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Genetic Molecular And Physiology Analysis Of Essential Genes In Microbiology Escherichia Colibacteria

Hayder Hatem Abdulwahhab[1] , Khansaa Basem Fadhil[2] , Abeer Talib Abdulqader[3] , Bushra Ismail Ibrahim[4]

[1]University of Çankırı Karatekin Department of Biology, Northern technical university, Al-dour technical institute

[2]Department of Technical medical laboratory, Northern technical university, Al-dour technical institute

[3]Biology /Physiology ,Department of medical laboratory Techniques, Northern technical university,Al-dour technical institute

[4]Department of Biology, College of Education, University of Tikrit,General Directorate of Education Salah Al-din

Abstract

The development of personalized medicine has created a strong demand for biomarkers that can be used in the context of patient diagnosis, monitoring and stratification. At the same time, the quest for new biomarkers has been revived by the emergence of new broadband technologies as well as the development of systemic approaches to study the pathophysiology of diseases such as cancer.According to the National Institutes of Health, a biomarker (or biological marker) is defined as "A biological characteristic measured objectively and evaluated as an indicator of normal or pathological biological processes or pharmacological responses to a therapeutic intervention". Escherichia coli is responsible for the development of various types of diseases, one of the most prevalent in society is urinary tract infection, which affects both sexes and all ages, with a higher prevalence among women. This bacterium can be classified according to clinical manifestations, epidemiology, virulence factor of the strain and anatomical site of the infection. This pathology can be asymptomatic or symptomatic. The complexity of this, in the case of uropathogenic E. coli , will vary according to the host's immunity, bacterial load and virulence factor, with fimbriae being the most important factor in the case of UTI, as they present end H adhesins that mediate the link between host receptor glycoproteins and the bacteria.A biomarker can therefore be molecular, anatomical,

physiological or biochemical.More precisely, a tumor marker is defined as "a molecule, a process or a substance altered quantitatively or qualitatively under precancerous or cancerous conditions, the modification being detectable by an analysis". These alterations can come from the tumor itself or from the surrounding normal tissue.Biomarkers are used in two main areas. In biomedical research, biomarkers can be used when developing and evaluating new therapies.

Kew words : Cystoscopy , biological marker , physiological , biochemical , Escherichia coli.

Introduction

In 80% of cases, bladder cancer is revealed by gross hematuria, often terminal. Signs of bladder irritation such as pollakiuria, urination or burning of the urine are present in 20% of cases. This is followed by interrogation (with the search for risk factors) and clinical examination, although the latter is usually normal for the early stages.

When suspected of bladder cancer, a diagnostic diagnostic assessment is performed including an ultrasound of the urinary tract, urine cytology and cystoscopy. Confirmation of the diagnosis is then made by histopathological examination based on a transurethral resection of the bladder (RTUV). An anatomo-pathological examination of the resection chips specifies the stage and grade of the tumor (Schrohl A-S, 2003).

Cystoscopy is an endoscopic examination that provides information on the number, location, appearance (papillary or solid) and size of the tumor. It is systematic in the face of any suspicion of bladder cancer. Urinary cytology is also systematically performed. It is done on fresh or fixed urine and aims to search for tumor cells.Cystoscopy has good sensitivity (62% - 84%) but can sometimes give false negatives by operator error or by difficulty detecting smaller tumors such as carcinoma in situ. Conversely, urinary cytology has a high specificity (78% - 100%) but lacks sensitivity (12% - 84.6%), especially for low grade tumors. In addition, this examination requires a qualified cytopathologist and the results can be operator-dependent (Kelloff GJ, 2012).

Subsequently, the extension assessment aims to seek a multifocality. Indeed, it is possible that foci are also present in the upper urinary tract which is also composed of urothelium. The analysis is done by a CT scan (uro-tomodensitometry or uro-CT) or an intravenous urography.If it is a TVIM, the extension assessment will then be done by thoraco-abdomino-pelvic CT. This examination makes it possible to assess the involvement of the upper urinary tract and neighboring organs as well as to detect possible lymphadenopathies and / or metastases (Biomarkers Definitions Working Group , 2001).

Proteomics therefore concerns the large-scale study of a proteome and the biological significance of proteins. Thus, proteomic studies can have different objectives such as understanding all aspects of proteins (their expression, function, interaction and structure) or leading to the discovery of new biological markers of disease states.

There are three main approaches for the discovery of new biological markers:

- The data-driven approach: it is an analysis of the whole proteome allowing to identify a link between the quantified proteins and a particular pathological state. This approach is unbiased because no assumptions are made about the proteins that could be involved.
- The knowledge-based approach: it is a targeted proteomic approach according to which potential biomarkers are determined thanks to the state of scientific knowledge for a pathology. Dependence on the state of knowledge can be a disadvantage because if it is not sufficiently developed, certain unknown markers cannot be identified.
- The approach based on systems biology: systems biology is an interdisciplinary field of research which studies the behavior and relationships of all the elements of a biological system and which develops models for predicting its behavior. This approach contrasts with the so-called reductionist approach which analyzes a complex system by dividing it into several parts and determining the relationships between them. This assumes that the study of isolated molecules is sufficient to understand the whole system, while systems biology is based on the integration of large amounts of genomic and proteomic data associated with calculation techniques (computational methods).

Bladder tumors are clinically characterized by their high recurrence rate and poor prognosis once they invade the muscle layer. T1G3 and in situ urothelial tumors are considered high risk because of their high spread of recurring and progressing to musculoskeletal and metastatic disease (Sanchez-Carbayo M, 2003). Intravesical immunotherapy with the Calmette-Guinin Bacillus (BCG) represents a highly successful empirical therapy in these patients, benefiting from its decrease in recurrence and progression rates. Despite the superior efficacy of BCG on transurethral resection alone or with q intravesical uimioterapia, over 50% of tumors noninvasive recur or persist (Ramachandran N, 2008). This is a prominent problem in high-risk patients with CIS, submucosa invasion (stage T1), and high-grade papillary as it increases the risk of progression and decreases the probability of cure with conservative bladder surgery and avoid cystectomy. A large number of these patients

do not respond to BCG immunotherapy; and their tumors not only persist and recur, but progress by investing and metastasizing. Despite the promising sensitivity and specificity to predict response to BCG in these three types of high-risk patients in non-muscle invasive disease, representing 80% of patients with bladder cancer, none of the predictive biomarkers described so far has been fully introduced into routine clinical practice (Sánchez-Carbayo 2012). Not only diagnostic markers are needed to detect the disease, but prognostic and predictive ones that can allow differentiating minor injuries from those lethal tumors to be able to provide therapeutic procedures adapted to the individual aggressiveness of each tumor. In this sense, we have described as identified candidates methylated in bladder cancer , such as Myopodin or PMF1, both the methylation and the expression of the protein by immunohistochemistry of these genes or others identified by high impact techniques such as ezrin, in tissue arrays play an important role as markers pro nostic and predictive of response to BCG in high risk patients with tumors T1G3.

Progress of the high - impact technologies for molecular analysis of tumors allow explore genetic profiles, epigenetic and protein characteristic different tumor subtypes and identify targets and molecular pathways that define a specific clinical behavior. Different groups, including ours, have used transcript, genomic and proteomic profiles of tumors and biological fluids to identify individual profiles and candidates that allow us to classify different bladder cancer subtypes, as well as molecular pathways involved in bladder cancer tumorigenesis and progression (Sharma S, 2009).

Objective

The central and specific objectives that were initially raised in this paper were the following:

The central scientific objective is to assess whether the characterization of clinically relevant epigenetic molecular rubrics in bladder cancer allows identification of individuals with bladder cancer or prone to disease in a high-risk population.

Materials and Methods

Urine collection: Urine samples were attempted to be collected arbitrarily from the sources. 50 urine samples have been collected from patient samples in the Urology services. The samples were frozen in situ in the hospitals at -80^{o}C, where the IP carried carbon dioxide snow in polystyrene boxes to collect the samples monthly. The specific objective we want is to select 50 candidates of genes and miRNAs matched differentially expressed in the urines with hematuria and bladder cancer confirmed to be employed in epigenetic techniques arrays of high impact. The CpG and miRNA arrays used include a number of candidates high

enough to identify at least 80-90 candidates differentially expressed among groups with p-values of significance less than 0.001. Thus, the number of 50 samples analyzed is acceptable and consistent with a known formula for calculating sample size. Assuming a type I error of 0.001 and a standard deviation of 0.45, the sample size of 50 could detect differences in expression of at least a range of> 2 of variation between groups with a statistical power of 95% (Segar, 2014).

Evaluation of microhematuria: The hidden presence of blood in all urine collected urine has been determined, since it is an initial sign of suspicion of the presence of bladder cancer. The evaluation of the presence of haemoglobin in urine was performed using dipsticks of Roche which are urine test strips that colorimetric method quantifies the degree of microhematuria based on the presence of hemoglobinuria. These test strips also allow the presence of leukocyturia and nitrites to be detected as signs of a potential urinary infection (Yu X, 2010).

Functional analysis to associate the hypermethylation of candidate genes with their silencing : The association of the methylation of candidates identified in the epigenetic profile by combining the discovery phase using CpG arrays in urine and tumors with the silencing of those genes has been started to evaluate in bladder cancer tumor lines . We would like to be able to evaluate and optimize 40-50 candidate genes, but due to both time and budget limitations, it has been possible to start characterizing at least one gene. The methylation status has been established by optimizing two methylation polymerase chain reactions (PCRs) on bisulfite-treated DNA. Primers have been designed to perform two procedures.

Identification of targets of the epigenetic profile miRNAs, and functional analysis to associate the silencing of targets with regulatory miRNAs : The association of the 40-50 miRNA candidates of the epigenetic profile identified after combining the identification phase using miRNA arrays in urine with the silencing of candidate targets, we would like to evaluate several lines of bladder cancer. It is desired to use massive sequencing (from Applied Biosystems using the Solid system) of these cell lines to determine the absolute abundance and profile of those miRNAs compared to the analyses obtained from the sample mixtures analysed in previous section, but there have been no sufficient funding for the accomplishment of this task. MiRNA sequences can be analyzed using the miRBase database and the University of California. The CpG island finder program will be used for miRNAs that are co-located on CpG islands , as 90% of promoters of human miRNAs have been predicted to locate 1,000 bp upstream of mature miRNAs (Lopez V, 2013).

For the determination of the genetic similarity of the uropathogenic E. coli isolates, the DNA macrorestriction method was used, followed by pulsed field electrophoresis. It was established, as a criterion for the genotypic analysis, isolates found in each patient in the two anatomical sites (urethral / periurethral or urine and feces) that presented the same phenotypic profile related to the tests of hemolysis, aerobactin, Congo red, pili 1 and P mobility (Lafi, Shehab , 2012).

The patients who reported improvement in their symptoms presented, in relation to those who were not better: a lower number of personal antecedents related to the development of UTI, fewer resistance to Escherichia coli, fewer episodes of UTI after being vaccinated, greater percentage decrease in UTI episodes, fewer positive urine cultures (before and after vaccination), lower total expenditure and higher percentage decrease in expenditure derived from recurrent UTI episodes. The percentage of women who reported improvement in their symptoms was higher among women with no personal history related to UTI, in whom UTI episodes decreased and the frequency of positive cultures after vaccination, in whom UTI episodes were only by Escherichia coli, and in those that decreased the total expenditure with vaccination derived from UTI episodes (Samarai, 2016).

Results and Discussion

In clinical practice, the various functions of biomarkers make it possible to establish a classification(Ahram M, 2008):

- Diagnostic biomarkers: they allow the identification of patients with an abnormal pathology or condition.
- Risk or susceptibility biomarkers: they make it possible to estimate the risk of developing a pathology.
- Prognostic biomarkers: they make it possible to determine the course of the pathology. In oncology, prognostic biomarkers give an estimate of the risk of recurrence.
- Predictive biomarkers: they make it possible to predict the response to a treatment and therefore to determine the most suitable treatment for a patient (strategy defined as "personalized medicine".
- Surveillance biomarkers: they are used during patient monitoring. In cancerology, they allow the detection of recurrence or remission.

Below is a summary of the strategy and the results that it has given time to carry out in this paper. After organizing the collection of urine samples and characterizing their microhematuria, Then it is optimized methodology of epigenetics to characterize the methylation by MS-PCR and to determine whether selected genes are methylated by techniques alternatives both tumor lines cellular and in future series of urines independent to those already collected. To assess whether hypermethylation of selected genes is associated with the loss of expression of the selected genes, has been treated bladder cancer cell lines with azacitidine a demethylating agent to retrieve the expression of the genes they are methylated if. It is confirmed to BDNF, the selected gene, the presence of methylation not only by the array of CpG, but PCRs methylation, sequencing bisulphite, and confirming the recovery of the expression of the gene by RT-PCR, western blot (WB) and immunofluorescence.

The results of serum BCA are demonstrated in Table (1). The frequency % level serum BCA was significantly (P=0.0001) highest in BCA group (15/50) (25.6%) compared to control group (1/35) (3.44%). For the mean titer, the BCA group exhibited the highest mean titer (15.27±27.90) in comparison with the control group (2.80±2.81) with significant differences (P=0.0001).

Table (1) : The values of bladder specific antigen (BCA) patients and control groups:

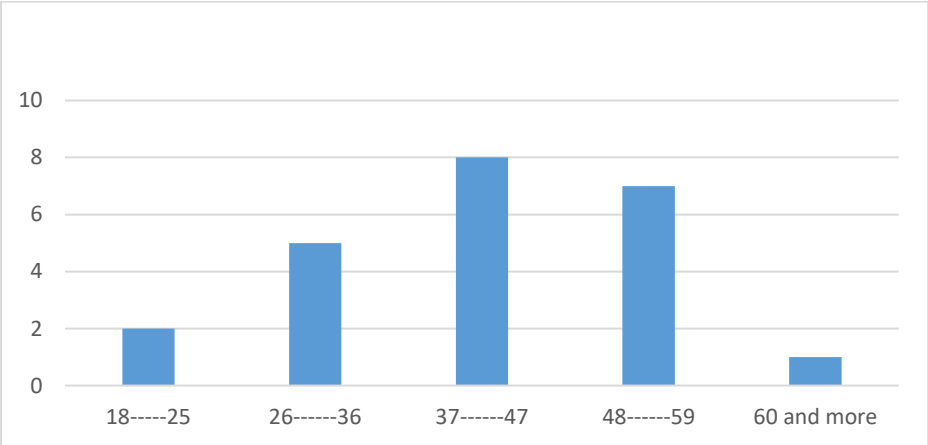
BCA	Groups ng/ml	No.	%Of No.	Mean±Std.D	Sum	Std.Error of Mean
Control	High > 10	1	35.3%	12.20±0.0	12.40	0.902
	Low < 3	24	73%	1.34±0.90	25.06	1.899
	Normal (3-10)	10	23.7%	5.35±1.71	47.76	2.815
	Total	35	100%	2.70±2.82	89.02	37.912
Patient	High > 10	15	27.7%	48.48±37.91	775.61	9.478
	Low < 3	10	31.7%	1.53±0.92	30.84	0.173
	Normal (3-10)	25	32.7%	5.58±2.20	119.69	0.471
	Total	50	100%	15.37±27.90	816.14	3.602
Total	High > 10	18	18.9%	46.40±37.69	768.81	9.142
	Low < 3	45	53.1%	1.28±0.85	57.90	0.126
	Normal (3-10)	27	30%	5.65±1.98	152.45	0.382
	Total	90	100%	11.11±23.53	1000.16	2.481
	Lsd= 40.62*	Df=5		Sig=0.0001		

After the introduction of the sublingual bacterial vaccine, a total of 50 urine cultures were performed in the 16 study patients. The mean number of urine cultures per patient and year after vaccination was 4.67 (SD: 3.58; 95% CI: 3.28 - 3.17), with a median of 1.93; significantly lower than those performed before vaccination (5.27), producing a mean decrease in urine cultures of 1.7(SD: 1.8; 95% CI: 1.31 - 1.87; p <0.0001). With vaccination there was a mean decrease in urine cultures of 37.5% (95% CI: 34.6% - 40.3%). In 15.7% patients there was no decrease in the number of previous cultures after vaccination (Abbas, 2018). There was a strong positive correlation between the number of urine cultures before and after vaccination (r = 0.849; p <0.0001). A greater decrease in the number of urine cultures with vaccination was correlated with a greater number of personal history (r = 0.238; p = 0.003), a greater number of UTI episodes prior to vaccination (r = 0.365; p <0 , 0001), a greater number of different antibiotics used before vaccination (r = 0.259; p = 0.001) and a greater number of packages of antibiotics consumed prior to vaccination (r = 0.332; p <0.0001) (Lehmann, 2011).

Data analysis:

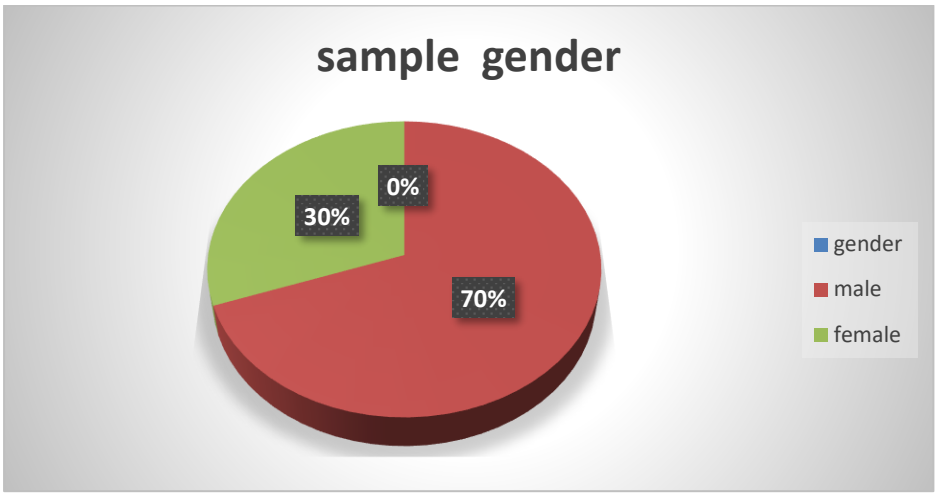
After data collection was completed, questionnaires were counted and checked for errors. The questionnaires were numbered and coded. A code book was created to facilitate data capturing and auditing of the captured data. The samples as they were sorted and analyzed by a statistician using the excel program Methodology.

Figure (1): Distribution of the study sample according demographic data (age).



The result of figure (1) shows that the most of the study sample their age at (37-47).

Figure (2): Distribution of the study sample according demographic data (gender).



The result of figure (2) reveals that the most of the study sample (70%) they were patients.

Assess the silencing of critical genes in tumorigenesis of the epigenetic profile by immunohistochemistry in tissue arrays (T.M.A). The tissues collected retrospectively prior to this paper have been reviewed by uropathologist. It was familiar with these techniques as can be seen in our publications. To construct the T.M.A s, representative areas of these tumors and the normal urotheliums have been selected and at least triplicates have been included, and whenever available, normal and dysplastic urothelium have been included. If in the future it is possible to obtain tissues from tumors evenly matched urines studied in this project, it will build arrays of tissue also with these samples. Silencing effects of one of the genes validity in vitro , being analyzed by immunohistochemistry in TMAs, and will relate to the results obtained by MS-PCR methylation. The miRNAs will be analyzed by specific Q-RT-PCR. Immunohistochemistry is being carried out entirely in the Translational Oncology Laboratory supervised by the IP.

Optimize & evaluate the epigenetic profile tests in the urine series of the Bladder cancer patients: The aim is to design an MS-MLPA assay selecting among the 50 methylated candidates in individuals with microhematuria in which the presence of bladder cancer is confirmed and controls without disease confirmed by cystoscopy. The selected candidates will have to be methylated in the urine, taking into account functional in vitro studies and independent serial clinical validation of tumors. For miRNAS, the 50 most differently expressed candidates with the same criteria would be selected to design a multiparametric assay based on q-RT-PCR. These tests would be evaluated in the urine series to be collected,

with a minimum of 50 samples from individuals working in industries at risk, including those in which the CpG arrays and miRNA arrays were performed in the previous secions. Sensitivity, specificity, positive and negative predictive values and the diagnostic accuracy of candidates Individual and multiparameter profiles will evaluate cases and controls (with and without disease) and with no risk together.

Conclusion

Bladder cancer occurs after environmental exposure to different carcinogenic agents such as tobacco and due to occupational exposure as well as the sequential accumulation of multiple genetic and epigenetic alterations. These molecular alterations result in uncontrolled cell proliferation, deregulation of the cell cycle and differentiation, decrease in cell death or apoptosis, invasion block and metastasis. The particular genetic alterations, epigenetic and protein expression occur as a pair like the interaction of these signalling pathways, and determine the biological behavior of the tumor, including its ability to grow, resort, progress and metastasize. Depending on the genetic profile, the clinical behavior, bladder tumors is can be assigned within the two main subgroups . The changes most common in tumors muscle Papillary invasive low grade include mutational activation of FGFR3, loss of heterozygosity (LOH) of the chromosome 9, mutational activation of PIK3CA and RAS genes and mutational inactivation of TSC1. Common alterations in muscle-invasive tumors include inactivation of TP53 and RB1, reduced expression of PTEN expression (via mutation, LOH, homozygous deletion, or other mechanisms), amplification of ERBB2, amplification of 6p22, chromosome deletions 8 and other Genomic alterations whose targets have not yet been characterized. Assigning to one of these subgroups already provides not only diagnostic but prognostic information.

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