

# Genetically engineered mucin mouse models for inflammation and cancer

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**Abstract** Mucins are heavily O-glycosylated proteins primarily produced by glandular and ductal epithelial cells, either in membrane-tethered or secretory forms, for providing lubrication and protection from various exogenous and endogenous insults. However, recent studies have linked their aberrant overexpression with infection, inflammation, and cancer that underscores their importance in tissue homeostasis. In this review, we present current status of the existing mouse models that have been developed to gain insights into the functional role(s) of mucins under physiological and pathological conditions. Knockout mouse models for membrane-associated (Muc1 and Muc16) and secretory mucins (Muc2) have helped us to elucidate the role of mucins in providing effective and protective barrier functions against pathological threats, participation in disease progression, and improved our understanding of mucin interaction with biotic and abiotic environmental components. Emphasis is also given to available transgenic mouse models (MUC1 and MUC7), which has been exploited to understand the context-dependent regulation and therapeutic potential of human mucins during inflammation and cancer.

**Keywords** Mucins · Cancer · Synteny · Transgenic mice models · Knockout models · Vaccines

## Abbreviations

MUC1	Human mucin
Muc1	Mouse mucin
KO	Knockout
KI	Knockin
Tg	Transgenic
KC	Cre-LSL-Kras <sup>G12D</sup>
KCM	KC mice with human MUC1 overexpression
KCKO	KC mice with Muc1 knockout
CF	Cystic fibrosis
SEA	Sperm protein, enterokinase, and agrin domain
EGF-L	Epidermal growth factor (EGF)-like domain
D domain	Dimerization domain
TR	Variable number of tandem repeats
Ser	Serine
Thr	Threonine
Pro	Proline
TM	Transmembrane
CT	Cytoplasmic tail
CTCK	Cystine knot-like domain
rCCSP	Rat Clara cell secretory protein
vWD	Von Willebrand
T-reg	Regulatory T cells
WT	Wild type
CTL	Cytotoxic T lymphocytes
APCs	Antigen-presenting cells
CSCs	Cancer stem cells

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## 1 Introduction

Mucins comprise a complex family of high-molecular-weight, membrane-bound, or secreted O-glycoproteins which are produced by glandular and ductal epithelial cells. Mucins play

critical roles in lubrication and protection of mucosa, renewal and differentiation of the epithelia, cell adhesion, and cellular signaling (Fig. 1a) [2–4]. So far, 21 mucins have been recognized in human, out of them 12 are attached to the cell membrane, whereas the others are secreted by the cells [2]. Multiple studies have shown the diverse and tissue-specific expression profile of mucins. Nonetheless, a single tissue can express number of different mucins (Table 1). Qualitative and quantitative alterations in mucins have been correlated with the inflammatory, pre-neoplastic, and neoplastic conditions [5, 7–12]. Studies have shown that some of the membrane-spanning mucins can serve as cell surface receptors and facilitate signal transduction in response to external stimuli that lead to cell proliferation, differentiation, apoptosis, migration, and invasion of cancer cells (Fig. 1b) [2, 13–18]. Despite ongoing research efforts, the structure and function of various mucins and mucin-mediated molecular mechanisms under normal and pathological conditions remain poorly understood. Moreover, the awry molecular and cellular mechanisms which lead to the aberrant expression and upregulation of various mucins under different disease conditions have not been completely comprehended [19].

Although cell-based *in vitro* studies have yielded crucial knowledge about the role of mucins under various pathological conditions, information regarding the physiologically relevant functions of mucins in context of the microenvironment which exists in tissue/tumor and complex interactions among different cell types remains obscure and underexplored. Therefore, the development of suitable animal models will provide an improved understanding of the consequence of dysregulation under normal and pathological conditions. Xenograft models have been helpful in validating the *in vitro* findings [20–24]; however, these models have several limitations including lack of competent immune system and factual microenvironment. Since recent reports have implicated the importance of mucins in immune system regulation, it is critical to study the role of mucins in the context of a functional immune system [25, 26]. These shortcomings could be overcome by utilizing transgenic (Tg), knockin (KI), and knockout (KO) mouse models, collectively known as genetically engineered mouse (GEM) models. Cre-Lox and Flp-FRT recombination systems are the most commonly used approaches for the development of conditional and inducible KO and KI mouse models [27, 28]. Some of the mucin mouse models have provided crucial information about the importance of mucins including their role under physiological and pathological conditions. In this review article, we intend to provide an overall picture of the currently available mucin mouse models and discuss their utility for understanding mucin function under inflammatory and malignant pathologies.

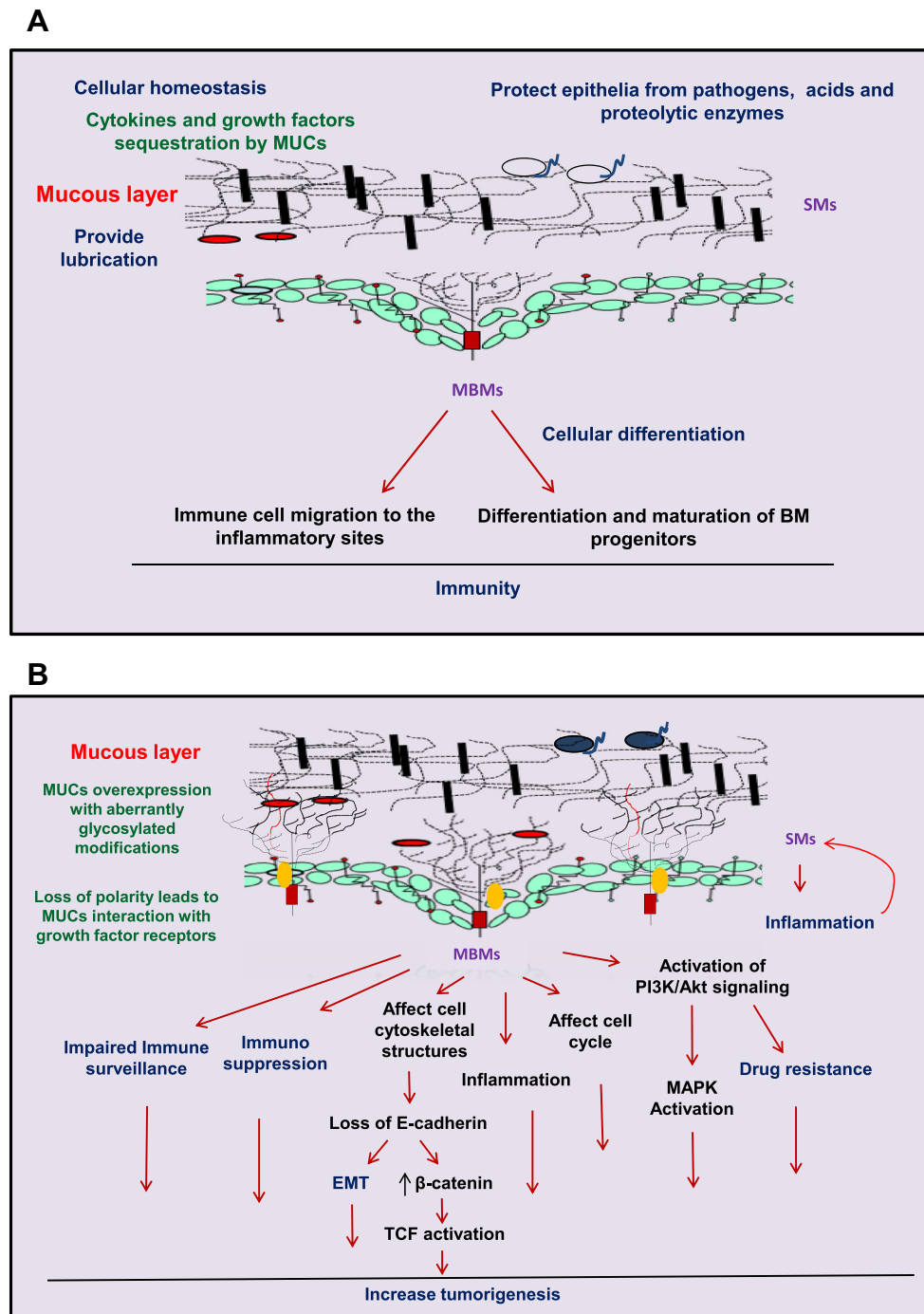
## 2 Comparative analysis and synteny of mouse and human mucins

Mice are frequently used as a model system to study the biology of human diseases because of their small size, ease of manipulation, short generation interval, and relatively inexpensive maintenance costs. Before describing the usefulness of murine models to understand the function of human mucins, it is important to emphasize the homology between murine and human mucins. Table 2 summarizes different types of human mucins, their homologues in mice, and their genomic localization. Mucins consist of multiple domains (Fig. 2): sperm protein, enterokinase, and the agrin domain (SEA) involved in protein interactions; epidermal growth factor (EGF)-like domain that can act as a ligand; cysteine-rich dimerization or D domain (including D1, D2, D', D3 similar to von Willebrand (vWD) domains) for oligomerization; variable number of tandem repeats (VNTR or TRs) rich in serine (Ser), threonine (Thr), proline (Pro) (collectively known as S/T/P) for O-linked glycosylation; the hydrophobic transmembrane (TM) domain for cell surface localization and cytoplasmic tail (CT) to facilitate signal transduction [31, 32]. The domain organization and expression pattern of mucins is largely conserved between human and mouse and is discussed below in detail.

### 2.1 Membrane-bound mucins

*Muc1* was the first murine mucin gene identified and characterized [33]. The human *MUC1* gene and its murine ortholog are 87 % identical in the non-TR domains and 74 % in the promoter regions. The VNTR region of human *MUC1* consists of 20 amino acid repeats, while that of mouse *Muc1* has 20 to 21 amino acids each [34]. The maximum similarity between *Muc1* and *MUC1* exists in their TM and cytoplasmic domains. The tissue-specific expression pattern of the mouse *Muc1* is also very similar to that of its human counterpart (Table 1). Similarities in the sequence and expression pattern of human *MUC1* and murine *Muc1* are indicative of their similarities in function(s), interacting partners, mode of internalization, subcellular localization, and routing to the plasma membrane during their recycling or after their synthesis.

*Muc4*, like its human ortholog, is encoded by 25 exons [35]. It consists of at least 20 TRs of 124–126 amino acids each, whereas human *MUC4* has 146–500 repeats of 16 amino acid residues. The Ser/Thr region located upstream of TRs in murine *Muc4* is significantly different and much smaller in size (63 amino acids) as compared to human sequence (951 amino acids) [35]. Interestingly, 12 potential N-glycosylation sites, which are downstream of TR region, are perfectly conserved in *Muc4* and *MUC4* and both orthologs exhibit similar expression patterns [35].



**Fig. 1** Illustration of the various physiological outcomes of aberrant mucin expression under normal and pathological conditions. **a** Under normal physiological condition, mucins provide lubrication and protection to the epithelial surface by providing a physical barrier from a hostile environment. Mucins shield the epithelium against the action of various pathogens (●), enzymes, gastric, and bile acids [1]. Mucins are involved in the cellular differentiation of epithelial and immune cells. The expression of mucins in bone marrow (BM) progenitors and mature immune cells is involved in hematopoiesis. **b** Under pathological conditions, mucins are aberrantly expressed and undergo differential posttranslational modifications. The mucous layer sequesters many molecules involved

in inflammation, cellular migration, and healing processes [2]. Mucins help transformed cells to avoid immune surveillance by masking epitopes of tumor antigens on the cell surface. Loss of apical–basolateral polarity allows interaction between membrane-bound mucins (MBMs) and growth factor receptors such as receptor tyrosine kinases (●), leading to sustained proliferative signaling cascades. Furthermore, the overexpression of mucins promotes cell motility and invasiveness and induces resistance to chemotherapeutic agents. Interestingly, the aberrant expression of secretory mucins (SMs) occasionally facilitates pathogenic infection, though the exact mechanism is still not understood

Two large exons of mouse *Muc16* at the N terminal region have sequence homology to exons 1 and 3 of human *MUC16*. Murine *Muc16* possesses only one SEA domain in its extracellular (EC) region, whereas the number of SEA domains in the EC region of human *MUC16* goes up to 60. *Muc16* also shares the similar characteristic repeat structure of human

**Table 1** Specific expression patterns of different mucins in the human body

Mucin	Normal expression pattern [2, 4–6]
MUC1	Expressed in the epithelial surfaces of the respiratory, female reproductive, and gastrointestinal tracts as well as in the middle ear, salivary, and mammary glands.
MUC2	Expressed in the intestinal and colonic goblet cells.
MUC3	MUC3 is the product of two genes, MUC3A and MUC3B, that are both present in the gastrointestinal epithelium.
MUC4	Mainly expressed by the epithelial surfaces of the eye, oral cavity, middle ear, lachrymal glands, salivary glands, mammary gland, prostate gland, stomach, colon, lung, trachea, and female reproductive tract.
MUC5	MUC5 is the product of two genes, MUC5AC and MUC5B. MUC5AC is primarily expressed in the tracheobronchial goblet cells and in the gastric epithelial cells, whereas MUC5B is present in the salivary, tracheobronchial, and esophageal mucous glands as well as in the pancreatobiliary and endocervical epithelial cells.
MUC6	Detected in the gastric and duodenal mucous glands, pancreatobiliary, and endocervical epithelial cells.
MUC7	Expressed in the oral cavity epithelial cells, minor salivary gland, and possibly in the respiratory tract. Its expression is also detected in the pancreas and bladder.
MUC8	Expressed in the airway and middle ear epithelial cells and male and female reproductive tracts.
MUC10	The expression pattern of MUC10 has not been determined to date.
MUC11	The MUC11 sequence is part of the very large VNTR domain of MUC12 and may represent a differential splice variant, which is normally expressed in the colon and stomach. Its expression has also been shown in the middle ear and lung epithelium.
MUC12	Normally expressed by the stomach and colon.
MUC13	Highly expressed in the epithelium of the gastrointestinal and respiratory tracts.
MUC15	Expressed in the lung, mammary gland, hematopoietic tissues, gonads, and gastrointestinal tract.
MUC16	Expressed in the ocular surface, respiratory tract, and female reproductive tract epithelia.
MUC17	Expressed in the gastrointestinal tract with the highest expression in the duodenum and conjunctival epithelium.
MUC19	Mainly expressed in the mucosal cells of major salivary glands and the epithelial cells from corneal, conjunctival, lacrimal gland, middle ear, and trachea.
MUC20	Highly expressed in the kidneys and moderately in the placenta, colon, lung, prostate, and liver.
MUC21	It is a novel transmembrane mucin and normally expressed in the lung, large intestine, thymus, and testis.

*MUC16* along with 66 % homology in their C-terminus [36]. The overall expression pattern of *Muc16* and *MUC16* is similar (Table 1). Both of them are expressed by the ovarian surface epithelial cells, though their cellular localization is different. Human *MUC16* is present on the cell surface and soluble fraction due to its shedding from the cell membrane, whereas murine *Muc16* has shown to be secreted by MOVCAR ovarian cells [37].

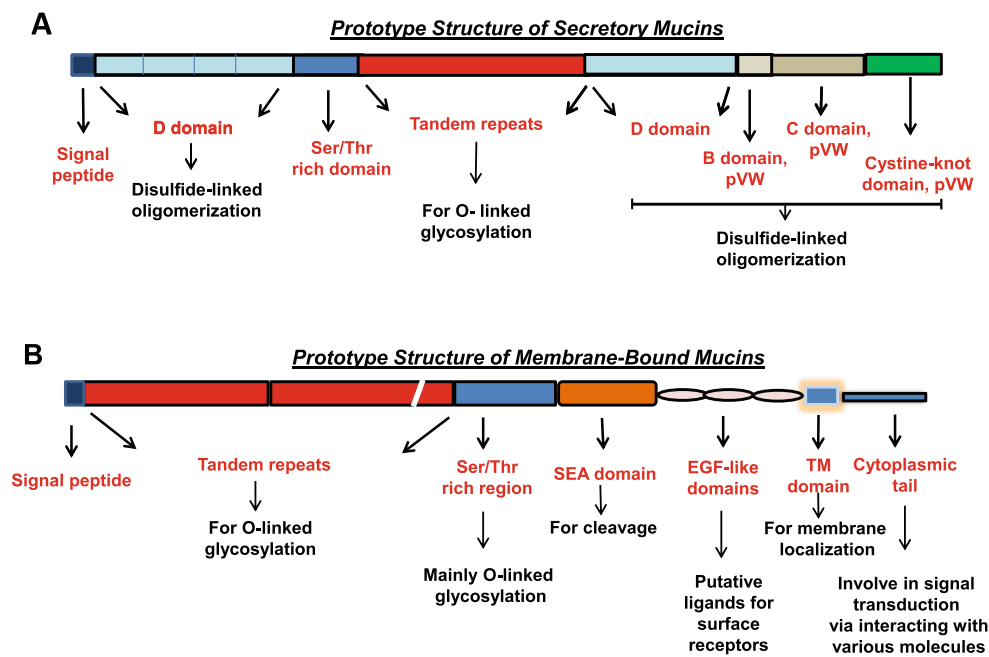
Other membrane-bound murine mucins including *Muc13*, *Muc15*, *Muc3*, *Muc20*, and *Muc21* are either partially characterized or have not been characterized yet. The C-terminus of *Muc13* shows 52 % identity to the human *MUC13* ortholog. However, the N-terminus of the *Muc13* mucin domain shows a significant divergence from the human *MUC13*, as the murine form has a nearly perfect repeat structure in contrast to the human form which retains many degenerate repeats [38, 39]. The carboxyl terminal of *MUC17* was found to be 59.6 % similar to murine *Muc3*, while there is only 46.4 % amino acid sequence similarity between murine and human *MUC3*. *MUC17* has 52 % similarity with the first EGF domain and 63.5 % similarity with the second EGF domain of *Muc3*. Altogether, there is greater similarity between *Muc3*

**Table 2** Different human mucin homologues of mice and their genomic localization

Human mucins	Type	Chromosomal location in human	Mouse homologue of human	Chromosomal location in mice
MUC1	TM	1q21	<i>Muc1</i>	3F1
MUC2	Gel	11p15	<i>Muc2</i>	7F5
MUC3A	TM	7q22	<i>Muc3</i> <sup>a</sup>	5G2
MUC3B	TM	7q22		
MUC4	TM	3q29	<i>Muc4</i>	16B3
MUC5AC	Gel	11p15	<i>Muc5ac</i>	7F5
MUC5B	Gel	11p15	<i>Muc5b</i>	7F5
MUC6	Gel	11p15.5–p15.4	<i>Muc6</i>	7F5
MUC7	Soluble	4q13–q21	<i>Muc7</i>	NP
MUC8	Gel	12q24.3	<i>Muc8</i>	NP
MUC10	NP	NP	<i>Muc10</i>	5qE1+
MUC11	TM	7q22	<i>Muc11</i>	NP
MUC12	TM	7q22	<i>Muc12</i>	NP
MUC13	TM	3q13.3	<i>Muc13</i>	16B3
MUC15	TM	11p14.3	<i>Muc15</i>	2E3
MUC16	TM	19p13.2	<i>Muc16</i>	9A3
MUC17	TM	7q22	<i>Muc3</i> <sup>a</sup>	5G2
MUC19	Gel	12q12	<i>Muc19</i>	15E3
MUC20	TM	3q29	<i>Muc20</i>	16B3
MUC21	TM	6p21	<i>Muc21</i>	17B1

TM transmembrane, NP not present

<sup>a</sup> *Muc3* is 46.4 and 59.6 % similar to human *MUC3* and *MUC17*, respectively [29, 30]. Therefore, murine *Muc3* is considered as true ortholog of human *MUC17*



**Fig. 2** Representation of the prototype structure of mucins along with the characterized and putative roles of their functional domains: MBMs and SMs have a TR domain with variable numbers and lengths of the repeats. They are predominantly get O-glycosylated and separated by unique sequences. They also have few N-glycosylation sites with varying localization with different mucins. Most of the MBMs possess SEA domains with a potential cleavage site (G/SVVV), except MUC4 where GDPH (also present in MUC2 and MUC5 AC secretory mucins) is considered to be a putative site for cleavage. Mucins have varying

lengths of CT (MUC4 CT is shortest with 22 amino acids), which are believed to facilitate signal transduction due to the presence of potential phosphorylation sites such as Ser, Thr, and Tyr residues. Other domains present in mucins include EGF-like motifs, nidogen and adhesion-associated NIDO and AMOP, and vWD domains. SMs are rich in cysteine-rich domains (D1, D2, D3, and D4), which are similar to the D domains of the vWD factor and flank the TR region. These domains are important for disulfide cross-linking to allow oligomerization between the mucin molecules required for gel-forming network

and MUC17 compared to Muc3 and MUC3, suggesting that MUC17 is the ortholog of Muc3 [29, 30]. Comparison of the amino acid sequences of human and mouse *Muc20* showed 48 % overall similarity [40]. Both mucins comprise several hydrophobic domains and three mucin-like repeats of 18 amino acid residues in their N-terminal regions and are expressed predominantly in the kidney.

## 2.2 Gel-forming mucins

Gel-forming mucins are the main components of mucus and consist of multiple “cysteine-rich” vWF C and vWF D domains in the flanking region of the mucin-like Thr/Ser-rich repeats and C-terminal cystine knot-like domain (CTCK) [41], which allow them to oligomerize by forming intermolecular disulfide bonds. Currently, five gel-forming murine mucins have been recognized: Muc2, Muc5ac, Muc5b, Muc6 and Muc19. Interestingly, four of these genes (*Muc2*, *Muc5ac*, *Muc5b*, and *Muc6*) are clustered on chromosome 7 F5 [42], a region that exhibits synteny with the human chromosome 11p15 [43]. The order of clustering of secretory mucin genes is Muc6–Muc2–Muc5ac–Muc5b, which is conserved in both human and mouse [43].

Muc2 forms the basic framework for the formation of an intraluminal mucus gel of various gastrointestinal (GI) organs

[44]. Apart from their 75 % homology at the N-terminus, mouse and human *MUC2* promoter regions also exhibit a strong sequence similarity which might subject them to similar transcriptional regulation [45]. Muc2, like its human counterpart, is predominantly expressed in the colon, to a lesser extent in the small intestine and undetectable in the stomach [46].

The TR of Muc5ac contains a 16-amino acid sequence, whereas the human MUC5AC has only eight amino acid residues per repeat [34, 47]. The TR domain of Muc5ac is followed by a 133-amino acid cysteine-rich non-repetitive region (CRR1), a 63-residue non-repetitive Ser/Thr-rich domain, and a second cysteine-rich region (CRR2) which share around 81 and 76 % similarity, respectively [34]. Despite the lack of sequence similarity between the TR units of the murine and human MUC5AC, their non-repetitive regions are nearly identical.

Alignment of the *Muc5b* gene with its human ortholog indicated few common features. Overall, there is 43 % identity between the murine Muc5b and human MUC5B which is predominantly contributed by N- and C-terminal regions which are 64 and 62 % similar, respectively [48]. However, the expression pattern of *Muc5b* does not match the human *MUC5B*, as the murine form is principally expressed in the laryngeal mucous glands and at a low level in the stomach and

duodenum, whereas the *MUC5B* gene is expressed in many tissues including the airway, gall bladder, and tongue [48].

The mouse *Muc19* gene is located on chromosome 15, which is homologous to human *MUC19* on chromosome 12 [41]. Like other gel-forming mucins, Muc19 also has vWD, vWC, and CTCK domains [41]. Paired analysis of mouse Muc19 and human MUC19 has shown 27 % homology [49], mainly at the C-terminus and the putative N-terminus of the peptide sequences, whereas the central repetitive regions did not show any homology [41]. Similar to the human MUC19, Muc19 is predominantly expressed in the salivary glands.

The murine *Muc6* is composed of 33 exons, and comparative analysis suggested that the human Muc6 and mouse Muc6 lack both the cysteine-rich domains and the cysteine-rich subdomains which are frequently found in the S/T/P-rich regions of other human and mouse secretory mucins (MUC2, 5AC, and 5B). The absence of these cysteine-rich domains and subdomains possibly makes them resistant to proteolytic degradation [50] and could be the reason for their high expression in the stomach in both humans and mice. In addition to the stomach, murine Muc6 also exhibits high expression in the duodenum, whereas it is expressed at low levels in the salivary glands [42].

### 3 Utilization of mucin mouse models for studying pathological conditions

Although studies of mucins under *in vitro* culture conditions have helped in elucidating the pathways operative in specific cell types, research in animal models incorporates the intricacies of the organ system in the context of dynamic immunological, hormonal, and physiological milieus. Mucin mouse models can provide improved understanding of the pathobiological implications of individual mucins in a more comprehensive and holistic manner in a physiologically relevant host microenvironment.

#### 3.1 Mucin KO mice and associated pathologies

To date, KO mouse models lacking the expression of Muc1, Muc2, and Muc16 have been generated. Strategies to generate these mice along with their utility to comprehend the role of these mucins in various diseases are discussed in detail in the following sections.

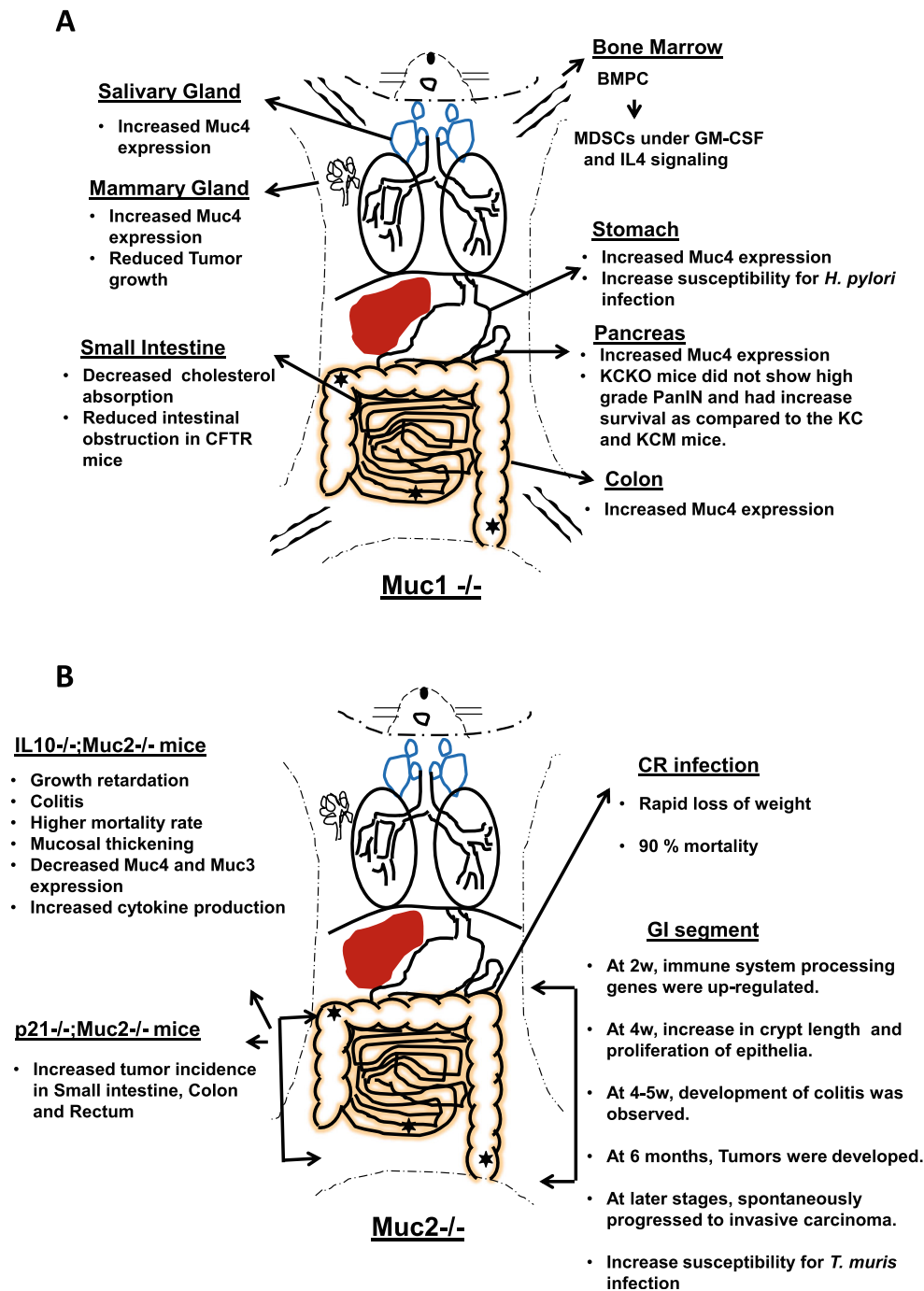
##### 3.1.1 The *Muc1* KO mouse model

The Muc1 KO mouse (*Muc1*<sup>-/-</sup>) was generated by targeted inactivation of *Muc1* gene by inserting a neomycin selection cassette within exon 2 [51]. Interestingly, *Muc1*<sup>-/-</sup> mice

displayed no alterations in survival rate and fertility and appeared phenotypically normal. In these mice, *Muc4* expression increased in lactating mammary gland, salivary gland, lung, stomach, and colon (Fig. 3a), but there was no significant difference in the expression levels of other mucin-like genes, including *ASGP-2*, *CD34*, *CD43*, *glycophorin*, and *Mad-CAM-1* [51]. *Muc1*<sup>-/-</sup> mice fed on lithogenic diet for 56 days exhibited significantly reduced secretion and accumulation of mucin (Muc1 and Muc5ac) in the gallbladder as compared to the wild-type (WT) mice, resulting in reduced crystallization of cholesterol and inhibition of gallstone formation (Fig. 3a) [52]. Like human disease, genetic mouse model of cystic fibrosis (CF), driven by CFTR S489X mutation, is characterized by high mucus production and accumulation which results in severe intestinal obstruction and death at weaning. CFTR mice in Muc1 null background exhibited significant reduction in the intestinal mucus accumulation, suggesting that Muc1 predominantly contributes to the mucinous obstruction of the GI tract during CF [53].

The *Muc1*<sup>-/-</sup> mouse model has helped in understanding the critical role played by MUC1 in cancer pathobiology. The growth of mammary tumors induced by the polyoma middle T antigen was significantly reduced in Muc1 null mice compared to WT littermates, although histologically, both groups developed poorly differentiated adenocarcinomas. The incidence and the metastasis were not remarkably different in the *Muc1*<sup>-/-</sup> and WT mice [51]. In a separate study, Cre-LSL-Kras<sup>G12D</sup> (KC) mouse expressing constitutively active Kras in pancreas was crossed with the Muc1 KO mouse to generate KC mice with Muc1 knockout (KCKO) mouse which showed higher survival and did not develop high-grade pancreatic intraepithelial neoplastic (PanIN) lesions even at 40 weeks of age, signifying the importance of Muc1 in pancreatic cancer progression (Fig. 3a) [54]. Only 10 % of KCKO mice exhibited metastasis to the lung, liver, and peritoneal sites, which was significantly less than KC and KC mice with human MUC1 overexpression (KCM) (generated by crossing KC mice with MUC1.Tg mice). Presence of MUC1 significantly accelerated the PC progression and metastasis. Thirty percent of the KC mice and 61 % of the KCM mice developed lung metastasis, and 20 % of the KC mice and 33 % of the KCM mice developed liver metastasis, whereas peritoneal metastasis was exhibited by 10 % of the KC mice and 23 % of the KCM mice [54]. Mechanistically, reduced tumorigenicity in KCKO mouse was associated with the absence of CDC25 and decreased phosphorylation of mitogen-activated protein kinase (MAPK) [54]. In addition, MUC1 resulted in the development of chemoresistance due to noticeable increase in multidrug resistance protein-1 expression in KCM as compared with KCKO tumors [55].

MUC1 has also been shown to regulate the development of the immune system. Myeloid-derived suppressor cells (MDSCs) play important role in suppressing the immune



**Fig. 3** Representation of the pathological phenotypes that arise upon the knockout of individual mucins in the mouse models: **a** Muc1 knockout (KO) mice express higher levels of Muc4 in the salivary gland, stomach, colon, and mammary glands, which could probably act as a compensatory mechanism. Muc1 null mice exhibited reduced breast tumor growth upon polyoma middle T antigen induction as compared to WT mice. The CFTR mouse when crossed with the Muc1<sup>-/-</sup> mouse showed less severe intestinal obstruction as compared to the control mice. Muc1<sup>-/-</sup> mice also demonstrated reduced cholesterol absorption, signifying the importance of mucin in cholesterol uptake in the small intestine. Furthermore, Muc1-deficient mice showed reduction in the formation of cholesterol crystals and, consequently, reduced incidence of gallstone formation when fed the lithogenic diet. MUC1<sup>-/-</sup> mice on a Kras<sup>G12D</sup>-Cre background demonstrated higher survival and reduced high-grade PanIN

lesions as compared to the KC and KCM mice. Muc1<sup>-/-</sup> mice exhibited increase susceptibility to get infected by *H. pylori*. In Muc1-deficient mice, increased differentiation of bone marrow progenitor cells (BMPC) to immature cell population of myeloid origin (MDSC) was observed upon GM-CSF and IL-4 stimulation. **b** Muc2<sup>-/-</sup> KO mice develop abnormalities predominantly in the lower gastrointestinal track (LGIT). There is an increase in crypt length and a higher proliferation rate at 4 weeks of age, which subsequently progresses to invasive carcinoma in older mice. IL-10 and p21 KO on a Muc2-deficient background further increased the incidence of tumor formation, mortality, and mucosal thickening in LGIT. IL-10<sup>-/-</sup>, Muc2<sup>-/-</sup> mice have shown a significant reduction in the expression of other mucins like Muc4 and Muc3 in the intestinal track, predisposing to environmental insult. Absence of Muc2 also increases the propensity for *T. muris* infection

system under cancerous conditions via various mechanisms [56]. Recently, Poh TW *et al.* demonstrated that compared to WT animals, bone marrow (BM) progenitor cells in Muc1<sup>-/-</sup> mice predominantly differentiated into MDSCs under granulocyte-macrophage colony-stimulating factor (GM-CSF) and IL-4 signaling (Fig. 3a). This was attributed to reduced  $\beta$ -catenin stability due to Muc1 deficiency, as *in vitro* studies revealed an inverse relationship between  $\beta$ -catenin stability and the expansion of CD11b+Gr1+ MDSCs [26]. Given this new role of Muc1 in the differentiation and proliferation of myeloid progenitors, it will be of interest to utilize the Muc1 mouse models to study hematological malignancies and evaluate efficacy of immunotherapies for various malignancies.

Besides participating in oncogenesis and differentiation of immune cells, Muc1 expression also impacts the outcome of pathogenic infections [57, 58]. In a recent study, *Helicobacter pylori* (*H. pylori*)—a pathogen regarded as an etiological factor for a number of pathologies including peptic ulcers, gastric mucosal-associated lymphoid tissue lymphoma, and gastric adenocarcinoma—was found to infect and colonize Muc1<sup>-/-</sup> mice with a 5-fold higher propensity as compared to the WT mice (Fig. 3a) [58]. This study further demonstrated the ability of *Helicobacter* strains (SS9 and J99) to bind to purified MUC1, which limits the access of *H. pylori* to the epithelial surface, and thereby providing protection from infection.

Altogether, studies on Muc1 KO mice have established that inhibition of Muc1 expression can reduce the tumorigenic properties of cancer and gallstone formation. Muc1 targeting can improve the clinical outcome of CF patients, though it can elevate the susceptibility of *H. pylori*-associated pathological conditions. In contrast, overexpression of MUC1 in mice promotes metastasis and drug resistance.

### 3.1.2 The Muc2 KO mouse model

Muc2 KO mice (Muc2<sup>-/-</sup>) were generated by substituting a genomic fragment spanning exons 2 to 4 of Muc2 with a phosphoglycerate kinase-neomycin (PGK-Neo) cassette [59]. Muc2<sup>-/-</sup> mice showed no significant developmental defects as compared to heterozygous and WT littermates till 12 months of age and showed no compensatory increase in the expression of other intestinal apomucins (e.g., Muc5ac, Muc3, and Muc13). Muc2<sup>-/-</sup> mice developed GI tumors by 6 months which spontaneously progressed to invasive carcinoma at an older age (Fig. 3b). Around 65 % of the Muc2<sup>-/-</sup> mice develop tumors by 24 months of age with the incidence of more than 1.5 tumors per mouse [59]. There was increased cellular proliferation, reduced goblet cell number, decreased apoptosis, and increased migration of intestinal epithelial cells. The most distinct difference between the WT and the Muc2<sup>-/-</sup> mice was the absence of distinguishable goblet cells in the Muc2<sup>-/-</sup> mice. Tff3 and SI, two important markers to check the functionality of goblet cells and enterocytes,

displayed no alteration in their expression levels in small intestine between WT and Muc2<sup>-/-</sup> mice [59, 60]. At a younger age (6 months), the Muc2 KO mice showed tumor development only in small intestine, whereas in older animals (1 year), tumors were observed in the small intestine (47 %), colon (21 %), and rectum (15.7 %). Similar to Muc2<sup>-/-</sup> mice, p21<sup>-/-</sup> (inhibitor of cyclin-independent kinase), Muc2<sup>-/-</sup> double-KO (DKO) mice also exhibited increased tumor formation in the small intestine, colon, and rectum but at a faster rate compared to p21<sup>-/-</sup> mice (Fig. 3b) [60]. Intestinal tumors size was 35 % larger in Muc2<sup>-/-</sup>, p21<sup>-/-</sup> ( $P=0.02$ ) or Muc2<sup>-/-</sup>, p21<sup>+/-</sup> ( $P=0.03$ ) mice than the tumors in Muc2<sup>-/-</sup>, p21<sup>+/-</sup> mice.

Muc2 deficiency has also been related with the different outcomes during both prenatal and postnatal stages and during the suckling-weaning transition [61]. Before birth, and before weaning, no significant differences in the body weights between Muc2<sup>-/-</sup> mice and WT mice were observed. However, after weaning, Muc2<sup>-/-</sup> mice had considerably less body weight compared to WT mice, suggesting growth retardation. Before weaning (P14), the levels of pro-inflammatory cytokines (IL-12 p35, IL-12 p40, and tumor necrosis factor alpha (TNF- $\alpha$ )) and immune cells influx (Foxp3-positive Treg cells and the immune suppressive cytokines TGF- $\beta$ 1 and IL-10) were significantly higher in Muc2<sup>-/-</sup> mice as compared to WT mice. After weaning (P28), the pro-inflammatory response remained active, but expression of immunosuppressive markers such as Foxp3 and TGF- $\beta$ 1 decreased [61]. This suckling-weaning transition exacerbated the development of colitis in Muc2-deficient mice, as reflected by reduced body weights, increase in distal colonic crypt lengthening, and influx of T cells in the mucosa. Altogether, these studies indicate that colitis in Muc2<sup>-/-</sup> mice is restricted before weaning but worsens in the distal colon after weaning, establishing the protective role of Muc2. Additionally, microarray analysis on 2–4-week-old Muc2<sup>-/-</sup> mice following colitis development revealed upregulation of genes involved in various immunological processes including the following: antigen processing/presentation, B cell receptor- and T cell receptor-mediated signal transduction, and Jak-STAT signaling (Fig. 3b) [62]. These mice expressed elevated levels of immunoglobulins, pro-inflammatory cytokines, chemokines, and antimicrobial proteins. Moreover, 4-week-old Muc2<sup>-/-</sup> mice showed significant alteration in the expression of certain cell structure maintenance genes, including upregulation of *claudin-10* and downregulation of *claudin-1* and *claudin-5* [62].

To gain insight into the protective functions of Muc2 and its possible role in intestinal bowel disease (IBD), Muc2 KO (Muc2<sup>-/-</sup>) and heterozygous (Muc2<sup>+/-</sup>) mice were utilized from birth to 16 weeks of age before the development of adenomas. The Muc2<sup>-/-</sup> animals exhibited significantly lower body weight at 5 weeks of age as compared to the WT and heterozygous (Muc2<sup>+/-</sup>) littermates, indicating a significant



growth retardation which has been associated with IBD and attributed to malnutrition [63]. *Muc2*<sup>-/-</sup> mice when challenged with colitis-inducing agent, dextran sulfate sodium, showed pathological signs of colitis by 5 weeks of age [64]. The microscopic analysis of the colon indicated mucosal thickening, increased proliferation, and superficial erosions. *Muc2*<sup>-/-</sup> mice also exhibited increase infiltration of lymphocytes in the colon as compared to WT mice. Further, a significant increase in the transcripts of the inflammatory cytokines TNF- $\alpha$  (5-fold) and IL-1 $\beta$  (4-fold) was observed in the colon of *Muc2*<sup>-/-</sup> mice as compared to WT mice. This study was further substantiated by the observations in interleukin-10 KO (IL-10<sup>-/-</sup>) mice, which spontaneously developed colitis by 3 months of age and displayed reduced *Muc2* levels [65, 66]. Further, KO of *Muc2* on IL-10<sup>-/-</sup> background, resulted in significant growth retardation, altered goblet cell morphology, crypt hyperplasia, infiltration of lymphocytes, flattening of epithelial cells, and alteration of the lamina propria and developed colitis by 4 weeks of age in the proximal colon (Fig. 3b). These mice also had increased circulating levels of inflammatory cytokines such as IL-12p70, TNF- $\alpha$ , IFN- $\gamma$ , MIP-1, and IL-6 compared to WT mice as early as 4 weeks, which remained persistently high at 5 weeks of age. Additionally, these animals exhibited apoptotic bodies along the crypts and had a higher mortality rate as compared to the WT or individual gene KO animals [67]. Thus, *Muc2* in the intestinal mucus layer appears to be essential for mucosal protection, and abrogation of mucus barrier function is a major cause of colitis development.

Mucositis is one of the most common and severe outcomes of anti-cancer chemotherapy and predisposes patients to life-threatening infections. Mucositis has been associated with an increased expression of MUC2 [68] and pro-inflammatory cytokines such as TNF- $\alpha$ , which exacerbates intestinal damage, and IL-10 [69, 70], which provides protection to the intestine. Due to the established protective role of *Muc2* in colitis as discussed earlier, De Koning *et al.* utilized *Muc2*<sup>-/-</sup> mice to understand the contribution of *Muc2* in providing the intestinal epithelial defense against methotrexate (MTX)-induced mucositis [71]. A significant increase in the expression of protective IL-10 in WT mice was observed, whereas *Muc2*<sup>-/-</sup> mice exhibited increased expression of damage inducing TNF- $\alpha$ , indicating that the lack of *Muc2* expression leads to an altered immune response prior to MTX challenge.

The presence of *Muc2* has also been found to impact host-pathogen interactions during infection [72]. Bergstorm *et al.* infected *Muc2*<sup>-/-</sup> mice with *Citrobacter rodentium*, a murine attaching-effacing (A/E) pathogen related to diarrheagenic A/E *E. coli* [72]. Upon infection, *Muc2*<sup>-/-</sup> mice exhibited a rapid loss of weight and high mortality of up to 90%. Mucin secretion was significantly higher in WT mice during infection as compared to the uninfected WT controls, signifying the importance of mucin production to clear the mucosal surface

from pathogenic bacteria (Fig. 3b). Protective function of *Muc2* was also demonstrated in *Trichuris muris* (*T. muris*) parasitic infection. WT mice showed 46% reduction in *T. muris* worm burden by day 15 and 84% reduction by day 20 after infection, whereas *Muc2*-deficient mice did not exhibit decrease in worm burden even at 20 days postinfection [73]. The nematodes also demonstrated noticeable decrease in their energy status in WT mice than that in the susceptible *Muc2*<sup>-/-</sup> mice. Reduced mucus permeability has been observed in *Muc2*<sup>-/-</sup> mice, suggesting the involvement of *Muc2* in the formation of effective physical barrier and network to concentrate and present defense proteins against pathogenic infection to protect gastric epithelia. Proteins required for the maintenance and restitution of GI tract, such as trefoil factor 3, angiogenin, and RELM- $\beta$ , might be enriched in the mucus layer formed by *Muc2* [73]. After infection, *Muc5ac* was found to be unexpectedly upregulated in the cecal mucus of WT and *Muc2*<sup>-/-</sup> mice, implying the importance of mucins in pathogenic infections.

Overall, studies on *Muc2* KO mice have established that *Muc2* not only functions as a tumor suppressor in colon cancer but it also provides protection against the development of inflammatory conditions, bacterial and parasitic infections.

### 3.1.3 The *Muc16* KO mouse model

*Muc16* KO mice (*Muc16*<sup>-/-</sup>) were generated by targeted inactivation of the gene by replacing a 6-kb genomic fragment, harboring the majority of exon 3 and a portion of intron 3 with a IRES-T-lacZ reporter cassette, followed by a PGK-NeobpA expression cassette flanked by the loxP sites [74]. The absence of any distinct phenotype in *Muc16* homozygous mutant mice suggested that CA125/*Muc16* is not essential for the normal development and reproduction. Similarly, overexpression of MUC16 carboxyl-terminus construct, also known as MUC16 $\Delta$ C354 as it includes 354 amino acids of the carboxy terminus, with chicken  $\beta$  actin (CAG) promoter had no effect on the viability, fertility, and development of mice [75]. Meanwhile, *Muc16*<sup>-/-</sup> male mice exhibited high reproductive ability, as their crossing with WT females yielded more progenies as compared to the ones crossed with WT male mice. *Muc16*<sup>-/-</sup> mice did not exhibit compensatory increase in other mucins genes; instead, there was a downregulation of *Muc1* in the uterus ( $p=0.01$ ) and lung of *Muc16*<sup>-/-</sup> mice [74].

Conditional *Muc16*<sup>-/-</sup> KO mice could be helpful in validating and defining the role of *Muc16* in oncogenesis in the background of p53<sup>-/-</sup> or constitutively active *Kras* mice in future studies, particularly in pancreatic, ovarian, and breast cancers, where MUC16 overexpression is frequently observed [76–78].

### 3.2 Mucin Tg mice and their phenotypes

To date, MUC1, Muc5ac, and MUC7 Tg mice models have been generated, characterized, and studied. Of these, MUC1 Tg mice have been extensively studied for evaluating immunotherapeutic approaches for various cancers.

#### 3.2.1 MUC1 Tg mouse

The MUC1.Tg mouse was generated by successfully inserting the entire MUC1 gene using either 40- or 10.6-kb vector constructs in the mouse genome. The 40-kb construct contained 4.5-kb upstream of the 5' sequence and 27-kb downstream of the 3' sequence, whereas the 10.6-kb construct included 1.6-kb upstream of the 5' sequence and 1.9-kb downstream of the 3' sequence of the MUC1 gene from the human genome [79]. MUC1.Tg mice expressed human MUC1 in a tissue-specific manner with a profile similar to that seen in humans [79]. Schroeder *et al.* further confirmed the role of MUC1 in tumorigenesis by generating the number of Tg animals overexpressing the full-length and deletion constructs of human MUC1 in the mouse mammary gland [80]. Interestingly, significantly high interaction between MUC1 and epidermal growth factor receptor (EGFR) was observed at the apical surface of lactating mammary gland due to presence of EGFR all over the alveolar lumen. Therefore, strong downstream activation of MAP kinases was observed upon EGF stimulation in the mouse mammary gland of MUC1.Tg mice as compared to the Muc1<sup>-/-</sup> and WT mice. Therefore, it is possible that the modulation of EGFR signaling and activation of MAPK are mechanistically important for MUC1-associated mammary gland tumorigenesis [80]. MMTV-MUC1.Tg mice exhibited a 62 % higher incidence of mammary tumor formation as compared to the WT mice [81]. The mammary glands in MMTV-MUC1.Tg mice were histologically atypical and hyperplastic and showed significantly reduced apoptosis and delayed involution. MUC1.Tg mice also showed the formation of pulmonary carcinomas, suggesting the requirement of MUC1 in the development of breast and lung tumors [81]. Increased tumor angiogenesis and increased tumor growth were also observed in a PyMT-MMTV/hMUC1.Tg mouse model [82]. The data demonstrated that both xenograft and double-Tg MMTV-PyMT/hMUC1 mice had larger tumors, along with the increased frequency of tumor formation as compared to the MMTV-PyMT control mice. Higher VEGF expression was also noticed in the tumors of the MMTV-PyMT/hMUC1 mice than the control animals, suggesting the pro-angiogenic role of MUC1 during tumor progression. Additionally, MUC1.Tg mice when crossed with IL-10<sup>-/-</sup> mice showed an early onset of inflammatory bowel disease (IBD), higher inflammation score, and a higher incidence of colon cancers compared to IL-10<sup>-/-</sup> mice [83], demonstrating

the ability of aberrant MUC1 expression to propagate chronic inflammation and tumor progression.

As mentioned earlier, MUC1.Tg mice (KCM) displayed increased metastasis, higher tumorigenic ability, and higher frequency of high-grade PanIN lesions as compared to the KCKO and KC mice along with significantly reduced survival rate as compared to the KCKO mice [54]. Subsequently, the same mice were used to study the influence of MUC1 on immune response during cancer progression to invasive adenocarcinoma [84]. Anti-MUC1 responses were observed in KCM (denoted as PDA.MUC1) mice in the earlier stages, but this effect is reduced at the later stages due to increment in the population of Tregs and MDSCs in the tumor microenvironment along with high tumor-draining lymph node (TDLNs) than KC mice [84]. This study was further substantiated when MUC1.Tg mice were utilized to examine the immunological tolerance to MUC1 and to test the efficacy of MUC1-targeted immunotherapy [85]. Upon immunization with MUC1 peptides, WT mice implanted with MUC1-expressing tumors produced IgM and IgG antibodies, whereas MUC1.Tg mice had strong IgM and feeble IgG antibody response, suggesting a significantly reduced class switching in B lymphocytes that resulted in failure to reject MUC1-expressing tumors. Mukherjee *et al.* further established MUC1 tolerance by crossing MUC1.Tg mice with ET mice (expressing the first 127 amino acids of SV40 T antigen under the control of the elastase promoter) to generate MET mice that developed pancreatic tumor [86]. These mice demonstrated acinar cell dysplasia at birth, which was ensued by the development of microadenomas and acinar cell carcinomas. Cytotoxic T cells (CTLs) were identified in all MET mice and were effective in eliminating implanted tumors *in vivo*, whereas the MUC1.Tg mice with the B16.MUC1 s.c. tumor elicited ineffective CTL response. These CTLs exhibited low affinity due to the presence of MUC1 in mouse thymus which led to the development of central tolerance toward MUC1. In spite of the stimulation of a tumor-specific immune response, MUC1 did not cause the rejection of a spontaneously occurring tumor, possibly due to the presence of elaborate means of escaping MHC class I-restricted immune response [86]. Altogether, these studies suggested that MUC1.Tg mice are a useful model for scrutinizing and comprehending the mechanistic aspect of central and peripheral tolerance to the tumor antigen MUC1.

In order to assess the efficiency of MUC1-specific immunotherapy on intestinal adenomas, Akporiaye *et al.* developed a bigenic MUC1.Tg/MIN mouse model by crossing Apc/(MIN/+) mice with MUC1.Tg mice [87]. The immunotherapy involved the combination of two MHC class I restricted MUC1 TR peptides and a class II pan helper peptide along with the biological adjuvants CpG-ODN and GM-CSF. This combination did not show change in the number of adenomas, but flattening of adenomas and reduction in the frequency of

large-sized adenomas were quite evident. Glycosylation of the mucins is a critical determinant of their immunogenicity [88], and the extent and pattern of glycosylation are altered during tumorigenesis. Most of the early efforts to develop MUC1-based cancer vaccines involved unglycosylated MUC1 TR peptides with variable lengths, conjugated to different carriers and/or co-administered with an adjuvant. Unfortunately, these strategies showed insignificant immune responses against MUC1-expressing cancer cells [89–92], which could possibly be due to the conformational discrepancies that arise due to differential glycosylation of MUC1 or the use of carrier proteins leading to immunosuppression [93]. Unlike MUC1 TR peptide, densely glycosylated MUC1 cannot be processed efficiently by antigen-presenting cells (APCs) [94], thereby compromising its presentation through the MHC class II and class I pathway. A recent study has shown that a covalent linkage between a glycosylated MUC1-derived glycopeptide and a Toll-like receptor (TLR) agonist can stimulate strong humoral and cellular immune responses and is efficacious in reversing tolerance and generating a therapeutic response [88]. The use of tripartite peptide vaccine composed of the TLR2 agonist Pam3CysSK4, a peptide T helper epitope and an aberrantly glycosylated MUC1 peptide had a significant therapeutic effect in MUC1.Tg mice due to the adjuvant mediated nonspecific antitumor responses and MUC1-peptide specific humoral and cellular immune responses. Glycosylation of the MUC1 peptide and its covalent attachment with TLR agonist was found to be critical for inducing CTLs and ADCC-mediating antibodies and had an optimal immunological response [88].

Studies comparing immunotherapy-induced anti-MUC1 responses in WT and Tg mice revealed that MUC1.Tg mice elicit reduced T helper responses, even in the presence of efficiently processed and well-presented MUC1 by APCs. MUC1 is a self antigen that is expressed under normal conditions, which makes MUC1.Tg mice tolerant to immunization against synthetic 100-mer MUC1 peptide-primed dendritic cells (DCs). Therefore, Turner MS *et al.* performed adoptive transfer of CD4+ T cells from WT mice to the MUC1.Tg mice, followed by the immunization with the same MUC1-loaded DCs [95]. Surprisingly, rather than expected increase in immune response, these mice showed decrease in cellular and humoral response, indicating the presence of immunosuppressive component in the WT CD4+ T cells. The assumption was further supported as the adoptive transfer after T-reg-depleted T helper cells derived from WT mice improved the efficacy of MUC1-specific vaccine and immunity in MUC1.Tg animals [95]. Beatty *et al.* have also demonstrated delayed progression of IBD to colitis-associated colon cancer (also known as CACC) upon administration of 100-mer TnMUC1 peptide-based immunotherapy in MUC1-positive IBD (generated by crossing IL-10<sup>-/-</sup> mice with MUC1.Tg mice). This delay in progression was attributed to the

induction of adaptive immunity (CTLs specific to MUC1), reduced neutrophil infiltration, and systemic reduction in MDSCs, leading to the depletion of abnormal MUC1-expressing cells in IBD colons [96]. On the other hand, IBD in control mice exhibited elevated population of immunosuppressive MDSC cells in the spleen. These studies in MUC1.Tg mice underscore the role of Tregs and MDSCs in limiting the adaptive immune responses following immunotherapy with self-antigens. Hence, the removal of these immunosuppressive components can possibly improve the efficacy of anti-MUC1 vaccines. Activation of adaptive immunity can modulate local and systemic microenvironment and combat the inability of the MUC1-targeted vaccines to elicit adaptive immune response to kill MUC1+ tumors, which is currently one of the major obstacles in MUC1-based vaccination in cancer patients.

### 3.2.2 *Muc5ac Tg mice*

Recently, rat *Muc5ac* cDNA was cloned with the GFP inserted immediately upstream of the 5' of the VNTR [97]. This fusion product of 9.1 kb was placed under the control of the rat Clara cell secretory protein (rCCSP) promoter. Murine airways harboring Clara cells were used to overexpress *Muc5ac*, as these cells contain the complete machinery required for the proper posttranslational modifications and secretion of mucins. The overexpression of rat *Muc5ac* did not affect fetal development, survival, and growth. No change in endogenous *Muc5b* expression was noticed, suggesting that in spite of their tandem localization on the same chromosome 7 F5, these mucins are possibly regulated by different mechanisms. Despite nearly 20-fold hypersecretion of rat *Muc5ac* compared to WT levels, *Muc5ac.Tg* mice model did not show any sign of obstruction in the small airways during physiological and inflammatory conditions. The *Muc5ac.Tg* mice exhibited significant decrease in PR8/H1N1 influenza virus susceptibility and neutrophil infiltration. Mechanistically, terminal  $\alpha$ 2,3-sialic acids present on *Muc5ac* have the ability to bind to the surface proteins of PR8/H1N1 influenza virus and thus act as a decoy receptors to prevent the viral entry into the respiratory epithelial cells [97]. Furthermore, the reduced neutrophilic response in *Muc5ac.Tg* mice following infection might restrict tissue damage to the lungs, thus providing additional protective advantage. Altogether, the studies in *Muc5ac.Tg* mice suggest that *Muc5ac* overexpression plays a protective role in respiratory infections.

### 3.2.3 *MUC7 Tg mice and its phenotype*

MUC7 is a small, soluble, and non-gel forming mucin. It is mainly involved in the clearance of various bacteria and inhibits bacterial colonization in the respiratory tract [98]. *MUC7* gene spans approximately 10 kb and comprises three

exons and two introns, which encode for a 39 kDa protein having six VNTRs with 23 amino acids each [99]. Bobek *et al.* have generated a 16-kb construct of MUC7 transgene having all of the three exons along with two introns and 3 kb of both downstream and upstream sequences [100]. MUC7 was found to be expressed in a tissue-specific manner in the sublingual glands of both male and female mice and at low levels in the submandibular glands of females. All other tested tissues did not show detectable MUC7 expression, though a separate study indicated its expression in the pancreas and bladder [101]. This model has been used to elucidate the role of MUC7 following cigarette extract exposure which resulted in the statistically significant increase in MUC7 transcripts in both the trachea and the lungs [102].

#### 4 Conclusions and perspectives

Currently available and established mucin KO mouse models have provided useful information regarding their role in carcinogenesis and inflammatory diseases. Muc1<sup>-/-</sup> mouse model has provided the information regarding the importance of Muc1 in mammary and pancreatic cancer, which has been further substantiated by using MUC1.Tg mice. Additionally, Muc2<sup>-/-</sup> mice showed the protective roles of Muc2 in GI segment, as Muc2 silencing abrogated the mucus barrier function of intestine, and thus made mice more susceptible for drug-induced or inflammation-induced damage. Muc2 KO mice also displayed tumor formation within just 6 months. Furthermore, Muc5ac.Tg mice revealed the protective function of Muc5ac against respiratory infection. Altogether, mucins express differentially under different pathological conditions including pathogenic infections, IBD, and cancer, which further emphasizes the need to generate additional mucin-targeted mouse models for their potential diagnostic and therapeutic implications.

Mucin overexpression in human cancers, like MUC4 in pancreatic cancer, appears very early during the pre-neoplastic lesions, suggesting its role as an oncogene, which is further corroborated by the utilization of spontaneous mouse models of several cancers [103]. Hence, mucin KO and/or expression of the mucin transgene in the background of spontaneous cancer models for pancreatic (Kras), colon (APC), lung (Kras), and prostate (PTEN) cancers will allow us to discern their pro- and anti-tumorigenic roles and provide a unique opportunity to test mucin-targeted combination therapies. In fact, such Tg models have been recently developed and utilized to study more precisely the oncogenic functions of mucins. For example, importance of MUC16 in tumorigenesis was confirmed when mice progenies resulting from the breeding of MUC16 carboxyl-terminus Tg mouse (MUC16ΔC354) mice with p53 heterozygotes showed high susceptibility to form spontaneous tumors. These mice

exhibited increase incidence of neoplastic lesion formation (particularly, sarcoma and lymphoma) as compared to the p53<sup>+/-</sup> heterozygote mice [104]. Additionally, these mouse models can also be helpful to understand the aberrant regulation of mucins in pathological conditions. We can highlight the involvement of extrinsic and intrinsic factors which are responsible for the aberrant expression and overexpression of mucins in different pathological conditions.

Another important aspect of the mucin biology is the compensatory mechanism where the loss of one mucin is compensated by the upregulation of another. These compensatory mechanisms may mask the significance of a given mucin in tissue homeostasis when studied using KO models. As discussed earlier, Muc1<sup>-/-</sup> mice exhibited a compensatory increase in Muc4 in mammary tissue [51], while Muc16<sup>-/-</sup> mice demonstrated reduced Muc1 expression in the uterus and lung [74]. Thus, KO of multiple mucins in the same animal model can potentially circumvent such compensatory mechanisms and provide insights into the interplay between their regulatory networks.

It is an established fact that the tumors are sustained by a relatively small population of cells called cancer stem cells (CSC) or cancer-initiating cells [105]. Monoclonality of the primary tumor and metastatic lesions also strengthens this belief. The MUC1 cleavage product (ectodomain) functions as a growth factor receptor on undifferentiated human embryonic stem cells and interacts with NM23 to maintain the stemness [106]. Similarly, our studies have shown that the interaction between MUC4 and HER2 is involved in the maintenance of stem cells in ovarian cancer cell lines and there was a significant increase in the stem cell population after MUC4 overexpression in the SKOV3 cell line [107, 108]. MUC1.Tg and KO mice have shown a drastic difference in the tumor progression and hematopoiesis abnormalities, again reinforcing their critical role in the CSC population [26, 109]. The involvement of mucins in the stemness has not been directly addressed in the mucin mouse models, but the aforementioned studies hint at their plausible involvement in the maintenance of the stem cell population in the tumor. It will be of immediate interest to undertake studies in GEM models to validate the involvement of mucins in the context of CSC origin, stemness, and clinical implications.

There is growing evidence supporting the role of mucins in modulating pathogenic infections [97]. Infection with *H. pylori* to the gastric mucosa has been positively associated with the aberrant expression of MUC13, and eradication of this pathogen leads to the reduction in various cell surface molecules including MUC13 [110, 111]. In contrast, expression of Muc1 has been shown protective effects for *H. pylori* infection, as it confines its colonization and resulting pathogen-mediated inflammation [58]. Interestingly, MUC4 overexpression has been observed in cervical cancer and cholangiocarcinoma [112, 113], though its expression has not been related with the

associated pathogens (HPV and HCV, respectively). Therefore, the availability of the relevant mouse models will allow the research community to understand the involvement of these mucins in the early stages of pathogen-induced carcinomas.

Mucin hypersecretion is believed to contribute to the morbidity and mortality in airway obstructive diseases such as asthma, COPD, or CF [114, 115] and anti-secretory therapies are often used in managing these patients. However, the aforementioned observations in the GEM models indicating the protective role of mucins in preventing pathogenic infections [58, 73] support the notion that frequently observed increase in mucin secretion might be occurring as a response rather than a cause of these airway obstructions. Thus, the strategies to utilize anti-secretory therapies for defensive and preemptive purposes need to be carefully examined and evaluated for the risk of epithelial infections by viruses and bacteria.

In human mucin genes, most of the polymorphism is TR region encoding DNA sequence, which leads to different numbers of these tandem repeats in mucins. VNTR polymorphism of the MUC1 mucin gene has shown to influence the susceptibility toward *H. pylori*-induced gastritis [116]. Additionally, a wide cohort study in northern Europe demonstrated a 100 % linkage disequilibrium between the frequently occurring MUC7\*5 (in the 5th VNTR) polymorphism and the single-nucleotide polymorphisms rs9982010 [117]. It has been associated with a reduced risk of being asthmatic with a better lung function. Similarly, MUC7 polymorphism has shown remarkably high incidence of asthma in African-Americans compared to Whites [118]. A lower predominance of the MUC7\*5 allele in asthma patients is suggestive of the protective functions of MUC7 in the respiratory system. Due to the established clinical association of MUC7 with asthma, MUC7 transgenic mouse model could also have high applicability to the study of cancer-associated inflammation, as recent studies have associated MUC7 expression in the pathogenesis of various cancers [119, 120]. Development of mucin mouse models can also highlight the importance of mucin polymorphism in clinical settings, which is currently at its infancy.

Lastly, studies have long demonstrated that mucin domains (vWD, SEA, EGF-like) and their glycosylation state dictate their function. Thus, it will be important to evaluate the contribution of individual domains in mucin biology by utilizing domain-deleted KO or knockin mucin mouse models. Developing the next-generation mucin mouse model should also entail combination with genes that allow for lineage tracing and real-time imaging for a better appreciation of the involvement of mucins in the etiologies and progression of pathologies.

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