review

Current and innovative approaches in the treatment of non-muscle invasive bladder cancer: the role of transurethral resection of bladder tumor and organoids

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Background. Bladder cancer is the 7th most common cancer in men. About 75% of all bladder cancer are non-muscle invasive (NMIBC). The golden standard for definite diagnosis and first-line treatment of NMIBC is transurethral resection of bladder tumour (TURB). Historically, the monopolar current was used first, today bipolar current is preferred by most urologists. Following TURB, depending on the tumour grade, additional intravesical chemo- or/and immunotherapy is indicated, in order to prevent recurrence and need for surgical resection. Development of new technologies, molecular and cell biology, enabled scientists to develop organoids – systems of human cells that are cultivated in the laboratory and have characteristics of the tissue from which they were harvested. In the field of urologic cancers, the organoids are used mainly for studying the course of different diseases, however, in the field of bladder cancer the data are scarce.

Conclusions. Different currents - monopolar and bipolar, have different effect on urothelium, that is important for oncological results and pathohistological interpretation. Specimens of bladder cancer can be used for preparation of organoids that are further used for studying carcinogenesis. Bladder organoids are step towards personalised medicine, especially for testing effectiveness of chemo-/immunotherapeutics.

Key words: bladder cancer; transurethral resection of bladder tumour; monopolar/bipolar current; organoids, mitomycin C; BCG

Introduction

Bladder cancer is 7th most common cancer in men. In the European Union (EU) age-standardised incidence rate is 19.1 for men and 4.0 in women.[1] In Europe, bladder cancer incidence is 27.1 and mortality 8.9.[2] The highest incidence rate in the EU is reported in Belgium and lowest in Finland. On the global level, incidence and mortality rates vary due to different methodologies and diagnostic practices.[1,2] Approximately three-quarters of all bladder cancer are non-muscle invasive (NMIBC) – the disease is confined to the mucosa (stage Ta, CIS) and submucosa (stage T1) (Table 1, Figure 1), in patients younger than 40 years this proportion is even higher.[1,3-5] According to the data from Cancer registry of the Republic of Slovenia, bladder cancer is the 8th most common cancer in men and the 13th most common when both genders are considered. Age standardised incidence rate is

11.74 in men and 2.97 in women. Age-standardised mortality rate is 5.46 in men and 1.52 in women.[6] Although many European countries experience incidence rise, the projected growth rate of bladder cancer incidence rates by 2030 in Slovenia is extremely high, i.e. 92% for men and 256% for women.

Standard treatment of NMIBC is transurethral resection of bladder tumour (TURB). Depending on the histopathological tumour characteristics, additional treatment with intravesical chemotherapeutics or immunotherapeutics is indicated. The aim of intravesical therapy is to decrease the rate of recurrence and need for surgical intervention.[1]

Organoids are 3D models which consist of cells derived from specific tissue or organ and are grown in the laboratory with the aim to study different cell biological mechanisms, homeostasis, development of disease and effect of different medications. Organoids have characteristics of the cells from which are derived, although differences could appear because of the effect of microenvironment.[7] There are some new data about organoids used to study oncogenesis and different treatments in urological cancers (prostate, kidney, bladder).[8]

Aim of this review is to present different modalities of TURB for treatment of NMIBC, and the role of bladder/urothelium organoids for studying oncogenesis, therapeutic modalities and personalised medicine in NMIBC.

Transurethral resection of bladder tumour (TURB)

TURB is a golden standard in diagnosis, treatment, and staging of NMIBC as well as in diagnosis of muscle invasive bladder cancer (MIBC).[1,9] The first published report involving the application of electric current for endoscopic resection of papillary bladder tumours through cystoscope originates in 1910s. The initial case was performed using water as medium. Since then, TURB has become standard in evaluation and treatment of patients with bladder cancer.[9] Guidelines of three urologic associations (European Association of Urology, EAU; American Urology Association, AUA; and Canadian Urology Association, CUA) emphasize the importance of TURB for diagnosis, staging and treatment of NMIBC (1, 9, 10, 11).[1,9,10,11]

TABLE 1. 2017 TNM classification of urinary bladder cancer [5]

T - primary tumour
TX Primary tumour cannot be assessed
T0 No evidence of primary tumour
Ta Non-invasive papillary carcinoma
Tis Carcinoma in situ: 'flat tumour'
T1 Tumour invades subepithelial connective tissue
T2 Tumour invades muscle
T2a Tumour invades superficial muscle (inner half)
T2b Tumour invades deep muscle (outer half)
T3 Tumour invades perivesical tissue
T3a Microscopically
T3b Macroscopically (extravesical mass)
T4 Tumour invades any of the following: prostate stroma, seminal vesicles, uterus, vagina, pelvic wall, abdominal wall
T4a Tumour invades prostate stroma, seminal vesicles, uterus or vagina
T4b Tumour invades pelvic wall or abdominal wall
N - regional lymph nodes
NX Regional lymph nodes cannot be assessed
N0 No regional lymph node metastasis
N1 Metastasis in a single lymph node in the true pelvis (hypogastric, obturator, external iliac, or presacral)
N2 Metastasis in multiple regional lymph nodes in the true pelvis (hypogastric, obturator, external iliac, or presacral)
N3 Metastasis in common iliac lymph node(s)
M - distant metastasis
M0 No distant metastasis
M1a Non-regional lymph nodes
M1b Other distant metastases

TURB is a relatively safe procedure with few complications, in some countries is performed in an outpatient setting. The most common complications are haematuria and urinary tract infection. One of the most important aims of TURB is to resect the whole tumour (depending on the size and invasion in muscle layer) and resect muscle layer in order to obtain the correct pathological stage.[1,10,11] Since its introduction in the 1910s, technology has developed and so has equipment and technique of TURB.[9] In 1900 Joseph Rivere discovered that a spark arcing from an electrode coagulates skin and he used it for treatment of skin lesions. In the following decades, this technique was used to treat skin lesions, lesions in the oral cavity, bladder, coagulation of vascular tumours and haemorrhoids. In 1920s Clark was one of the first who observed the tissues exposed to current under a microscope and found that they shrink from dehydration. Bovie constructed the first diathermy unit that was used for cutting, coagulation, and dissection and was first used on October 1st 1926 in Boston. Since then, this instrument is used in everyday surgical practice (12).[12]

The clinical effect of electrocautery is a consequence of heat. When oscillating current is applied to the tissue, the rapid movement of electrons through the cytoplasm causes an increase of intracellular temperature. The effect on the tissue depends upon the amount of thermal energy delivered and the time rate of delivery. Temperature below 45°C causes reversible thermal damages, when increased it causes denaturation and loss of protein structure, above 90°C liquid evaporates, resulting in vaporization if heated rapidly or desiccation if heated slowly. Temperatures over 200°C cause carbonization.[12]

Electric energy could be monopolar or bipolar. Monopolar energy delivery requires the current to pass from the generator to the active electrode through the patient and out of the body through a dispersive electrode pad which is connected to the generator in order to complete the circuit. On the other hand, bipolar delivery does not require a dispersive return electrode because both active and return electrodes are integrated into energy delivery forceps with target tissue between them (Figure 2).[12]

Monopolar electrocautery for TURB

In urology, TURB was introduced in the 1910s by Beer using monopolar current for fulguration (Figure 2).[13,14] Monopolar electrocautery requires high energy and voltage to allow current transmission from the loop to the tissue. In monopolar electrocautery glycine is used as a medium that is associated with limitations, such as short resection time due to the risk of development of TURB syndrome. The heat generated while cutting the tissue causes damage to the surrounding cells. Because of the thermal artefacts, pathologic assessment of the specimen is sometimes difficult.[13] There are studies which compared monopolar and bipolar elec-

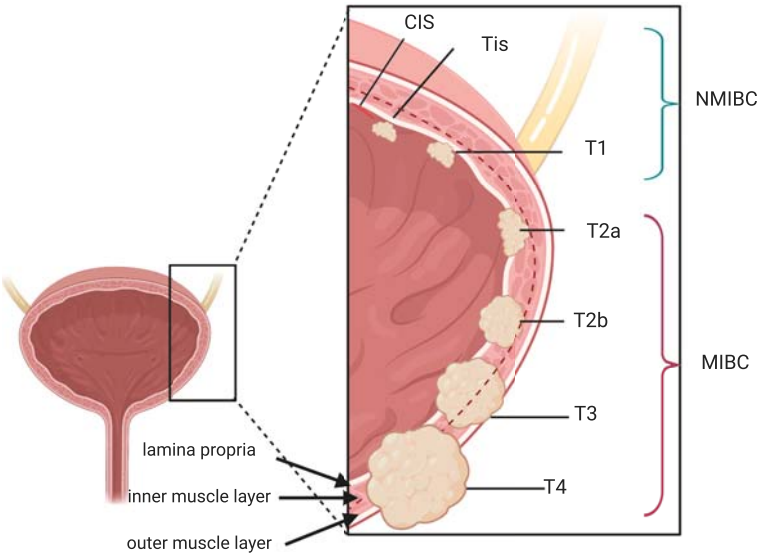

FIGURE 1. Classification of bladder cancer.

MIBC = Muscle invasive bladder cancer; NMIBC = Non muscle invasive bladder cancer

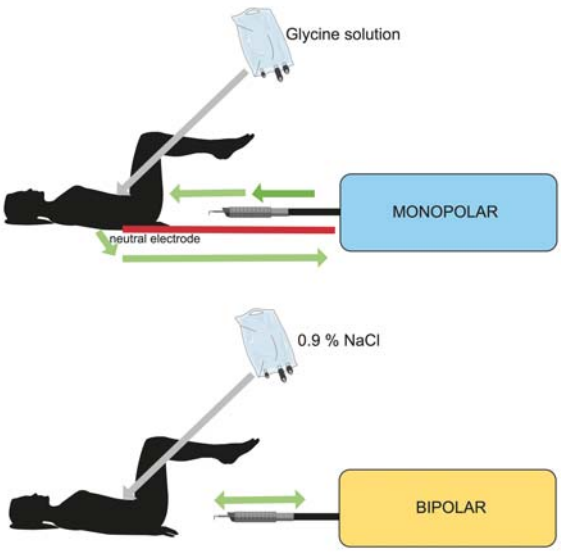

FIGURE 2. Monopolar vs. bipolar transurethral resection of bladder tumor.

TABLE 2. Comparison of monopolar and bipolar current for TURB[1,9-14]

Variable	Monopolar	Bipolar
Dispersive electrode pad	yes	no
Energy	high	low
Voltage	high	low
Working medium	glycine	saline
Temperature at thermal effect (°C)	400	40–70
Time of resection	limited	extended (not strictly limited)
TUR syndrome	common	rare
Obturator jerk	common	rare
Quality of haemostasis and coagulum	poor	good

trocautery for TURB and concluded that there is very little or no difference in thickness of thermal artefacts.[14] One study has shown mean depth of thermal artefacts of 0.237 mm when using bipolar electrocautery and 0.26 mm when using monopolar electrocautery.[9] Monopolar electrocautery is not inferior to bipolar electrocautery regarding intra and postoperative bleeding, and perforation of bladder wall (Table 2).[14]

Bipolar electrocautery for TURB

Bipolar electrocautery has been introduced about 30 years ago and a few years after its introduction has gained popularity over monopolar electrocautery.[12] Bipolar electrocautery was first used for transurethral resection of the prostate (TURP) for benign prostate enlargement and obstruction. It was quickly adopted for TURB because of advantages: (1) isotonic medium could be used (such as saline), (2) electric circuit is completed using only resection loop and sheath of the device itself, so the patient is not included in the circuit, (3) risk for TUR syndrome is low, (4) time for resection is extended, which is of essential importance in case of large bladder tumours, (5) incidence of obturator jerk is lower, (6) there are fewer bladder perforations. It is also related to fewer postoperative complications such as clot retention and contracture of the bladder neck (Table 2).[9]

One of the main differences between monopolar electrocautery and bipolar electrocautery systems is the coagulation mechanism. In bipolar electrocautery system voltage is low and energy dissipates as heat in the tissue leading to the formation of coagulum and haemostasis.[13]

In monopolar electrocautery, electrical injury directed into the tissues and electrical resistance creates temperature as high as 400°C which causes tissue desiccation and collateral tissue damage. Radiofrequency energy of bipolar electrocautery systems converts the conductive medium into a plasma field with highly ionised particles that disrupt the organic bonds between tissues and allows thermal effect to occur at lower temperatures (between 40 and 70°C). Pathologists sometimes classify thermal damage into three categories: (1) cautery artefact less than 1/3 of the specimen, (2) cautery artefact of 1/3 to 2/3 of the specimen and (3) over 2/3 of the specimen.[13] Although different electrocautery systems are used for more than 30 years we have no data on the effect of different electrocautery systems on cellular level.

Adjuvant treatment of NMIBC

Intravesical chemotherapy

TURB is used as definitive therapy in TaT1 tumours. The adjuvant treatment of NMIBC is indicated when dealing with high grade NMIBC. Immediate single intravesical instillation of chemotherapy is used to destroy circulating tumour cells after TURB and has an ablative effect on residual tumour cells at the resection site and on small overlooked tumours. Immediate single intravesical instillation should be performed within the first 24 hours after TURB to maximise its effect. Meta-analyses were performed which have shown that after immediate single intravesical instillation the recurrence rate is lower. Recent reviews and meta-analysis have shown that immediate single intravesical instillation also reduces 5-year recurrence rate.[1,15]

The most commonly used chemotherapeutics for immediate single intravesical instillation are mitomycin C (MMC), epirubicin and pirarubicin.[1] In Slovenia, we use MMC for immediate single intravesical instillation. In literature, there is data that further repeat instillations of chemotherapy also have an impact on the recurrence rate. The length and frequency of chemotherapy instillation are still controversial. According to data available, the length of this treatment should not exceed one year.[1,16]

There is evidence that intravesical chemotherapy combined with microwave-induced hyperthermia in high-risk patients has enhanced efficacy – it has improved recurrence-free survival at 24 months. There are also undergoing trials regarding use of different methods of hyperthermic intravesical chemotherapy and electromotive drug administration but data about their efficacy is still lacking.[1,17] In Slovenia, we are not using combinations with microwave, hyperthermia or electromotive drug

administration, since the use of these combinations is not confirmed by randomised controlled trials.

Intravesical bacillus Calmette-Guerin (BCG) immunotherapy

There is evidence in the literature that BCG after TURB is superior to TURB alone or in combination with chemotherapy for preventing recurrence of NMIBC. BCG maintenance therapy reduces the recurrence rate for 32% in comparison to MMC, but increases risk for 28% in patients who did not receive maintenance therapy with BCG.[1,18] The main disadvantage of BCG intravesical immunotherapy is in its side effects. According to data in the literature, serious side effects are encountered in less than 5% of treated patients. Side effects are a consequence of systemic absorption of BCG.[1,19] Caution is needed in immunocompromised patients, although some studies have not shown that immunocompromised patients are more prone to experience side effects.[1] For BCG instillation is used 6-week schedule introduced by Morales.[1,20] Many studies were conducted but none has shown advantages or disadvantages of this schedule compared to others.[1,21] Meta-analysis has shown that at least one year of maintenance BCG is required to obtain superiority of BCG over MMC for prevention of recurrence or progression. Regarding BCG dose, studies have shown that one-third dose is required to be effective for intermediate-risk tumors, full dose is needed for high-risk tumors.[1,22]

According to the EAU guidelines on NMIBC, BCG intravesical immunotherapy is recommended to patients with intermediate and high-risk tumors, three year maintenance therapy is more effective in patients with high-risk tumors to prevent recurrence.[1]

There are cases when BCG intravesical chemotherapy fails: (1) muscle invasive bladder cancer (MIBC) detected during follow-up, (2) BCG-refractory tumor - (a) if high-grade NMIBC is detected at three months, (b) CIS is present at three and six months and (c) high grade tumor is detected after BCG therapy. Therefore, radical cystectomy is indicated.[1]

Personalized treatment - precision medicine in the treatment of bladder cancer

The efficacy of cancer management is challenging and depends upon genomic, molecular and immunologic characteristics of cancer. New discoveries in these fields are making cancer treatment more targeted and efficient. Concerning bladder cancer, mutations in genes of DNA repair pathway (e.g. *ERCC2, FANCC, ATM, RB1* and etc.) can predict response to neoadjuvant platinum-based systemic chemotherapies. Therapies that influence the immune system such as immune checkpoint inhibitors (PDA, PDL1, CTLA4) are approved for use in treatment of bladder cancer and represent renaissance in medical oncological treatment of this disease.[23]

Cancer treatment based on the response of bladder cancer organoid could contribute to more personalized approach in the treatment of this disease.

Role of organoids in studying bladder cancer

Organoids

In order to understand the role of cancer-specific genetic alterations in tumorigenesis, maintenance of tumor and sensibility age-standardized response to different therapeutics, development of *in vitro* and *in vivo* model systems that accurately reflect genetic diversity and lineage specificity of cancer was required.[7,8] For this purpose, cell lines are used. Their disadvantages is that they are mainly long term 2D cultures, and there is a lack of clinical data regarding the organ of origin. To overcome these disadvantages *in vivo* models are used.[8] The advantage of i*n vivo* models is that they are able to recapitulate histological and therapeutic response but there are species-specific differences and inaccurate recapitulation of *in vivo* human tumour biology that are main disadvantage.[7,8] In combination with organoids derived from normal cells, tumour cell organoids can be used to study transformation from normal to malignant, when exposed to different carcinogens.[8]

Sato *et al*. in 2009 discovered that single leucine-rich repeat containing G-protein coupled receptor 5-positive intestinal stem cell is able to generate a continuously expanding, self-organizing, physiological epithelial structure that was similar to normal gut tissue. It was named organoid culture.[24] Liu *et al*. demonstrated that combination of ROCK inhibitor and feeder fibroblast culture conditions enables the infinite growth of multiple primary human epithelial cell types. Based on this, organoids from normal and tumour cells might be able to proliferate indefinitely *in vitro*, with no need to transduct exogenous viral or cellular genes

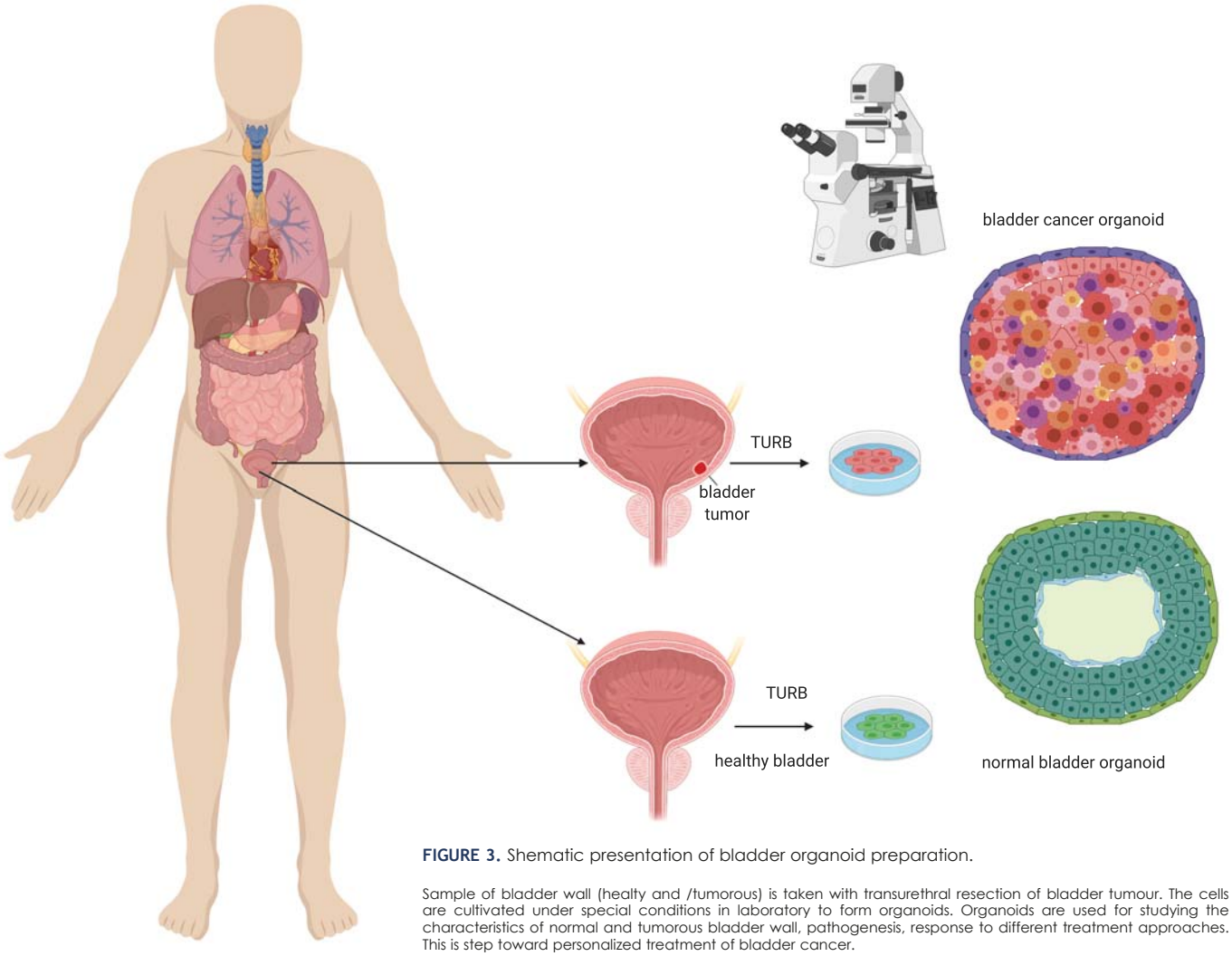

FIGURE 3. Shematic presentation of bladder organoid preparation.

Sample of bladder wall (healty and /tumorous) is taken with transurethral resection of bladder tumour. The cells are cultivated under special conditions in laboratory to form organoids. Organoids are used for studying the characteristics of normal and tumorous bladder wall, pathogenesis, response to different treatment approaches. This is step toward personalized treatment of bladder cancer.

(Figure 3).[8,25,26] Organoids are nowadays used for research of different cancers.[7,8] In the field of urology there are studies/reports on kidney, prostate and bladder organoids.[8,27,28]

3D organoids could be derived from cell lines, primary tissues, induced pluripotent stem cells, embryonic stem cells and embryonic whole organs such as organ explants that consist of many tissue types. Organoid is composed of multiple cell types and contains multicellular organ structures, which mimic the tissue of origin and functions in a similar manner. Organoids can be generated in different manners.[7,8]

Cell lines

In 3D culture conditions, some immortalized cell lines are able to form polarized 3D structures.[8] Smith *et al*. cultured human bladder cells that were used to investigate how terminally differentiated human urothelial cells interact with uropathogenic *E.coli*. They cultured 5637 cells under microgravity conditions within a rotating wall vessel bioreactor. Under these conditions, cells remain in suspension and form organoids that reflect characteristics of *in vivo* tissue-specific determination. Human bladder cells in this model had developed into a model that expresses specific markers and structures, characteristic of differentiated human urothelium.[8,29]

Lee *et al*. have shown that patient-derived bladder tumour organoids have most characteristics of parental tumour, although in a culture they may change their marker phenotype. Organoids could be used for studying the biology of the tumour and the effect of different therapies on tumour of individual patient.[7] In the future, these models could be used for personalized treatment of the patient according to tumour characteristics.[7]

Primary adult stem cells

Primary adult stem cells could also be used for organoids.[8] Matulay *et al*. collected bladder tumour specimens during cystoscopy, enzymatically digested them into single cells and cell clusters and then cultured them in organoid-prompting, embedded-cell culture conditions. They used these bladder organoids to analyse genetic mutations in bladder cancer.[8,30]

Pluripotent stem cells

Pluripotent stem cells have the ability to form all cellular components of an organ (epithelial, stromal and endothelial cells). Organoid could be generated from embryonic or induced pluripotent stem cells (iPSC). Using organoid 3D system, scientists are able to induce pluripotent stem cells to develop into organoids of the desired organ. Until 2014 there were two protocols to induce human embryonic stem cells or human iPSC into urothelium.[8] Osborn *et al*. have developed *in vitro* culture system that was matrix-free and cell-contact-free, and was able to induce human embryonic stem cells or human iPSC to differentiate into definitive endoderm and then urothelium using directed differentiation in urothelium specific medium.[8,31] Kang *et al*. have developed a protocol that used a chemically defined culture system to induce human iPSC to differentiate into endoderm and bladder urothelial cells.[8,32]

Application of organoids in diagnosis and management of bladder cancer

Organoids could be used to predict the response to treatment and guide the medicine regimen. For colorectal cancer, 3D organoid based drug sensitivity screen was developed. It identifies molecular signatures associated with altered drug responses. A similar strategy could be used with bladder cancer organoids.[33,34] Resistance to chemotherapy is promoted by cancer stem cells. Models with origin from cancer stem cells may be helpful for identifying effective prognostic biomarkers and for individual treatment.[8] In case of prostate cancer, there are already organoids being used to test different drugs, even further, there are models developed to screen for prostate cancer instead of cored biopsy, so-called liquid biopsy.[8,30,36]

Matulay *et al*. have established urothelial cancer organoids from patient-derived tissue samples. Using DNA sequencing analysis, they have shown that organoid lines have similar mutational profiles to those of tumour sample and can provide a platform for personalized drug-response assays in urothelial cancers.[8,30]

There are few studies aim of which was to identify bladder cancer stem cells, but the results are inconclusive.[8] In one study, researchers have described Sonic hedgehog (Shh) expressing and cytokeratin-5 (Ck5) expressing basal urothelial cells from ShhCreER/WT; R26mTmG/WT mice in organoid culture. Those were multipotent stem cells capable of self-renewal and regeneration into all cell types within urothelium in response to chemical injury or bacterial infection. Individual Shh-expressing cells formed cyst-like organoids after 5–7 weeks of 3D culture. CK5 was expressed in the outer layer. They also formed a luminal space in which CK5 and Shh were not expressed. Individual cells of these organoids were capable of self-renewal and differentiation, however, the origin and formation of bladder cancer stem cells remain unknown.[8,37]

Mullenders *et al*. collected samples of tumours of MIBC and NMIBC patients who underwent radical cystectomy and TURB, and normal macroscopically looking urothelium and established a sample of 50 human bladder organoids. Besides histological and functional investigations, they also used organoids for testing the efficacy of different intravesical chemotherapeutics. They applied different concentrations of anticancer drugs for time period of 5 days and found different responses in different organoids.[38]

Neal *et al*. purposed an *in vitro* model of different cancers for studying immunotherapy. One of them was also bladder cancer and responsiveness to immunotherapy with checkpoint inhibitors.[34]

Limitations of organoids

Organoids have a potentially important role in urological research, clinical decision-making, and treatment of urological cancers. Limitations of organoids are that the spatial orientation of tissues is random, also in many cases, the cellular components that are present in *in vivo* systems such as stromal, vascular endothelial and immune cells are missing.[8]

Conclusions

Bladder cancer presents public health problem because incidence and mortality are constant despite

the development of technology, molecular and cell biology, pharmacology and improvement of surgical technique. TURB is the golden standard for diagnosis, staging, and treatment of NMIBC. Both monopolar and bipolar current are equally effective, bipolar current having fewer complications. Development of molecular and cell biology leads to the construction of organoids which are a step towards personalized medicine. We expect that they will enable us to treat our patients based on the data acquired from organoids, regarding oncogenesis, responsiveness to different therapeutic modalities and possibilities for reconstruction.

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