social and economic losses in societies. Cancer causes the death of 8.2 million people in the world every year and affects all people by infecting 14 million people (Ergin et al., 2019).

Bladder cancer is a global health problem with incidence and prognosis that vary by gender. This type of cancer has a number of different molecular subtypes depending on whether the disease is non-muscle invasive or

muscle invasive. It is known that the mutation burden is higher in muscle-invasive disease than in non-muscle-invasive disease. Genes such as TERT, FGFR3, TP53, PIK3CA, STAG2, as well as genes involved in chromatin modification, are among those frequently mutated (Dyrskjøt, 2023). Bladder cancer can be divided into two categories based on genetic instability at the nucleotide and chromosomal levels. In order to understand the basic mechanisms of cancer development, it is necessary to know the DNA missmatch repair (MMT) system and its correlation with other systemic pathways. Zigeuner et al. (2006), they stated that cancer suppressor genes, namely the retinoblastoma (Rb) gene on chromosome 13q and the p53 gene on chromosome 17p, play an important role in the formation and progression of bladder cancer. In a study on bladder cancer, the finding of a deletion on chromosome 9 suggested that there were other cancer suppressor genes effective on chromosomes 3, 4, 8, 11, and 14 (Edge et al., 2010).

Two different groups of bladder cancer have been identified in the molecular pathogenesis of the disease. First group; They are localized, papillary and low-grade cancers resulting from urothelial hyperplasia. These cancers also exhibit one or more mutations along with the fibroblast growth factor receptor 3 (FGFR3) gene mutation. This histological type generally has a better prognosis; There is a relationship between mutant FGFR3 gene expression and lack of muscle invasion. The second group is; Bladder cancer consists of non-papillary and high-grade cancers (Ojea et al., 2007). The second group is; They defined bladder cancer as non-papillary and high-grade cancers.

This group of cancers develop on the basis of severe dysplasia and carcinoma in situ. They often exhibit multiple chromosomal anomalies, such as genetic alterations in the p53 pathway and loss of heterozygosity on chromosome 9. The long-term survival of these cancers is low due to the high rate of progression and muscle invasion (Johar et al., 2013). The main treatment for non-muscle invasive bladder cancer is transurethral resection; As standard treatment, radical cystectomy treatment is performed together with neoadjuvant chemotherapy. Accordingly, immune checkpoint inhibitors have been shown to help treat metastatic bladder cancer in both groups (Dyrskjøtet al., 2023).

The incidence of bladder cancer in men is 5 times higher than in women, and the average age of diagnosis is 65 years. More than 80% of these do not spread to the muscles. The remaining 20% of tumors are muscle-invasive tumors and have a less favorable prognosis. 5-year survival is 50%. When diagnosed early, the 5-year survival rate of bladder cancer is approximately 94%, so timely intervention can significantly increase the patient's possıblty of survival (Goodison et al., 2013).

While radical surgery is required for muscle-invasive bladder cancer, non-muscle-invasive bladder cancer can be treated more conservatively with transurethral resection of the tumor. However, more than 70% of bladder cancer patients experience recurrence of the disease within the first two years after diagnosis. If left untreated, this initially non-muscle-invasive bladder cancer may turn into muscle-invasive (Goodison et al., 2013). The possibility of recurrence of non-muscle-invasive bladder cancer makes it one of the most common cancers worldwide. Once treated, bladder cancer patients need to be under constant surveillance with routine cystoscopy examinations and cytology for early detection of new cancer development. The gold standard for the initial clinical diagnosis of bladder cancer is cystoscopic examination of the bladder. Cystoscopy is an invasive procedure that may require anesthetizing the patient and then performing a biopsy for histopathological diagnosis and staging (Trivedi, 2009). Voiding urinary cytology (VUC) currently remains the preferred method for non-invasive evaluation of patients other than cystoscopy (Trivedi, 2009). VUC is based on microscopic visualization of cancer cells shed in voided urine. Low-grade tumors and lower-stage tumors shed fewer cancer cells into the urine, and therefore the sensitivity of detecting these early-stage tumors with VUC ranges from 20% to 40% (Tetu, 2009). However, in this method, results cannot be obtained quickly because there may be differences between microscopic observers and diagnosis is difficult and relatively expensive.

What is miRNA? and its relationship with Bladder Cancer

miRNAs were first identified as genes that play a regulatory role in developmental timing events in a model organism, C. elegans (Kato & Slack, 2008). miRNAs are small non protein coding RNA molecules approximately 18-25 nucleotides long. These molecules affect many biological processes, including cellular. It is involved in proliferation, differentiation and apoptosis and plays important roles in normal development, physiology and disease (Celik et al., 2013; Chandrasekaran et al., 2019). Outside of these features, it has been determined that more than half of miRNA molecules are located in cancer-related gene regions or fragile regions in the human genome. In cancer development, miRNAs can act as oncogenes or tumor suppressors

depending on the mRNAs they target, so miRNAs appear to be regulators of tumor progression, metastasis, and invasion. (Celik et al., 2013; Saydam et al., 2011).

Uncovering new mechanisms and relationships will also contribute to the development of diagnosis and treatment methods. Urinary bladder cancer is the fourth most common cancer in the Western world. This cancer is initially limited to the mucosa or submucosa in approximately 75% of cases, and these cases are grouped as non-muscular invasive bladder cancer (NMIBC), while approximately 25% are referred to as muscle-invasive bladder cancer (MIBC). Standard initial diagnostic and prognostic evaluation of bladder cancer patients includes cystoscopy and histopathological analysis of biopsy samples. However, current prognostic markers such as tumor grade, stage, and size may not accurately reflect the clinical outcome. Therefore, new biomarkers need to be identified to improve the diagnosis and prognosis of different types of bladder cancer. One of these biomarkers is miRNA. miRNAs are small non-protein coding RNAs consisting of 19 to 24 nucleotides that regulate gene expression by degrading mRNAs post-transcriptionally or impairing translation abilities. The involvement of miRNAs in gene regulatory processes and their role in many diseases, including cancer, make them very attractive for diagnosis, prognosis and treatment in clinical practice. The increasing number of studies investigating bladder cancer specific miRNA expression profiles indicates increasing interest in searching for specific miRNAs to serve as diagnostic or prognostic biomarkers. All miRNA studies were based on analysis of miRNA expression and comparisons with reference genes. In bladder cancer profiling studies, reference genes RNU6B and RNU48 were used. In our study, RNU6B will be used as the reference gene. The RNU6B reference gene has been used and validated in most studies. Selection of validated reference miRNAs is important to obtain reliable miRNA expression data. The levels of miRNA 22 5p in mesenchymal stem cells and myocardial infarction, and the levels of miRNA 337 5p in gastric cancer and breast cancer were investigated. For this reason, considering the relationship of the two miRNAs with other types of cancer, it has been observed that their levels in bladder cancer have not been investigated. For this purpose, we aim to determine the expression levels of miRNA 22 5p and miRNA 337 5p in Bladder Cancer patients by Real-Time PCR method.

Conclusion

This study will be carried out to determine whether these two miRNAs can guide early diagnosis and diagnosis in Bladder Cancer patients and will provide preliminary information by providing preliminary information to clinicians and contributing to the literature on this subject.

Scientific Ethics Declaration

The authors declare that the scientific ethical and legal responsibility of this article published in EPHELS Journal belongs to the authors.

Acknowledgements or Notes

* This article was presented as an oral presentation at the International Conference on General Health Sciences (www.icgehes.net) held in Alanya/Turkey on May, 02-05, 2024.

*This study was supported by Gaziantep University Scientific Research Project Unit. Project number: FEF.DT.22.27

References

Antoni, S., Ferlay, J., Soerjomataram, I., Znaor, A., Jemal, A., & Bray, F. (2017). Bladder cancer incidence and mortality: A global overview and recent trends. *European Urology*, *71*(1), 96-108.

Celik, D. A., Kosar, P. A., & Ozcelik, N. (2013). MikroRNA lar ve kanser ile iliskisi. *SDU Tıp Fakultesi Dergisi*, *20*(3), 121-127.

Chandrasekaran, A. R., MacIsaac, M., Dey, P., Levchenko, O., Zhou, L., Andres, M., & Halvorsen, K. (2019). Cellular microRNA detection with miRacles: microRNA-activated conditional looping of engineered switches. *Science Advances*, *5*(3), eaau9443.

Dyrskjøt, L., Hansel, D. E., Efstathiou, J. A., Knowles, M. A., Galsky, M. D., Teoh, J., & Theodorescu, D. (2023). Bladder cancer. *Nature Reviews Disease Primers*, *9*(1), 58.

Edge, S. E., Bryd, D. R.,. & Trittz, A. G.(2010). *AJCC cancer staging manual* (pp.347-376). New York, NY: Springer.

Ergin, A., Ozdilek, R., & Dutucu, N. (2019). 2012-2017 yılları arasında kadınlarda gorulen kanser turleri ve dağılımları: Bir universite hastanesi ornegi. *Kadın Saglıgı Hemsireligi Dergisi*, *5*(1), 1-21.

Goodison, S., Rosser, C. J., & Urquidi, V. (2013). Bladder cancer detection and monitoring: Assessment of urine and blood based marker tests. *Molecular Diagnosis & Therapy*, *17*, 71-84.

Johar, R.S., Hayn, M.H.,…& Stegemann, A.P. (2013). Compli- cations after robot assisted radical cystectomy: Results from the International Robotic Cystectomy Consortium. *European Urology*,*64*(1), 52-57.

Kato, M., & Slack, F. J. (2008). microRNAs: small molecules with big roles - C. elegans to human cancer. *Biology of The Cell*, *100*(2), 71–81.

Kaufman, D.S., Shipley, W.U., Feldman, A.S.(2009). Bladder cancer. *Lancet, 374*(9685), 239–249

Klug, W. S., Cummings, M. R., & Spencer, C.A. (2011). *Genetik kavramlar*. Ankara: Palme Yayıncılık.

Kumar, A., Kumar, R., Gupta, N.P.(2006). Comparison of NMP22 Bladder Chek test and urine cytology for the detection of recurrent bladder cancer. *Japanese Journal of Clinical Oncology, 36*(3),172–175.

Millan-Rodriguez F., Chechile-Toniolo G., Salvador-Bayarri J., Palou J,. Algaba F., Vicente-Rodriguez, J. (2000). Primary superficial bladder cancer risk groups according to progression, mortality and recurrence. *Journal of Urology, 164*(3), 680–684.

Nakamura, K., Kasraeian, A., Iczkowski, K. A., Chang, M., Pendleton, J., Anai, S., & Rosser, C. J. (2009). Utility of serial urinary cytology in the initial evaluation of the patient with microscopic hematuria. *BMC Urology*, *9*, 1-6.

Ojea, A., Nogueira, J. L., Solsona, E., Flores, N., Gómez, J. M. F., Molina, J. R., ... & CUETO Group. (2007). A multicentre, randomised prospective trial comparing three intravesical adjuvant therapies for intermediate-risk superficial bladder cancer: low-dose bacillus Calmette-Guerin (27 mg) versus very low-dose bacillus Calmette-Guerin (13.5 mg) versus mitomycin C. *European Urology*, *52*(5), 1398-1406.

Têtu, B. (2009). Diagnosis of urothelial carcinoma from urine. *Modern Pathology*, *22*(2), 53-59.

Trivedi D., & Messing, E.M.(2009) Commentary: the role of cytologic analysis of voided urine in the work-up of asymptomatic microhematuria. *BMC Urology*, *9*,13.

Zigeuner, R. E., Hutterer, G., Chromecki, T., Rehak, P., & Langner, C. (2006). Bladder tumour development after urothelial carcinoma of the upper urinary tract is related to primary tumour location. *BJU International*, *98*(6), 1181-1186.

Author Information

Mehmet Aydin Dagdeviren
University of Gaziantep, Department of Biology, 27310 Gaziantep, Turkiye
Contact e-mail: *aydin_dagdeviren@hotmail.com*

Mehmet Ozaslan
University of Gaziantep, Department of Biology, 27310 Gaziantep, Turkiye

To cite this article:

Dagdeviren, M.A., & Ozaslan, M. (2024). Determination of miRNA expression levels in bladder cancer *The Eurasia Proceedings of Health, Environment and Life Sciences (EPHELS),*128-131