

## Membrane-bound mucin modular domains: from structure to function.

Nicolas Jonckheere, Nicolas Skrypek, Frédéric Frénois, Isabelle van Seuningen

► **To cite this version:**

Nicolas Jonckheere, Nicolas Skrypek, Frédéric Frénois, Isabelle van Seuningen. Membrane-bound mucin modular domains: from structure to function.. Biochimie, Elsevier, 2013, 95 (6), pp.1077-86. 10.1016/j.biochi.2012.11.005 . inserm-00807818

**HAL Id: inserm-00807818**

**<https://www.hal.inserm.fr/inserm-00807818>**

Submitted on 4 Apr 2013

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

**Membrane-bound mucin** modular domains: from structure to function

**Membrane-bound mucin modular domains: from structure to function**

1  
2  
3  
4 Nicolas Jonckheere<sup>1, 2, 3</sup>, Nicolas Skrypek<sup>1, 2, 3</sup>, Frédéric Frénois<sup>1, 2, 3</sup> and Isabelle Van  
5  
6  
7 Seuningen<sup>1, 2, 3</sup>  
8  
9

10  
11  
12 1 Inserm, UMR837, Jean Pierre Aubert Research Center, Team #5 “Mucins, epithelial  
13  
14 differentiation and carcinogenesis”, rue Polonovski, 59045 Lille Cedex, France  
15

16  
17 2 Université Lille Nord de France, 1 Place de Verdun, 59045 Lille cedex, France  
18

19  
20 3 Centre Hospitalier Régional et Universitaire de Lille, Place de Verdun, 59037 Lille cedex,  
21  
22 France.  
23

24  
25  
26  
27  
28  
29 Corresponding author : Dr Nicolas Jonckheere, Inserm UMR837/JPARC, rue Polonovski,  
30  
31 59045 Lille Cedex, France, Phone: +33 3 20 29 88 50, Fax: +33 3 20 53 85 62, E-Mail:

32  
33  
34 [nicolas.jonckheere@inserm.fr](mailto:nicolas.jonckheere@inserm.fr)  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

## Abstract

1  
2  
3  
4 Mucins belong to a heterogeneous family of large *O*-glycoproteins composed of a long  
5 peptidic chain called apomucin on which are linked hundreds of oligosaccharidic chains.  
6  
7 Among mucins, membrane-bound mucins are modular proteins and have a structural  
8 organization usually containing Pro/Thr/Ser-rich *O*-glycosylated domains (PTS), EGF-like  
9 and SEA domains. *Via* these modular domains, **the membrane-bound** mucins participate in  
10 cell signalling and cell interaction with their environment in normal and pathological  
11 conditions. Moreover, the recent knowledge of these domains and their biological activities  
12 led to the development of new therapeutic approaches involving mucins. In this review, we  
13 show 3D structures of EGF and SEA domains. We also describe the functional features of the  
14 evolutionary conserved domains of **membrane-bound** mucins and discuss consequences of  
15 splice events.  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44

45  
46 Keywords: mucin, evolution, structure-function, EGF-like, 3D structure  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

## 1. Introduction

1  
2 Mucins belong to a heterogeneous family of large *O*-glycoproteins composed of a long  
3  
4 peptidic chain called apomucin on which are linked hundreds of oligosaccharidic chains.  
5  
6 Based on biochemical and molecular biology studies, mucins were separated into two  
7  
8 structurally distinct **categories**: the secreted and the membrane-bound mucins. Mucins have a  
9  
10 cell- and tissue-specific pattern of expression that is profoundly altered in epithelial cancers  
11  
12 (loss of expression, over-expression, aberrant expression, neo-expression) suggesting their  
13  
14 implication in tumourigenesis [1, 2]. They play roles in cell signalling, cell proliferation,  
15  
16 tumour progression or cell polarity, and mediate immune evasion. Mucins are considered as  
17  
18 potent new therapeutic targets in mucosal biology, in malignant and inflammatory diseases of  
19  
20 the epithelial tissues [3-6].  
21  
22  
23  
24

25  
26 **Secreted mucins**, gel-forming components of viscoelastic mucus gels protecting the epithelia,  
27  
28 include **the 11p15 secretory mucins**: MUC2, MUC5AC, MUC5B, MUC6. **Next to that**  
29  
30 **family of genes, a fifth secretory mucin was described that is MUC19**. Their main  
31  
32 function is to participate in mucus formation by forming a three-dimensional network *via*  
33  
34 oligomerization domains in order to protect underlying epithelia against various injuries  
35  
36 (inflammation, bacteria, virus, pollutants, pH, etc). MUC7 and MUC9 are smaller secreted  
37  
38 mucins that do not oligomerize and do not form gels [3, 7].  
39  
40  
41  
42

43 The membrane-bound mucins belong to an ever increasing **group** of type I membrane-  
44  
45 anchored proteins. Based on their structure and localization at the cell surface they are thought  
46  
47 to act in cell-cell and cell-matrix interactions and in cell signalling. The biological roles they  
48  
49 play in cellular interactions and in cell signalling indicate that they are involved in regulating  
50  
51 biological properties of epithelial cells [4, 5]. **Among the membrane-bound mucins, some**  
52  
53 **conform to the mucin definition (presence of a Pro/Thr/Ser (PTS) region in the peptidic**  
54  
55 **sequence) that are** MUC1, MUC3A/3B, MUC4, MUC12, MUC16, MUC17, MUC21 and  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

1 MUC22 [8-11]. **Others were assigned the term MUC despite the absence of that PTS**  
2 **domain that are MUC13, MUC15 and MUC20.** Because of their specific pattern of  
3  
4 expression during the different steps of carcinogenesis, membrane-bound mucins stay under  
5  
6 intense investigation as both potent new biomarkers and therapeutic targets in epithelial  
7  
8 cancers [2-4].  
9

10  
11 Analysis of the peptidic sequences of mucins allowed description of their modular  
12  
13 organization. Membrane-bound mucins are modular proteins which share conserved domains  
14  
15 such as epidermal growth factor-like (EGF) **or** Sea urchin sperm protein Enterokinase and  
16  
17 Agrin (SEA) **domains** (Figure 1) [8, 9, 12]. **The PTS domain, which is a common feature**  
18  
19 **between mucins is the only domain not conserved at the genomic level although**  
20  
21 **similarities exist at the amino acid level.** The human MUC1, MUC3A/3B, MUC12,  
22  
23 MUC13, MUC16, and MUC17 mucins contain the SEA domains. Some domains are specific  
24  
25 to one mucin. For **example**, the MUC4 mucin contains NIDO, AMOP, and VWF-D domains  
26  
27 that are unique in the apomucin family. MUC22 (also called as Panbronchiolitis-related  
28  
29 mucin-like protein 1, PBMUCL1) has recently been discovered in the disease-susceptibility  
30  
31 locus for diffuse panbronchiolitis and contains both the typical PTS and a transmembrane  
32  
33 (TM) domains [10]. Existence of other conserved domains in MUC22 remains to be  
34  
35 demonstrated. **At this time, MUC15 is considered as a mucin-like since it lacks the**  
36  
37 **characteristic tandem repeat (TR) region.**  
38  
39  
40  
41  
42  
43  
44  
45

46 Understanding the structure and the function of membrane-bound mucin domains will bring  
47  
48 new information about their biological roles in epithelium homeostasis as well as in  
49  
50 pathological situations such as carcinogenesis or inflammatory processes in which membrane-  
51  
52 bound mucin expression **and localization are** often altered. In this review, we will discuss the  
53  
54 structural and functional features of the evolutionary conserved domains of **membrane-**  
55  
56 **bound** mucins and their abilities to modulate the biological properties of epithelial cells.  
57  
58  
59  
60  
61  
62  
63  
64  
65

## 2. *Mucins and evolution*

Phylogenetic analyses have shown that the membrane-bound mucins are only found in mammals with the exception of MUC16 and MUC4 [13]. Mucin domains are shared among most of the MUC family members, suggesting common ancestors **or adoption of functional modules during evolution**. MUC1, MUC3, MUC12, MUC13 and MUC17 **share a peptidic sequence similar to** heparan sulfate proteoglycan of basement membrane (HSPG2) (Figure 2). *MUC3*, *MUC12* and *MUC17* genes are present contiguously on chromosome 7 (7q22) supporting the hypothesis of a process of duplication. *MUC16* evolved separately from agrin. MUC4 arose from two evolutionary events involving (i) NIDO and EGF-like domains from a common ancestor to nidogen and (ii) AMOP and VWF-D domains from a common ancestor to the Susd2 [13]. Beside its homology through the SEA domain, MUC1 has sequence similarities with other membrane-bound mucins and in fact evolved from repeated sequences of MUC5B secreted mucin [14]. Based on available sequence information, one can hypothesize that membrane-bound mucins evolved from several distinct ancestor genes and could be divided in different subgroups based on their conserved domains. Interestingly, mucins arising from a common ancestor gene are frequently clustered in a chromosome locus as illustrated on figure 2.

### 3. *The membrane-bound mucin prototype domains*

A prototype membrane-bound mucin is **characterized** by the presence of an extracellular *O*-glycosylated PTS domain, a TM domain and a cytoplasmic tail. Two EGF-like and one SEA domains are commonly found in most of membrane-bound mucins (Figure 1A).

#### 3. 1 *The PTS domain*

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

The hallmark of membrane-bound mucins is the large extracellular subunit that protrudes at the cell surface mainly composed by the PTS domain. The PTS domain is the substrate of important post-translational *O*-glycosylation modifications. This central domain is encoded by a large intronless genomic DNA sequence (>10 kb), typically characterized by a variable number of tandem repeat (VNTR) polymorphism [15]. PTS domain harbours extensive *O*-glycosylation (up to 80% of the total weight of the mature mucin) that forms chains of varying lengths, sequences, and compositions. The biosynthesis of mucin *O*-glycan chains is a step-by-step process occurring in the Golgi apparatus, involving specific glycosyl- and sulfo-transferases expressed in a tissue-specific manner [16, 17]. The *O*-glycans on PTS domain play major roles in the conformation and stability of the mucin and participate in defense of mucosae covering and protecting the underlying epithelium against various types of aggression (mechanical and chemical stress). In diseases such as cancer and inflammation, mucin *O*-glycosylation is altered, modifying the antigenic and adhesive properties of the glycan epitopes they bear [18]. Tumour-associated carbohydrate antigens (TAAs) are produced *via* incomplete synthesis of carbohydrate chains. TAA notably involve Tn and T antigens and their sialylated counterparts, sialyl-Tn and sialyl-T antigens. Cancer-associated *O*-glycans are often highly sialylated and less sulfated compared with *O*-glycans from normal mucins [19, 20].

Because of its extracellular localization **at the apical pole of the cell, the** MUC<sub>PTS</sub> domain is mainly thought to act **as a sensor of the microenvironment. In the normal polarized epithelial cell, it may interact with immune cells, antibodies, viruses, bacteria to help maintain the epithelial homeostasis. In a depolarized cancer cell, the membrane-bound mucin is then expressed circumferentially and is going to then play different roles in cell-cell or cell-extracellular matrix interactions.** For example, **the** MUC1<sub>PTS</sub> domain **is able** to interact with adhesion molecules such as the endothelial protein ICAM-1 and

1 consequently may assist **the** cancer cell during heterotopic adhesion and invasion into the  
2 endothelium and reattachment at distant metastasis sites [21, 22].  
3

4 Galectins belong to a family of carbohydrate binding proteins ( $\beta$ -galactoside-specific lectins).  
5

6 Galectin-1 was shown to interact with MUC16. Galectin-3 interacts with MUC1, MUC4 and  
7 MUC16. *O*-glycans on MUC4<sub>PTS</sub> interact with galectin-3 at the cell surface and mediate  
8 docking of tumour cells on endothelial cells [22, 23]. Galectin-3-MUC1 interaction alters  
9 MUC1 cell surface polarization, enhances tumour cell homotypic aggregation, prevents  
10 anoikis [24, 25], and regulates EGFR internalization, subcellular localization and ERK  
11 signalling pathway activation [26].  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23

### 24 **3. 2 The epidermal growth factor (EGF)-like domain**

25 EGF-like domains are 30-40 residue-long and evolutionarily well-conserved. The EGF-like  
26 domain contains six cysteine residues that form three different disulfide bonds within the  
27 domain (C1–C3, C2–C4, and C5–C6). The first cysteine exhibits a mild conservation between  
28 the different mucins whereas the five others are highly conserved suggesting that the resulting  
29 tertiary structure leads to biological functions (Figure 3A/3B).  
30  
31  
32  
33  
34  
35  
36  
37

38 The EGF-like domains of membrane-bound mucins are believed to direct the interactions with  
39 different proteins. Mucin EGF-like domains are thought to act as ligands with membrane  
40 receptor such as those of the ErbB family mostly based on the work conducted on  
41 MUC4/rMuc4 biological roles. It has been speculated that EGF domains located in **the**  
42 released extracellular domain of mucins resulting from cleavage could also act as growth  
43 factors and serve as indicators of alteration of epithelial surfaces [5].  
44  
45  
46  
47  
48  
49  
50  
51  
52

53 Experimental evidence suggests that rat homologues of Muc4 and ErbB2 are regulators of  
54 signalling related to growth, motility or differentiation of the cell *via* the EGF-like domains  
55 [27]. Human MUC4 and ErbB2 were shown to physically interact *via* a peptidic region  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65



1 including the three EGF-like and intermediate domains of MUC4 [28]. Recombinant human  
2 MUC3A TM-EGF1/EGF2 protein led to reduced apoptosis induced either by TNF- $\alpha$  or Fas  
3 receptor stimulation. MUC17 EGF-like domains (also designated as Cys-rich domains, CRD)  
4 were shown to have anti-apoptotic and pro-migratory activity *via* ERK phosphorylation but  
5 did not stimulate cell proliferation [29, 30]. *In vivo* rectally administered MUC17 CRD, as  
6 well as its mouse homologue, accelerated healing in an experimental mouse model of colitis  
7 [29], suggesting a potential therapeutic use of EGF-like domains as treatment of mucosal  
8 inflammatory diseases.

9 The deduced amino-acid (aa) sequences of the EGF-like domains of MUC4, MUC3A,  
10 MUC12, MUC13, MUC16 and MUC17 (Table1) were used to predict their 3D structures  
11 using the Phyre 2 server (Protein Homology/analogy Recognition Engine V2.0) [31]. The  
12 predicted 3D structures of each EGF-like domain was compared with the resolved 3D  
13 structure of one monomer of the human EGF available through RCSB (Research  
14 Collaboratory for Structural Bioinformatics) protein data bank (pdb)  
15 (<http://www.pdb.org/pdb/home/home.do>) (pdb code : 1j19) and with the other EGF domains  
16 3D predicted structures using the molecular visualization system, PyMOL ( The PyMOL  
17 Molecular Graphics System), version 1.5.0.1 Schrödinger, LLC). PyMOL did not allow us to  
18 predict the secondary structure of the third EGF domain of MUC4 (MUC4<sub>EGF3</sub>) nor of the  
19 EGF domain of MUC12. To determine whether the EGF-like domains of two membrane-  
20 bound mucins were structurally homologous, PyMOL was used to measure the root-mean-  
21 square-deviation (RMSD) of superimposed 3D structures. Human EGF resolved structure  
22 consists notably of two anti-parallel  $\beta$  sheets. This topology is conserved in MUC<sub>EGF</sub>  
23 predicted structures as illustrated on Figure 4A. The RMSD illustrates the average distance  
24 value between the backbone alpha carbons of two structures and consequently, the RMSD of  
25 two aligned structures indicates their divergence from one another. If the RMSD value is

1 below 1.5Å, two 3D structures whose sequence alignment is over 30% can be considered as  
2 almost homologous. The most significant result is then correlated with the highest number of  
3 residues aligned. Regarding the mucin EGF domains, there is strong structural homology  
4 between the 3D structure of the human EGF and the 3D predicted structure of MUC4<sub>EGF1</sub>,  
5 MUC3A<sub>EGF</sub> and MUC13<sub>EGF1</sub> (Table 1). We also found high homology between MUC13<sub>EGF1</sub>  
6 and MUC3A<sub>EGF</sub> and MUC12<sub>EGF</sub>. The 3D predicted structure of MUC4<sub>EGF1</sub> was shown **to be**  
7 homologous to MUC16<sub>EGF</sub>.  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18

### 19 **3. 3 The Sea urchin sperm protein Enterokinase and Agrin (SEA)**

20 The SEA module is a 120 aa domain found in many membrane-associated proteins at the cell  
21 surface. SEA domains were initially described as extracellular domains associated with *O*-  
22 glycosylation but more recently they have also been implicated in both cleavage events and  
23 association of the subunits [32-34]. Mucins usually contain one SEA domain in their  
24 extracellular region. However, MUC16 contains multiple SEA domains that were repeated up  
25 to 56 times through duplication events [13]. Alignment analysis indicated a mild conservation  
26 of the SEA domains of the different membrane-bound mucins (Figure 3C). We confirmed that  
27 MUC3, MUC12 and MUC17, that are located in a cluster on the human 7q22 chromosome,  
28 share the highest identity of SEA sequences (Figure 3D).  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42

43 The MUC1<sub>SEA</sub> module is a self-cleaving domain [35]. Computer modeling of MUC1<sub>SEA</sub>  
44 domain initially suggested that it consists in three  $\alpha$ -helices and six  $\beta$ -strands forming an  $\alpha/\beta$   
45 sandwich fold [33]. Macao *et al.* later determined that SEA consists **of** four-stranded  
46 antiparallel  $\beta$ -sheets and four  $\alpha$ -helices occurring in the  $\beta 1-\alpha 1-\alpha 2-\beta 2 \uparrow \beta 3-\alpha 3-\alpha 4-\beta 4$  order  
47 and with the helices packed against the concave surface of the  $\beta$ -sheet [35]. Moreover,  
48 determination of the SEA domain structure from the murine Muc16 homologue using multi-  
49 dimensional NMR spectroscopy allowed Maeda *et al.* [36] to propose the GSVVV motif as a  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

1 proteolytic cleavage site located in the short loop connecting  $\beta 2$  and  $\beta 3$  sheets. Levitin et al.  
2 proposed that this module likely functions as a site for proteolytic cleavage in all SEA  
3 module-containing proteins [37]. Cleavage of membrane-bound mucins in the SEA domain is  
4 not entirely sequence dependent, but may be related to structural features of the SEA domain.  
5 Exposure of the cleavage site protruding outward from a compact structure enhances its  
6 recognition and cleavage by cellular proteases [33]. In addition to its cleavage involvement,  
7 MUC17 EGF1-linker-EGF2 recombinant protein induces anti-apoptotic activity that requires  
8 an intact SEA module [29].

9 The deduced aa sequences of the SEA domains of MUC1, MUC3A, MUC12, MUC13,  
10 MUC16 and MUC17 (Table 2) were used to predict their 3D structures using the Phyre 2  
11 server. The 3D predicted structures of each SEA domain was compared with the 3D resolved  
12 structure of the human mucin MUC1 available in the protein data bank (pdb code : 2ACM)  
13 and with the 3D predicted structures of the other SEA domains using the molecular  
14 visualization system, PyMOL. In the same manner and with the same criteria as for the 3D  
15 prediction structures of the EGF domains, we looked at the 3D structural homology thanks to  
16 the RMSD calculation. Bioinformatic analysis indicates that the 3D resolved structure of  
17 MUC1<sub>SEA</sub> is homologous with MUC17<sub>SEA</sub>. MUC17<sub>SEA</sub> is itself structurally close to  
18 MUC3A<sub>SEA</sub> and MUC12<sub>SEA</sub>. Superimposition of the 3D structures showed that the  $\alpha/\beta$   
19 sandwich fold of resolved MUC1<sub>SEA</sub> is conserved in predicted structures of MUC3A<sub>SEA</sub>,  
20 MUC12<sub>SEA</sub>, MUC17<sub>SEA</sub> (Figure 4B) [33].

#### 21 **4. The unique domains of membrane-bound mucin MUC4**

22 Among membrane-bound mucins, MUC4 contains NIDO, AMOP, and VWF-D domains that  
23 are unique in the apomucin family.  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

#### 4. 1. *The Nidogen-like (Nido) domain*

1  
2 NIDO nidogen-like domain is an extracellular domain of unknown function found in nidogen  
3  
4 and other hypothetical proteins such as sushi nidogen and EGF-like domains 1 (SNED1),  
5  
6 plexin domain containing-1/-2 (PLXDC-1/-2) and tectorin alpha (TECTA). Phylogenetic  
7  
8 analysis revealed the origin of the MUC4<sub>NIDO</sub> domain from an ancestor common to the NIDO  
9  
10 protein [13]. Recent work demonstrated for the first time that MUC4<sub>NIDO</sub> does not alter the  
11  
12 motility of pancreatic cancer cells but promotes invasion and extravasation. The MUC4<sub>NIDO</sub>  
13  
14 domain, which is similar to the G1-domain of fibulin-2 or extracellular matrix protein  
15  
16 Nidogen/entactin, contributes to the interaction properties of MUC4. Senapati *et al.*  
17  
18 hypothesized that MUC4<sub>NIDO</sub> disrupts the interaction between NIDO/entactin and fibulin-2  
19  
20 [38], a major component of murine liver blood vessels [39], in a competitive inhibition  
21  
22 manner and thus may create a favorable environment in liver for the extravasation of  
23  
24 metastatic pancreatic cancer cells. MUC4<sub>NIDO</sub> domain is thus proposed as a mediator of  
25  
26 protease-independent cell invasion in pancreatic cancer metastasis [38].  
27  
28  
29  
30  
31  
32  
33  
34  
35

#### 4. 2 *The Adhesion-associated domain in MUC4 and Other Proteins (AMOP)*

36  
37 AMOP and vWF-D domains originated from a common ancestor to the Sushi-domain  
38  
39 containing protein 2 (SUSD2) [13]. AMOP domain is uncommon in the human genome as it  
40  
41 is described only in four proteins (Isthmin-1, -2, SUSD2 and MUC4) using Simple Modular  
42  
43 Architecture Research Tool (SMART) database. The role of AMOP domain is unclear.  
44  
45 Presence of AMOP in cell adhesion molecules could be indicative of a role for this domain in  
46  
47 adhesion. Indeed, mouse Isthmin, an angiogenesis modulator, disrupts endothelial cell (EC)  
48  
49 capillary network formation on Matrigel through its C-terminal AMOP domain [40]. From  
50  
51 there, one could hypothesize such role for MUC4 in angiogenesis.  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

#### 4.3 The Von Willebrand Factor-D (vWF-D) domain

1  
2 The human gel-forming mucins contain vWF-D and C-terminal cysteine-knot domains that  
3  
4 are responsible for their oligomerization [9, 41, 42]. Only MUC4 membrane-bound mucin  
5  
6 contains a vWF-D in its intracellular region [9]. VWF domains usually participate in forming  
7  
8 disulfide bonds. However, the cysteine residues, conserved in secreted mucins, are not  
9  
10 conserved in MUC4 suggesting a loss of function of this domain in membrane-bound mucins  
11  
12 during evolution [43]. The crystal structures of three vWF domains are known. All three  
13  
14 domains share identical three-dimensional fold with a  $\alpha$ - $\beta$ - $\alpha$  sandwiched model [44].  
15  
16  
17  
18  
19  
20  
21

#### 5. The cytoplasmic tail

22  
23  
24 Cytoplasmic tails (CT) of mucins are poorly conserved ruling out a potential common  
25  
26 evolution. Size and aa sequence greatly vary among membrane-bound mucins leading to a  
27  
28 great variety of functions in cell signalling (Table 3). Indeed, MUC4 acts as a receptor partner  
29  
30 at the membrane through its extracellular domain because of its short CT whereas MUC1 acts  
31  
32 as an intracellular docking protein for signalling molecules. Among membrane-bound mucins,  
33  
34 MUC1 has been under intense investigation.  
35  
36  
37

38  
39 MUC1<sub>CT</sub>, is 72 aa long, contains seven tyrosine residues, an Src Homology 2 (SH2)  
40  
41 interaction domain, and is phosphorylated by several kinases [4, 22, 45, 46]. The MUC1<sub>CT</sub>  
42  
43 allows the direct interaction of MUC1 with a wide array of signalling pathways in tumour  
44  
45 cells. MUC1<sub>CT</sub> was shown to interact with Src family kinase including c-Src, Lyn and Lck  
46  
47 and promotes phosphorylation of Tyr46 involved in protein-protein interaction. MUC1<sub>CT</sub> is  
48  
49 also targeted by kinases  $\zeta$  chain associated protein kinase of 70 kDa (ZAP70), and the  $\delta$   
50  
51 isoform of protein kinase C (PKC $\delta$ ) [22, 47]. Interaction between MUC1<sub>CT</sub> and proapoptotic  
52  
53 kinase cAbl leads to phosphorylation of Tyr60 by its binding to the c-Abl SH2 domain [48].  
54  
55  
56 Binding of scaffolding protein Grb2 on MUC1<sub>CT</sub> activates MAPK pathway, involved in  
57  
58  
59  
60  
61  
62  
63  
64  
65

1 proliferation of tumour cells [49]. MUC1<sub>CT</sub> interacts with components of the IκB kinase  
2 (IKK) complex enhancing IKKα-IKKβ interaction, promoting IKKβ phosphorylation, IKKα  
3 degradation, NFκB-p65 targeting to the nucleus that leads to NF-κB p65 transcriptional  
4 activity [50, 51]. MUC1<sub>CT</sub> interacts with chaperone heat shock proteins (HSP) HSP70 and  
5 HSP90 leading to mitochondrial translocation of MUC1<sub>CT</sub> and therefore inhibiting apoptosis  
6  
7 [52]. MUC1<sub>CT</sub> binds to Wnt signalling pathway components β-catenin and APC. MUC1<sub>CT</sub>-β-  
8 catenin interaction enhances the levels of nuclear β-catenin during disruption of cadherin-  
9 mediated cell-cell adhesion and promotes expression of Wnt target genes [46, 53]. The  
10 MUC1<sub>CT</sub>-β-catenin binding is regulated by GSK3β and HSP90 in a competitive inhibition  
11 manner [54]. MUC1<sub>CT</sub> is phosphorylated by several cell surface receptors such as fibroblast  
12 growth factor receptor 3 (FGFR3), platelet-derived growth factor receptor (PDGFR) and ErbB  
13 receptor family altering interaction with proteins mentioned above. FGF1 induces Tyr46  
14 YEKV phosphorylation whereas PDGF catalyses phosphorylation of Tyr35 and enhances  
15 invasion *in vitro*, tumour growth and metastasis *in vivo* [55].

16 MUC1<sub>CT</sub> also regulates its own nucleo-cytoplasmic transport by binding importin-β and  
17 nucleoporin p62 (nup62) *via* a CQC motif and therefore alters transcriptional regulation of  
18 numerous target genes [56]. In the cytoplasm of breast and lung cancer cells, MUC1<sub>CT</sub> forms  
19 dimers that are necessary for its nuclear localization *via* the CQC motif [56, 57]. In the  
20 nucleus, MUC1<sub>CT</sub> associates with transcription factors such as β-catenin/TCF4, p53,  
21 CDKN1A (p21), nuclear factor-κB p65 or STATs [3]. The MUC1<sub>CT</sub> domain also stabilizes  
22 estrogen receptor-α (ERα) and is necessary for its nuclear localization [58].

23 MUC1<sub>CT</sub> has been extensively characterised whereas other mucin CT are scarcely described.  
24 MUC3<sub>CT</sub> contains an YVAL aa motif which is similar to motifs recognized by the SH2  
25 domain [59]. MUC13<sub>CT</sub> contains a PKC phosphorylation site [60]. MUC16<sub>CT</sub> contains  
26 polybasic aa that are predicted to interact with ezrin/radixin/moesin (ERM) actin-binding  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

1 proteins [61] and is proposed to cross-link MUC16 with actin-cytoskeleton. MUC16<sub>CT</sub> is also  
2 proposed to interact with JAK2 *via* its ERM domain and mediate breast cancer cell  
3 proliferation [62]. PDZ-interacting domains are observed within MUC3<sub>CT</sub>, MUC12<sub>CT</sub> and  
4 MUC17<sub>CT</sub> but only phosphorylation sites of MUC17<sub>CT</sub> exhibit a strong binding to PDZ  
5 domain of PDZK1 [63]. It was also shown using *Pdzk1*<sup>-/-</sup> mice that *Pdzk1* plays a specific role  
6 in stabilizing mMuc3 (MUC17 mouse homologue) at the apical pole of polarized enterocytes.  
7 MUC20<sub>CT</sub> binds to a multifunctional docking site of hepatocyte growth factor (HGF) receptor  
8 Met and therefore suppresses the Grb2-Ras pathway [64].  
9

10 The tremendous variability of MUC<sub>CT</sub> is thus associated with a wide array of signalling  
11 pathways regulated by membrane-bound mucins and much is still to be discovered to fully  
12 characterize their biological activities and effects on epithelial cell behavior.  
13  
14  
15  
16  
17  
18

## 19 **6. Mucin splice variants**

### 20 **6. 1. MUC1**

21 Membrane-bound mucin isoforms or alternative splicing events have been reported for  
22 MUC1, MUC3A, MUC4 and MUC17 that lead to new apomucin polypeptides possibly  
23 lacking functional domains and presumably showing altered cellular/biological functions.  
24

25 MUC1/X is an alternate isoform of MUC1 in which the extracellular domain is comprised of  
26 the SEA module in addition to thirty MUC1 N-terminal aa residues [37]. MUC1/Z and  
27 MUC1/Y are two isoforms lacking the PTS domain. MUC1/Z isoform is proteolytically  
28 cleaved after its synthesis and generates the two subunits MUC1 $\alpha$  and MUC1 $\beta$ . On the  
29 contrary, MUC1/Y contains a truncated SEA domain and therefore lacks the cleavage site,  
30 resulting in a single uncleaved apomucin. MUC1/Y also contains the TM and cytoplasmic  
31 domains. Interestingly, MUC1/Y isoform expression increases the tumorigenicity of DA3  
32 mouse mammary epithelial cells [65]. An alternate isoform MUC1/ZD results from a reading  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

frameshift caused by a splicing event that deletes the TR-PTS domain but contains the signal peptide and a subsequent stretch of 30 aa [66]. Variant MUC1/SEC lacks the TM and cytoplasmic sequences and has the potential to be directly secreted out of the cell. Interestingly, tumour cells expressing MUC1/SEC fail to develop tumours in immunocompetent mice. MUC1/SEC may inhibit tumour development and may support antitumour immune responses *via* the downregulation of Urokinase-Type Plasminogen Activator (uPA) and Signal Transducer and Activator of Transcription 1 (STAT1) [67]. Moreover, MUC1/SEC is capable of blocking expression of arginase 1 and production of reactive oxygen species (ROS) in myeloid-derived suppressor cells MDSCs that play a critical role in tumour-induced immunosuppression [68].

## 6.2. *MUC4*

Multiple splicing events have been described for *MUC4* [69]. Among them, MUC4/Y and MUC4/X lack the central TR/MUC4<sub>PTS</sub> domain, the main feature of membrane-bound mucins [69]. Some MUC4 isoforms are also lacking TM domain and might be secreted. One could hypothesize that the EGF-like domains of MUC4 act as growth factors when released in the extracellular environment. Globally, more than twenty cDNA isoforms have been described for *MUC4* that are generated by several mechanisms (alternative use of cassette exons, exon skipping or use of cryptic splice donor/acceptor sites) [70]. However, till now no splice variants for Muc4 have been observed in mouse tissues [9]. So far, the existence at the protein level and the biological roles of the different MUC4 isoforms remain to be determined.

## 6.3. *Other mucin variants*

Transcription variants of *MUC3* encode truncated proteins, suggesting the possibility of expression of soluble forms [71]. Williams *et al.* confirmed the existence of two secreted



1 isoforms of MUC3 that lack the TM domain [72]. In a similar manner, an alternative MUC17  
2 splice event occurs and lacks the second EGF domain, the TM domain, and the cytoplasmic  
3 tail and leads to a secreted form MUC17/SEC [73].  
4  
5  
6  
7  
8

## 9 **7. Using MUC domains as therapeutic targets/tools**

10 Membrane-bound mucins display major biological activities in epithelium homeostasis and  
11 pathologies and hold promise as biological tools for therapy in cancer or inflammatory  
12 diseases [4].  
13  
14  
15  
16  
17

18 The extended knowledge of MUC1 domains and their biological significance led to the  
19 development of peptide-based therapies that may have important clinical implications [1, 3, 4,  
20 74]. As an example, MUC1 inhibitory peptide (MIP) was designed to block the intracellular  
21 interactions between MUC1/ $\beta$ -catenin and MUC1/EGFR. MIP was then fused to the protein  
22 transduction domain, PTD4 (PMIP) in order to increase cellular uptake. Exogenous treatment  
23 of PMIP led to a significant reduction in aggressiveness of metastatic breast cancer cells *in*  
24 *vitro*, and inhibition of growth and recurrence of breast tumour in an *in vivo* transgenic model  
25 [75]. Moreover, PMIP selectively inhibits lung adenocarcinoma proliferation, and inhibits ER  
26 responses *via* the blocking of the MUC1-ER $\alpha$  interaction [76]. MUC1<sub>CT</sub> dimerization may  
27 also be targeted by reducing agents. Similarly, cell-penetrating peptide containing the  
28 CQCRRKN sequence binds directly to the endogenous MUC1<sub>CT</sub> and blocks its ability to  
29 dimerize [57]. That blockage induces death of human breast cancer cells *in vitro* and in  
30 xenograft tumour models [77].  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50

51 Since MUC1 displays ubiquitous expression in a wide variety of tumour types, numerous  
52 studies are targeting MUC1. The MUC1 peptide core or glycopeptide has been used in  
53 immunotherapy for immunization. The different strategies showed T cell- and antibody-  
54 responses and then were challenged for tumour protection [78, 79]. Recently, immunization  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

by a tripartite vaccine based on a synthetic 60-mer MUC1<sub>PTS</sub> derived peptide covalently linked to a Toll-like receptor (TLR) agonist could elicit IgG antibodies that efficiently lysed MUC1-expressing cancer cells, stimulated cytotoxicity of T lymphocytes, and activated innate immune responses [80].

Trastuzumab (also called herceptin®), a monoclonal antibody, targets ErbB2-expressing tumours such as breast cancers and leads to inhibition of intracellular signalling and induction of an immune system-mediated antitumour response [81]. Human MUC4 and ErbB2 do physically interact *via* MUC4<sub>EGF3+1+2</sub> region that contains the three EGF-like domains [28]. MUC4 overexpression decreases cancer cell sensitivity to trastuzumab, possibly by steric hindrance [82]. MUC4 is thought to impair access of trastuzumab to ErbB2 *via* its large PTS domain that protrudes outside the glycocalyx. More recently, we also showed an effect of MUC4 on gemcitabine sensitivity in pancreatic ductal adenocarcinoma [83] but via domains and partner still to identify.

MUC17<sub>EGF</sub> domains display a strong potential in the treatment of mucosal inflammatory diseases as healing is accelerated in acetic acid and dextran sodium sulfate (DSS)-induced colitis in mice [29]. MUC1 also has a protective effect against DSS-induced colitis in mice. Since Muc1 is lacking EGF-like domains, this effect is most likely mediated by another mechanism. MUC1 could act through its ability to increase the mucus barrier but also by decreasing T cell recruitment to the afflicted site [84].

## 7. Conclusions

Membrane-bound mucins are characterized by a multi-domain organisation with a **PTS domain**, conserved EGF or SEA domains and unique domains such as AMOP, NIDO or vWF-D that could mediate specific functions for some membrane-bound mucins. Understanding the structure and the biological functions of each domain might help explain

the major roles of mucins in inflammatory and cancerous diseases of the epithelium. In the future, the better structural and functional characterization of these domains should give rise to new mucin-based therapeutic targets/tools.

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

## Acknowledgments

1  
2  
3  
4  
5 Dr Nicolas Jonckheere is a recipient of a postdoctoral fellowship from the Ligue Nationale  
6  
7 Contre le Cancer (LNCC). Nicolas Skrypek is a recipient of a PhD fellowship from the Centre  
8  
9 Hospitalier Régional et Universitaire (CHRU) de Lille/région Nord-Pas de Calais. Dr Frédéric  
10  
11 Frénois is a recipient of a postdoctoral fellowship from the Fondation pour la Recherche  
12  
13 Médicale (FRM). This work is supported by a grant from la Ligue Nationale Contre le Cancer  
14  
15 (Equipe Labellisée Ligue 2010, IVS). Isabelle Van Seuningen is the recipient of a “Contrat  
16  
17 Hospitalier de Recherche Translationnelle”/CHRT 2010, AVIESAN.  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

## References

- 1 [1] Jonckheere N., Skrypek N., van Seuning I., Mucins and pancreatic cancer, *Cancers* 2  
2 (2010) 1794-1812.
- 3 [2] Jonckheere N., Van Seuning I., The membrane-bound mucins: From cell signalling to  
4 transcriptional regulation and expression in epithelial cancers, *Biochimie* 92 (2010) 1-11.
- 5 [3] Kufe D.W., Mucins in cancer: function, prognosis and therapy, *Nat Rev Cancer* 9 (2009) 874-  
6 885.
- 7 [4] Jonckheere N., Van Seuning I., The membrane-bound mucins: how large O-glycoproteins  
8 play key roles in epithelial cancers and hold promise as biological tools for gene-based and  
9 immunotherapies, *Crit Rev Oncog* 14 (2008) 177-196.
- 10 [5] Hollingsworth M.A., Swanson B.J., Mucins in cancer: protection and control of the cell surface,  
11 *Nat Rev Cancer* 4 (2004) 45-60.
- 12 [6] Van Seuning I., Pigny P., Perrais M., Porchet N., Aubert J.P., Transcriptional regulation of  
13 the 11p15 mucin genes. Towards new biological tools in human therapy, in inflammatory diseases and  
14 cancer?, *Front Biosci* 6 (2001) D1216-1234.
- 15 [7] Linden S.K., Sutton P., Karlsson N.G., Korolik V., McGuckin M.A., Mucins in the mucosal  
16 barrier to infection, *Mucosal Immunol* 1 (2008) 183-197.
- 17 [8] Jonckheere N., Van Seuning I., The ever growing family of membrane-bound mucins, in:  
18 Van Seuning I. (Ed.), *The Epithelial Mucins: Structure/Function. Roles in Cancer and Inflammatory  
19 Diseases*, Research Signpost, Kerala, India, 2008, pp. 17-38.
- 20 [9] Desseyn J.L., Tetaert D., Gouyer V., Architecture of the large membrane-bound mucins, *Gene*  
21 410 (2008) 215-222.
- 22 [10] Hijikata M., Matsushita I., Tanaka G., Tsuchiya T., Ito H., Tokunaga K., Ohashi J., Homma S.,  
23 Kobashi Y., Taguchi Y., Azuma A., Kudoh S., Keicho N., Molecular cloning of two novel mucin-like  
24 genes in the disease-susceptibility locus for diffuse panbronchiolitis, *Hum Genet* 129 (2011) 117-128.
- 25 [11] Dekker J., Rossen J.W., Buller H.A., Einerhand A.W., The MUC family: an obituary, *Trends  
26 Biochem Sci* 27 (2002) 126-131.
- 27 [12] Moniaux N., Escande F., Porchet N., Aubert J.P., Batra S.K., Structural organization and  
28 classification of the human mucin genes, *Front Biosci* 6 (2001) D1192-1206.
- 29 [13] Duraisamy S., Ramasamy S., Kharbada S., Kufe D., Distinct evolution of the human  
30 carcinoma-associated transmembrane mucins, MUC1, MUC4 AND MUC16, *Gene* 373 (2006) 28-34.
- 31 [14] Duraisamy S., Kufe T., Ramasamy S., Kufe D., Evolution of the human MUC1 oncoprotein, *Int  
32 J Oncol* 31 (2007) 671-677.
- 33 [15] Fowler J., Vinall L., Swallow D., Polymorphism of the human muc genes, *Front Biosci* 6 (2001)  
34 D1207-1215.
- 35 [16] Groux-Degroote S., Harduin-Lepers A., Delannoy P., Biosynthesis of mucin O-glycan chains in  
36 normal and pathological states, in: Van Seuning I. (Ed.), *The Epithelial Mucins: Structure/Function.  
37 Roles in Cancer and Inflammatory Diseases*, Research Signpost, Kerala, India, 2008, pp. 39-54.
- 38 [17] Silverman H.S., Sutton-Smith M., McDermott K., Heal P., Leir S.H., Morris H.R., Hollingsworth  
39 M.A., Dell A., Harris A., The contribution of tandem repeat number to the O-glycosylation of mucins,  
40 *Glycobiology* 13 (2003) 265-277.
- 41 [18] Robbe-Masselot C., Michalski J.C., Capon C., Tumour-associated antigens of mucin O-  
42 glycans. Clinical relevance of glycobiology in cancer and inflammatory diseases of the epithelium, in:  
43 Van Seuning I. (Ed.), *The Epithelial Mucins: Structure/Function. Roles in Cancer and Inflammatory  
44 Diseases*, Research Signpost, Kerala, India, 2008, pp. 55-74.
- 45 [19] Brockhausen I., Pathways of O-glycan biosynthesis in cancer cells, *Biochim Biophys Acta*  
46 1473 (1999) 67-95.
- 47 [20] Brockhausen I., Sulphotransferases acting on mucin-type oligosaccharides, *Biochem Soc  
48 Trans* 31 (2003) 318-325.
- 49 [21] Rahn J.J., Chow J.W., Horne G.J., Mah B.K., Emerman J.T., Hoffman P., Hugh J.C., MUC1  
50 mediates transendothelial migration in vitro by ligating endothelial cell ICAM-1, *Clin Exp Metastasis* 22  
51 (2005) 475-483.
- 52 [22] Senapati S., Das S., Batra S.K., Mucin-interacting proteins: from function to therapeutics,  
53 *Trends Biochem Sci* 35 (2010) 236-245.
- 54 [23] Senapati S., Chaturvedi P., Chaney W.G., Chakraborty S., Gnanapragassam V.S., Sasson  
55 A.R., Batra S.K., Novel INTeraction of MUC4 and galectin: potential pathobiological implications for  
56 metastasis in lethal pancreatic cancer, *Clin Cancer Res* 17 (2011) 267-274.

- [24] Zhao Q., Guo X., Nash G.B., Stone P.C., Hilkens J., Rhodes J.M., Yu L.G., Circulating galectin-3 promotes metastasis by modifying MUC1 localization on cancer cell surface, *Cancer Res* 69 (2009) 6799-6806.
- [25] Zhao Q., Barclay M., Hilkens J., Guo X., Barrow H., Rhodes J.M., Yu L.G., Interaction between circulating galectin-3 and cancer-associated MUC1 enhances tumour cell homotypic aggregation and prevents anoikis, *Mol Cancer* 9 (2010) 154.
- [26] Merlin J., Stechly L., de Beauce S., Monte D., Leteurtre E., van Seuningen I., Huet G., Pigny P., Galectin-3 regulates MUC1 and EGFR cellular distribution and EGFR downstream pathways in pancreatic cancer cells, *Oncogene* 30 (2011) 2514-2525.
- [27] Carraway K.L., Ramsauer V.P., Haq B., Carothers Carraway C.A., Cell signaling through membrane mucins, *Bioessays* 25 (2003) 66-71.
- [28] Jonckheere N., Skrypek N., Merlin J., Dessein A.F., Dumont P., Leteurtre E., Harris A., Desseyn J.L., Susini C., Frenois F., Van Seuningen I., The Mucin MUC4 and Its Membrane Partner ErbB2 Regulate Biological Properties of Human CAPAN-2 Pancreatic Cancer Cells via Different Signalling Pathways, *PLoS One* 7 (2012) e32232.
- [29] Luu Y., Junker W., Rachagani S., Das S., Batra S.K., Henrikson R.L., Shekels L.L., Ho S.B., Human intestinal MUC17 mucin augments intestinal cell restitution and enhances healing of experimental colitis, *Int J Biochem Cell Biol* 42 (2010) 996-1006.
- [30] Ho S.B., Luu Y., Shekels L.L., Batra S.K., Kandarian B., Evans D.B., Zaworski P.G., Wolfe C.L., Henrikson R.L., Activity of recombinant cysteine-rich domain proteins derived from the membrane-bound MUC17/Muc3 family mucins, *Biochim Biophys Acta* 1800 (2010) 629-638.
- [31] Kelley L.A., Sternberg M.J., Protein structure prediction on the Web: a case study using the Phyre server, *Nat Protoc* 4 (2009) 363-371.
- [32] Parry S., Silverman H.S., McDermott K., Willis A., Hollingsworth M.A., Harris A., Identification of MUC1 proteolytic cleavage sites in vivo, *Biochem Biophys Res Commun* 283 (2001) 715-720.
- [33] Palmari-Pallag T., Khodabukus N., Kinarsky L., Leir S.H., Sherman S., Hollingsworth M.A., Harris A., The role of the SEA (sea urchin sperm protein, enterokinase and agrin) module in cleavage of membrane-tethered mucins, *FEBS J* 272 (2005) 2901-2911.
- [34] Bork P., Patthy L., The SEA module: a new extracellular domain associated with O-glycosylation, *Protein Sci* 4 (1995) 1421-1425.
- [35] Macao B., Johansson D.G., Hansson G.C., Hard T., Autoproteolysis coupled to protein folding in the SEA domain of the membrane-bound MUC1 mucin, *Nat Struct Mol Biol* 13 (2006) 71-76.
- [36] Maeda T., Inoue M., Koshiba S., Yabuki T., Aoki M., Nunokawa E., Seki E., Matsuda T., Motoda Y., Kobayashi A., Hiroyasu F., Shirouzu M., Terada T., Hayami N., Ishizuka Y., Shinya N., Tatsuguchi A., Yoshida M., Hirota H., Matsuo Y., Tani K., Arakawa T., Carninci P., Kawai J., Hayashizaki Y., Kigawa T., Yokoyama S., Solution structure of the SEA domain from the murine homologue of ovarian cancer antigen CA125 (MUC16), *J Biol Chem* 279 (2004) 13174-13182.
- [37] Levitin F., Stern O., Weiss M., Gil-Henn C., Ziv R., Prokocimer Z., Smorodinsky N.I., Rubinstein D.B., Wreschner D.H., The MUC1 SEA module is a self-cleaving domain, *J Biol Chem* 280 (2005) 33374-33386.
- [38] Senapati S., Gnanapragassam V.S., Moniaux N., Momi N., Batra S.K., Role of MUC4-NIDO domain in the MUC4-mediated metastasis of pancreatic cancer cells, *Oncogene* 31 (2012) 3346-3356.
- [39] Piscaglia F., Dudas J., Knittel T., Di Rocco P., Kobold D., Saile B., Zocco M.A., Timpl R., Ramadori G., Expression of ECM proteins fibulin-1 and -2 in acute and chronic liver disease and in cultured rat liver cells, *Cell Tissue Res* 337 (2009) 449-462.
- [40] Xiang W., Ke Z., Zhang Y., Cheng G.H., Irwan I.D., Sulochana K.N., Potturi P., Wang Z., Yang H., Wang J., Zhuo L., Kini R.M., Ge R., Isthmin is a novel secreted angiogenesis inhibitor that inhibits tumour growth in mice, *J Cell Mol Med* 15 (2011) 359-374.
- [41] Lang T., Hansson G.C., Samuelsson T., Gel-forming mucins appeared early in metazoan evolution, *Proc Natl Acad Sci U S A* 104 (2007) 16209-16214.
- [42] Desseyn J.L., Gouyer V., Tetaert D., Architecture of the gel-forming mucins, in: Van Seuningen I. (Ed.), *The Epithelial Mucins: Structure/Function. Roles in Cancer and Inflammatory Diseases*, Research Signpost, Kerala, India, 2008, pp. 1-16.
- [43] Desseyn J.L., Clavereau I., Laine A., Cloning, chromosomal localization and characterization of the murine mucin gene orthologous to human MUC4, *Eur J Biochem* 269 (2002) 3150-3159.
- [44] Hassan M.I., Saxena A., Ahmad F., Structure and function of von Willebrand factor, *Blood Coagul Fibrinolysis* 23 (2012) 11-22.
- [45] Hatstrup C.L., Gendler S.J., Structure and function of the cell surface (tethered) mucins, *Annu Rev Physiol* 70 (2008) 431-457.
- [46] Carson D.D., The cytoplasmic tail of MUC1: a very busy place, *Sci Signal* 1 (2008) pe35.



- [47] Kufe D.W., Functional targeting of the MUC1 oncogene in human cancers, *Cancer Biol Ther* 8 (2009) 1197-1203.
- [48] Raina D., Ahmad R., Kumar S., Ren J., Yoshida K., Kharbanda S., Kufe D., MUC1 oncoprotein blocks nuclear targeting of c-Abl in the apoptotic response to DNA damage, *Embo J* 25 (2006) 3774-3783.
- [49] Pandey P., Kharbanda S., Kufe D., Association of the DF3/MUC1 breast cancer antigen with Grb2 and the Sos/Ras exchange protein, *Cancer Res* 55 (1995) 4000-4003.
- [50] Ahmad R., Raina D., Trivedi V., Ren J., Rajabi H., Kharbanda S., Kufe D., MUC1 oncoprotein activates the I $\kappa$ B kinase beta complex and constitutive NF- $\kappa$ B signalling, *Nat Cell Biol* 9 (2007) 1419-1427.
- [51] Ahmad R., Raina D., Joshi M.D., Kawano T., Ren J., Kharbanda S., Kufe D., MUC1-C oncoprotein functions as a direct activator of the nuclear factor- $\kappa$ B p65 transcription factor, *Cancer Res* 69 (2009) 7013-7021.
- [52] Ren J., Bharti A., Raina D., Chen W., Ahmad R., Kufe D., MUC1 oncoprotein is targeted to mitochondria by heregulin-induced activation of c-Src and the molecular chaperone HSP90, *Oncogene* 25 (2006) 20-31.
- [53] Wen Y., Caffrey T.C., Wheelock M.J., Johnson K.R., Hollingsworth M.A., Nuclear association of the cytoplasmic tail of MUC1 and beta-catenin, *J Biol Chem* 278 (2003) 38029-38039.
- [54] Ren J., Raina D., Chen W., Li G., Huang L., Kufe D., MUC1 oncoprotein functions in activation of fibroblast growth factor receptor signaling, *Mol Cancer Res* 4 (2006) 873-883.
- [55] Singh P.K., Wen Y., Swanson B.J., Shanmugam K., Kazlauskas A., Cerny R.L., Gendler S.J., Hollingsworth M.A., Platelet-derived growth factor receptor beta-mediated phosphorylation of MUC1 enhances invasiveness in pancreatic adenocarcinoma cells, *Cancer Res* 67 (2007) 5201-5210.
- [56] Leng Y., Cao C., Ren J., Huang L., Chen D., Ito M., Kufe D., Nuclear import of the MUC1-C oncoprotein is mediated by nucleoporin Nup62, *J Biol Chem* 282 (2007) 19321-19330.
- [57] Raina D., Ahmad R., Rajabi H., Panchamoorthy G., Kharbanda S., Kufe D., Targeting cysteine-mediated dimerization of the MUC1-C oncoprotein in human cancer cells, *Int J Oncol* 40 (2012) 1643-1649.
- [58] Wei X., Xu H., Kufe D., MUC1 oncoprotein stabilizes and activates estrogen receptor alpha, *Mol Cell* 21 (2006) 295-305.
- [59] Songyang Z., Shoelson S.E., McGlade J., Olivier P., Pawson T., Bustelo X.R., Barbacid M., Sabe H., Hanafusa H., Yi T., et al., Specific motifs recognized by the SH2 domains of Csk, 3BP2, fps/fes, GRB-2, HCP, SHC, Syk, and Vav, *Mol Cell Biol* 14 (1994) 2777-2785.
- [60] Maher D.M., Gupta B.K., Nagata S., Jaggi M., Chauhan S.C., Mucin 13: structure, function, and potential roles in cancer pathogenesis, *Mol Cancer Res* 9 (2011) 531-537.
- [61] Blalock T.D., Spurr-Michaud S.J., Tisdale A.S., Heimer S.R., Gilmore M.S., Ramesh V., Gipson I.K., Functions of MUC16 in corneal epithelial cells, *Invest Ophthalmol Vis Sci* 48 (2007) 4509-4518.
- [62] Lakshmanan I., Ponnusamy M.P., Das S., Chakraborty S., Haridas D., Mukhopadhyay P., Lele S.M., Batra S.K., MUC16 induced rapid G2/M transition via interactions with JAK2 for increased proliferation and anti-apoptosis in breast cancer cells, *Oncogene* 31 (2012) 805-817.
- [63] Malmberg E.K., Pelaseyed T., Petersson A.C., Seidler U.E., De Jonge H., Riordan J.R., Hansson G.C., The C-terminus of the transmembrane mucin MUC17 binds to the scaffold protein PDZK1 that stably localizes it to the enterocyte apical membrane in the small intestine, *Biochem J* 410 (2008) 283-289.
- [64] Higuchi T., Orita T., Katsuya K., Yamasaki Y., Akiyama K., Li H., Yamamoto T., Saito Y., Nakamura M., MUC20 suppresses the hepatocyte growth factor-induced Grb2-Ras pathway by binding to a multifunctional docking site of met, *Mol Cell Biol* 24 (2004) 7456-7468.
- [65] Baruch A., Hartmann M., Zrihan-Licht S., Greenstein S., Burstein M., Keydar I., Weiss M., Smorodinsky N., Wreschner D.H., Preferential expression of novel MUC1 tumor antigen isoforms in human epithelial tumors and their tumor-potentiating function, *Int J Cancer* 71 (1997) 741-749.
- [66] Levitin F., Baruch A., Weiss M., Stiegman K., Hartmann M.L., Yoeli-Lerner M., Ziv R., Zrihan-Licht S., Shina S., Gat A., Lifschitz B., Simha M., Stadler Y., Cholostoy A., Gil B., Greaves D., Keydar I., Zaretsky J., Smorodinsky N., Wreschner D.H., A novel protein derived from the MUC1 gene by alternative splicing and frameshifting, *J Biol Chem* 280 (2005) 10655-10663.
- [67] Ilkovitch D., Handel-Fernandez M.E., Herbert L.M., Lopez D.M., Antitumor effects of Mucin 1/sec involves the modulation of urokinase-type plasminogen activator and signal transducer and activator of transcription 1 expression in tumor cells, *Cancer Res* 68 (2008) 2427-2435.
- [68] Ilkovitch D., Lopez D.M., Urokinase-mediated recruitment of myeloid-derived suppressor cells and their suppressive mechanisms are blocked by MUC1/sec, *Blood* 113 (2009) 4729-4739.

- 1 [69] Moniaux N., Escande F., Batra S.K., Porchet N., Laine A., Aubert J.P., Alternative splicing  
2 generates a family of putative secreted and membrane-associated MUC4 mucins, *Eur J Biochem* 267  
3 (2000) 4536-4544.
- 4 [70] Escande F., Lemaitre L., Moniaux N., Batra S.K., Aubert J.P., Buisine M.P., Genomic  
5 organization of MUC4 mucin gene. Towards the characterization of splice variants, *Eur J Biochem* 269  
6 (2002) 3637-3644.
- 7 [71] Crawley S.C., Gum J.R., Jr., Hicks J.W., Pratt W.S., Aubert J.P., Swallow D.M., Kim Y.S.,  
8 Genomic organization and structure of the 3' region of human MUC3: alternative splicing predicts  
9 membrane-bound and soluble forms of the mucin, *Biochem Biophys Res Commun* 263 (1999) 728-  
10 736.
- 11 [72] Williams S.J., Munster D.J., Quin R.J., Gotley D.C., McGuckin M.A., The MUC3 gene encodes  
12 a transmembrane mucin and is alternatively spliced, *Biochem Biophys Res Commun* 261 (1999) 83-  
13 89.
- 14 [73] Moniaux N., Junker W.M., Singh A.P., Jones A.M., Batra S.K., Characterization of human  
15 mucin MUC17. Complete coding sequence and organization, *J Biol Chem* 281 (2006) 23676-23685.
- 16 [74] Kufe D.W., MUC1-C oncoprotein as a target in breast cancer: activation of signaling pathways  
17 and therapeutic approaches, *Oncogene* doi: 10.1038/onc.2012.158. (2012).
- 18 [75] Bitler B.G., Menzi I., Huerta C.L., Sands B., Knowlton W., Chang A., Schroeder J.A.,  
19 Intracellular MUC1 peptides inhibit cancer progression, *Clin Cancer Res* 15 (2009) 100-109.
- 20 [76] Klinge C.M., Radde B.N., Imbert-Fernandez Y., Teng Y., Ivanova M.M., Abner S.M., Martin  
21 A.L., Targeting the intracellular MUC1 C-terminal domain inhibits proliferation and estrogen receptor  
22 transcriptional activity in lung adenocarcinoma cells, *Mol Cancer Ther* 10 (2011) 2062-2071.
- 23 [77] Raina D., Ahmad R., Joshi M.D., Yin L., Wu Z., Kawano T., Vasir B., Avigan D., Kharbanda S.,  
24 Kufe D., Direct targeting of the mucin 1 oncoprotein blocks survival and tumorigenicity of human  
25 breast carcinoma cells, *Cancer Res* 69 (2009) 5133-5141.
- 26 [78] Tang C.K., Katsara M., Apostolopoulos V., Strategies used for MUC1 immunotherapy: human  
27 clinical studies, *Expert Rev Vaccines* 7 (2008) 963-975.
- 28 [79] Tang C.K., Apostolopoulos V., Strategies used for MUC1 immunotherapy: preclinical studies,  
29 *Expert Rev Vaccines* 7 (2008) 951-962.
- 30 [80] Lakshminarayanan V., Thompson P., Wolfert M.A., Buskas T., Bradley J.M., Pathangey L.B.,  
31 Madsen C.S., Cohen P.A., Gendler S.J., Boons G.J., Immune recognition of tumor-associated mucin  
32 MUC1 is achieved by a fully synthetic aberrantly glycosylated MUC1 tripartite vaccine, *Proc Natl Acad  
33 Sci U S A* 109 (2011) 261-266.
- 34 [81] Mukohara T., Mechanisms of resistance to anti-human epidermal growth factor receptor 2  
35 agents in breast cancer, *Cancer Sci* 102 (2011) 1-8.
- 36 [82] Nagy P., Friedlander E., Tanner M., Kapanen A.I., Carraway K.L., Isola J., Jovin T.M.,  
37 Decreased accessibility and lack of activation of ErbB2 in JIMT-1, a herceptin-resistant, MUC4-  
38 expressing breast cancer cell line, *Cancer Res* 65 (2005) 473-482.
- 39 [83] Skrypek N., Duchêne B., Hebbar M., Leteurte E., van Seuninghen I., Jonckheere N., The  
40 MUC4 mucin mediates gemcitabine resistance of human pancreatic cancer cells via the Concentrative  
41 Nucleoside Transporter family, *Oncogene* doi: 10.1038/onc.2012.179 (2012).
- 42 [84] Petersson J., Schreiber O., Hansson G.C., Gendler S.J., Velcich A., Lundberg J.O., Roos S.,  
43 Holm L., Phillipson M., Importance and regulation of the colonic mucus barrier in a mouse model of  
44 colitis, *Am J Physiol Gastrointest Liver Physiol* 300 (2011) G327-333.
- 45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65



## Figure legends

1  
2  
3  
4  
5 **Figure 1: Membrane-bound mucin prototype.** (A) Membrane-bound mucins are modular  
6  
7 proteins sharing conserved domains such as epidermal growth factor-like (EGF), Sea urchin  
8  
9 sperm protein Enterokinase and Agrin (SEA) or Pro/Thr/Ser (PTS) domains or MUC4-  
10  
11 specific domains (AMOP, Nido and vWF-D). (B) Alternative splicing events have been  
12  
13 reported and lead to new secreted or membrane-bound apomucin polypeptides possibly  
14  
15 lacking functional domains and presumably showing altered cellular/biological functions.  
16  
17  
18  
19  
20  
21

22 **Figure 2: Evolution of the membrane-bound mucins.** Phylogenetic trees of membrane-  
23  
24 bound mucins were built after aligning of amino acid sequences of various domains  
25  
26 conserved in mucin families. Three independent trees were deduced clustering (A) MUC1,  
27  
28 MUC3, MUC12, MUC13, MUC17 evolving from SEA-containing protein HSGP2. MUC3<sub>EGF</sub>  
29  
30 domains display the highest identity with EGF. MUC1 cytoplasmic tail (CT) peptide  
31  
32 sequence is related to MUC5B tandem repeat (TR) (B) MUC4 arises from two ancestor  
33  
34 proteins common to Nidogen (Nido and EGF-like domains) and Susd2 (AMOP and vWF-D  
35  
36 domains) (C) MUC16 arises from multiplication of SEA domain related to Agrin proteins.  
37  
38  
39  
40  
41  
42  
43

44 **Figure 3: EGF-like and SEA domains of membrane-bound mucins.** (A) Alignment of  
45  
46 EGF domains of membrane-bound mucins and EGF. Peptidic sequences of the domains were  
47  
48 identified by Protein Knowledgebase (UniprotKB, <http://www.uniprot.org>) (B) Phylogenetic  
49  
50 tree of identity of EGF domains. (C) Alignment of SEA domains of membrane-bound mucins.  
51  
52 Peptidic sequences of the domains were identified by Protein Knowledgebase. \* MUC16  
53  
54 contains up to 56 SEA domains. For clarity purpose, we decided to use the SEA1 domain as a  
55  
56 template for alignment analysis. (D) Phylogenetic tree of identity of SEA domains.  
57  
58  
59  
60  
61  
62  
63  
64  
65

1  
2 **Figure 4: Three dimensional structure of EGF and SEA domains of membrane-bound**  
3 **mucins.** (A) Ribbon diagram showing the superimposition of the 3D structure of the  
4 EGF1 and EGF2 domains of MUC4 and the human EGF. (The PyMOL Molecular Graphics  
5 System, Version 1.5.0.1 Schrödinger, LLC). (B) Ribbon diagram showing the  
6 superimposition of the 3D structure of MUC1<sub>SEA</sub> with the 3D predicted structure of  
7 MUC3A<sub>SEA</sub>, MUC12<sub>SEA</sub> and MUC17<sub>SEA</sub> (The PyMOL Molecular Graphics System, Version  
8 1.5.0.1 Schrödinger, LLC).  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21

22 **Table 1. RMSD values of mucin EGF domains superimposition on alpha carbons.** The  
23 3D structure predictions of the mucins EGF domains was carried out with the Phyre 2 server  
24 (Protein Homology/analogy Recognition Engine V 2.0) by using the deduced amino-acids  
25 sequences of each domain. The predicted 3D structure of each EGF domain was compared  
26 with the resolved 3D structure of the human EGF (PDB code : 1j19) and with the other EGF  
27 domains predicted structures using the molecular visualization system ,PyMOL (The PyMOL  
28 Molecular Graphics System, Version 1.5.0.1 Schrödinger, LLC). To determine whether the  
29 EGF domains were structurally homologous, PyMOL was used to measure the root-mean-  
30 square-deviation (\*RMSD): the average distance between the alpha carbons atoms (the  
31 backbone atoms) of superimposed proteins. n.s:no 3D structure prediction. \*RMSD : Root  
32 Mean Square Deviation is the square root of the mean of the square of the distances between  
33 the matched atoms.  $RMSD = \sqrt{(\sum(d_{ii})^2)/N}$ .  $d_{ii}$  is the distance between the  $i$ th atom  
34 of structure 1 and the  $i$ th atom of structure 2 and N is the number of atoms matched in each  
35 structure  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

**Table 2: RMSD values of mucin SEA domains superimposition on alpha carbons.** The

3D structure predictions of the mucin SEA domains was carried out with the Phyre 2 server (Protein Homology/analogy Recognition Engine V 2.0) by using the amino-acids sequence of each domain. The predicted 3D structure of each SEA domain was compared with the resolved 3D structure of the human SEA domain of MUC1 (PDB code : 2ACM) and with the others SEA domains predicted structures using the molecular visualization system ,PyMOL (The PyMOL Molecular Graphics System, Version 1.5.0.1 Schrödinger, LLC). To determine whether the SEA domains were structurally homologous, PyMOL was used to measure the root-mean-square-deviation (\*RMSD).

**Table 3: Sequence and functions of the cytoplasmic tails of membrane-bound mucins.**

Table 1

Table 1. RMSD values of mucin EGF domains superimposition on alpha carbons









<b>EGF</b>	<b>Human EGF</b>	<b>MUC4_EGF1</b>	<b>MUC4_EGF2</b>	<b>MUC4_EGF3</b>	<b>MUC3A_EG F</b>	<b>MUC17_EGF</b>	<b>MUC12_EGF</b>	<b>MUC13_EGF 1</b>	<b>MUC13_EGF 2</b>	<b>MUC13_EGF 3</b>	<b>MUC16_EG F</b>
<b>MUC4_EGF1</b>	1.272 (25 atoms)		3.708 (21 atoms)	n.s	0.502 (15 atoms)	0.876 (13 atoms)	1.947 (14 atoms)	3.698 (12 atoms)	n.s	5.958 (30 atoms)	1.128 (27 atoms)
<b>MUC4_EGF2</b>	3.929 (24 atoms)	3.708 (21 atoms)		n.s	4.664 (24 atoms)	5.175 (35 atoms)	8.013 (16 atoms)	5.163 (26 atoms)	n.s	3.188 (14 atoms)	3.082 (30 atoms)
<b>MUC4_EGF3</b>	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	ns
<b>MUC3A_EG F</b>	0.502 (15 atoms)	2.177 (17 atoms)	4.664 (24 atoms)	n.s		4.776 (19 atoms)	7.122 (23 atoms)	0.148 (7 atoms)	n.s	8.884 (19 atoms)	2.559 (16 atoms)
<b>MUC17_EGF</b>	2.235 (22 atoms)	0.876 (13 atoms)	5.175 (35 atoms)	n.s	4.776 (19 atoms)		3.195 (23 atoms)	0.226 (4 atoms)	n.s	3.490 (18 atoms)	1.747 (21 atoms)
<b>MUC12_EGF</b>	2.093 (24 atoms)	1.947 (14 atoms)	8.013 (16 atoms)	n.s	7.122 (23 atoms)	3.195 (23 atoms)		1.455 (12 atoms)	n.s	1.812 (8 atoms)	2.643 (25 atoms)
<b>MUC13_EGF 1</b>	0.793 (22 atoms)	3.698 (12 atoms)	5.163 (26 atoms)	n.s	0.148 (7 atoms)	0.226 (4 atoms)	1.455 (12 atoms)		n.s	3.272 (27 atoms)	2.087 (24 atoms)
<b>MUC13_EGF 2</b>	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	ns
<b>MUC13_EGF 3</b>	1.178 (7 atoms)	5.958 (30 atoms)	3.188 (14 atoms)	n.s	8.884 (19 atoms)	3.490 (18 atoms)	1.812 (8 atoms)	3.272 (27 atoms)	n.s		2.867 (28 atoms)
<b>MUC16_EGF</b>	2.803 (41 atoms)	1.128 (27 atoms)	3.082 (30 atoms)	n.s	2.559 (16 atoms)	1.747 (21 atoms)	2.643 (25 atoms)	2.087 (24 atoms)	n.s	2.867 (28 atoms)	

Table 2. RMSD values of mucin SEA domains superimposition on alpha carbons






<b>SEA</b>	<b>MUC1_SEA (2ACM)</b>	<b>MUC3A_SEA</b>	<b>MUC12_SEA</b>	<b>MUC13_SEA</b>	<b>MUC16-SEA</b>	<b>MUC17_SEA</b>
<b>MUC3A_SEA</b>	3.812 (61 atoms)		4.615 (87 atomes)	11.740 (20 atoms)	1.759 (66 atoms)	0.004 (48 atoms)
<b>MUC12_SEA</b>	5.363 (66 atoms)	4.615 (87 atomes)		10.773 (45 atoms)	8.417 (82 atoms)	0.004 (56 atoms)
<b>MUC13_SEA</b>	11.740 (20 atoms)	13.996 (75 atoms)	10.773 (45 atoms)		3.361 (40 atoms)	6.417 (30 atoms)
<b>MUC16_SEA</b>	0.912 (44 atoms)	1.759 (66 atoms)	8.417 (82 atoms)	3.361 (40 atoms)		7.400 (48 atoms)
<b>MUC17_SEA</b>	1.204 (52 atoms)	0.004 (48 atoms)	0.004 (56 atoms)	6.417 (30 atoms)	7.400 (48 atoms)	

Table 3

apomucin	Cytoplasmic tail sequence	Protein binding-domains and phosphorylated tyrosines
<b>MUC1</b>	CQCRRKNYGQLDIFPARDTYHPMSEYPT YHTHGRYVPPSSTDRSPYEKVSAGNGGS SLSYTNPAVAATSANL	7 Tyr SH2 domain c-Src, Lyn, Lck, ZAP70, c-Abl, Grb2, IKK, HSP70, HSP90, b- catenin, GSK3, FGFR3
<b>MUC3</b>	AVRSGWWGGQRRGRSWDQDRKWFET WDEEVVGTFSNWFEDDGTDKDTNFH VALENDTTMKVHIKRPEMTSSSV	SH2 domain
<b>MUC4</b>	LRFWGCSGARFSYFLNSAEALP	1 Tyr
<b>MUC12</b>	SQRKRHREQYDVPQEWKKEGTPGIFQK TAIWEDQNLRESRFLENA YNNFRPTLE TVDSGTELHIQRPEMVA STV	2 Tyr
<b>MUC13</b>	VTARSNNKTKHIEEENLIDEDFQNLKLR TGFTNLGAEGSVFPKVRITASRDSQMQN PYSRHSSMPRPDY	2 Tyr PKC
<b>MUC15</b>	GKRKTDSFSHRRLYDDRNEPVLRLDNAP EPYDV SFGNSSY YNPTL NDSAMPESEEN ARDGIPMDDIPPLRTSV	4 Tyr
<b>MUC16</b>	VTTRRRKKEGEY NVQQQCPGY YQSHLD LEDLQ	3 Tyr moesin
<b>MUC17</b>	SIYSNFQPSLRHIDPETKIRIQRPQVM TTS F	1 Tyr PDZK1
<b>MUC20</b>	ENGGFLLRLSVASPEDLTDPRVAERLM QQLHRELHAHAPHFQVSLLRVRRG	c-Met
<b>MUC21</b>	RNSLSLRNTFNTAVYHPHGLNHGLGPGP GGNHGAPHRPRWSPNWFWR RPVSSIAM EMSGRNSGP	1 Tyr
<b>MUC22</b>	SFCLRNLFPLRYCGIYYPHGSHSLGLD LNLGLGSGTFHSLGNALVHGGELEMGH GGTHGFGYGVGHGLSHIHGDGYGVNHG GHYGHGGGH	6 Tyr

**Table 3:** Sequence and functions of the cytoplasmic tails of membrane-bound mucins.  
Tyrosines that can be phosphorylated are bold.

**Figure 1**  
[Click here to download high resolution image](#)

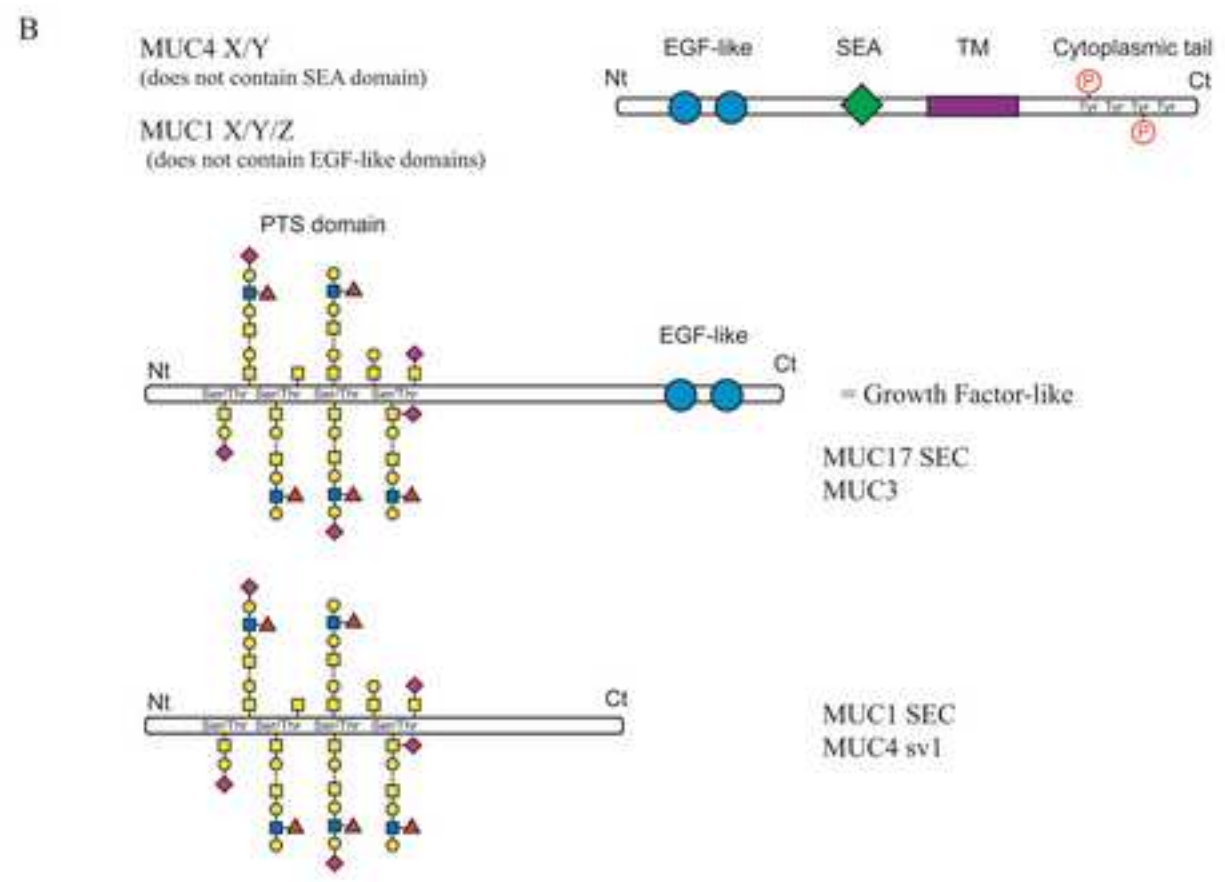
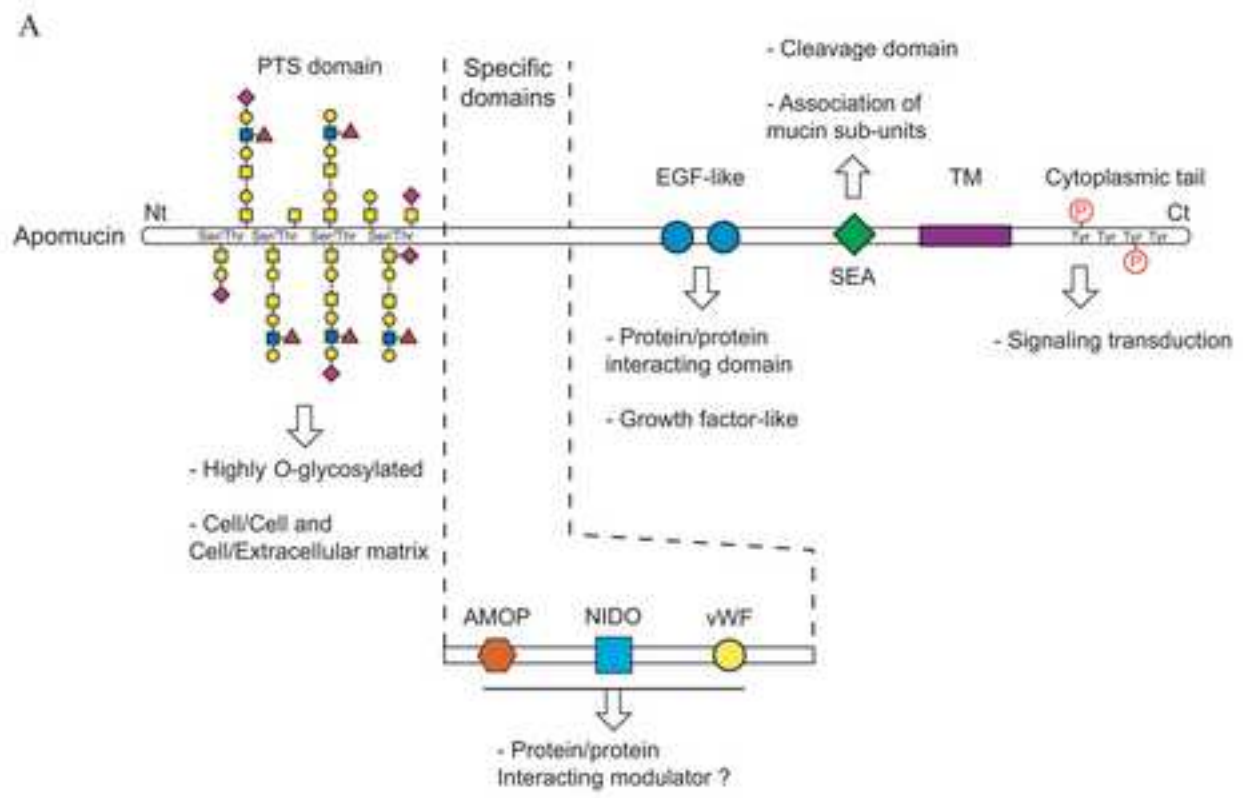
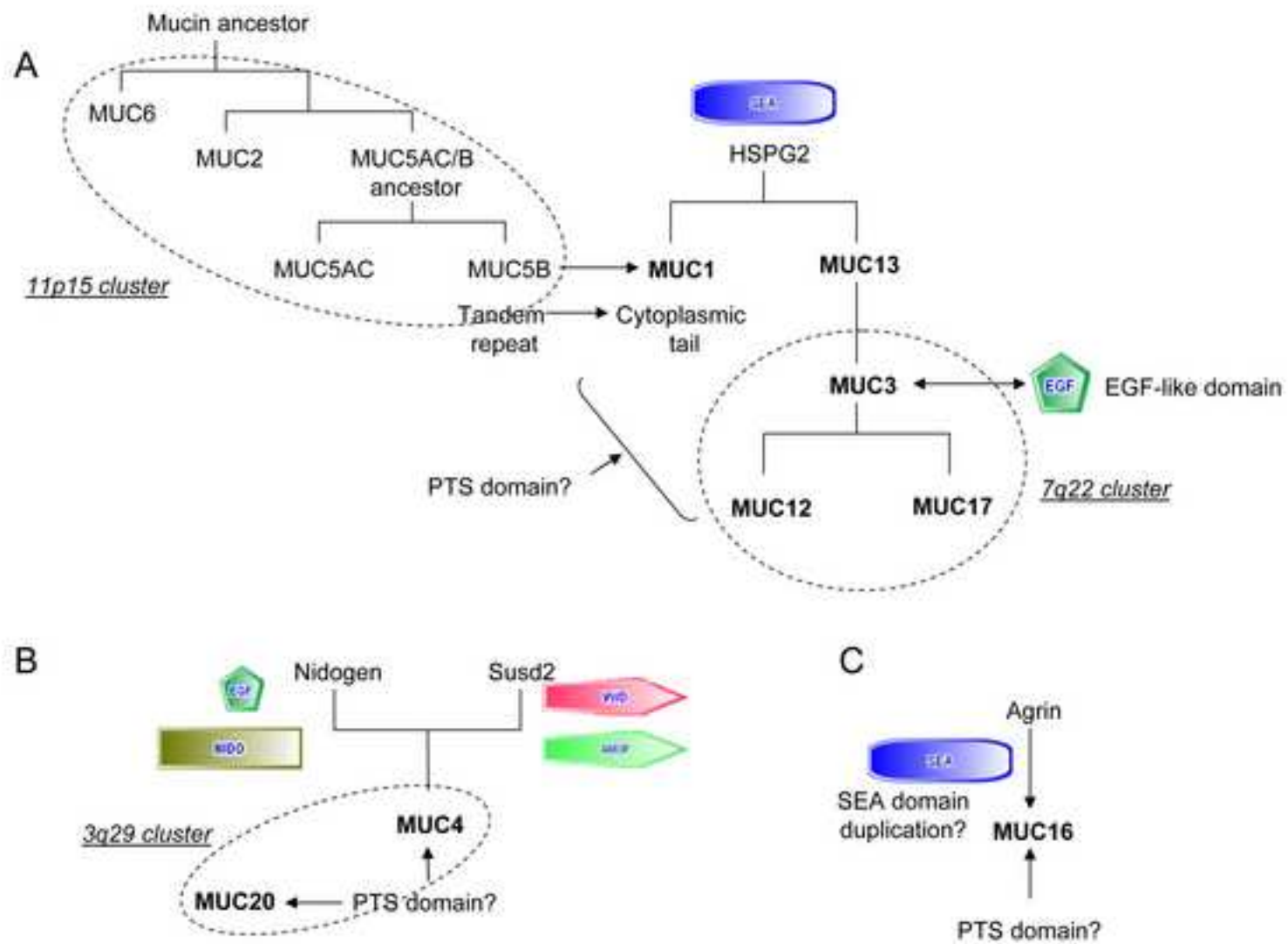




Figure 2  
[Click here to download high resolution image](#)



**Figure 3**  
[Click here to download high resolution image](#)

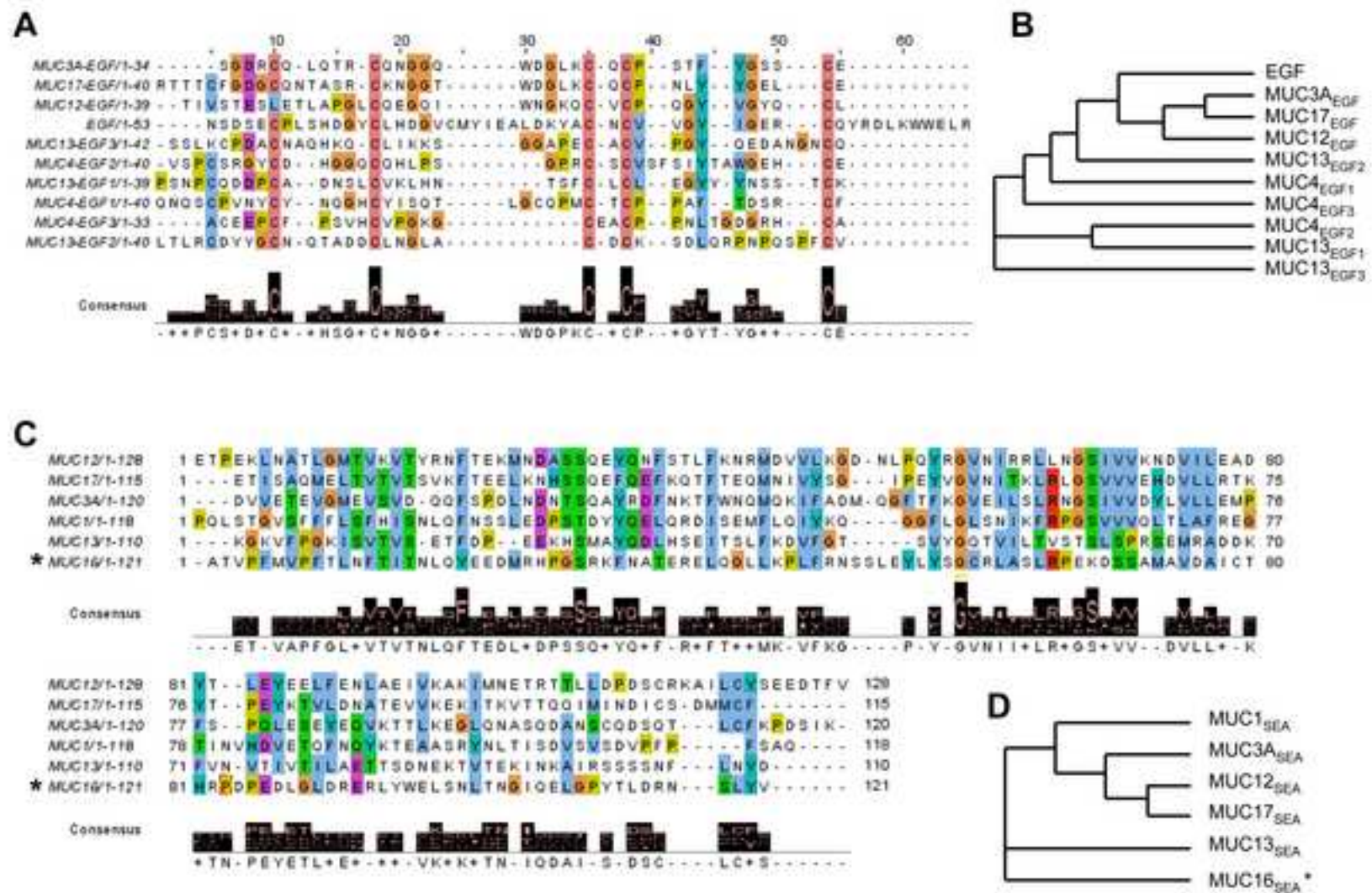



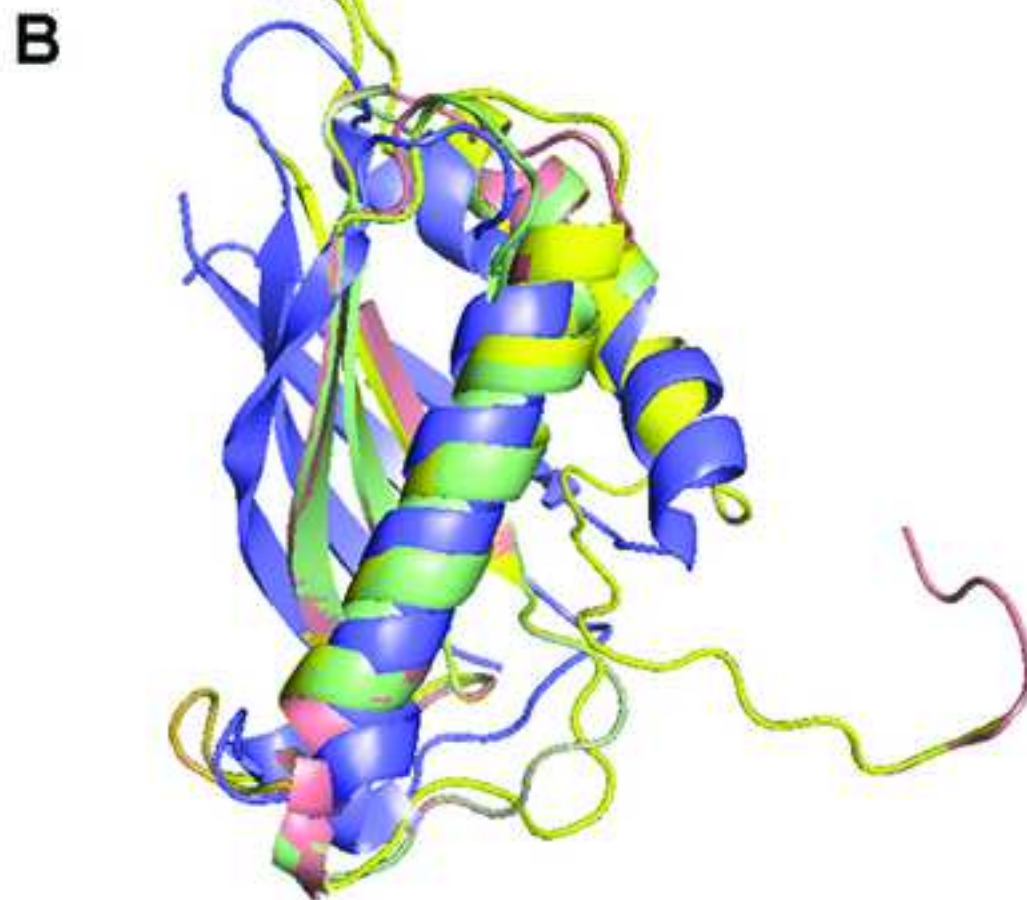






Figure 4  
[Click here to download high resolution image](#)



	Predicted 3D structure of MUC4 <sub>EGF1</sub>
	Predicted 3D structure of MUC4 <sub>EGF2</sub>
	3D structure of human EGF



	Predicted 3D structure of MUC3A <sub>SEA</sub>
	Predicted 3D structure of MUC12 <sub>SEA</sub>
	Predicted 3D structure of MUC17 <sub>SEA</sub>
	3D structure of MUC1 <sub>SEA</sub>