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Review article

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Mechanisms and implications of epithelial cell plasticity in the bladder

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Abstract

Cellular plasticity, the ability of cells to reprogramme and alter their fate, has a pivotal role in maintaining homeostasis and facilitating tissue regeneration after injury. The bladder urothelium, a dynamic transitional epithelial layer, displays a highly plastic phenotype that enables its remarkable regenerative capacity in response to wounding. During both development and repair, urothelial cells exhibit considerable plasticity through processes such as dedifferentiation, transdifferentiation and epithelial-to-mesenchymal transition. Urothelial plasticity is not only crucial for healthy tissue repair but is also involved in pathological conditions, including cancer. In bladder tumorigenesis, urothelial cells exploit plasticity to acquire new phenotypic and functional characteristics, transitioning between distinct cellular states. This plasticity contributes to tumour heterogeneity, subtype switching, progression, metastasis and resistance to therapies. These dynamic cellular transitions are regulated by intrinsic and extrinsic factors, including transcriptional and epigenetic mechanisms, as well as microenvironmental influences. Targeting urothelial plasticity could offer novel therapeutic strategies for bladder-related diseases.

Sections

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Key points

- Epithelial cell plasticity contributes to bladder repair, but leads to metaplastic changes, fibrosis and malignancy risk under pathological conditions such as inflammation or mechanical irritation.
- Cellular plasticity drives bladder cancer heterogeneity, progression and therapy resistance through dynamic lineage transitions, epithelial-to-mesenchymal transition and stem-like traits, underscoring its pivotal role in disease evolution and treatment challenges.
- Epithelial plasticity in bladder repair and cancer is regulated by transcription factors, signalling pathways, epigenetic modifications and microenvironmental cues, driving cell transitions and phenotypic adaptations.
- Targeting epithelial cell plasticity offers promising avenues for bladder disease, regenerative medicine and cancer treatment.

Introduction

The bladder urothelium is a specialized multilayered epithelium that serves as a crucial barrier, separating urine from underlying tissues and maintaining homeostasis[1]. Dysregulation of urothelial biology is implicated in various bladder diseases, including bacterial cystitis, interstitial cystitis/bladder pain syndrome (IC/BPS) and neoplasms[2,3]. Anatomically, the urothelium comprises three distinct layers: a basal cell layer, an intermediate layer and a superficial luminal layer formed by terminally differentiated umbrella cells[4]. Under physiological conditions, the bladder urothelium remains largely quiescent, with a slow turnover rate of ~1 year in humans[2,5]. However, upon injury, the urothelium exhibits remarkable regenerative capacity, with active proliferation observed across all layers[6]. Lineage tracing studies have revealed distinct progenitor populations within the basal and intermediate layers that possess self-renewal capabilities, which contribute to urothelial development, homeostasis and repair[2].

Historically, the differentiation hierarchy of urothelial cells was considered a unidirectional and irreversible process, progressing from basal to intermediate and then to umbrella cells[7]. However, emerging evidence challenges this idea, suggesting that lineage hierarchy is more flexible than previously thought[3,8,9]. Under certain conditions, cells can deviate from established differentiation pathways to adopt alternative fates in response to environmental challenges and genotoxic stresses, a phenomenon termed 'cellular plasticity'[10]. This adaptability has a crucial role in maintaining tissue homeostasis following damage, inflammation or cellular stress, and it is increasingly recognized as a feature of various epithelial tissues such as the lung, intestine and breast as well as the bladder[11–13].

Epithelial cell plasticity manifests in several forms, including dedifferentiation, in which differentiated cells revert to a more progenitor-like state; transdifferentiation, which involves the direct conversion of one differentiated cell type into another, often serving as a precursor for metaplasia; and epithelial-to-mesenchymal transition (EMT), characterized by the loss of epithelial traits — such as cell polarity and adhesion — alongside the acquisition of mesenchymal properties, including increased motility and invasiveness[11]. In bladder biology, cellular plasticity is primarily perceived as a characteristic of stem and progenitor cells or as a transient process in which epithelial cells acquire EMT-like features during tissue repair and inflammation[2,4,14]. This narrow perspective is probably a result of decades of research focused on identification of urothelial progenitor cells and elucidation of their roles in bladder urothelium regeneration. However, emerging evidence shows that under certain conditions, all urothelial cell types, whether basal or non-basal populations, can regenerate a fully functional, hierarchically structured urothelium[8,9,15]. This finding challenges the traditional stem cell-centric perception of urothelial regeneration and suggests that mature urothelial cells retain greater plasticity than previously assumed.

Beyond maintaining healthy tissue repair, cellular plasticity is also implicated in pathological processes, particularly in tumorigenesis[16]. Malignant cells exploit plasticity to transition between different cellular states, enhancing their adaptability to selective pressures from the tumour microenvironment (TME) and therapeutic interventions[17]. This phenotypic flexibility promotes intratumour heterogeneity, growth, metastasis and therapy resistance[16–18]. In bladder cancer, cellular plasticity is understood to be malignant cells oscillating between epithelial and mesenchymal phenotypes, frequently accompanied by the acquisition of stem cell-like properties and increased motility[3,19,20]. However, this oversimplified understanding does not account for the full spectrum of phenotypic transitions observed in bladder cancer, including partial EMT states, neuroendocrine differentiation and squamous differentiation. Advances in multidimensional sequencing and lineage tracing technologies have uncovered novel transitional cell states that blur the boundaries between traditional molecular subtypes, indicating that bladder cancer plasticity is more dynamic than previously recognized[21–25].

A comprehensive understanding of cellular plasticity in the bladder can provide new insights into the mechanisms that drive bladder-related pathologies.

In this Review we synthesize current knowledge on cellular plasticity in the bladder urothelium, emphasizing its role in bladder repair and tumorigenesis. Furthermore, we explore the molecular mechanisms of urothelial plasticity and discuss its potential as a novel therapeutic target for bladder-related diseases.

Historical and current insights

The bladder urothelium, initially described in the late nineteenth and early twentieth centuries, comprises three distinct layers: basal, intermediate and superficial umbrella cells, each characterized by specific molecular markers and morphology[4] (Fig. 1). Basal cells, located along the basement membrane, are small and undifferentiated, expressing markers such as keratin 5 (KRT5), KRT14, TP63, sonic hedgehog (SHH), CD44, CD49f and β4 integrin[2,26,27]. Intermediate cells form a transitional layer between basal and umbrella cells, with variable marker expression of KRT5, TP63, SHH, CD49f or uroplakins (UPKs)[28,29]. Superficial umbrella cells are large and terminally differentiated with polyhedral morphology and can be distinguished by the expression of KRT20 and UPKs[30,31]. These cells form the urine permeability barrier through urothelial plaques and tight junctions, whereas progenitor cells in the basal and intermediate layers drive urothelial regeneration after injury[4,32].

Throughout the twentieth century, extensive research focused on identifying context-specific progenitor populations and their roles in urothelial patterning and repair. Techniques such as Cre–LoxP recombination, label-retaining cell assays and organoid formation aided identification of several progenitor lineages marked by TP63, SHH, UPK, KRT5, KRT14 and CD49f expression[2,4,19,26,27] (Fig. 1b). The proliferative and differentiation status of progenitor cells is highly influenced

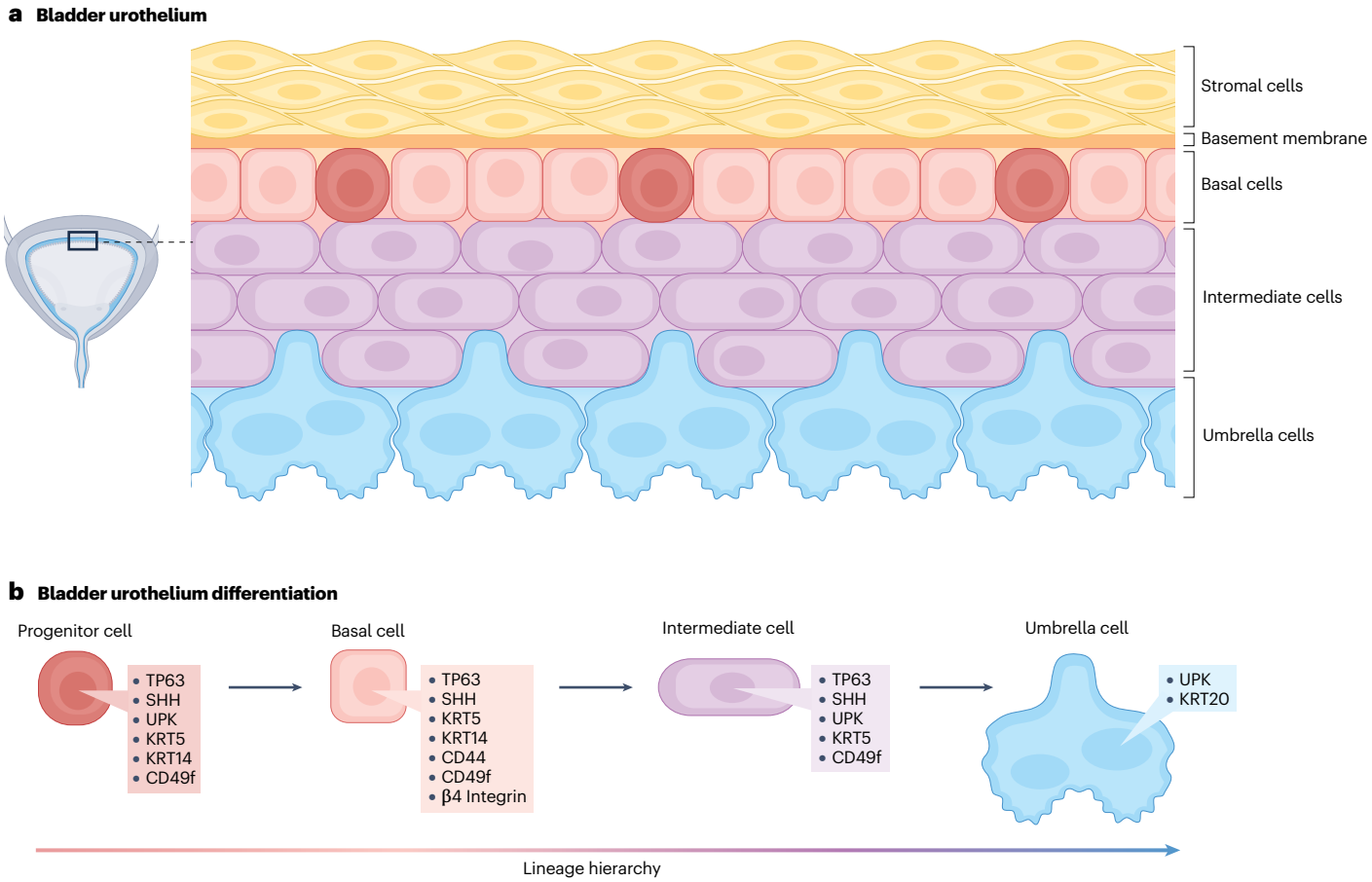

Fig. 1 | The hierarchical structure of bladder urothelium. a, The bladder urothelium consists of three distinct cell types: basal cells, intermediate cells and superficial (umbrella) cells. The basal layer contains a population of urothelial progenitor cells that are tightly regulated by the surrounding stromal elements within their niche. **b**, During urothelium development and homeostasis, self-renewing progenitor cells undergo division, generating basal cells. These basal cells then give rise to intermediate cells, which subsequently differentiate into a single layer of umbrella cells. The figure also shows multiple lineage-specific progenitor cells involved in this process, as well as the known markers expressed by each layer of cells. KRT, keratin; SHH, sonic hedgehog; UPK, uroplakin.

by crosstalk between the urothelial cells and adjacent mesenchymal or stromal cells[8,9,33]. Results of tissue recombination experiments have demonstrated that stromal signals, particularly via WNT, BMP and NOTCH pathways, can shape urothelial differentiation trajectories, highlighting the plasticity of progenitor populations[13,33,34]. In some contexts, urothelial progenitors can undergo transdifferentiation, switching between epithelial and mesenchymal phenotypes[35]. Furthermore, results of studies in which basal and non-basal urothelial subpopulations were isolated have shown that both populations can exhibit similar long-term growth and differentiation potential. Remarkably, non-basal urothelial cells can regenerate a fully organized, hierarchically structured urothelium comparable to that derived from basal progenitors[8]. This observation challenges the long-standing assumption that only basal cells serve as stem-like progenitors, suggesting instead that the urothelium harbours multiple, context-dependent progenitor pools.

An examination of the evolving model of bladder urothelial hierarchy must consider advances in delineating cellular heterogeneity during bladder development and injury using single-cell genomics. This powerful tool has facilitated the discovery of cellular hierarchies and developmental trajectories across multiple organ systems, including the lung, kidney and intestine[36–38]. Similarly, advances in single-cell genomics have revolutionized understanding of urothelial heterogeneity and developmental trajectories. Single-cell RNA sequencing (scRNA-seq) analyses have provided comprehensive transcriptome profiles of bladder urothelial populations, uncovering previously unrecognized urothelial subpopulations ($ASPM^+$ basal-like cell) with plasticity-related characteristics[9]. This cell subset exhibits distinct gene signatures associated with proliferative and regenerative capacity and is substantially upregulated following urinary tract infection with uropathogenic *Escherichia coli*, suggesting a dynamic role in urothelial repair during injury or infection. Unlike canonical basal cells, $ASPM^+$ basal-like cells might transition between basal, progenitor and differentiated states depending on environmental cues, further supporting the concept of urothelial plasticity[9].

Overall, these findings reveal a previously unappreciated heterogeneity and complexity within the urothelium and challenge the traditional hierarchical model[8,9,15,39]. Urothelial plasticity is a highly dynamic and context-dependent process, influenced by interactions between epithelial and stromal cells and shaped by local microenvironmental cues.

This plasticity is essential to maintain bladder homeostasis and enable urothelial regeneration after injury, inflammation or genotoxic stress.

Plasticity in development and repair

Understanding of the complexity of bladder urothelial hierarchy has increased with the identification of cellular states that exhibit unique or mixed gene expression profiles. However, the developmental trajectories of these newly identified states remain unclear, raising questions about their hierarchical positioning and whether they arise from progenitor differentiation or the plasticity of mature cells. In urothelial biology, 'plasticity' has traditionally been used to describe only variations in stem and/or progenitor cell differentiation along epithelial lineages, deviations from canonical cellular states and experimentally induced state transitions[2,3]. However, evidence now challenges this restricted definition, instead suggesting that mature urothelial cells retain the ability to undergo fate transitions in response to injury, inflammation or external stimuli[8,9].

Emerging experimental evidence indicates that cellular lineage trajectories are reversible, enabling reprogrammed mature cells to acquire characteristics of tissue-resident unipotent or multipotent progenitors through dedifferentiation[8,11,19] (Fig. 2). For example, non-basal NGFR-negative urothelial subpopulations can regenerate a hierarchically organized, differentiated tissue, closely resembling native urothelium, including reconstituting the NGFR-positive basal layer under experimental conditions[8]. These findings challenge the notion that urothelial regeneration relies solely on distinct progenitor populations, instead supporting a plasticity model in which urothelial cell phenotype is dynamically shaped by local microenvironmental cues. This plasticity enables non-basal cells to dedifferentiate and re-enter the progenitor-like state and contribute to tissue repair, further adding current knowledge of bladder urothelial biology.

Dedifferentiation and transdifferentiation are not commonly observed under physiological conditions, but urothelial plasticity becomes pronounced during tissue repair after injury[40,41]. Upon chronic inflammation or mechanical irritation, urothelial cells can undergo morphological transitions to squamous or glandular epithelium, a process known as metaplasia, which is a histological form of transdifferentiation[40] (Fig. 2). Various forms of bladder metaplasia, including von Brunn nests, cystitis cystica, cystitis glandularis, intestinal metaplasia, squamous metaplasia and nephrogenic metaplasia, are recognized[40,42–44]. The frequent occurrence of these metaplastic changes highlights the role of cellular plasticity as a common adaptive mechanism in response to bladder injury, triggered by systemic and local factors such as hormones, inflammation and irritation[40]. Notably, most metaplastic changes are reversible and distinct from dysplasia, which involves genetic alterations that lead to neoplastic transformation. Unlike dysplasia, metaplasia is an adaptive response without irreversible genetic changes, and its reversibility offers potential for therapeutic intervention[42]. Upon removal of the eliciting stimulus, the urothelium can revert to expected differentiation patterns[42,45]. However, some forms, such as keratinizing squamous metaplasia, have a heightened risk of malignancy, warranting early intervention[43].

Another hallmark of urothelial plasticity is EMT, a key process in epithelia-derived cancers that is also observed in urothelial cells during bladder repair[46] (Fig. 2). During EMT, urothelial cells lose their characteristic cell–cell adhesion and polarity, acquire mesenchymal traits such as enhanced migration and invasion, and are marked by changes in gene expression, including downregulation of epithelial markers (such as E-cadherin and cytokeratins) and upregulation of mesenchymal markers (such as N-cadherin, vimentin, αSMA and MMP9)[46,47]. Experimental models have shown these EMT-associated changes in urothelial cells during repair, with the TGFβ1 and TNF signalling pathways having key roles[41,46]. For example, TGFβ1 treatment induces a fibroblast-like morphology in urothelial cells, leading to a decrease in E-cadherin expression and an increase in N-cadherin, αSMA and MMP9 levels[46]. The EMT-associated pro-fibrotic phenotype of urothelial cells has also been observed in bladder diseases such as bladder outlet obstruction (BOO) and IC/BPS, in which it contributes to fibrosis and structural remodelling. In BOO models, urothelial cells showed elevated expression of EMT and pro-fibrotic markers, alongside increased soluble collagen production, contributing to bladder wall stiffness[14]. Similarly, in IC/BPS, TNF-mediated chronic inflammation promotes fibrogenesis through sustained EMT activation, as demonstrated in in vitro models[41]. These findings highlight the dual role of urothelial plasticity, which is essential for injury repair but can also contribute to pathological remodelling in chronic disease conditions.

Together, these insights refine understanding of bladder urothelial plasticity, moving beyond a strict hierarchical framework to recognize the dynamic and context-dependent nature of urothelial cell fate decisions. This evolving perspective has important implications for regenerative medicine and disease management, as targeting urothelial plasticity could offer novel therapeutic strategies to enhance tissue repair while preventing fibrosis and metaplasia.

Plasticity in bladder cancer

Cellular plasticity is fundamental for tissue regeneration and also has a crucial role in cancer biology, enabling cells to adopt new phenotypic and functional characteristics by transitioning between distinct cellular states[16,17]. In bladder cancer, lineage plasticity – driving histological subtype switching – has emerged as a hallmark of disease progression[20,22,24]. This adaptability supports tumour heterogeneity, progression and metastasis, contributing to the aggressive nature and therapeutic resistance of bladder cancer (Fig. 3).

Heterogeneity driven by plasticity

Bladder cancer is characterized by substantial histological, molecular and clinical heterogeneity. In the widely accepted molecular classification, six molecular subtypes are defined: luminal papillary (LumP), luminal non-specified (LumNS), luminal unstable (LumU), stroma-rich, basal/squamous (Ba/Sq) and neuroendocrine-like (NE-like)[48]. These subtypes differ in gene expression profile, histopathological features and clinical behaviour, reflecting diverse pathways of tumour evolution and plasticity. Similarly, most bladder cancers are urothelial carcinomas but often present with divergent histomorphologies, such as sarcomatoid, small-cell carcinoma, micropapillary and plasmacytoid variants, which are associated with poor outcomes[49].

Bladder cancer subtypes exhibit distinct molecular and histological characteristics that reflect varying degrees of plasticity[48,50,51]. For example, luminal subtypes express urothelial differentiation markers (such as UPK3A and KRT20) and show PPARγ pathway activation. LumP tumours frequently harbour *FGFR3* mutations and exhibit papillary histology, have favourable prognosis and are identified by immunohistochemistry (IHC) markers (GATA3, FOXA1, KRT20, UPKs)[52]. LumNS displays intermediate features and retained luminal markers, indicating a transitional phenotype, whereas LumU tumours have EMT activation, *TP53* mutations and genomic instability, suggesting an aggressive phenotype with potential for basal or mesenchymal transitions[48]. The Ba/Sq subtype, characterized by basal markers (KRT5/6, KRT14, TP63, CD44),

exhibits squamous differentiation, high EMT activity and histological variants (squamous, sarcomatoid, small-cell carcinomas)[52], reflecting urothelial-to-mesenchymal and urothelial-to-neural plasticity. Similarly, stroma-rich tumours, marked by abundant stromal content, show TGFβ1 activation and mixed epithelial–mesenchymal traits (vimentin, desmin and SMA), contributing to resistance to conventional therapies[48,53]. Neuroendocrine-like tumours characterized by neuroendocrine markers (CHGA, SYP, CD56) and *TP53* and/or *RB1* mutations, closely resemble small-cell carcinomas and exhibit an aggressive phenotype[48,50,54] (Fig. 3).

Evidence from histopathological and genomic studies suggests that these aggressive variants evolve from precursor populations of conventional urothelial carcinomas through distinct but overlapping trajectories[55]. Specifically, micropapillary and plasmacytoid subtypes follow a luminal trajectory, whereas small-cell and sarcomatoid variants evolve along a basal pathway, suggesting considerable urothelial plasticity in bladder cancer[56,57] (Fig. 3). Research on squamous differentiation — one of the most prevalent variants of bladder cancer[58] — has provided new insights into lineage plasticity[22]. Integrated genomic and transcriptomic analyses have revealed that urothelial and squamous regions within heterogeneous bladder tumours share a common precursor, despite displaying divergent morphologies[22]. These findings suggest that squamous differentiation does not arise from distinct genomic alterations but instead from epigenetic and transcriptional reprogramming. Downregulation of key urothelial transcription factors disrupts urothelial identity and promotes basal-like differentiation, underscoring the role of lineage-specific transcriptional control in bladder cancer plasticity[22].

Collectively, histological and genomic evaluations have elucidated key aspects of cellular heterogeneity and plasticity, including histological variability at disease onset, subclonal mutations and molecular clustering based on gene expression[22,48,59]. However, traditional methods using bulk gene expression signatures to classify bladder cancers might overlook the complexities of intratumoural heterogeneity and the dynamic nature of cellular plasticity during disease evolution, thereby limiting understanding of the molecular drivers of heterogeneity and plasticity. Advances in scRNA-seq and lineage tracing have provided improved insights into cellular plasticity during tumour evolution[60]. For example, non-stem cells in urothelial cancers can acquire stem-like properties and develop self-renewal capabilities, supporting the notion that tumour initiation and progression involve dynamic interconversions between basal, luminal and mesenchymal states[61]. Subsequent exploration of the relationship between tumour heterogeneity and lineage plasticity using scRNA-seq in a chemically induced transplantable mouse model of muscle-invasive bladder cancer (MIBC)[24] showed that tumour cells from various lineage subtypes cluster closely at the transcriptional level, suggesting extensive transcriptional plasticity. Notably, many tumour cells can simultaneously express mRNA from multiple subtypes, reflecting hybrid cellular states with basal, luminal and mesenchymal characteristics. Functional studies further demonstrated that tumour initiation and cellular plasticity could originate from diverse lineage components, emphasizing the dynamic interplay between epithelial, basal, luminal and mesenchymal traits during disease evolution[24] (Fig. 3). Use of surface markers (CD49f and EPCAM) revealed multidirectional plasticity, with various cell populations capable of transitioning to alternative tumour lineages depending on microenvironmental or genetic cues[24]. Importantly, evidence from patient-derived xenograft models supported the relevance of these findings in human bladder cancer. CD49f^{low} cells, identified as basal-like progenitor cells, exhibited the ability to generate both CD49f^{low} and CD49f^{hi} progeny, further underscoring lineage plasticity in human bladder cancer[24]. Additionally, a multi-omics single-cell atlas approach further highlighted the role of transcriptional plasticity in early oncogenesis, enabling identification of a TM4SF1-positive subpopulation with stem cell-like properties and dynamic transcriptional plasticity. This subpopulation, emerging from basal urothelial progenitors, undergoes EMT and contributes to aggressive tumour behaviour and metastasis by developing transcriptionally heterogeneous lineages[62].

Overall, these findings highlight the dynamic capacity of bladder cancer cells to undergo subtype switching, contributing to tumour heterogeneity, and emphasize the crucial role of lineage plasticity in bladder cancer biology.

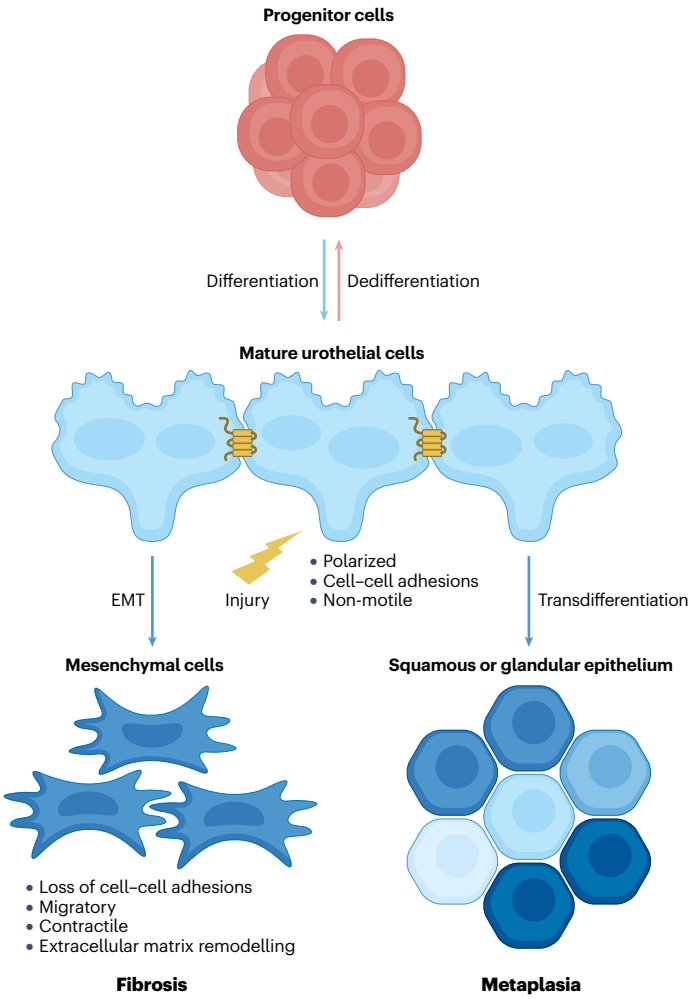

Fig. 2 | Manifestations of urothelial plasticity during bladder development and repair. Dedifferentiation: after bladder injury, mature urothelial cells exhibit remarkable plasticity by dedifferentiating back to a basal-like or progenitor-like state. Transdifferentiation: in response to chronic injury or inflammation, urothelial cells can change their cell fate and transform into squamous or glandular epithelium, leading to metaplastic changes. Epithelial-to-mesenchymal transition (EMT): urothelial cells lose their cell polarity and adhesion properties and acquire mesenchymal traits, gaining increased motility and fibroblast-like phenotype.

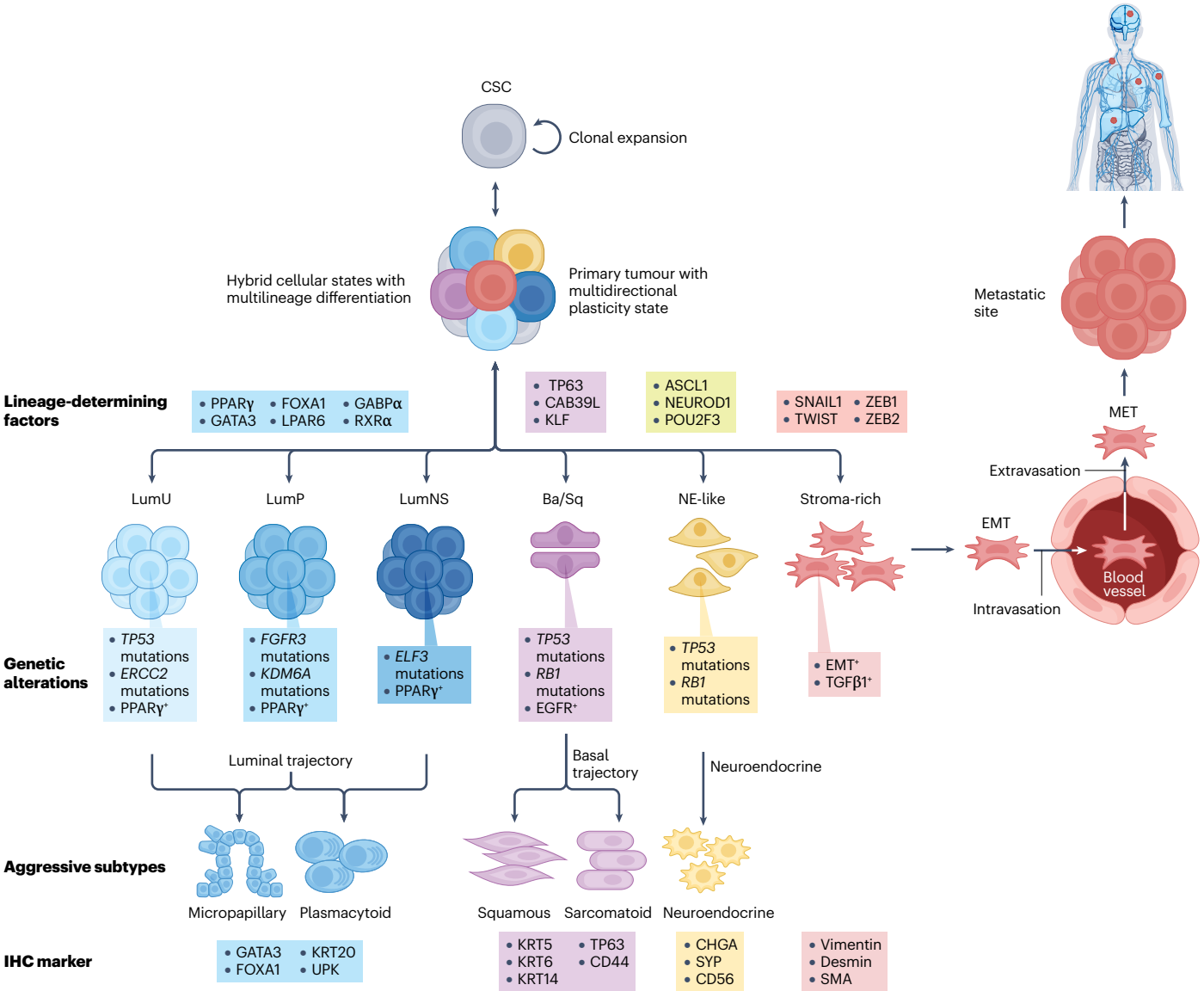

Fig. 3 | Lineage plasticity triggers the heterogeneity and aggressive nature of bladder cancer. During bladder tumour initiation, malignant cells emerge from a diverse array of lineage origins, including luminal papillary (LumP), luminal unstable (LumU), luminal non-specified (LumNS), basal/squamous (Ba/Sq), neuroendocrine-like (NE-like) and stroma-rich subtypes, each exhibiting remarkable cellular plasticity[24,48]. This inherent plasticity enables malignant epithelial cells to enter hybrid states with multilineage differentiation and acquire cancer stem cell (CSC)-like features. These transitional states can facilitate molecular subtype switching, contributing to intratumoural heterogeneity, histological diversity, progression and metastasis. In the process of epithelial-to-mesenchymal transition (EMT), mesenchymal cells in the surrounding stromal tissues enter the bloodstream (intravasation) and travel to distant organs where they extravasate, colonize and establish metastatic foci. Subsequently, at metastatic sites, mesenchymal-to-epithelial transition (MET) can occur, enabling cancer cells to revert to an epithelial phenotype, facilitating regrowth and secondary tumour formation. IHC, immunohistochemistry; KRT, keratin; UPK, uroplakin.

Plasticity in progression and metastasis

Cellular plasticity in bladder cancer primarily denotes the capacity of malignant epithelial cells to acquire mesenchymal and stem-like characteristics, which are pivotal for tumour progression and metastasis[3] (Fig. 3). Results of studies in which transplanted tumour fragments and xenografted cell lines were used have demonstrated that urothelial cancer stem cells (CSCs) have a central role in this process, and epithelial plasticity enables CSCs to respond dynamically to environmental cues, including those promoting EMT, facilitating shifts between epithelial and mesenchymal states[63]. Thus, EMT promotes a stationary-to-migratory phenotype transition in CSCs, empowering these cells to enter the circulation, extravasate and establish metastases[3,63]. EMT involves the loss of epithelial features and the acquisition of mesenchymal traits, promoting cancer cell migration. Conversely, metastatic colonization often requires a mesenchymal-to-epithelial transition (MET), enabling metastatic cells to revert to epithelial phenotypes and

contributing to metastatic recurrence[64]. Key regulatory factors, such as N-cadherin and zinc-finger transcription factors (such as SNAIL1 and TWIST), mediate these transitions, and their upregulated expression correlates with increased tumour aggressiveness and enhanced invasiveness[65]. scRNA-seq evidence suggests that EMT might not be a binary switch but instead a dynamic and reversible process that occurs through intermediate hybrid states that combine both epithelial and mesenchymal traits[16,66]. These hybrid states enhance the invasive potential of bladder cancer cells and underscore the complexity and dynamic nature of cellular plasticity in tumour evolution. Similarly, studies of bladder cancer cell lines have enabled identification of dynamic phenotypic states — holoclones, meroclones and paraclones — each with distinct proliferative capacities and plasticity. Holoclones possess stem-like features and high self-renewal potential, whereas meroclones and paraclones exhibit more-differentiated characteristics but retain the ability to transition back to stem-like states under certain conditions, reflecting dynamic phenotypic flexibility during tumour progression[67]. This work reveals how non-genetic plasticity, characterized by cell state transitions and phenotypic heterogeneity, has a crucial role in shaping bladder cancer progression, invasion and therapeutic adaptation, offering options to target plasticity in metastatic bladder cancer. Beyond EMT, cellular plasticity in bladder cancer also encompasses molecular subtype switching, whereby urothelial cells lose epithelial traits and adopt other new expression profiles (Fig. 3). This subtype switching contributes to the diversity of molecular signatures and clinical behaviour in bladder cancer. Basal tumours, often characterized by squamous differentiation, exhibit more aggressive disease than luminal tumours, which are typically papillary in morphology and show less-aggressive behaviour[55]. Despite maintaining similarities to non-malignant urothelial cells, bladder cancer cells can also differentiate into squamous, glandular, trophoblastic or Müllerian lineages, contributing to histological heterogeneity and aggressive behaviour[55–57].

Patient-derived bladder tumour organoids have provided insights into bladder cancer plasticity and subtype switching. These organoid models faithfully retain parental tumour heterogeneity while exhibiting luminal-to-basal transitions during in vitro culture. Remarkably, this phenotypic plasticity seems to be reversible, as luminal characteristics can be re-established in xenograft models, reflecting the inherent plasticity of bladder cancer cells during tumour evolution and adaptation[68]. This reversible subtype switching underscores the nonlinear and flexible nature of bladder cancer differentiation, driven by both microenvironmental cues and intrinsic plasticity programmes. In vivo transplantation models further demonstrate the presence of multiple lineage subtypes within bladder tumours, with dynamic lineage expression during tumour progression. Notably, the ability of luminal and mesenchymal cells to revert to basal phenotypes after progression highlights the potential for reverse EMT and the adaptability of tumour cells[24].

Analysis of matched primary and metastatic bladder tumours has provided new insights into the role of cellular plasticity in metastatic evolution. Notably, molecular subtype can influence metastatic recurrence patterns, and transformation can occur during metastasis. For example, subtype-specific metastatic tropism was identified using RNA-based and IHC-based classification, showing that Ba/Sq tumours favour lymph node metastases but rarely spread to bone, whereas urothelial-like tumours are enriched in bone metastases[69]. Genomically unstable tumours exhibit a propensity for atypical sites, such as the brain and central nervous system, but are under-represented in lung metastases[69]. These findings highlight a potential link between cellular plasticity and organotropism, suggesting that plasticity-driven adaptations might determine metastatic site preference. Interestingly, protein-based molecular subtypes (luminal, basal and neuroendocrine) remain largely stable between primary and metastatic tumours, but transcriptomic profiles often undergo dynamic shifts, probably owing to interactions with the metastatic microenvironment[70]. For example, stroma-rich metastases often display transcriptomic masking of luminal traits despite retaining luminal IHC markers such as CK20 and FOXA1, underscoring the need to combine RNA-based and IHC-based profiling to capture the full complexity of bladder tumour plasticity. Multi-omics analysis further highlighted the evolutionary trajectory of metastatic bladder cancer, revealing that primary tumour mutations (such as those in *FGFR3*, *TP53*, *RB1*) persist in metastatic lesions, but additional genomic and transcriptomic alterations emerge owing to selective pressures imposed by treatment and the metastatic niche[71]. This observation suggests that metastatic evolution is not solely a consequence of pre-existing subclonal diversity but also involves plasticity-driven reprogramming in response to external cues. Notably, in contrast to primary tumours, molecular subtypes in metastatic settings seem to exhibit weaker correlation with immune infiltration[71], raising important questions about the immune landscape of metastatic bladder cancer and the potential effects of plasticity-driven immune evasion.

Collectively, bladder cancer plasticity is a highly dynamic and reversible process that has a pivotal role in tumour progression and metastasis. Advances in single-cell and organoid-based models have substantially improved understanding of these processes, but further research is needed to translate these findings into clinically actionable interventions.

Plasticity and bladder cancer therapy

Despite advances in targeted therapies and immunotherapy, cisplatin-based chemotherapy remains the mainstay for unresectable and metastatic MIBC. Unfortunately, in most patients this disease eventually develops chemoresistance[72]. CSCs and mesenchymal-like cells are central to this resistance, exhibiting greater resilience to chemotherapy than more-differentiated tumour cells[73]. Notably, treatment can enrich CSC populations via symmetrical division or the conversion of non-CSCs into a CSC state, highlighting the dynamic nature of resistance[3,16,74]. This occurrence suggests that chemoresistance is not driven solely by clonal selection but also by non-genetic plasticity, enabling tumour cells to transition between CSC-like and differentiated states. However, emerging evidence suggests that most tumour cells could possess the capacity for CSC-like behaviour depending on environmental conditions.

Beyond the CSC perspective, lineage plasticity has been proposed as a fundamental mechanism of treatment resistance across multiple cancer types[16,17]. For example, prolonged EGFR inhibitor therapy in EGFR-mutant lung adenocarcinoma can induce a neuroendocrine small-cell phenotype, reflecting therapy-driven epithelial-to-neuroendocrine plasticity[75]. Similarly, anti-androgen treatment in luminal prostate cancer can promote neuroendocrine differentiation by driving lineage plasticity, contributing to therapy resistance and tumour progression[76]. These observations suggest that epithelial plasticity is not only a passive consequence of treatment but also an active adaptation mechanism that facilitates tumour survival and recurrence. In bladder cancer, chemotherapy-induced subtype transitions also suggest a potential role for epithelial plasticity in driving chemoresistance[77]. However, the underlying mechanisms that drive these transitions remain poorly understood, underscoring the need for further research to develop strategies to overcome plasticity-driven resistance.

In this context, investigations using an orthotopic MIBC mouse model with gene-edited organoids have provided crucial insights into plasticity-driven chemoresistance. A key finding was the identification of semi-squamatization, a partial squamous differentiation process linked to acquired chemoresistance in both mouse and human MIBC tumours[21]. Clonal barcoding assays demonstrated that tumour cells transition into chemoresistant states predominantly through lineage plasticity instead of clonal selection, highlighting the dynamic nature of cell fate changes under therapeutic pressure. Live-cell tracking also indicated increased squamous marker expression following chemotherapy, supporting the hypothesis that lineage plasticity contributes to treatment resistance. Importantly, patient-derived xenograft models validated these findings, displaying enhanced squamous characteristics and plasticity following chemotherapy. These results suggest that semi-squamatization is a crucial form of lineage plasticity that facilitates tumour adaptation and acquired chemoresistance. This discovery has important implications for therapeutic strategies, underscoring the need to target plasticity-driven pathways to overcome treatment resistance and improve clinical outcomes in MIBC[21].

Epithelial plasticity also underpins resistance to radiotherapy. The plasticity of bladder tumours during radiotherapy has been investigated, focusing on transcriptional state dynamics and their role in mediating transient resistance at the tumour cell population level[67]. Using the T24 bladder cancer cell line, the effects of irradiation on DNA damage and survival between mesenchymal and epithelial phenotype cells were compared. Notably, mesenchymal cells exhibited a more efficient DNA damage response than epithelial cells, enabling faster double-strand break repair and reduced replication errors following irradiation. This enhanced DNA repair capacity contributed to radioresistance. However, mesenchymal cells retained the ability to transition to epithelial-like states, maintaining a dynamic balance in transcriptional states within the tumour cell population. The scRNA-seq of irradiated and control colonies further revealed distinct transcriptional adaptations, with DNA damage response pathways being upregulated in epithelial cells, whereas homologous recombination pathways were more active in mesenchymal cells, enhancing their post-irradiation survival[67]. These findings suggest that radiation treatment drives tumour cell populations towards a transient equilibrium characterized by intermediate EMT states, complicating efforts to eradicate resistant tumour populations. Thus, targeting EMT-associated transcriptional reprogramming and DNA repair pathways might offer a promising strategy to enhance radiosensitivity in bladder cancer.

Clinical data further underscore the influence of epithelial plasticity on therapeutic outcomes and resistance. Cisplatin-based chemotherapy often induces subtype switching, reflecting dynamic tumour adaptability under therapeutic pressure. These transitions are linked to altered transcriptional programmes with increased expression of EMT markers (such as TWIST and ZEB1), basal markers (such as CK5 and CD44) and stem cell-related factors (such as SOX2 and NANOG), enhancing mesenchymal traits, invasiveness and survival[77,78]. Additionally, neoadjuvant chemotherapy induces proteomic and histological plasticity, such as reduced keratinization and increased ECM remodelling[79,80]. Similar trends have been observed in immunotherapy, whereby molecular subtypes influence the response to immune checkpoint inhibitors. For example, the genomically unstable Lund subtype and neuronal-like tumours exhibit more favourable responses to immunotherapy than tumours with highly plastic phenotypes that can develop immune evasion mechanisms[81,82].

Together, these findings underscore the crucial role of tumour plasticity in driving therapeutic resistance and emphasize the urgent need to develop strategies to target plasticity-related pathways, such as EMT inhibition, CSC eradication and immune modulation, to improve treatment outcomes. These findings collectively emphasize that epithelial plasticity is not merely a correlative phenomenon but also an active driver of therapeutic resistance in bladder cancer. The ability of tumour cells to undergo EMT, CSC transitions and lineage plasticity confers substantial survival advantages, enabling adaptation to chemotherapy, radiotherapy and immunotherapy. Improved mechanistic understanding of plasticity will be essential for the development of effective therapeutic strategies that can overcome tumour adaptability and improve patient outcomes.

Mechanisms regulating plasticity

Urothelial plasticity is governed by complex networks integrating intrinsic and extrinsic factors, including lineage-specific transcription factors, signalling pathways, epigenetic modifications and microenvironmental influences, which together regulate cell transitions and phenotypic adaptations (Fig. 4).

Lineage-specific transcription factors

Understanding the lineage-specific transcription factor profile in non-malignant bladder urothelial cells is essential to elucidate the urothelial differentiation programme and the molecular mechanisms that underlie urothelial plasticity. Studies involving differentiated urothelial cells, progenitor cells and embryonic models have enabled identification of several key lineage-specific transcription factors, including PPARγ, RAR–RXR, FOXA1, GATA3, KLF4, KLF5, GRHL3 and ELF3 (refs. 2,4,83,84) (Fig. 4). Under physiological conditions, these transcription factors control urothelial differentiation and maintain cellular identity, through formation of tightly regulated networks. However, in pathological states, disruptions in transcription factor regulation profoundly affect urothelial plasticity and the repair process. For example, PPARγ, a nuclear receptor expressed throughout the urothelium[85], is pivotal for regulation of urothelial differentiation. Activation of PPARγ promotes the differentiation of urothelial cells, organoids and pluripotent stem cells, which are induced to adopt a urothelial fate[45]. However, loss of PPARγ activity leads to chronic inflammation and basal cell misprogramming, resulting in squamous differentiation and impaired maturation of umbrella cells, particularly during urinary tract infections[83,86]. Conversely, ectopic expression of PPARγ in basal cells can reverse squamous phenotypes and induce terminal differentiation markers (KRT13, KRT20 and UPKs), without causing tumour formation[42]. However, under carcinogenic stress, such as exposure to *N*-butyl-*N*-(4-hydroxybutyl)-nitrosamine (BBN), PPARγ activation in basal progenitors drives luminal tumour formation[87]. Further investigations have revealed that overexpression of GATA3 and FOXA1 collaborates with PPARγ activation to facilitate the transdifferentiation of basal subtype to a luminal phenotype, indicating a complex regulatory network involving these transcription factors in determining luminal cell fate[88]. In bladder cancer, mutations and amplifications of *PPARG* are frequently identified in MIBC, resulting in the overexpression of PPARγ and its target gene[89]. Notably, these mutations and amplifications are particularly associated with luminal tumours characterized by an active *PPARG* regulon[59]. Moreover, a subset of luminal tumours seem to lose their canonical identity, acquiring basal features as *PPARG* expression declines[87], suggesting a role for PPARγ in maintaining luminal identity. Beyond bladder cancer, PPARγ

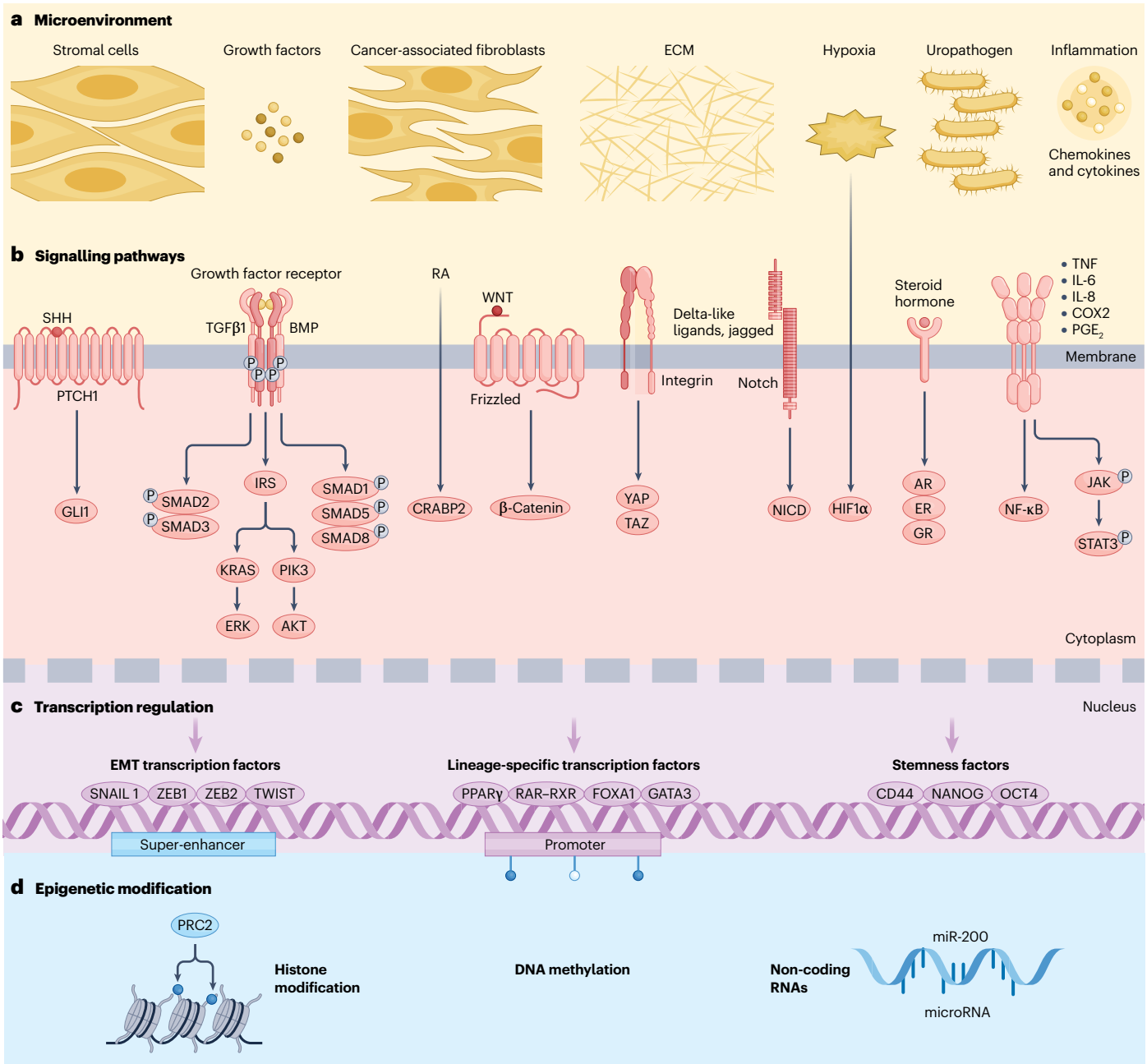

Fig. 4 | Molecular mechanisms regulating bladder urothelial plasticity. Urothelial plasticity is regulated by extracellular signals from the microenvironment and intrinsically, through lineage-specific transcription factors, signalling pathways and epigenetic modifications. Key signalling pathways, including TGFβ1, EGFR, FGF, sonic hedgehog (SHH), retinoic acid (RA), BMP4, NOTCH and WNT, drive urothelial development and plasticity, often through epithelial–mesenchymal communication with stromal cells or cancer-associated fibroblasts. Disruption of these pathways can induce epithelial-to-mesenchymal transition (EMT) and stem cell-like phenotypes, contributing to abnormal bladder repair and tumorigenesis. Hypoxia stabilizes HIF1 and HIF2, further promoting EMT and stemness, whereas pro-inflammatory cytokines such as COX2 enhance inflammation-driven plasticity through the JAK–STAT3 pathway. Steroid hormone signalling – especially mediated by androgen receptor (AR), oestrogen receptor (ER) and glucocorticoid receptor (GR) pathways – has a pivotal role in modulation of urothelial plasticity. These nuclear receptors influence cellular differentiation states, EMT and stemness properties. The extracellular matrix (ECM) also regulates plasticity by driving EMT transcriptional programmes and supporting stem-like traits through integrin–YAP signalling. These pathways affect transcriptional programmes regulated by key transcription factors involved in EMT (such as SNAIL1, ZEB1, ZEB2 and TWIST), lineage-specific subtype (for example, PPARγ, RAR–RXR, FOXA1 and GATA3) and stemness (for example, CD44, NANOG and OCT4). Their action can also be modulated by epigenetic mechanisms, including histone modifications, DNA methylation and non-coding RNAs (such as microRNAs). Collectively, these factors form a dynamic network that controls urothelial plasticity, influencing bladder development, repair and cancer. P, phosphate; PRC2, Polycomb repressive complex 2.

has also been shown to regulate epithelial plasticity in other epithelial tissues, such as breast cancer. Evidence has demonstrated that using a combination of PPARγ agonists and MEK inhibitors can promote transdifferentiation of breast cancer cells into postmitotic adipocytes. This adipogenic reprogramming is associated with the upregulation of PPARγ and C/EBPα, cytoskeletal rearrangement and lipid droplet accumulation[90]. This observation highlights the broader functional capacity of PPARγ as a transcriptional regulator capable of reprogramming malignant epithelial cells towards terminal differentiation. In the context of bladder cancer, it suggests that pharmacological activation of PPARγ might not only support maintenance of luminal identity but could also serve as a therapeutic strategy to induce differentiation and suppress plasticity-associated phenotypes.

The loss of developmental transcription factors that define cell lineage is a key driver of cell plasticity. A prominent example is the loss of luminal epithelial lineage factor FOXA1, which is essential to maintain urothelial cell identity[84]. This loss can facilitate squamous transdifferentiation and contribute to increased immunological heterogeneity in bladder cancer[22]. *FOXA1* mutations, present in ~5% of bladder cancers, are common in luminal tumours but are often absent in Ba/Sq or neuroendocrine tumours[91]. Mouse models further illustrate the effect of FOXA1 loss on subtype switching. Conditional inactivation of *Foxa1* and *Pten* in intermediate and/or luminal cells induces bladder cancer with squamous features and increased sensitivity to carcinogens[92]. Interestingly, ubiquitin–Cre-mediated ablation of *Foxa1* in the urothelium of mice results in sex-specific histological alterations, with male mice developing urothelial hyperplasia, whereas female mice exhibit keratinizing squamous metaplasia[84]. This observation underscores the influence of hormonal or epigenetic factors in dictating lineage plasticity. Similarly, in mammary glands, loss of luminal transcription factors (such as FOXA1 and GATA3) enhances basal transdifferentiation[93], mimicking basal-to-luminal plasticity seen in bladder cancer. Moreover, FOXA1 is essential for luminal differentiation in prostate cancer, and its loss induces neuroendocrine phenotypes[94]. FOXA1 preferentially binds to a non-canonical motif (GTAAAG/A), leading to altered transcriptional programmes that drive neuroendocrine lineage plasticity in advanced and metastatic prostate cancer. In KRAS-driven lung adenocarcinoma, FOXA1 collaborates with FOXA2 to mediate a pulmonary-to-gastric lineage switch[95]. This switch involves FOXA1 and FOX2-dependent epigenetic reprogramming, characterized by DNA demethylation, histone H3K27 acetylation (H3K27ac) and altered 3D chromatin interactions. These findings collectively emphasize the central role of FOXA1 as a lineage-defining transcription factor across multiple epithelial tissues. In the context of bladder cancer, its loss not only facilitates subtype switching, but also enables increased cellular plasticity, immune evasion and therapeutic resistance[22]. The cross-cancer evidence suggests that modulation of FOXA1 activity — or restoration of its regulatory network — could be a viable strategy to stabilize epithelial identity and limit malignant plasticity in urothelial carcinoma.

The acquisition of new transcription factor activities in bladder cancer often coincides with the activation of stem cell pluripotency factors such as CD44, NANOG and OCT4, as well as EMT regulators such as SNAIL1, ZEB1, ZEB2 and TWIST[3,20] (Fig. 4). These stemness factors drive dedifferentiation into CSC-like states and promote transdifferentiation, exemplified by the role of CD44 in epithelial–mesenchymal plasticity[96]. Consequently, pluripotency transcription factors orchestrate a complex interplay of transdifferentiation and dedifferentiation, fostering highly plastic cellular states. Additionally, EMT-associated transcription factors further enable epithelial cells to acquire mesenchymal traits by repressing epithelium-specific genes, such as *CDH1* (encodes E-cadherin), while activating mesenchymal genes such as *CDH2* (encodes N-cadherin) and *VIM* (encodes vimentin)[3,17,97]. This dual regulation underscores the intricate molecular pathways that underpin lineage plasticity and the dynamic nature of cellular identity in the context of cancer.

In addition to the definitive binary lineage switching, cancer cells frequently adopt highly plastic hybrid states, wherein the interplay and opposing actions of various transcription factors serve as crucial mediators[17,98]. For example, single-cell analyses revealed that human MIBCs harbour individual epithelial cells that exhibit gene expression patterns characteristic of multiple mRNA subtypes[24]. These cells can simultaneously or independently express high levels of markers associated with basal, luminal, EMT and claudin states, thereby indicating the coexistence of tumour cells within both epithelial and mesenchymal-like transcriptional states, along with bidirectional transitions occurring within and between tumour subclones[24,67]. Similar hybrid states have been observed in prostate cancer models undergoing epithelial–neuroendocrine transdifferentiation, in which transcription factors associated with luminal epithelial identity co-express with neuronal and stemness transcriptional programmes[99].

Beyond the gain or loss of transcription factor functions, the modulation of transcription factor expression and activity is crucial in facilitating cell lineage plasticity. A whole-organ mapping strategy has highlighted the roles of LPAR6 and CAB39L as essential regulators of urothelial differentiation, functioning as upstream modulators of luminal transcription factors (such as GATA3 and PPARγ) and basal transcription factors (such as TP63)[25]. Experimental models demonstrate that silencing *LPAR6* and *CAB39L* disrupts the basal-to-luminal differentiation programme, leading to hyperplastic changes characterized by distinct luminal and basal subtypes, respectively. This dysregulation sensitizes the urothelium to BBN-induced carcinogenesis, recapitulating luminal and basal subtypes observed in human bladder cancers[25]. Furthermore, GABPα has also been identified as a crucial upstream activator of *FOXA1* and *GATA3* transcription in bladder cancer, with a pivotal role in orchestrating luminal differentiation while simultaneously suppressing stem cell characteristics and invasive potential[100]. Notably, *GABPA* expression shows a positive correlation with luminal molecular signatures and improved patient survival, underscoring its importance in bladder cancer progression and outcomes[100]. However, whether modulating GABPα activity can stably reprogramme tumour cells remains an open question.

Taken together, these findings suggest that the transcriptional programme that governs urothelial plasticity is severely dysregulated under pathological conditions, emphasizing the crucial role of transcription factors as both guardians of tissue homeostasis and facilitators of pathological transformation. However, instead of being dictated by a single master regulator, plasticity emerges from the interplay of multiple transcription factors, signalling pathways and epigenetic modifications.

Signalling pathways

Urothelial plasticity is tightly regulated by a complex network of signalling pathways essential for development and regeneration, including FGF, retinoic acid, BMP, NOTCH and WNT signals, many of which involve epithelial–mesenchymal communication[4,19] (Fig. 4). Importantly, these signals can precisely target specific progenitor populations under various injury conditions, enabling tailored regenerative responses while minimizing aberrant repair such as fibrosis, squamous metaplasia or

tumorigenesis. This capacity to direct distinct cellular programmes is central to both effective tissue repair and the prevention of pathological plasticity. For example, FGF signalling is crucial for urothelial stratification during embryogenesis, with its loss resulting in the absence of intermediate bladder cells[101]. Results of functional studies demonstrate that FGF administration stimulates basal urothelial proliferation in vitro, whereas FGFR deficiency leads to increased cell cycle activity and pathological endoreplication of KRT14$^+$KRT5$^+$ basal cells[2,102]. Similarly, WNT signalling promotes KRT14$^+$ basal cell proliferation after chemical injury[29], highlighting its role in injury-induced regeneration. Also, BMP4 signalling, which is active in the sub-urothelial stroma[103], regulates KRT5$^+$ cell proliferation during infection[104], raising the possibility that different signalling pathways coordinate responses based on the nature of injury. This signalling specificity underscores the importance of tightly controlled plasticity, as inappropriate pathway activation can shift repair towards maladaptive lineages, contributing to disease progression or malignant transformation. Understanding these context-dependent responses might help to identify therapeutic points of intervention to promote regeneration while limiting pathological plasticity in bladder disorders.

Beyond homeostasis, abnormal alterations in signalling pathways also substantially contribute to bladder-related diseases and tumorigenesis. Pathways such as TGFβ1, EGFR, FGF, retinoic acid, NOTCH, SHH and WNT can facilitate epithelial–mesenchymal plasticity and stem cell-like phenotypes during bladder injury and cancer progression[3,4,20] (Fig. 4). Among these pathways, NOTCH signalling is particularly notable for its context-dependent effects. Under healthy conditions, NOTCH signalling, active across urothelial layers during homeostasis, is crucial for luminal cell differentiation. Its inhibition leads to TP63 upregulation and a reduction in luminal markers[26]. Inducible tissue-specific inactivation of *Notch* in mouse urothelium results in hyperplasia, inflammation and mucosal sloughing, ultimately compromising barrier function and correlating with interstitial cystitis in humans[105]. In bladder cancer, NOTCH pathway mutations (for example, in *NOTCH1*, *NOTCH2*, *NOTCH3*, *NCSTN* and *PSEN1*) are frequent and particularly associated with Ba/Sq tumours, underscoring its plasticity-regulating role[106,107]. Mice with NOTCH pathway inactivation show increased carcinogen-induced bladder tumours with squamous features and reduced survival[106]. Additionally, studies indicate that retinoic acid signalling is necessary to suppress squamous differentiation and promote urothelial cell identity, whereas inhibition of retinoic acid signalling impairs urothelial specification and results in squamous metaplasia[108]. Similarly, vitamin A (the inactive precursor of retinoic acid) deficiency leads to squamous metaplasia in bladder urothelium, a risk factor for squamous cell carcinoma[109]. Moreover, *RXRA* hotspot mutations, present in ~5% of MIBCs, are predominantly found in the luminal subgroup[91], suggesting a potential role for retinoic acid signalling in maintaining luminal differentiation. However, direct evidence of RAR–RXR heterodimers regulating luminal cell identity remains lacking. Further studies are needed to clarify whether *RXRA* mutations confer a selective advantage by altering urothelial differentiation states or by modulating interactions with other signalling pathways.

Emerging evidence indicates that steroid hormone signalling, particularly the androgen receptor and oestrogen receptor pathways, has a crucial role in urothelial plasticity and bladder cancer progression[110,111]. Androgen receptor signalling interacts with EMT-associated transcription factors such as ZEB1, TWIST and SNAIL to suppress epithelial markers (such as E-cadherin) while promoting mesenchymal traits (for example, N-cadherin and vimentin)[112]. Additionally, androgen receptor signalling amplifies TGFβ1-induced EMT and activates WNT–β-catenin signalling, facilitating CSC-like phenotypes[113]. Interestingly, similar mechanisms are observed in prostate cancer, in which androgen receptor inhibition through androgen deprivation therapy leads to the loss of luminal markers (such as FOXA1) and increased EMT-associated transcription factors driving epithelial–neuroendocrine transition and EMT[94]. This observation suggests a broader paradigm in which androgen receptor signalling maintains epithelial differentiation across multiple tissues, and its loss enables plasticity and tumour aggressiveness.

Conversely, elevated oestrogen receptor expression has been observed in metaplastic tissues, suggesting a role in squamous differentiation and keratinization[114]. Reduced progesterone receptor expression in invasive carcinoma further suggests that the loss of protective hormone signalling could contribute to malignant transformation[114]. Additionally, glucocorticoid receptor signalling has been linked to EMT regulation in aggressive sarcomatoid urothelial carcinoma[23]. Evidence from organoid studies demonstrates that glucocorticoid receptor activation with glucocorticoids such as dexamethasone reverses EMT, reduces invasion and restores epithelia-like features[23].

Collectively, these findings underscore the intricate network of hormone-driven signalling pathways that govern bladder urothelial plasticity and identify potential therapeutic targets for bladder diseases.

Epigenetic mechanisms

The epigenome coordinates spatiotemporally specific gene expression during development and adulthood, for the maintenance of tissue homeostasis and cellular identity[115]. In the bladder urothelium, epigenetic mechanisms such as histone modifications, DNA methylation, chromatin remodelling and non-coding RNAs regulate lineage-specific gene expression, influencing bladder repair and carcinogenesis[19,116] (Fig. 4).

In bladder urothelium, epigenetic pathways, particularly the Polycomb repressive complex 2 (PRC2), are pivotal for cell specification and progenitor differentiation[117]. PRC2 subunits, such as EZH2 and EED, catalyse H3K27me3 histone modifications, thereby repressing differentiation-associated genes and sustaining progenitor cell states[118]. Results of functional studies have demonstrated that EZH2 deletion induces UPK3A$^+$ superficial cell expression, whereas EED and EED–EZH2 double-mutant models show delayed superficial cell differentiation[117]. Furthermore, loss of EED in embryonic urothelial progenitors reduces proliferation and dysregulates gene expression (such as of *CDKN2A*, *CDKN2B* and *SHH*), leading to premature differentiation of KRT5$^+$ basal cells and ectopic expression of squamous markers. EED also sustains the proliferative and regenerative potential of adult urothelial progenitors, preventing precocious differentiation, whereas mutants exhibit squamous differentiation and downregulation of key urothelial differentiation pathways, including SHH, retinoic acid and PPARγ[117].

In bladder cancer, mutations in chromatin-modifying genes — particularly histone methyltransferases and demethylases — are highly prevalent, more so than in other cancer types, underscoring the crucial role of epigenetic alterations[89]. These modifications, in concert with subtype-specific genes, drive cancer cell plasticity and neoplastic subtype formation. Histone modifications, particularly the formation of bivalent chromatin states, contribute to cancer cell plasticity by keeping lineage-specific genes transcriptionally 'poised'. This chromatin state, characterized by co-enrichment of active marks (such as H3K4me3) and repressive marks (such as H3K27me3), facilitates rapid cell state transitions[115]. A comprehensive genome-wide analysis provided strong evidence to support this model in primary bladder

tumours, revealing distinct histone methylation patterns between basal and luminal subtypes, predominantly localized to enhancer regions[119]. Specifically, luminal tumours exhibited H3K4me1 peaks enriched for PPARγ and RXRα binding motifs, reinforcing the role of PPARγ in luminal subtype regulation and the importance of enhancer-mediated epigenetic control in cancer cell plasticity and subtype switching[119]. Similarly, in prostate cancer, androgen withdrawal increases the H3K27me3-to-H3K4me3 ratio at epithelial genes and decreases it at neuronal genes, driving neuroendocrine differentiation[120]. These findings suggest that targeting enhancer-mediated epigenetic regulation could offer novel therapeutic avenues for subtype-specific interventions.

An integrated approach combining genome-wide mapping to investigate subtype-specific regulation in bladder cancer was subsequently undertaken[121]. Distinct enhancer landscapes and specific open chromatin patterns enriched for lineage-specific transcription factor motifs for luminal and basal subtypes, such as *GRHL2*, *TP53* and *TP63* in luminal cells, and *TEAD1*, *TEAD4* and *KLF* factors in basal cells, were identified. Further analysis of distal enhancers revealed the involvement of luminal-specific transcription factors such as FOXA1 and GATA3 in regulating gene expression associated with luminal differentiation, whereas 3D chromatin landscape analysis demonstrated subtype-specific chromatin loops, with more enhancer–promoter interactions in luminal models than in basal models[121]. Moreover, the first comprehensive epigenetic map of bladder cancer has been generated[122]. This work showed distinct super-enhancer activation patterns specific to basal and luminal subtypes, with key master transcription factors such as FOXA1 driving subtype-specific transcriptional programmes. Functional studies using CRISPR–Cas9 to mutate *FOXA1* demonstrated a shift from luminal to basal phenotypes, further implicating FOXA1 in luminal identity maintenance[122]. These enhancer landscapes are reminiscent of those observed in prostate and breast cancers, in which lineage-specific enhancers regulate subtype identity and plasticity[12,17]. These findings reinforce the notion that each bladder cancer subtype is not merely defined by genetic alterations but is driven by distinct epigenetic and transcriptional networks, offering new insights into the molecular regulation of this disease and potential therapeutic targets.

Beyond histone modifications, DNA methylation patterns have an important role in bladder cancer stratification. Results of an important study based on The Cancer Genome Atlas database revealed that 34% of bladder tumours exhibit a CpG island methylator phenotype, prompting further investigation into the relationship between DNA methylation and molecular subtype[89]. Analysis of multilevel genomic data enabled identification of three distinct DNA methylation patterns linked to clinicopathological features and gene expression subtypes[123]. Importantly, a methylation-driven epigenetic switch at the *HOXA–HOXB* loci was discovered, linked to tumour differentiation and aggressiveness, exhibiting subtype-specific expression. This switch was associated with retinoic acid-responsive genes, which demonstrated coordinated changes in promoter methylation and mRNA expression, consistent with retinoic acid as a key mediator of urothelial differentiation[109,123]. This epigenetic switch, involving coordinated changes in promoter methylation and mRNA expression, parallels findings in triple-negative breast cancer, in which TET1-mediated demethylation enhances self-renewal and CSC expansion[124]. Such observations highlight the pivotal role of dynamic DNA methylation remodelling in enabling tumour cell plasticity. This plasticity underlies reversible shifts between differentiated and stem-like states, influencing tumour progression and therapeutic response.

Integrative studies have enabled further dissection of DNA methylation interplay with chromatin accessibility in bladder cancer. Assay for transposase-accessible chromatin using sequencing (ATAC-seq), combined with DNA methylation, and gene expression data, were used to uncover subtype-specific regulatory patterns in bladder cancer[125]. Neuronal subtypes exhibited the lowest DNA methylation in neuronal regulatory regions but showed hypermethylation in non-neuronal regions. Notably, neuronal active regulatory regions were associated with β-catenin and TCF and LEF family target genes, such as *NKD1*, a WNT signalling inhibitor that was hypomethylated and upregulated in this subtype[125]. These findings highlight aberrant β-catenin and WNT activation as drivers of neuronal differentiation in bladder cancer. In parallel, results of experimental studies have demonstrated that *FOXA1* inactivation, associated with squamous differentiation and the basal subtype, is mediated by promoter hypermethylation. DNA methyltransferase inhibitors can reverse this process, restoring *FOXA1* expression[92]. Similarly, decitabine, a DNA-demethylating agent, can induce hypomethylation of the *NOTCH1* promoter, leading to increased NOTCH1 expression and reduced basal marker KRT5 levels[126].

MicroRNAs (miRNAs) add another layer of epigenetic regulation by targeting EMT-associated transcription factors and plasticity-related pathways[17,20]. For example, the miR-200 family functions as a crucial suppressor of EMT in bladder cancer by repressing ZEB-mediated transcriptional reprogramming[127]. Notably, miRNA dysregulation has also been implicated in CSC maintenance and therapy resistance, suggesting that targeting miRNA networks could be an effective strategy to modulate tumour plasticity[73].

Furthermore, the importance of genetic and non-genetic factors needs to be considered in understanding the plasticity and progression of bladder cancer[128]. Genetic instability, characterized by mutations that affect DNA repair, chromatin remodelling and transcriptional regulation, drives genomic diversity, leading to clonal evolution. Simultaneously, non-genetic instability, manifested as reversible phenotypic switches between epithelial and mesenchymal states or CSC-like traits, has an equally crucial role by promoting heterogeneity within clonal populations.

Together, these instabilities enable tumour cells to navigate an irregular epigenetic landscape, transitioning between stable attractors that define distinct cancer cell phenotypes[128]. In the context of bladder cancer, compelling evidence for the interplay between genetic and epigenetic instability was provided by mapping the evolution of bladder carcinogenesis through whole-organ histological and genomic profiling[129]. The findings demonstrate that bladder cancer arises from early field effects — subtle molecular alterations in seemingly healthy urothelium — which eventually evolve into distinct luminal and basal subtypes driven by unique genetic and epigenetic landscapes[129]. These findings further reinforce the notion that bladder cancer does not arise from a singular initiating mutation but from a stepwise accumulation of genetic and epigenetic disruptions, ultimately culminating in clonal expansion and intratumoural heterogeneity.

Microenvironment

Urothelial plasticity is also profoundly regulated by the surrounding microenvironment, which modulates cell fate through inductive and inhibitory signals from adjacent mesenchyme and stroma[4,13,46,130] (Fig. 4). Evidence from tissue recombination studies has highlighted the importance of stromal interactions in urothelial plasticity, with basal urothelial cells secreting SHH to activate stromal WNT signalling,

promoting urothelial proliferation and regeneration[29,34,131]. However, SHH gradients also influence mesenchymal cell fate, directing stromal cells towards smooth muscle or subepithelial mesenchyme[35]. This bidirectional communication is essential for urothelial proliferation and differentiation during bladder development and repair. The extracellular matrix (ECM), composed primarily of collagen and laminin, provides both structural and biochemical cues that regulate urothelial integrity and plasticity[132]. Beyond its role as a scaffold, the ECM actively modulates cellular adhesion, migration and differentiation through integrin-mediated interactions[105,133]. Notch signalling in mice further regulates cell–cell and cell–ECM communication, maintaining urothelial integrity and mediating repair. Its inactivation causes structural defects and inflammation, resembling bladder pain syndrome, whereas reactivation restores barrier function[105]. This phenomenon raises important therapeutic considerations such as whether targeting NOTCH or ECM–integrin interactions could restore healthy urothelial differentiation and mitigate inflammation-driven plasticity.

The ECM also modulates cancer cell plasticity through biophysical cues that influence key transcriptional programmes[134] (Fig. 4). For example, bladder cancer cells exhibit a highly plastic phenotype in 3D tissue-like cultures, in which malignant traits such as invasion can be suppressed by specific ECM components[133]. Mechanically, integrin-mediated mechanotransduction activates plasticity-associated regulators such as ZEB1 and YAP, driving EMT-associated transcriptional programmes, and enhances stem-like traits through the COX2–PGE_2–SOX2 axis, linking ECM signalling to cancer cell plasticity[74]. Similar ECM-mediated plasticity mechanisms have been reported in breast and pancreatic cancers, in which matrix stiffness enhances stemness via YAP–TAZ mechanotransduction signalling[135]. These findings reinforce the notion that the ECM is not merely a passive component of the TME but an active driver of tumour progression.

In bladder cancer, the TME also considerably influences cancer cell lineage plasticity via paracrine signalling, mechanical cues and metabolic reprogramming (Fig. 4). Growth factors secreted by cancer-associated fibroblasts (CAFs), such as HGF, EGF, TGFβ1, SHH and FGF, profoundly affect transcriptional regulators of cell plasticity by activating EMT-associated transcription factors such as SNAI1, SNAIL2, ZEB1, ZEB2 and TWIST[136,137]. These transcription factors trigger transcriptional programmes linked to stemness and EMT plasticity, enhancing aggressiveness in bladder cancer cells[3,17,130]. Results from an scRNA-seq study revealed a unique subpopulation of bladder cancer-associated fibroblasts overexpressing *SLC14A1*, induced by interferon signalling, that promote bladder cancer cell stemness through the WNT5A paracrine pathway[138]. This CAF-driven modulation of EMT and cancer plasticity is not unique to bladder cancer. Similar paracrine mechanisms have been observed in liver cancer, in which HGF release by CAFs induces FRA1, which, in turn, activates NOTCH signalling via HEY1, enhancing cancer cell plasticity and stem-like properties[139], underscoring the conserved role of TME-mediated plasticity across different cancer types.

Another major component of the microenvironment — hypoxia — further induces urothelial plasticity by stabilizing HIF1α, a master regulator of cellular responses to low oxygen levels[140,141]. HIF1α activates EMT mediators and enhances epithelial–mesenchymal plasticity, increasing invasive and metastatic potential[142,143] (Fig. 4). Hypoxic cancer cells also exhibit increased VEGF, ZEB1 and MCT1 expression, along with suppressed E-cadherin, promoting angiogenesis and metastasis[140,144,145]. Beyond bladder cancer, hypoxia-induced HIF1α interacts with FOXA2 in prostate cancer, driving neuroendocrine plasticity and metastasis, illustrating a broader relevance of hypoxic adaptation in shaping tumour cell identity[146].

Inflammation is another crucial microenvironmental factor linked to urothelial plasticity. Chronic inflammatory and recurrent infections can induce squamous differentiation in bladder urothelium, a well-documented precursor to bladder carcinogenesis[40]. The epigenetic and transcriptional mechanisms that underlie the inflammation–plasticity relationship remain to be fully understood, but emerging evidence suggests that pro-inflammatory chemokines and cytokines (such as TNF, IL-6 and IL-8) activate key transcriptional regulators such as NF-κB, JAK–STAT3 and NOTCH[147] (Fig. 4), which enhance stemness and plasticity. For example, the COX2–PGE_2 pathway is implicated in CSC repopulation and inflammation-related carcinogenesis through JAK2–STAT3 signalling[148,149]. Notably, evidence has shown that nuclear COX2 localization correlates with upregulated OCT3, OCT4 and CD44 in bladder cancer, indicating a role for COX2 in inflammation-driven stemness[150]. In parallel, studies on *Stat3*-transgenic mice have shown that *Stat3* activation bypasses noninvasive tumour stages, driving invasive cancer and enriching stem cell populations[151]. Comparable JAK–STAT signalling mechanisms in prostate cancer promote NE-like and stem-like phenotypes, facilitating resistance to androgen receptor-targeted therapies[76]. This observation underscores the broader implication that JAK–STAT3-driven plasticity not only accelerates tumour progression and cellular dedifferentiation across multiple cancer types but also poses a considerable therapeutic challenge by promoting treatment-resistant subpopulations.

Evidence also implicates microbial infections as novel modulators of urothelial plasticity. In bladder cancer, *E. coli*, a common uropathogen, can foster epithelial–mesenchymal plasticity and stemness-like phenotype through metabolic reprogramming[152]. This finding is particularly compelling because it suggests an unrecognized dimension of TME-mediated plasticity regulation. Whether microbial infections could serve as an initial trigger for urothelial reprogramming, creating the conditions for carcinogenesis, is an apt question. Further studies are needed to determine whether targeting infection-induced metabolic changes could provide new preventive strategies against bladder cancer initiation.

Crucially, the interplay between the TME and cancer cell plasticity is bidirectional and dynamic, with subtype transitions substantially reshaping the TME and, in turn, influencing cancer cell behaviour. For example, squamous transdifferentiation of pancreatic cancer cells induces the reprogramming of pancreatic stellate cells into inflammatory CAFs, which, in turn, secrete pro-inflammatory factors that further enhance cancer cell plasticity[153]. Similarly, in bladder cancer, Ba/Sq subtype tumours exhibit extensive CAF activation, ECM remodelling and immune infiltration[48], which support epithelial–mesenchymal plasticity and enhance tumour invasion and metastasis.

Collectively, these findings highlight the intricate and multifaceted role of the microenvironment in regulating urothelial plasticity, both in healthy tissue homeostasis and in cancer progression. The convergence of stromal interactions, ECM remodelling, inflammatory signalling and hypoxic adaptation creates a permissive landscape for plasticity-driven tumour evolution. Notably, these mechanisms are conserved across multiple cancer types, underscoring the broad relevance of TME-mediated plasticity beyond bladder cancer and providing insights for potential therapeutic interventions targeting cancer cell plasticity and the TME.

Implications and future directions

The capacity of urothelial cells to adopt alternative phenotypes is fundamental to both tissue regeneration and disease progression. Advances in understanding urothelial plasticity have highlighted its dual role: supporting repair after injury, whereas when dysregulated, driving maladaptive responses such as fibrosis, metaplasia and cancer[2,7,14,25,40]. The ability of urothelial cells to transition between distinct states underpins both healthy healing and pathological processes. Elucidation of the mechanisms that govern these dynamic transitions provides a foundation for development of therapeutic strategies to modulate plasticity in benign and malignant bladder conditions, offering new avenues for targeted intervention.

Potential applications of plasticity

Urothelial plasticity underpins the pathogenesis and repair mechanisms of various bladder diseases, including interstitial cystitis, fibrosis and inflammation. This plasticity is essential for tissue regeneration, but its dysregulation can lead to maladaptive repair processes, including metaplasia and fibrosis, which compromise bladder function. Therapeutic modulation of epithelial plasticity holds promise for reversal of maladaptive repair processes and improvement of outcomes in these conditions (Fig. 5).

In response to injury, urothelial plasticity facilitates repair through normal differentiation and proliferation programmes. However, inflammation or mechanical stress can disrupt this process, leading to metaplasia and EMT-mediated fibrosis owing to the inherent plasticity of bladder urothelium[14,40]. Metaplastic changes, such as keratinizing squamous metaplasia, are strongly associated with bladder cancer and require long-term surveillance[43]. Other forms, such as intestinal metaplasia, carry uncertain malignancy risks but also necessitate monitoring[44]. Chronic fibrosis impairs bladder compliance, reduces capacity and causes urinary retention, considerably affecting urinary function[154]. Mechanistic insights reveal that lineage-specific transcription factors, signalling pathways (for example, TGFβ1) and epigenetic factors, along with the microenvironment, regulate urothelial plasticity and maladaptive remodelling. Targeting these mechanisms offers therapeutic potential for various bladder diseases (Fig. 5). For example, results of preclinical studies suggest that activation of pathways such as PPARγ or EGFR could restore normal urothelial differentiation and function[45]. Blockade of pro-inflammatory cytokines, including TGFβ1 and TNF, could prevent urothelial cells from adopting a mesenchymal phenotype, thereby reducing fibrosis and inflammation[41]. Small molecules or biologics designed to inhibit EMT and enhance urothelial integrity could alleviate symptoms in conditions such as interstitial cystitis and BOO. Moreover, therapeutic strategies aimed at prevention or reversal of squamous metaplasia could potentially reduce the risk of bladder cancer. Inhibition of key plasticity-associated pathways, such as EGFR, or the use of differentiation therapies to revert squamous differentiation could also reduce the risk of bladder cancer[42] (Fig. 5). Thus, targeting the regulatory networks of epithelial plasticity presents a promising therapeutic strategy for a range of bladder disorders, potentially reversing maladaptive repair responses and improving patient prognosis.

Urothelial plasticity also holds promise for regenerative therapies in end-stage bladder diseases. Current surgical approaches, such as ileocystoplasty and cystectomy with urinary diversion using bowel, face limitations owing to the inability of the bowel epithelium to withstand prolonged urine exposure, leading to complications such as electrolyte imbalance, infections and malignancy[155]. Consequently, tissue engineering efforts aim to generate autologous urothelial cells for bladder reconstruction[156]. Advances in urothelial culture techniques, such as serum-free and enzyme-free media, have enabled the expansion of human urothelial cells for up to 16 passages, but these cells often fail to form functional barriers, highlighting the need for progenitor cells with the ability to generate all urothelial layers[157]. Research on lineage transcription factors has increased understanding of urothelial differentiation, with evidence pointing to a basal progenitor population marked by KRT5 and TP63 expression[4,131,158]. Emerging technologies, including Cre–LoxP recombination, CRISPR–Cas9 and organoids, are enabling precise in vivo and in vitro studies of urothelial progenitors[2,26]. scRNA-seq has revealed regulatory networks governing urothelial cell identity[9]. However, the precise identification of progenitor cells remains elusive, underscoring the complexity of urothelial regeneration and the need for optimized differentiation protocols in tissue engineering.

Evidence has revealed the remarkable plasticity of urothelial cells, showing that urothelium, under optimized culture conditions, can undergo dedifferentiation, acquire basal-like properties and form functional urothelial barriers, opening new avenues for tissue regeneration[8] (Fig. 5). These findings challenge traditional hierarchical models of urothelial regeneration, suggesting that regeneration is not limited to a specific progenitor population. This possibility has important implications for urothelial tissue engineering, suggesting that cultivating the entire urothelial cell population might be more efficient than selecting specific progenitor cells. However, urothelial regeneration might be compromised in disease states, as cells from diseased bladders exhibit limited proliferative capacity[159]. Additionally, efforts to generate urothelial cells from non-urothelial stem cell sources, such as adipose-derived stem cells, urine-derived stem cells, amniotic fluid stem cells and induced pluripotent stem cells, offer promising therapeutic avenues for urothelial regeneration[19]. In summary, these advances in urothelial plasticity and stem cell technologies provide exciting opportunities to improve regenerative therapies in urology.

Strategies for targeting plasticity

Modulation of lineage plasticity in bladder cancer remains a key therapeutic challenge owing to the dynamic and adaptive nature of tumour cells[24,71]. Current approaches to modulation of lineage plasticity are diverse, including transcriptional regulation, epigenetic therapies, TME modulation and differentiation therapies (Fig. 5). Lineage-specific transcription factors have a pivotal role in regulation of epithelial plasticity, making them attractive therapeutic targets. For example, PPARγ and RXRα are integral to maintaining cellular homeostasis and driving tumorigenesis and subtype switching through transcriptional regulation[45,87]. Pharmacological or genetic inhibition of PPARγ has been shown to reduce bladder cancer cell proliferation, migration and invasion, particularly in tumours with *PPARG* amplification or *RXRA* mutations, positioning PPARγ as a potential therapeutic target for luminal subtypes[59,87]. However, directly targeting most oncogenic transcription factors remains challenging owing to their undruggable structures. Advances in drug development, such as proteolysis-targeting chimeras (PROTACs) and molecular glues, offer new avenues to address these challenges[160]. PROTACs are small, bifunctional molecules that induce target protein degradation via E3 ubiquitin ligase recruitment, and they have been used to successfully target oncogenic proteins, including transcription factors such as androgen and oestrogen receptors[161]. Similarly, molecular glues, which enhance protein–protein interactions to promote degradation, are showing promise in clinical trials for prostate and breast cancer[162,163].

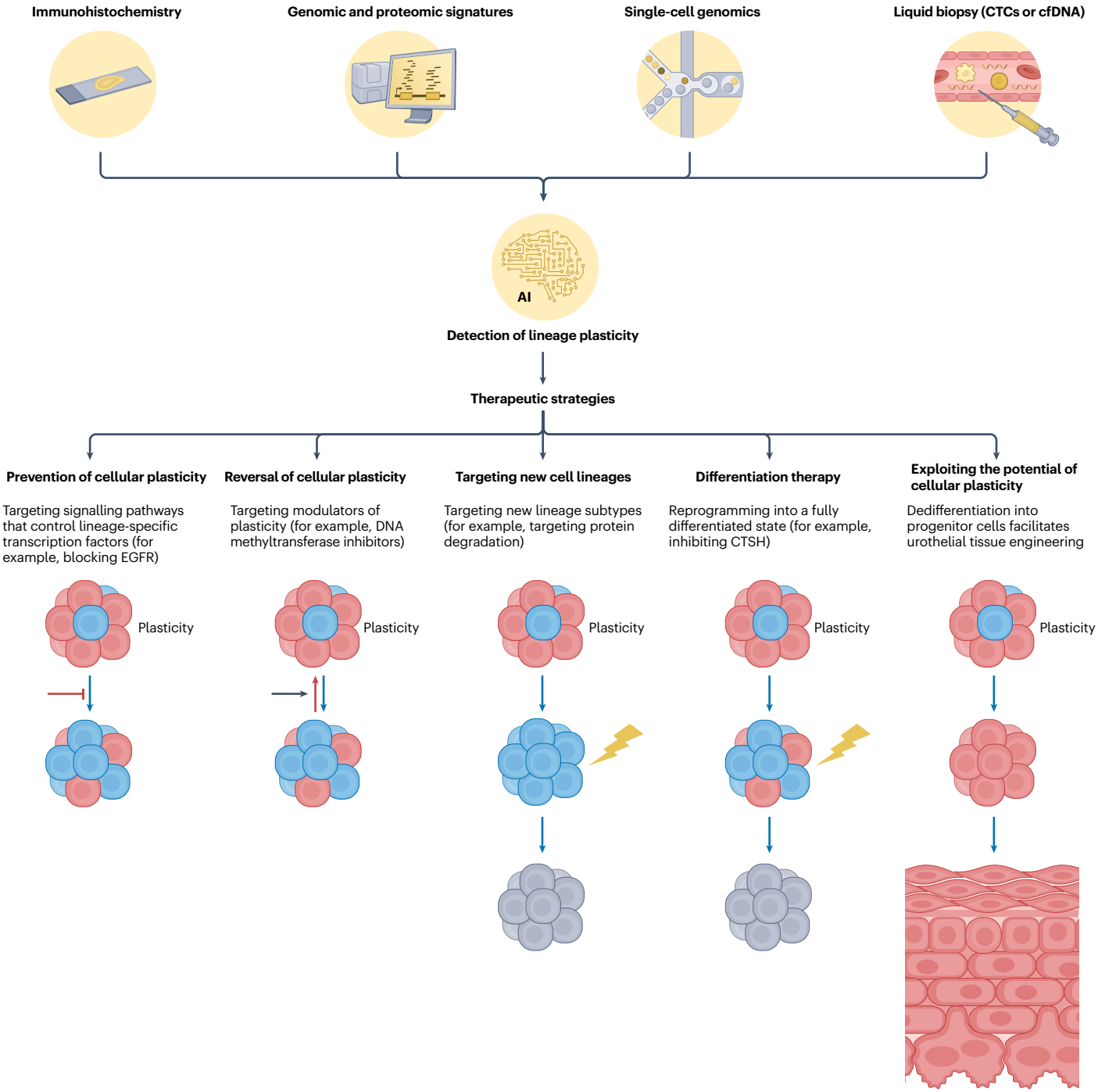

Fig. 5 | Detection of and therapeutic strategies for lineage plasticity. Various methods are available to detect lineage plasticity, including immunohistochemical staining of tissue samples, genomic and proteomic assays, single-cell omics and noninvasive liquid biopsies that enable analysis of circulating tumour cells (CTCs) or cell-free DNA (cfDNA). Integrated analysis of multi-omics data through an artificial intelligence (AI)-integrated platform enables real-time monitoring of lineage plasticity and precise therapeutic strategies. Lineage plasticity can be prevented by targeting signalling pathways that control lineage-specific transcription factors. Another approach is to reverse the lineage plasticity by targeting modulators of plasticity (such as DNA methyltransferase inhibitors that can reverse squamous differentiation). The third approach is to eliminate the new cell lineages by targeting key protein degradation. The fourth method is to reprogramme highly plastic cells to a fully differentiated state by differentiation therapy (for example, inhibition of CTSH can drive full squamous differentiation, overcoming chemoresistance). Finally, the inherent plasticity of cells can be exploited for urothelial tissue engineering.

Despite these advances, challenges such as off-target effects and potential toxic effects must be addressed. The selectivity of PROTACs needs refinement to minimize unintended protein degradation, which could have substantial clinical implications. Beyond targeting oncogenic transcription factors, restoration of tumour suppressor function through mimetic strategies is a promising avenue for differentiation therapies[164]. For example, mimetic approaches aim to stabilize epithelial differentiation by restoring WNT signalling tumour suppressors or by modulating aberrant WNT signalling. This method can involve small molecules, peptides or compounds designed to inhibit β-catenin-dependent transcription, enhance β-catenin degradation or strengthen WNT pathway interactions[165]. By re-establishing WNT signalling balance, mimetics could complement other differentiation and epigenetic therapies, improving therapeutic efficacy and reducing resistance to conventional treatments. Future studies should focus on identification of suitable transcription factor targets for these emerging therapies, optimization of delivery mechanisms and mitigation of toxic effect-related concerns.

Targeting the signalling pathways and epigenetic regulators that control lineage-specific transcription factor expression presents another promising strategy to manage cell plasticity and combat therapeutic resistance[17]. Advances in molecular biology have enabled identification of key targets, such as EGFR, FGFR, VEGFR, WNT, PI3K–AKT–mTOR and COX2, which can reverse EMT, suppress CSC-like phenotypes and inhibit metastasis, potentially optimizing patient outcomes. Epigenetic therapies are also emerging as a means to reverse lineage plasticity and induce redifferentiation, which could resensitize tumours to standard treatments. In bladder cancer, DNA methyltransferase inhibitors have shown promise in reversing squamous differentiation and reactivating FOXA1, potentially restoring chemosensitivity[92]. Preclinical studies with epigenetic drugs have yielded encouraging results, but their clinical success remains variable[166], emphasizing the need to understand how these therapies modulate lineage plasticity and induce redifferentiation. Co-targeting epigenetic pathways alongside lineage-specific drivers in patients at high risk of lineage plasticity could be a promising future approach in cancer treatment.

The microenvironment is another therapeutic target to influence tumour cell plasticity and sensitize tumours to treatment. Specifically, the COL4A1–ITGB1 interaction, enriched in endothelial cells and CAFs, drives EMT phenotypes in bladder cancer cells[167]. Blockade of this interaction with specific antibodies has been shown to suppress EMT-inducing transcription factors, inhibit angiogenesis, reduce proliferation and alleviate resistance to gemcitabine[167]. However, TME-targeted interventions face challenges, including heterogeneity in CAF populations and the dynamic nature of immune interactions within the tumour[168,169]. Future studies should explore precision-based TME therapies tailored to specific molecular subtypes of bladder cancer, potentially incorporating single-cell transcriptomics and spatial profiling to refine therapeutic strategies.

Emerging evidence suggests that highly plastic malignant cells could be reprogrammed into differentiated states, providing a novel therapeutic approach through differentiation therapy[170]. Historically, differentiation therapy has been successful in treating acute promyelocytic leukaemia using retinoic acid and arsenic, but its application in solid tumours has had limited success[170]. Despite this limitation, differentiation therapies targeting CSCs have gained renewed interest, particularly in the context of MET, which converts phenotypically mesenchymal CSCs into epithelial cells, inhibits self-renewal and resensitizes tumours to chemotherapy[171]. In bladder cancer, differentiation therapies have shown potential to resensitize tumours to chemotherapy by restoring EGFR dependency, as observed with the anti-EGFR antibody cetuximab, which is effective in bladder cancer cell lines with epithelial phenotypes[172].

Manipulation of miRNAs has emerged as a powerful strategy to reverse EMT and improve chemosensitivity. For example, restoration of miR-200 expression in mesenchymal bladder cancer cell lines can reduce migration, induce differentiation and enhance responsiveness to EGFR inhibitors by modulating regulators such as ZEB1 and ZEB2 (ref. 173). The novel concept of semi-squamatization shows that cisplatin induces partial squamous differentiation that correlates positively with chemoresistance in bladder cancer[21]. Inhibition of CTSH, a chemotherapy-upregulated protease, drives full squamous differentiation, overcoming resistance via the TNF pathway and triggering pyroptosis (Fig. 5). Cathepsin inhibitors such as E64d and RWJ-445380, which exhibit low toxic effects in clinical trials, are a promising therapeutic strategy for chemoresistant tumours[21]. These findings highlight the potential of differentiation therapy in solid cancers and suggest that targeting lineage plasticity could offer a novel approach to treatment of chemoresistant epithelial cancers.

However, detection of lineage plasticity, which involves dynamic shifts in cellular phenotype, remains a challenge, as these changes are often identified late and require genomic assays or IHC to confirm alterations in gene expression and lineage markers. Noninvasive liquid biopsies from which circulating tumour cells or cell-free DNA (cfDNA) are analysed have emerged as valuable tools for real-time monitoring of lineage plasticity and plasticity-related epigenetic changes, enabling early intervention[17,174,175]. Furthermore, multi-omics approaches powered by high-throughput technologies and artificial intelligence (AI) facilitate the identification of key plasticity targets and enable personalized treatment strategies[176]. For example, AI-integrated platforms such as CancerSEEK, in which cfDNA and proteomics are combined, exemplify the potential for early diagnosis, prognosis and therapy optimization (Fig. 5). AI-based models hold promise, but their clinical implementation requires extensive validation to ensure reliability and reproducibility across diverse patient populations.

Conclusions

Bladder urothelial research has largely focused on identifying progenitor cells and mapping the hierarchical development of urothelial lineages. The early emphasis on these progenitor populations and their roles in bladder repair and tumorigenesis shaped perceptions of urothelial plasticity as limited to stem cell activity or EMT, with little use of terms such as dedifferentiation and transdifferentiation. However, emerging evidence now reveals that urothelial cells exhibit remarkable plasticity, particularly during injury-induced repair and chronic inflammation. Processes such as transdifferentiation, metaplastic changes and EMT contribute to bladder pathologies such as BOO and IC/BPS. Notably, mature urothelial cells can dedifferentiate into progenitor-like states, challenging the traditional hierarchical understanding. Similarly, in bladder cancer, dynamic shifts between luminal and basal subtypes, alongside EMT, highlight the role of epithelial plasticity in tumour heterogeneity, progression, metastasis and therapy resistance.

Advances in single-cell technologies and epigenomic mapping have provided improved insights into urothelial plasticity, enabling identification of crucial lineage-specific transcription factors such as PPARγ and FOXA1, signalling pathways, epigenetic regulators and microenvironmental cues, as key drivers of cellular transitions. These findings have clarified the molecular and cellular dynamics that drive

urothelial plasticity and opened new therapeutic avenues. Strategies that target lineage-specific transcription factors, signalling pathways and epigenetic modifiers might offer promising treatments for chronic bladder conditions and cancer. Differentiation therapies, aimed at reprogramming cancer cells into differentiated states, are emerging as promising approaches to overcome chemoresistance. Furthermore, advances in the understanding of urothelial plasticity are driving the development of autologous bladder substitutes, highlighting the potential for regenerative medicine. Advances in noninvasive methods of monitoring biomarkers, such as liquid biopsies, and the integration of multi-omics technologies and AI could enhance early detection, refine mechanistic insights and facilitate personalized treatment approaches to target urothelial plasticity in bladder diseases and tumours.

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Author contributions

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Competing interests

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