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REVIEW

Why are epididymal tumours so rare?

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Epididymal tumour incidence is at most 0.03% of all male cancers. It is an enigma why the human epididymis does not often succumb to cancer, when it expresses markers of stem and cancer cells, and constitutively expresses oncogenes, pro-proliferative and pro-angiogenic factors that allow tumour cells to escape immunosurveillance in cancer-prone tissues. The privileged position of the human epididymis in evading tumourigenicity is reflected in transgenic mouse models in which induction of tumours in other organs is not accompanied by epididymal neoplasia. The epididymis appears to: (i) prevent tumour initiation (it probably lacks stem cells and has strong anti-oxidative mechanisms, active tumour suppressors and inactive oncogene products); (ii) foster tumour monitoring and destruction (by strong immuno-surveillance and -eradication, and cellular senescence); (iii) avert proliferation and angiogenesis (with persistent tight junctions, the presence of anti-angiogenic factors and misplaced pro-angiogenic factors), which together (iv) promote dormancy and restrict dividing cells to hyperplasia. Epididymal cells may be rendered non-responsive to oncogenic stimuli by the constitutive expression of factors generally inducible in tumours, and resistant to the normal epididymal environment, which mimics that of a tumour niche promoting tumour growth. The threshold for tumour initiation may thus be higher in the epididymis than in other organs. Several anti-tumour mechanisms are those that maintain spermatozoa quiescent and immunologically silent, so the low incidence of cancer in the epididymis may be a consequence of its role in sperm maturation and storage. Understanding these mechanisms may throw light on cancer prevention and therapy in general.
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INTRODUCTION

Cancer is not common, despite the constant genetic insults on cells, because of the effectiveness of endogenous tumour suppression.[1] Although cancers occur in the testis of young men, and in the prostate of old men, malignant neoplasms of the epididymis are rare.[2–4] There has been surprisingly little interest in this intriguing observation. The epididymis lies over the testis and receives the non-functional spermatozoa produced by the testis (**Figure 1**). The epithelia in its different regions (**Figure 2**) provide luminal environments that foster sperm maturation and permit quiescent sperm storage.[5] As knowledge about the organ's evasion of tumours could be useful for the design of cancer therapies in general, this review explores the current understanding of tumour initiation and suppression, the general hallmarks of cancer and their relevance to the epididymis. Epidemiological data were extracted from cancer registries in the public domain and calculated from cases reported in the China Hospital Knowledge Database (CHKD) under China National Knowledge Infrastructure (http://www.chkd.cnki.net) from 1979 to 2010.

In most cancer registry reports, the epididymis is not entered as a separate tumour site. In some, it is under 'male genital other than testis, prostate and penis' (**Table 1**) or not at all. Rare as they are, statistics show epididymal cancer incidence rates up to 0.03% of all male cancers, in sharp contrast to almost 20% in Western countries for the most common, prostate cancer. The tumour incidence in the epididymis is about 50 times as rare as that in the testis, whereas the kidney, with the same embryonic origin as the epididymis, is about 100 times more likely to develop tumours (**Table 1**). Such data are not available in China, but screening of the CHKD for men from 1979 to 2010 revealed 328, 18 387 and 54 550 reports of cases of tumours in the epididymis, testis and kidney, respectively. The relative numbers of these published cases for these three organs resemble the relative incidences above.

The CHKD reports that epididymal tumours can occur unilaterally or bilaterally, at any age after puberty (13–83 years) with mean ages in the thirties and forties, and 82% of cases are not malignant. Of the 328 cases, 55% belonged to the 'adenomatoid' type (occurring in the epithelial compartment), 15% were smooth muscle tumours and 5% were in blood or lymph vessels. In 73% of cases, a primary tumour was stated in the diagnosis, only two stated metastasis. Both the invasion of primary epididymal tumours into other organs, and metastases of tumours in other organs spreading to the epididymis have been reported.[3] In a Western survey of 257 benign epididymal tumours, 73% were adenomatoid, whereas of 84 malignant cases, 51% were primary or metastatic carcinomas (epithelial) and 44% were sarcomas (stromal[4]). The most studied human epididymal cancer is cystadenoma, a benign cystic tumour of the gland.

CELLS-OF-ORIGIN OF EPIDIDYMAL TUMOURS

Epididymal stem cells

Tumour cells often originate from normal tissue stem cells.[6] OCT4, SOX2, NANOG, KLF4 and NOTCH1 are factors that maintain

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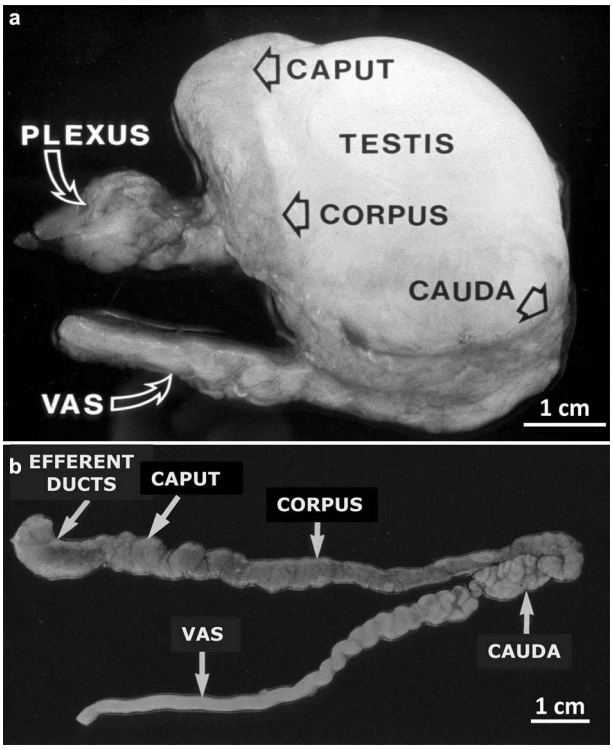

Figure 1 Macroscopic appearance of the human epididymis. (**a**) Photograph of a human epididymis attached to the testis from a 74-year-old man with prostatic cancer. The head of the epididymis (comprising efferent ducts and caput epididymidis (CAPUT)) is partially hidden behind the testis, the entire corpus epididymidis (CORPUS) is visible, and the cauda epididymidis (CAUDA) is partially hidden behind the testis but is continuous with the vas deferens (VAS). PLEXUS indicates the site of entry and exit of blood and lymphatic vessels. Scale bar=1 cm. (**b**) The same organ dissected from the testis showing the general regions of efferent ducts, caput, corpus and cauda epididymidis and vas deferens. Unlike that of other species, the human epididymis does not conform to the usual mammalian model of a thin capsule revealing the outline of the underlying convoluted tubules in clear-cut caput, corpus and cauda regions. The efferent ducts within the proximal head regions are often dark. Scale bar=1 cm.

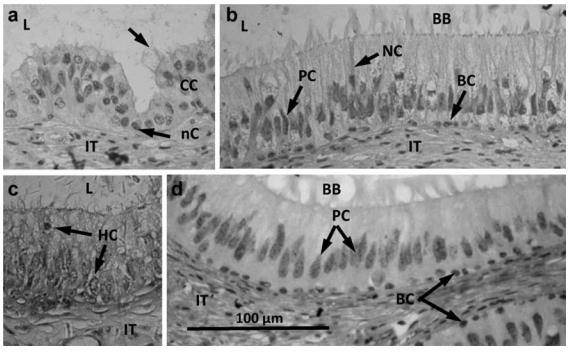

Figure 2 Photomicrographs of the human post-testicular duct system revealing various cell types present in the epithelium. (**a**) Efferent ducts. Arrows indicate an nC and the cilia of a CC. (**b**) Caput epididymidis. Arrows indicate the cytoplasm of an NC and nuclei of PCs and BCs. (**c**) Caput epididymidis. Arrows indicate HCs among principal cells. (**d**) Corpus epididymidis. Arrows indicate nuclei of PCs and BCs. Scale bar in (D) 100 μm, applicable to all micrographs. BB, brush border; BC, basal cell; CC, ciliated cell; HC, halo cell; IT, interstitium; L, lumen; nC, non-ciliated cell; NC, narrow cell; PC, principal cell.

pluripotency in embryonic stem cells. Whereas human embryonic and adult stem cells, and cancer stem cells express OCT4, differentiated normal cells in adults have lost this expression.[7] Both principal and basal cells of the human adult epididymal epithelium express OCT4 and SOX2 in the nuclei and NANOG in the cytoplasm.[8] KLF4 is highly expressed by the whole epithelium of the epididymis of the mouse.[9] *Notch1* mRNA is in all epididymal regions, without cellular identity;[10] *Notch1* is only detectable by *in situ* hybridisation in hyperplastic epididymal epithelial cells of constitutively active *Notch1*-transgenic mice.[11] These embryonic stem cell factors may have other functions, such as cellular differentiation, in the adult epididymis.

Markers of adult stem cells found in other organs are also present in the epididymis (**Table 2**), but as they are expressed by most epithelial cells, no particular type is a likely candidate, for which only a small positive population is anticipated (<2% in the kidney[12] and <5% in the prostate[13]). However, the formation in human epididymal cell culture of epithelial spheres,[8] a characteristic of 'cancer stem cells' or 'tumour initiating cells',[14] suggests that these cells do exist.

Epididymal cancer progenitor cells

In the absence of epithelial stem cells, cells giving rise to tumours can only be mutated progenitor cells located in the epithelium or stroma. Epithelial cells are more likely than stromal cells to be the cancer cells-of-origin, since 55% of all epididymal tumours are adenomatoid, of which 88% are adenomas and 12% cystadenomas (CHKD). Both epithelial and peritubular progenitor cells can be the cancer cell-of-origin in mouse models of prostatic cancer, whereas peritubular cells have been implicated in the human.[6] In the *Pten+Vhl* double gene-knockout mouse model of epididymal cystadenoma, an increase in epithelial basal cells suggested to Frew *et al.*[15] that the neoplasm was generated by basal cell proliferation without differentiation.

Table 1 Statistics on tumour incidence from three cancer registries[a], (i) as a percentage of total male cancers and (ii) as number of cases per 100 000 of the male population

ICD code	*Primary site of tumour*	*Dhaka (i)*	*Ireland (i)*	*New York (ii)*
C60.9	Penis	0.28	0.16	1.0
C61.9	Prostate	1.64	19.07	166.9
C62.9	Testis	1.63	1.19	5.6
C63.0	**Epididymis**	**0.03**		
C63.1	Spermatic cord	0.04		
C63.2	Scrotum	0.06		
C63.7	Seminal vesicle	0.01		
C63	**Other male genital[b]**		**0.02**	**0.2**
C64.9	Kidney	0.99	1.87	21.7
C66.9	Ureter	0.07	0.06	0.7
C67.9	Bladder	1.97	2.34	42.5
C68.0	Urethra	0.05		
Comparison with epididymal tumours (n-fold)[c]				
	Testis/epididymis	**59**	**75**	**28**
	Prostate/epididymis	**55**	**954**	**835**
	Kidney/epididymis	**36**	**117**	**109**

Abbreviation: ICD, International Classification of Diseases.
[a] Sources: Dhaka: World Health Organization cancer registry report for National Institute of Cancer Research and Hospital in Dhaka, Bangladesh in 2005–2007; Ireland: Ireland national cancer registry report, yearly averaged rate in 2005–2009; New York: Cancer registry report for whole of New York State in 2004–2008.
[b] Male genital organs (including epididymis) other than penis, prostate and testis.
[c] The entry for C63 was used for calculation when epididymis was not a separate entry.
Bold entries highlight values used in comparison of epididymis with other organs.

REGULATION OF CELL PROLIFERATION

Growth suppressors

Tumour progression is sustained by cell proliferation following the loss of normal cell controls, which could reflect responses to excessive proliferation signals or evasion of growth suppressors. Retinoblastoma protein 1 (RB1) is a nuclear transcriptional suppressor that interacts with many regulatory proteins, depending on its phosphorylation state.[16] Dysfunction of RB1 is associated with many human cancers.[17]

Rb1 RNA is expressed in the mouse,[18] rat[19] and human epididymis.[20] The expression of the tumour suppressor genes *Pten* and *Rb1* counter-balances the activity of pro-proliferative signal proteins in the proximal epididymis of mice.[21] Xu *et al.*[21] have suggested that the rarity of epididymal cancer could be attributable to growth suppressor genes, including DUSP6 (dual specificity phosphatase 6) and related proteins. DUSPs inactivate phosphorylated proteins in the mitogen-activated protein kinase signal pathway and terminate proliferative mechanisms, and DUSP6 exerts anti-tumour effects on lung cancer cell lines.[22] DUSP6 mRNA is found in the human epididymis,[20] but its comparative expression in other cancer-prone organs is not known.

Cell proliferation rate

High proliferation rates are likely to be associated with high chances of unregulated cell division. In the rat epididymis and prostate, such rates estimated from ^{3}H-thymidine labelling of DNA, decrease rapidly after birth to similar rates for the two organs in the adult, slightly higher than those of liver and skeletal muscle.[23] In the epididymal epithelium, proliferation rates decline during early adulthood from around 2% to stabilize at around 0.5% of labelled cells.[24,25] Similarly low proliferation rates (1.3%) have been confirmed in the adult rat prostate.[26] It seems unlikely that proliferation rates alone can account for the far higher tumour incidence in the prostate than that of the epididymis.

Table 2 Molecular markers of normal adult stem cells in various organs, and their expression in the epididymis without evidence that they are stem cell markers

	Brain[a]	*Liver*	*Intestine*	*Kidney*	*Prostate*	*Epididymal transcript*[b]	*Epididymal protein*[c]
	Mouse/human	*Human*	*Mouse/human*	*Human*	*Mouse/human*	*Human*	*Mouse/bull/human*
Abcg2	+					+ (Ref. 20)	Mouse caput epithelial microvilli, caput and corpus interstitial endothelial cells[165]
Ascl 2			+			+ (Ref. 20)	
Bmi-1	+		+				
CD9	+					+ (Ref. 20)	Bull corpus and cauda epithelium[166]
CD24				+		+ (Refs. 20 and 38)	
CD29		+					Human cultured cells[8]
CD44					+	+ (Refs. 20 and 38)	Human cultured cells; mouse basal cells[8]
CD49b					+		
CD49f		+					
CD73		+					
CD81	+					+ (Refs. 20 and 38)	
CD90		+					
CD95	+						
CD117					+		
CD133	+	+		+	+		Human cultured cells (j); mouse principal cells microvilli except initial segment[167]
CD144	+						
CD146	+	+					
CD184	+						
CK6a					+		
DCAMKL-1			+			+ (Ref. 20)	
EpCAM		+					
Lgr5			+			+ (Ref.20)	
Musashi-1			+				
mTERT			+				
Nestin	+					+ (Ref. 38)	
OLFM4			+			+ (Ref. 20)	
PTEN			+			+ (Ref. 20)	Mouse caput epithelium apical surface, especially initial segment stereocilia[21]
Sca-1					+		
sFRP5			+			+ (Ref. 20)	
SOX4			+			+ (Ref. 20)	
SOX9			+			+ (Ref. 20)	
WIP1			+				
Reference	6	161	162	12	Murine,[163,164] human[13]	20, 38	8, 21, 165–167

[a] In neural stem cells not exclusive to adults.
[b] Expression reported in human epididymal transcriptome.[20,168] No listed markers are found in the proteome.[39]
[c] Protein localized by immunohistology in epididymal cells.

Growth promoters

Wingless/int (Wnt) signalling pathways are important for normal cell proliferation, differentiation and maintenance of cellular homeostasis. Disruption of these, especially the canonical β-catenin-dependent pathway, has profound effects on human cancer development.[27] The binding of Wnt to the frizzled receptors (Fzd) releases β-catenin from a destructive complex; the stabilized β-catenin then enters the nucleus and activates pro-proliferative target genes.[28] Humans and mice share the same gene components of Wnt and Fzd (http://www.stanford.edu/group/nusselab/cgi-bin/wnt/) and almost all Wnt signalling pathway component genes are transcribed in the adult mouse epididymis (http://www.mrg.genetics.washington.edu/index.cgi); many are in the adult human epididymis.[20]

β-catenin is expressed in the rat epididymis at the junctional complexes between epithelial cells,[29] in association with E-cadherin, which is also expressed in the human epididymis.[30] Rat epididymal β-catenin released from E-cadherin following castration accumulates in the cytoplasm, not the nucleus, and there is no subsequent hyperplasia.[29] A high expression of the non-canonical pathway Wnt4, which can inhibit the canonical Wnt pathway through competition for common components,[31] has been reported in the epididymis.[32] The regulation and role of Wnt pathways in epididymal tumourigenesis remain unexplored.

ENDOGENOUS PROTECTION AGAINST MUTAGENIC MICROENVIRONMENTS

Tumourigenic transformation requires a stressful mutagenic microenvironment causing abnormal DNA transcription and uncontrolled cell division, which can be brought on by oxidative attack on DNA itself or by the activation of oncogenes.

Antioxidant protection of epididymal cell DNA

The production of reactive oxygen species (ROS) is normally limited by the low oxygen tension in epididymal tissue[33] and epididymal lumen.[34] This may stem from the epithelial principal cells' expressing high activities of indoleamine-2,3-dioxgenase (IDO) and tryptophan-2,3-dioxgenase (TDO), both of which can incorporate molecular oxygen into their substrates,[35] and from the high number of luminal spermatozoa, which consume oxygen even when concentrated and immotile.[36] Complementing this non-oxidative environment are anti-oxidant enzymes and components removing superoxide radicals and hydrogen peroxide,[37] some of which are reported in the human epididymal proteome.[38,39] These anti-oxidant strategies make excessive oxidative damage to epididymal cell DNA unlikely.

Oncogene expression in the normal epididymis

Naturally occurring cancers and cancerous growths induced *in vitro* are associated with the expression of oncogenes, which are often mutated versions of normal proteins not normally expressed in adult tissues. When present in non-metastatic tissue, the non-mutated forms (proto-oncogenes) may have other cellular roles. For example, c-*ros* in many developing mouse organs declines in expression as differentiation proceeds and stops in the adult, but is upregulated in many tumours.[40] Activation of the mutated oncogenes or overexpression of proto-oncogenes in tissues can give rise to tumours within them (see section on 'Dormancy of early tumour cells').

Many proto-oncogenes are expressed in the normal adult epididymis. In contrast to the common postnatal downregulation of c-*ros*, it is upregulated in the proximal caput epididymidis around puberty, when the initial segment differentiates, and sustained in adulthood.[41] c-*Ros* deletion leads to male-specific infertility because of epididymal maldevelopment.[41] c-*ROS* has been identified in the human epididymis.[42] In adult mice, epididymal expression of c-*Mos*, A-*raf*, B-*Raf*, c-*Raf*, L-*Myc*, N-*Myc*, B-*Myc*, C-*Myc*, c-*Ret* and *Met* have been identified.[43,44]

Explanations for epididymal oncogene expression not being accompanied by typical oncogenic activity include: (i) the counter-balancing of antiproliferative gene expression (see above)—the expression of antiproliferative B-Myc protein exceeds that of pro-proliferative c-Myc;[45] (ii) the triggering, by high expression of some oncogenes (e.g., *Ras* family and C-*Myc*), of fail-safe mechanisms that induce senescence or apoptosis (see section on 'Intrinsic barriers to tumour formation'), instead of inducing proliferation;[46] (iii) the provision of protection against tumours by endogenous oncogenes. The expression of K-*Ras*, B-*Raf* and *Myc*, expressed in the adult human epididymis,[20] enhances the basal expression of the transcription factor Nrf2 (nuclear factor E2-related factor 2), which controls intracellular levels of ROS through an inducible antioxidant programme.[47] *Nfe2l2* (*Nrf2*) mRNA is highly expressed throughout the rat[19] and mouse epididymis[18] and also found in that of man.[20] Adult $Nrf2^{-/-}$ male mice suffer reduced antioxidants and raised lipid peroxidation in the epididymis, accompanied by reduced epididymal sperm motility.[48] Whether the constitutively expressed oncogenes in the epididymis contribute to its especially strong anti-oxidation defence mechanisms (see section on 'Antioxidant protection of epididymal cell DNA') warrants investigation; and (iv) non-oncogenic functions of oncogene proteins in the epididymis. The regulation of A-*Raf* and B-*Myc* by androgens, and of B-*Myc* and *c-Ros* (in the initial segment) but not A-*Raf* (in the caput) by testicular fluid,[49,50] mirrors that of cancer-unrelated epididymal proteins. Similarly, 'cancer markers' such as metastasis-associated protein 1[51] and human epididymal protein 4,[52,53] which are normally expressed, may serve other functions.

CELL–CELL JUNCTIONS AND CANCER

Tight junctions

Tight junctions are the most distinctive cell junctions in epithelia[54] and constitute an inherent barrier to aberrant cell proliferation by mediating the meiotic block upon cell contact,[55] maintaining adhesion with adjacent cells and generating a barrier that restricts the paracellular passage of fluid and separates luminal and basolateral compartments. Tight junctional disruption allows luminal growth factors to interact with their basolateral receptors, which encourages mitogenesis.[55] Expression of the main junction-associated proteins (occludins, claudins) is altered in cancerous tissue.[56] Of more than 100 tight junction proteins, some are themselves tumour suppressors,[57] whereas others are hubs for signal transduction leading to cell proliferation and tumourigenesis.[58]

Tight junctions in the epididymis are the physical component of the blood–epididymis barrier[59] and the major tight-junction proteins have been localized in the human epididymis.[60] When Sertoli cell tight junctions undergo seasonal regression, epididymal tight junctions remain intact in the mink[61,62] and are even reinforced in bats.[63] In a related situation in men (non-obstructive azoospermia), caput epididymidal junctions appear normal, with no change in expression and location of most claudins and tight junction proteins.[64] Stable and persistent tight junctions may be one component of epididymal resistance to cancer.

Other junctions

Epithelial cell adhesion is also mediated by adherens junctions and desmosomes, both of which contain cadherins,[65] whereas gap

junctions, formed from connexins, provide intercellular communication. Cadherin and catenin expression is altered in cancers[66] and there is evidence for an inhibitory role of connexins in the progression of primary tumours, despite suggestions of their enhancing tumour cell adhesion and migration in cancer invasion and metastasis.[67] Adherens junctions, desmosomes and gap junctions are found in the epididymis.[61,62,68] Of the connexins considered primary tumour suppressors, Cx26, Cx32[69] and Cx43[70] have been localized in the gap junctions of the rat epididymis.

INTRINSIC BARRIERS TO TUMOUR FORMATION

Apoptosis

Tumour suppression mechanisms include the prevention of cellular proliferation by apoptosis, which can be activated by intrinsic and extrinsic factors. Apoptosis can be induced by the oncogenes C-*Myc*, *E1A* and *E2F*, and regulated by the p53 (tumour suppressor protein 53) pathway.[71] Epididymal apoptosis is normally non-detectable by TUNEL and DNA ladder analysis,[72] and is rare in the epithelium.[73] Such low apoptosis may reflect the presence of anti-apoptotic B-cell lymphoma-2 (Bcl-2) and absence of pro-apoptotic Fas.[74] The normal mouse epididymis expresses p53 and the apoptosis effector caspase 3,[75] yet apoptosis in this organ following cryptepididymis and castration is independent of p53 and Fas/FasL,[75,76] as is rat epididymal apoptosis after efferent duct ligation.[73]

Senescence

Another mechanism of intrinsic tumour suppression is age-independent 'senescence', a signal transduction programme leading to irreversible arrest of cell proliferation, accompanied by distinct changes in cellular phenotype.[77] Senescence can be induced by oxidative, bacterial, genotoxic and oncogenic stresses; for example, continuous overexpression of *Ras* leads to irreversible cell arrest.[77,78] In mouse prostatic tumours induced by *Pten* inactivation, and in human prostatic tumours,[79] oncogene-induced senescence is detected before neoplasia develops, but not afterwards when this barrier is overcome.

The most common and consistent molecular marker of oncogene-induced senescence in mouse and man is the enzyme 'senescence-associated β-galactosidase' (SABG), with cytoplasmic activity detectable at pH 6, contrasting with the lysosomal optimum of pH 4.[77,80] Among the factors regulating this senescence programme, p53 is the best established.[71] In mice, many prostatic cells become positive for p53 and SABG during cancer development following *Pten* inactivation.[79]

β-galactosidase (β-Gal) with neutral pH activity is constitutively expressed in the caput and corpus epididymidal epithelium of young adult rats and mice.[81] It is located in the supranuclear region of principal cells in the initial segment, but not in adjacent caput regions,[82] and in the corpus.[42] It is not known if this β-Gal is identical to SABG in oncogene-induced senescence. The SABG-encoding gene *GLB179* mRNA is found in the human epididymal transcriptome.[20] A rat epididymis-specific β-galactosidase-like protein (GLB1L4), absent from the initial segment, has immunoreactivity in the remaining principal cells of the caput,[83] but has no enzymatic activity.

The dual role of p53 in apoptosis and senescence makes p53-null mice excellent models for the study of cancers resulting in various tissues.[84] Epididymides from such mice exhibit cribriform hyperplasia, which is characterized by intraepithelial protein-filled vacuoles, infrequent apoptosis and rare mitotic figures.[84] These changes are not considered neoplastic, but a variant of normal histology that can be seen in aging mice[85] and occasionally in the human epididymis.[86] Such abnormal intraepithelial vacuoles can be induced in the epithelium of prepubertal, but not adult, mice following deprivation of upstream regulatory luminal factors by ductal ligation.[87] It may reflect the presence of an occult cancer.[1] The lack of relationship of epididymal p53 with apoptosis (see section on 'Apoptosis'), the possible role of p53 in senescence, and the constitutive activity of β-Gal in the epididymis support the hypothesis that senescence, induced by the constitutively expressed oncogenes (see section on 'Oncogene expression in the normal epididymis'), constitutes a persistent barrier to cancer in the epididymis.

ENDOGENOUS BARRIERS TO TUMOUR FORMATION: IMMUNOSURVEILLANCE

Upon failure of intrinsic protective mechanisms, when cellular transformation to tumourigenesis occurs, a second line of defence, 'immunoediting', is triggered. Most cancer-forming cells are immunogenic and routinely eliminated in immunocompetent hosts by this process.[88,89]

Eradication of early tumour cells

The molecular signals from early tumour cells that trigger their eradication remain to be identified, but such cell removal involves innate and adaptive immunocompetent cells,[89] including macrophages, natural killer (NK) cells and NK T lymphocytes (NKT), dendritic cells, as well as $CD4^+$ and $CD8^+$ T lymphocytes. Leukocytes within the normal epididymal epithelium are considered to block the development of immunity against luminal spermatozoa. In human epididymides from organ donors, no B lymphocytes are found but T cells are numerous, making up around 13% of epithelial cells.[90] Of these, the cytotoxic T cells are predominant in the epithelium, suggesting selective recruitment from the interstitium where the much lower cytotoxic/regulatory T-cell ratio reflects that of blood.

In epididymides from young and old men, intraepithelial macrophages are reported, some in direct contact with lymphocytes.[91] When young adult rats age, the numbers of $CD4^+$ T cells, $CD8^+$ T cells and macrophages in the epithelium change, leading to the proximal regions having more intraepithelial immunocompetent cells than the distal.[92] Although quantitative data are lacking for men, the same may be true, as the cauda epididymidis is the site with the highest tumour incidence: of the 218 cases of epididymal cancer in the CHKD where the site of occurrence was given, 30% were in the caput, 8% in the corpus and 60% in the cauda, with $<3\%$ occurring throughout the whole epididymis.

A chemokine secreted by activated T cells to induce movement of immune cells from the bloodstream, RANTES (Ccl5), is expressed at the RNA and protein levels in all cells of the normal mouse epididymis and efferent ducts.[93] Its function here is unclear and the authors suggested a reproductive rather than immune role in view of its presence on luminal spermatozoa.

Dendritic cells are antigen-presenting cells mediating communication between the innate and adaptive immune cells. Cells with dendritic phenotype forming a dense network have been described at the base of the mouse epididymal epithelium, sending cytoplasmic extensions between epithelial cells towards the lumen.[94] NK and NKT cells, innate immune cells participating in the elimination phase of tumour immunoediting,[89] have not been studied in the epididymis, other than in its associated adipose tissue. In contrast to ~2% of all lymphocytes in blood or lymph nodes being NK or NKT cells, these cells account for ~12% in inguinal fat and exceed 30% in the epididymal fat pad.[95] Although their presence may explain the low cancer rates in adipose

tissue, there is no prominent epididymal fat pad in the human, as there is in laboratory rodents, and no attention has been paid to any adipose tissues adhering tightly over the caput and corpus.

In many organs, the innate immune system operates in non-immune epithelial cells. These recognize bacterial, fungal and viral components through membrane-bound and intracellular Toll-like receptors (TLRs), retinoic acid-inducible-gene-1-like receptors and Nod-like receptors, and respond by killing the pathogens or secreting cytokines to attract destructive immune cells. TLR are constitutively present within the rat epididymal epithelium,[96] whereas Nod2 is upregulated in the mouse epididymis upon TLR stimulation by lipopolysaccharide.[97] The rodent epididymis responds to lipopolysaccharide challenge by upregulation of tumour necrosis factor[97] and secretion of interleukins.[98] Lipopolysaccharide-binding protein is present within the apex of principal cells of the human epididymis.[99]

Dormancy of early tumour cells

When the elimination of early tumour cells is incomplete, the surviving cells go through a 'dormancy' phase when the adaptive immunity of the tissue suppresses tumour outgrowth. This is achieved by $CD4^+$ and $CD8^+$ T cells, interleukin-12, interferon-γ and other factors.[89] This equilibrium phase can last as long as the host lives, or the dormant tumour can remain undetected until it escapes suppression upon acquiring the ability to circumvent immune recognition and destruction (see section on 'Escape of early tumour cells from immunological surveillance').

Evidence that transformed cells experience dormancy in the epididymis is provided by reports of the resistance of the epididymis to tumourigenesis, when other organs succumb, in response to the imposition of tumourigenic challenges, whether they be oncogene activation, downregulation of regulators or overexpression of growth factors: (i) *in vivo* activation of c-*erbB-2* (the human homologue of c-*neu*) leads to preneoplastic tumours in the kidney and lung, yet only hyperplasia and hypertrophy in the epididymis;[100] (ii) activation of c-*neu* itself,[101] and of *Notch*-related *int-3*,[102] also lead only to epididymal hyperplasia in animals that develop mammary adenocarcinoma; (iii) activation of H-*ras* produces only epididymal hyperplasia in animals that develop hepatocarcinoma and polycystic kidney disease;[103] (iv) constitutive expression of stabilized β-catenin leads to hyperplasia in both epididymis and prostate, but only to transdifferentiation in the latter;[104] (v) silencing the p53 gene induces only hyperplasia in the epididymis of mice that develop other tissue cancers;[84] (vi) targeting the simian virus 40 large T antigen to the caput epididymidis results in the GPX5Tag-1 line to full tumours in the prostate but only non-malignant hyperplasia and dysplasia of the epididymis;[105] (vii) overexpressing transforming growth factor alpha (TGF-α) leads to hyperplasia of p63-positive basal cells in the prostate but not in the epididymis;[106] and (viii) overexpressing vascular endothelial growth factor (VEGF) leads to epididymal hyperplasia without malignancy.[107]

All these experimental models indicate that while the epididymis may succumb to unregulated proliferation, the resulting hyperplastic tissue is prevented from further development into tumours, which could represent a condition of dormancy (**Figure 3**).

Escape of early tumour cells from immunological surveillance

To circumvent immunosurveillance and rejection, tumour cells downregulate expression of classical tumour antigens (HLA class I) and upregulate non-classical (HLA-G) antigens, which prevent stimulation of cytotoxic $CD8^+$ T cells and NK cells. They also induce changes in their microenvironment (e.g., upregulating IDO, VEGF, TGF-β, cathepsin D) that render it hostile to the cells responsible for the immunosurveillance.[108] In tumour niches, VEGFs inhibit immune dendritic cells and recruit myeloid-derived suppressor cells that block T-cell function, whereas IDO has a dual action, both removing the amino-acid tryptophan essential for the survival of immune cells, and converting it to products that are toxic to these cells. TGF-β inhibits the proliferation of T cells and NK cells, and suppresses their cytotoxic function against tumour cells.[109] Independent of its proteolytic activity, cathepsin D attenuates the effect of anti-tumour cytokines secreted by dendritic cells and is highly expressed in prostate and breast cancer cells.[110]

The epididymis expresses many of the above factors, employed by tumour cells to evade immunological attack, but a pro-tumour role for them in the epididymis is unlikely. VEGF is constitutively expressed in the normal adult epididymal epithelium of rodents and men (see section on 'Atypical responses to hypoxia in the epididymis'). TGF-β expression has been reported in the epithelium (TGF-β3) and interstitium (TGF-β1) of the rat epididymis;[111] in the marmoset, TGF-β1 is located in apical cells and its receptor in the principal cells.[112]

In contrast to its interferon-γ-induced expression in most tumourigenic organs, IDO is constitutively expressed in the epididymis[113] with an activity far exceeding that of many other organs.[114] IDO1 is located in principal cells,[114] as well as in basal and apical cells, but not in the interstitium. Enzyme expression is highest in the caput and corpus, but absent from the cauda epididymidis. There is an increased expression of inflammatory markers in the caput region of IDO1-null mice,[115] attesting to a role for this enzyme in regulating local immune status. IDO-like proteins (INDOL, IDO2) have been found in man and mouse, with highest activity in the epididymis after the kidney.[116]

Although TGF-α, VEGF, IDO and cathepsin D, which act against anti-tumour immune cells in cancers, are expressed by the epididymis, immune cells persist there (see section on 'Eradication of early tumour cells'). This may reflect the absence of necessary coeffectors for attacking anti-tumour immune cells in the healthy organ. Alternatively, the constitutive and persistent, rather than inducible and transient, expression of these factors in the epididymis may lead to the unresponsiveness of the targeted immune cells, *via* exhaustion or clonal anergy. The retention of active immune cells, keeping check on any neoplastic epididymal cells, would prevent progression to full tumours, consistent with the observations cited in the section 'Dormancy of early tumour cells'.

ANGIOGENESIS AND ITS ENDOGENOUS INHIBITORS IN TUMOURIGENESIS

Pro-angiogenic factors

Angiogenesis is considered essential for tumour progression and metastasis; the process may be an early tumourigenic event. In healthy adult organs, there is a balance between anti- and pro-angiogenic factors to maintain vasculature stability. The activation of the vasculature in pre-malignant lesions, mainly by overexpression of VEGF by hyperplastic or neoplastic epithelial cells, is characterized by endothelial cell proliferation and sprouting of vessels from pre-existing vessels, resulting in increased microvessel density.[117]

In the normal human epididymis, VEGF is expressed in basal and principal cells, and in peritubular myoid cells.[118] Since neovascularisation is rarely observed, a paracrine role for epididymal VEGF in regulating vessel permeability is possible. In adult rats, VEGF is localized in the principal cells of the whole epididymis.[119] Interestingly, in addition to the main VEGF isoforms, the mRNA of the splice variant $VEGF_{144}$ is detected, which is rarely expressed in adult organs except when they are

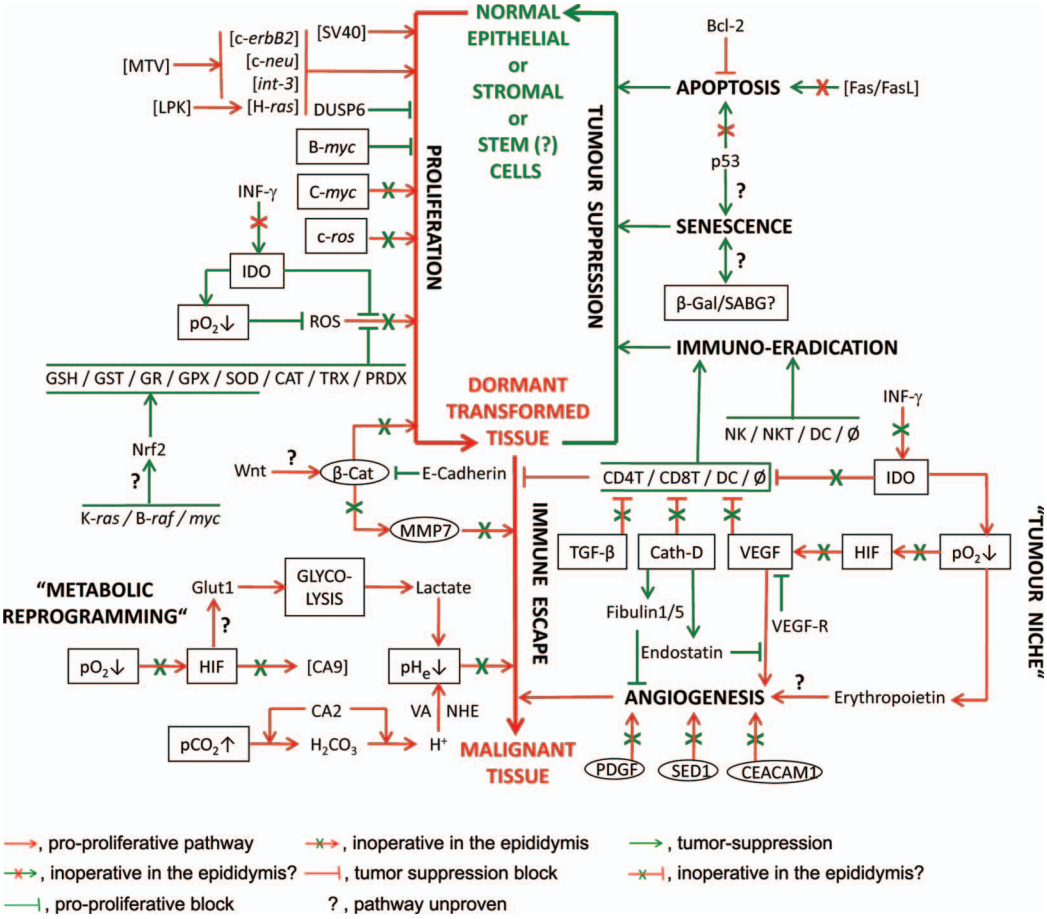

Figure 3 Representative factors found in the epididymis that are involved in pro-cancer and tumour suppression mechanisms in cancer-prone tissues. Tumours follow transformation of a susceptible cell type to a potentially cancerous form (*via* proliferation) that can either be suppressed (immune suppression) or remain dormant until conditions allow progression into malignant tissues (by immune escape). Pro-cancer forces are shown in red; anti-cancer in green. Boxed factors are constitutively expressed in the epididymis, but only upregulated in cancer cells upon induction by pro-tumour factors. Circled factors are in the epididymal epithelium but not localized where they are in cancerous tissues. Square brackets enclose factors that have not been proven to be in the epididymis or non-endogenous factors that have been used to drive tumourigenesis. Factors not in parentheses are present in the epididymis as RNA or protein (see text for details). The major causes of tumourigenicity, ROS and oncogene expression, are either heavily suppressed or inoperative, respectively. There is no evidence that the boxed factors elicit the responses that occur in cancer-prone tissues. Even when induction of tumourigenesis is attempted (e.g., by introduction of SV40 or driving exogenous oncogenes by MTV or LPK), no progression beyond epididymal hyperplasia occurs, suggesting that immunosurveillance maintains the tissue in a dormant state. Bcl-2, B-cell lymphoma-2; β-CAT, beta-catenin; β-Gal, beta-galactosidase; CA2,9, carbonic anhydrase 2,9; CAT, catalase; Cath-D, cathepsin D; CD4T/8T, CD4-/8-positive T-lymphocytes; CEACAM1, carcino-embryonic antigen-related cell adhesion molecule; DC, dendritic cells; DUSP6, dual-specificity phosphatise 6; Fas, Fas receptor CD95; FasL, Fas ligand CD95L; Glut1, glucose transporter 1; GPX, glutathione peroxidases; GR, glutathione reductase; GSH, glutathione; GST, glutathione S-transferase; HIF, hypoxia-induced factor; IDO, indoleamine 2,3-dioxygenase; INF-γ, interferon gamma; LPK, L-type pyruvate kinase gene promoter; MMP7, matrix metalloproteinase 7 (matrilysin); MTV, mouse mammary tumour virus long terminal repeat; NHE, sodium-hydrogen exchanger; Nrf2, nuclear factor E2-related factor 2; NK, natural killer cells; NKT, natural killer T-lymphocytes; p53, tumour suppressor protein 53; $pCO_2\uparrow$, high tissue carbon dioxide; PDGF, platelet-derived growth factor; $pH_e\downarrow$, high extra-cellular acidity; $pO_2\downarrow$, low tissue oxygen tension; PRDX, peroxiredoxins; ROS, reactive oxygen species; SABG, senescence-associated beta-galactosidase; SED1, secreted protein 1 with two EGF repeats and discoidin domains; SOD, superoxide dismutase; SV40, Simian virus 40 large T antigen; TGF-β, transforming growth factor beta; TRX, thioredoxin peroxidases; VA, vacuolar ATPase; VEGF, vascular endothelial growth factor; VEGF-R, VEFG receptor; Wnt, Wingless/int pro-tumour protein; ϕ, macrophage.

cancerous.[120] In mice, overexpression of VEGF leads to epididymal hyperplasia associated with increased capillary density, fenestrations and permeability.[107] Isoforms of another pro-angiogenic factor, platelet-derived growth factor (PDGF) and its receptor[121] are present in the epididymis of adult animals, not in the stroma, but in the epithelium,[122] a site inconsistent with a vascular role.

The adhesion molecule CEACAM1 has pro-angiogenic properties in endothelial cells[123] and can promote cell migration.[124] It is expressed in normal human epididymal tissue at the luminal surface of the epithelium.[125] The luminal rather than stromal protein location casts doubt on a pro-angiogenic function. Similarly, SED1 in rodents and its homologue Del1 in man, are associated with tumour neovascularisation,[126] but both are located in the epididymal epithelium, not stroma.[127]

By digesting the stromal matrix, matrix metalloproteinases (MMPs) have been considered angiogenic, although they also act as tumour suppressors.[128] *MMP7* (matrilysin) is a target gene of the Wnt/β-catenin pathway[129] (see section on 'Growth promoters') and is expressed in the efferent ducts and initial segment of mice.[130] This unusual presence in epithelial cells suggests that the protein is involved in tissue development rather than tumourigenesis;[131] indeed, transgenic mice overexpressing MMP7 in the epididymis exhibit the protein in a structurally normal organ.[132] Erythropoietin promotes tumour growth

by stimulating angiogenesis.[133] It is present in the epididymal interstitium, but whether there is an angiogenic role is uncertain.[134]

As well as mediating peritubular contractility and sperm passage through the epididymal lumen, endothelins and their receptors (ER) are associated with cancer.[135] ER-A is involved in cell proliferation, migration, invasion and angiogenesis, and ER-B in countering tumour progression by promoting apoptosis and binding ET-1.[136] Endothelin-1 and these receptors are present in the human epididymal epithelium and peritubular muscle, respectively.[137]

Anti-angiogenic factors

Many endogenous inhibitors of angiogenesis are fragments of extracellular matrix molecules making up the vascular basement membrane. They are released upon proteolysis of the extracellular matrix by enzymes such as MMPs, cathepsins and elastases,[138] and one such proteolytic product is endostatin, the C-terminal fragment of collagen 18. In the human epididymis, collagen 18 is synthesized by the epithelial cells and secreted to form the basement membrane. Endostatin is highly expressed in the epithelial cells, but only weakly expressed in the interstitium of the normal organ, and is absent from human epididymal adenomatoid tissue, except in blood vessels within and around the tumour.[139]

Cathepsin D degrades endothelial basement membrane into fibulin-1 fragments and fibulin-5, which inhibit angiogenesis and suppress tumour growth.[140] In mice, the epididymis has the highest expression of fibulin-1D and fibulin-2, possibly attributable to their interaction with interstitial sex hormone-binding globulin.[141] The transcript of fibulin-5, which antagonizes VEGF signalling and inhibits endothelial sprouting, is expressed in the entire epididymis of rats and mice, especially in the corpus region (http://www.mrg.genetics.washington.edu/). Cathepsin D, fibulin-1 and -5 are reported in the human epididymal transcriptome.[20]

Non-matrix-derived angiogenesis inhibitors, interferons and certain interleukins,[138] are present in the rat epididymis,[98] human epididymal cysts and spermatocoele fluid.[142] Vasostatin, a potent inhibitor of neovascularisation in tumour growth, acting by preventing endothelial cell attachment to laminin, is the N-terminal domain of human calreticulin.[138,143] Although calreticulin is highly expressed in rodent (http://www.mrg.genetics.washington.edu/) and the human epididymis,[38] its fragment, vasostatin, is unreported.

Non-endothelial cell VEGF receptor (VEGFR) is considered an inhibitor of angiogenesis by binding to its ligand, thereby preventing VEGF signalling. Whereas VEGF is localized in the principal cells of the whole epididymis, VEGFR1 (FLT-1) is found in the caput and cauda in the rat.[119] Human FLT-1 has high expression in the efferent ducts and colocalizes with VEGF in epithelial cells and in luminal macrophages, but in the epididymis proper it is only confined to the interstitial lymph vessels, and non-detectable in the epithelium where VEGF is positive.[118] Interstitial FLT-1 is well situated to antagonize any VEGF secreted there. VEGFR2 (KDR) is localized in efferent duct epithelial cells and in basal cells of the epididymal tubule in addition to endothelial cells.

Angiogenesis in epididymal tumours

A rare quantitative study of the angiogenic status of epididymal adenomatoid tissues revealed blood vessel densities similar to, or less than, those of normal tissues[125] and almost the whole tumour tissue was negative for the pro-angiogenic factor CEACAM1, in contrast to the upregulation of endothelial expression reported in cancers of other organs such as the prostate. Whether poor angiogenesis is a common feature of epididymal tumours awaits more evidence; nevertheless, it may provide one explanation of the rarity of epididymal tumours.

THE METABOLIC CHARACTERISTICS OF CANCER CELLS

A hallmark of cancer cells is their switch to enhanced glycolysis under aerobic conditions, which generates energy and intermediate metabolites needed for tumour growth and proliferation.[144] This is effected through the signalling of oncogenes, including AKT kinase that upregulates hypoxia-induced factors (HIF-1α and HIF-1β), which in turn upregulate glycolysis. Under normal oxygen tension, HIF-1α is degraded after polyubiquitination by the VBC complex; in hypoxia, the VBC complex dissociates, allowing intact HIF-1α to interact with the constitutively present HIF-1β/ArNT and become an active dimer. Unless hypoxia sets in, the VBC complex keeps HIF inactive and suppresses both angiogenesis[145] and the upregulation of glycolysis, which would favour tumour cell metabolism.[88]

Active HIFs enable normal cells to adapt to hypoxic conditions, by binding to sites in the promoter regions of genes involved in energy metabolism, angiogenesis, stress and O_2 delivery. In cancers, HIF upregulates the glucose transporter GLUT-1 which provides glucose to the tissue, carbonic anhydrase 9 which produces carbonic acid from respiratory carbon dioxide[146] and VEGF which stimulates angiogenesis and thereby improves blood flow.[147,148] The combination of low oxygen tension, increased aerobic glycolysis and the production of extracellular carbonic and lactic acids causes a reduction in extracellular pH, which selects and retains cancer cells in the tumour niche.[149] Proton export by the tumour cells raises their intracellular pH, which is an effective proliferative trigger.[150]

Atypical responses to hypoxia in the epididymis

VHL, a protein component of the VBC complex, is encoded by a cancer suppressor gene, since its mutation in patients with von Hippel–Lindau (VHL) disease is responsible for a certain group of tumours found at various sites including the epididymis.[4] The multifocal epithelial tumourlets in the epididymis associated with VHL disease are confined to the efferent ducts,[146,148] which comprise much of the head of the human epididymis[151] (**Figure 1b**). Thus, even with a non-functional *VHL* gene, only the efferent ducts develop non-malignant tumourlets, and the epididymis proper is even more resistant to tumourigenesis. Upregulation of HIF1, HIF2, VEGF, GLUT1 and CAIX (CA9) occurs in human efferent duct cystadenomatous tissue.[146,148]

Whereas VHL expression in the mouse epididymis is marginal, elongin B (Tceb2, the second component of the VBC complex) expression is distinctively positive (http://www.mrg.genetics.washington.edu/), and cullin 2, the third component of the VBC complex, is absent. The presence of a VBC complex in the epididymis is thus doubtful; its absence would permit constitutive expression of intact HIF-1α and render the epididymis unresponsive to changes in local oxygen tension. Indeed, the transcription of HIF-1α in normoxic rats does occur in all regions of the epididymis, to an extent similar to that in the liver and kidney,[152] with less in the caput than the cauda.[153] In contrast to the ischemic testis, where HIF protein increases over that of the normoxic, neither caput nor cauda HIF content changes in experimental ischemia *in vivo* in rats[153] or mice.[154] The pro-angiogenic erythropoietin is, however, upregulated by acute hypoxia in the mouse epididymis.[132]

Constitutively-expressed HIF protein in the epididymis should enhance glycolysis under normal situations, rather than in hypoxia as occurs in other tissues. The epididymis does glycolyse aerobically and the rate does not increase under anaerobic conditions—it secretes lactate under both conditions.[155] A target gene of HIF, *Glut1* (*Slc2a1*), is especially expressed in the rodent cauda epididymidis (http://www.mrg.genetics.washington.edu/), and *Glut1* mRNA is found in the human epididymis.[20] Another target of HIF, *CA9*, is not present in the rat epididymis.[156]

Epididymal fluid has a higher osmolality, lower ionic strength, lower Na^+/K^+ ratio than blood plasma,[157] a low pH,[158,159] low pO_2,[34] and high pCO_2.[159] Many of these characteristics mirror those of the tumourigenic niche.[149] Whereas such a niche promotes tumours in neoplastic cells in other organs, epididymal cells normally survive in this environment.

SUMMARY

The privileged position of the human epididymis in evading tumourigenicity, confirmed in transgenic mouse models, appears to reflect a continuum of prevention, suppression and lowered sensitivity to otherwise tumourigenic insults. These may combine to create: (i) a low chance of cells becoming cancerous (no stem cells, strong anti-oxidative mechanisms, active tumour suppressors, and inactive oncogene products) with (ii) immune eradication and senescence of any affected cells (strong immunosurveillance that restricts hyperplastic cells to dormancy), and (iii) blockade of mechanisms favouring metaplasia (persistent tight junctions, presence of anti-angiogenic factors, misplaced pro-angiogenic factors and inoperative immune escape) (**Figure 3**). In response to the constitutive expression of normally inducible pro-proliferative factors, and an environment mimicking that of the tumour niche, epididymal cells may have evolved to be less responsive than others to tumourigenic stimuli, thereby raising the threshold for tumour initiation above that of other organs. As an anti-oxidative and immune-suppressive environment is also what maintains high numbers of spermatozoa quiescent and immunologically silent, the low incidence of cancer in the epididymis may be a direct consequence of its role in sperm maturation and storage. Such hypotheses are open to experimentation, but they may not explain the similarly low incidence of tumours in the seminal vesicles[160] (**Table 1**), which also deserves study. Further investigation into the regulation of the epididymal anti-tumourigenic activity may well offer insights that can be used in the prevention or treatment of cancers in general.

AUTHOR CONTRIBUTIONS

CHY initiated and designed the study, collected and analysed data, planned and wrote the review. KW searched and analysed the Chinese databases and wrote parts of the review. TGC initiated and designed the study, planned and wrote the review.

COMPETING FINANCIAL INTERESTS

The authors have no competing financial interests.

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