Modeling and simulation
of a low-grade urinary bladder carcinoma

Svetlana Bunimovich-Mendrazitsky
Department of Computer Science and Mathematics, Ariel University, Ariel, 40700, Israel

Vladimir Pisarev
V.A.Negovsky Research Institute for General Reanimatology,
25 Petrovka, Bldg.2, Moscow, 107035, Russian Federation,
D. Rogachev Federal Clinical Research Center for Children Hematology, Oncology and Immunology,
1 Samory Mashela St., Moscow, 117198 Russian Federation,
University of Nebraska Medical Center, Omaha, NE 68198, USA

Eugene Kashdan*
School of Mathematical Sciences, University College Dublin, Belfield, Dublin 4, Ireland

Abstract
In this work, we present a mathematical model of the initiation and progression of a low-grade urinary bladder carcinoma. We simulate the crucial processes involved in tumor growth, such as oxygen diffusion, carcinogen penetration, and angiogenesis, within the framework of the urothelial cell dynamics. The cell dynamics are modeled using the discrete technique of Cellular Automata, while the continuous processes of carcinogen penetration and oxygen diffusion are described by nonlinear diffusion-absorption equations. As the availability of oxygen is necessary for tumor progression, processes of oxygen transport to the tumor growth site seem most important. Our model yields a theoretical insight into the main stages of development and growth of urinary bladder carcinoma with emphasis on two most common types: bladder polyps and carcinoma in situ. Analysis of histological structure of bladder tumor is important to avoid misdiagnosis and wrong treatment and we expect our model to be a valuable tool in the prediction of tumor grade and progression patterns, based on the exposure to carcinogens and an oxygen dependent expression of genes promoting tumor growth. Our numerical simulations have good qualitative agreement with in vivo results reported in the corresponding medical literature.

Keywords: Bladder cancer, cellular automata, nonlinear diffusion-absorption equation

*corresponding author
Email addresses: svetlanabu@ariel.ac.il (Svetlana Bunimovich-Mendrazitsky), vpisarev@gmail.com (Vladimir Pisarev), ekashdan@maths.ucd.ie (Eugene Kashdan)
1. Introduction

Bladder cancer (BC) represents an increasing health problem worldwide. It is estimated that around 400,000 new cases are diagnosed annually and 150,000 people die directly from BC each year. The highest incidence of BC occurs in the industrialized and developed countries in Europe, North America, and Northern Africa. According to the current statistics urinary bladder carcinoma is the fourth most common new cancer in men and ninth in women [1].

A number of risk factors have been strongly linked to the development of BC. Roughly 20% of all BC cases have been related to occupational exposure to chemicals, mainly in industrial areas processing paint, dye, metal, and petroleum products. Tobacco smoking is the main BC risk factor, accounting for at least 30% of BC cases. Epidemiological and experimental evidence has also implicated environmental carcinogens in the aetiology of BC. Exposure to arsenic in drinking water has been recognized as a cause of BC, for instance, in a study of long-term impact of arsenic pollution observed in Chile. BC mortality was significantly higher in affected regions more than 20 years after cessation of pollution [2].

Based on the evidence of BC origins and the biological properties of the urothelium, the first model of carcinogen penetration and BC initialization was proposed by Kashdan and Bunimovich-Mendrazitsky [3]. In this model, the authors employed a porous-medium-type equation to model carcinogen penetration and combined it with the Cellular Automata (CA) simulation of cell dynamics. The original model [3] assumed that the tissue was well oxygenated and the angiogenesis had not started. Thus it aimed to simulate the BC initiation and the first steps of the superficial polyp growth. It laid a basis of the high fidelity model presented in this manuscript that reproduces the state and behavior of multiple mechanisms involved in BC development. The new model introduces continuous oxygen and nutrient diffusion, and addresses hypoxia as one of the regulating factors in the cell cycle for both normal and cancerous cells. In the new model both carcinogen penetration under chronic exposure and oxygen diffusion depend on the absorption properties of the particular cells. A mechanism of angiogenesis has been embedded in the new model allowing us to simulate tumor growth beyond its initial stages.

Our research is based on the hypothesis that BC development is a multiscale multi-level process. The “building blocks” of our model are: (i) carcinogen penetration into the urothelium and (ii) oxygen diffusion, influenced by (iii) angiogenesis. These processes are embedded into the CA model of urothelial cells dynamics. Our approach to BC modeling corresponds with the multiscale studies of brain gliomas [4] and colorectal cancer [5] that eventually led to novel therapeutic strategies to treat these diseases.

The first goal of our new mathematical model is to analyze various tumor development scenarios leading to the development of the neovascular network (a process known as angiogenesis). The second goal is to provide a basis for BC therapy personalization through the simulation of BC progression under various conditions. In the numerical experiments presented in this manuscript, we discuss a number of scenarios involving low-grade urothelial carcinomas such as superficial bladder polyp and carcinoma in situ (CIS). However, our model is not limited to these cases and it could simulate the invasive form of BC in conjunction with the model of tumor invasion.
The mathematical model of invasive BC is based on the interaction (and the competition) between the matrix metalloproteinases (MMP), produced by the cancerous cells, and the tissue inhibitors of metalloproteinases (TIMP), secreted by the tissue to confront the tumor progression, was introduced and studied in [6].

The manuscript is organized as follows: In Section 2, we present general information about the BC and the biological background of our model. After which we provide the reader with the model framework, divided into three consecutive sections:

(a) The CA model of the urothelial cell dynamics (Section 3);
(b) Continuous processes, which correspond to oxygen diffusion and carcinogen penetration (Section 4); and
(c) Modeling angiogenesis (Section 5).

Section 6 is dedicated to numerical simulations and a discussion of a number of scenarios leading to tumor development and progression. We conclude our manuscript in Section 7.

2. Biological background

2.1. Normal urothelium

A human bladder consists of the following layers: bladder lumen, urothelium, lamina propria, muscle and fat [7, 8] as sketched in Fig. 1.

The urothelium is a highly specialized layer of epithelial cells lining the bladder. It has to maintain a tight barrier against urea and other toxins, while accommodating large changes in bladder volume. Under normal conditions the urothelium has a very low cell turnover rate, however, when the urothelium is damaged it has to be able to repair itself rapidly [7, 9]. The balance between the cell proliferation and differentiation controls the cell replacement process.

The urothelium functions as a highly efficient barrier to the movement of water and ionized substances across the bladder wall. The epithelial cells of the urothelium form part of an integrated network, which plays a central role in bladder wall sensation, local blood flow modulation, pathogen removal and active barrier provision.

The urothelium is composed of three to six layers of cells including basal cells, intermediate cells and superficial (umbrella) cells (Fig. 2). Basal cells are germinal in
nature and approximately 5 \( \mu m \) to 10 \( \mu m \) in diameter. The basal cells are the only cells in a normal urothelium that could proliferate and move between layers after differentiation (if needed). The basal cells replace dead intermediate and umbrella cells by accepting the phenotype corresponding to each layer [7]. The cell replacement process is discussed in more detail in the Section 3. The layer containing basal cells includes rare normal stem cells, which have a potential to initiate tumor growth [10, 11]. It has been well-established that tumor-initiating cells (stem cells, or stem-like cells), originating from normal stem cells or from cells, which have transformed back from differentiated cells due to mutations, are responsible for initiation, maintenance and metastasis of numerous tumors including urothelial cancer (see review in [11]). Accumulation of carcinogenic mutations in normal stem cells due to continuous action of environmental carcinogenic compounds is crucial to initiate and drive the carcinogenesis due to the altered expression of genes, affecting key signaling pathways of the cell. Most recent studies have demonstrated that abnormal overexpression, even of one protein in stem cells, within the basal urothelium in mice might generate an invasive lesion resembling all patterns of human bladder carcinoma in situ [12].

Intermediate cells are located above the basal cells and are approximately 20 \( \mu m \) in diameter. A layer of umbrella cells forms the luminal surface of the urothelium. These cells are the largest epithelial cells in the body, measuring from 100 \( \mu m \) to 200 \( \mu m \) in diameter. The bladder urothelium shrinks (20–50%) during the urination process. When the bladder fills the number of cell layers reduces as the cells flatten to accommodate the stretching of the bladder wall [13].

As with any epithelial tissue, the normal (healthy) urothelium obtains oxygen and nutrients through diffusion from the capillary network located in the lamina propria and is separated from the urothelium by the basement membrane [7].

2.2. BC classification and grading

The most recent 2004 WHO classification distinguishes four grades of urothelial tumors in accordance with their malignancy (from low to high) [7]:

1. Urothelial papilloma.
2. Urothelial neoplasm of low malignant potential.
3. Papillary urothelial carcinoma, low-grade.
4. Papillary urothelial carcinoma, high-grade.

In this work, we model the low-grade papillary urothelial carcinoma, however, our approach is not limited to this type of BC and the model could be generalized to the simulation of high-grade carcinoma as well.

There are four morphological patterns of bladder tumors (Fig. 3):

- Our particular interest is with the non-invasive papillary carcinoma (polyp) and the flat non-invasive carcinoma (carcinoma in situ – CIS). The non-invasive polyp and CIS carcinomas shown in Fig. 3 are responsible for approximately 75% of diagnosed BC cases [14]. As sketched in Fig. 3(a), the noninvasive papillary carcinoma (polyp) passes through the bladder wall into the bladder lumen but goes no deeper than the lamina propria. On the other hand, CIS (Fig. 3(c)) remains within the urothelium. If not treated properly, both polyp and CIS could transform into the far more dangerous invasive forms. In Fig. 3, this corresponds to the (a) → (b) and (c) → (d) transformations. It is estimated that between 15% and 30% of low-grade carcinomas (Figs. 3(a) and 3(c)) will progress to the high-grade (Figs. 3(b) and 3(d)) correspondingly [14]. Statistically, CIS has more chance to progress to high-grade (invasive) urinary bladder carcinoma than the bladder polyp [15].

2.3. Tumor dynamics and carcinogenesis pathways

The main factor responsible for BC initiation is an accumulation of genetic alterations (DNA mutations) in stem-like cells within the bladder urothelium, capable of triggering aberrant signaling through key molecular networks involved in proliferation, differentiation and survival. Similar to other malignancies, it is likely that bladder carcinogenesis involves aberrations in cell differentiation and proliferation, often with the derangement in the genetic composition of malignant cells. Conversion of normal urothelial cells to cancer cells and progression from the low-grade to the high-grade muscle-invasive cancer are the result of the sequential acquisition of somatic gene mutations [16]. We refer the reader to Table 1 in [18] for the list of the gene alterations...
common in bladder cancer and to the scheme of molecular and genetic pathways for bladder tumor classification in the reference [19].

Different carcinogens act on cells in different ways [20, 21]. However, they all have certain aspects in common:

1. First, carcinogens all have the ability to damage the genetic composition of the cell, either directly through DNA modifications, or through inducing gross chromosomal abnormalities.

2. Second, the initial action of the carcinogen does not immediately cause the cell to lose its growth controls (contact inhibition and organized growth patterns with specific cell-cell adhesion). Such a pre-cancerous cell must undergo at least one cell division in order to express the cancerous behavior condition. This DNA and chromosome replication thus “sets up” or initiates the carcinogenic behavior.

In case of BC, there are two pathways of carcinogen activation [22]. For several compounds with carcinogenic or co-carcinogenic activities their accumulation could be critical for dose-dependent, sequential accumulation of mutations in tumor-initiating cells. Metal ions, proved to be accumulated in human tissues and to induce tumors in vitro or in vivo, are prime candidates for such effects [23]. Prolonged exposure to heavy metals including arsenic and cadmium were found to enhance mutagenicity and carcinogenicity, or, even directly to induce malignant transformation of non-malignant immortalized cells [23, 24, 25]. Evidence is growing that prolonged exposure to heavy metals of gene expression profiles of epithelial cells is consistent with the malignant phenotype of cells [26, 27, 28]. The metal traces quantification in bladder biopsies from tumoral, non-cancerous areas, adjacent to tumors, and normal tissues, revealed significantly elevated levels of two carcinogens, arsenic and cadmium, in adjacent to tumor areas [29]. The accumulation of metals and possibly other carcinogenic compounds in the microenvironment might increase the likelihood of genetic instability and multiple mutations of tumor-initiating cells located within the field of cancerization.

Another pathway is exposure to chemical carcinogens, for instance, aromatic amines, associated with smoking [22]. These carcinogens are excreted in the urine and stored temporarily in the bladder, where they have an extended opportunity to interact with the urothelium. The gradual accumulation of chromosomal damage in the urothelial cells eventually results in phenotypic expression and transformation into overt neoplasm. These carcinogens form highly reactive electrophilic species that bind nucleic acid and induce structural mutations within bladder epithelial cells. The observed changes (which may vary in different cells) include allelic deletions, loss of heterozygosity, inactivation of tumor suppressor genes or activation of proto-oncogenes.

One should note that increased carcinogen concentration could yield non-cancerous gene alterations as well. However, in this manuscript we consider the mutation sequence that leads to the malignancy only. In fact, invasive and non-invasive forms of BC correspond to the different sequences of the DNA mutations [17, 19]. According to [17] it takes between three to five mutations for a cell to obtain a cancerous phenotype that corresponds to the superficial polyp or carcinoma in situ. This result agrees with [18, 19], where four mutations are considered necessary to initiate development of bladder cancer.
As highlighted above, BC is a complex phenomenon, which involves the number of physical and biological processes. The main features responsible for BC development and progression are:

(i) The alteration of tumor-initiating cell DNA due to the carcinogen penetration from the bladder; and

(ii) The angiogenesis – a process leading to the development of a neovascular system to provide growing tumor with additional oxygen and nutrients necessary for their continuous proliferation [16].

Both these processes crucial for tumor development and growth are modeled in our work. In our computational experiments, we investigate the possible role of insufficient oxygen supply (hypoxia inducible factor – HIF) in the progression of the low-grade urinary bladder carcinoma, and examine its expression in relation to the cell turnover and the proliferation status.

3. Computational model of urothelial cell dynamics

We have chosen the Cellular Automata (CA) formulation for representation of cell dynamics in the urothelium. CA allows us to model intuitively the critical parameters governing tissue regulatory mechanisms: cell proliferation, apoptosis (cell death), and dynamics of both normal and cancer cells [30]. All these mechanisms include a number of sub-processes (i.e. maturation) that have also been taken into account. The basis of our model is a slice of the bladder tissue as shown in Fig. 4 (a). Bladders size estimations vary in the literature. The anatomy and physiology textbook [8] reports average volume of bladder as 600mL. The approximate size of the urothelium cross-section that we are looking on is 330mm × 0.2mm. In the numerical simulations we consider a 4mm × 0.2mm fragment of the urothelium cross-section.
The CA model consists of a two-dimensional array of automaton elements, which will be eventually identified with the real urothelial cells, shown in Fig. 4 (b). The cell characteristics change along the vertical axis representing the layers of the urothelium. In our simulations, we consider the following morphological structure:

- The lumen layer is located in the top 9 rows. The bladder polyp cells grow there vertically upwards by breaking through the bladder wall.
- The urothelium layer comprises the next ten rows of the lattice (i.e., rows 10–19).
- The lamina propria, where blood vessels reside, occupies two rows (20–21).
- The rows 22–30 represent fat and muscle layers to simulate the invasive type of the BC.

The state vector represents cell dynamics of each CA cell. In the model implemented in this work, the state vector has six components: (i) the site of the cells: lumen, umbrella and intermediate layers (that we address as ordinary urothelial cells), basal layer, lamina propria (where blood vessels reside), fat and muscle layer; (ii) occupation status, i.e. whether the element is occupied by the normal cell, the mutated cell, by the “empty space” or by the blood vessel; (iii) the cell status, i.e. whether the basal cell is in the proliferative or in the interphase state; (iv) the carcinogen concentration; (v) cancerous mutation counter of the cell; and (vi) oxygen and nutrients level.

The state vector evolves in time according to the prescribed local rules, used to update any given element from its own state and that of its neighbors on the previous time step. The structure called “urothelium state” characterizes every cell and it is filled during the numerical simulation. The CA model presented in this manuscript is the enhanced and expanded version of the CA approach proposed in [3, 6]. The general flowchart of the algorithm is shown in Fig. 5.

Our model represents differences in the behavior of normal (basal and ordinary) and cancer cells as follows:

- **Normal basal cells containing tumor-initiating cells:** The stem cells of the urothelium are located within the basal layer. The hypothesis that the basal cells are routinely lost and replaced had been experimentally confirmed. Stem cells behave as an equipotent population in which the balance between proliferation and differentiation is achieved through frequent stem cell loss and replacement. The cell division depends on the dynamics of clonal population and anatomic constraints [32, 33].

  Cell proliferation produces two cells from one, and it requires cell growth followed by cell division. Uncontrolled cell proliferation is a hallmark of cancer. Multiple mutations that accumulate in somatic cells over many years eventually remove an elaborate set of controls that would otherwise prevent cancer cells from dividing unchecked. In normal tissues, cell proliferation is generally restricted to cells that replenish the tissue. Stem cells (including tumor-initiating cells) are self-renewing cells that can divide asymmetrically to yield a new stem cell and a progenitor cell [10]. Progenitor cells may or may not undergo further
divisions, ultimately leading to terminal differentiation. Once cells have terminally differentiated, they have a specialized function and are no longer dividing. Most tissues are made up of such non-dividing cells. Thus proliferation is normally tightly controlled so that only particular cells in the body are dividing [34]. The average length of the cell cycle is 1 day, which corresponds to one iteration in the CA algorithm.

The following CA rules (see also flowchart in the Fig. 6) are applied to each basal cell as the algorithm progresses from the time-step \( i \) to the time-step \( i + 1 \):

1. Cell death in urothelium (either necrosis or apoptosis) initiates the signal for proliferation and provides nutritional support that reaches the basal level containing stem cells. The experiments confirm that the injured or dying cells might produce or liberate molecules signaling to the rare subsets of stem or progenitor cells from the basal layer [35]. Provided that the signal for empty place exists, and the cell is ready to proliferate, we turn the proliferation flag on for the next iteration. A cell must replicate DNA (and its various organelles) before it can divide, and there are many ways that a cell can monitor its nutritional status and oxygen concentration; if nutrients and oxygen are insufficient, it typically will not divide [34].

2. If more than one cell is ready for proliferation then the closest cell to the empty space will be chosen; if more than one cell could fill the empty space then the choice is made by “throwing the dice”. The process is detailed as follows:
   - If the oxygen level at the element occupied by the cell is above the threshold \( L_1 \), then we sample the region around the element in order to determine whether the cell division occurs or not;
   - If there is empty space, then the cell divides, and the new cell occupies the empty space;
   - If there is no empty space, the cell fails to divide, and will try to do it on the next iteration.

3. If the cell is in the proliferative state, it will divide to form two daughter cells, of which one will replace (renew) it, and another will move into the “empty” site.

4. Every basal cell accumulates carcinogen; if carcinogen concentration passes the threshold \( C_1 \) we add one to the cancerous mutation counter; as soon as the BC mutation counter is equal to four the cell obtains cancer phenotype and behaves according to the cancer cell mode described below. Carcinogens yield various DNA mutations, many of which are not cancerous. However, the mutation process is not entirely random. In [21], the authors used the DNA sequence and copy number information to trace the mutational evolution of individual tumors and to reconstruct the order of abnormalities as individual tumors evolve for two separate cancer types. Our assumption is that the concentration of carcinogen above the threshold leads to the first of the mutations in the BC sequence and consequently to the rest of mutations down the road. As confirmed by the experiments,
the time-line of the mutations accumulation process could vary from few
days as result of a massive urothelial cell loss due to the bladder injury or
inflammation, to a number of years in case of the regular turnover of the
urothelial cells.

5. The daughter cells inherit the mutation counter and the carcinogen concen-
tration from their mother cell. There is no information, regarding propor-
tion of carcinogen inheritance between the daughter cells. However, as a
result of the chronic exposure to the carcinogens coming from outside this
value is not important. Due to the diffusive properties of the continuous
model of carcinogen penetration discussed in Subsection 4.2, the carcino-
gen concentration in every single cell is similar to the concentration in its
neighbors and the outliers are fixed within a few very short time-steps of
the continuous model.

In fact, the carcinogen penetration increases the probability of maligniza-
tion, but does not force it. The major reason behind BC is the accumulation
of genetic damage under effect of carcinogens that lead to the mutations. In
some cases, the DNA could be repaired and the cells could either survive or
die in this process. However, when the mutations necessary for carcinogen-
esis build up, and there exists a micro-environment (e.g., an inflammation
leading to the massive cell death), the tumor growth starts.

6. The basal cell has two oxygen concentration thresholds: $L_1$ (enough for
proliferation) and $L_2$ (sufficient for life, but insufficient for proliferation);
the oxygen level below $L_2$ leads to the cell death from the hypoxia.

7. In order to differentiate, the basal cell should satisfy the following condi-
tions:
   (a) The cell should be in the proliferative state;
   (b) The cell should have sufficient oxygen concentration (above $L_1$);
   (c) The cell proliferation flag is turned on (as result of the appropriate
       signal from the ordinary cell as described below).

• Ordinary (umbrella and intermediate) cells:

During physiological cell turnover, older differentiated cells are typically elim-
inated by apoptosis and replaced by the division progeny of stem cells. In fact,
the urothelium has one of the lowest turnover rates among mammalian epithelia,
estimated as once a year [36]. We set the threshold of the oxygen concentration
for the ordinary cell equal to $L_2$ (same as for the basal cell). The CA rules are
applied to the ordinary cells as follows:

1. The dying cell sends a signal to the basal (stem) cells that the empty space
   has emerged and could be filled;

2. The ordinary cell could die in three cases:
   (a) *apoptosis* – natural death after 12 months [36];
   (b) *apoptosis under hypoxia* – death due to the lack of oxygen (the oxygen
       level is below $L_2$) [37];
   (c) *necrosis* – when the carcinogen concentration is above the threshold
       $C_1$ [38].
• **Cancer cells:**

At each time step, the CA algorithm checks the carcinogen concentration for every cell. If the concentration passes the threshold level the first mutation is registered in accordance with [39]. Different carcinogens have different mutation threshold values. Therefore simultaneous exposure to multiple carcinogens could significantly accelerate mutations rate. The DNA mutations caused by carcinogens may occur in any cell of the urothelium. However, in ordinary cells they yield the cell death. Only stem cells located in the basal layer can transfer their genetic alterations to the daughter cells, which in their turn might eventually become cancerous following the number of cell divisions [38]. Thus we examine mutations on the basal layer only.

The cancer cell behaves according to the following set of rules:

1. The cancer cells can differentiate anywhere in urothelium and they compete with the normal cells for empty space;
2. A tumor cell could survive with concentration of carcinogen above $C_1$;
3. Cancer cells have their own oxygen thresholds: if the oxygen level in the cancer cell is less than $L_3$ it produces a signal, which is sent to the vascular system in order to start sprouting of blood cells towards the area with the lack of oxygen. This process is known as an angiogenesis – see Section 5. In this case, the cancer cell cannot proliferate;
4. If the oxygen concentration is below $L_4$ the cancer cell enters the hypoxia-induced dormancy state for one year and only after that, if the oxygen supply does not increase above the threshold, the cancer cell dies.

This rule corresponds to a well-known, but not exclusive, feature of cancer cells – their ability to enter a quiescent state under hypoxic conditions, in which they suspend all activity, including any cell division that is not essential for survival [40]. The duration of the hypoxia-induced dormancy state varies between approximately 2 months and 1 year depending on the cancer. There is also difference between in vivo and in vitro experiments [41]. However, we have found numerically that varying this parameter between 9 months and 1 year (an estimated range for tumor-initiating epithelial cells) has very little impact on tumor progression due to renewed oxygen supply via the angiogenic network of blood vessels.

To summarize behavior of each type of cells we include the flowcharts describing the corresponding CA rules.

**3.1. Interaction between the models**

At each time step the CA model interacts with the continuous models of carcinogen penetration and oxygen diffusion by supplying them with the state vector information and obtaining the levels of carcinogen and oxygen concentration correspondingly as shown in Fig. 8.

Before we move to the next section describing continuous processes we want to point out that the CA state vector does not address the cell size. On the other hand,
Figure 5: The CA rules corresponding to the state vector evolution. \textit{Car} and \textit{MutN} represent the carcinogen concentration level and the mutation counter respectively; \textit{C1} is the carcinogen threshold from Table 1.

Figure 6: The CA rules corresponding to the basal cell proliferation rules. \textit{O2} is the oxygen concentration. \textit{L1} and \textit{L2} correspond to the oxygen thresholds for normal cells, given in Table 1. \textit{L1} is the proliferation threshold and \textit{L2} is the apoptosis under hypoxia threshold.
Figure 7: The CA rules corresponding to the cancer cell proliferation rules. $O_2$ is the oxygen concentration level. $L_3$ and $L_4$ correspond to the oxygen thresholds for cancer cells, given in Table 1. $L_3$ is the proliferation threshold and $L_4$ is the dormancy state threshold.

Figure 8: Combination of the discrete CA model of urothelial cell dynamics with the continuous models of carcinogen penetration and oxygen diffusion. $\Delta t$ is a period of time between updates of the cell states in accordance with the CA rules. $i$ is the number of iteration (update). The data transferred between the models is detailed in Section 6 dedicated to numerical experiments.
the simulation of continuous processes runs on a separate rectangular grid, where the actual urothelial cell could correspond to one or more grid cells.

4. Continuous processes

4.1. Oxygen diffusion

One of the major features of the normal urothelium (and any other transitional epithelium) is the absence of blood vessels. The blood vessels are located in the lamina propria, underneath the epithelial tissue. The lamina propria is separated from the epithelial tissue by the basement membrane. The oxygen and nutrients reach the urothelial cells through passive diffusion.

We describe the process of oxygen diffusion using the nonlinear diffusion-reaction equation, based on the porous-medium equation with the absorption term:

\[
\begin{align*}
\partial_t u - \nabla \left[ D(\vec{x}, t) \nabla \left( |u|^{m-1} u \right) \right] + h(t) |u|^{q-1} u &= f(\vec{x}, t) \quad \text{in} \quad Q_T^N, \\
u(\vec{x}, 0) &= k \delta_0.
\end{align*}
\]

We call \( u(\vec{x}, t) \) the concentration of oxygen measured in \([\text{amount of oxygen per cell volume}]=[\text{mol/L}]\). The diffusion coefficient \( m D(\vec{x}, t) |u|^{m-1} > 0 \) has dimensions of \([\text{cell area on lattice per unit of time}]=[\text{cm}^2/\text{s}]\) and it is proportional to the degree \( m - 1 \) of \( u \), where \( m > 1 \). When \( m = 1 \), we have linear diffusion, which has infinitely fast speed (impossible from the biological point of view). While \( D \) is usually known or could be measured, the value of \( m \) corresponds to the speed of diffusion and it is adjusted using numerical experiments. Cells can absorb water, oxygen or even salt through passive transport. The plasma membrane allows relatively small molecules to enter and exit the cell, but does not allow larger molecules to pass. To simulate this process we add non-differentiated absorption term \( q h(t) |u|^{q-1} > 0 \), the units of which are \([\text{s}^{-1}]\). The rate of absorption is proportional to the degree \( q - 1 \) of concentration \( (q > 1) \), which is also adjusted in numerical experiments. Initially oxygen concentration is random within the urothelium and is maximal in the blood vessels within lamina propria. This situation is represented by the initial condition and the source term \( f(x, t) \). The changes in oxygen diffusion that correspond to angiogenesis are discussed in Section 5.

We assume that both \( D \) and \( h \) are piecewise continuous, non-negative functions, representing the diffusion and the absorption coefficients correspondingly; \( m \) and \( q \) are positive real numbers representing the speed of diffusion and absorption in their turn; \( k \) is a non-negative constant matrix of the same size as the lattice and \( \delta_0 \) is the Dirac delta function that represents the source at time \( t = 0 \). The evolution of source in time and in space is given by \( f(\vec{x}, t) \). Our domain of interest \( Q_T^N \) is a two-dimensional space-time continuum \((N = 2)\). Equation (1) has a non-negative solution associated with the physics of the model. Another important feature of this equation is the finite speed of diffusion, which cannot be simulated in the linear model [42].

Equation (1) was studied theoretically in [43]. The authors first considered the case when \( D \equiv 1, h \equiv 0 \) and \( m > (N-2)/2 \) and defined a corresponding solution \( B_k(x, t) \)
for any \( k > 0 \) (here \( k \) corresponds to the initial condition of (1)):

\[
B_k(x, t) = t^{-l} \left( C_k - \frac{(m - 1)l|x|^2}{2mNt^{2l/N}} \right)^{1/(m-1)}
\]  

(2)

where

\[
l = \frac{N}{N(m-1)+2}, \quad C_k = a(m, N)k^{2(m-1)/N},
\]

and \( a(m, N) \) is a free parameter. Note that \( B_k(x, t) \) is also known as “Barenblatt solution” [42].

Since \( B_k \) is a supersolution for problem (1), a sufficient condition for existence (and uniqueness) of \( u_k \) is

\[
\iint_{Q^T} B_k(x, t)h(t)dxdt < \infty.
\]

(3)

By change of variable \( y = t^{1/N}x \), this condition becomes independent of \( k > 0 \). The authors in [43] have proved that if

\[
\int_0^1 h(t)t^{-1/q}dt < \infty,
\]

(4)

when \( m > 1, q > 0 \), then the problem (1) admits a unique positive solution \( u = u_k \).

The oxygen diffusion model allows us to investigate the possible role of HIF in the recurrence and the progression of low-grade urinary bladder carcinoma, and to examine its expression in relation to proliferation status, cell death and angiogenesis [45].

4.2. Carcinogen penetration

The carcinogens mixed with the solute, in the form of biofluid, penetrate through the bladder wall into the deep layers of urothelium. If the carcinogen concentration inside the basal cells passes certain threshold, it triggers the chain of mutations, leading potentially to the cells with the cancerous phenotype. Here we summarize the number of assumptions we made to model the penetration process in accordance with [46]:

1. The carcinogen is accumulating on the bladder wall;
2. The carcinogen penetrates slowly through the layers of urothelium and its concentration reaches mutation threshold level in the years-long span;
3. Cell loss (both apoptosis and necrosis) leads to the “empty space” in the lattice with no carcinogen [44].

In this work we generalize the model of carcinogen penetration suggested in [3]. Carcinogen penetration is modeled within the same mathematical framework as oxygen diffusion (equation (1)) and defined on the same physical domain – two-dimensional lattice \( Q^T_N \), which corresponds to the one-cell-thick slice of the urothelium. However, diffusion and absorption coefficients for carcinogen penetration are different and correspond to the values obtained from the toxicology studies as detailed in Table 2. The
carcinogen concentration is measured as \([\text{amount of solute per cell volume}] = \text{mol/L}\).

The speed of carcinogen penetration is much slower than the speed of oxygen diffusion, as reflected in the corresponding coefficients in Table 2. It has been proven experimentally (see, for instance, [47]) that majority of carcinogens are absorbed through simple passive diffusion. The degrees of proportionality \(m - 1\) and \(q - 1\) of diffusion and absorption coefficients are adjusted experimentally as well. The concentration of carcinogens on the bladder wall is random throughout the simulation and it is updated at the beginning of each CA time-step.

5. Angiogenesis

The tumor cannot grow beyond the certain size (usually, 1–2 \(mm^3\)) by exploiting resources of the urothelium [48]. Its further growth requires additional oxygen and nutrients provided by the neovascular network developing towards the tumor (a process known as angiogenesis). These new blood vessels have a very chaotic structure and have a higher permeability compared to the regular vascular system. The new blood vessels are more leaky and carry with them less oxygen and nutrients than the regular blood vessels. However, they provide surrounding normal and cancer cells with easier access to oxygen and nutrients than the blood vessels lying in the lamina propria. Angiogenesis is playing a very important role in both low-grade and high-grade BC [45, 49].

Responding to the insufficient oxygen supply the cancer cells overexpress and activate HIF (specifically, HIF-1\(\alpha\)). The latter activates expression of vascular endothelial growth factor (VEGF), the secreted protein capable of signaling towards the lamina propria. VEGF is one of the key angiogenic factors that stimulates formation of the new blood vessels and tumor growth [50]. Elevated levels of VEGF expression were detected in the urine samples from the BC patients and correlated with the disease recurrence and progression [49]. In agreement with this study, a high level of the VEGF expression that was present in tumors and in serum samples from patients with BC, also predicted a poorer prognosis and an increased frequency of disease recurrence [45]. Interestingly, simultaneously to promoting angiogenesis via VEGF, HIF-1\(\alpha\) promotes invasion of malignant cells through tissues via other mechanisms. Therefore, the increased angiogenesis might associate with both increased rate of tumor growth and promotion of invasion processes, two main markers of a disease progression.

Our approach to the simulation of angiogenesis could be described as follows:

1. The mutated cell has obtained cancer phenotype, but it does not have sufficient amount of nutrients and oxygen necessary for proliferation (oxygen level below \(L3\)).
2. The cell sends the VEGF signal to the vascular network located in the lamina propria.
3. As soon as the VEGF signal reaches the closest cell in lamina propria; the blood vessels start to branch away from this cell and work as a source of oxygen and nutrients for all surrounding cells with increased diffusion coefficient due to its high permeability [48].
4. On each CA time step, the blood vessels grow towards the cancer cells by capturing empty space or replacing (killing) basal and ordinary cells located on their path. The tip cells of the angiogenic sprouts secrete enzymes such as matrix metalloproteinases (i.e., MMP-9) that lead to the degradation of the extra-cellular matrix (ECM) and eventual death of the cells lying on the pass between the newly formed blood vessels and the developing tumor [51, 52]. One should also notice that MMPs help degrade the proteins that keep the vessel walls solid [53].

5. Other cancer cells experiencing lack of oxygen send the VEGF signal that could reach either lamina propria or newly formed vessels and start new sprouts, effectively building the angiogenesis tree.

The structure of the neovascular network depends on the type of BC [54]. The new vessels are confined within the polyp when it grows into the bladder lumen and they eventually kill the tumor cells.

As a result of the angiogenic activity the urothelium looses its elasticity resulting in blood leaking into the bladder that is usually detected through the discovery of blood traces in the urine sample.

6. Numerical simulations

6.1. Settings

In order to simulate BC growth on computer we make the number of assumptions in addition to ones discussed in the previous sections:

- We simulate general characteristics of carcinogen penetration and tumor development process on the one-cell-thick layer of the urothelium, which is, in fact, a two-dimensional lattice, however, we ignore the geometry of the cell.

- We assume that an ordered sequence of four specific mutations is sufficient for basal cell to obtain a BC phenotype [17, 18, 19].

- In our experiments, angiogenesis is regulated by HIF [49]. We simulate the effect of hypoxia on ordinary, basal and cancer cells by setting the corresponding oxygen level thresholds (see Table 1).

The threshold values in Table 1 are given as references. They had been estimated for the US Environmental Protection Agency (USEPA) using the methodology detailed in [55].

Table 1 is used to connect between the CA and the models of continuous processes as shown in Fig. 8. On each CA iteration, the actual values of oxygen level and carcinogen concentration are checked against the threshold values for each cell. The changes in the cell state vector (e.g., cell status and mutation counter) are introduced then.

In the simulation of continuous processes, we consider an \(xy\)-plane, such that the horizontal \(x\)-axis goes along the layers and the vertical \(y\)-axis goes down through the layers of urothelium. We assume that the carcinogens penetrate from top to bottom.
Name of the threshold | Value, [mol/L] | References
--- | --- | ---
Oxygen concentration level $L1$ *(above: sufficient for basal cell proliferation)* | $4.5 \times 10^{-4}$ | [56]

$L2$ *(above: sufficient for life for both ordinary and basal cells, but insufficient for basal cell proliferation; below: ordinary and basal cell apoptosis under hypoxia)* | $1.5 \times 10^{-4}$ | Estimated as $1/3$ of $L1$ following [34]

$L3$ *(above: sufficient for cancer cell proliferation)* | $4.5 \times 10^{-5}$ | [30]

$L4$ *(above: sufficient for cancer cell life, but insufficient for its proliferation; below: cancer cell enters the sleeping state for 1 year and dies after that.)* | $1.5 \times 10^{-5}$ | [30]

Carcinogen concentration level $C1$ *(mutation threshold in the basal cells and necrosis threshold in the ordinary cells)* | $1.5 \times 10^{-4}$ | [57]

Table 1: The threshold values used in the CA model.

(with exception of bladder polyps); the oxygen diffusion starts from bottom, but could eventually start and go in any direction following development of the neovascular network due to the angiogenesis. In the BC scenarios discussed in this work, the tumor grows towards the bladder lumen and yields a polyp or remains within the urothelium forming a CIS.

The diffusion coefficients for both carcinogen penetration and oxygen diffusion models (summarized in the Table 2) are piecewise constant, and their values depend on the cell type. Newly formed blood vessels have very high permeability [48], as result they do not carry as much oxygen and nutrients as regular vessels. Using the available data (see also [58]) we estimate their oxygen concentration is half that of the oxygen concentration in regular blood vessels.

The absorption (uptake) coefficients are equal to $0.1s^{-1}$ for oxygen diffusion model [59] and to $1s^{-1}$ for carcinogen penetration model [60].

There is no clear rule on choosing parameters $m$ and $q$ for each of the models. So we consider $m = q = 2$ for carcinogen penetration, which brings it in line with the classical Porous Medium Equation. Oxygen diffusion is modeled with $m = 2$ and $q = 3$. In practice, these two processes have a different time scale: carcinogen penetration is a very slow process, while oxygen diffusion is supposed to be much faster [61].

In our numerical experiments, we consider a small part of the urothelium – a rectangular slice that corresponds to $\sim 2000$ cells (roughly 2% of the urothelium cross-section). In order to avoid numerical artifacts leading to increased carcinogen concentration and fast tumor growth near the boundaries we add so called *buffer layers* from both sides of the computational domain.
In normal urothelium, the carcinogens are almost not penetrating below the lamina propria. The effect of carcinogen penetration into the fat and muscle layers and further deep into the surrounding tissues is related to the MMPs secreted by the cancer cells following a specific chain of mutations modeled in [6].

We consider the oxygen concentration within the regular blood vessels to be equal to $5.175 \times 10^{-3}\text{mol/L}$ at the beginning of each CA time-step [62]. The carcinogen on the bladder wall is uniformly distributed and its maximum is estimated as $3 \times 10^{-6}\text{mol/L}$, which corresponds to the average cotinine concentration in the urine of the active smokers [63]. Cotinine, a nicotine metabolite, is a biomarker of tobacco, nicotine, and carcinogen exposure.

The equation (1) is solved numerically by the 4th order finite difference algorithm for integration in space and the 4th order Runge-Kutta scheme for advancing in time [64]. The algorithm is able to handle a situation when the carcinogen concentration within the solute changes rapidly and sharply. We also did a perturbation analysis to confirm that small changes in parameter values do not lead to significant changes in the outcome of our numerical simulations. The temporal resolution of continuous models is chosen to ensure the stability of numerical solution. In physical terms it is equal to $\sim 4\text{ min.}$ (duration of one CA iteration is 24 hours). A very small artificial viscosity is added through the fourth order spatial derivative (with coefficient of order $10^{-9}$).

The CA simulation of cell dynamics and models of carcinogen penetration and oxygen diffusion could have the same (one cell) spatial resolution. However, in general, a single cell of urothelium (a basic unit of the CA model) could be represented by the number of nodes on the grid used for carcinogen penetration or/and oxygen diffusion.
modeling. In such a case, the levels of carcinogen concentration and oxygen diffusion transferred to the CA model are averaged inside the corresponding urothelial cell.

The time span of both continuous models is corresponding to the single time-step of the CA, which is equal to one day in our experiments. As shown in Fig. 8, the continuous models update the cell state vector in CA model and obtain the initial conditions, the values of coefficients and the values of carcinogen and oxygen concentration levels from the previous time step.

In the next sections we discuss numerical simulations representing two common scenarios of BC progression: bladder polyp and CIS.

6.2. Bladder polyp

Bladder polyps are the most common form of the urinary bladder carcinoma. Their main macroscopic features are single or multiple exophytic papillary masses [65]. In our simulations, we follow the process of polyp formation starting from the clones of mutated cells within the urothelium. Growth of polyps is always supported by the angiogenesis [16] and it is also reflected in our experiments. As we have already stated in Section 2, BC development and progression is a long multi-year process. In the bladder polyp scenario presented in this work we make the 85 months snapshots of cell status (Fig. 9(a)) and carcinogen penetration and oxygen diffusion (Figs. 10(a) and 10(b) respectively). By that time we have two full grown polyps within the bladder lumen and the number of smaller polyps of various sizes. The similar scenarios are often observed during bladder polyp removal surgery.

The neovascular network (shown in red in Fig. 9(a)) grows through the urothelium toward the polyp and eventually ruins the layered structure of urothelium and changes its physical characteristics. Our simulation reflects an important morphological feature of angiogenic behavior in the bladder polyp form of BC: the blood vessels grow within the polyp [54]. One more observation corresponds to the particular BC scenario run in the simulation: most basal cell have already obtained the cancer phenotype by the
6.3. Carcinoma in situ (CIS)

The CIS is caused by the different chain of mutations than the polyp [19]. In Fig. 11, we show the CIS modeled using our approach (a) and the cross-section of CIS from the cystoscopy (b). On the in vivo images the CIS is marked by brown color and one can see that it spreads within the urothelium barely leaving the umbrella cells. According to [65], the major macroscopic properties of the CIS are granular or cobblestone appearance (as seen in Fig. 11(b)) and multifocality.

One can also see that our simulation has also captured a major morphological characteristics of CIS: its “mushroom-like shape”.

7. Discussion and future work

In this work, we have presented an in silico model of urinary bladder carcinoma development and growth. In our simulations, we demonstrated the cell dynamics corresponding to the development of low-grade BC in form of bladder polyps and CIS.
The main purpose of our numerical experiments was to study the effects of carcinogen penetration and hypoxia and to understand the role the angiogenesis is playing in the BC progression.

In our previous work [3, 6], we introduced a framework that combines discrete and continuous models to describe quantitatively the BC initiation. However, the current model represents a major revision of our approach and its significant extension. It allows us to simulate bladder carcinogenesis from the initial mutations to the full-size tumor. The nonlinear submodel of oxygen diffusion and the CA-based submodel of angiogenesis, presented in this manuscript, could be used for modeling and simulation of various cancers, originating in epithelial cells.

One of the major challenges in cancer urology is to provide a prognosis of tumor development for a patient based on the information collected from the cystoscopy and the initial treatment. If such a prognosis could be done early in the course of disease, those with a high likelihood of BC recurrence or progression could be treated more aggressively. A histological examination is used to predict the probability of recurrence and the progression of low-grade urinary bladder carcinoma. From the analysis of the cell structure, the pathologist determines the stage, grade, and multiplicity of tumor. However, the accuracy of such an examination depends on the pathologist’s experience.

The current consensus is that these tumors evolve down one of two major genetic pathways. The first is associated with low-grade papillary and non-invasive cancers that infrequently progress to invasive BC. The other is typified by in situ or higher grade superficial tumors that frequently recur and progress to higher grade and muscle invasive disease [66]. Given individual patient data and genetic analysis of the biopsy, our model will be able to comprehend the diagnostics process by analyzing various scenarios of tumor behavior and make both qualitative and good first-order quantitative predictions of tumor development.

Bladder tumors have diverse morphology [14] and this situation significantly complicates their diagnosis. In many cases, BC may contain variable proportions of different histologic patterns. The clinical outcome of some variants differs from that of
typical urothelial carcinoma and may call for different therapeutic approach. Pathologists should also be aware of the incidence and histologic appearances of secondary neoplasms of the urinary bladder, with emphasis on the points of distinction from primary tumors and their histologic variants [67]. Comparison of the computer-predicted and actually observed tumor patterns may help clinicians with better understanding of tumor histology and planning of treatment strategy.

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