MUC18, a marker of tumor progression in human melanoma, shows sequence similarity to the neural cell adhesion molecules of the immunoglobulin superfamily

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ABSTRACT The MUC18 antigen is an integral membrane glycoprotein of 113 kDa whose expression on primary human melanomas correlates with poor prognosis and the development of metastatic disease. MUC18 is expressed only sporadically in benign melanocytic nevi and thin primary melanomas that have a low probability of metastasizing. However, with increasing tumor thickness, MUC18 expression becomes more frequent and it is found on 80% of advanced primary tumors and metastases. MUC18-encoding cDNA clones were obtained by screening a human melanoma phage λ expression library with monoclonal antibodies produced against the denatured antigen. The deduced sequence of 603 amino acids consists of a signal peptide, five immunoglobulin-like domains, a transmembrane region, and a short cytoplasmic tail. The highest sequence similarity is with a group of nervous system cell adhesion molecules, which includes neural cell adhesion molecule (N-CAM). The close structural relationship with these molecules suggests that MUC18 may also be a developmentally regulated cell adhesion molecule.

Malignant cells are not inherently metastatic but appear to develop this capacity during tumor progression as they acquire new characteristics through a multistep process of selection from continuously arising variants (1). Analysis of in situ phenotypic changes that correlate with the progression of cutaneous malignant melanoma has led to the identification of molecules that may contribute to the metastatic potential of this tumor (2-5). Two of these molecules are HLA-DR and intracellular adhesion molecule 1, which are not found on thin tumors that have a good prognosis but begin to be expressed on primary tumors ≥ 1 mm thick and appear to be coordinately expressed (6). A third melanoma antigen whose expression has been shown to correlate with tumor progression is the 113-kDa cell surface glycoprotein MUC18 (7). Although the expression of MUC18 is independent of HLA-DR and intracellular adhesion molecule 1, it is also not expressed on benign melanocytic lesions or thin tumors with a good prognosis. Here, we report the cDNA cloning and sequencing of MUC18 from melanoma cells.[‡] The derived amino acid sequence indicates that MUC18 is a member of the immunoglobulin superfamily and shows the greatest sequence similarity to a group of neural cell adhesion molecules expressed during organogenesis. On the basis of this sequence similarity, we speculate that MUC18 may also be developmentally regulated and mediate intercellular adhesion. Since the expression of MUC18 in normal adult tissues appears limited to vascular smooth muscle (7), expression of this molecule by melanoma cells may contribute to their interaction with elements of the vascular system, an essential step in the dissemination of tumor cells.

MATERIALS AND METHODS

Antibodies and Cells. Monoclonal antibody (mAb) MUC18 (IgG2a) has been described (7) and mAb 96.5 (antimelanotransferrin, ref. 8) was kindly provided by I. Hellström (Oncogen, Seattle), and the myeloma protein UPC10 (IgG2a) was purchased from Sigma. Cell lines were obtained from the American Type Culture Collection, through exchange, or were established in our laboratory.

Immunohistochemistry and Flow Cytometry. Indirect immunoperoxidase staining on frozen tissues was performed as described (7). Tissues were regarded as positive when $\geq 5\%$ of the tumor cells were stained. The following numbers of primary melanomas were examined: 17 at ≤ 0.75 mm and 15 at 0.76–1.5 mm, 15 at 1.51–3.0 mm, and 15 at ≥ 3.0 mm. For flow cytometry, cells were incubated with mAb followed by fluorescein isothiocyanate-conjugated second antibody and analyzed using a FACScan (Becton Dickinson).

Selection of the Antibodies for Western Blot Analysis. mAbs MUCBA18.1-18.5 (all IgG1) were produced from a (C57BL/6 \times BALB/c)F₁ mouse injected i.p. with 100 mg of lipid A (9) followed 14 days later by heat-denatured immunoprecipitated MUC18 in 0.1% SDS. Three injections of immunoprecipitates from 3×10^9 Mel-JuSo cells with Freund's complete adjuvant, Freund's incomplete adjuvant, and Bordetella pertussis adjuvant, respectively, were spaced over 6 weeks. The spleen cells were fused with P3×63Ag8.63 myeloma cells and culture supernatants were screened on MUC18 glycoprotein-loaded nitrocellulose strips. Immunoprecipitates from Mel-JuSo cells $(5 \times 10^8$ cells per cm of nitrocellulose) were separated by SDS/PAGE under reducing conditions and electrophoretically transferred to nitrocellulose membranes (BA85; Schleicher & Schüll). The nitrocellulose strips were incubated with the mAb followed by peroxidase-conjugated rabbit antiserum to mouse immunoglobulins (Dakopatts, Copenhagen) as described (4).

Isoelectric Focusing and Peptide Mapping. Cells were labeled using lactoperoxidase–glucose oxidase-catalyzed surface iodination (10) and immunoprecipitates were prepared as described (7). Isoelectric focusing was performed essentially as described by Neefjes *et al.* (11). Peptide mapping by partial proteolysis in gel slices (12) was performed using 100 ng of *Staphylococcus aureus* V8 protease (Sigma).

cDNA Libraries and DNA Sequencing. cDNA expression libraries were constructed in the $\lambda gt11$ and λZAP vectors (Strategene). cDNA was prepared from Mel-JuSo cells (cDNA synthesis system; BRL) and size-selected [≥ 1200 base pairs (bp)] by agarose gel electrophoresis. Fusion proteins were induced, blotted on nitrocellulose filters, and

Abbreviation: mAb, monoclonal antibody.

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[‡]The sequence reported in this paper has been deposited in the GenBank data base (accession no. M28882).

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FIG. 1. Reaction pattern of mAb MUC18 with primary melanoma *in situ*. Open bars, percentage of lesions that contained $\geq 5\%$ stained tumor cells; solid bars, 5-year mortality rate in each group (17).

screened with mAbs MUCBA18.1–18.5 (13). Reactive plaques were detected using peroxidase-coupled second antibody. For sequencing, suitable restriction fragments were subcloned in pUC18 and double-stranded cDNA was sequenced using the dideoxynucleotide chain-termination method (14) with Sequenase (United States Biochemical).

Northern and Southern Blot Analysis. RNA was denatured in formaldehyde, separated on a 1% agarose/formaldehyde gel (15), and transferred to Hybond-N (Amersham). The 1200-bp Sst I-Pst I cDNA fragment labeled with $[\alpha^{-32}P]dCTP$ (Amersham) by random-priming was used as probe. Membranes were hybridized overnight at 65°C in 6× SSC/5× Denhardt's solution/0.5% SDS/salmon sperm DNA (20 µg/ ml). (1× SSC = 0.15 M NaCl/0.015 M sodium citrate, pH 7.0; 1× Denhardt's solution = 0.02% polyvinylpyrrolidone/ 0.02% Ficoll/0.02% bovine serum albumin.) The filters were washed at 65°C in 3× SSC/0.1% SDS, 1× SSC/0.1% SDS, 0.3× SSC/0.1% SDS, and 0.1× SSC/0.1% SDS. DNA was digested with restriction endonucleases, electrophoresed,



FIG. 2. Biochemical comparison of the molecules recognized by mAbs MUC18 and MUCBA18.1–18.5. (A) Immunoblots. The blotted MUC18 immunoprecipitates were probed with mAbs MUCBA18.1 (lane 1), MUCBA18.2 (lane 2), MUCBA18.3 (lane 3), MUCBA18.4 (lane 4), MUCBA18.5 (lane 5), and UPC10 (lane 6). (B) Isoelectric focusing banding pattern of the MUC18 immunoprecipitate (lane 1) and the MUCBA18.1 immunoprecipitate (lane 2). Immunoprecipitates were prepared from Mel-JuSo cells labeled by surface iodination. The pH gradient is indicated on the left. (C) Peptide mapping. MUC18 immunoprecipitate (lane 2), and, as control, the melanotransferrin immunoprecipitate (p97, lane 3) were partially digested with 100 ng of Staphylococcus aureus V8 protease. Immunoprecipitates were prepared from Mel-JuSo cells labeled by surface iodination. The locations of molecular mass standards are indicated on the left in kDa.



FIG. 3. Flow cytometric analysis of the MUC18 glycoprotein expression and Northern blot analysis. (A) Immunofluorescence staining of the melanoma cell lines Mel-JuSo, SK-Mel-28, SK-Mel-1, SK-Mel-25, A375, and B16F1 by mAb MUC18 (dashed lines) and isotype control UPC10 (dotted lines). (B) Total RNA ($20 \mu g$) from the melanoma cell lines Mel-JuSo (lane 1), SK-Mel-18 (lane 2), SK-Mel-1 (lane 3), SK-Mel-25 (lane 4), A375 (lane 5), and B16F1 (lane 6) was electrophoresed, blotted on nylon membranes, and hybridized with the ³²P-labeled MUC18 cDNA probe.

transferred to Hybond-N nylon membranes, and hybridized as described above.

RESULTS AND DISCUSSION

Correlation of MUC18 Expression with the Development of Human Metastatic Melanoma. mAb MUC18 produced against human malignant melanoma was selected because it does not react with benign melanocytic lesions. In contrast 45% of the 62 primary melanomas and 71% of the 31 metastatic melanomas examined expressed the MUC18 antigen (7). The vertical thickness of primary melanomas (the Breslow index) is directly correlated with the probability of metastatic disease (16). Division of the primary melanomas into four evenly



matched groups according to their vertical thickness reveals that expression of MUC18 shows a linear correlation with this parameter (Fig. 1). The association of MUC18 expression with tumor thickness suggests that the appearance of this molecule reflects changes in the tumor cells that may contribute to the development of metastatic capacity.

Characterization of the Antibodies for Western Blot Analysis. To isolate MUC18-encoding cDNA clones, five mAbs (MUCBA18.1–18.5) reactive with the denatured MUC18 glycoprotein were produced (Fig. 2A). These mAbs precipitated a single protein of 113 kDa from surface-labeled Mel-JuSo cells (data not shown). In addition, MUC18 and MUCBA18.1 immunoprecipitates had identical isoelectric points and the same pattern of microheterogeneity (Fig. 2B) as well as identical peptide maps (Fig. 2C), indicating that both antibodies recognize the same molecule.

Isolation of MUC18 cDNA Clones and Identification of MUC18 mRNA. Three immunopositive cross-hybridizing clones were isolated from two human melanoma cDNA λ expression libraries. In addition to reactivity with mAbs



FIG. 5. Southern blot analysis. (A) Genomic DNA from Mel-JuSo cells was digested with Taq I, Pvu II, Pst I, EcoRI, and Bgl II (lanes 1–5, respectively). (B) Genomic DNA from peripheral blood lymphocytes of a normal donor was digested with EcoRI (lane 1). Genomic DNA from Mel-Ho cells (lanes 2 and 4) and the autologous B-lymphoblastoid cell line (lanes 3 and 5) were digested with Pvu II (lanes 2 and 3) or Taq I (lanes 4 and 5) and hybridized to the ³²P-labeled MUC18 cDNA probe.

FIG. 4. Structure of the MUC18 glycoprotein mRNA and relationship between the cDNA clones (A), sequencing strategy (B), and localization of 1300-bp Sst 1–Pst 1 MUC18 cDNA probe used for Northern and Southern blot analysis (C). Three cDNA clones, zapy1, drop1, and drop4, were isolated by using the antibodies used in Western blot analysis as probes and the clones drop4.2, drop4.7, and drop4.11 were isolated by using a ³²P-labeled MUC18 cDNA probe derived from the 5' end of clone drop4. The divergent nucleotide sequence of drop4.7 in the 3' region is indicated by dashed line. The 1809-bp open reading frame is indicated by the open box. Various restriction sites were used to generate subclones for sequencing. P, Pvu II; T, Taq I; Ps, Pst I; K, Kpn I; S, Sst I.

MUCBA18.1, -18.2, -18.3, and -18.5, the 3.3-kilobase mRNA detected by these clones demonstrates an expression pattern in human melanoma cell lines that mirrors exactly the binding of the MUC18 mAb (Fig. 3). High levels of MUC18 antigen and the 3.3-kilobase mRNA were seen in Mel-JuSo and Sk-Mel-28 cells, whereas lower levels were seen in Sk-Mel-1 cells and no products were detected in Sk-Mel-25 and A375 cells. The detection of a 3.3-kilobase mRNA in the mouse melanoma cell line B16F1 suggests that the MUC18 gene may be conserved across species, although the particular epitopes defined by the MUC18 antibodies are absent.

Inspection of the cDNA sequence revealed that it was incomplete in the 5' region. Therefore, the melanoma library was screened with a cDNA probe derived from the 5' end of clone drop4. Three additional clones were identified and the six overlapping clones correspond to the size of the observed mRNA. The relationship of the various cDNA clones is schematically depicted in Fig. 4A.

Analysis of Mel-JuSo DNA digested with various restriction endonucleases showed a pattern consistent with a singlecopy gene, as only a single hybridization fragment was obtained (Fig. 5A). Comparison of the restriction patterns in melanoma and autologous B-lymphoblastoid cell lines from different patients provides no indication that the gene is rearranged in melanomas or polymorphic in the population (Fig. 5B).

Nucleotide and Amino Acid Sequence. The MUC18 cDNA sequence (Fig. 6A) reveals a 7-bp 5' untranslated region, a single open reading frame of 1809 bp, and a 1487-bp 3' untranslated region, which includes the consensus polyade-nylylation signal (AATAAA) at position +3264 followed 15 bases later by a poly(A) tail.

In contrast to this sequence, the cDNA clone drop4.7 contains an unrelated 92-bp 3' untranslated region that begins at position +2847 (Fig. 6B) and lacks the consensus polyadenylylation signal. If not a cloning artifact this cDNA clone may indicate that the expression of the MUC18 molecule is regulated by the production of mRNAs with different 3' untranslated regions (18).

The translation initiation site was assigned to the first ATG codon of the single open reading frame at position +1, which is surrounded by a nucleotide sequence corresponding to the consensus sequence proposed for putative initiation codons (GGAAGCATGG) (19, 20). Hydrophobicity analysis (21) suggests that this sequence is succeeded by a 28-residue signal peptide sequence, which may be cleaved between alanine-28 and glutamic acid-29 (22). The open reading frame ends with the termination codon TAA at position +1810. Thus, the mature MUC18 molecule appears to consist of 575 amino acid

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~	-7	GGGAAG	C ATG Met	GGG Glv	CTT Leu	CCC Pro	AGG Arg	CTG Leu	GTC Val	TGC Cvs	GCC Ala	TTC Phe	TTG Leu	CTC Leu	GCC	GCC	TGC Cvs	TGC Cvs	TGC CV5	TGT Cvs	CCT	CGC	GTC	GCG	GGT	GTG Val	CCC	GGA	GAG	GCT	28
	85	GAG CA	G CCT	GCG	CCT	GAG	CTG	GTG	GAG	GTG	GAA	GTG	GGC	AGC	ACA	GCC	CTT	CTG	AAG	TGC	GGC	стс	TCC	CAG	TCC	CAA	GGC	AAC	стс	AGC	
	175	CAT GT	GAC	TGG	TTT	тст	GTC	CAC	AAG	GAG	AAG	CGG	ACG	TCA	TCT	TCC	GTG	TGC	GCC	AGG	GCC	AGG	GCC	AGA	GCG	AAC	CTG	Asn ★ GGG	Leu AGT	Ser	58
	265	His Va	L Asp G CGG	Trp	Phe	Ser	Val CAG	His GAC	Lys AGA	Glu	Lys	Arg	Thr	Ser	Ser	Ser	Val	Cys	Ala	Arg	Ala CAA	Arg	Ala	Arg	Ala	Asn TTC	Leu	Gly	Ser	Thr	88
		Ser Ly	s Arg	Leu	Ser	Leu	Gln	Asp	Arg	Gly	Ala	Thr	Leu	Ala	Leu	Thr	Gln	Val	Thr	Pro	Gln	Asp	Glu	Arg	Ile	Phe	Leu	Cys	Gln	Gly	118
	300	Lys Ar	g Leu	GGT Gly	Pro	AGG Arg	AGT Ser	Thr	Ala	Ser	AGC Ser	Ser	Ala	TCT Ser	ACA Thr	AAG Lys	CTC Leu	CGG Arg	ATG Met	Pro	AAC Asn	ATC Ile	CAG Gln	GTC Val	AAC Asn	CCC Pro	CTG Leu	GGC Gly	ATC Ile	CCT Pro	148
	445	GTG AAG Val As	C AGT n Ser	AAG Lys	GAG Glu	CCT Pro	GAG Glu	GAG Glu	GTC Val	GCT Ala	ACC Thr	TGT Cys	GTA Val	GGG Gly	AGG Arg	AAC Asn	GGG Gly	TAC Tyr	CCC Pro	ATT Ile	CCT Pro	CAA Gln	GTC Val	ATC Ile	TGG Trp	TAC Tyr	AAG Lys	AAT Asn	GGC Gly	CGG Arg	178
	535	CCT CT Pro Le	G AAG J Lys	GAG Glu	GAG Glu	AAG Lys	AAC Asn	CGG Arg	GTC Val	CAC His	ATT Ile	CAG Gln	TCG Ser	TCC Ser	CAG Gln	ACT Thr	GTG Val	GAG Glu	TCG Ser	AGT Ser	GGT Gly	TTG Leu	TAC Tyr	ACC Thr	TTG Leu	CAG Gln	AGT Ser	ATT Ile	CTG Leu	AAG Lys	208
	625	GCA CA Ala Gl	G CTG	GTT Val	AAA Lys	GAA Glu	GAC Asp	AAA Lys	GAT Asp	GCC Ala	CAG Gln	TTT Phe	TAC Tyr	TGT Cys	GAG Glu	CTC Leu	AAC Asn	TAC Tyr	CGG Arg	CTG Leu	CCC Pro	AGT Ser	GGG Gly	AAC Asn	CAC His	ATG Met	AAG Lys	GAG Glu	TCC Ser	AGG Arg	238
	715	GAA GT	C ACC	GTC Val	CCT	GTT Val	TTC Phe	TAC	CCG	ACA Thr	GAA	AAA	GTG Val	TGG Trp	CTG	GAA	GTG	GAG	CCC	GTG	GGA	ATG Met	CTG	AAG	GAA	GGG	GAC	CGC	GTG	GAA	268
	805	ATC AG	G TGT	TTG	GCT	GAT	GGC	AAC	ССТ	CCA	CCA	CAC	TTC	AGC	ATC	AGC	AAG	CAG	AAC	ccc	AGC	ACC	AGG	GAG	GCA	GAG	GAA	GAG	ACA	ACC	200
	895	AAC GA	C AAC	GGG	GTC	CTG	GTG	CTG	GAG	CCT	GCC	CGG	AAG	GAA	CAC	AGT	GGG	CGC	ASN X TAT	GAA	Ser TGT	CAG	GCC	GIU TGG	ALA	TTG	GLU	ACC	Thr ATG	ATA	298
	985	Asn As	p Asn G CTG	Gly AGT	Val GAA	Leu CCA	Val CAG	Leu GAA	Glu CTA	Pro CTG	Ala GTG	Arg	Lys TAT	Glu GTG	His TCT	Ser GAC	Gly GTC	Arg CGA	Tyr GTG	Glu AGT	Cys CCC	Gln GCA	Ala GCC	Trp	Asn GAG	Leu AGA	Asp CAG	Thr GAA	Met GGC	Ile :	328
	1075	Ser Le	Leu	Ser	Glu	Pro	Gln	Glu	Leu	Leu	Val	Asn	Tyr	Val	Ser	Asp	Val	Arg	Val	Ser	Pro	Ala	Ala	Pro	Glu	Arg	Gln	Glu	Gly	Ser :	358
		Ser Le	1 Thr	Leu	Thr	Cys	Glu	Ala	Glu	Ser	Ser	Gln	Asp	Leu	Glu	Phe	Gln	Trp	Leu	Arg	Glu	Glu	Thr	Asp	Gln	Val	Leu	Glu	Arg	Gly	388
	1165	Pro Val	L Leu	Gln	TTG Leu	CAT His	GAC Asp	CTG Leu	AAA Lys	CGG Arg	GAG Glu	GCA Ala	GGA Gly	GGC Gly	GGC Gly	TAT Tyr	CGC Arg	TGC Cys	GTG Val	GCG Ala	TCT Ser	GTG Val	CCC Pro	AGC Ser	ATA Ile	CCC Pro	GGC Gly	CTG Leu	AAC Asn	CGC Arg	418
	1255	ACA CAG Thr Glu	G CTG	GTC Val	AAG Lys	CTG Leu	GCC Ala	ATT Ile	TTT Phe	GGC Gly	CCC Pro	CCT Pro	TGG Trp	ATG Met	GCA Ala	TTC Phe	AAG Lys	GAG Glu	AGG Arg	AAG Lys	GTG Val	TGG Trp	GTG Val	AAA Lys	GAG Glu	AAT Asn	ATG Met	GTG Val	TTG Leu	AAT Asn	448
	1345	CTG TC Leu Sei	f TGT Cys	GAA Glu	GCG Ala	TCA Ser	GGG Gly	CAC His	CCC Pro	CGG Arg	CCC Pro	ACC Thr	ATC Ile	TCC Ser	TGG Trp	AAC Asn	GTC Val	AAC Asn	GGC Gly	ACG Thr	GCA Ala	AGT Ser	GAA Glu	CAA Gln	GAC Asp	CAA Gln	GAT Asp	CCA Pro	CAG Gln	CGA Arg	478
	1435	GTC CTC Val Lev	G AGC	ACC Thr	CTG Leu	AAT Asn	GTC Val	CTC Leu	GTG Val	ACC Thr	CCG Pro	GAG Glu	CTG Leu	TTG Leu	GAG Glu	ACA Thr	GGT Gly	GTT Val	GAA Glu	TGC Cys	ACG Thr	GCC Ala	TCC Ser	AAC Asn	GAC Asp	CTG Leu	GGC Gly	AAA Lys	AAC Asn	ACC Thr	508
	1525	AGC ATC Ser Ile	C CTC	TTC Phe	CTG Leu	GAG Glu	CTG Leu	GTC Val	AAT Aşn	TTA Leu	ACC Thr	ACC Thr	CTC Leu	ACA Thr	CCA Pro	GAC Asp	TCC Ser	AAC Aşn	ACA Thr	ACC Thr	ACT Thr	GGC Gly	CTC Leu	AGC Ser	ACT Thr	TCC Ser	ACT Thr	GCC Ala	AGT Ser	CCT Pro	538
	1615	CAT ACC His Th	AGA	GCC Ala	AAC Asn	AGC Ser	ACC Thr	TCC Ser	ACA Thr	GAG Glu	AGA Arr	AAG Lys	CTG Leu	CCG Pro	GAG Glu	CCG Pro	GAG Glu	★ AGC Ser	CGG	GGC Glv	GTG Val	GTC Val	ATC Ile	GTG Val	GCT	GTG Val	ATT	GTG Val	TGC Cvs	ATC	568
	1705	CTG GT	CTG	GCG	H GTG	CTG	GGC	GCT	GTC	стс	TAT	TTC	стс	TAT	AAG	AAG	GGC	AAG	GCT	GCC	GTG	CAG	GCG	стс	AGG	GAA	GCA	GGA	GAT	CAC	
	1795	GCT GCC		GTC	TCG	TAA	GACO	GAAC	TTG	TAGT	IGAAG	TTA/	GTC/	IYI AGATA	Lys	CCC	AGAA	GAGA1	IGGGG	CTC	TGC/	GIN	GCAG	CGG:	IGAC/	AGA	GGC1	CCGG	ASP GAGA	ACCA	298
	1908	Ala Ala	A Pro	Val ACATO	Ser CGATO	END	GCA	ITAGO		GAATO	CACTI	CAGO	TCCC	CTTCC	CTGC	CTG	GACC	ATTCO	CCAGO	TCC	TGC	CAC	CTTC	TCTO	CAGCO	CAAA	CTC/	AAGO	GACI	TAGA	603
	2027	GAGAAG	CTCC	TGCT	ccci	rcgco	CTGC/	ACACO	ccc:	ITTC/	AGAGO	GCC/	CTG	GTT/	AGGAC	CTG	GGA	CTC	ACTTO	GCC	CTGC/	AGGO	ccòc	TTT	ICAGO	GACO	AGTO	CACO	ACCA	ATCT	
	2146	CCTCCA	GTTG.	AGTG	AAGC	CATO	CCA	AGCA	GGAG	SCCCC	CAGTO	TCCC	GAGO	GGG	CAGG/	GAG	TTC	TGC/	AGAA	GTG	TTTT	TCT	TACA	CAC	ATTA	IGCTO	TAA/	TAC	CTC	STCC	
	2265	TGCCAG	CAGCT	GAGC	IGGGI	AGCO	CTCTC	CTGAC	SCTG	GTTTC	CTG	:ccc/	AAGO	SCTGO	SCAT 1	CCAC	CAT	CAG	GTGC/	ACCA	CTGA	GTG	GGAC		CCGG	AGCC/	GGCC	CCTO	CTC/	ATGT	
	2384	TGAAGTO	CGCT	GTTC/	ACACO	CGC1	ICCGO	GAGAG	SCACO	CCCAC	GCAGO	ATCO	CAGA	GCAC	SCTGC	AGTO	SCAA	CTTO	GCAT	SCCT	SCGTO	STTGO	TGCA	CCA	CCCTO	CTG	CTGC	CTCI	TCA/	AAGT	
	2503	CTCCTG	GACA	TTTT	TCT	TGG	ICAG/	AGGCC	CAGG	AACTO	GTGTC	ATTO	CTT/	AAGA	ATACO	TGCO	CGGGG	SCCA	GTG	IGGC:	ICACO	ССТО	TAAT	CCC1	AGCAC	TTTC	GGA	GCCC	GAGGC	CGGC	
	2622	GGATCA	CAAAG	TCAG	ACGAC	SACC/	ATCCI	IGGC1	CAAC/	ACGG1	IGAA/	CCC1	GTC	CTAC	CTAA/	AATA	CAA	-	AAAT	TAGC:	TAGGO	GTA	TGGI	TGGG	CACCI	TATA	TCCF	AGC	ACTO	CGGA	
	2741	AGGCTG	AGCA	GGAG	AATGO	TATO	GAATO	CCAGO	GAGG:	IGGAC	SCTTO	CAGI	GAGO	CGA	GACCO	TGCC	CACTO	SCAC1	ICCA	CCT	GGGC/	ACAG	AGCO	AGAG	CTCCC	STCTO	GAG	GAAA/	AAA/	AAT	
	2860	CGTGCT	GTAG	CAGCI	rGGC1	CTG	TTCO	GAGTO	AGG!	IGAA!	TAGO	CTCA	ATCO	CCG	GTTC	ACTI	GCT	CCA	TAGCO	CTC	TGA	GGA1	CACG	TAA	ACTO	GAAA	GCAC	CGGC	GAGO	CAGA	
	2979	CAAAGA	GAGG		CCCC	-1001 	TTCA!	IGGGG	SATT/	AAGO	TATO	GTT	TAT		ACCAP	ACTI	ICTA(CAA	JCTC/	AGGAC		ACCCT		AGGGG	CCA	ATGA	GAGA	ATGO	TAC	
	3217	ATATAT	TATC		.0000C		501A(GIUI	TAA	1016] 10777		-ATA(AIA				1001]	I I I G	CAGG	91GT(JIAA /	AI ITO	-CAA/	1161	TTCC	LITT	
P	5411				.nini	am		-111	aid.										ഹസ		•										
D	2847	CCGGCCG	GTTG	CGCGC	GCCC	TCGO	GACCO	CTCAG	GAGAG	GCG/	AGGGT	TCG	GGGG	CACGA	GTTC	GAGO	CCA/	ACCTO	GTC		rggg1	TGA		AA							

FIG. 6. Nucleotide and deduced amino acid sequence of the MUC18 glycoprotein. (A) Nucleotides are numbered at left and amino acids are at right of the sequence, starting with the initiation codon and the corresponding methionine, respectively. The hydrophobic putative signal peptide and transmembrane sequences are underlined. The potential signal peptide cleavage site between Ala-28 and Glu-29 is marked with an arrow. Nine potential asparagine-linked glycosylation sites are indicated by asterisks. The consensus polyadenylylation site AATAAA at position +3264 is marked with a bold line. (B) Divergent 3' nucleotide sequence of cDNA clone drop4.7, which begins at position +2847 (marked with an arrow in A).

residues with a calculated molecular weight of 63,195. This is in excellent agreement with the observed size of 65 kDa for the deglycosylated MUC18 (glycopeptidase F digestion, data not shown). The deduced amino acid sequence includes a second stretch of 24 hydrophobic residues characteristic for a membrane-spanning region and eight sites for putative asparaginelinked glycosylation (Asn-Xaa-Ser/Thr) that are located in the extracellular region of the molecule. Due to proline in the Asn-Pro-Ser sequence at positions 286–288, this site will not be used for glycosylation (23).

MUC18 Is a Member of the Immunoglobulin Superfamily. The MUC18 molecule fulfills all criteria proposed for membership in the immunoglobulin superfamily (24). The entire extracellular part of MUC18 is constructed from five domains, all homologous to each other and to immunoglobulinlike domains (Fig. 7). Domains I and II of the MUC18 molecule belong to the V-set sequences and the domains is approximately 100 amino acids long and contains two appropriately spaced cysteines as well as conserved residues flanking the cysteines. The observed change in the apparent molecular mass from 113 kDa under reducing conditions to 100 kDa under nonreducing conditions in SDS/PAGE (7) strongly suggests the presence of intrachain disulfide bonds in the MUC18 molecule. The algorithim of Chou and Fasman (25) to predict protein secondary structure indicates predom-



FIG. 7. Sequence homology between five repeated domains in the MUC18 molecule and relationship to two immunoglobulin-like domains of neural cell adhesion molecule (N-CAM) and myelin-associated glycoprotein (MAG). Sequences were visually aligned to show maximum internal homology. Boxed amino acids correspond to residues shared by three or more sequences. The middle portion of each domain was omitted for simplicity, and the number of amino acids not shown is indicated. The position of the putative disulfide bond is indicated by S . . . S. The location of the known β -strands in immunoglobulin domains is marked below the aligned sequences with dashed lines. The single-letter amino acid sequence is used.

inantly β -strands in each of the MUC18 domains, fulfilling the prediction for an immunoglobulin domain.

A search of the Max Planck and Martinsried Institute Protein Sequence Data Bank (MIPSZ; release 9) using the FASTP program (26) detected no significant similarities outside the immunoglobulin superfamily. Among the immunoglobulin-related molecules, MUC18 shows the greatest similarity to the cell adhesion molecules of the nervous system neural cell adhesion molecule (N-CAM) (27), L1 (28), amalgam (29), fasciclin II (30), contactin (31), and myelinassociated glycoprotein (MAG) (32) and to human carcinoembryonic antigen (CEA) (33), which has also been shown to mediate cell adhesion (34) (Table 1). The immunoglobulinrelated molecules are thought to have been derived by gene duplication and divergence from an archetypal gene. Within the superfamily, some molecules show greater than average similarity suggesting a more recent common origin. One such group consists of the neural cell adhesion molecules that share functional as well as structural properties. The close structural relationship of MUC18 to these adhesion molecules predicts that MUC18, like these molecules, may also be developmentally regulated and involved in intercellular adhesion. Melanocytes are derivatives of the neural crest and MUC18 expression by melanomas may reflect a stage in their ontogeny. If the MUC18 molecule is indeed involved in adhesive interactions, its expression may allow melanoma cells to interact with cellular elements of the vascular system (where MUC18 is also expressed) thereby enhancing hematogeneous tumor spread.

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Table 1. Proteins with sequence similarity to MUC18 glycoprotein

Molecule	Identity, %	Overlap amino acids, no.	Optimized score	SD value
N-CAM	25.5	133	133	22.1
CEA	20.2	297	160	19.7
L1	22.6	288	172	17.2
Amalgam	26.0	169	133	15.4
Fasciclin II	16.2	229	132	14.7
Contactin	22.2	117	79	13.3
MAG	21.8	362	130	11.9

Comparisons were performed using the FASTP and RELATE programs (26). The optimized score is obtained when one takes deletions and insertions into account (that is, gaps). It is the usual output from the FASTP program and is discussed in ref. 26. SD values greater than 10 are considered to indicate significant sequence homology. N-CAM, neural cell adhesion molecule; CEA, carcinoembryonic antigen; MAG, myelin-associated glycoprotein.

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