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# CELL ADHESION AND UROTHELIAL BLADDER CANCER:

# THE ROLE OF CADHERIN SWITCHING AND RELATED PHENOMENA

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**ABSTRACT**

Cadherins are mediators of cell-cell adhesion in epithelial tissues. E-cadherin is a known tumour suppressor and plays a central role in suppressing the invasive phenotype of cancer cells. However, the abnormal expression of N- and P-cadherin ("cadherin switching") has been shown to promote a more invasive and malignant phenotype of cancer, with P-cadherin possibly acting as a key mediator of invasion and metastasis in bladder cancer. Cadherins are also implicated in numerous signalling events related to embryonic development, tissue morphogenesis, and homeostasis. It is these wide-ranging effects and the serious implications of cadherin switching that make the cadherin cell adhesion molecules and their related pathways strong candidate targets for the inhibition of cancer progression, including bladder cancer. This review will focus on cadherin switching in the context of bladder cancer and in particular the switch to P-cadherin expression, and will discuss other related molecules and phenomena, including EpCAM and the development of the cancer stem cell phenotype.

**MEDIA SUMMARY**

Cadherins are mediators of cell-cell adhesion in epithelial tissues. E-cadherin is a tumour suppressor and plays a central role in suppressing the invasive phenotype of cancer cells. However, the abnormal expression of other cadherins ("cadherin switching") has been shown to promote a more invasive and malignant phenotype of cancer. Cadherins are also implicated in numerous signalling events related to embryonic development, tissue morphogenesis, and homeostasis. It is these wide-ranging effects and the serious implications of cadherin switching that make the cadherin cell adhesion molecules and related pathways attractive targets for the inhibition of cancer progression, including bladder cancer.

## BLADDER CANCER

### Introduction

Urothelial bladder cancer (UBC) is the fifth most common cancer in Western society, with a global incidence of over 356,000 and a prevalence estimated at 2.7milion [1;2]. The burden of the disease is predicted to increase significantly in the foreseeable future as a result of population aging and the increasing world population, together with the progression of the tobacco epidemic and increasing exposure to occupational carcinogens in developing countries [2]. In the UK there are approximately 10,200 new cases and 5,000 deaths attributed to bladder cancer per year [3]. In Western populations over 90% of bladder cancers are transitional cell carcinomas of urothelial origin (urothelial cancers, UCs), and at presentation 75-85% will be non-muscle-invasive tumours (NMIBC, stages Ta/T1/Tis), with the remainder being muscle-invasive (MIBC, stages T2-4) [1;4-6].

NMIBC is a heterogeneous disease typified by a high rate of recurrence (15-61% at one year, depending upon risk category [7]) and so long-term, even lifelong, surveillance with outpatient flexible cystoscopy is the mainstay of subsequent management [6;8]. Progression to MIBC is also a concern for high-risk NMIBC patients, occurring in up to 17% of patients at one year [7]. However, the overall prognosis is good with 65-85% of patients surviving for 5 years or more [5].

Progression to (or presentation with) MIBC represents the critical step in the disease course, necessitating more radical therapies and carrying a 5-year survival rate of only 25-50% [5;9]. For curative intent, patients who present with or progress to MIBC are treated by radiotherapy [6;10], chemoradiotherapy [11], radical cystectomy, or neoadjuvant chemotherapy followed by radical cystectomy [6;9;10].

The cumulative cost of treating UBC exceeds all other forms of human cancer, the majority of which is attributable to the long-term treatment and surveillance of NMIBC [12-14]. Despite this, there is only modest research funding for UBC compared to other malignancies [15], and as a result there has been a lack of scientific advancement in the field [15-17], with no major new drugs approved for UBC in over 10-years [17;18].

Cadherins are mediators of cell-cell adhesion in epithelial tissues [19;20]. We have previously demonstrated that the abnormal expression of P-cadherin (an example of “cadherin swithching”) is associated with an invasive and

aggressive phenotype of UBC [21], and have hypothesized that P-cadherin may act as a key effector of muscle-invasion [22]. The cadherins are involved in a number of important phenomena related to cancer progression, including epithelial-to-mesenchymal transition (EMT) and the development of a cancer stem cell phenotype [22;23]. It is these wide-ranging effects and the serious implications of cadherin switching that make the cadherins and their related pathways strong candidate targets for the inhibition of cancer progression, including UBC. This review will focus on cadherin-based cell adhesion in the context of UBC and the switch to P-cadherin expression, and will discuss other related molecules and phenomena, including EpCAM and the development of the cancer stem cell phenotype.

## METHODS

Our group has been working in the field of cadherin biology for a number of years [24;25], and we regularly review the literature on these molecules and their associated pathways [22]. Specifically, this review was written utilising papers obtained following *PubMed* searches and with the following structure: bladder cancer background, epidemiology and molecular pathogenesis; cadherin background and biology; cadherins in epithelial malignancies, cadherin switching, and cadherins in bladder cancer. The background to cadherins and cadherin biology presented here has been derived from key papers by workers who initially characterised and described these molecules, and then who subsequently investigated cadherin expression and function in various epithelial malignancies and model systems. We updated the field for cadherin switching to describe this process in the context of malignancy and related phenomena (eg. epithelial-to-mesenchymal transition, cell migration, metastasis, cancer stem cells, EpCAM signalling), utilising papers written by significant workers in this field. The data, findings and information contained within these publications were then assimilated to create a review of cadherin switching in bladder cancer and including some of our own interpretations.

## MOLECULAR PATHWAYS TO NON-MUSCLE-INVASIVE & MUSCLE-INVASIVE BLADDER CANCER

Different approaches have been taken to describe the molecular alterations involved in bladder tumorigenesis [26-31]. We have previously described such pathways based upon the six “hallmarks of cancer” described by Hanahan

and Weinberg in 2000 [32-35]. In 2011 Hanahan and Weinberg updated their original landmark review, describing genome instability and inflammation as underlying these hallmark changes, and proposed “reprogramming of energy metabolism” and “evading immune destruction” as two emerging hallmarks with potential for generality [35]. In addition, they described that tumors exhibit another dimension of complexity by containing a repertoire of recruited, ostensibly normal cells that contribute to the acquisition of hallmark traits by creating the "tumor microenvironment" [35], and our own research has demonstrated the apparent importance of the immunological milieu of the bladder tumour microenvironment [RT Bryan et al - unpublished data]. In their 2011 update, Hanahan and Weinberg also introduced the concept of “cancer stem cells” [35], a concept that has existed for a number of years in haematopoietic malignancies [36;37]. Cancer stem cells (CSCs) are a subset of tumor cells that have the ability to self-renew and to generate all of the heterogeneous cells that comprise a tumor (properties that are analogous to a stem cell, the original cell of an organ and responsible for organogenesis and organ maintenance) [23;36;38-40]. In the setting of UBC, CSCs appear to play a role in a subset of tumors, but their true significance is yet to be clarified [23].

Other authors have reviewed the field of UBC molecular pathogenesis in detail [26-31], and there has been general consensus on a divergent pathway for the development of Ta/T1 disease and Tis/T2+ disease [29;41-46]. However, Dancik et al recently identified a cell of origin gene signature for basal cells and umbrella cells of the urothelium [47]. By utilising this cell of origin signature in UBCs from 874 patients, it appeared that NMIBCs and MIBCs developed from distinct progenitor cells [47], possibly shifting our understanding of urothelial carcinogenesis away from the classical two pathway model. Further detailed genomic and epigenomic studies of both MIBCs and NMIBCs are thus required to clarify our understanding of the pathogenesis of these tumours [48].

Although a detailed examination of these pathways is beyond the scope of this review, this is a rapidly changing field and new developments appear frequently with the advent of high-throughput experimental platforms including “deep sequencing” [49], proteomics [50-52] and metabolomics [53]. Most recently, The Cancer Genome Atlas (TCGA) Research Network undertook the comprehensive molecular characterization of 131 MIBCs [49]. With regard to somatic DNA mutations, a notable finding was the significant enrichment of non-silent mutations in chromatin regulatory genes compared to other epithelial cancers studied: 76% of the tumours (MIBCs) had an inactivating

mutation in one or more of these genes, and 41% had at least two such mutations [49]. *TP53* mutations were also common (49%), as were amplification and overexpression of *MDM2*, suggesting that TP53 function was inactivated in 76% of tumours [49]. There were a large number of previously undescribed mutations, and viral DNAs and transcripts were also indentified [49]. RNA-seq data identified 4 tumour clusters and pathway analysis demonstrated three frequently dysregulated pathways [49]: cell-cycle regulation (altered in 93% of cases); kinase and phosphatidylinositol-3-OH kinase (PI(3)K) signaling (72%); and chromatin remodelling (89%). A number of the genomic alterations indentified are theoretically amenable to therapeutic targeting [49], and such new therapeutics are desperately needed for UBC [17;18;54].

Choi et al also utilised whole genome mRNA expression profiling to cluster MIBCs into 3 distinct groups, based upon the established molecular subtypes of breast cancer [55]: basal MIBCs shared biomarkers with basal breast cancers and were characterized by p63 activation, squamous differentiation, and more aggressive disease; luminal MIBCs contained features of active PPARγ and oestrogen receptor transcription and were enriched with activating FGFR3 mutations and potential FGFR inhibitor sensitivity; p53-like MIBCs were consistently resistant to a number of chemotherapeutics, including cisplatin; and all chemoresistant tumours adopted a p53-like phenotype after therapy [55]. These findings have important implications for the clinical management of MIBC: they include not only prognostic information, but also suggestions for subtype-directed targeted therapy and potential to predict response to cisplatin-based chemotherapy (although further work is needed to elucidate other biomarkers of resistance) [56]. It is, however, disappointing that NMIBCs were not analysed in the same way by either the TCGA Research Network or Choi et al [48], especially as these tumours represent the vast majority (>75%) of bladder cancer patients [57;58].

## CADHERINS

The classical cadherins are calcium-dependent transmembrane glycoproteins found at the adherens junction and are mediators of cell-cell adhesion in epithelial tissues [19;20]. E-cadherin is a tumour suppressor, playing a central role in suppressing the invasive phenotype of UBC cells [59]. The abnormal expression of other “classical” cadherins (P- and N-cadherin) has been shown to promote a more invasive and malignant phenotype of UBC [24], possibly acting

as key mediators of invasion and metastasis. With such a large difference in UBC outcomes between early stage disease (stage Ta) versus MIBC (stages T2+) it is reasonable to assume that cell adhesion molecules, and in particular cadherins, play a fundamental role in the spread of bladder tumours, initially from the urothelium into the lamina propria (through the basement membrane) and subsequently into the detrusor muscle [22]. Therefore, the classical cadherins and their related molecular pathways represent attractive therapeutic targets for the inhibition of progression in bladder cancer patients [19;59-61].

Cadherins comprise of extracellular (EC1-5), transmembranous, and cytoplasmic domains, with the cytoplasmic domain anchored to the cell cytoskeleton by catenin family members (α-, β-, γ-catenin and p120) [19;61-65]. P21-activated kinase 5 (PAK5) also appears to associate with β-catenin and p120 to stabilise the adherens junction in order to maintain normal cell-cell adhesion [66]. Traditionally, cell-cell adhesion is described as being achieved by the symmetric interactions of the first extracellular domains (EC1) of cadherins on neighbouring cells (trans-interaction) [64;67]; cadherins on the same cell also interact with each other (cis-interaction) through the EC1 domain of one and the EC2 domain of the other [64;67;68]. More recently, it has been described that optimal cell-cell adhesion (50-70pN) is achieved by all 5 EC domains of E-cadherin, and with a cell-cell separation of 5-11nm [65]. See **Figure 1**. E-, P- and N-cadherin were the first cadherins identified, and can all mediate cell-cell adhesion in this fashion [63;69]:

- E-cadherin (CDH1, 120kDa): the main mediator of cell-cell adhesion in epithelial tissues and expressed by most normal epithelial cells [19;61;62;69-71].
- N-cadherin (CDH2, 130kDa): expressed by neural, endothelial, and muscle cells, but not normally by epithelial cells [62;69].
- P-cadherin (CDH3, 118kDa): normally only weakly expressed in the basal layers of stratified epithelia such as oesophagus, bronchus and bladder [24;69;71].

Epithelial malignancies, including bladder cancer, typically show loss of E-cadherin expression as grade and stage progress, and this is often accompanied by increased expression of N- or P-cadherin. This phenomenon is described as “cadherin switching” [19;61;69;71-73], illustrated in the bladder cancer setting in **Figure 2**. Excellent reviews of

the field have been published recently [74;75], and we have previously reviewed this field for bladder cancer [22]; we provide an overview below.

## CADHERIN SWITCHING

Cadherin switching (CS) is a hallmark of epithelial-to-mesenchymal transition (EMT) [76], the process by which epithelial cells lose their characteristic polarity, disassemble cell junctions, and become more migratory as a precursor to invasion and metastasis (they acquire properties analagous to mesenchymal cells) [19;25;61;77-82]. In this setting, CS typically describes a process where the normal expression of E-cadherin is replaced by the abnormal expression of N-cadherin, or where N-cadherin expression is increased and E-cadherin levels remain unchanged [19;61;76]. CS appears to play a role late in many malignancies (including breast, prostate, pancreas, ovarian, bladder and melanoma), resulting in a more invasive and malignant phenotype of disease with a worse outcome [19;24;61;74-76;83-89]. The regulation of CS is yet to be fully elucidated, but most likely involves transcriptional and post-transcriptional events, possibly influenced by cytokines or growth factors [19;61]. Recently, Slug (*SNAI2*, a member of the Snail family of zinc-finger transcription factors) has been identified to play a critical role in EMT by control of the E-cadherin to N-cadherin switch in UBC [90].

In UBC, ourselves and others have described CS, demonstrating increased expression of both P- and N-cadherin in late stage high-grade disease (**Figure 2**) [24;69;89;91;92]. We studied 153 bladder tumours and utilised a variety of cell lines and functional in vitro models [24]: increased membranous P-cadherin expression was observed in almost half of all MIBCs and almost 40% of grade 3 UBCs, accompanied by significantly reduced expression of E-cadherin [24]. Increased P-cadherin expression was associated with worse bladder cancer-specific survival, and P-cadherin status was an independent prognostic factor (alongside grade and stage) [24]. Functional in vitro experiments showed that altering the balance of E- and P-cadherin in favour of P-cadherin expression enhanced anchorage-independent growth, and that P-cadherin alone was unable to mediate normal cell-cell adhesion [24]. We concluded that P-cadherin expression promoted a more malignant and invasive phenotype of bladder cancer (even in the presence of E-cadherin), and appeared to have a novel role late in the disease process [24].

Mandeville et al also demonstrated similar findings [92]. In their in vitro studies, utilising P-cadherin transfection and knockdown, they demonstrated that P-cadherin induced a significant increase in migratory capacity (although with no accompanying change in invasive potential) [92]. The authors suggested that P-cadherin may have a role in regulating the migration of basal cells to the intermediate cell layer in normal urothelium, as well as a role in neoplastic progression [92]. More recently, Wang et al have demonstrated similar findings [89].

Ourselves and others have postulated that a subgroup of aggressive P-cadherin-expressing tumours may be derived from the normally weakly P-cadherin-expressing basal layer of the urothelium [22]. In support of this hypothesis, Van Batavia et al recently demonstrated that papillary and CIS lesions were derived from different urothelial populations, with intermediate cells contributing to non-invasive papillary lesions and basal cells representing the origin of CIS (which ultimately leads to MIBC) [93]. These findings support a model in which the heterogeneity observed in bladder cancers is determined both by genetic changes and the cell lineage from which the tumour originates [93].

However, despite P-cadherin expression being associated with a more aggressive phenotype in many cancers, such behaviour is not ubiquitous and is context dependent [75]. For example, in malignant melanoma, which commonly demonstrates a cadherin switch to N-cadherin expression [22], P-cadherin promotes adhesion and inhibits invasion in a similar fashion to E-cadherin [75], and E-cadherin negative breast cancer cells show many similarities when subsequently transfected with E- or P-cadherin [74;94]. Ribeiro et al investigated these phenomena in detail in a breast cancer model, demonstrating that P-cadherin co-localizes with E-cadherin, and promotes cell invasion by disrupting E-cadherin/catenin interactions [95]. E- and P-cadherin co-expressing tumour cells showed enhanced in vivo tumour growth compared with those expressing only E- or only P-cadherin, and co-expression of E- and P-cadherin in breast tumours correlated with high-grade biologically aggressive tumours accompanied by poor patient survival [95]. It is therefore feasible that P-cadherin only promotes invasion in tissues that endogenously express E-cadherin [74], with heterodimerisation between E- and P-cadherin disrupting the formation of functional cadherin-catenin complexes [75].

It is likely that the key mechanisms involved in P-cadherin's deregulation largely occur in the promoter region of *CDH3* and not by structural alterations of its coding sequences [74]: in 2005, Paredes at al demonstrated

hypomethylation of the *CDH3* gene promoter correlated with P-cadherin overexpression in breast cancer [74;96], and other workers have described this phenomenon in pancreatic [74;97] and colorectal cancers [74;98]. Our own data suggest differential *CDH3* promoter methylation between bladder cancer cell lines and tumours, and normal urothelium [RT Bryan - unpublished data]. Furthermore, the balance of E- and P-cadherin expression impacts the overall genetic programme [74], altering the expression of genes involved in signal transduction and growth factors, cell cycle, cell adhesion and the extracellular matrix, cytokines and inflammation [74;94]. In addition, P-cadherin can provoke the secretion of pro-invasive factors such as the matrix metalloproteinases MMP1 and MMP2 [74;75;99]. The role of p120 also appears important, with P-cadherin probably interfering with the normal binding of p120 to E-cadherin at the adherens junction [74;100]. In a pancreatic cancer model, accumulation of p120 in the cytoplasm (and not bound to E-cadherin at the membrane) appeared to induce the increased cell migration seen following P-cadherin expression via the Rho GTPases, Rac1 and Cdc42 [74;101]. P-cadherin-induced increase in Rac1 and Cdc42 activity (mediated via p120) has also been observed in ovarian cancer [74;102]. Specifically, insulin-like growth factor 1 receptor (IGF1R) can seemingly form a complex with P-cadherin, resulting in the tyrosine phosphorylation and activation of cytoplasmic p120 to promote invasion [75;102;103]; this pathway appears specific to P-cadherin and not the other classical cadherins [75;103].

Taken together, all of the data above emphasise that P-cadherin represents a very attractive target for novel anti-cancer therapeutics [74], and phase I trials of a P-cadherin inhibitor (PF-03732010, a human monoclonal antibody against P-cadherin) have been undertaken [104], although its development now seems to have stalled.

## CADHERINS AND CANCER STEM CELLS

Although solid tumours can be reduced in size or eradicated by chemotherapy, radiotherapy or surgery (alone or in combinations), disease relapse or progression often occurs [105;106]. Such relapse or progression may be explained by the persistence of residual tumour-initiating cells and tumour-maintaining cells, and such cells have been reported in a variety of malignancies (breast, brain, prostate, lung, pancreas, etc) since they were first identified in leukaemia [79;105;107]. Such “cancer stem cells” (CSCs) theoretically have the ability to self-renew and to generate

the heterogeneous cells that comprise a tumour [105-110], and thus need to be eradicated to provide long-term disease-free survival (although it appears that CSCs are more resistant to conventional therapies) [108;110-112]. CSCs may either develop following genetic or epigenetic events in normal stem cells or from differentiated tumour cells that develop the capability for unlimited growth [23;82]. Cellular markers of "stemness" are still under debate, but include CD44, CD24, CD133 and EpCAM [82]: in breast, prostate and oral squamous carcinomas, CSCs are likely identified as $CD44^{+}/CD24^{-}$, whereas CD133 appears to be a CSC marker in gliomas and in colon and pancreatic carcinomas [82].

In a previous review we suggested that the evidence supports the CSC paradigm for UBC, as in other epithelial malignancies [23]. As discussed above, in normal urothelium P-cadherin is only expressed in the basal cell layer (the assumed urothelial stem cell niche) and in a subset of more aggressive UBCs [21-23;92;113]. It is therefore tempting to assume that P-cadherin is a marker of urothelial stem cells and UBC CSCs. Although E-cadherin intercellular adhesion is considered important for the survival of human embryonic stem cells (hESCs) and induced pluripotent stem cells (iPSCs) [82], Kolle et al recently identified *CDH3* (P-cadherin) and *TACSTD1* (EpCAM) as genes encoding hESC markers (antibodies for EpCAM were also able to enrich for pluripotent hESCs) [114]. Vieira et al have also demonstrated that P-cadherin mediates stem cell properties in basal-like breast cancer [115]. P-cadherin therefore appears promising as a potential marker of CSCs in UBC, and similar work is required to confirm these findings in UBC [23]. The fact that *CDH3* (P-cadherin) did not appear in Dancik et al's cell of origin signature described earlier is somewhat surprising since it is normally expressed by basal urothelial cells and in a subset of aggressive UBCs that may also harbour CSCs; however, as described above, P-cadherin's deregulation is most likely governed by epigenetic phenomena rather than structural alterations in its coding sequences [74]. Characterisation of the UBC epigenome/methylome may thus be required to elucidate P-cadherin's role in these UBC subtypes.

It is highly feasible that treatment-resistant cells develop via other mechanisms and pathways, with CSCs being responsible only for a minority [105;116]. Heterogeneity within some tumours may result from selective pressure during tumorigenesis [35;112]. See **Figure 3**. It has been suggested that UBCs arise from more differentiated cells, and self-renewal capacity may be acquired secondarily by inactivation of *p53* and *RB1* function [105;116]. The tumour microenvironment may also play an important role [108], potentially inducing a transitory or reversible CSC-

like state [117]: although EMT may drive the development of CSCs [35], EMT itself is reversible with mesenchymal-to-epithelial transition (MET) favouring a cell's colonisation of distant sites to generate metastases [35]. Whether the CSC state reverses in a similar setting and fashion remains unknown, but such interactions highlight the importance of the tumour microenvironment for all cancer cells, not just CSCs [35].

## CADHERINS AND EPCAM

EpCAM is a type-1 membrane protein that functions as a cell adhesion molecule [118]. It is overexpressed in many epithelial malignancies, including bladder CIS [119] and high grade and advanced stage UBCs [120]. The tumour-specific expression of EpCAM has led to its use for capturing circulating tumour cells by the FDA-approved *CELLSEARCH* system [121], and also for directing therapies to bladder tumours [122]. High tissue levels of EpCAM are associated with a poor prognosis in UBC [120]. However, the role of EpCAM remains elusive: both tumour suppressor and oncogenic properties have been reported. In 2009, Maetzel et al demonstrated that EpCAM could be sequentially cleaved to release extracellular and intracellular domains, 'EpEX' and 'EpICD', respectively [123]; EpICD diffuses into the nucleus and activates oncogenic signalling events by associating with FHL2, β-catenin and Lef-1 [123;124]. See **Figure 4**.

In 2014, as part of our de novo urinary biomarker discovery programme [125], we demonstrated that elevated urinary EpCAM was observed in patients with grade 3 NMIBCs and MIBCs [51;52]. EpCAM was a significant independent prognostic factor for UBC-specific survival, with elevated urinary levels resulting in an increased risk of dying from bladder cancer (hazard ratio 1.76). The predominant form of EpCAM in the urine was a soluble and stable form comprised of the entire extracellular domain, and not the intact protein [52]. Our data therefore suggested that the cleavage of EpCAM into EpEX and EpICD could also occur in UBC [52;123], and further evidence supports this: Ralhan *et al* recently demonstrated that 9 out of 10 cases of UBC were positive for EpICD [126]. However, our work demonstrated that the extracellular domain of EpCAM was released by cleavage immediately adjacent to the cell membrane [52]; the exact location of cleavage was not described by Maetzel et al [123], but the protease

involved (TACE or ADAM 17) usually cleaves membrane proteins 10-15 residues away from the membrane surface [127], suggesting atypical cleavage or an alternative mechanism of extracellular domain release in UBC [52].

Notably, there are important relationships between EpCAM and classical cadherins, although this relationship appears to be tissue- and tumour-specific [128]. In 1997, Litvinov et al suggested that EpCAM has a role in the development of a proliferative and malignant phenotype of epithelial cell [129]: increasing the expression of EpCAM in cadherin-positive cells led to the gradual abrogation of adherens junctions [129]. Although EpCAM had no influence on the total amount of cellular cadherin, it affected the interaction of the cadherins with the cytoskeleton and, as cadherin-mediated cell-cell adhesion diminished, EpCAM-mediated intercellular connections predominated [129]. In a murine fibroblast model, Winter et al subsequently demonstrated that this may occur by disruption of the link between α-catenin and F-actin, probably by EpCAM's disruption of the actin cytoskeleton or possibly via p120 [130]. In later work on human breast epithelial cells, the same authors demonstrated that EpCAM cross-signaling with N-cadherin resulted in the abrogation of cadherin adhesion complexes, mediated by PI(3)K [131]. In breast cancer cell lines, Martowicz et al showed that epithelial cells need EpCAM to promote growth and invasion, yet mesenchymal tumour cells are independent of EpCAM for invasion and progression [132]; the same authors also demonstrated that overexpression of EpCAM in human mammary epithelial cells led to a more proliferative phenotype and downregulation of E-cadherin [133].

Conversely, in a zebrafish model, Slanchev et al demonstrated that EpCAM was indispensible for skin epithelial integrity, and that *epcam* mutant embryos displayed reduced levels of membranous E-cadherin [134]. Guerra et al also postulated an important role for EpCAM in the maintenance of normal intestinal architecture and function in congenital tufting enteropathy, utilising an *mTrop1/Epcam* knockout mouse model of the disease [135]. Other model systems have also demonstrated a direct association between loss of EpCAM expression and loss of cadherin-mediated adhesion [136].

Seemingly, EpCAM has dual functions in normal and cancerous cells with regard to cadherin regulation, cell-cell adhesion and epithelial integrity: EpCAM may be essential for normal epithelial tissue integrity and cell-cell adhesion, but there also appears to be a role for EpCAM in the disruption of normal cell-cell adhesion to initiate EMT, with the subsequent transformed cells acting independently of EpCAM signaling for invasion and progression.

Interestingly, Zeb1 (a known transcription factor inducing EMT) represses both E-cadherin and EpCAM by binding to the EpCAM promoter [137], yet the expression of E-cadherin and EpCAM is related to a stem cell-like phenotype [138;139]; in basal-like breast cancer EpCAM and P-cadherin both appear to be associated with the CSC phenotype [115]. As described for the hallmarks of cancer [34], the timing and ordering of these events appears to differ between normal and tumerous tissues, between different tissue and tumour types, and most likely within the same tumour. It is feasible that during EMT in some malignancies, EpCAM may stimulate the dissolution of E-cadherin/catenin complexes and so permit P- and N-cadherin complexes to predominate (cadherin switching) and β-catenin-mediated oncogene transcription to be upregulated; yet in other tumour types, EpCAM and E-cadherin may be downregulated in parallel, with EMT being driven by alternate pathways. Conversely, EpCAM may stabilise E-cadherin/catenin complexes in some tumours, possibly providing a "stable" and less chaotic cellular milieu unaffected by EMT, in which the development of a CSC phenotype can be "nurtured" by alternative pathways (as described above, EpCAM is a cell surface marker of hESCs, and can be used to isolate a pluripotent subpopulation from hESC culture [114]). If the latter model is correct, then the corollary would potentially be the normalisation of β-catenin-mediated transcription in CSCs; evidence to date in other malignancies suggests that this is not the case [140-142]. However, these are dynamic processes, and even within the same tumour all of these proposed phenomena may be unfolding simultaneously; in the future, single cell genomics may resolve these issues [143;144]. It is important to note that CSC-like treatment-resistant disease may develop via alternate pathways (Figure 3), and there is likely to be considerable plasticity [142], with cells reverting to a less aggressive state by mesenchymal-to-epithelial transition (MET) or by the reversal of the CSC phenotype. Furthermore, the influence of EpCAM on P-cadherin is yet to be elucidated. Our current research is attempting to resolve some of these mechanisms.

## DISCUSSION & CONCLUSION

P-cadherin seemingly has a number of fundamental roles in bladder cancer and other malignancies, including mediating the development of CSCs and EMT, both of which lead to more aggressive disease and worse survival. The mechanisms of these phenomena have been well-described in other malignancies, but remain to be elucidated in UBC. Although we have assumed some crossover of P-cadherin's function between tumour and tissue types, we know that many of P-cadherin's actions are tumour- and tissue-specific. Therefore, such findings from other malignancies need to be reproduced in UBC if we are to genuinely understand P-cadherin's role in this setting. However, given the genomic characterizations of MIBC described above [47;49;55], it is unlikley that P-cadherin represents a "driver" of urothelial carcinogenesis [145]; P-cadherin is more likely to represent an important downstream effector of such driver mutations, with multiple influences on important pathways and phenomena that determine outcomes in advanced disease (eg. EMT, CSCs), probably mediated by PI(3)K [49]. Moreover, it appears that P-cadherin plays a fundamental role in the cell surface and cell adhesion phenomena that permit tumour cells to migrate and invade, and possibly to metastasize.

In conclusion, P-cadherin represents a highly attractive therapeutic target, alongside N-cadherin [146-148]. However, given P-cadherin's complex interactions described above (and undoubtedly many yet to be discovered), P-cadherin inhibition may have far more wide-reaching effects than those directly related to tumour invasion and progression. The difficulties of taking an anti-P-cadherin agent through clinical trials and into clinical use should therefore not be underestimated. Furthermore, the association of classical cadherins with EpCAM is particularly fascinating and requires further elucidation in UBC, and our work in this area is ongoing.

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**LEGENDS FOR FIGURES**

**Figure 1: Cell-cell adhesion in epithelial tissues** (taken from [22]). **a)** overview of cell-cell adhesion complexes; **b)** pictorial representation of cell-cell interactions on neighbouring cells; **c)** molecular structure of the adherens junction, showing the relationship between E-cadherin molecules on neighbouring cells, and between E-cadherin, the catenins (α, β, γ, p120) and the cell cytoskeleton. Traditionally, cadherins on neighbouring cells adhere via EC1 domains, although more recent research suggests that all 5 EC domains are required for optimal adhesion [65].

**Figure 2: Cadherin switching in bladder UCs** (taken from [22]). **a)** E-cadherin is strongly expressed at the cell membrane throughout the normal urothelium. Reduced expression is observed in a proportion of NMIBCs, and the majority of MIBCs demonstrate either reduced expression or a complete absence of E-cadherin; **b)** P-cadherin is expressed in the basal 1-2 layers of normal urothelium, and this pattern is preserved in the majority of NMIBCs. The majority of MIBCs demonstrate strong P-cadherin expression throughout the tumour mass; **c)** N-cadherin is not expressed in normal urothelium or the majority of NMIBCs. However, the majority of muscle-invasive UCs express N-cadherin throughout the tumour mass.

**Figure 3: Proposed pathways for the development of a bladder cancer stem cell phenotype and the relationship with EpCAM** (adapted from [23]). Cancer stem cells (CSCs) result in the development of treatment resistant disease in some cancer settings, and this diagram proposes potential pathways for their development in UBC. There is likely considerable plasticity in these pathways [142], with cells reverting to a less aggressive state by mesenchymal-to-epithelial transition (MET) or by the reversal of the CSC phenotype, and most likely influenced by the tumour microenvironment [23]. We also propose a model whereby EpCAM modulates the development of EMT and/or CSCs (see text).

**Figure 4: EpCAM's relationship with E-cadherin** (adapted from [123;149])**.** The dual role of EPCAM in epithelial tissues is demonstrated. EpCAM can either disrupt the adherens junction, resulting in the release of β-catenin **(a),** or stabilise the adherens junction to maintain E-cadherin's anchorage to the cell cytoskeleton **(b)**. In (a), released β-catenin subsequently forms a complex with EpICD and the transcriptional co-factor FHL2 [150], either at the cell membrane or in the cell nucleus. The EpICD/FHL2/β-catenin complex then interacts with the Lef-1 transcription

731 factor in the cell nucleus to activate the transcription of various target genes, including known oncogenes. In UBC we
732 demonstrated that the extracellular domain of EpCAM is released by cleavage immediately adjacent to the cell
733 membrane [52]. The exact location of cleavage was not described by Maetzel et al [123], but the protease involved
734 (TACE or ADAM 17) usually cleaves membrane proteins 10-15 residues away from the membrane surface [127],
735 suggesting atypical cleavage or an alternative mechanism of extracellular domain release in UBC. (α=α-catenin, β=β-
736 catenin).

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**Figure 1: Cell-cell adhesion in epithelial tissues.**

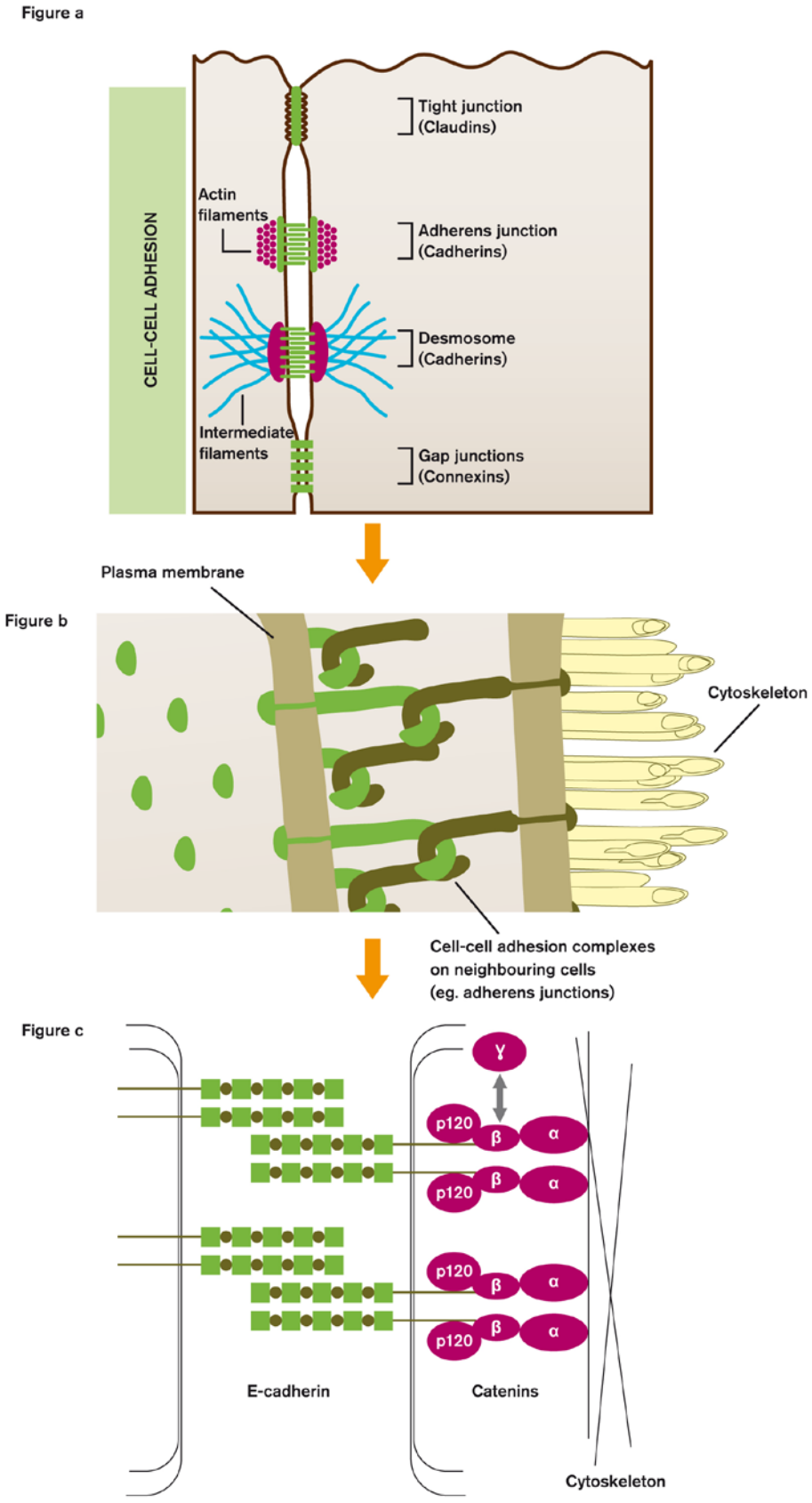
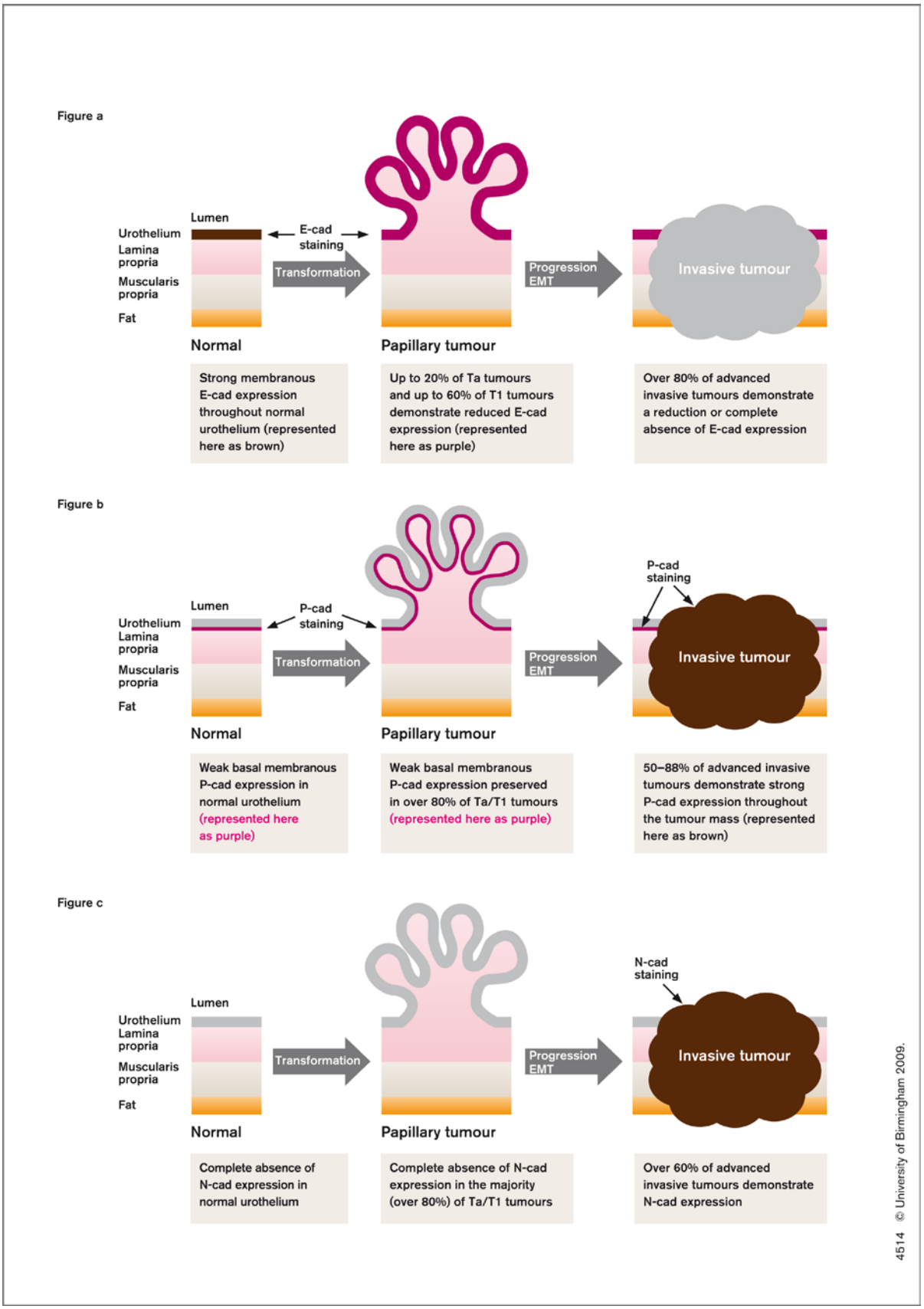


**Figure 2: Cadherin switching in bladder UBCs.**



744 **Figure 3: Proposed pathways for the development of a bladder cancer stem cell phenotype and the relationship with EpCAM.**

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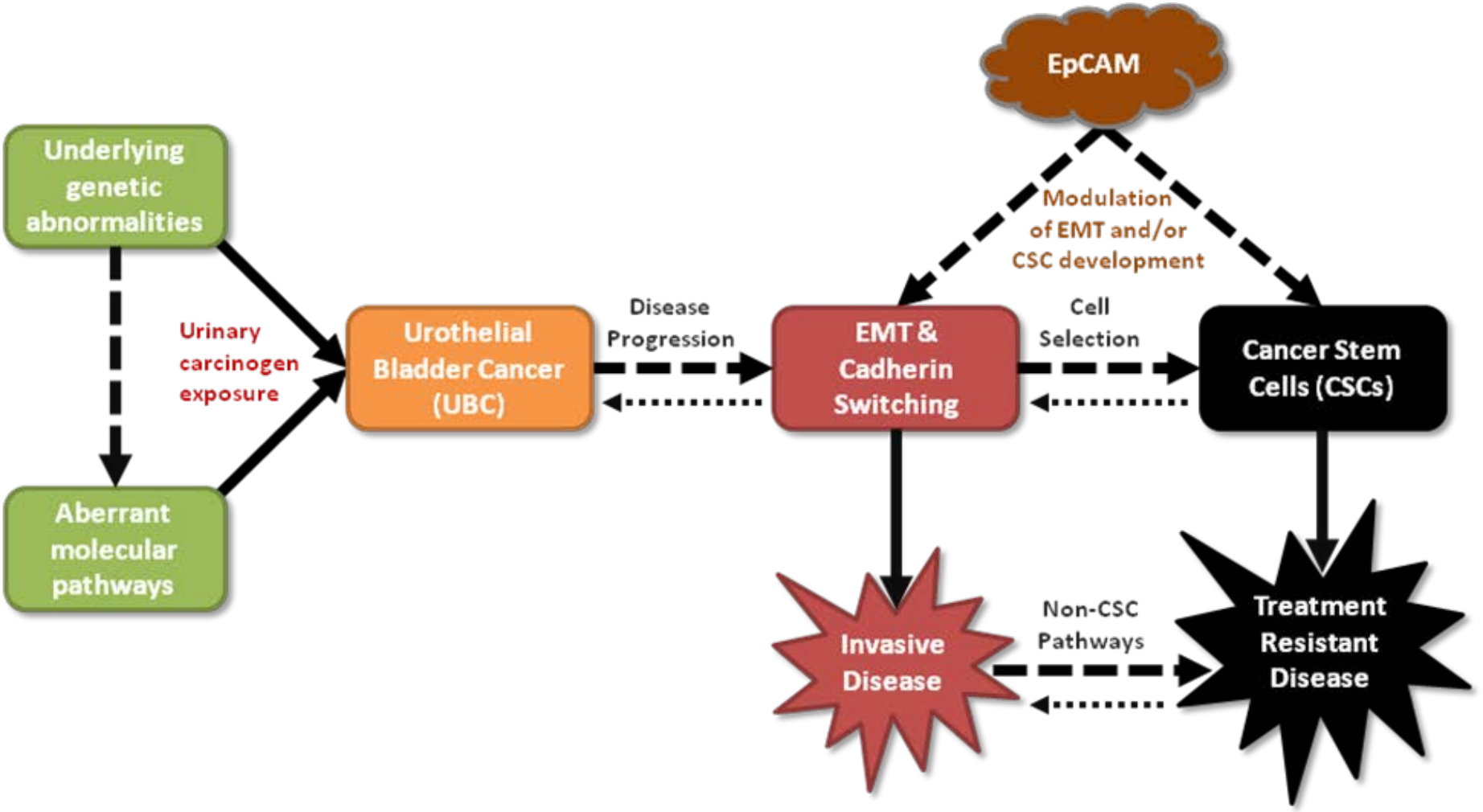


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747 **Figure 4: EpCAM's relationship with E-cadherin.**

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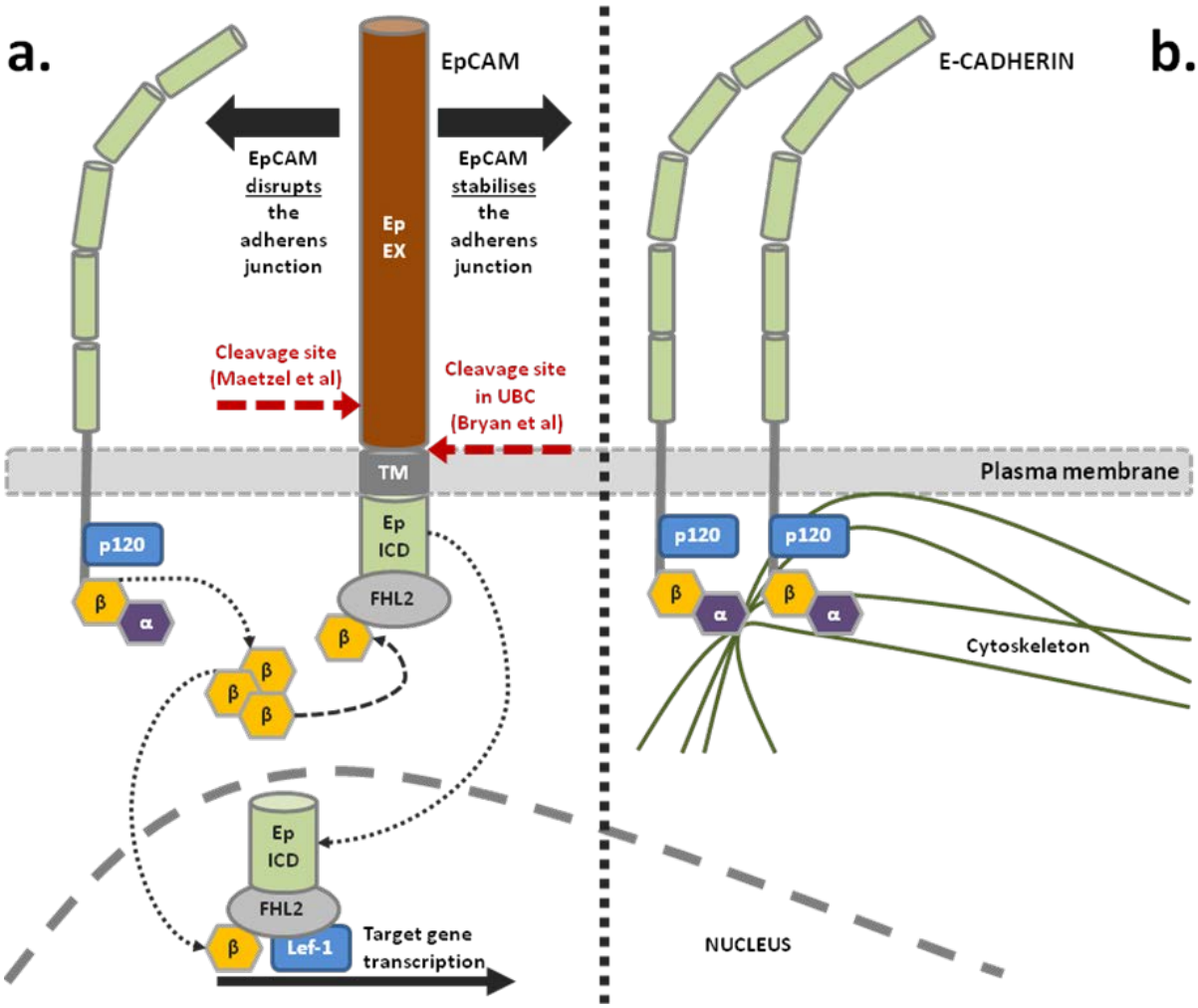


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