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Mucin expression, epigenetic regulation and patient survival: A toolkit of prognostic biomarkers in epithelial cancers

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Conflict of interest

Authors declare no conflict of interest

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Abstract

Twenty mucin genes have been identified and classified in two groups (**encoding** secreted and membrane-bound **proteins**). Secreted mucins participate in mucus formation by **assembling** a 3-dimensional network *via* oligomerization, whereas membrane-bound mucins are anchored to the outer membrane **mediating** extracellular interactions and cell signaling. Both groups have been associated with carcinogenesis progression in epithelial cancers, and are therefore considered as potential therapeutic targets. In the present review, we discuss the link between **mucin expression patterns** and patient survival **and** propose mucins as prognosis biomarkers of epithelial cancers (esophagus, gastric, pancreatic, colorectal, lung, breast or ovarian cancers). We also investigate the relationship between mucin expression and overall survival in **the** TCGA dataset. **In particular, epigenetic** mechanisms regulating mucin gene expression, such **as aberrant DNA methylation and histone modification**, are **interesting** as they are also associated with diagnosis or prognosis significance. **Indeed**, mucin hypomethylation has been shown to be associated with carcinogenesis progression and was linked to prognosis in colon cancer or PDAC patients. Finally we describe the relationship between mucin expression and non-coding RNAs that **also** may serve as biomarkers. **Altogether the concomitant knowledge of specific mucin-pattern expression and epigenetic regulation could be translated as biomarkers with a better specificity/sensitivity performance in several epithelial cancers.**

1. Introduction

Mucus is a secreted biological fluid that has rheological properties (elasticity and viscosity) enabling protection of the underlying epithelia (respiratory, digestive, reproductive) against various kinds of aggression [1]. Nicolas Theodore de Saussure introduced the term mucins to designate the main component of the secreted mucus in 1835 [2]. Mucins are biochemically defined as a peptide core (called apomucin) on which a **huge** carbohydrate-load is linked (**thousands of kDa with mainly O-glycan chains**). Initially **restricted to** secreted glycoproteins, the molecular biology era led to the discovery of a second group of mucins tethered to the cell membrane called membrane-bound **or transmembrane** mucins.

In total, 20 mucin genes are listed by the Human Genome Organization/Gene Nomenclature Committee (HUGO/GNC) (Table 1). Genes encoding secreted mucins include *MUC2*, *MUC5AC*, *MUC5B*, *MUC6* (clustered at 11p15.5 chromosome **arm**) and *MUC19* [3], **which mainly** participate in mucus formation by **assembling** a 3-dimensional network *via* oligomerization domains [4, 5]. *MUC7* and *MUC9/OVGP1* are smaller secreted mucins that do not oligomerize [6].

Membrane-bound mucins include *MUC1*, *MUC3A/B*, *MUC4*, *MUC12*, *MUC13*, *MUC15*, *MUC16*, *MUC17*, *MUC20*, *MUC21* and *MUC22* [7-9]. Membrane-bound mucins are anchored to the membrane, **with** a similar structural organization, **which** usually **includes** a Pro/Thr/Ser rich O-glycosylated domain (PTS), Epidermal Growth Factor (EGF)-like and Sea urchin sperm protein Enterokinase and Agrin (SEA) domains [10].

Other **membrane-bound** mucins, **such as** *Muc10*, *MUC14/EMCN*, *MUC18/MCAM* are **shorter** glycoproteins that are less characterized and are considered as atypical or mucin-like [11, 12].

Both secreted and membrane-bound mucins have been linked with carcinogenesis progression in a wide array of cancers and are therefore considered as potential therapeutic targets [13-18]. It has been projected that **out** of the 1.4 million tumors diagnosed each year

in the US, about two thirds (900,000) harbor overexpression of MUC1 [19] suggesting that membrane-bound mucins, such as MUC1, could be highly attractive therapeutic targets or potential diagnosis/prognostic biomarkers. Among membrane-bound mucins, MUC1 and MUC4 have been **extensively** described [14-16, 20, 21]. More than 200 clinical trials are currently ongoing either targeting MUC1 dimerization (**e.g. with GO203**, NCT02204085) or using MUC1 peptide-based vaccine in solid tumors (**e.g.** NCT03300817).

The mucin gene expression pattern in respiratory, digestive, reproductive tracts is complex and tightly regulated. Each mucin has an expression pattern that is organ and cell type specific [3, 22]. Moreover, **these** patterns are heavily deregulated during the histological changes accompanying carcinogenesis [22, 23]. After thirty years of research **showing** that mucins can be bona fide actors or passengers of carcinogenesis, we exhaustively describe in this review the link between mucin expression and cancer patient survival, in order to propose mucins as prognosis biomarkers in cancers.

2. Mucins and overall survival in The Cancer Genome Atlas (TCGA)

We have recently investigated the relationship between *MUC4* expression and patient overall survival (OS) in the TCGA dataset [24]. *MUC4* expression is associated with poorer survival in several cancers in TCGA database including pancreatic, bladder, gastric, colon and skin cancers as well as, lung adenocarcinoma, lung squamous adenocarcinoma. We have also evaluated a *MUC4/MUC16/MUC20* combination signature that is associated with poorer outcome notably in pancreatic, colon and gastric cancers. In the present manuscript, we extended this analysis to the OS for each mucin gene in every TCGA dataset using the SurvExpress online tool (bioinformatica.mty.itesm.mx/SurvExpress [25]). **We have analyzed mucin gene expression with Hazard Ratios (HR) representing the probability of event occurrences (patient death), where a HR of two means that the patient from *MUC* high expression group has twice the probability of dying than a patient from low expression group.**

We have extracted HRs for membrane-bound mucins (*MUC1*, *MUC4*, *MUC12*, *MUC13*, *MUC15*, *MUC16*, *MUC17*, *MUC20* and *MUC21*) (Figure 1A), secreted mucins (*MUC2*, *MUC5B*, *MUC6* and *MUC7*) (Figure 1B), and atypical mucins (*MUC14/EMCN* and *MUC18/MCAM*) (Figure 1C) (Table 1). No information is available regarding *MUC3*, *MUC5AC*, *MUC8*, *MUC19* and *MUC22* expression.

MUC1 expression is correlated with an increased HR in 15 different TCGA datasets. Acute Myeloid Leukemia (AML), which is, unlike most of the TCGA documented cancers, not an solid tumor, is the only dataset in which *MUC1* is associated with a better prognosis (HR=0.6, p=0.0228). In adrenocortical carcinoma, cholangiocarcinoma, pancreatic adenocarcinoma and thymoma melanoma high *MUC1* expression presented the most important hazard ratio (HR>4). It was recently shown that *MUC1* expression is negatively associated with OS in glioblastoma (GBM) TCGA dataset [26]. Among the membrane-bound mucins, *MUC20* expression is also associated with a poorer prognosis in 13 different TCGA datasets. For uveal melanoma and thyroid carcinoma, *MUC20* expression presented the most important hazard ratio (HR=5.41 and HR=8.65, respectively). High expression of *MUC15* is correlated with worst outcome in 8 different TCGA datasets, notably in thymoma melanoma (HR=15.02) and uterine corpus endometrial carcinoma (HR=3.99). High expression of *MUC16* is associated with several digestive cancers (stomach, pancreatic, colorectal; HR=1.72-2.53) and head and neck cancer (HR=1.39). Other membrane-bound mucins were more restricted to different types of cancers. *MUC12* expression is notably associated with poorer prognosis of adrenocortical carcinoma (HR=4.56) and glioblastoma (HR=2.95). High *MUC13* expression leads to a poorer outcome in adrenocortical carcinoma (HR=4.23) and to a lower extent in esophageal carcinoma, lung adenocarcinoma and stomach adenocarcinoma (HR=1.48-1.79). *MUC17* expression is associated with poorer survival in pancreatic, gastric adenocarcinoma and uterine corpus endometrial carcinoma. *MUC21* expression is highly associated with poorer survival in prostate adenocarcinoma (HR=9.27) and to a lower extent with cervical squamous cell carcinoma and endocervical adenocarcinoma, head and neck

squamous cell carcinoma, pancreatic adenocarcinoma and skin cutaneous melanoma (HR= 1.74-2.11).

We observed fewer correlations between secreted mucin expression and OS in TCGA datasets. High *MUC5B* expression is the most correlated secreted mucin, as it is associated with poorer outcome in 6 different TCGA datasets, including adrenocortical carcinoma (HR= 4.66) and pancreatic adenocarcinoma (HR=4.38). On the other hand, *MUC5B* is associated with a better prognosis in uterine corpus endometrial carcinoma (HR=0.43). *MUC2* expression is associated with poorer prognosis in bladder urothelial carcinoma (HR=2.51), lung adenocarcinoma (HR=1.58), prostate adenocarcinoma (HR=6.94) and uterine corpus endometrial carcinoma (HR=2.41). High *MUC6* expression leads to a poorer outcome in uterine corpus endometrial carcinoma (HR=9.66) and to a lower extent in liver hepatocellular carcinoma and lung squamous cell carcinoma (HR= 1.9-1.94).

Atypical mucins *MUC14/EMCN* and *MUC18/MCAM* were associated with patient prognosis in a wide variety of cancers. *MUC14* expression is highly correlated with poor prognosis of thymoma melanoma (HR= 21.39) and at a lower extent with OS in uveal melanoma, prostate adenocarcinoma and adrenocortical carcinoma datasets. *MUC18* expression is associated with poorer prognosis in glioma, breast invasive carcinoma, cervical squamous cell carcinoma and endocervical adenocarcinoma and uterine corpus endometrial carcinoma.

The limit of this kind of analysis from TCGA datasets is that expression data were only extracted from RNAseq analyses in order to go through standardized software pipelines. It does not take into account the level of protein expression. On the other hand, RNAseq has the advantage of not being biased by the insufficient performance of antibodies in IHC experiments in regard to their eventual sub-optimal specificity/sensitivity balance. The analyses were generated by the SurvExpress tool that uses an algorithm that splits the cohort into two groups with a maximal statistical significant difference (high and low HR). This is realized by performing log-rank tests with the split point varying from same-size

groups to a point where the tested p-value between groups is minimal [25]. We used this web tool on TCGA datasets to evaluate mucin expression as prognosis biomarkers. Moreover, discrepancies can be observed between alteration at the RNA and proteins levels showing the importance of the methodology of biomarker detection. Validation of these putative biomarkers on independent cohorts remains mandatory before any routine testing of specific mucin signatures and specific cancers can be envisioned.

Additionally to gene expression, TCGA database was also analyzed regarding somatic gene mutation and relationship with OS. *MUC4* and *MUC17* germline mutations were associated with both better and worse survival rates depending on the organs [27]. *MUC4*, *MUC5B* and *MUC16* mutations are associated with better patient survival in kidney cancer, eso-gastric and gastric cancer, respectively [28-30]. However, caution should be kept regarding the detection of mutation spot within mucins; notably in the PTS-rich region that is repeated up to several hundred times; and that is therefore difficult to sequence and prone to errors.

3. Mucins as prognostic markers

3.1. Esophageal cancer

In normal esophagus, MUC1 and MUC4 are expressed in the epithelium and MUC5B is expressed in submucosal glands [31-33]. There are two major subtypes of esophageal cancer: esophageal squamous cell carcinoma (ESCC) and adenocarcinoma (EAC) that are epidemiologically and biologically distinct [34]. EAC occurrence has risen in industrialized countries during the last 40 years [31]. Niv and colleagues have performed a meta-analysis showing that mucin expression is significantly higher in esophageal lesions than in normal esophageal mucosa [35]. MUC2, MUC3, MUC5AC and MUC6 expression is higher in EAC than in high grade dysplasia lesions (HGD), and higher in HGD than in LGD low grade dysplasia (LGD) tissue. On the contrary, MUC1 and MUC4 higher expression was observed in less advanced lesions (EAC) than in HGD and LGD. However, no correlation was found

between their expression level and histopathological variables, recurrence or survival. Up-regulation of membrane-bound MUC16 is thought to be a late event of EAC progression [36]. Kaplan-Meier analysis showed a trend of worse survival for patients with moderate/diffuse MUC16 expression. However, because of a low number of samples, this trend was not significant. Interestingly, MUC2 expression is associated with intestinal metaplasia and Kaplan Meier curve shows that intestinal-type EAC has a worse outcome than the gastric-type. Multivariate analysis showed that the MUC2 expression is associated with poor prognosis in EAC patients independently of node stage and other prognostic factors [37].

In esophagus squamous cell carcinoma, which is more common in low and middle income countries, the 5-year survival rate is associated with MUC1 mRNA and protein levels, as well as mucin-associated carbohydrate epitopes (TF, sialyl-Tn) [38, 39]. Interestingly, two other membrane-bound mucins, MUC13 and MUC20, were not associated with OS when considered alone, but the combination of MUC13/MUC20 expression (MUC13^{high}/MUC20^{low} vs MUC13^{low}/MUC20^{high}) was an independent prognostic factor for ESCC patients in the postoperative specimens of patients who received neoadjuvant chemotherapy [40].

3.2. Gastric cancer

Gastric adenocarcinoma (GAC) is the most common histological type (95%) of malignancies originating in the stomach [41]. Two classifications are used by pathologists: (1) The WHO divides GAC as tubular, papillary, mucinous, mixed and poorly cohesive carcinomas whereas (2) the Lauren classification divides GACs into intestinal, diffuse and mixed types. MUC2 and MUC5B expression seems to be a favorable prognostic factor in all type GC patients [42-44]. Interestingly, high MUC5B expression was deleterious in the intestinal subtype and not in the diffuse subtype [43], whereas low MUC5AC expression is an independent poor prognostic factor in diffuse-type gastric cancer [45]. Other conflicting results showed that MUC5AC

immuno-staining is associated with a poorer survival [46]. A meta-analysis performed on more than 2000 patients from 11 studies confirmed that decreased MUC5AC expression is significantly correlated with poor OS of gastric cancer patients [47].

Several membrane-bound mucins are proposed as prognosis biomarkers for gastric cancer [23]. A meta-analysis conducted by Wang and collaborators showed that the MUC1 expression, detected by immunohistochemistry (IHC), is significantly higher in intestinal-type than that in diffuse-type carcinomas and that MUC1 is highly correlated with poor survival in GAC [48]. Another meta-analysis also concluded that MUC1 predicted an unfavorable prognosis in gastric cancers [49]. MUC13 was shown to be an independent prognostic factor of early-staged gastric cancer. Redistribution of MUC13 pattern from membrane localization to cytoplasmic staining is associated with poor outcome intestinal type GC [50]. In addition, MUC16 is a prognostic marker independent of other prognostic indicators. Detection of MUC16 in patient's serum is associated with occurrence of metastases, lymph node metastasis, and shorter survival time [36].

Moreover, gastric signet ring cell (SRC) adenocarcinoma is an emerging histological gastric cancer sub-type with cells containing abundant intracytoplasmic mucin (mainly MUC2 and MUC5AC) and is associated with a poor prognosis [51, 52].

3.3. Pancreatic cancer

Pancreatic Ductal Adenocarcinoma (PDAC) presents one of the most dramatic mortalities with an extremely short survival curve at five years (<6 months) and lacks both early diagnostic and prognostic markers [53, 54]. The most frequent form of PDAC (85%) develops from pancreatic intraepithelial neoplasia (PanIN) precursor lesions. PDACs harbor altered expression of both membrane mucins (MUC1, MUC4, MUC13, MUC16 and MUC20) and secreted mucins (MUC2, MUC5AC and MUC6). Notably, the roles and regulation of MUC1 and MUC4 membrane-bound mucins have been extensively studied in pancreatic cancer

[55, 56]. PDAC showing aggressive biological behavior commonly express MUC1 [57]. However, MUC1 is already expressed in centro-acinar cells and intercalated duct of the normal pancreas reducing its potential as a prognostic marker. Several reports have suggested MUC4 as a prognostic marker. Survival of patients with high MUC4 expression (31% at 1 year after diagnosis) is significantly worse than patients with low MUC4 expression (64% at 1 year) [58]. Increased MUC16 expression is a late event during the pancreatic carcinogenesis. Moderate diffuse staining of MUC16 is strongly associated with poor patient outcome [36]. Multivariate analysis of endoscopic ultrasound-guided fine-needle aspiration (EUS-FNA) biopsy samples showed that cytoplasmic expression of MUC16 is an independent predictor of a poor prognosis regardless of other clinico-pathologic factors [59]. Interestingly, analysis of long term survivor showed that the enrichment of neoantigens derived from MUC16 is associated with decreased relapse and prolonged survival, highlighting its potential as biomarker for immunogenic tumors eligible for immunotherapies [60]. By analyzing RNA-seq data of TCGA public database, we observed that *MUC4* and the combination *MUC4/MUC16/MUC20* expression are a poor prognostic signature in PDAC [24]. Furthermore, hierarchical clustering analysis of PDAC TCGA dataset led us to propose a membrane-bound mucin signature (*MUC1/4/16/17/20/21*) as a prognostic biomarker for OS. RNA-seq [61] TCGA analysis also revealed that high *MUC20* mRNA expression is correlated with poor survival in PDAC [62]. MUC13 expression and localization is altered in PanIN lesion and is associated with progression towards carcinoma. IHC analysis on 204 patients showed that nuclear MUC13 expression is positively correlated with metastasis to lymph node and poorer patient survival [63].

Besides PanIN, Intraductal Papillary Mucinous Neoplasms (IPMNs) are another type of pancreatic cancer precursors, but **their occurrence** is indicative of a better clinical **outcome** course. MUC1 and MUC2 may have a diagnostic and prognostic value as markers for this indolent kind of tumor compared to PDAC arising from PanIN [23, 64]. Most IPMN express MUC2. MUC1 expressing IPMN are invasive carcinoma with a short survival rate and bad

prognosis. MUC5AC expressing tumors are slow-growing adenomas with a good prognosis [65, 66]. MUC13 expression is more common in IPMN that have an associated invasive carcinoma with a poorer prognosis [67].

3.4. Colorectal cancer

Colorectal cancer is a predominant cancer accounting for 10% of cancer-related mortality [68]. In the classic colorectal cancer formation model, the majority of cancers arise from a polyp evolving into an early adenoma, progresses to an advanced adenoma and finally becoming a colorectal cancer [69].

MUC2 is the major gel-forming secretory mucin expressed by intestinal goblet cells. Its protective role was clearly demonstrated as Muc2 knock-out mice harbored colon adenocarcinomas [70]. The potential of MUC2 as a prognostic marker is controversial as several meta-analyses did not show a significant association with patient survival [44, 71, 72], whereas a recent meta-analyses showed that a low MUC2 level in CRC tissues is a poor prognostic factor independently of disease stage [73]. Another recent analysis showed that low MUC2 immunostaining is associated with longer disease-free survival, but is not associated with OS [74]. Overexpression of other secreted mucins, such as MUC5AC and MUC6 (occurring in less than 10% of tumors), significantly predicts favorable outcome [75]. Imai and collaborators investigated the relationship between mucin expression and the outcome in different histopathological CRC subtypes. MUC2 and MUC5AC expression were associated with a better prognosis in well-to-moderately differentiated and poorly differentiated adenocarcinoma [76].

High expression of membrane-bound mucins (MUC1, MUC4, MUC13 or MUC20) was shown to be associated with poorer prognosis for CRC patients [24, 72, 77-79]. Independently of MUC4 expression, two single nucleotide polymorphisms (rs3107764 and rs842225) in the *MUC4* gene are associated with overall and event-free survival of CRC patients [80].

Conflicting results were published regarding MUC16: Streppel and collaborators observed that MUC16 expression (no staining vs positive staining) was significantly correlated with a better CRC patient outcome [36] whereas Bjorkman et al. performed a protein profiling (relative quantification) showing that patients with high MUC16 had a poor prognosis in CRC and notably in non-mucinous adenocarcinoma-type tumors [81].

Mucinous adenocarcinomas (MAC), up to 25% of CRC and defined by the extracellular deposition of mucus (>50%) by the tumor cells, are associated with MUC2 and MUC5AC overexpression and with a worse survival [82, 83].

MUC3 is an independent factor for poor prognosis in appendiceal carcinoma that shares pathological features with colorectal cancer [84].

3.5. Lung cancer

Lung cancer is the leading cause of cancer-related deaths worldwide with non-small-cell lung cancer (NSCLC) representing approximately 85% of these cancers [85]. MUC1 predicts an unfavorable prognosis in NSCLC [49, 86, 87]. MUC1 cell topology in cancer cells (depolarization from apical staining to basolateral/cytoplasmic staining), or protein detection in blood samples are also associated with worse OS [23, 88-90]. MUC4 is overexpressed in more than 90% of lung cancer [91]. Several studies suggested that MUC4 expression is correlated with the histological type of lung carcinoma [92], **although conflicting evidence were reported** regarding its impact on patient survival. MUC4 is usually described as a mucin promoting carcinogenesis in most cancers, but surprisingly, high MUC4 score is associated with a better OS **in NSCLC** [93]. On the contrary, Yonezawa's laboratory observed that small-sized lung adenocarcinoma patients with high MUC4 expression have a lower survival rate than those harboring low MUC4 expression [94]. Survival analysis showed that high expression of MUC5AC is associated with a poorer outcome in NSCLC. Moreover, patients combining high MUC5AC and sialyl-LewisX glycan epitopes had a worst survival in the

adenocarcinoma subtype [95]. High expression of MUC5B was also associated with a poorer outcome in NSCLC and high MUC5B expression combined with TTF-1 negative group chains was significantly associated with poorer survival [96].

Aggressive mucinous adenocarcinomas harbor a **high expression of the MUC2, MUC5AC, and MUC6 secreted mucins** [92].

3.6. Breast cancer

Breast cancer is the most common malignancy in women. Worldwide, 1.7 million patients are diagnosed per year [97, 98]. The vast majority of normal breast tissues express MUC1 and MUC4, whereas a small portion of ductal epithelium (up to 10%) express MUC5AC and MUC6 [99]. The majority of breast carcinomas express the membrane-bound mucins MUC1, MUC3 and MUC4 and to a lower extent the secreted mucins MUC2, MUC5AC and MUC6 [100]. MUC1 expression, occurring in more than 90% of tumors, was not associated with OS in 1400 consecutive breast cancer patients. However, subcellular MUC1 localization could be used as a prognostic feature as topological cytoplasmic and membrane expression patterns (compared to the normal apical localization) have poorer overall and disease free survival (DFS) [100]. Analysis of prognoScan database showed that the *MUC1* mRNA level is associated with decreased overall, disease specific and relapse-free survival in breast cancer [101]. Breast cancers overexpressing MUC2 have a shorter survival than those who do not express MUC2. On the other hand MUC6 expression, occurring in 20% of BC patients, improves patient survival [99].

Invasive micropapillary carcinoma of the breast (IMPC) is a low frequency tumor overexpressing MUC4. MUC4⁺ IMPC patients tend to have a lower DFS than MUC4 negative patients [102].

3.7. Ovarian cancer

Epithelial ovarian cancer (EOC) is the most common cause of gynecological cancer related death [103]. CA125 is a peptide epitope of the mucin MUC16 and has been used in the clinic as a prognostic marker since the early 1980s [104, 105]. Serum levels of CA125 are routinely monitored in patients with ovarian cancer for EOC diagnosis, prognosis (distinguishing between benign and malignant) and for monitoring response to treatment (indicating recurrence) [105, 106]. **In contrast, the circulating MUC1 prognostic marker CA15-3 predicts a negative outcome in treatment-resistant ovarian cancers [106].** Membrane-bound MUC4 is also upregulated in early stages of EOC, but is decreased in advanced-stage ovarian tumors. However, no significant correlation was demonstrated and only a trend toward improved survival was observed in MUC4 expressing patients [107, 108]. MUC20 expression is associated with poor prognosis only in advanced stage EOC (III and IV) [109].

MUC2 expression was shown to increase concomitantly during the progression from mucinous adenoma to mucinous adenocarcinoma (10-15% of ovarian cancer). Low MUC2 expression in ovarian mucinous adenocarcinoma is correlated with a better long-term survival [110].

3.8. Other cancers

As mucins are commonly deregulated in the majority of epithelial cancers, numerous studies were conducted in biliary tract, liver, prostate, head and neck or renal cancers. **In particularly, membrane-bound mucins MUC1 and MUC4 were the most studied.**

Large meta-analyses (>2000 patients) showed an association between increased membrane-bound mucins MUC1 and MUC4 expression and poorer OS. Additionally to other cancers described in the previous sections, significant associations were also observed in biliary tract carcinomas for MUC4 and in cholangiocarcinomas for MUC1 [49, 56, 111].

Yonezawa *et al.* have investigated the role of mucins as **prognostic** factors in different histological **forms** of liver cancers. In mass forming type intrahepatic cholangiocarcinoma (ICC-MF, the most common ICC) and Extrahepatic Bile Duct Carcinoma (EHBDC), MUC1, MUC4 and MUC16 are independent factors for poor prognosis [112-116]. The non-sialylated forms of MUC2 are related with a better outcome in ICC and EHBDC [111, 117]. Regarding mucins that are usually less studied, overexpression of MUC13 was identified as an independent **prognostic** factor for overall and DFS in hepatocellular carcinoma (HCC) [118]. MUC15 is reduced in HCC; decreased MUC15 expression is correlated with shorter OS and poor clinical prognosis [119].

In prostate cancer, MUC1 is aberrantly expressed and is associated with higher Gleason score (GS) usually observed in more aggressive tumors [120-122]. MUC1 expression has been assessed by IHC, which showed that MUC1 protein staining correlates with a slightly worse OS and strongly correlates with DFS [121]. High MUC1 expression was shown to be an independent **prognostic** factor for prostate cancer death [123] and for prostate-specific antigen (PSA) failure (rise of PSA level occurring in patients without symptoms) [124]. Tang's laboratory also determined a nine-gene signature derived from a MUC1-regulated network (APC, CTNNB1, GALNT10, GRB2, LYN, SIGLEC1, SOS1, ZAP70 and FAM84B) that presents an independent risk factor for prostate cancer recurrence [125, 126]. MUC4 was shown to be downregulated in prostate cancer but its impact on patient survival remains to be determined [127].

Expression of MUC1, MUC2 and MUC4 is observed in gallbladder carcinoma, where MUC4 is an independent risk factor. The high MUC4/low MUC2 group showed a significantly worse outcome than the low MUC4 group or the high MUC4/ high MUC2 co-expression group [23, 128].

A meta-analysis was conducted to analyze the association between MUC gene expression and **clinico-pathological** features of head and neck cancers. MUC1 and MUC4 expression

was shown to be associated with worse prognosis. Subgroup analysis showed that this worse survival rate is mainly observed in Asian patients [129].

In the main subtype of kidney cancer, clear cell renal cancer (ccRC), patients with tumor containing >70% of MUC1 positive cells have a poorer survival prognosis. MUC1 membranous staining is associated with high-grade tumors that have a higher visceral metastatic potential and therefore with a poorer outcome [122, 130, 131]. MUC3 is also overexpressed in ccRC, but its expression was not correlated with a prognosis [132]. On the contrary, MUC13 expression is associated with worse OS. Two independent analyses showed that MUC13 can predict DFS and OS of ccRC patients [133, 134]. In addition, MUC5AC and MUC7 secreted mucins are independent prognostic factors for overall and DFS in ccRC [135, 136]. The atypical MUC18 mucin expression is an independent marker of good prognosis as a lower MUC18 expression in ccRCC predicted a shorter survival time [137].

MUC1, MUC2 MUC4, MUC5AC and MUC6 IHC staining is observed in urothelial bladder cancer (UBC) [138, 139]. Only MUC2 and MUC6 high expression are associated with low pathological stage of urothelial bladder cancer and better survival [139].

4. Mucin regulation as epigenetic biomarker?

The term “epigenetic” is defined as heritable changes in gene expression patterns that are driven by mechanisms other than alterations in the primary nucleotide sequence of the gene [140]. The strict interpretation of the prefix “*epi-*” suggests mechanisms directly altering chromatin, *i.e.* DNA methylation or histone post-translational modifications. Such epigenetic marks that constitute the epigenome are affixed during development in order to mold cell functions. The discovery of profound alterations of epigenetic landscapes at early stages during carcinogenesis led very quickly to the hypothesis that epigenetic changes could be

used as useful biomarkers. Indeed, according to the National Institutes of Health (NIH) Biomarker Definition Working Group a biomarker is defined as a biological characteristic that can be objectively measured and indicates pathogenic processes or pharmaceutical response to therapeutic strategies [141].

4.1. DNA methylation or histone modifications on mucin genes

Histone post-translational modifications, and especially global acetylation and methylation of Histones H3 and H4, analyzed using immunohistochemistry, have been proposed as an independent prognostic marker in prostate cancers [142]. Similarly, detection of circulating nucleosomes in cancer patient serum could be promising [143]. However, precise and targeted analysis of histone post-translational modifications does not seem to be adaptable to routine clinical use yet. On the contrary, DNA methylation is stable and does not require extreme precaution when it comes to preserve patient samples. DNA methylation status can be assessed through tumor biopsies or even noninvasive sampling, including blood (circulating tumor DNA), stool and urines [144, 145]. DNA methylation levels vary amongst tissues and amongst patients, especially with age. It is therefore difficult to use global hypomethylation observed in cancer cells as an ubiquitous marker, although it has been proposed for some cancers [145]. On the contrary, variations of methylated DNA levels at precise locations in the epigenome are easily and accurately measurable. They proved to be tumor-type specific and could be used as potential biomarkers to evaluate cancer risk, prognosis, diagnosis and response to treatment [146] in order to reach a higher level of precision medicine. As a matter of fact, among the multiple candidates that have been proposed over the past two decades, some were validated in the clinic and are commercially available for the diagnosis of colon, prostate and lung cancers [146, 147].

The location of the secreted mucin genes *MUC2*, *MUC5AC*, *MUC5B* and *MUC6* on chromosome region 11p15.5 is of particular interest when it comes to epigenetic

mechanisms of regulation [3, 148, 149]. Indeed, aberrant DNA methylation in this region is associated with severe developmental abnormalities [150, 151] and cancer [152]. Therefore, only shortly after the first study reporting specific DNA hypermethylation in colon cancer by the Baylin's team [153], DNA methylation was shown to be associated with *MUC2* silencing in non-mucinous colon cancer cells [154]. Thereafter, several studies of our laboratory and others were able to demonstrate aberrant DNA methylation at *MUC2* but also *MUC5B* and *MUC5AC* promoters in colon, gastric and pancreatic cancers [3, 155-159]. However, only few studied big enough patient cohorts to associate these profiles with diagnosis or prognosis significance. In 2014, Yokoyama et al developed methylation-specific electrophoresis (MSE) to evaluate *MUC2* methylation status in the pancreatic juice samples of 45 patients with various pancreatic lesions [160]. Their results showed that *MUC2*, in combination with *MUC1* and *MUC4* methylation, was useful for the differential diagnosis of human pancreatic neoplasms (with specificity and sensitivity of 87% and 80% for PDAC; 100% and 88% for intestinal-type IPMN; and 88% and 77% for gastric-type IPMN, respectively).

In 2015, we were **however** the first to publish the use of pyrosequencing to study the DNA methylation status of both *MUC2* and *MUC5AC* mucin genes in a cohort of colon cancer patients [161]. This quantitative and accurate method [146, 162], routinely used in the clinic, **allowed** us to anticipate the use of methylation profiles of mucin genes as biomarkers. Using two cohorts of CRC patients we showed, for the first time, that *MUC5AC* hypomethylation is an early event of carcinogenesis, and is associated with *BRAF* mutations, the CpG Island Methylator Phenotype and microsatellite instability (MSI) (characteristic of the serrated neoplasia pathway) in colon cancer patients [161, 163]. Since MSI-phenotyped patients present a better outcome, *MUC5AC* hypomethylation may be linked to better prognosis in these patients [161].

Because *MUC1* and *MUC4* gene promoters carry CpG islands, hot spots of aberrant DNA methylation in cancers, they are also epigenetically regulated [164] and of interest as hypermethylated biomarkers. Indeed, several studies have associated the expression of

these membrane mucins to the methylation status of their gene locus in digestive and breast cancers [164-169]. For instance, *MUC4* DNA-methylation status was associated with pancreatic cancer progression [170]. More interestingly, *MUC1* and/or *MUC4* aberrant methylation status was correlated, for the first time in 2016, with decreased OS in stage-matched PDAC tissues [164, 166].

No methylation studies were conducted yet for other mucin genes, although *MUC12* and *MUC17* mucin expression was correlated with their gene methylation profile using TCGA data analysis in colorectal cancers [171].

Hence, although numerous studies have now shown a clear correlation between mucin gene expression and epigenetic status, occasionally depending on tumor stage, the use of mucin gene epigenetic profile as a cancer biomarker is still at its dawn [149].

4.2. Mucins and long non coding RNA

In addition to genomic histone modifications and DNA methylation, MUC expression may be affected by non-coding RNAs (such as lncRNAs and miRNAs) that may also serve as biomarkers. Several work showed that mucin (*MUC1*, *MUC4*, *MUC6*, *MUC13*, *MUC16* and *MUC19*) expression is directly regulated by numerous miRNA in different cancers (extensively reviewed in table 2). Furthermore, several competing endogenous RNA (CeRNA) complexes were identified in which lncRNA act as a sponge for miRNA that bind on mucin 3'UTR (Table 2).

Among miRNA, several potential regulatory antisense transcripts were observed in close vicinity of *MUC* gene regions (Table 3). A first example studied is the antisense transcript *MUC5B-AS1*, which was shown to be highly co-expressed with *MUC5B*. This transcript increased *MUC5B* expression levels in lung adenocarcinoma, and is associated with poor outcomes in patients with lung adenocarcinoma [172]. In addition, the miRNA-6756 located

within the *MUC18* gene was proposed as a biomarker to differentiate between indolent and castration-resistant prostate cancer [173], [174]. One study showed that polymorphisms at miRNA binding sites of *MUC21* and *MUC17* may predict clinical outcome in colorectal cancer patients [175]. Also low miR-150 expression affecting *MUC4* expression is associated with higher mortality rates in pancreatic cancer [176]. The association with patient survival of antisense transcripts, lncRNAs close to the *MUC* genes, as well as *MUC*-regulating miRNAs [174] remains to be fully assessed.

5. Conclusions

According to the definition of a biomarker mucins are bona fide biomarkers for carcinoma because of their commonly **altered expression patterns** in epithelial carcinoma, and because mucins are biological molecules that promote cancer aggressiveness [3, 22, 23, 149]. The membrane-bound mucins subgroup is **notably** fit for routine analysis by immunohistochemistry, because of their cellular localization and strong expression. Actually, MUC16/CA125 is implemented in clinical practice for ovarian cancer. Circulating CA19-9 (sialyl-Lewis a, sLe^a) and CA15.3 (Sialyl Lewis x, sLe^x) glycan epitopes, harbored by MUC1 are widely-used as serum prognostic markers in pancreatic and breast cancer [177] and are also found in numerous gastrointestinal diseases [178]. This **review highlights** additional membrane-bound mucins that could be used as biomarkers for specific cancers.

Membrane-bound mucin overexpression is generally associated with a poorer survival. On the contrary, the association between secreted mucin expression and OS appears to be much more specific of the tissue origins of the cancer. For example, MUC2 is a favorable prognosis factor in gastric cancer [42], and is associated with worsen survival in breast and ovarian cancer [99, 110]. Overexpression of MUC5AC predicts better survival in IPMN and in colorectal cancer, where it is associated with promoter hypomethylation in the MSI subtype. However, MUC5AC expression is associated with poorer outcome in NSCLC and gastric

cancer [45, 65, 76, 95]. This impact on survival is generally related to the state of differentiation occurring during the carcinogenesis, since secreted mucins are considered as markers of terminal differentiation. Additionally to the specificity of the organ affected and the interpatient heterogeneity, it is important to take into account the intra-tumoral heterogeneity. Transcriptional and epigenetic regulation of the genome, including mucin genes, contributes to the tumor heterogeneity and associated resistance to therapy. The related cellular plasticity contributes to the tumor progression and thus alters the patient survival [179, 180]. Among mechanisms underlying this plasticity, microbiota and tumor-related dysbiosis emerge as mediators of the heterogeneity and plasticity [181]. Interestingly, membrane-bound mucins have been involved in barrier function with the micro-environment and interactions with mucosal pathogenic and commensal bacteria [17]. Mucin expression was shown to be regulated by surrounding bacteria in the intestine and in colorectal cancer and is able to participate to the induced-phenotypic heterogeneity [182, 183]. Development of an efficient biomarker or tailored panel of biomarkers will be a major challenge for the development of personalized treatment strategies.

Unsupervised hierarchical clustering analysis of TCGA datasets based on genetic and epigenetic alterations demonstrated the existence of four main breast cancer classes reinforcing the possibility to molecular classification based on the appropriate biomarker signatures [184]. As we previously suggested, the specific expression patterns of several mucins can be combined in order to improve the area under curve (AUC) score (showing the ability to distinguish normal and tumor tissues) and to obtain a better specificity/sensitivity performance as marker in several digestive cancers. [24]. The concept of combined mRNA gene expression is already proven effective for precision medicine, as illustrated by Oncotype DX (commercialized by Genomic Health) that is a 21-gene recurrence-score assay providing prognostic information in hormone-receptor-positive breast cancers. Using Oncotype DX score, clinicians may identify up to 85% of early breast cancer patients who can be spared adjuvant chemotherapy [185]. We recently highlighted that the combination of

MUC4, *MUC16* and *MUC20* mRNA score is associated with statistically significant reduced OS and increased hazard ratio in different digestive cancers [24]. In addition, we have in this review discussed several other mucins that may be considered for use as biomarkers and included in prognostic signatures.

6. References

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Figure legend

Figure 1 : Correlation of Mucin and patient survival in TCGA datasets. Forest plot showing the hazard ratio and 95% confidence intervals in high and low expression levels of membrane-bound (A), secreted (B) and atypical (C) mucin genes in every TCGA cohorts. Hazard ratio was calculated in population designated as high risk and low risk (higher value of mucin gene of interest for higher risk) based on individual mucin expression in TCGA datasets by SurvExpress optimized algorithm available in bioinformatica.mty.itesm.mx/SurvExpress. The datasets analysed were Adrenocortical carcinoma (ACC); Cholangiocarcinoma (CHOL); Bladder Urothelial Carcinoma (BLCA) ; Gliomas (GBMLGG) ; Breast invasive carcinoma (BRCA) ; Cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC) ; Colon and Rectum adenocarcinoma (COADREAD) ; Esophageal carcinoma (ESCA) ; Uveal Melanoma (UVM) ; Head and Neck squamous cell carcinoma (HNSC) ; Acute Myeloid Leukemia (LAML) ; Kidney PAN cancer (KIPAN) ; Liver hepatocellular carcinoma (LIHC) ; Lung adenocarcinoma (LUAD), Lung squamous cell carcinoma (LUSC) ; Ovarian serous cystadenocarcinoma (OV) ; Pancreatic adenocarcinoma (PAAD) ; Prostate adenocarcinoma (PRAD) ; Skin Cutaneous Melanoma (SKCM), Stomach adenocarcinoma (STAD) ; Testicular Germ Cell Tumors (TGCT) ; Thymoma (THYM) ; Thyroid carcinoma (THCA) ; Uterine Corpus Endometrial Carcinoma (UCEC). P-value is indicated on the graph (** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$).

Figure 2: Schematic representation of the link between mucin expression and patient survival in different epithelial cancers. Mucin expression associated with poorer overall survival (OS) is indicated in red font. Mucins associated with better OS are indicated in green font. Conflicting results are indicated in blue font. Results obtained from TCGA datasets are marked by an asterisk*. Esophageal carcinoma (ESCA), Esophageal Squamous Cell

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Carcinoma ESCC, gastric adenocarcinoma (GAC), Signet Ring Cell carcinoma (SRC), pancreatic ductal adenocarcinoma (PDAC), Intraductal Papillary Mucinous Neoplasm (IPMN), colorectal cancer (CRC), Breast Cancer (BC), Epithelial Ovarian Cancer (EOC), Urothelial Bladder Cancer (UBC), Clear Cell Renal Cancer (ccRC), Prostate Cancer (PC), Non Small Cell Lung Cancer (NSCLC), Cholangiocarcinoma (CHOL). MB-mucins designates the MUC1/4/16/17/20/21 membrane-bound mucin signature.

Table 1: List of membrane-bound, secreted and atypical mucin genes and association of mRNA expression with TCGA patients survival using SurvExpress optimized algorithm.

HGNC ID	Symbol	Approved name	Chromosome	Ensembl gene ID	Group	correlated with survival (p<0.05) in TCGA	
						good prognosis	bad prognosis
HGNC:7508	MUC1	mucin 1, cell surface associated	1q22	ENSG00000185499	membrane-bound	LAML	ACC, CHOL, GBM, BRCA, CESC, UVM, Kidney, LIHC, LUSC, OV, PAAD, SKCM, STAD, THYM, UCEC
HGNC:7513	MUC3A	mucin 3A, cell surface associated	7q22.1	ENSG00000169894	membrane-bound	nd	nd
HGNC:7514	MUC4	mucin 4, cell surface associated	3q29	ENSG00000145113	membrane-bound	none	BLCA, COADREAD, LUAD, LUSC, PAAD, SKCM, STAD,
HGNC:7519	MUC8	mucin 8	12q24.33		membrane-bound	nd	nd
HGNC:8524	MUC9/OVGP1	oviductal glycoprotein 1	1p13.2	ENSG00000085465	membrane-bound	nd	nd
HGNC:7510	MUC12	mucin 12, cell surface associated	7q22.1	ENSG00000205277	membrane-bound	none	ACC, GBM, LUAD, STAD
HGNC:7511	MUC13	mucin 13, cell surface	3q21.2	ENSG00000173702	membrane-	none	ACC, ESCA, LUAD,

		associated			bound		STAD
HGNC:14956	MUC15	mucin 15, cell surface associated	11p14.3	ENSG00000169550	membrane- bound	None	ACC, BRCA, CESC, HSNC, LUAD, STAD, THYM, UCEC
HGNC:15582	MUC16	mucin 16, cell surface associated	19p13.2	ENSG00000181143	membrane- bound	None	COADREAD, HNSC, STAD,
HGNC:16800	MUC17	mucin 17, cell surface associated	7q22.1	ENSG00000169876	membrane- bound	None	PAAD, STAD, UCEC
HGNC:23282	MUC20	mucin 20, cell surface associated	3q29	ENSG00000176945	membrane- bound	None	ACC, BLCA, GBM, CESC, COADREAD, UVM, HNSC, Kidney, LUAD, LUSC, PAAD, STAD, THCA
HGNC:21661	MUC21	mucin 21, cell surface associated	6p21.33	ENSG00000204544	membrane- bound	None	CESC, HNSC, PAAD, PRAD, SKCM
HGNC:39755	MUC22	mucin 22	6p21.33	ENSG00000261272	membrane- bound	Nd	nd
HGNC:7512	MUC2	mucin 2, oligomeric mucus/gel- forming	11p15.5	ENSG00000198788	secreted	None	BLCA, LUAD, PRAD, UCEC
HGNC:7515	MUC5AC	mucin 5AC, oligomeric mucus/gel-	11p15.5	ENSG00000215182	secreted	Nd	nd

		forming					
HGNC:7516	MUC5B	mucin 5B, oligomeric mucus/gel-forming	11p15.5	ENSG00000117983	secreted	UCEC	ACC, BRCA, ESCA, Kidney, LUAD, PAAD
HGNC:7517	MUC6	mucin 6, oligomeric mucus/gel-forming	11p15.5	ENSG00000184956	secreted	None	LIHC, LUSC, UCEC
HGNC:7518	MUC7	mucin 7, secreted	4q13.3	ENSG00000171195	secreted	None	none
HGNC:14362	MUC19	mucin 19, oligomeric	12q12	ENSG00000205592	secreted	Nd	nd
	MUC10	mucin 10, mouse			atypical	Nd	nd
HGNC:16041	MUC14/EMCN	endomucin	4q24	ENSG00000164035	atypical	None	ACC, BLCA, BRCA, CESC, COADREAD, ESCA, UVM, HNSC, Kidney, LIHC, LUAD, LUSC, OV, PAAD, PRAD, STAD, THYM
HGNC:6934	MUC18/MCAM	melanoma cell adhesion molecule	11q23,3	ENSG00000076706.17	atypical	None	BLCA, GBM, BRCA, CESC, COADREAD, Kidney, LIHC, LUAD, SKCM, STAD, UCEC

Table 2: Mucin and direct regulation by miRNA or ceRNA network in epithelial cancers

Mucin gene	miRNA or ceRNA network (miRNA/lncRNA)	Cancer	References
<i>MUC1</i>	miR-1182/circWHSC1	Ovarian cancer	[186]
	miR-1226-3p/miR-210HG	Breast cancer	[187]
	miR-1291	Esophageal cancer	[188]
	miR-136	Esophageal cancer	[189]
	miR-140-5p	Breast cancer	[190]
	miR-145/circWHSC1	Ovarian cancer	[186]
	miR-145	Ovarian cancer	[191]
	miR-194/LINC00909	Glioma	[192]
	miR-206	Gastric cancer	[193]
	miR-29a	Pancreatic cancer	[194]
	miR-326	Prostate cancer	[195]
	miR-330-5p	Pancreatic cancer	[194]
	miR485-5p	Breast cancer	[196]
	miR485-5p/CircHECTD1	Hepatocarcinoma	[197]
	miR-512-5p	Cervical cancer	[198]
<i>MUC4</i>	miR-150	Pancreatic and colorectal cancers	[176, 199, 200]
	miR-200c	Pancreatic cancer	[201]
	miR-211	Cervical cancer	[202]
	miR-219-1-3p	Pancreatic cancer	[203]
<i>MUC6</i>	miR-24/CASC2	Pancreatic cancer	[204]
<i>MUC13</i>	miR-132-3p	Gastric cancer	[205]
	miR-145	Pancreatic cancer	[206]
<i>MUC16</i>	miR-200c	Pancreatic cancer	[201]
<i>MUC19</i>	miR-593/hsamirc0007534	Breast cancer	[207]

Table 3 Mucin genes and overlapping/flanking ncRNA

Overlapping Mucin gene	ncRNA name (AntiSense, Overlapping Transcript)	Ensemble annotation	RefSeq (Genbank)
<i>MUC5B</i>	<i>MUC5B-AS1</i>	ENST00000532061.2	NR_157183
<i>MUC3A</i>	<i>MUC3A-AS</i>	ENST00000618276.1	XR_001745304.1 (RP11-395B7.2/AC254629.1)
<i>MUC12</i>	<i>MUC12-AS1</i>	ENST00000448513.1	NR_120519 (RP11-395B7.4/AC105446.1)
<i>MUC13</i>	<i>MUC13-OT</i>	ENST00000497378.1	
<i>MUC14/EMCN</i>	<i>EMCN-AS</i>	ENST00000652064.1	(AC097459.1)
	<i>EMCN-OT</i>	ENST00000510657.1	
<i>MUC18/MCAM</i>	miR-6756	ENST00000616240.1	
<i>MUC19</i>	<i>MUC19-AS</i>	ENST00000552757.1	(RP11-115F18.1/AC107023.1)
	<i>snRNA</i>	ENST00000384761.1	
Flanking a Mucin gene	ncRNA name	Ensemble annotation	RefSeq (Genbank)
<i>MUC6 - MUC2</i>	<i>LINC02688</i>	ENST00000534584.2	NR_160890
<i>MUC4 -</i>	<i>LINC01983</i>	ENST00000444346.1	NR_134939.1
<i>MUC9 -</i>	<i>LINC</i>	ENST00000564771.1	(AL390195.2)
<i>MUC14/EMCN -</i>	<i>LINC01216</i>	ENST00000515728.1	
	<i>LINC01217</i>	ENST00000514427.1/ ENST00000657311.1	
	<i>LINC01218</i>	ENST00000515150.1	
<i>- MUC20</i>	<i>MIR570HG</i> (miR host gene; MUC20-OT)	ENST00000630170.2	NR_134940.1

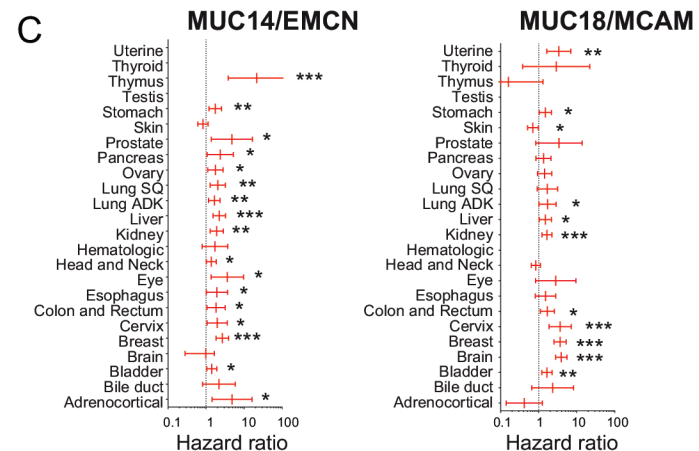
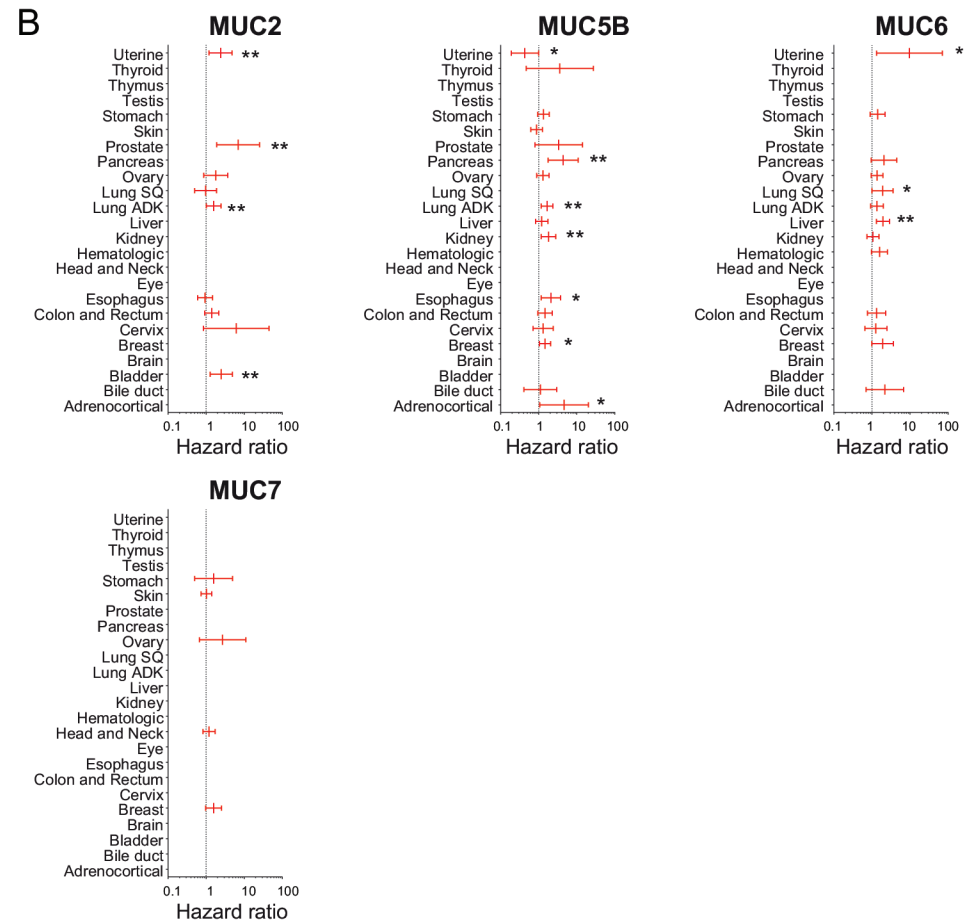


Figure 2

