FEBS Letters 446 (1999) 292–298 FEBS 21698

TM7XN1, a novel human EGF-TM7-like cDNA, detected with mRNA differential display using human melanoma cell lines with different metastatic potential

Albert J.W. Zendman[a,*], Ine M.H.A. Cornelissen[a], Ulrich H. Weidle[b], Dirk J. Ruiter[a], Goos N.P. van Muijen[a]

[a] *Department of Pathology, University Hospital, P.O. Box 9101, 6500 HB Nijmegen, The Netherlands*
[b] *Roche Diagnostics GmbH, Pharma Research, Nonnenwald 2, D-82377 Penzberg, Germany*

Received 12 November 1998; received in revised form 10 February 1999

Abstract We have identified a novel 3845 bp cDNA differentially expressed in a human melanoma metastasis model. Northern blot analysis showed expression in the poorly and intermediately metastasizing cell lines and a marked down-regulation in the highly metastatic cell lines. Using RT-PCR expression was also seen in several other tumor cell lines and normal cell types of human origin. cDNA sequence analysis revealed an ORF of 687 amino acids containing seven putative transmembrane domains C-terminally and a long N-terminus. The gene was mapped to 16q13. Highest homology was observed with members of the EGF-TM7 subfamily of the secretin/calcitonin receptor family. We propose the delineation of a subfamily of TM7 proteins, LN-TM7, containing seven transmembrane proteins with a long N-terminal extracellular part.

Key words: mRNA differential display; Melanoma; TM7 protein; cDNA cloning

1. Introduction

Several genes or gene products have been identified which are differentially expressed during melanocytic tumor progression [1]. To identify differentially expressed genes various methods have been used at the DNA, mRNA and protein levels. Among the strategies used differential hybridization, subtractive hybridization, mRNA differential display and serial analysis of gene expression [2] are the most common. In oncobiology these techniques have been successfully used in the identification and subsequent isolation of differentially expressed genes in several types of tumors [3–10]. To pick up possible human melanocytic tumor progression markers we performed mRNA differential display [11], comparing two human melanoma cell lines: 1F6 and a spontaneous mutant of this cell line, 1F6m. Whereas 1F6 only sporadically gives rise to metastases after s.c. inoculation into nude mice, 1F6m metastasizes very frequently and quickly [12].

Here we describe a new cDNA, TM7XN1, which inversely correlates with the metastatic potential in a panel of human melanoma cell lines. Sequence analysis and database searching showed highest homology to members of the EGF-TM7 (seven transmembrane molecules containing epidermal growth factor-like domains) subfamily of the secretin peptide hormone receptor family [13]. Based on extensive sequence and homology searching, we argue for the delineation of a novel subfamily of seven transmembrane receptors, named LN-TM7 (long N-terminus), including the novel TM7XN1 and the EGF-TM7 molecules.

*Corresponding author. Fax: (31) (24) 3540520.
E-mail: h.zendman@pathol.azn.nl

EMBL/Genbank data library accession number for TM7XN1: AJ011001.

2. Materials and methods

2.1. Melanoma cell lines

Twenty-three human melanoma cell lines were used. A panel of eight melanoma cell lines containing 530, 1F6, MV1, M14, Mel57, BLM, MV3, and 1F6m was described earlier [12]. In this panel of cell lines 530 and 1F6 are poorly metastatic, while MV3 and BLM are highly metastatic cell lines. MV1, M14, and Mel57 are cell lines with an intermediate metastatic capacity. 1F6m, a metastatic subline of 1F6, can be placed between the intermediate and highly metastatic cell lines. All other human melanoma and non-melanoma cell lines used are listed in Table 1 [12,14–20].

All cell lines were grown in Dulbecco's modified Eagle's medium as described earlier [14]. Cultured pericytes, smooth muscle cells and endothelial cells were a kind gift from Dr. M. Verbeek [21]. Cell line U2OS and dendritic cells were a generous gift from Dr. F. Hartgers (Department of Tumor Immunology, Academical Hospital Nijmegen, The Netherlands). Keratinocytes, melanocytes and nevus cells were cultured as described previously [22]. Fibroblasts were grown on Eagle's minimum essential medium (BioWhittaker, Walkersville, MD, USA) supplemented with 20% human serum, 10% fetal calf serum, and antibiotics.

2.2. Human tissues

Lesions from all stages of melanocytic tumor progression (common nevi, atypical nevi, primary melanoma and melanoma metastases) were excised from patients at the University Hospital Nijmegen, The Netherlands. As normal human tissues we used disease-free samples from surgically removed tissues or from autopsies with post-mortem delays shorter than 4 h. Tissue samples were snap-frozen in liquid nitrogen and stored at −80°C until use.

2.3. RNA isolation

From cultured cells total RNA was isolated using the RNeasy kit (Qiagen, Hilden, Germany) following the manufacturer's protocol. From tissue samples total RNA was isolated (following the manufacturer's protocol) by disrupting about 25 frozen sections of 20 μm thickness in 1 ml RNAzol B (Campro, Veenendaal, The Netherlands) using a pestle. The RNAzol B method was followed by an additional RNeasy cleaning step of the RNA.

2.4. mRNA differential display

Before starting differential display PCR, DNase I treatment was performed on the RNA samples using the Message-Clean kit (GenHunter Corporation, Brookline, MA, USA). For differential display the RNAmap protocol (GenHunter) was used with some minor modifications. Differing from the original protocol, we used [^{32}P]dATP instead of [^{35}S]dATP. For the PCR, combinations of the four T_{12}MN primers together with six arbitrary primers, $AP_{1,2,6,7,11,12}$ [23], were used.

PII: S0014-5793(99)00230-6

2.5. Northern blot analysis

Total RNA (10 μg) was treated with glyoxal/DMSO or with formaldehyde [24], separated on a 1.2% agarose gel and blotted onto a Hybond N^+ membrane (Amersham, Aylesbury, UK). cDNA probes were radiolabeled by [^{32}P]dATP incorporation using a random-primed DNA labeling kit (Roche Diagnostics GmbH, Penzberg, Germany). Membranes were hybridized overnight with the radiolabeled probes at 65°C in a hybridization mix (0.25 M sodium phosphate buffer pH 7.2, 7% SDS, 1% BSA, 1 mM EDTA, 0.1 mg/ml single stranded salmon sperm DNA). Afterwards membranes were washed at 65°C with wash buffers containing decreasing amounts of salt (1% SDS, 1 mM EDTA and 0.25–0.05 M sodium phosphate pH 7.2), and autoradiographed using Kodak Xomat-S films.

2.6. cDNA library screening, sequencing and homology searching

cDNA probes were labeled as described before and hybridized [24] to a λZAP cDNA library of human melanoma cell line 530, kindly provided by Dr. G. Swart, Department of Biochemistry, University of Nijmegen, The Netherlands. After isolation of the full length cDNA, sequencing on both strands was performed by Roche Diagnostics GmbH, Penzberg, Germany. Homology searches were performed using BLAST [25] and software on all kinds of public servers of DNA and protein databases on Internet (http://www.genome.ad.jp/; http://www.ncbi.nlm.nih.gov/; http://expasy.hcuge.ch/; http://dot.imgen.bcm.tmc.edu:9331/; http://www.genome.wi.mit.edu/).

2.7. RT-PCR

Synthesis of cDNA (10 min at 25°C, followed by 59 min at 42°C) was performed on 0.5–1.0 μg of total RNA using the AMV RT kit (Roche Diagnostics GmbH, Penzberg, Germany). The reaction mixture was supplemented with 0.04 U of random hexadeoxynucleotide primers, 2 μl 25 mM $MgCl_2$, 1 μl 10 mM dNTPs, 1 μl of RT buffer (100 mM Tris-HCl pH 8.3, 500 μM KCl), 25 U RNasin, 10 U AMV reverse transcriptase and water to a final volume of 10 μl. For amplification one tenth of the cDNA was supplemented with 2.5 μl of PCR buffer (200 mM $(NH_4)_2SO_4$, 750 mM Tris-HCl pH 9, 0.1% Tween), 5 μl 1 M dNTPs, 10 pmol of each primer, 2.5 μl 15 mM $MgCl_2$, 0.15 U of Thermoperfectplus DNA polymerase (Integro, Zaandam, The Netherlands) and water to a final volume of 25 μl. PCR conditions were 45 s at 94°C, 1 min at 59°C and 1 min 30 s at 72°C for 30 cycles. These cycles were preceded by 3 min denaturation at 94°C and followed by a 5 min elongation step at 72°C. Primer combinations and PCR product lengths were: β_2-microglobulin (136 bp): sense: 5′-CTCGCGCTACTCTCTCTTTCT-3′; antisense: 5′-TGTCGGATTGATGAAACCCAG-3′; TM7XN1 cDNA (474 bp): sense: 5′-CCATCTTTCTGGTGACGC-3′; antisense: 5′-GAGCTGATGGGGAGCCTG-3′. DNA molecular weight markers were from Roche Diagnostics GmbH, Penzberg, Germany.

2.8. Chromosomal localization

Chromosomal localization of the corresponding gene was determined by genomic PCR on a panel of hamster/human and mouse/human hybrid cell lines [26]. DNA of these cell lines, each specific for one human chromosome, was kindly provided by Dr. A. Simons, Department of Human Genetics, University Hospital Nijmegen, The Netherlands. PCR conditions were the same as used for the cDNA primer combinations described above. Intron spanning primers and PCR product length were: TM7XN1 DNA (526 bp): sense: 5′-GCTCTGTCTCTCGTGGTC-3′; antisense: 5′-GCATGGTCCACAGTTCTTG-3′.

3. Results and discussion

3.1. Isolation and cloning of TM7XN1 cDNA

With mRNA differential display, using primer combination $T_{12}MG$ and AP_7, we detected a 250 bp cDNA (clone 25) abundantly present in 1F6 and almost absent in 1F6m (not shown). Using this 250 bp cDNA clone as a probe a Northern blot, containing RNA from a panel of human melanoma cell lines with varying metastatic potential, revealed two bands at 4.2 kb and 1.0 kb (Fig. 1A). Marked expression of the 4.2 kb transcript was only seen in the poorly and intermediately metastatic cell lines 530, 1F6, MV1 and M14. No expression could be detected in the highly metastatic cell lines MV3 and BLM. Very weak expression was seen in 1F6m cells. To investigate the specificity of the 1.0 kb band we did Northern blot analyses of one poorly (530), one intermediately (MV1) and two highly (MV3, BLM) metastatic cell lines with three different PCR probes throughout the full length cDNA. The probes represented the N-terminal part of the open reading frame (ORF) (bp 351–979), the seven transmembrane region (bp 1878–2352) and the 3′-UTR (untranslated region) (bp 3339–3689). With all three probes we could no longer detect the 1 kb band, while the 4.2 kb band showed the same expression pattern. As an example we show the results for the probe corresponding to the 3′-UTR (Fig. 1B). From these results we concluded that the 1 kb signal was a background hybridization of clone 25, the 250 bp probe (corresponding to the last part of the 3′-UTR and part of the poly-A tail). Using the 250 bp cDNA clone as a probe we screened a λZAP cDNA library of human melanoma cell line 530, in which marked expression of the 4.2 kb transcript was detected on Northern blot. We isolated a 3845 bp cDNA and sequenced this clone (EMBL: AJ011001). The original 250 bp clone matched completely with the 3′ part of the full length cDNA. A variable poly-A tail may be the explanation for the difference in length of the estimated 4.2 kb on Northern and the 3845 bp cloned cDNA. The cDNA contains an ORF resulting in a protein of 687 amino acids (Fig. 2) including seven putative C-terminal transmembrane domains (aa 405–

Table 1
Expression of TM7XN1 in human tumor cell lines, primary cell cultures and organ tissues determined by RT-PCR[a]

	Strong	Moderate	Weak	Negative
Melanoma cell lines	1F6 [12]; 451Lu [19]; 518A2 [14]; 530 [12]; 603 [14]; A375m*; A375p*; Bowes*; M24met [14]; Mel57 [12]; MKR [20]; MV1 [12]; OCM1 [17]; OMM1 [18]; WM164 [19]	1F6m [12]; E10 [14]; M14 [12]; ZKR [20]	BLM [12]; BRO [16]; MV3 [12]; SK-Mel-28*	
Other tumor cell lines	A431*; CaCo2*; HT29 [15]; Molt4*; Umscc2 [14]	Hep/G2 [15]; HT1080 [15]	U2OS [15]	Daudi*; Jurkat*; K562 [15]; U937 [15]
Primary cell cultures	keratinocytes; nevus cells; endothelial cells; melanocytes	fibroblasts	PBMCs[b]; pericytes; smooth muscle cells	dendritic cells
Organ tissue		kidney; prostate; testis	bladder; brain; lungs; thyroid gland; uterus	bone marrow; colon; heart; ileum; liver; lymph node; pancreas; spleen; stomach

[a]All samples had a positive control PCR (β2M).
[b]PBMC, peripheral blood mononuclear cells; *cell lines from the American Type Culture Collection (Rockville, MD).

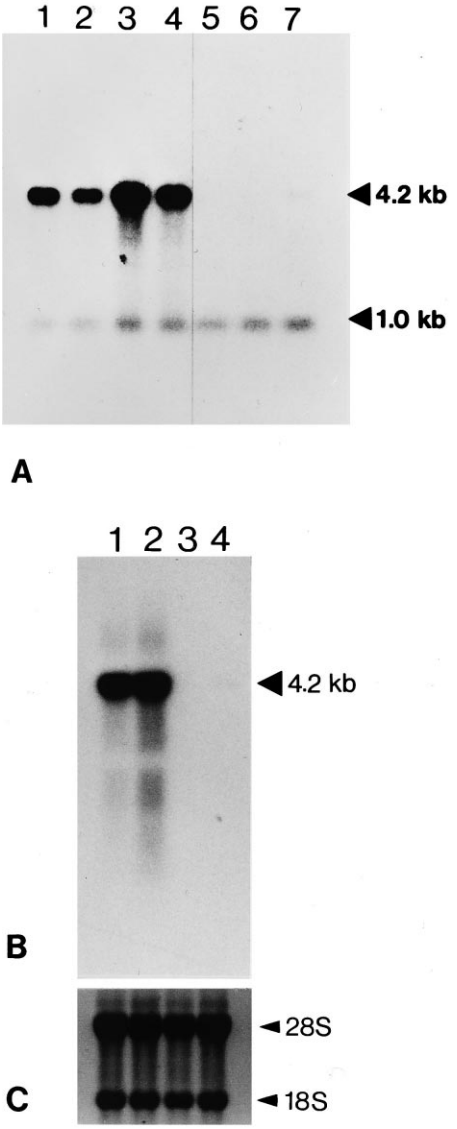

Fig. 1. Northern blot analysis of clone 25 on a panel of human melanoma cell lines with different metastatic capacity after subcutaneous inoculation into nude mice [12]. A: Glyoxal/DMSO blot hybridized with clone 25, the 250 bp differential display cDNA. Lane 1: 530; lane 2: 1F6; lane 3: MV1; lane 4: M14; lane 5: MV3; lane 6: BLM; lane 7: 1F6m. B: Formaldehyde blot hybridized with PCR fragment corresponding to the 3′-UTR (bp 3339–3689) of the full length cDNA. Lane 1: 530; lane 2: MV1; lane 3: MV3; lane 4: BLM. C: 28S and 18S rRNA bands of ethidium-stained formaldehyde gel.

656) and an N-terminal signal peptide (aa 1–25). The protein has a very high leucine and serine content of respectively 14.8 and 11.1%. Using MOTIFFINDER (http://www.motif.genome.ad.jp) we found, besides several phosphorylation sites, seven potential *N*-glycosylation sites in the N-terminal part. The short C-terminus contains some phosphorylation sites and a putative AMP binding domain (aa 675–686) which, together with a potential tyrosine kinase phosphorylation site (aa 546) between TM4 and TM5, may be indicative for interaction with signalling components. It also has a microbodies C-terminal targeting site (aa 685–687). This site is normally involved in guidance of proteins towards single membraned organelles. The PSORT II server (http://psort.nibb.ac.jp) predicted TM7XN1 to be present in the membrane of the endoplasmic reticulum or in the plasma membrane. Determination of the specific localization of TM7XN1 protein needs further studies.

3.2. Homology search

Homology and domain searches revealed a perfect homology with many human ESTs from several types of both normal and tumor tissues. No functional clues from homology to known domains were found in the long N-terminal part. We could place the gene in the secretin/calcitonin receptor family because of its strong C-terminal homology, especially towards members of the recently described EGF-TM7 subfamily [13] and other non-classical TM7 proteins. Next to the 30% identity in the C-terminal seven transmembrane part it also had a long N-terminal part, though it lacked the EGF-like domains which characterize the EGF-TM7 family. Based on these finding we named the molecule TM7XN1, in which the X represents the lack of known domains (no EGF-like domains) and unclear function of the long N-terminal part of the protein.

3.3. TM7XN1 expression

Expression of TM7XN1 was tested on a great number of human cell types and tumor cell lines using RT-PCR. On a melanoma cell line panel with known metastatic behavior, we found a clear expression in the poorly and intermediately metastatic cell lines 530, 1F6, MV1 and Mel57 (Fig. 3). Expression in M14 and 1F6m was somewhat lower. Though Northern blot was negative for MV3 and BLM (Fig. 1), using the more sensitive RT-PCR we could detect some weak expression in these highly metastatic cell lines (Fig. 3). Expression in 1F6m is higher than we expected on the basis of the expression detected with the more quantitative Northern blot. We think this is due to instability of the 1F6m cell line, as the RNA used for the RT-PCR was isolated from 1F6m cells of a much later passage than the one used for differential display and Northern blot. Based on the Northern blot results with the melanoma cell line panel TM7XN1 expression inversely correlates with the metastatic potential after s.c. inoculation

```
  1 MTPQSLLQTTLFLLSLLFLVQGAHGRGHREDFRFCSQRNQTHRSSLHYKP  50
 51 TPDLRISIENSEEALTVHAPFPAAHPASRSFPDPRGLYHFCLYWNRHAGR 100
101 LHLLYGKRDFLLSDKASSLLCFQHQEESLAQGPPLLATSVTSWWSPQNIS 150
151 LPSAASFTFSFHSPPHTAAHNASVDMCELKRDLQLLSQFLKHPQKASRRP 200
201 SAAPASQQLQSLESKLTSVRFMGDMVSFEEDRINATVWKLQPTAGLQDLH 250
251 IHSRQEEEQSEIMEYSVLLPRTLFQRTKGRRGEAEKRLLLVDFSSQALFQ 300
301 DKNSSHVLGEKVLGIVVQNTKVANLTEPVVLTFQHQLQPKNVTLQCVFWV 350
351 EDPTLSSPGHWSSAGCETVRRETQTSCFCNHLTYFAVLMVSSVEVDAVHK 400
401 HYLSLLSYVGCVVSALACLVTIAAYLCSRRKPRDYTIKVHMNLLLAVFLL 450
451 DTSFLLSEPVALTGSEAGCRASAIFLHFSLLTCLSWMGLEGYNLYRLVVE 500
501 VFGTYVPGYLLKLSAMGWGFPIFLVTLVALVDVDNYGPIILAVHRTPEGV 550
551 IYPSMCWIRDSLVSYIITNLGLFSLVFLFNMAMLATMVVQILRLRPHTQKW 600
601 SHVLTLLGLSLVLGLPWALIFFSFASGTFQLVVLYLFSIITSFQGFLIFI 650
651 WYWSMRLQARGGPSPLKSNSDSARLPISSGSTSSSRI              687
```

Fig. 2. Protein sequence of TM7XN1. Indicated are the signal peptide (bold; arrow), potential glycosylation sites (arrowhead), cysteine box (open box) and transmembrane regions (underlined).

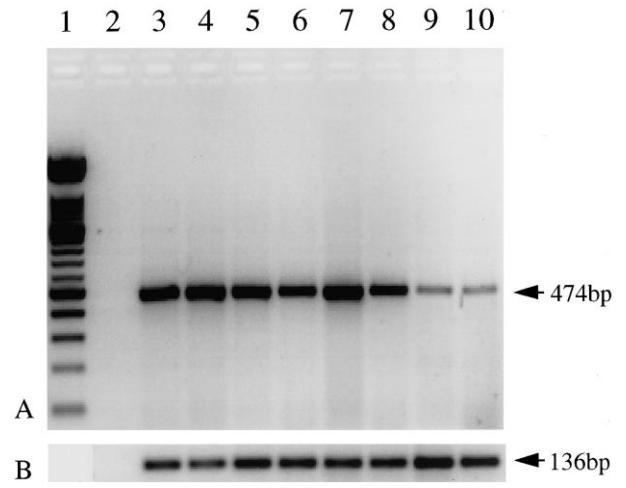

Fig. 3. A: Expression of TM7XN1 in human melanoma cell lines with different metastatic potential using RT-PCR. Lane 1: DNA molecular weight marker XIV (100 bp ladder); lane 2: water control; lane 3: 530; lane 4: 1F6; lane 5: MV1; lane 6: M14; lane 7: Mel57; lane 8: 1F6m; lane 9: MV3; lane 10: BLM. B: β_2-Microglobulin control RT-PCR.

into nude mice. RT-PCR on RNA isolated from corresponding xenografts of the cell line panel matched completely with the results of the cultured cells (not shown). RT-PCR results on expression of TM7XN1 in a great number of other cultured normal human cells and cell lines are shown in Table 1. All other human melanoma cell lines showed TM7XN1 expression, being weak in only four of all melanoma cell lines tested (MV3, SK-mel-28, BLM and its parental cell line BRO). Most cell lines derived from other tumor types also expressed TM7XN1. In primary cell cultures strong expression was observed in keratinocytes, melanocytes, nevus cells and endothelial cells (Table 1), while weak expression was found in fibroblasts, smooth muscle cells, pericytes, and peripheral blood mononuclear cells. Dendritic cells (non-stimulated) were negative. In normal human tissues clear expression was seen in kidney, prostate and testis, weak expression in bladder, brain, lungs, uterus and thyroid glands and no expression in bone marrow, colon, heart, ileum, liver, lymph node, pancreas, spleen, and stomach. We also studied TM7XN1 mRNA expression in fresh human melanocytic lesions with RT-PCR. TM7XN1 expression was detected in all stages of melanocytic tumor progression (not shown). Since not only melanocytic cells but also other cell types present in these lesions (keratinocytes, fibroblasts, endothelial cells) may express TM7XN1 (see Table 1) we need additional studies to determine whether there are differences in expression between melanocytic cells in the different stages of tumor progression. For this purpose we are currently raising antibodies against TM7XN1 to be able to study expression of the corresponding protein at the single cell level.

Our data indicate that TM7XN1 is widely distributed and expressed in a great number of both normal and tumor cells and tissues. This finding is in line with the perfect homology of the gene with numerous human ESTs. Expression of the best studied EGF-TM7 molecules (CD97 and EMR1) is

Table 2
Characteristics of TM7XN1 homologues

	Protein	Species	EMBL accession number	length (aa)	Signal peptide (aa)	N-terminus (aa)	EGF domain	cys-box	Miscellaneous domains[a]	Ref.
Secretin rec.	Calcitonin r.	*H. sapiens*	L00587	490	22	132	–	−		[29]
	Secretin r.	*H. sapiens*	U20178	440	22	121	–	−		[30]
LN-TM7	**TM7XN1**	*H. sapiens*	**AJ011001**	687	26	381	–	+		-
	HE6	*H. sapiens*	X81892	1014	37	587	–	+		[31]
	Latrophilin	*R. norvegicus*	U78105	1466	24	831	–	+		[32]
	KIAA0786*	*H. sapiens*	AB018329	>1021	–	>410	?	+	?	[33]
	KIAA0768*	*H. sapiens*	AB018311	>872	–	>290	?	+	?	[33]
	BAI-1	*H. sapiens*	AB005297	1584	25	924	–	+	RGD; TSP1 (5×)	[34]
	BAI-2	*H. sapiens*	AB005298	1572	23	900	–	+	extra TMs; L-zipper; TSP1 (5×)	[35]
	BAI-3	*H. sapiens*	AB005299	1522	24	855	–	+	TSP1 (5×)	[35]
	B0286.2	*C. elegans*	U39848	1582	–	907	–	+	extra TM	[36]
	B0457.1	*C. elegans*	Z54306	1014	29	522	–	+		[36]
	F31D5.5	*C. elegans*	U28941	1817	50	1473	–	−	RGD	[36]
	BOSS	*D. melanogaster*	X55887	896	37	498	–	−		[37]
	GRL101	*L. stagnalis*	Z23104	1115	22	747	–	−	LDL-RA (12×); LRR (6×)	[38]
EGF-TM7	CD97	*H. sapiens*	X84700	742	20	440	3–5	+	RGD	[39]
	EMR1	*H. sapiens*	X81479	886	17	583	1–6	+		[40]
	KIAA-0279*	*H. sapiens*	D87469	2408	21	1842	6	+	cadherin (4×); L-zipper	[28]
	CELSR1	*M. musculus*	AF031572	3034	26	2459	7	+	cadherin (7×); laminin AG (2×)	[41]
	R29368.2*	*H. sapiens*	AC004262	>344	–	>58	?	+	?	[42]

[a]RGD = Arg-Gly-Asp; TSP1 = thrombospondin type 1; TM = transmembrane; L-zipper = Leucine zipper; LDL-RA = low density lipoprotein class A; LRR = leucine-rich repeats; cadherin = cadherin-like domain; laminin AG = laminin A G-domain.
*Only partial protein sequence known.

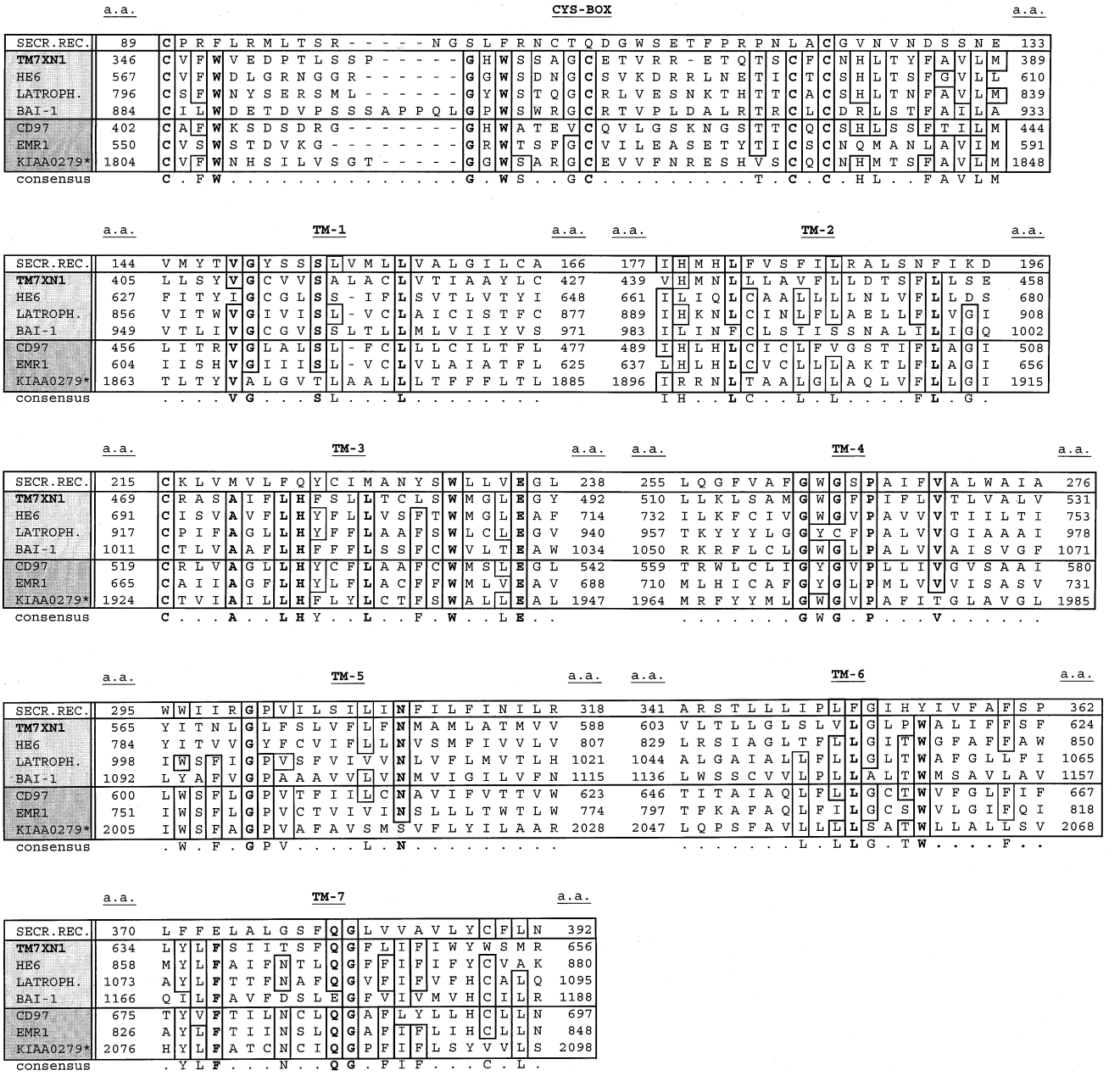

Fig. 4. Multiple protein alignment of the cys-box and the transmembrane regions of the secretin receptor (white), LN-TM7 family members (light gray), and EGF-TM7 family members (gray). Strongly conserved amino acid residues are boxed and bold; frequently homologues residues are only boxed. Gaps (-) are introduced to allow maximal alignment. * only partial protein is known.

mainly seen in cells of the hematopoietic lineage and in solid-tumor-derived cell lines [13]. They are described as leukocyte antigens with a possible immunologic role. Recently CD97 expression has also been described in thyroid tumors [27]. As for CD97, expression in (para)thyroid tumors is also the case for KIAA0279 (AA843289; EMBL), which like TM7XN1 is mainly expressed in testis, brain, kidney, and prostate [28].

3.4. Chromosomal localization

Inspection of the human gene map (NCBI) showed perfect homology of TM7XN1 with an unidentified transcript. This STS (sequence tagged site) was called STSG1704 and located on 16q13 between microsatellite markers D16S419 and D16S408 (65–72 cM). To confirm localization of TM7XN1 on chromosome 16, we performed an intron spanning PCR on DNA of a human chromosome specific hybrid cell panel (hamster/human or mouse/human). We could only detect the 526 bp specific product in the DNA sample of the chromosome 16 specific hybrid cell line (not shown). Some EGF-TM7 molecules map on chromosome 19p13, indicating the position of a EGF-TM7 gene cluster on the short arm of chromosome 19.

3.5. Alignment: LN-TM7

To get more insight into the relationship between TM7XN1, the secretin-like receptors and EGF-TM7 molecules, we compared TM7XN1 with several other family mem-

bers that were found previously and with other proteins that we additionally found during homology searches with parts of TM7XN1 (Table 2) [29–42]. Some proteins of non-human origin were used when no human homologue was known. All sequences aligned have seven C-terminal transmembrane domains. The proteins vary markedly in length, with the secretin and calcitonin receptors (G-protein-coupled receptor family 2) being the smallest. These peptide hormone receptors have a relatively short N-terminus (<200 aa), containing their hormone binding domain. The recently described EGF-TM7 subfamily members have a much longer extracellular part containing several EGF-like domains [13]. Several splice variants of the EGF-TM7 molecules with variable numbers of EGF-like domains have been reported [43]. Our TM7XN1 protein is very homologous to the secretin receptor and to CD97 and EMR1, but lacks any known domain in its long N-terminus. In Table 2 we have made a separation between EGF-TM7 members and molecules with a long N-terminus lacking EGF-like domains, LN-TM7. Their N-terminus is longer than 300 bp and most proteins contain a so-called cysteine box (cys-box): C-x_{14-24}-C-x_{11}-C-x-C [31]. Cleavable signal peptides were found in all proteins of which the start of translation is known [44], except for B0286.2. Additional EGF-TM7 molecules are the human KIAA0279 and R29368.2 as well as mouse CELSR1. Because R29368.2 has 50% identity with EMR1 and because the gene is located on 19p13, as are CD97 and EMR1, we suspect this gene to be an EGF-TM7 family member. KIAA0279 is not located on chromosome 19p13, but on chromosome 1 [28], while CELSR1 was mapped to human 22qter [41].

Instead of EGF-like domains some of the LN-TM7 molecules possess other known domains (Table 2) in their long N-terminus. These putative dual-function proteins are believed to be formed by genetic rearrangements, like exon shuffling, by which TM7 proteins gained additional domains from other genes [45–47]. Almost all LN- and EGF-TM7 molecules have a highly glycosylated mucin-like region within their N-terminal extracellular part between the membrane-spanning domain and the N-terminal conformational domain(s). This region is often lacking cysteine residues. It was already postulated that the long N-termini capture a ligand and then present this ligand to the binding pocket situated inside the TM7 structure. The peptide hormone receptors do not need such a long N-terminus because of the small peptide ligand [38].

Fig. 4 shows an alignment of the seven transmembrane domains and the cys-box preceding the first membrane domain of some representatives of the subfamilies investigated. All proteins have the characteristic conserved cysteines in loops 2 and 4 (data not shown) which are thought to be necessary for stabilization of the transmembranal conformation of G-protein-coupled receptors [48]. From the alignment of the cysteine box a LN-TM7 specific protein consensus sequence for the cys-box can be distilled: C-x_2-W-x_{6-16}-W-x_4-C-x_{11}-C-x-C. Next to the consensus described earlier [31], some very highly conserved tryptophan residues are also present. Some less well-conserved but very frequent amino acids, such as the FAVLM sequence in the last part of the cys-box, are also indicated in Fig. 4. Based on the comparisons we argue for the delineation of a novel LN-TM7 subfamily of the secretin receptor family. For most members there is no experimental evidence for G-protein coupling. Only latrophilin has been shown to be linked to G-proteins ($G\alpha_0$) and inositol polyphosphate production [32,49]. Since TM7XN1 does not have any known specific functional domain with regard to a possible ligand structure, all reflections about its function are speculative. However, if TM7XN1, like CD97 [50], should function as an adhesion molecule, downregulation as seen in the most aggressive melanoma cell lines might explain its involvement in the metastatic capacity of these tumor cells.

3.6. Summary

TM7XN1 is a novel protein placed in a novel LN-TM7 family of seven transmembrane proteins, probably G-protein-coupled, with long N-terminal domains. These domains are presented to the environment by a long rod-like glycosylated spacer. The whole extracellular domain seems to be carried and stabilized by a cysteine box, serving as a statue foot on top of the seven transmembrane anchor. Although we found a marked downregulation of TM7XN1 expression in the highly metastatic melanoma cell lines, a possible role of TM7XN1 in melanocytic tumor progression needs further studies which are in progress now.

Acknowledgements: We would like to acknowledge Dr. T. Wobbes (Department of Surgery, University Hospital Nijmegen) and Dr. M. de Rooij (Department of Dermatology, University Hospital Nijmegen) for providing fresh surgical specimens. We thank Dr. G. Swart (Department of Biochemistry, University of Nijmegen) for providing the cDNA library of human melanoma cell line 530. Dr. M. Herlyn (The Wistar Institute, Philadelphia, PA, USA) is acknowledged for providing cell lines 451Lu and WM164. For generously supplying cell lines MKR and ZKR we thank Dr. U. Keilholz (Department of Internal Medicine, University of Berlin, Berlin, Germany). We thank Dr. F. Hartgers (Department of Tumor Immunology, University Hospital Nijmegen) who supplied us with RNA of dendritic cells and cell line U2OS, and Dr. A. Simons (Department of Human Genetics, University Hospital Nijmegen) who generously provided DNA from human chromosome specific somatic hybrid cells.

References

[1] Weterman, M.A., van Muijen, G.N., Bloemers, H.P. and Ruiter, D.J. (1994) Lab. Invest. 70, 593–608.
[2] Velculescu, V.E., Zhang, L., Vogelstein, B. and Kinzler, K.W. (1995) Science 270, 484–487.
[3] Shen, R., Su, Z.Z., Olsson, C.A. and Fisher, P.B. (1995) Proc. Natl. Acad. Sci. USA 92, 6778–6782.
[4] Lee, J.H. and Welch, D.R. (1997) Int. J. Cancer 71, 1035–1044.
[5] Zhang, L., Zhou, W., Velculescu, V.E., Kern, S.E., Hruban, R.H., Hamilton, S.R., Vogelstein, B. and Kinzler, K.W. (1997) Science 276, 1268–1272.
[6] van Groningen, J.J., Bloemers, H.P. and Swart, G.W. (1995) Cancer Res. 55, 6237–6243.
[7] Yeatman, T.J. and Mao, W. (1995) Nucleic Acids Res. 23, 4007–4008.
[8] Jiang, H., Lin, J.J., Su, Z.Z., Goldstein, N.I. and Fisher, P.B. (1995) Oncogene 11, 2477–2486.
[9] Francia, G., Mitchell, S.D., Moss, S.E., Hanby, A.M., Marshall, J.F. and Hart, I.R. (1996) Cancer Res. 56, 3855–3858.
[10] Duncan, L.M., Deeds, J., Hunter, J., Shao, J., Holmgren, L.M., Woolf, E.A., Tepper, R.I. and Shyjan, A.W. (1998) Cancer Res. 58, 1515–1520.
[11] Liang, P. and Pardee, A.B. (1992) Science 257, 967–971.
[12] Westphal, J.R., van't Hullenaar, R.G., van der Laak, J.A., Cornelissen, I.M., Schalkwijk, L.J., van Muijen, G.N., Wesseling, P., de Wilde, P.C., Ruiter, D.J. and de Waal, R.M. (1997) Br. J. Cancer 76, 561–570.
[13] McKnight, A.J. and Gordon, S. (1996) Immunol. Today 17, 283–287.

[14] de Vries, T.J., Fourkour, A., Wobbes, T., Verkroost, G., Ruiter, D.J. and van Muijen, G.N.P. (1997) Cancer Res. 57, 3223–3229.
[15] Quax, P.H., van Leeuwen, R.T., Verspaget, H.W. and Verheijen, J.H. (1990) Cancer Res. 50, 1488–1494.
[16] Lockshin, A., Giovanella, B.C., De Ipolyi, P.D., Williams Jr., L.J., Mendoza, J.T., Yim, S.O. and Stehlin Jr., J.S. (1985) Cancer Res. 45, 345–350.
[17] Danen, E.H., ten Berge, P.J., van Muijen, G.N., Jager, M.J. and Ruiter, D.J. (1996) Melanoma Res. 6, 31–35.
[18] Luyten, G.P., Naus, N.C., Mooy, C.M., Hagemeijer, A., Kan Mitchell, J., Van Drunen, E., Vuzevski, V., De Jong, P.T. and Luider, T.M. (1996) Int. J. Cancer 66, 380–387.
[19] Juhasz, I., Albelda, S.M., Elder, D.E., Murphy, G.F., Adachi, K., Herlyn, D., Valyi-Nagy, I.T. and Herlyn, M. (1993) Am. J. Pathol. 143, 528–537.
[20] Scheibenbogen, C., Keilholz, U., Meuer, S., Dengler, T., Tilgen, W. and Hunstein, W. (1993) Int. J. Cancer 54, 494–498.
[21] Verbeek, M.M., Otte Holler, I., Wesseling, P., Ruiter, D.J. and de Waal, R.M. (1994) Am. J. Pathol. 144, 372–382.
[22] Danen, E.H., Jansen, K.F., Klein, C.E., Smit, N.P., Ruiter, D.J. and van Muijen, G.N. (1996) Eur. J. Cell Biol. 70, 69–75.
[23] Bauer, D., Muller, H., Reich, J., Riedel, H., Ahrenkiel, V., Warthoe, P. and Strauss, M. (1993) Nucleic Acids Res. 21, 4272–4280.
[24] Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, 2nd edn., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
[25] Altschul, S.F., Madden, T.L., Schaffer, A.A., Zhang, J., Zhang, Z., Miller, W. and Lipman, D.J. (1997) Nucleic Acids Res. 25, 3389–3402.
[26] Geurts van Kessel, A.H., Tetteroo, P.A., von dem Borne, A.E., Hagemeijer, A. and Bootsma, D. (1983) Proc. Natl. Acad. Sci. USA 80, 3748–3752.
[27] Aust, G., Eichler, W., Laue, S., Lehmann, I., Heldin, N.E., Lotz, O., Scherbaum, W.A., Dralle, H. and Hoang Vu, C. (1997) Cancer Res. 57, 1798–1806.
[28] Nagase, T., Ishikawa, K., Nakajima, D., Ohira, M., Seki, N., Miyajima, N., Tanaka, A., Kotani, H., Nomura, N. and Ohara, O. (1997) DNA Res. 4, 141–150.
[29] Gorn, A.H., Lin, H.Y., Yamin, M., Auron, P.E., Flannery, M.R., Tapp, D.R., Manning, C.A., Lodish, H.F., Krane, S.M. and Goldring, S.R. (1992) J. Clin. Invest. 90, 1726–1735.
[30] Jiang, S. and Ulrich, C. (1995) Biochem. Biophys. Res. Commun. 207, 883–890.
[31] Osterhoff, C., Ivell, R. and Kirchhoff, C. (1997) DNA Cell Biol. 16, 379–389.
[32] Lelianova, V.G., Davletov, B.A., Sterling, A., Rahman, M.A., Grishin, E.V., Totty, N.F. and Ushkaryov, Y.A. (1997) J. Biol. Chem. 272, 21504–21508.
[33] Nagase, T., Ishikawa, K., Suyama, M., Kikuno, R., Miyajima, N., Tanaka, A., Kotani, H., Nomura, N. and Ohara, O. (1998) DNA Res. 5, 277–286.
[34] Nishimori, H., Shiratsuchi, T., Urano, T., Kimura, Y., Kiyono, K., Tatsumi, K., Yoshida, S., Ono, M., Kuwano, M. and Nakamura, Y. et al. (1997) Oncogene 15, 2145–2150.
[35] Shiratsuchi, T., Nishimori, H., Ichise, H., Nakamura, Y. and Tokino, T. (1997) Cytogenet. Cell Genet. 79, 103–108.
[36] Wilson, R., Ainscough, R., Anderson, K., Baynes, C., Berks, M., Bonfield, J., Burton, J., Connell, M., Copsey, T. and Cooper, J. et al. (1994) Nature 368, 32–38.
[37] Hart, A.C., Harrison, S.D., Van Vactor Jr., D.L., Rubin, G.M. and Zipursky, S.L. (1993) Proc. Natl. Acad. Sci. USA 90, 5047–5051.
[38] Tensen, C.P., Van Kesteren, E.R., Planta, R.J., Cox, K.J., Burke, J.F., van Heerikhuizen, H. and Vreugdenhil, E. (1994) Proc. Natl. Acad. Sci. USA 91, 4816–4820.
[39] Hamann, J., Eichler, W., Hamann, D., Kerstens, H.M., Poddighe, P.J., Hoovers, J.M., Hartmann, E., Strauss, M. and van Lier, R.A. (1995) J. Immunol. 155, 1942–1950.
[40] Baud, V., Chissoe, S.L., Viegas Pequignot, E., Diriong, S., N'Guyen, V.C., Roe, B.A. and Lipinski, M. (1995) Genomics 26, 334–344.
[41] Hadjantonakis, A., Sheward, W.J., Harmar, A.J., de Galan, L., Hoovers, J.M. and Little, P.F. (1997) Genomics 45, 97–104.
[42] Mohrenweiser, H., Olsen, A., Archibald, A., Beattie, C., Burmeister, M., Lamerdin, J., Lennon, G., Stewart, E., Stubbs, L. and Weber, J.L. et al. (1996) Cytogenet. Cell Genet. 74, 161–186.
[43] McKnight, A.J. and Gordon, S. (1998) J. Leukocyte Biol. 63, 271–280.
[44] von Heijne, G. (1983) Eur. J. Biochem. 133, 17–21.
[45] Probst, W.C., Snyder, L.A., Schuster, D.I., Brosius, J. and Sealfon, S.C. (1992) DNA Cell Biol. 11, 1–20.
[46] van Lier, R.A., Eichler, W. and Hamann, J. (1996) Immunol. Lett. 54, 185–187.
[47] Hamann, J., Hartmann, E. and van Lier, R.A. (1996) Genomics 32, 144–147.
[48] Watson, S. and Arkinstall, S. (1994) The G-protein Linked Receptor Facts Book, Academic Press, Londen.
[49] Davletov, B.A., Meunier, F.A., Ashton, A.C., Matsushita, H., Hirst, W.D., Lelianova, V.G., Wilkin, G.P., Dolly, J.O. and Ushkaryov, Y.A. (1998) EMBO J. 17, 3909–3920.
[50] Hamann, J., Vogel, B., van Schijndel, G.M. and van Lier, R.A. (1996) J. Exp. Med. 184, 1185–1189.