The secret identities of TMPRSS2: Fertility factor, virus trafficker, inflammation moderator, prostate protector and tumor suppressor

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Abstract. The human TMPRSS2 gene is pathogenetically implicated in both coronaviral lung infection and prostate cancer, suggesting its potential as a drug target in both contexts. SARS-COV-2 spike polypeptides are primed by the host transmembrane TMPRSS2 protease, triggering virus fusion with epithelial cell membranes followed by an endocytotic internalisation process that bypasses normal endosomal activation of cathepsin-mediated innate immunity; viral co-opting of TMPRSS2 thus favors microbial survivability by attenuating host inflammatory responses. In contrast, most early hormone-dependent prostate cancers express TMPRSS2:ERG fusion genes arising from deletions that eliminate the TMPRSS2 coding region while juxtaposing its androgen-inducible promoter and the open reading frame of ERG, upregulating pro-inflammatory ERG while functionally disabling TMPRSS2. Moreover, inflammatory oxidative DNA damage selects for TMPRSS2:ERG-fused cancers, whereas patients treated with antiinflammatory drugs develop fewer of these fusion-dependent tumors. These findings imply that TMPRSS2 protects the prostate by enabling endosomal bypass of pathogens which could otherwise trigger inflammation-induced DNA damage that predisposes to TMPRSS2:ERG fusions. Hence, the high oncogenic selectability of TMPRSS2:ERG fusions may reflect a unique pro-inflammatory synergy between androgenic ERG gain-of-function and fusogenic TMPRSS2 loss-of-function, cautioning against the use of TMPRSS2-inhibitory drugs to prevent or treat early prostate cancer.

Keywords: Prostate cancer, host defense, proteases, inflammation, innate immunity, SARS-COV-2, COVID-19

1. Introduction

Of the twenty thousand genes in the human genome, one has gained recent recognition for two distinct reasons: transmembrane serine protease 2 (TMPRSS2) is one of two key genes encoding membrane proteins that mediate infectivity of the COVID-19 coronavirus (CV) SARS-COV-2, whilst also being one of two (fused) genes implicated in 50% of primary prostate cancers [1–3]. Is this a coincidence, or could there be a function of the TMPRSS2 gene product that accounts for its involvement in both pathogenetic contexts?

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Two percent of human genes encode proteases, the functions of which remain incompletely understood; part of this interpretational difficulty lies in the cascade-like feedback interactions between proteases and their substrates [4, 5] which hamper clarifying the role of any single protease-encoding gene. Examples include androgen-inducible kallikrein-related peptidases in seminal fluid such as KLK2 and prostate-specific antigen (PSA), which have been implicated as either tumor-suppressive or oncogenic in different contexts [6–9]. The TMPRSS2 gene on chromosome 21q22.3 encodes another androgen-inducible serine protease found in semen; this gene is most highly expressed in the adult prostate [10], and at lower levels in the gut, breast, ovary, kidney, heart and lung [11, 12], in which latter organ it is targeted by SARS-COV-2 [13]. Some research, though not all [14], has raised hopes about the value of androgen-deprivation therapy for blocking lung TMPRSS2 expression and thus reducing SARS-COV-2 infectivity [3, 15–19], while similar interest has focused on using TMPRSS2 inhibitors to prevent or treat this infection [20].

The frequent involvement of TMPRSS2 in prostate cancer gene fusions [21] has likewise led to suggestions that oral drug inhibitors of the TMPRSS2 protease may benefit patients with, or at risk of, this disease [10, 12, 22, 23]. The hypothesis of TMPRSS2 being an actionable driver of prostate cancer [24] has also received support from preclinical models [22, 25]. Here this thesis is revisited in the light of recent cross-disciplinary insights from SARS-COV-2-related research.

2. **TMPRSS2 structure and function**

TMPRSS2 encodes a type II transmembrane serine protease (TTSP) that includes three adjacent extracellular domains – the juxtamembrane LDL receptor A (LDLRA) domain, an intermediary scavenger receptor cysteine-rich (SRCR) domain, and a carboxyterminal serine protease (SP) domain – which modular structure is tightly conserved across phylogeny [26], even extending to invertebrates [1] (Fig. 1A). In addition, human TMPRSS2 expression varies markedly between fetal and adult life in a bidirectional and tissue-dependent manner [26] (Table 1). These findings imply that TMPRSS2 serves an important, albeit not yet sharply defined, biologic function.

The extracellular location of the tripartite LDLRA-SRCR-SP cassette suggests a transmembrane signaling function, such as proteolytic activation of extracellular matrix proteins [22] or growth factors [25], and/or a defense function targeting extrinsic pathogens, as suggested by the role of TMPRSS2 in SARS-COV-2 infection [27] (see below). Mutagenesis studies have shown that the SRCR domain adjacent to the SP domain is essential for TMPRSS2 catalytic activity, whereas the LDLRA domain is dispensable [28]. Evolutionary conservation of the SRCR domain is consistent with, but does not prove, an affinity for microbial polyanionic molecules including proteins, lipopolysaccharides – which activate other proteases implicated in prostate cancer invasion [29] – or polynucleotides [30]. Another androgen-inducible serine protease, PSA, has been implicated in prostatic innate defence [31], consistent with PSA rises associated with either microbial [32] or non-microbial [33, 34] prostatic inflammation.

TMPRSS2 is synthesized as a latent zymogen which is first expressed as an intact 58–70 kDa transmembrane protein, but may later undergo autocatalytic secretion into apical glandular lumina as the 32–42 kDa carboxyterminal SP domain [10, 35] (Fig. 1B). Both the androgen-inducibility and immunohistochemical location of the latter process suggest a proteolytic role for TMPRSS2 in semen [35]. This raises further questions: is autocatalytic cleavage and secretion of the SP fragment confined to prostatic tissues? Does androgen-dependent TMPRSS2 activation occur due to overexpression [10], with the protein’s unpaired extracellular cysteine-140 in the SP domain [35] enabling disulfide bond formation between adjacent protease moieties, facilitating enzymatic cross-cleavage followed by secretion? If so, could this represent a mode of negative feedback on the sequelae of androgenic signaling, rather than simply a protease activation event? Does the transmembrane TMPRSS2 holoprotein containing the
Fig. 1. Structure and posttranslational modification of prostatic TMPRSS2. A, The zymogen includes three adjacent extra-cellular subdomains, LDLRA, SRRC, and SP, in addition to transmembrane (TM) and a short intracellular (IC) domain. B, In response to androgen receptor (AR) stimulation, TMPRSS2 protein becomes overexpressed at the cell surface, followed by catalytic cleavage and secretion of proteolytically active SP domains into seminal fluid.

<table>
<thead>
<tr>
<th>TMPRSS2 expression</th>
<th>Fetal</th>
<th>Adult</th>
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<td>Lung</td>
<td>++++</td>
<td>+</td>
</tr>
<tr>
<td>Brain</td>
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1As measured by Northern blotting, in human tissues sampled in fetal and adult life, per the cited study by Paoloni-Giacobino et al. [26]. Such contrasting changes of expression suggest conserved functional roles not expected of a gene exhibiting true evolutionary redundancy.

3. TMPRSS2 as a fertility factor

Pertinent to these knowledge gaps, TMPRSS3 is another TTSP containing an LDLRA-SRCR-SP cassette. Germline TMPRSS3 mutations affecting either the LDLRA or SRCR domains impair the proteolytic function of the SP domain via unknown mechanisms, with these non-SP mutations abolishing amiloride-sensitive sodium channel (ENaC) function and thereby causing autosomal recessive nerve deafness [38]. Wild-type TMPRSS2 downregulates ENaC activity [39], opposing the normal effect of TMPRSS3; however, gene-targeted knockout mice lacking only the SP domain of TMPRSS2 appear viable, fertile, and without any abnormal phenotype [40]. Null mutations knocking out all domains
of the TTSP matriptase gene – *MT-SP1*, which encodes a TMPRSS2 substrate [22] – are lethal [41]. Since sodium channel function is important for sperm fertility [42], and similar sperm-regulatory channelpathies cause male infertility [43], one possibility is that incomplete *TMPRSS2* knockouts involving only SP domain loss are fertile due to compensatory retention of ENaC, analogous to hearing retention in pseudohypoaldosteronism patients who, like *TMPRSS3* knockouts, have genetic loss of cochlear ENaC expression [44].

Another explanation beyond redundancy for the failure to demonstrate a *TMPRSS2* knockout phenotype, even in whole-gene knockouts [45], is that a further stressor is needed to reveal the gene’s function [46], with viral infection being one candidate. For example, the mumps paramyxovirus (MuV) is a common cause of orchitis and male sterility [47], such as could select for genes that prevent these outcomes. MuV increases its infectivity via inhibition of cytokine pathways mediating innate immunity [48], whereas other viruses including influenza and SARS-COV-2 evade the antiviral innate inflammatory responses by hijacking TMPRSS2 as an entry portal [49] (see below). Since TMPRSS2 is expressed in the testis together with its SARS-COV-2 co-receptor angiotensin-converting enzyme 2 (ACE2) [50, 51], and given that SARS-COV-2 viremia is often associated with mild orchitis [52], germline *TMPRSS2* losses could predispose to more severe orchitis with higher risk of sterility. Moreover, since mumps (also known as epidemic parotitis) causes prominent salivary gland inflammation, and given that both *TMPRSS2* and ACE2 are expressed in salivary glands [53], the possibility is raised by analogy that germline *TMPRSS2* or *ACE2* aberrations might affect fertility outcomes of orchitis caused not only by CVs but also by MuV.

In common with *TMPRSS2* – and hence plausibly related to their joint role in enabling extrinsic pathogens to bypass endosomal innate immunity (see below) – ACE2 expression is associated with reduced inflammation [54, 55], although it is not yet clear whether this phenotype is independent of co-localised *TMPRSS2* [56]. Against the latter possibility are reports that ACE2 activates inflammasomes on SARS-COV-2 binding [57], and that pro-inflammatory cytokines upregulate TMPRSS2 while downregulating ACE2 [58]. Nonetheless, since decreased expression of *ACE2* also characterises Sertoli cells in patients with non-obstructive azoospermia [59], it seems possible that pre-existing *ACE2* defects could unmask *TMPRSS2* hypofunction (e.g., due to heterozygous germline defects or promoter methylation) as a predisposition to infertility.

**4. TMPRSS2 as a virus trafficker**

New insights into the function of *TMPRSS2* have been gained from its exploitation by RNA viruses, including influenza A – the hemagglutinin of which is cleaved by *TMPRSS2* [60] – parainfluenza viruses [61], hepatitis C [62], and CVs such as SARS-COV-2; indeed, *TMPRSS2* polymorphisms are reported to be predictors for the susceptibility and severity of such viral diseases [63]. The spike (S) proteins of SARS-COV-2 contain two *TMPRSS2* cleavage sites, S1 and S2; proteolytic *TMPRSS2* priming of S1 enables viral binding to human ACE2 receptors in bronchial secretory cells, then activation of S2 permits fusion of viral and cell membranes, allowing viruses entry to host cytoplasm [13]. Of note, the priming of S1 in this lung context implies that androgen-inducible catalytic cleavage and secretion is not essential for *TMPRSS2* enzymatic activity; however, cells or organisms lacking *TMPRSS2* are resistant to CVs [64], implying that the role of transmembrane *TMPRSS2* in CV infection is indeed essential.

The evolutionarily deselected pathway of interest in this context appears to be endosomal endocytosis involving pathogen processing by intracellular cathepsins B/L, which is the entry pathway for CVs that infect cells lacking *TMPRSS2* [65] (Fig. 2A). Consistent with this, *TMPRSS2* inhibitors reduce CV pathogenicity whereas cathepsin inhibitors do not [66]. Since cathepsin-linked endosomes host Toll-like receptor (TLR) recognition proteins which mediate innate immunity [67], and given also that
Fig. 2. Schematic depiction of internalisation pathways with dissimilar inflammatory effects triggered by different viruses. A, Activation of the TLR-mediated endosomal cathepsin pathway, initiating innate immune signaling, thus leading to higher cell inflammation and lower viral survival. ROS, reactive oxygen species (which damage DNA, thus predisposing to potentially carcinogenic genetic changes, including but not limited to TMPRSS2:ERG fusions). B, Activation of TMPRSS2/ACE2 intracellular trafficking, which bypasses endosomal capture and innate immunity, leading to lower cell inflammation and higher viral survival.

TLR-deficient mice are hypersusceptible to CVs [68], such viruses appear to have selected TMPRSS2 binding affinity in order to evade cathepsin-mediated inflammatory responses [69–72] (Fig. 2B). Viral subversion of inflammatory innate host cell defense via TMPRSS2-mediated endosomal TLR bypass thus provides a teleologic rationale: TMPRSS2 balances the strength of non-adaptive endocytotic pathogen defense against the severity of innate immune inflammatory reactions, such as could damage fertility in early adult life (see above) or predispose to multistep carcinogenesis during later life (see below). Of note, human papillomavirus (HPV)-positive head and neck cancers upregulate TMPRSS2 when compared with HPV-negative tumors [73], consistent with a defensive role in HPV infections, notwithstanding that E6/E7 expression may be driven by viral integration rather than replication.

5. TMPRSS2 as an inflammation moderator

The function of TMPRSS2 has sometimes been assumed to be that of a monofunctional protease, with a spectrum of substrates having been proposed: these include matriptase [22], PAR-2 [74], precursors of uroplasminogen activator and PSA [12], and of the c-Met ligand (pro-hepatocyte growth factor, HGF) [25]. No key physiologic proteolytic function of TMPRSS2 has yet been experimentally established, however – not even in the prostate, where the androgen-inducibility of TMPRSS2 coupled to the secretion of its SP domain strongly implies a role in seminal fluid.

Recognition functions of the transmembrane zymogen could also contribute to a host defense mission of TMPRSS2. In the prostate, for example, androgenic induction of wild-type TMPRSS2 would enable certain phagocytosed antigens to bypass endosomal cathepsin L, thereby reducing the intensity of inflammatory damage, and maintaining tissue androgen-dependence by permitting survival of oxidant-stressed epithelial cells which could otherwise (e.g., in the presence of TMPRSS2 gene loss) transform into prostate inflammatory atrophy (PIA) [75], prostatic intraepithelial neoplasia (PIN) or cancer [76].
Moreover, polynucleotide activation of prostatic endosomal TLR3 receptors causes innate NFκB-mediated inflammatory responses that trigger apoptosis of androgen-dependent cells, as may lead to androgen-independence [77, 78].

This is consistent with the established role of innate immunity in inflammatory [79, 80] and neoplastic processes [81, 82] in the prostate and other organs. In the prostate, for example, non-adaptive endocytotic reactions to non-self antigens can cause apoptotic loss of androgen-dependent cells in normal young adult males, suppressing progression to neoplastic invasion while maintaining androgen responsiveness of the remaining cell population [83–85]. With aging, however, prostatic senescence may give rise to an androgen- and cytokine-mediated senescence-associated secretory phenotype (SASP, ‘inflammaging’) that comes to exceed the anti-inflammatory capacity of TMPRSS2, and could thus play a role in benign prostatic hypertrophy (BPH) [86] or PIA [87]. Short-term SASP-driven inflammaging may inhibit neoplastic progression of immortalized (but not metastatic) cells [88], suggesting a protective effect of senescent inflammation against early cancer. However, more chronic androgen-dependent SASP selects for loss-of-function mutations affecting the tumor suppressor gene PTEN [89], driving tumor progression towards androgen-independence [90].

Ubiquitous skin microbiota such as Propionibacterium acnes (formerly Corynebacterium parvum, now reclassified as Cutibacterium acnes) have been implicated in chronic prostatic infections, BPH [91] and prostate cancer [92], possibly via stimulation of innate immunity-mediated inflammatory responses [93]. Phagosomic elimination of this bacterium itself appears independent of cathepsin-mediated lysosomal fusion [94], however, raising the possibility of prostatic vulnerability to this infection due to androgen-inducible TMPRSS2 expression. Other sources of chronic intraprostatic inflammation include dietary carcinogens and estrogens [75], sexually transmissible bacterial infections such as Chlamydia and Neisseria spp., and viral infections such as herpes simplex-2 and HPV [95]. Hence, if TMPRSS2 does indeed have the function of moderating inflammation, the prostate appears to be an ideal tissue expression site.

6. TMPRSS2 as a prostate protector

The prostate (‘protector’ - Greek προστάτης) gland optimises male reproductive function by expediting outgoing sperm delivery, as well as by shielding from infection the seminal vesicles and epididymis; the latter organs are 100,000-fold less prone to cancer than the prostate, despite similar vulnerability to infection [96], reliance on innate immune responses [97], and DNA damage handling [95]. The hallmark androgen-inducibility of TMPRSS2 implies that a symbiotic balance between host defense optimisation and inflammation moderation could be most needed in the prostate, where androgen-driven sexual activity predisposes to microbial, xenobiotic or other pro-inflammatory exposures. The importance of this balance is suggested by an association of defective interferon-dependent functioning with prostate cancer risk in male cohorts with high numbers of lifetime sexual partners [98]. The gene of interest in this respect, IFNL4, plays a counterintuitive pro-viral anti-inflammatory role [99], reminiscent in this respect of TMPRSS2.

The pathogenetic importance of the endosomal pathway in the prostate is further supported by the finding that NADPH oxidase (NOX) proteins generating oxidant DNA damage are overexpressed in the endosomal compartments of prostate cancers [100]. Conversely, downregulation of tumor-promoting androgenic signaling occurs via endosomal/lysosomal degradation of androgen receptors induced by the suppressor gene TSG101 [101]. Hence, for prostate cancers which have progressed beyond early hormone-dependent growth, endosome-based innate immunity-mediated inflammatory positive feedback loops now represent a rational new therapeutic target [102].
Remarkably, the \textit{TMPRSS2:ERG} fusion occurs in around 50\% of primary prostate cancers, making it one of the most consistent genetic aberrations in oncology, and in turn raising questions as to why this chromosomal fusion is so avidly selected. The androgen response element (ARE) in the \textit{TMPRSS2} 5’-untranslated region is typically fused to the ETS-encoding \textit{ERG} gene – conferring androgen-inducibility on the latter, and explaining both the association of \textit{TMPRSS2:ERG} with hormone-dependent rather than castrate-resistant cancers [103], and the lack of potent transforming activity of the fusion gene [104].

These fusions often arise from \(\sim 2.8 \text{ mB} \) deletions extending from the \(21q22.2\) \textit{ERG} proto-oncogene to the telomeric \textit{TMPRSS2} gene; as such, the deleted region usually extends from the first intron of \textit{TMPRSS2} to the functional 3’ \textit{ERG} exons [21], excluding the \textit{TMPRSS2} coding region containing and beyond exon 2 [105]. These fusogenic deletions also tend to involve sixteen interstitial genes – some of which have tumor-suppressor activity, loss of which leads to progression that is independent of \textit{ERG} [106] – with such deletions being commoner than fusogenic translocations in castrate-resistant metastases [107].

Since \textit{TMPRSS2} protein translation is silenced in promoterless \textit{TMPRSS2}-fused cells [1, 108] and given that the fused/deleted protein lacks protease activity [109], the tumor-specific selection of \textit{TMPRSS2} dysfunction is consistent with the hypothesis that this loss promotes tumor growth. The fact that some \textit{TMPRSS2:ERG} fusion transcripts encode \textit{ERG} proteins which are \textit{N}-truncated due to premature stop codons [110] further implies that the selectability of these fusions could arise from \textit{TMPRSS2} loss of function \textit{per se} rather than solely from \textit{ERG} gain of function. These conclusions are also compatible with the findings that non-\textit{TMPRSS2:ERG} prostate cancers are associated with autocatalytic \textit{TMPRSS2} processing that leads either to secretion of inactive fragments, and/or cytoplasmic mislocalization of dysfunctional full-length proteins [10, 35, 37].

Inflammation-induced oxidant DNA damage causing loss of \textit{TMPRSS2} protective function at the cellular level [36] predisposes at the chromosomal level to \textit{TMPRSS2:ERG} fusion events [111]. Androgen-inducible \textit{ERG} expression drives prostaglandin-mediated inflammation both in \textit{TMPRSS2:ERG}-expressing prostate cancer cells [112] and tumors [113], a phenotype that is reinforced by \textit{ERG}-inducible downregulation or loss of heterologous tumor suppressor genes such as \textit{NKX3.1} [114]. This androgenic addiction cycle seems only breakable by evolution to androgen-independence [115]. Furthermore, anti-inflammatory cytokines repress both androgen receptor (AR) and \textit{TMPRSS2} expression in prostate cancer cells [116], and \textit{TMPRSS2:ERG}-expressing incident prostate cancers occur less often in patient cohorts receiving anti-inflammatory drugs [117]. These findings imply that downregulation of \textit{TMPRSS2} in prostatic tissues, including but not limited to those containing \textit{TMPRSS2:ERG} fusions, promotes androgen-driven prostate cancer by removing a negative constraint on endosomally-mediated inflammation, thereby synergizing with \textit{ERG}-driven inflammation [113, 118].

Long-term non-senescent (e.g., microbial) inflammation may not only select for \textit{TMPRSS2:ERG} fusions [111], but may also exacerbate prostatitis and promote evolution of androgen-dependent invasive cancer [119] or progression via epithelial-mesenchymal transition [120]. Such tumorigenicity can occur irrespective of \textit{ERG} upregulation – which downregulates \textit{PTEN} expression [121] – via direct selection for \textit{TMPRSS2} dysfunction [122, 123]. This predicts that if drug inhibitors are used to block prostatic \textit{TMPRSS2} with the aim of cancer prevention, inadvertently increasing inflammation, reduced selection for \textit{TMPRSS2}-deleted fusion genes could result – i.e., despite the fact that in the absence of \textit{TMPRSS2} drug inhibition, inflammation selects for \textit{TMPRSS2:ERG} fusions [111] – since in this drug-treated context there is no additional tumor-selecting advantage for a translocation that abrogates \textit{TMPRSS2} function. This reasoning predicts that \textit{TMPRSS2} protease-inhibitory drugs could reduce short-term low-grade androgen-dependent neoplasia but at the same time predispose to high-grade androgen-independent tumors.
Carcinogenic inflammation in the prostate has been linked to high-fat diets [124, 125]. Dietary estrogens have been implicated as a specific cause of tumorigenic prostatitis [75], and estradiol-activated estrogen receptors (ERα) induce TMPRSS2 expression in extra-prostatic androgen-insensitive tissues such as the heart [126]. In prostate cancers the tumor-promoting function of TMPRSS2:ERG fusions appears mediated by ERα expression in basal epithelial cells, but may be repressed during early hormone-dependent disease by ERβ splice variants [127]. Since antiandrogen treatment of prostate cancer cells reduces TMPRSS2 expression but also transactivates ERα expression via ESR1 induction [128], a synergistic tumor-promoting effect of ERα expression and TMPRSS2 functional loss is a possibility. During progression to castrate-resistant prostate cancer (CRPC), non-canonical estradiol signaling via ERβ may supervene, triggering TMPRSS2:ETV5 fusions in AR-null cells which – unlike TMPRSS2:ERG fusions in AR-expressing cells – drive NF-κB-dependent metastasis in the absence of innate immune signaling upregulation [129].

7. TMPRSS2 as a tumor suppressor

The heterogeneity of TMPRSS2 and ERG-like aberrations in prostate cancers [2] creates challenges for etiopathogenetic assessment [130]. Cancer-associated fusion genes may arise via intra- or interchromosomal rearrangements, with such clonal rearrangements implying tumor selection for a driver genotype [24]; in most instances the 5′ gene sequence drives expression of the 3′ gene partner, with ‘actionable’ inhibitors of the latter gene product – or downstream targets thereof [131] – often able to slow tumor growth [132]. Since the TMPRSS2:ERG genotype is an upregulating ARE-ERG fusion accompanied by loss of normal TMPRSS2 function, with the latter gene contributing only untranslated sequences to the fusion [104], data showing TMPRSS2 gene induction in such tumors [12, 18, 133] should not be interpreted as signifying overactivity of the normal functional protein. Moreover, in one study reporting prostate cancer-specific TMPRSS2 overexpression based on in situ hybridization, the only TMPRSS2 gene aberration was a protease-inactivating null mutation [134]. This and other groups also reported that prostate cancer cell progression to androgen-independence is associated with TMPRSS2 downregulation [135].

Upregulation of a given wild-type transcript or protein in tumor tissue, unassociated with selectable hardwired genotypes such as gene amplification, is hard to interpret, since it could imply either a primary oncogenic driver or secondary (i.e., reactive, compensatory or suppressor) regulatory role. Relevant to this, one study showed that TMPRSS2 expression is upregulated in a subset of tumors including not only prostate cancer and HPV-positive cervix cancer, but also cancers of the rectum, colon and stomach; this transcriptional upregulation was associated with promoter hypomethylation, but not with increased TMPRSS2 copy number [136]. A further study likewise showed ACE2 transcriptional upregulation due to promoter hypomethylation in rectal, colon and stomach tumors, as well as similar methylation-induced downregulation in testicular and thyroid cancers [137]. Considered in the context of their collaborative endosome-bypassing anti-inflammatory roles, these studies suggest that the previously reported tumor-suppressive properties of ACE2 [138] may be supplemented in tandem by its membrane partner TMPRSS2.

The ERG fusion event involving TMPRSS2 loss of function is implicated in early, but not late, prostatic neoplasms [108]; moreover, judged by immunohistochemical expression, there is no difference in TMPRSS2 protein expression between malignant and benign tumors [37], nor between poorly-and well-differentiated neoplasms [22, 35]. Although these findings do not suggest a proto-oncogenic function for TMPRSS2, support for a pro-metastatic action has been forthcoming from studies using transformed model systems such as xenografts [22] or SV40-driven tumor-bearing TRAMP mice [25]. In the latter study the protease inhibitor bromhexine blocked TMPRSS2 activity while also
reducing prostate cancer metastasis in vivo; more recent research reported that bromhexine does not inhibit TMPRSS2, however, and also noted that the TRAMP mouse study used a TMPRSS2 recombinant lacking the LDLRA domain [20]. Another intriguing finding of the TRAMP study was that Tmprss2−/− mice with deletions of TMPRSS2 produced tumors over double the size of those occurring in Tmprss2+/+ mice [25], suggesting a tumor-suppressor function. The lack of TMPRSS2 gene amplification events reported in prostate tumors, as well as the reported homozygous loss of TMPRSS2 in cancer cell lines [139], further favor a suppressor role. Additional support for this interpretation comes from the original subtractive cloning of TMPRSS2 as a gene downregulated in bone metastases [26], as well as from frameshift loss-of-function TMPRSS2 mutations occurring in metastatic prostate tumors [140].

These findings do not exclude the possibility that TMPRSS2 plays different roles in different contexts, e.g., in the normal prostate, in the development of hormone-dependent invasive cancer, and/or in progression to castrate-resistant metastatic disease. Since cancer evolution may be associated with a gene changing its function due to epistatic effects [141] – with such genes comparable to electronic components, the effect of which depends upon placement in a circuit [142] – it is possible that in the metastatic context TMPRSS2, either independently or via interaction with other TTSPs [109], could drive tumor dissemination by a path involving, say, proteolytic activation of pro-HGF in extracellular matrix [22, 25]. One study supporting this hypothesis showed that a recombinant TMPRSS2 inhibitor reduced prostate cancer cell invasion and metastasis [143]; however, the same inhibitor had earlier been reported to inhibit invasion and metastasis of lung cancer cells [144] for which TMPRSS2 is not a co-factor.

A key concern relating to the notion of TMPRSS2 as a tumor suppressor in the prostate is that selection for the TMPRSS2:ERG fusion removes only one functional TMPRSS2 copy. Hence, given that even homozygous knockouts have shown no discernible phenotype [40, 45], it is fair to ask whether or not such a heterozygous loss could plausibly manifest as haploinsufficiency and thus contribute to pro-inflammatory cancer progression ‘beyond ERG’, as it were [145]. We previously reported that homozygous knockouts of caretaker tumor suppressor genes – which maintain cell and genomic integrity, unlike gatekeeper suppressor genes which regulate apoptotic threshold and cell-cycle traverse – generally yield viable offspring of normal phenotype but impaired male fertility [146]. As noted earlier, male infertility as a haploinsufficient phenotype of TMPRSS2 loss could well be conditional on thresholds for sterility-inducing testicular inflammation, such as may vary with epidemic viral orchitis or ACE2 hypofunction. The high frequency of TMPRSS2:ERG fusion events in prostate cancer mirrors this conditionality by creating the precise circumstances needed to unmask the modest 50% decline in TMPRSS2 function predicted by protein alone; for this 20–100-fold androgen-driven ERG fusion/upregulation event [147] induces exactly the prostaglandin- [112, 148] and innate immunity-mediated inflammatory insults [79, 102] that wild-type TMPRSS2 ameliorates both in lung infections [149] and in the prostate [80, 116]. Hence, in this context the TMPRSS2:ERG gene fusion represents the perfect gene imbalance to reveal TMPRSS2 haploinsufficiency [46]. Similarly, an ideal environment to promote selection for TMPRSS2 dysfunction (i.e., such as could in due course undergo fixation as a TMPRSS2:ERG fusion) would be prostatitis of a subtype that is usually controllable by TMPRSS2 transcriptional upregulation alone, but which in severe or prolonged cases stochastically or stoichiometrically overwhelms normal capacity (Table 2).

A limitation of this model is that there is as yet no definitive evidence whether the putative TMPRSS2 molecular targets in the normal prostate – involving either proteolytic or non-proteolytic interactions – are microbial antigens [120, 150], inflammation-associated molecules [151], or tumor neoantigens [152]. Further work is needed for elucidation, including controlled comparisons of null vs. SP-only TMPRSS2 knockout mice under stressed conditions, such as microbial infections, short- and long-term inflammation, and/or high testosterone levels.
Table 2

Genetic or environmental variables that could unmask TMPRSS2 haploinsufficiency\(^2\)

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<tr>
<th>Phenotype: TMPRSS2 loss</th>
<th>Conditional haploinsufficiency</th>
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<tr>
<td>Genetic</td>
<td>Environmental</td>
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ACE2 loss          Viral orchitis
ARE:ERG fusion    Chronic prostatitis

\(^2\)Based on references in the text, hypothesised polygenic or extrinsic triggers that could convert TMPRSS2 from apparent redundancy or heterozygous haplosufficiency to conditional haploinsufficiency, with respect either to male infertility or prostate cancer development. ARE, functional 5’ androgen response element of exon-deleted TMPRSS2.

8. Conclusions

The recently recognised role of TMPRSS2 in SARS-COV-2 infection has spawned hypotheses that antiandrogens and/or TMPRSS2 inhibitors could reduce viral infectivity. The credibility of these hypotheses has since been supported by studies which indicate that SARS-COV-2 and other RNA viruses have evolved their predilection for TMPRSS2-mediated cell entry as a way of evading the host’s innate immune inflammatory reactions, thus enhancing viral infectivity. Clinical trials will soon assess the utility of targeted drug antagonists in this disease setting.

The notably high frequency of fusions between the TMPRSS2 and ERG genes in early hormone-dependent prostate cancers has likewise suggested that one or both of these gene products could be useful anticancer drug targets. However, data confirming a targetable driver role for TMPRSS2 in the setting of primary prostate cancer – this being the clinical context in which TMPRSS2:ERG gene fusions are most relevant – remain weak. In contrast, there is growing albeit still circumstantial evidence as to a role for wild-type TMPRSS2 in moderating the infertility and/or neoplastic consequences of uncontrolled inflammation in the testis and/or prostate. Since worsening of inflammatory damage could be counterproductive, caution remains appropriate for applying TMPRSS2 inhibition to the preventive or early cancer treatment settings.

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Conflict of interest

The author has no conflicts of interest to report.

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