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MUC7 gene expression and genetic polymorphism

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This study examined differential expression of several mucin genes in the human submandibular gland and trachea, *MUC7* tissue and species specificity, and *MUC7* genetic polymorphism. Mucin gene expression examined by RT-PCR indicated that *MUC1*, *MUC4* and *MUC7* are expressed in the human submandibular gland, while *MUC1*, *MUC2*, *MUC4*, *MUC5* and *MUC7* are expressed in the human trachea. Northern blot analysis confirmed the expression of *MUC7* in the human trachea and *MUC4* in the human submandibular gland. Northern blot analysis also demonstrated that *MUC7* is not expressed in the submandibular/sublingual gland complexes of hamster, mouse and rat. Southern blot analysis suggested the presence of a *MUC7* homologue in monkey genomic DNA. Genetic polymorphism studies of *MUC7* by PCR and Southern blot analysis revealed the presence of a limited variable number of tandem repeats (VNTR) polymorphism.

***Keywords*: *MUC7*, gene expression and genetic polymorphism**

***Abbreviations*: RT-PCR, reverse transcriptase polymerase chain reaction; DNA, deoxyribonucleic acid; RNA, ribonucleic acid; ORF, open reading frame; VNTR, variable number of tandem repeats; HSMSL, human submandibular-sublingual saliva; MG1, high molecular weight mucin glocoprotein; MG2, low molecular weight mucin glycoprotein; SDS, sodium dodecyl sulfate; NBT, nitro blue tetrazolium; BCIP, 5-bromo-4-chloro-3-indolyl phosphate; Na_2EDTA, disodium ethylenediamine tetra-acetic acid; dGTP, 2′-deoxyguanosine-5′-triphosphate; dCTP, 2′-deoxycytidine-5′-triphosphate; dATP, 2′-deoxyadenosine-5′-triphosphate; dTTP, 2′-deoxythymidine-5′-triphosphate; TE buffer, (10 mM Tris-HCl, 1 mM Na_2EDTA, pH 8.0); RE, restriction endonuclease.**

Introduction

Mucins are a subclass of glycoproteins expressed at most host/environmental interfaces by specialized epithelial cells and are involved in the maintenance of mucosal surfaces [1–3]. Eight distinct human mucin genes encoding for the mucin protein cores have been reported, and these have been designated *MUC1*, *MUC2*, *MUC3*, *MUC4*, *MUC5AC*, *MUC5B*, *MUC6* and *MUC7* [4–18]. The entire open reading frame (ORF) sequences and their derived amino acid sequences have been reported for *MUC1*, *MUC2* and *MUC7* while only partial sequence data are available for *MUC3*, *MUC4*, *MUC5AC*, *MUC5B* and *MUC6*. The *MUC5* family is apparently comprised of two distinct genes, designated *MUC5AC* and *MUC5B* [17]. The *MUC5B* gene appears to be genetically distinct from *MUC5AC* based on differential physical mapping and tissue specific expression [17]. Recently identified cDNA clones, designated NP3a and L31, have been suggested to encode for the 3′ end of the *MUC5AC* gene [16, 18]. Although these clones share 98.6% DNA sequence homology, their exact relationship remains undetermined [18]. Historically, mucin genes have been associated with the tissues from which they were cloned; *MUC1* (pancreatic), *MUC2* (intestinal), *MUC3* (intestinal), *MUC4* (tracheobronchial), *MUC5AC* (tracheobronchial), *MUC5B* (tracheobronchial), *MUC6* (gastric) and *MUC7* (salivary). However, it has been recently reported that most of these mucins are expressed in a wide range of tissues and exhibit patterns of overlapping expression [14, 19, 20]. For example, *MUC2* is expressed in human intestine, bronchus, cervix, gall bladder and mammary gland tissues; while *MUC5AC* is expressed in human trachea, stomach, gall bladder and terminal ileum tissues [14, 19]. Expression of these seven mucin genes, with the exception of *MUC7*, has not been examined in human submandibular gland tissue.

Two mucins designated MG1 (high molecular mass mucin glycoprotein) and MG2 (low molecular mass mucin glycoprotein) have been purified and characterized from human submandibular-sublingual gland saliva (HSMSL) [21–23]. The *MUC7* gene has been shown to encode for the protein core of MG2 [15], however, the mucin gene encoding for MG1 is presently unknown. In contrast to other

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mucin genes, *MUC7* expression appears to be limited to human submandibular and sublingual gland tissues [15]. By Northern blot analysis, *MUC7* was not detected in human ovary, parotid gland, stomach, tonsil, uterus, or placenta tissue [15]. In contrast, very little is known about the presence of a *MUC7* gene in non-human species.

A major conserved structural feature of mucin genes is the presence of a centrally located region of DNA encoding for a tandem repeat domain [1, 2]. It has been proposed that this tandem repeat domain may function as a structural scaffolding that ensures conservation of specific glycosylation motifs [2]. The amino acid sequence and number of amino acids per tandem repeat vary significantly among the seven mucin genes. *MUC1*, *MUC2*, *MUC3* and *MUC4* all exhibit genetic polymorphism that is related to a variable number of tandem repeats (VNTR) among subjects [2, 4, 7]. *MUC1* and *MUC2* alleles having $\sim$25–125 and $\sim$50–115 VNTR units, respectively, have been identified [4, 7]. Preliminary genomic Southern blot analysis of DNA from two subjects suggests that *MUC7* also exhibits genetic polymorphism [15]. However, detailed studies examining whether *MUC7* genetic polymorphism is related to differences in the quantity of tandem repeats have not been reported. The purpose of this study was to examine expression of several human mucin genes in human submandibular gland and trachea, determine the tissue and species expression patterns of *MUC7*, and examine *MUC7* genetic polymorphism with regard to VNTR.

Materials and methods

Materials

The following materials were obtained from the indicated sources: RNAgents total RNA isolation kit, agarose, nitro blue tetrazolium (NBT), and 5-bromo-4-chloro-3-indolyl phosphate (BCIP) (Promega, Madison, WI); Immobilon N membranes (Millipore Corp., Bedford, MA); Sequenase di-deoxynucleotide DNA sequencing kit and Genius non-radioactive DNA labeling kit (US Biochemical Corp., Cleveland, OH); Perkin-Elmer 9600 thermocycler, EZ rTth RNA PCR kit, and *Ampli*Taq DNA polymerase (Applied Biosystems Inc., Foster City, CA); ^{35}S labeled αdATP (>1000 Ci mM^{-1}) and ^{32}P labeled αdCTP ($\sim$3000 Ci mM^{-1}) (Amersham Corp., Arlington Heights, IL); PCR oligonucleotide primers (Bio-Synthesis Inc., Houston, TX); human genomic DNA, monkey genomic DNA, human trachea poly A RNA (Lot no. 43766, normal tissue from 13 donors), human submandibular gland poly A RNA (Lot no. 28578, normal tissue from three donors), human small intestine poly A RNA (Lot no. 26147, normal tissue from one donor) and human cerebral brain poly A RNA (Lot no. 35291, normal tissue from one donor) (CLONTECH Lab., Palo Alto, CA); *Sfi*I, *Not*I, *Hind*III, *Hinc*II, *Eco*RI, *Hae*III and *Bam*HI restriction endonucleases (Boehringer Mannheim, Indianapolis, IN); random primer DNA labeling kit (GIBCO BRL, Grand Island, NY); and Puregene DNA isolation kit (Gentra Systems, Minneapolis, MN). The *MUC4* containing JER64 clone [12] was kindly provided by Dr J.P. Aubert, Unite INSERM, Lille de Cidex, France.

Total RNA and genomic DNA preparation from mammalian tissues

Rat, hamster and mouse submandibular-sublingual gland complexes and liver tissues were obtained by dissection following euthanasia of the animals and immediately frozen in liquid nitrogen. Normal human submandibular and parotid glands were obtained from an individual following prophylactic radical neck dissection and immediately frozen in liquid nitrogen. Total RNA from the salivary gland tissues was purified using a RNAgents total RNA isolation kit (Promega) following the manufacturer's instructions. Collection and handling of human and animal tissues followed the guidelines of the Human Subject's Review Board and the Institutional Animal Care and Use Committee, respectively, at SUNY/Buffalo. Genomic DNA from rat, hamster and mouse was extracted from the animal liver samples by a modified proteinase K method as described by Enrietto *et al.* [24]. For genomic polymorphism studies, human genomic DNA was extracted from fresh whole blood using the Puregene DNA isolation kit following the manufacturer's instructions. Whole blood samples (5 ml) were obtained from human subjects by venipuncture collection or alternatively 50 µl whole blood samples were obtained by finger lance collection. Blood samples were collected in the presence of 0.2% Na_2EDTA to prevent clotting.

Reverse transcriptase polymerase chain reaction (RT-PCR)

Differential mucin expression was examined by RT-PCR. Human submandibular gland, trachea and brain poly A RNAs purified from normal tissues were obtained commercially. Oligonucleotide primers for RT-PCR were designed based on the DNA sequences of *MUC1*, *MUC2*, *MUC3*, *MUC4*, *MUC5AC*, *MUC6* and *MUC7* and obtained from Bio-Synthesis Inc. (Table 1A). The *MUC5AC* primers used in the RT-PCR experiments were designed from DNA sequence common to both NP3a and L31 cDNA clones, which likely encodes the 3′ end of the *MUC5AC* gene [16, 18]. RT-PCR was performed using the EZ rTth PCR kit following the manufacturer's guidelines. These experiments were optimized for each set of primers under a variety of temperatures and conditions. Since the primers were designed to have a similar GC content, the optimal conditions for each RT-PCR reaction were similar. Briefly, the following standard optimized reaction mixture was prepared: 10 µl of 5X EZ buffer, 1.5 µl of 10 mM dGTP, 1.5 µl of 10 mM dATP, 1.5 µl of 10 mM dTTP, 1.5 µl of 10 mM dCTP, 2 µl (5 U) of rTth DNA polymerase, 5 µl of 25 mM $Mn(OAc)_2$ solution, 0.45 µM of both sense and

Table 1A. RT-PCR primers.

Mucin gene		*RT primer sequence*	*Primer position*
MUC1 [5]	sense	5′ GCCAGGGCTACCACAACCCCAGCC	727–751
	antisense	5′ CACCCCAGCCCCAGACTGGGCAGA	1235–1259
MUC2 [8]	sense	5′ CATGGAGGCCCCCGGGGAGTGCTG	2352–2376
	antisense	5′ TGTCCCGCAGGACCCGGAGCAATG	2702–2726
MUC3 [10]*	sense	5′ ACCACCTCTGAGACCCCCTCACACAG	9–32
	antisense	5′ AGCTGGGAGTACTGTGTGAGGGGGTC	244–268
MUC4 [12]*	sense	5′ GGCCACGCCACCTCTCTTCTTGTC	19–42
	antisense	5′ GGATGCTGAGGAAGCGTCGGTGAC	473–496
MUC5AC [16]	sense	5′ CACCGGCCTCACCCGACGCCCACC	1467–1490
	antisense	5′ GATGGGGCCGGCCTCCCGGAGAGC	1881–1904
MUC6 [14]	sense	5′ GCCACCAGCAGCAGGCCACCACCA	340–363
	antisense	5′ GTGGGTCACAGGGGTGCTGGTGTC	814–837
MUC7 [15]	sense	5′ CTGGACTGCTAGCTCACCAGAAGCCG	244–267
	antisense	5′ GGGTGGGGCAGCTGTGGTGTCTTG	582–605

* Variable size (smear or ladder), primers anneal at multiple sites in tandem repeat DNA.

antisense primers, 0.02 μg of poly A RNA, and 24 μl of RNAse free deionized water. The reaction mixture was incubated for 45 min at 62 °C for the reverse transcription step. The mixture was then incubated for 60 s at 95 °C, followed by 40 cycles of 15 s at 95 °C, 30 s at 62 °C, and 15 s at 65 °C, followed by 7 min at 62 °C for the DNA amplification step. DNA RT-PCR products (20 μl/50 μl) were separated on 2% agarose gels and visualized by ethidium bromide staining. The specificity of RT-PCR amplified products was further evaluated by transferring NaOH denatured DNA to Immobilon N hybridization membranes and hybridization with Genius labeled *MUC7* and *MUC4* DNA probes following the manufacturer's instructions.

Northern blot analysis

Poly A RNA (~1 μg) or total RNA samples (~15 μg) were denatured by a formamide/formaldehyde procedure and separated on 2% agarose/6% formaldehyde agarose gels at 70 V for 3 h. The separated RNA was then transferred to Immobilon N membranes as described by the manufacturer. The blots were prehybridized, hybridized, and washed using high stringency hybridization conditions as previously reported [25]. *MUC7* (~0.9 kb *Eco*RI/*Bam*HI fragment) and *MUC4* (~1.8 kb *Eco*RI fragment) nucleic acid probes were labeled with a Genius non-radioactive DNA labeling and detection kit or with ^{32}PαdCTP using the random primer DNA labeling kit following the manufacturer's instructions.

PCR screening of genomic DNA for *MUC7* VNTR polymorphism

A typical PCR reaction mixture contained 1 μl genomic DNA (0.5 μg); 10 μl of 10X PCR polymerase buffer (100 mM Tris-HCl, 500 mM KCl, pH 8.3, 15 mM $MgCl_2$ and 0.01% gelatin); 10 μl of 2.5 mM mix of dGTP, dATP, dCTP, and dTTP (0.2 mM final concentration); 1 μM each of sense and antisense primers (Table 1B), 0.5 μl (2.5 U) of *Ampli*Taq DNA polymerase and sterile deionized water to a final volume of 100 μl. Following a 2 min denaturation step at 94 °C, 25 cycles of three cycle amplification were performed under the following parameters: denaturation at 94 °C for 15 s, annealing at 55 °C for 30 s, and extension at 72 °C for 30 s. This amplification cycle was followed by an extension step of 6 min at 72 °C. The PCR reactions were separated on 2% agarose gels and visualized by ethidium bromide staining.

Southern blot analysis

Genomic DNA samples were digested and the fragments were separated on 1% agarose gels. The separated DNA was transferred to Immobilon N membranes as described by the manufacturer. The blots were prehybridized, hybridized, and washed using high stringency hybridization conditions as previously reported [15]. *MUC7* cDNA fragments (~890 bp *Eco*RI/*Bam*HI fragment and 2.4 kb *Sfi*I/*Not*I fragment) were labeled with ^{32}P αdCTP using a random primer DNA labeling kit following the manufacturer's instructions and used to probe the Southern blots.

Table 1B. *MUC7* genomic PCR primers.

MUC7 *Primer*	*Primer sequence*
MUC7 F sense	5′ CTGGACTGCTAGCTCACC AGAAGCCG
MUC7 R antisense	5′ TTCAGAAGTGTCAGGTGCAAG

Results

Differential mucin expression

The poly A RNA from the human submandibular gland and trachea was used to examine *MUC1–MUC7* gene expression by RT-PCR. Poly A RNA from brain served as a negative control. All experiments were repeated in triplicate with identical results. As shown in Figure 1, *MUC1*, *MUC4* and *MUC7* expression was detected in the human submandibular gland (lanes H, K and N, respectively). In human trachea, *MUC1*, *MUC2*, *MUC4*, *MUC5AC* and *MUC7* expression was observed (lanes A, B, D, E and G, respectively). *MUC4* products appeared as a smear rather than a distinct size product because, as pointed out in Table 1A, *MUC4* primers are derived from the tandem repeat DNA [12]; the only DNA sequence available for *MUC4*. The primers thus anneal at multiple sites and variable size (smear or ladder) RT-PCR products were expected from the trachea RNA and were also obtained from the submandibular gland RNA. Because of the novel nature of the *MUC4* and *MUC7* expression patterns, the same RT-PCR products were probed with *MUC4* and *MUC7* cDNA clones. The *MUC4* and *MUC7* probes only hybridized to the RT-PCR products from human submandibular gland and trachea which were amplified with the *MUC4* and *MUC7* primers, respectively (data not shown). This confirmed the specificity of the *MUC4* and *MUC7* RT-PCR products. *MUC2* and *MUC5AC* were not expressed in human submandibular gland tissue; while *MUC1*, *MUC2*, *MUC4*, *MUC5AC*, and *MUC7* were not expressed in human brain tissue. RT-PCR results pertaining to *MUC3* and *MUC6* expression were not interpreted since the specificity of the *MUC3* (no negative control) and *MUC6* (no positive control) primer sets could not be confirmed in PCR control reactions. Previously, we reported *MUC7* expression in the submandibular and sublingual salivary glands but not stomach, ovary, placenta, uterus or parotid gland [15]. The results of the present study suggest that *MUC7* is also expressed in human trachea, but not brain. This is also the first report of *MUC4* expression in human submandibular gland. In addition, these RT-PCR results using human submandibular gland poly A RNA were confirmed with human submandibular gland total RNA from a second source.

MUC7 expression in human tissues

To confirm and to quantitate the observations from the RT-PCR studies, the *MUC7* and *MUC4* expression in human tissues was also examined by Northern blot analysis. A *MUC7* DNA probe hybridized to a discrete ~2.4 kb RNA transcript from both human submandibular gland and trachea poly A RNA (Figure 2, lanes A and B, respectively). Besides the discrete ~2.4 kb transcript, several minor bands were also detected in the submandibular gland RNA. The significance of the minor bands is presently unknown. Since the blot was prehybridized, hybridized, and washed using high stringency hybridization conditions [25], the minor bands cannot be explained by low hybridization stringency. The hybridization signal was considerably stronger in human submandibular gland RNA compared to an equal concentration of human trachea RNA. The 2.4 kb transcript was not, however, detected in human small intestine or brain poly A RNA. These experiments were

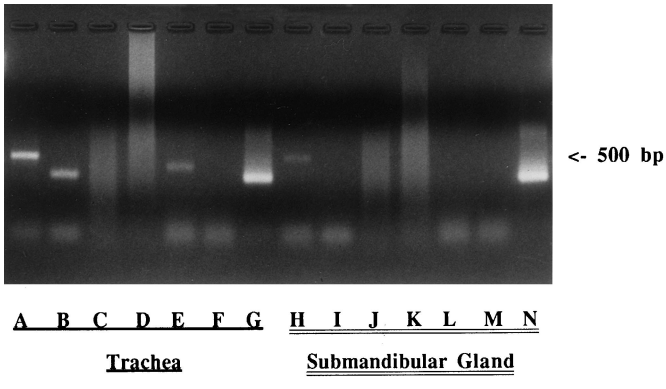

Figure 1. Human mucin expression in the submandibular gland and trachea. Agarose gels (2%) of RT-PCR reaction products were stained with ethidium bromide. Lanes A–G contain RT-PCR products from human trachea polyA RNA amplified with different *MUC* primers. Lanes H–N contain RT-PCR products from human submandibular gland polyA RNA amplified with different *MUC* primers. Lanes A and H, *MUC1* primers; Lanes B and I, *MUC2* primers; Lanes C and J, *MUC3* primers; Lanes D and K, *MUC4* primers; Lanes E and L, *MUC5A,C* primers; Lanes F and M, *MUC6* primers; Lanes G and N, *MUC7* primers.

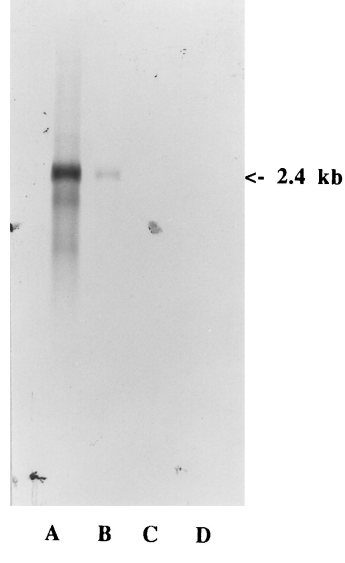

Figure 2. Northern blot analysis of *MUC7* expression in human tissues. Equal amounts of polyA RNA (1.3 μg) were analyzed using a *MUC7* *Eco*RI/*Bam*HI fragment (~890 bp) as the hybridization probe. Lane A, submandibular gland; Lane B, trachea; Lane C, small intestine; Lane D, brain. All experiments were performed in duplicate.

conducted in duplicate with identical results. These results confirm that the *MUC7* gene is expressed in trachea tissues and has a wider distribution of tissue expression than previously reported. In the trachea, it is possible that *MUC7* is expressed by a minor cell population such as the mucous secreting goblet cells as opposed to constitutive expression by all trachea epithelial cells.

The JER64 *MUC4* DNA probe [12] hybridized very strongly to a polydisperse transcript in human trachea poly A RNA (Figure 3, lane B). This signal was most intense at ~9.0 kb. This result is consistent with the original findings where the JER64 probe hybridized to polydisperse messages on the Northern blot analysis of tracheo-bronchial mucosa RNA [12]. The *MUC4* probe also hybridized to human submandibular and small intestine poly A RNA (Figure 3, lanes A and C, respectively). These are independent signals from the trachea since on the shorter exposure, there was no overlap of signals from the three lanes and the experiments were conducted in duplicate with identical results. The *MUC4* transcript was not detected in human brain poly A RNA. The hybridization signal was considerably stronger in trachea RNA as compared to an equal concentration of submandibular gland and small intestine RNA. In addition to the ~9.0 kb transcript, a smaller and much weaker ~2.4 kb transcript was also detected in submandibular gland RNA. The significance of this 2.4 kb transcript is presently unknown. Since the Northern blot in Figure 3 was a separate blot from the one in Figure 2 (probed with *MUC7* probe) this band does not represent a residual hybridization signal from the *MUC7* probe. Collectively, these results confirm that *MUC4* is expressed in submandibular gland tissues.

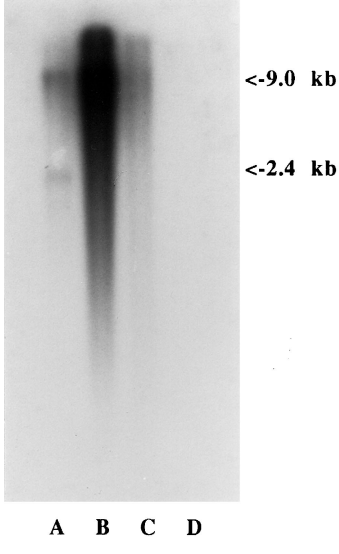

Figure 3. Northern blot analysis of *MUC4* expression in human tissues. Equal amounts of polyA RNA (1.3 μg) were analyzed using a *MUC4* *Eco*RI fragment (~1.8 kb) as a hybridization probe. Lane A, submandibular gland; Lane B, trachea; Lane C, small intestine; Lane D, brain. All experiments were performed in duplicate.

MUC7 expression in mammalian submandibular glands

MUC7 expression was examined in mammalian submandibular gland tissue by Northern blot analysis. A *MUC7* DNA probe hybridized to RNA transcripts in human submandibular gland RNA, but not to human parotid gland or mouse, rat, or hamster submandibular-sublingual gland complex total RNA (Figure 4). A discrete hybridization signal with an apparent size of ~2.4 kb was observed in human submandibular gland RNA. In addition, a larger polydisperse hybridization signal, consistent with previously published data using total RNA, was also observed [25]. However, based on the Northern blot analysis of *MUC7* expression using poly A RNA (see Figure 2), where a distinct 2.4 kb transcript was detected, this large polydisperse signal appears to be artifact since it was only detected in Northern blots examining total RNA. This artifact could be a result of the formation of higher molecular weight RNA aggregates or complexes. Similar results were obtained using hybridization conditions of high and low stringency.

In addition, *MUC7* species specificity was examined by Southern blot analysis of human, monkey, hamster, mouse and rat genomic DNA using *MUC7* cDNA as a probe. Under high stringency hybridization conditions, the *MUC7* probe hybridized to restriction enzyme fragments from both human and monkey genomic DNA digests, but not to mouse, hamster, or rat genomic DNA (Figure 5). A single

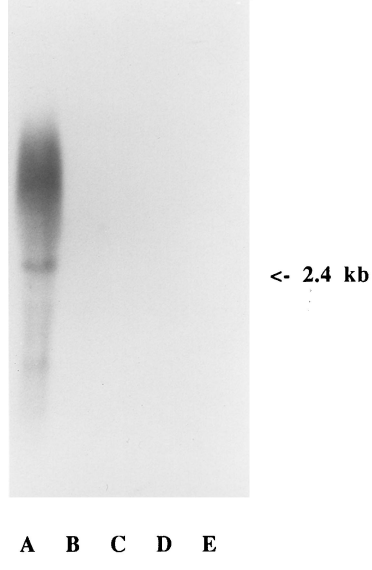

Figure 4. Northern blot analysis of *MUC7* expression in mammalian salivary gland tissues. Equal amounts of total RNA (15 μg) were analyzed using *MUC7* *Sfi*I/*Not*I fragment (~2.4 kb) as a hybridization probe. Lane A, human submandibular gland; Lane B, human parotid gland; Lane C, hamster submandibular-sublingual gland complex; Lane D, mouse submandibular-sublingual gland complex; Lane E, rat submandibular-sublingual gland complex.

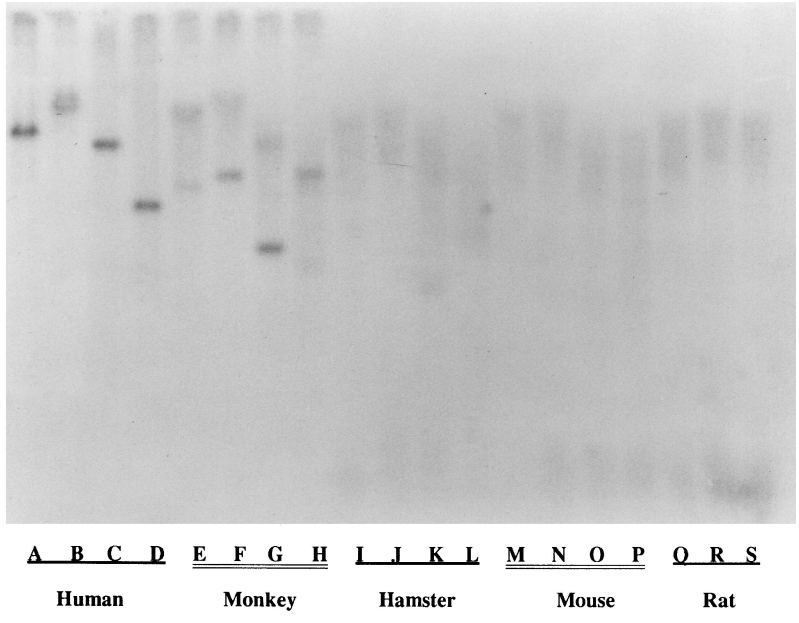

Figure 5. Southern blot analysis of *MUC7* genetic homology in mammalian genomic DNA. Equal amounts of RE digested genomic DNA (15 μg) were analyzed using *MUC7 Sfi*I/*Not*I fragment (~2.4 kb) as a hybridization probe. Lanes A–D, human genomic DNA; Lanes E–H, monkey genomic DNA; Lanes I–L, hamster genomic DNA; Lanes M–P, mouse genomic DNA; and Lanes Q–S, rat genomic DNA. Lanes A, E, I, M, and P, *Eco*RI digest; Lanes B, F, J, N, and R, *Bam*HI digest; Lanes C, G, K, O, and S, *Hind*III/*Bam*HI digest; Lanes D, H, L, and P, *Eco*RI/*Hind*II digest.

hybridization band was detected in each digest of the human genomic DNA, while two bands were generated in each digest of the monkey DNA. This suggests that the structural organization of the monkey *MUC7* genomic homologue is different. To substantiate this observation, monkey genomic DNA was amplified by PCR using *MUC7* specific forward and reverse primers derived from the 3′ untranslated region. The predicted 1.1 kb fragment was amplified and showed ~90% DNA homology with human *MUC7* (data not shown). At low stringency conditions, the *MUC7* probe also hybridized to hamster genomic DNA fragments.

MUC7 genetic polymorphism with respect to VNTR

Many mucin genes demonstrate genetic polymorphism which has been attributed to variation in the number of tandem repeats (VNTR). The *MUC7* cDNA clones [15] and genomic clones [26] that we have previously characterized contained six tandem repeats of 69 bp (23 aa). A Southern blot analysis of genomic DNA from two subjects, however, indicated a possible *MUC7* genetic polymorphism with respect to VNTR [15]. Thus, a PCR amplification scheme was developed to allow for the screening of *MUC7* genetic DNA VNTR polymorphism in large patient populations. PCR primers were designed from the *MUC7* translated region to amplify an ~852 bp region of DNA containing the six tandem repeat nucleotide sequence. This region of *MUC7* genomic DNA is free of introns [26]. Amplification of genomic DNA from 14 subjects demonstrated two distinct patterns (Figure 6) that are probably representative of two different size alleles. In one patient population, 10 out of 14 subjects had a single ~852 bp amplification product corresponding to the presence of six tandem repeats. In contrast, amplification of genomic DNA from the other four subjects produced a doublet of equal intensity (~780 bp and ~852 bp) suggesting the presence of a size polymorphism at one allele, corresponding to five and six tandem repeats, respectively. This is very similar to the genetic polymorphism seen in rat submandibular gland mucin [27]. Additional, weaker bands were also seen in the PCR product of some samples.

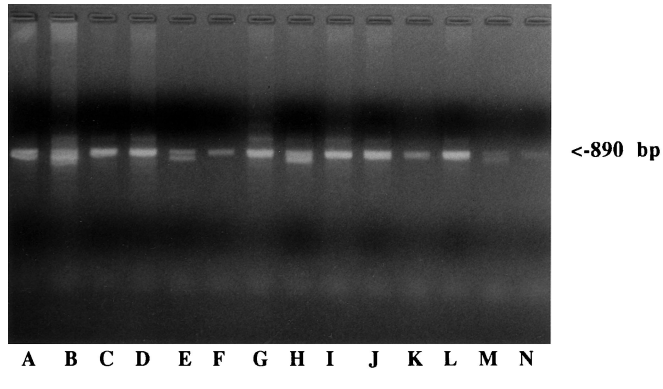

Figure 6. *MUC7* genetic polymorphism, A 2% agarose gel of PCR reaction products was stained with ethidium bromide. Lanes A–N, PCR amplification of genomic DNA from 14 human subjects using *MUC7* primers that span the VNTR region.

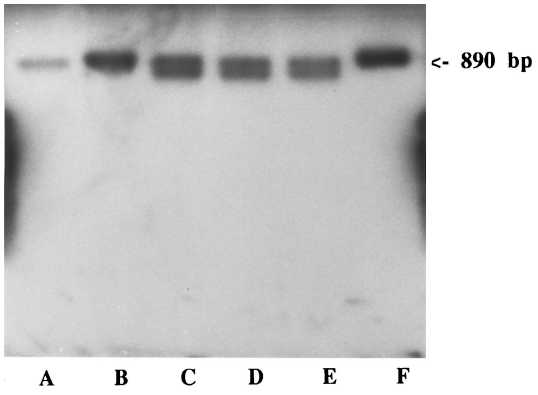

Figure 7. Southern blot analysis of *MUC7* genetic polymorphism. Equal amounts of *Eco*RI/*Bam*HI digested genomic DNA (12 μg) were analyzed for VNTR using *MUC7 Eco*RI/*Bam*HI fragment (~890 bp) as a hybridization probe. Lanes A–F, human genomic DNA from subjects A, C, H, M, E, and F in Figure 6.

To confirm the PCR observations, and to address the weaker bands also seen with the PCR product, VNTR polymorphism was examined by Southern blot analysis of genomic DNA from six of the 14 subjects tested previously by PCR. Three of these subjects demonstrated a single PCR product and three a double PCR product. Genomic DNA from these subjects was digested with *Eco*RI/*Bam*HI and hybridized with an 890 bp *Eco*RI/*Bam*HI fragment of *MUC7* as a probe to produce a 890 bp hybridization fragment in the subjects with six tandem repeats. At high stringency, this probe hybridized in two distinct patterns (Figure 7) confirming the PCR results. As expected, in three subjects, the *MUC7* probe hybridized to a single ~890 bp DNA fragment corresponding with the presence of six tandem repeats (Figure 7, lanes A, B and F, corresponding to individuals A, C and F in Figure 6). In the other three subjects, the *MUC7* DNA probe hybridized, also as expected, to ~820 and ~890 bp DNA fragments that correspond to the presence of two alleles with five and six tandem repeats, respectively (Figure 7, lanes C, D and E, corresponding to individuals H, M and E, respectively, in Figure 6). Similar findings were obtained by Southern blot analysis of *Eco*RI/*Hae*III digested genomic DNA. A 583 bp *Eco*RI/*Hae*III *MUC7* cDNA fragment used as a probe hybridized, as expected, to a single 583 bp fragment in the three subjects with six tandem repeats. In the three subjects with six and five tandem repeats, this probe hybridized to 583 bp and 514 bp fragments, respectively (data not shown). These results also demonstrated that there is no significance to the additional weaker bands seen in the PCR reactions.

Discussion

This report examined the differential *MUC1–MUC7* gene expression in human submandibular gland and trachea tissues. We observed that *MUC1*, *MUC4* and *MUC7* are expressed in the human submandibular gland. The gene product of *MUC1* has been previously detected by immunocytochemistry in human submandibular gland [28]. *MUC1* encodes for a cell surface associated mucin that is present in many epithelial tissues. Based on its size, amino acid composition and cell associated nature, it is not a likely candidate gene for the high molecular weight human salivary mucin, MG1. The expression of *MUC4* in human submandibular gland is an interesting observation. *In situ* hybridization studies have demonstrated that the *MUC5B* RNA message is detected with intermediate labeling intensity in human submandibular gland, while *MUC4* is not [20]. Our studies show that *MUC4* is expressed in human submandibular gland tissue, albeit in relatively small amounts.

This study also shows *MUC7* expression in trachea. The *MUC7* gene product, MG2, has been shown to bind to *Pseudomonas aeruginosa* and *Staphylococcus aureus*, which function as opportunistic respiratory pathogens [29, 30]. MG2 is thought to facilitate bacterial clearance in the oral cavity and may well serve that function in the trachea [31]. *In vitro* and *in vivo* models have shown that mucin binding to *P. aeruginosa* inhibits this bacteria's ability to adhere to host epithelium [32–34]. Mucin binding to *Escherichia coli* has also been shown to inhibit *in vitro* adhesion of bacteria to host tissues [35, 36]. In addition, MG2 has been shown to complex with potent antimicrobials, including secretory IgA and lysozyme, which further supports its antibacterial function [21, 29]. Collectively, these data coupled with the finding of *MUC7* expression in the trachea suggests that MG2 participates in modulating the colonization of human tracheal tissues by *P. aeruginosa* and *S. aureus*.

Our earlier study comparing genomic DNA digests of two subjects by Southern blot analysis indicated a possible genetic polymorphism in the *MUC7* gene [15]. The present study confirms the existence of at least two different size alleles with respect to the tandem repeat domain in the *MUC7* gene. In a population of 14 subjects, 10 exhibited an allele with six tandem repeats and the other four exhibited two different size alleles, corresponding to five and six tandem repeats, respectively. Genetic polymorphism seen with other mucin genes, such as *MUC1* and *MUC2*, have been attributed to VNTR [4, 7]. Thirty different alleles in 69 subjects, ranging in size from 20–125 VNTR, have been described for the *MUC1* gene [4]. The *MUC2* gene also exhibits VNTR polymorphism, having alleles of 50–115 tandem repeat units [7]. The majority of these alleles contain ~100–115 tandem repeat units [7]. *MUC3* and *MUC4* also appear to exhibit genetic polymorphism, manifesting multiple alleles, which is probably related to VNTR [12, 37]. These VNTR polymorphisms are thought to occur by unequal but homologous cross-over events [8, 38]. Compared to these mucin genes, *MUC7* has a minimal degree of VNTR genetic polymorphism. Recently, the rat

submandibular gland apo-mucin gene has been reported to have an allelic variation very similar to that of *MUC7* [27]. Using Southern blot analysis, six of 10 animals tested showed alleles of the same length, corresponding to 11 tandem repeats, and the other four animals showed a doublet pattern, corresponding to 10 and 11 tandem repeats [27].

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