Bladder cancer, a two phased disease?

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Abstract

The processes of intraepithelial migration, intraluminal seeding, and field cancerization as models for initiation, spread, and recurrences of urothelial cell carcinoma are reviewed in light of recent molecular investigations. The accumulated molecular data on synchronous and metachronous tumors indicate that the majority of recurrent and multiple tumors are monoclonal. Molecular data has also shown the presence of chromosomal and genetic changes in precursor lesions as well as in normal urothelial cells. Genetic-histological mapping of cystectomized bladders has shown that overt tumors occur as local events in areas of genetically altered urothelium. A model is put forward in which the tumor process is initiated by genetically altered but histologically normal cells that produce fields of altered cells by intraepithelial displacement. By the accumulation of further genetic changes the fields of altered urothelium reaches a state of criticality which locally may produce frank tumors.

Keywords: field cancerization; urothelial cell carcinoma; urinary bladder; transitional cell carcinoma; stem cells

Contents

1. Urothelial cell carcinoma

Urothelial cell carcinomas (UCC) originate from the epithelial cells of the inner lining of the bladder wall. Seventy percent of the tumors are papillary and confined to the urothelial mucosa (stage Ta) or to the lamina propria (stage T1) whereas the remaining invade the muscle (T2), perivesical fat (T3) or surrounding organs (T4) (Figure 1). It is not uncommon for patients with Ta/T1 tumors to show multiple, synchronous tumors. Most Ta tumors are of low grade (G1 or G2), rarely progress, and are associated with a favorable prognosis whereas high grade Ta (TaG3) and T1 tumors represent a significant risk of tumor progression. Carcinoma in situ, Tis, is a flat lesion commonly found in association with malignant tumors and is generally believed to be the precursor of invasive cancer. UCC is characterized by a number of chromosomal and genetic alterations. Cytogenetic loss and loss of heterozygosity (LOH) of chromosome 9 is particularly frequent occurring in 40-50% of the cases [1, 2]. The most commonly lost region in 9p includes *CDKN2A* that frequently also shows homozygous losses. Several regions of chromosome arm 9q have been suggested to harbor tumor suppressor genes but no definite gene has so far been identified [2]. The receptor gene *FGFR3* is activated by mutations in up to 70% of the Ta tumors but less frequently in invasive tumors [3]. The reverse pattern is seen for *TP53*. This has led to the suggestion that UCC may constitute two entities of tumors developing through two different genetic pathways [4]. Superficial tumors, Ta and T1, are mostly treated by transurethral resection, in many cases combined with subsequent intravesical chemo- or immunotherapy. However, up to 70% of the patients show recurrences after treatment making a lifelong follow-up by regular cystoscopy necessary. Patients with muscle-invasive disease are commonly treated by cystectomy sometimes accompanied by systemic neo adjuvant or adjuvant chemotherapy.

The biology of UCC gives an opportunity to investigate the early stages of tumor development as both recurrences and multiple tumors frequently are available. This makes it possible to study "re-initiation" of the transforming process in the same genetic and environmental background. Furthermore, the topology of the bladder organ has made it feasible to analyze non-cancerous tissue in a systematic way. In the present review the data on genetic changes in normal, premalignant, as well as in synchronous and metachronous UCC tumors will be examined. Suggested hypotheses for the origin of synchronous and metachronous tumors will be evaluated in view of the existing data and possible alternative explanations will be put forward taking the recent findings of cancer stem cells into consideration.

2. Intraepithelial migration, intraluminal seeding, and field cancerization

Two major hypotheses have been proposed to explain the origin of synchronous and metachronous tumors in patients with UCC (Figure 2). One assumes a monoclonal origin either through intraepithelial migration of tumor cells or by intraluminal seeding from a primary carcinoma. The second, the field cancerization model, proposes a field change and that individual cells in these fields are transformed to overt tumors and states, in the stronger version, that independent genetic events will produce multiple or recurrent tumors. In this scenario the genomic changes in neighboring cells will be unrelated and the resulting tumors consequently oligoclonal. In the original version [5], however, the field cancerization model did not include independent cellular origin of tumors. The leading idea behind intraepithelial migration is that individual tumor cells migrate and spread in the otherwise normal epithelium and subsequently form a new tumor at a distant site. Intraepithelial migration is expected to result in continuous areas of transformed cells. As the distance a neoplastic cell may migrate is limited, the intraepithelial migration predicts localized recurrences. Furthermore, the primary and the recurrent tumors are expected to show clonal relationships. The hypothesis of intraluminal seeding states that recurrent and multiple tumors arise from the shredding of cells from a primary tumor and that shred cells are implanted in the urothelium resulting in the growth of new tumors. It has been suggested that the inflammatory reaction and/or the wound healing response induced by tumor resection would facilitate the implantation and growth of seeded cells. As the shred cells are distributed in the bladder through the urine, recurrent tumors may be induced at some distance from the primary tumor. Hence, multiple tumors would show a clonal relationship but mucosa located between the tumors would not show the presence of neoplastic cells.

3. Premalignant lesions and normal urothelium show genetic changes found in cancer cells.

There is growing evidence for the presence of chromosomal and genetic changes characteristic of UCC already in premalignant lesions. Hartmann et al. [6] showed that 10 out of 14 hyperplasias showed monosomies or partial loss of chromosome 9. In seven out of eight patients with genetic alterations in the hyperplasias the genetic changes were also present in the concomitant papillary tumors. In two out of six investigated patients chromosome 9 deletions were also detected in biopsies of normal epithelium. The authors concluded that simple hyperplasia may be a precursor lesion for bladder cancer. Oberman et al. 2003 [7] showed by comparative genome hybridization (CGH) and LOH analyses that flat urothelial hyperplasia showed chromosomal imbalances shared by concomitant papillary tumors. The number of imbalances found in hyperplasias did not differ significantly from the numbers found in papillary tumors. In a similar analysis Chow et al. [8] found evidence for clonality with the concomitant papillary tumor in 10 out of 15 papillary hyperplasias using LOH. These findings were verified by fluorescent in situ hybridization (FISH) using probes for chromosome 9 which also detected losses of chromosome 9 in normal epithelium. In addition, the papillary hyperplasias also showed LOH at other chromosomal regions than seen in the tumors indicating that the genome had undergone a diversification already at the stage of hyperplasia. Hartman et al. [9] analyzed microdissected dysplasias and Tis (CIS) using FISH with probes for 9p, 9q, and 17p, LOH analyses of chromosome 9, and mutation analyses of *TP53*. Deletion of chromosome 9 was detected in 86% of the Tis and 75% of the dysplasias. Deletions of 17p at the *TP53* loci were seen in 84% of the Tis and 53% of the dysplasias, and *TP53* mutations were seen in 72 % of the Tis and 67% of the dysplasias. Due to the similar genetic profiles of dysplasia and Tis it was concluded that dysplasia is a precursor for carcinoma in situ.

As noted, the presence of cancer related mutations is not limited to pre-cancerous lesions but is also seen in morphologically normal urothelium in patients with UCC. Cianciulli et al. [10] showed by FISH analyses that morphologically normal urothelium from patients with UCC demonstrated genetic aberrations common to those of bladder cancer. Particularly -9 and +7 was seen in the tumors and the proximal as well as in the distal mucosa. Steidl et al. [11] showed by FISH analyzes that in 8 of 11 cases both the tumors and their adjacent urothelium were affected by chromosomal aberrations. In five of the eight cases at least one identical chromosomal aberration was observed in both the urothelium and the corresponding tumor suggesting a clonal relationship. In two of the cases the normal urothelium contained changes not seen in the adjacent tumor, demonstrating that karyoptypic evolution takes place within the normal urothelium. Genomic imbalances in normal urotheliun have also been shown by CGH and LOH [12-14]. Stoehr et al. [15] found LOH of chromosomes 9 and 8 in histologically normal epithelium in five out of fifteen patients. Thirty cases with no history of UCC were used as controls and no LOH was found.

The genetic alterations seen in normal and premalignant cells are not limited to chromosomal changes but also include gene mutations and epigenetic changes. Pycha et al. [16] found TP53 to be overexpressed as determined by immunohistochemistry (IHC) in similar rates in both malignant and nonmalignant cells. Simon et al. [17] showed that normal mucosa adjacent to the tumors as well as the tumors showed positive IHC staining for TP53. The IHC staining was located in continuous areas and not scattered as would have been expected if intraluminal seeding had occurred. The authors conclude that their findings may be explained by intraepithelial migration. Stoehr et al. [18] showed that normal urothelium, preneoplastic lesions, as well as the tumors shared the same *TP53* mutation and that regions of normal epithelial cells were TP53 positive by IHC. Muto et al. [13] showed that epigenetic changes, methylation, of the tumor suppressor gene *CDKN2A* also took place in normal mucosa.

It may concluded that both chromosomal and gene alterations occurs in normal and premalignant urothelial cells. Even though many investigations have been based on the analysis of individual cells using FISH the identification of genomic changes using LOH and CGH show that substantial proportions, and not just occasional cells, of the tissue may show similar genomic alterations. Hence, genetically aberrant but morphologically non-cancerous cells appear to surround the growth of carcinomas at high densities. The adjacent cells frequently share many unique genetic alterations with the corresponding tumor but can also show signs of genomic evolution. Even though these findings may be explained by an intraepithelial migration model, such a model has to account for migration at high rates as large proportions of the surrounding tissue may contain aberrant cells.

4. Recurrences, multifocal tumors, and the question of clonality

To investigate the possible multiclonality among multiple tumors from the same patient Sidransky et al. [19] analyzed 13 tumors from 4 different patients by chromosome X-inactivation analysis. In each case the tumors showed X-inactivating patterns consistent with a monoclonal origin. Li et al. [20] studied 10 patients with both synchronous and metachronous Ta/T1 tumors by X-chromosome inactivation analyses. Tumors from the same patient showed the same X-inactivation pattern indicating a mono-clonal origin in all investigated cases. Cheng et al. [21] studied 11 patients with muscle invasive UCC from which samples from different regions were taken. Nonrandom X-chromosome inactivation patterns were found in nine cases (82%), whereas two cases showed different patterns in different sites suggesting an oligoclonal origin.

Hartman et al. [22] analyzed nine cases of superficial low grade tumors showing multiplicity using LOH and FISH analyses of chromosomes 9 and 17. Four cases showed identical patterns of changes and five cases showed genetic heterogeneity. The heterogeneity was however compatible with clonal divergence and selection of different cell populations derived from a common progenitor cell. Simon et al. [23] investigated 32 multifocal tumors from six patients using CGH, *TP53* mutation and IHC analyses. They demonstrated a close karyotypic relationship among the multiple tumors from the same

patient indicating a monoclonal origin. However, the authors also showed that individual tumors from the same patient could either show a wild-type or a mutated *TP53*. Dalbagni et al. [24] investigated metachronous and synchronous tumors from seven patients and all patients displayed identical *TP53* mutations in their tumors. TP53 overexpression could however not be established in the morphologically normal epithelium. Takahashi et al. [25] characterized meta- and/or synchronous multifocal urothelial cancers from 25 patients by allelotyping 20 loci on eight different chromosome arms. In twenty (80%) of the cases the patients were considered to have tumors of clonal origin. Hafner et al. [26] investigated synchronous and metachronous tumors from patients with at least one upper urinary tract tumor using LOH and *TP53* mutation analysis with the motivation that overgrowth by one clone or intra epithelial migration is unlikely when examining upper and lower urinary tract tumors. The pattern of deletions revealed monoclonality of all tumors in nine patients whereas five patients showed evidence for oligolonality. It was concluded that the patients with a monoclonal origin supported intra luminal seeding and that the patients with oligoclonal origin suggested the hypothesis of field effect cancerization.

Based on accumulated data one may conclude that the majority of multiple and recurrent tumors show monoclonality indicating a common cellular origin whereas only a minority show oligoclonality and independent origins. However, to establish "monoclonality" different means may be used [27]. Demonstrating monoclonality using the X-inactivation pattern indicates that e.g., preneoplastic and neoplastic cells, originates from the same precursor cell but does not exclude the possibility that descendants of this precursor have accumulated different aberrations during tumor evolution. On the other hand, the term "clonality" may be used to indicate that the recurrent tumor derives from cells in a primary overt tumor e.g., by intraluminal seeding. In this case the absence of relatively late genetic changes e.g., a *TP53* mutation or LOH of a given DNA marker, in a recurrence when present in a previous tumor, would indicate "oligoclonality" but would be inconclusive if referring to a common precursor cell. Hence, as divergent LOH patterns in meta- and synchronous tumors have been used as an argument for diverse cellular origins, the reported frequency of oligoclonality may be an overestimation of the actual incidence of oligoclonal progenitor cells.

5. Histologic-genetic mapping

Using X chromosome inactivation analysis of cells microdissected from histological slides from a normal female human bladder Tsai et al. [28] showed that the normal urothelium was organized in patches of monoclonal segments. These patches were about 120 mm^2 and estimated to contain approximately 2 x 10^6 cells. Tsai et al. suggested that the patches were composed of descendants of an original founder cell, a stem cell, and estimated the number of such cells to 200-300 per bladder. This suggests that the bladder mucosa is divided into a large number of segments responsible for

maintaining the integrity of the cell layer by replacing damaged cells and that these segments to some extent behave independently.

With the aim to characterize the extent and distribution of genetic and histological alterations in bladders from patients with UCC Chaturvedi et al. [29] and Czerniak et al. [30] superimposed histological and genetic maps from cystectomized bladders. This was accomplished by dividing the entire bladder mucosa into a large number of segments and by examining each segment both histologically and genetically. The segments were analyzed for chromosome aberrations using LOH of chromosome 17 and for mutations in *TP53* [29] or by LOH of chromosome 9 and mutations in *CDKN2A* and *CDKN2B* [30]. LOH analyses revealed that for many markers the same allele was lost in all samples with LOH indicating a clonal relationship among samples. By superimposing the LOH data for specific markers over the histological maps two patterns were seen; a scattered pattern with several isolated regions showing LOH for specific markers and a plaque like pattern consisting of a continuous areas showing LOH for the same marker [Figure 3]. Some of the plaque like alterations involved large areas of bladder mucosa encompassing various histological precursor lesions and areas of morphologically normal urothelium. LOH of chromosome 17 was seen in the invasive and in the preinvasive phases of the urothelial neoplasia, as well as in the microscopically normal urothelium. This indicates that LOH of chromosome 17, associated with invasive tumors, preceded the development of morphologically recognizable changes. It was also found that LOH of chromosome 9 preceded the development of microscopically identifiable urothelial abnormalities. In the study by Chaturvedi et al. [29], *TP53* mutations were mapped to early stages of utothrelial neoplasia. One of the investigated cases showed three separate foci of tumors all exhibiting the same *TP53* mutation. The same *TP53* mutation was also seen in areas of intraurothelial precursor conditions separating the three foci. Czerniak et al. [30] showed the presence of homozygous deletions of the *CDKN2A* gene in morphologically normal mucosa. Furthermore, gradual expansion of the deleted region occurred in the progression to low grade and subsequently to high grade intraurothelial neoplasia, and in the transition to carcinoma. In an extended and analogous study Czerniak et al. [31] showed that LOH for chromosomes 4, 8, 9, 11, and 17 existed in the normal urothelium showing that extensive karyotypic evolution already occurs in morphologically normal cells, including changes that are associated with advanced tumors. Stoehr et al. [18] performed histological-genetic mapping of cystectomized bladders from patients with invasive tumor. *TP53* mutations were detected in regions with preneoplastic lesions as well as in physiologically normal urothelium. In a similar way Simon et al. [17] showed that synchronous tumors with *TP53* mutations were located within continuous areas of normal urotheliun with *TP53* mutations, whereas tumors with *wtTP53* were located within areas of urothelium showing no mutations.

Histologic and genetic mapping of bladders with UCC have thus shown that areas with LOH and gene mutations may cover large parts of the urothelium and that these regions are larger than the normal patches of monoclonal cells described by Tsai et al. [28]. In fact the genetically altered regions

almost completely covered the inner surface of the bladder in some cases [29, 30]. This indicates that a massive intraepithelial spread of genetically altered cells has taken place. Some markers showed LOH in almost all of the mapped segments, indicating large clonal segments, whereas others were scattered and showed “islands” of LOH. This shows that the cells evolve locally during spreading, resulting in a genetically heterogeneous field of cells. The *TP53* mutation mapping of cystectomized bladders demonstrates further that tumors are located in fields of normal/preneoplastic cells showing the same gene mutation as in the tumor proper (Figure 3). The accumulated histologic-genetic mapping data thus favors intraepithleial migration and makes intraluminal seeding less likely as the most prominent mechanism for tumor spread or recurrence.

6. The chronology of tumor presentation does not parallel the genetic evolution

van Tilborg et al. [32] studied 11 patients with five or more recurrences using LOH and mutation analyses. For each patient tumor progression trees were constructed based on the accumulating number and sizes of the genetic changes, creating a chronology of the recurrences based on genetic events. By comparing the genetic chronology with the chronology of tumor appearance it was found that the genetic progression trees better reflected the tumor evolution than their chronologic order of presentation. In fact this was the leading principle rather than the exception. Hence, recurrences with more evolved genomes could appear long before clonally related recurrences with less evolved genomes. The suggested lack of correlation between the chronological appearance of tumors and genetic progression is also supported by the fact that recurring tumors may be of lower grade than the preceding ones. Borhan et al. [33] showed that grade regression occurred in almost 50% of the investigated cases and that only 33% of *TP53* positive cases were positive at the recurrence. Data presented by Dahse et al. [34] supports that *TP53* mutations present in a primary tumor may be absent in the recurrent tumor. Thus the chronology of tumor presentation does not reflect the genetic progression suggesting that cells or segments of the urothelium with differently progressed but clonally related genomes may co-exist and produce overt tumors independently.

7. Two phases of bladder cancer.

The data favoring the presence of genetic changes characteristic of frank carcinomas in morphologically normal urothelium, including LOH, chromosomal changes, and gene mutations, is more than convincing. The use of microdissection has successfully showed that premalignant lesions, hyperplasias, papillary hyperplasias, and dysplasias, adjacent to tumors show genetic aberrations similar to those in overt tumors. The detailed histologic-genetic mapping shows that regions with shared genetic aberrations can in fact cover a large part of the bladder urothelium. Furthermore, islands of cells containing additional genetic markers indicate local genomic divergence and hence that

affected fields may be genetically heterogeneous. The affected regions were also characterized by morphological heterogeneity in that they demonstrated several different preneoplastic and neoplastic lesions. One may conclude with some confidence that tumors are located within continuous fields of genetically and sometimes also histologically aberrant cells.

There remain two major alternative explanations to the source of such fields, the commonly suggested intraepithelial migration of cancer cells, *the tumor-first-field-later model*, and alternatively the *field-first-tumor-later model* in which nonmalignant but genetically modified cells spread though the epithelium and eventually develop to frank tumors. In the case of intraepithelial migration of cells from existing tumor foci, one has to explain a switch from an adherent to a migrating phenotype. Furthermore, migrating cells have to behave as dormant cancer cells as they may be located in the urothelium for an extended period of time without resulting in overt tumors. As most primary and recurrent Ta tumors are of low grade and thus exhibit epithelial or epithelial like characteristics, the path from a primary to a migrating tumor showing dormancy, and the initiation of a second tumor again showing epithelial like characteristics, would include both an epithelial-mesenchymal transition (EMT), to facilitate migration [35], as well as the reverse transition. These cellular transitions are in many ways complex and involve several genetic regulatory systems such as alterations of cell adhesion genes and the TGFB signaling pathway, as well as of the micro environment of the cells, systems that have to be turned on and off in a reversible and coordinated way.

An alterative explanation for meta- and synchronous tumors could involve stem cells. Several investigators have shown the importance of cancer stem cells in the development of hematological malignancies [36] and recently cancer stem cells have been revealed in breast cancer [37] and in glioblastoma [38]. Self-renewing cancer cells may either derive from stem cells or from restricted progenitor or differentiated cells that acquire a self-renewal potential. A possible scenario could thus be that a self-renewing cell, of either origin, acquires genetic alterations that partly blocks differentiation, and then colonizes the patches described by Tsai et al. [28]. All cells in a patch will eventually become genetically altered daughter cells. As a result of subsequent genetic alterations successive cells escape normal growth control by neighboring cells and develop into expanding clones that invade adjacent patches and laterally displace the normal epithelium resulting in fields of premalignant cells [39]. Thus the spreading of premalignant cells would involve intraurothelial displacement, rather than intra epithelial migration, replacing the genetically normal epithelium. The outcome of this process would be a sheet of epithelial cells with cancer-associated genetic alterations, but without invasive or exophytic growth making it a histopathologically benign lesion.

The process of multistep carcinogenesis is most likely operating already during field extension as several chromosomal changes and gene mutations may be present in morphologically normal urothelium in patients with UCC. As a consequence of accumulated genetic changes, cells will eventually develop into frank carcinomas, *the field-first-tumor-later model*. It is conceivable that the likelihood for a transformation to occur is related to the number of acquired genetic aberrations; cells

with highly evolved genomes would be more liable to transform than cells with less evolved genomes. However, as shown by van Tilborg et al. [32] the chance for a transformation event to occur does not seem to be related to karyotyopic complexity. No relationship between karyotypic evolution and the chronology of appearance is seen in patients with several recurrences [32], suggesting that the transformation event is, at least to some extent, uncoupled to previous changes (Figure 4). At the time of tumor presentation the urothelium most likely already is in a state of criticality, or "field cancerization", showing several genetically evolved and divergent segments of the urothelium. It could be envisaged that a transforming event, e.g., a profound increase in proliferation, in an individual cell in such a field may result in a local cancerous growth but leaving the neighboring cells in their respective states. In this scenario bladder cancer would not be a local disease but "a local manifestation of a diffuse abnormality of the urothelium" [40].

Several investigations have shown that tumor foci are located within areas of premalignant and normal urothelium showing several genetic changes. Most probably these fields expand over a period of time, up to several years, creating no or only mild symptoms. In light of this, the expanding field of hyperplasia or dysplasia by intra urothelial displacement may be compared to a chronic phase and the local transitions to cancerous growth to an acute phase. The chronic phase would involve the spreading of mildly transformed, premalignant cells throughout the epithelium that eventually develop overt tumors, in analogy with the transition of chronic myeloid leukemia (CML) to acute myeloid leukemia (AML). CML is generally characterized by granulocytic and megakaryocytic hyperplasia in which the maturation proceeds in an orderly manner without any arrest or block. After the initial relatively benign chronic phase, which may last for some years, the disease typically enters an accelerated phase and eventually become indistinguishable from AML. It seems that UCC pass through two similar phases, one that involves the induction of self-renewal capacity and ultimately in a field defect, and a second in which a local transformation of preneoplastic cells results in overt tumors.

A corollary of the histologic-genetic mapping and several LOH, FISH, and CGH investigations of premalignant lesions is that karyotypic evolution appears to occur already in premalignant cells. Furthermore, some level of chromosomal instability may have been acquired already before the transition to overt cancer. An indication of this is that premalignant and normal urothelium may show chromosomal changes associated with advanced and invasive tumors such as losses of 3p, 5q, 6q, 8p, 17p, and 18q [1, 6, 7, 8, 13, 29]. Clearly, cells with chromosomal changes associated with late and invasive stages may remain dormant in the sense that they do not produce frank tumors. Even though chromosomal changes alone may not be sufficient for cancerous growth, the accumulated evidence for an association between tumor stage and grade, and karyotype pattern is convincing [1, 41-44]. This opens for the possibility that a local event initiating the "acute phase", apart from changing the growth properties of the cells, transforms the cells into tumor stages and grades determined by the genetic changes acquired during the "chronic phase" (Figure 4). Consequently, when a recurrent tumor "progresses" to a higher grade or stage, cells in a different segment of altered urothelium, with a

different set of genetic changes, may have been activated, and conversely in cases of tumor regression (Figure 3). Furthermore, this segment could have been present already at the presentation of the primary tumor. The activation of different segments of clonally related but diverged patches of cells could, at least to some extent, explain cases of grade regression [33], that multiple tumors may show different grades, stages, or mutations [17, 45, 46], and the lack of correlation between the chronology of presentation and genetic evolution [32].

8. Consequences for treatment

The concept of expanded fields of preneoplastic cells that may function as a potent source for initiation of new tumor foci has important clinical consequences. One of the key challenges in the management of UCC is the high frequency of recurrences. Some success in prolonging the recurrence free period after transurethral resection (TUR) have been accomplished by combining TUR with intravesical chemo- or immunotherapy. It is conceivable that the selective action of many chemotherapeutic agents is due to the dedifferentiated state and to the impaired production of membrane plaques, tight junctions, and other surface structures present in normal urothelium making neoplastic cells more permeable to chemotherapeutic agents. Still, even if adjuvant treatment, functionally directed towards fully transformed cells, will eradicate any remaining tumor cells due to shredding, insufficient resection, or remaining microscopic tumors not seen by conventional cystoscopy, the major source for recurrences will remain: an urothelium in a state of criticality that locally may transform into an overt tumor.

A promising method to evaluate the presence and extension of preneoplastic lesions is the use of fluorescent agents that specifically labels segments of hyperplasia and dysplasia but not normal urothelium [47]. This approach has led to the identification of affected segments of the urothelium not observed by normal cystoscopy. However, even if the use of fluorescent agents will lead to more complete resections, a combination with adjuvant chemotherapy to eradicate remaining hyperplastic/preneoplstic cells may be necessary. In this scenario the active agent has to discriminate between normal and hyperplastic/preneoplstic urothelium in order to avoid side effects caused by reactions in the normal urothelium; a none-trivial task given the similarity of normal and hyperplastic cells. An alternative is to target genetic changes present in hyperplastic/preneoplstic but not in normal urothelium with chemotherapeutic agents, in analogy with the treatment of CML expressing the fusion gene *BCR/ABL1* with Imatinib (Glivec) [48]. A possible target in UCC could be *FGFR3* as this gene is mutated in about 70% of papillary tumors [49] and drugs specifically inactivating the corresponding protein have been described [50]. If, however, the field defect is a consequence of the activity of cancer stem cells, cancer stem cells should be the ultimate target for therapy. Thus a major future goal in the analysis of UCC is to identify and characterize possible bladder cancer stem cells.

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Figure legends

Figure 1. A schematic representation of UCC staging. Carcinoma in situ, Tis or cis, are flat lesions showing dysplasia and are believed to be precursors to invasive UCC. Ta tumors represent the mildest form and show exophytic growth but do not engage the lamina propria. T1 tumors have transversed the basal membrane and engage the lamina propria. These tumors may also show a more solid growth pattern. Invasive tumors engage the underlying muscles and the surrounding organs in the most severe forms. Ta and T1 tumors are occasionally grouped together and characterized as superficial.

Figure 2. Two major explanations for syn- and metachronous UCC. The first model includes either migrating tumor cells or shed cells that re-implant in the bladder mucosa. Migration of tumor cells could occur at the surface or within epithelium. Both processes will however result in recurrences (T_R) or synchronous tumors that show a clonal relationship with the primary tumor (T_P). In the field cancerization model synchronous tumors would each be caused by a unique molecular event and represent a group of concomitant primary tumors ($T_{P1,}$ $T_{P2,}$ and T_{P3}).

Figure 3. Genetic-histological mapping. The graph represents a generalization of the findings in references 29, 30, 31, 32, and 40 and the suggested model for UCC development. A large field with a common genetic change (genetic change 1) has evolved in the urothelium. The genetic change could be a chromosomal change, LOH, or gene mutation. Some cells in this field has acquired further changes (genetic changes 2a and 2b) and produced smaller areas with more evolved genomes, and finally, within one of these areas a third genetic event has taken place resulting in a patch of cells with even more evolved genomes. These areas represent regions of “diffuse abnormalities” [40]. A primary overt tumor (T_P) has occurred in a field with two genetic changes, the first recurrence (T_{R1}) in the field with one genetic change, and the second recurrence (T_{R2}) in the field with three genetic changes. Hence, in this case, the produced genetic tree would not coincide with the tree produced by the chronologic appearance [32]. An assumed starting point for the spreading of genetically aberrant cells by epithelial displacement is indicated by a filled square.

Figure 4. A schematic representation of the normal-chronic-acute phase model. A cell within a patch of monoclonal cells acquires self renewal capacity and colonizes the patch. The self-renewing cells acquire further genetic aberrations and invade neighboring patches. The expansion of the field with genetically aberrant cells constitutes the chronic phase. As a result of further genetic divergence areas with different genetic changes evolve, resulting in a urothelium in a “critical” state. Each box with chromosomal and genetic changes represents an individual sub-field. Crucial changes (indicated with star like structures) within a limited number of cells, or within a single cell in a sub-field result in the

growth of overt tumors. T_P, a primary tumor (chronologically first) originating from a sub-field with few changes; T_R, a recurrence originating from a neighboring sub-field with a related but different set of genetic changes.

Figure 1

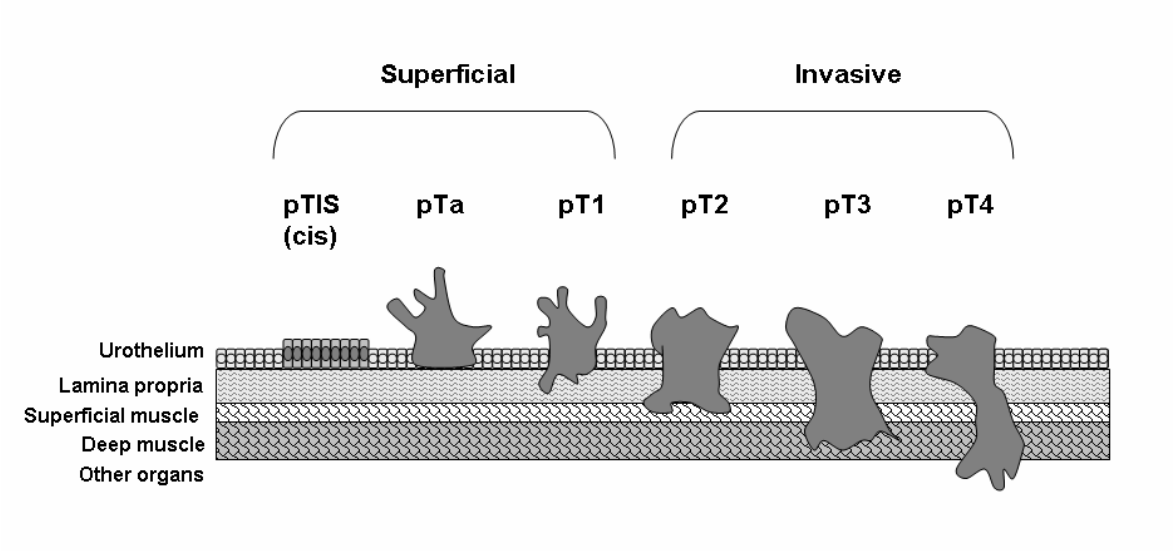

Figure 2

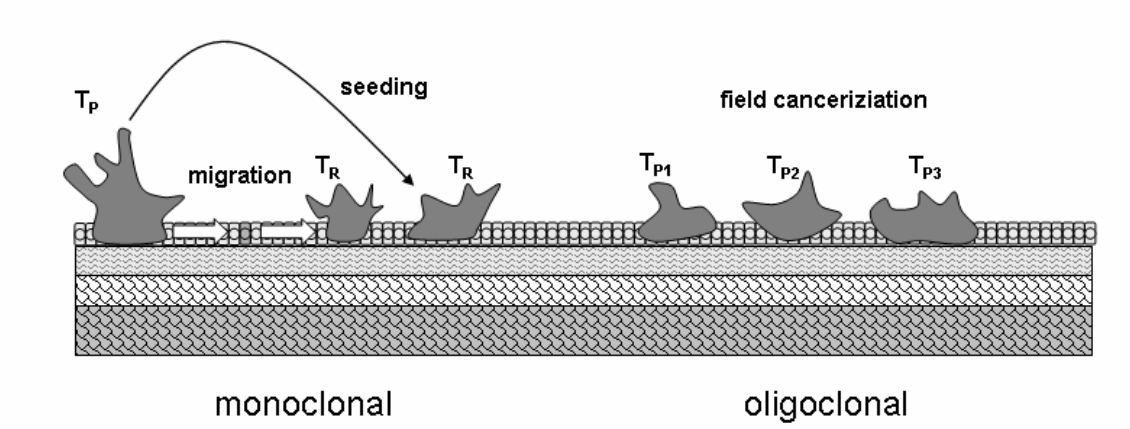

Figure 3

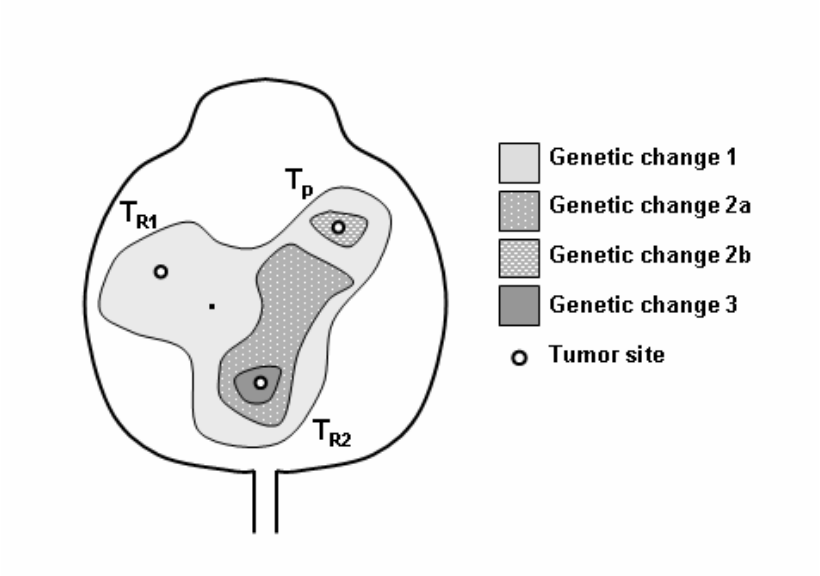

Figure 4