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Review Article

Therapeutic significance of *ARID1A* mutation in bladder cancer

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Abstract

Bladder cancer (BC) develops from the tissues of the urinary bladder and is responsible for nearly 200,000 deaths annually. This review aims to integrate knowledge of recently discovered functions of the chromatin remodelling tumour suppressor protein ARID1A in bladder urothelial carcinoma with a focus on highlighting potential new avenues for the development of personalised therapies for *ARID1A* mutant bladder tumours. ARID1A is a component of the SWI/SNF chromatin remodelling complex and functions to control many important biological processes such as transcriptional regulation, DNA damage repair (DDR), cell cycle control, regulation of the tumour microenvironment and anti-cancer immunity. *ARID1A* mutation is emerging as a truncal driver mutation that underlies the development of a sub-set of urothelial carcinomas, in cooperation with other driver mutations, to cause dysregulation of a number of key cellular processes. These processes represent tumour drivers but also represent potentially attractive therapeutic targets.

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Keywords: Bladder cancer, ARID1A, Synthetic lethalities, Immune checkpoint therapy, Clonal expansions

Introduction

The genetics of bladder cancer

Bladder cancer (BC) refers to any of several types of cancer that arises from the tissues of the urinary bladder. It is the 10th most common form of cancer worldwide and responsible for nearly 200,000 deaths annually [1]. More than 95% of all bladder tumours are urothelial carcinomas, which can be further subdivided into non-muscle invasive bladder cancer (NMIBC) and muscle invasive bladder cancer (MIBC) (Figure 1). NMIBC include papillary and flat tumours, classified according to their growth properties [2]. Papillary tumours which are limited to the mucosa and invading the lamina propria are classified as stage Ta and T1, respectively [3]. Flat, high-grade tumours that are confined to the mucosa are classified as carcinoma *in situ* (CIS). NMIBC can be treated by transurethral resection of bladder tumour (TURBT), combined with intravesical chemotherapy or immunotherapy (mitomycin or *Bacillus Calmette-Guerin*). When flat urothelial carcinomas or papillary urothelial carcinomas invade the sub-mucosal muscle layer they are defined as MIBC. For these patients, the treatment of choice is the removal of the bladder (radical cystectomy), preferably with platin-based neoadjuvant chemotherapy, or in very selected cases trimodal therapy ([4] and 2019 eUpdate of [5]). Patients with advanced metastatic disease are treated with chemotherapy as first-line treatment and immune checkpoint therapy as maintenance therapy, or in PD-L1-positive, chemotherapy-ineligible cases with first line immune checkpoint therapy ([4] and 2019 eUpdate of [5]). Despite the availability of these different therapeutic modalities, many patients are currently not cured of their disease and these therapies do not exploit the specific genetic alterations in BCs, or the novel classifications based on gene expression changes. It will be essential to use and expand upon these genetic and molecular classifications to obtain a better cellular and functional understanding of the underlying causes of BC in order to improve and increase therapeutic possibilities for urothelial carcinomas.

Recurrent genetic alterations in at least 52 genes have been identified through sequencing of large cohorts of MIBC tumours. MIBCs are broadly

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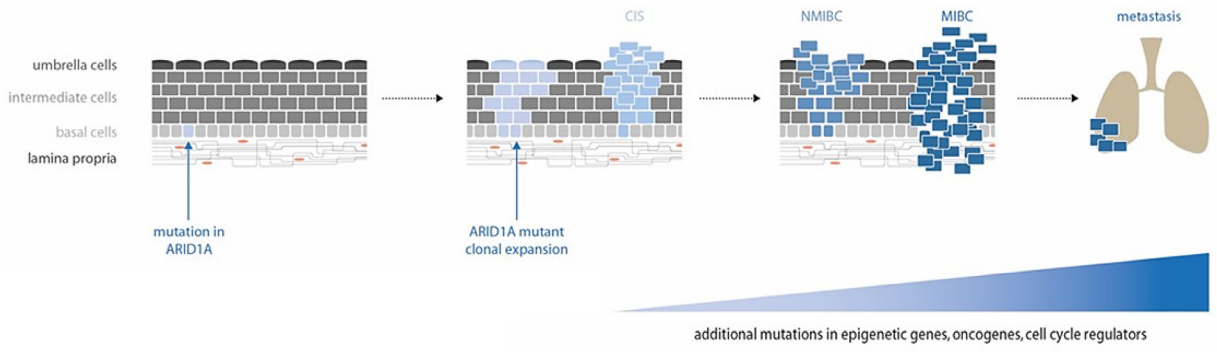

Figure 1. Schematic model of the process of evolution of bladder cancer involving *ARID1A* mutation from truncal mutation in a single progenitor cell through clonal expansion, carcinoma *in situ* (CIS), non-muscle invasive bladder carcinoma (NMIBC), muscle invasive bladder carcinoma (MIBC) to metastatic dissemination.

characterised by genetic alterations that fall into three distinct functional classes: i) epigenetic regulation (including *ARID1A, KMT2C, KMT2D, KDM6A* and *EP300*), ii) cell cycle control (including *TP53, MDM2, RB1* and *CDKN2A*) and iii) oncogenic signalling (including *FGFR3, FGFR1, PIK3CA, PTEN, RAS* and *RAF*). The mutation spectrum highlights the importance of the processes that are altered as a consequence of these mutations in the pathogenesis of MIBC. Importantly, two recent studies [6],[7] demonstrated that genetic alterations in very specific epigenetic pathways in urothelial cells frequently represent the earliest events in the development of urothelial carcinomas. By sequencing multiple regions of normal urothelium, these studies identified many discrete regions of clonally expanded cells that harbour mutations in several epigenetic regulatory genes that had previously been found to be recurrently mutated in established tumours, most frequently *KMT2D, KDM6A* and *ARID1A*. Strikingly, these clonal expansions do not show enrichment for the other classes of genes regulating cell cycle control or oncogenic signalling pathways that are found in established BC. It is therefore believed that BC formation is initiated by spontaneous or mutagen-induced mutations in epigenetic genes in basal or intermediate cells that drive clonal expansions, giving rise to pools of genetically-sensitized cells that then accumulate additional mutations in genes controlling the cell cycle (*TP53, CDKN2A, RB1*), oncogenic signalling (*EGFR, FGFR*, RAS-MEK pathways) or in other epigenetic regulatory genes. These drive the transition to CIS and cancer in cooperation with the already altered epigenetic state of the cell. Given that mutations in epigenetic regulatory genes frequently represent truncal tumour mutations, it is imperative to better understand the diverse molecular and cellular consequences of these mutations in order to define their contributions to different stages of the development and progression of bladder cancer and to investigate the potential to therapeutically target these alterations with the aim of developing future personalised therapies for BC patients.

In addition to the inter-patient mutational heterogeneity exhibited by MIBC, numerous large-scale transcriptomic studies of MIBC have defined different molecular subclasses of urothelial carcinomas based on different mRNA expression patterns [8]. A study pooling the data from these different studies [9] led to a new consensus classification into 6 different molecular subtypes of MIBC based on the different gene expression profiles. These subtypes include luminal papillary, luminal non-specified, luminal unstable, stroma-rich, basal/squamous, and neuroendocrine-like tumour categories. Genome-wide DNA methylation analyses of large numbers of MIBC identified five different patterns of hypo- and hyper-methylation, also implicating aberrant DNA methylation as a factor in the pathogenesis of urothelial carcinoma [10]. The identified mRNA and methylation signatures appear to reflect differences in the biology of the tumour cells and in the tumour microenvironment. However, the biological meaning, the clinical significance, and the potential therapeutic relevance of these different molecular MIBC classes remains unclear. All but the rare neuroendocrine-like subset (which has a relatively poorer prognosis) have 5-year survival rates ranging between approximately 35-55 % [1],[9], implying rather aggressive diseases in the context of current therapeutic treatments. It appears that there are no clear genetic drivers of particular molecular subtypes of MIBC and that the same combinations of mutations may give rise to apparently different clinical manifestations in different patients [9]. It remains to be clarified how different genetic mutations and/or different molecular subtypes of MIBC may respond to particular types of current therapy and an ongoing challenge will be to design new therapies that are tailored to each patient's individual tumour.

The ARID1A tumour suppressor protein

Several genes that encode chromatin modifying tumour suppressor proteins [11] are recurrently mutated in BC [6],[7] pointing to the fact that loss of normal epigenetic regulation is a driving force for BC progression. One important class of epigenetic regulator that is frequently dysregulated due to mutation in urothelial carcinomas are the SWI/SNF (SWItch/Sucrose Non-Fermentable) nucleosome remodeling complexes. *ARID1A* is the most commonly mutated SWI/SNF subunit encoding gene across all human cancers [11–13] and is mutated in approximately 18-25% of urothelial carcinomas [10]. ARID1A (AT-rich interactive domain-containing protein 1A, also known as BAF250a), belongs to one of the two major subclasses of SWI/SNF complexes: the BRG1-associated factor (BAF) [14]. The other subclass, which contains ARID2, is the polybromo BRG1-associated factor (PBAF). ARID1A is the largest, non-catalytic BAF subunit and it confers target specificity on the SWI/SNF complex and directs the ATPase activity [15],[16]. ARID1A likely plays a multi-factorial and complex role in tumour suppression in BC. In other tumour types, mutational inactivation or silencing of ARID1A has been linked not only to dysregulation of transcriptional programs but also to classical mechanisms of tumour suppression including control of the cell cycle, DDR and checkpoint signalling, regulation of p53 targets and telomerase activity [11],[17]. The consequences of the disruptions in these pathways together with abnormal expression of other regulators that ARID1A cooperates with or the aberrant epigenetic regulation of gene expression may lead to mutagenesis and carcinogenesis [17]. In turn, these dysregulated cellular processes also represent potential specific therapeutic vulnerabilities of *ARID1A* mutant cells (Figure 2).

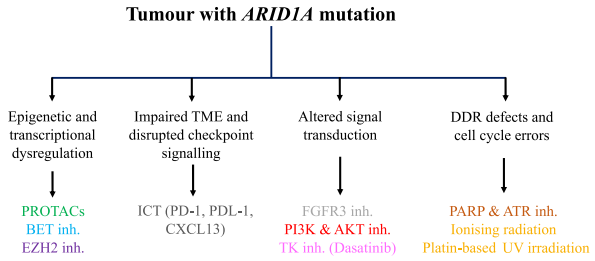

Figure 2. Summary of the biological processes that are altered as a consequence of ARID1A mutation and the therapeutic opportunities that arise and that could be tested in bladder cancer. TME: tumour microenvironment; DDR: DNA damage repair; PROTACs: proteolysis targeting chimeras; ICT: immune checkpoint therapy.

ARID1A mutations give rise to synthetic lethalities

Mutations in and loss of the *ARID1A* gene mostly lead to its inactivation and ARID1A protein loss [18]. Inactivating mutations or low expression of tumour suppressor genes are very common genetic events in cancer and are sometimes deemed as being "undruggable" [19]. However, synthetic lethal interactions (i.e. the situation where simultaneous mutation of a pair of genes leads to death, but mutation of either gene alone does not significantly affect survival) can be identified by genetic screening using shRNA or CRISPR-Cas9 libraries, or using chemical libraries. The idea is to identify synthetic lethal partner proteins for the mutated proteins of interest and hence kill the cancer cells carrying the mutant gene by specifically targeting the function of the synthetic lethal partner [20].

ARID1B, another BAF member of the SWI/SNF complex, has been identified as a specific synthetic lethal partner of *ARID1A* mutant cancer cell lines [11],[21]. Either one or the other is always present in the SWI/SNF complex. However, these complexes are mutually exclusive of one another and moreover they appear to differently regulate a number of biological processes. Knockdown of ARID1A modestly accelerates cell cycle re-entry (after serum-deprived cell-cycle arrest) whilst knockdown of ARID1B markedly delays the re-entry. ARID1A coimmunoprecipitates with HDAC1/2-containing complexes, while ARID1B is associated with HDAC3 and they also interact differently with E2F family members [22]. Mechanistically, the basis of synthetic lethality relies on the fact that ARID1A maintains chromatin accessibility at enhancers while the impact of ARID1B is only apparent in the context of *ARID1A* mutation [23]. ARID1B-containing SWI/SNF complexes continue to be intact in *ARID1A*-mutant tumours and act at a group of enhancers to maintain their activity. Synthetic lethality is therefore due to this dependence of *ARID1A*-mutant cancers on this group of enhancers [11]. PBAF is the other subclass of the SWI/SNF complexes, and it contains ARID2. Mutations of both *ARID1B* and *ARID2* have been found in some types of cancers that also contain *ARID1A* mutations [7],[24]. However, it has not been yet systematically studied if these genes are mutually exclusive or do have some extent of overlap or even if they have tumour suppressor activity. Analogous synthetic lethal relationships have also been observed between other pairs of SWI/SNF subunits such as the two mutually exclusive ATPase subunits SMARCA4 and SMARCA2 [25],[26]. It is therefore possible that targeting other members of the SWI/SNF complex, for example through the design of specific PROTACs (proteolysis targeting chimeras: small molecules composed of two active domains and a linker, capable of removing specific targeted proteins), may also offer opportunities for therapeutic targeting of *ARID1A* and SWI/SNF-mutant cancers [25].

An shRNA kinome library screen identified the BET domain protein BRD2 as a synthetic lethal partner in ARID1A mutant ovarian clear cell carcinoma and the BET inhibitors JQ-1 and iBET762 were shown to be effective in cell culture, cell line xenograft and patient-derived xenograft pre-clinical models [27]. At least part of the therapeutic effects of these drugs may relate to the reduction in levels of ARID1B induced by BET inhibitors. Whether this relationship also exists in *ARID1A*-mutant bladder cancer remains to be tested.

ARID1A mutations frequently occur together with activating mutations of *PIK3CA* and/or loss of PTEN function or expression, leading to the activation of the PI3K/AKT pathway [28]. In a mouse model of endometrial cancer, it was found that conditional deletion of *Arid1a* alone in endometrial epithelial cells was insufficient to induce neoplastic transformation. However, tumour progression was strongly increased when *Arid1a* deletion was coupled with *Pten* deletion [29]. PIK3CA was identified as another synthetic lethal vulnerability across *ARID1A*-mutant cancers [21]. Loss of ARID1A protein expression was also found to increase the sensitivity of cancer cells to PI3K- and AKT- inhibitors, measured by an augmented apoptosis in treated ARID1A-depleted cells [30]. These results demonstrate that genetic inactivation of *ARID1A* alone is insufficient for tumour initiation, but it requires additional genetic alteration (s) such as *PTEN* deletion to drive tumorigenesis. It will be interesting to study the effects of introduction of loss-of-function mutation of *Pten* or gain of function mutation of *Pik3ca* together with *Arid1a* loss in mouse models of BC.

Direct interactions between the SWI/SNF complex and Polycomb complexes have also been suggested [31],[32]. It is known that these complexes have opposing functions on chromatin: chromatin accessibility at enhancers is increased by the SWI/SNF complexes which also act to maintain H3K27 acetylation, whereas Polycomb complexes compact chromatin and catalyze H3K27 trimethylation [25]. It is also known that the EZH2 methyltransferase (the functional enzymatic component of the Polycomb complex 2) acts in a synthetic lethal manner in *ARID1A*-mutated ovarian cancer cells and that the mutational status of *ARID1A* correlates with response to the EZH2 inhibitor PIK3IP1. This inhibitor is a direct target of ARID1A and EZH2 and it is upregulated by EZH2 inhibition, contributing to synthetic lethality by inhibiting PI3K-AKT signalling [33].

ARID1A has been shown to act in concert with p53 to regulate target gene expression, at least in gynaecological cancers [34]. Recently, genetic mouse model experiments proved that despite accounts of mutual exclusivity observed in primary tumours, in endometrial cancer the co-existence of *TP53* and *ARID1A* mutations were tolerated *in vivo* and promoted more aggressive cancer phenotypes than either mutation independently [35]. In BC samples no clear co-occurrence or a mutual exclusivity pattern for *ARID1A* and *TP53/RB1* genetic alterations were identified using sequencing data derived from independent studies [10],[36]. These studies again suggest that the inactivation of *ARID1A* on its own is not sufficient to initiate tumour development but an additional inactivation (e.g. *TP53*) might be necessary to drive malignant transformation in urothelial cells [13]. In line with this, *Arid1a*-knockout mice do not develop tumours in the bladder [12] and *ARID1A* mutations can be found in non-malignant clonal expansions in human urothelium [6],[7]. Furthermore, the inactivation of ARID1A in TERT-immortalized normal human urothelial cells caused the induction of p53/p21 expression, the deregulation of the cell cycle and the enhancement of cellular growth probably by regulating and antagonizing c-Myc activity [37],[38]. A synthetic lethal relationship between *ARID1A* mutation and the tyrosine kinase inhibitor dasatinib was identified that is associated with G1-S cell-cycle arrest and dependent upon p21 and Rb [39]. The basis of this synthetic lethality is likely to be the inhibition of the dasatanib target protein YES1, a signalling kinase whose genetic knockdown selectively inhibits proliferation of *ARID1A* mutant cells [39].

Apart from its canonical functions in regulating transcriptional programs, the chromatin remodelling function of ARID1A is necessary for several aspects of DDR and of DNA damage signalling. ARID1A-containing BAF complexes remodel chromatin at sites of DNA damage, are necessary

for efficient repair by non-homologous end joining, interact with the mismatch repair protein MSH2 to suppress a hypermutability phenotype, suppress DNA replication stress, and interact with ATR to sustain DNA damage signalling that is necessary to enforce DNA damage cell cycle checkpoint [17],[40]. Taking advantage of defects in these fundamental DNA repair processes, several studies identified that *ARID1A* mutant cells are highly sensitive to poly ADP-ribose polymerase (PARP) inhibitors, ionising radiation, cisplatin and UV irradiation, as well as to inhibitors of the DNA damage sensing kinase ATR [41].

ARID1A alterations have also been found to generally occur in *FGFR3* wildtype, poor-prognosis bladder tumours. An inverse association between *ARID1A* and *FGFR3* mutations has been found as well as the predominance of *ARID1A* mutations in more aggressive tumours [42]. It will be interesting to observe if alterations in *ARID1A*, and in other chromatin remodelers or histone modifiers, might contribute to the multiple regional epigenetic silencing phenotype that occurs in FGFR3 wildtype BC [43].

Collectively, these studies of a range of different *ARID1A* mutant tumour types have identified a promising series of agents that will now need to be extensively tested in pre-clinical therapeutic studies to determine if these compounds represent new potential therapies for BC. An attractive idea will be to combine different agents that target mechanistically distinct aspects of ARID1A-dependent synthetic lethality. This approach may on the one hand achieve more efficient therapeutic targeting and on the other hand reduce the possibility that cancer cells can develop resistance to individual targeted therapies. This will require a concerted effort using complementary model systems for bladder carcinoma, including genetically modified cancer cell lines, patient-derived urothelial carcinoma organoid cultures, patient derived-xenograft models as well as the development of new autochthonous mouse models that accurately reproduce the mutational spectra of *ARID1A* mutation in combination with other driver mutations that recur in human MIBC.

Immune checkpoint therapy, a promising approach

The above-described studies focused on identifying cell-autonomous therapeutic vulnerabilities of *ARID1A* mutation in cancer cells. However, an equally promising approach is to target the distinct tumour microenvironment (TME) in *ARID1A* mutant BC. This concept builds upon the success of immune checkpoint therapy (ICT), which has transformed the therapeutic landscape of several types of human tumours. A number of different ICT inhibiting PD-1 (programmed cell death-protein 1) or PD-L1 (programmed cell death-ligand 1) have been approved as first- or second-line therapy for metastatic urothelial carcinoma, with response rates of approximately 30% [44]. Recently, an inverse relationship between *ARID1A* mutation and the immune cytokine CXCL13 in metastatic urothelial carcinoma (mUCC) was identified. CXCL13-/- tumour-bearing mice were resistant to ICT, whilst ARID1A knockdown increased sensitivity to ICT in a mouse model of BC, revealing that the combination of CXCL13 expression and *ARID1A* mutation may serve as predictive markers for patients that receive ICT [45]. Ongoing research efforts now aim to identify biomarkers (such as specific gene mutations) or aspects of the TME that predict good responses to ICT, as well as to develop new combination therapies that aim to modify the TME to improve clinical responses in more patients. A number of recent studies support the notion that cancer cell epigenetic status affects the anti-tumour immune response in urothelial carcinomas. Mutations in *ARID1A, KMT2A/C, EP300* and *KDM6A* have all been shown to regulate the TME and/or to predict responses to ICT in bladder and other human cancers [46–49]. A number of interesting findings link *ARID1A* mutation and the above-described resulting defects in DDR to good responses of BC to ICT. Cancers with high mutational burden respond very positively to immune checkpoint inhibitors and MIBC has one of the highest mutational rates of all cancers [50]. Meta-analyses have shown higher levels of microsatellite instability and tumour mutation load in patients with BC and genetic mutations of *ARID1A* that correlates with greater progression-free survival rates after immunotherapy [51].

Furthermore, the association between ARID1A deficiency and high tumour mutational burden (TMB) has also been demonstrated by clinical studies, mainly in gastrointestinal (GI) carcinomas. These studies suggested that there was a correlation between selected DDR defects and a high TMB in more than 20% of cases [52]. The elevated immune activity of *ARID1A*-mutated GI cancers also correlated with higher tumour mutation load and lower aneuploidy levels [53]. It has also been observed that cancers with *ARID1A* deficiency normally do not show generalized genomic instability, measured through copy number alterations (CNAs) [54]. Despite the fact that CNAs are positively associated with high TMB, increased somatic CNAs are linked to worse response to anti-PD-1 therapy [55]. This paradoxical situation in BC – on the one hand the role of ARID1A in maintaining mitotic integrity and on the other hand the lack of genomic instability in *ARID1A*-mutated cancers - has recently been reconciled by the discovery that ARID1A inactivation impairs telomere cohesion, selectively eliminating gross chromosome aberrations by apoptosis [56]. ARID1A inactivation appears to generate a favourable situation for ICT by inducing an elevated TMB but low CNAs.

Another intriguing link between DNA damage responses and the TME in BC comes from a recent study that identified that a signaling axis comprising CXCL9, CXCL10, CXCL11 and the CXCR3alt receptor induces the recruitment of a specific subpopulation of $CD8^+$ T cell, namely stem-like T cells, in MIBC and that the presence of these cells correlates with good responses to chemotherapy [57]. An epigenetic regulation of cytokine expression may be hypothesized given that cytokine activation can be, amongst other possibilities, a consequence of DNA damage-induced STING pathway activation. It will be interesting to investigate whether mutations in *ARID1A*, or any of the other epigenetic tumour suppressor proteins that also regulate DNA damage responses, induce activation of pro-inflammatory innate immune signaling pathways via STING and whether this may shape the TME.

For MIBC there is currently no formal maintenance therapy, and hopefully it is here where ICT can play an important role [58],[59]. The identification of strong biomarkers can help predict which patients respond to specific ICT therapies and which ones may be assigned to novel therapies. It will also be necessary to dissect the TME using single cell RNA sequencing and/or mass cytometry to gain insight into different types of TME and the different potential crosstalk between cancer cells and cells of the TME. This will hopefully help to understand the underlying molecular mechanisms to be able to design better combination therapies (for example, to enhance T cell recruitment/activation; or with targeted therapies to improve ICT), or to remove other barriers of an immunosuppressive TME. For example, very recently a study proved that the combination therapy with lentiviral interferon-α and an anti-PD1 antibody effectively removed bladder tumour burden in an intravesical mouse model, being more effective than either therapy alone [60].

Targeting *ARID1A* mutant cells in clonal expansions to prevent cancer development

It has been recently described that somatic mutations in *ARID1A, KMT2D* and *KDM6A* can be observed in clonal expansions in the normal urothelium [6],[7]. How and why do these mutations occur? In the specific case of the bladder, due to its direct contact with urine, the urothelium is constantly exposed to a series of potential carcinogens and their metabolic products as well as to environmental factors (mainly tobacco smoking and aristolochic acid [61]) that can cause genotoxic stress to urothelial cells [7]. This likely results in the accumulation in the histologically normal urothelium of clones of cells that are believed to represent an expanded pool

of cells that are further subjected to mutagenic forces. The occurrence of additional somatic mutations and a positive proliferation selection pressure is thought to eventually lead to malignant transformation and BC [7]. This model may explain the sequence of events underlying bladder tumour formation and has been supported by different mouse models [62–64]. Urothelium-specific *Kdm6a*-mutant female mice, for example, are susceptible to develop tumours upon treatment with BBN mutagen whilst under normal conditions no apparent phenotype is observed [64]. The effect of the mutagen probably stimulates additional mutations which then drive the transition of *Kdm6a* mutant clonally expanded cells to cancer.

The specific role of mutation in *ARID1A* in driving clonal expansion in normal urothelium remains unclear. It could be hypothesized that mutations in *ARID1A* contribute to the ongoing transition to malignancy due to the loss of ARID1A's functions in DNA repair. ARID1A loss represents the removal of a barrier to the maintenance of genomic stability. This may lead to an increased rate of accumulation of secondary mutations or copy number alterations in other genes that play an important role in DDR or in coordinating cellular responses to DNA damage [10], which are frequently found in BC, resulting in a further increase in the mutation rate. The subsequent occurrence of genetic changes in proliferation-promoting genes such as cell cycle and oncogenic signalling genes likely then drive tumour formation from clonal expansions. The genetic heterogeneity of BC shows that there are many different genetic evolutionary trajectories that can be taken by normal urothelium cells on the path to becoming tumour cells. It will be imperative to understand not only how different sets of mutations cooperate mechanistically with *ARID1A* mutation (and similarly with mutation of the other epigenetic regulatory genes that arise in clonal expansions) but also to investigate the contributions of mutational pressures such as APOBEC activity, tobacco smoking and aristolochic acid [65] to tumour development from clonal expansions. This understanding will not only inform about basic tumour biology but also have important future implications for prevention, diagnosis and potentially the application of synthetic lethal-based therapeutic concepts described above, that aim to eliminate *ARID1A* mutant cells from the normal urothelium at pre-malignant stages [66].

More research involving the study of the normal and diseased urothelium using human cellular systems (human bladder cancer 2D cells lines, patient clonal expansions and culture systems of primary human bladder 3D organoid cultures) is necessary to try to answer the question of how mutations in different epigenetic regulatory genes including *ARID1A* contribute to the earliest steps in BC appearance and development. Human and also mouse studies will help to gain deeper insight in the understanding of the effect of each of the mutations alone or in combination with other mutations in the earliest stages of cancer development. Currently available *in vivo* models of BC cancer include carcinogen-based and genetically-engineered mouse (GEM) models, as well as orthotopic and renal grafting [67]. Due to the fact that the mouse bladder may be relatively uncooperative to developing invasive tumours, it has been difficult to develop GEM BC models, especially those that represent a spectrum of BC phenotypes and are informative as preclinical models [67]. Future mouse modelling experiments should be guided by the recent knowledge about the timing of different genetic events in the development of human bladder cancer by developing models that combine mutations in the genes that arise in clonal expansions with those that arise later in tumour formation, ideally with temporal control of the timing of the different gene mutations to better mimic the long-term process of BC development in humans.

Conclusion

The outcomes of BC therapy are improving but the 5-year survival rate is still around 50 % [1],[9]. It is clear that classical BC treatment (mainly TURBT, BCG vaccine, chemotherapy, radiotherapy and cystectomy) is not sufficient for all BC patients. Hope for future therapeutic approaches lies in taking advantage of the underlying molecular features of patients' tumours. Genomic studies have revealed the diverse genetic mutational landscapes of BC [10] and identified novel classifications based on gene expression changes [8], providing a molecular roadmap of candidate proteins and pathways that can be explored as therapeutic targets. One prominent line of therapeutic investigation centres around *ARID1A* [11],[14]. Mutation of *ARID1A* or of other components of the BAF complex arise in over 40% of MIBC patients [13] and mechanistic studies in a number of different tumour types have uncovered several different molecular functions of this tumour suppressor gene that appear to be relevant to the biology of BC. High-throughput screens have discovered several specific vulnerabilities of ARID1A deficient cells including ARID1B [21],[22], ARID2 [24],[53], PIK3CA [68], EZH2 [33], p53 [34],[37], c-Myc [38], DNA damage checkpoint proteins [17],[63] and FGFR3 [44] that represent candidates for the development of new therapies that might directly target *ARID1A*-mutant BC cells. Building upon discoveries of the role of ARID1A in maintaining DNA fidelity, the high mutational burden seen in MIBC [50], the importance of the TME [69] and clinical successes in BC with immune checkpoint therapy, there appears to be possibility for the further development of therapies that target the cross talk between BC tumour cells and immune cells in the TME. For example, synergistic therapies that manipulate immune sensing mechanisms (such as the c-GAS/STING axis) and BCG/IFN pathway genes [70]. Finally, it has become apparent that *ARID1A* mutation may represent a valid therapeutic target at the earliest stage of the development of some tumours, namely at the stage of clonal expansion [6],[7]. This type of therapeutic intervention could be envisaged as preventative therapy.

Author contributions

MC and IJF contributed to the conceptualisation and writing of the manuscript as well as the preparation of the figures.

CRediT authorship contribution statement

Marina Conde: Conceptualization, Writing – original draft, Writing – review & editing. **Ian J. Frew:** Conceptualization, Writing – original draft, Writing – review & editing.

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