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Identification of a Novel Transmembrane Protein (UKW): Association with Invasive Status of Mammary Carcinoma Cell Lines and Expression in Pancreatic Carcinoma

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Abstract. *A novel transmembrane-glycoprotein, referred to as UKW, was identified by expression profiling of a metastatic versus a non-metastatic pancreatic carcinoma cell line (S2-007 and S2-028). UKW is strongly expressed only in the metastatic cell line. The corresponding cDNA encodes for a protein of 374 amino acids. UKW is located on chromosome 11q24.1, a locus which is frequently amplified in pancreatic cancer. Bioinformatic analysis revealed that UKW corresponds to a putative transmembrane-glycoprotein composed of an extracellular domain of 215 aa containing two C2-type Ig folds, a transmembrane region of 23 aa and a highly acidic cytoplasmic region of 117 aa. Sequence alignment revealed homology with human, murine and zebrafish coxsackievirus and adenovirus receptor of 35%, 33% and 36% and colon-related A33 antigen of 32%, respectively. Murine adipose-specific protein 5 (Asp-5), however, exhibited the closest homology (93%), suggesting that UKW represents the human orthologue of Asp-5. Multiple tissue expression array analysis revealed UKW expression in gastrointestinal tissues, brain, aorta and uterus and absence in most other human tissues. In a panel of invasive and non-invasive mammary carcinoma cell lines, relative overexpression of UKW was observed in the invasive cell lines. In addition, increased expression of UKW mRNA was found in pancreatic adenocarcinoma compared to tissues derived from patients with chronic pancreatitis and normal pancreatic tissue.*

Abbreviations: aa, amino acids; bp, base pairs; cDNA, complementary DNA; EST, Expressed Sequence Tag; IgSF, Immunoglobulin supergene family; kb, kilo base(s); kD, kilo Dalton; MTE, Multiple Tissue Expression.

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Neoplasms of the exocrine pancreas may arise from ductal, acinar or stromal cells. Eighty % of pancreatic carcinomas display a ductal epithelial phenotype. Sixty % of these tumors are located in the head of the pancreas, 10% in the tail and 30% are located in the body of the pancreas or are diffuse (1). Histologically, these tumors are graded as well-differentiated, moderately-differentiated and poorly-differentiated. Some tumors are classified as adenosquamous, mucinous, undifferentiated or undifferentiated with osteoblast-like giant cells (2). Since the disease is asymptomatic in its early stages, due to the lack of any test for early diagnosis and due to its aggressive character with respect to local invasion and metastasis to visceral organs, this disease is associated with a dismal prognosis. Only 20% of the tumors are resectable and the survival benefit of approved chemotherapy regimens is rather poor (3).

The identification of new targets for early diagnosis and a more effective therapeutic intervention is of paramount importance. A promising technique for achieving this objective is the Affymetrix GeneChip® analysis for transcriptional profiling (4, 5). The cell lines used for transcriptional profiling in this study are derived from SUIT-2, a human pancreatic cancer cell line which was obtained from a liver metastasis of a human pancreatic cancer (6). Subline S2-028 is non-metastatic, whereas subline S2-007 exhibits a high potential of metastasis to the lymph nodes and to the lungs after subcutaneous injection (7). We identified a new transmembrane-glycoprotein of the immunoglobulin superfamily, referred to as UKW, whose up-regulated expression correlated with increased invasiveness and metastasis.

Materials and Methods

Human cell lines and tissue samples. For transcriptional profiling, the pancreatic cancer cell line SUIT-2 derived clones, S2-007 and S2-028, were used. The cell culture medium for both cell lines was DMEM supplemented with 10% FCS and 1% glutamine. For expression analysis of gene UKW, the following mammary

carcinoma cell lines were investigated: MDA-MB-157, MDA-MB-175, MDA-MB-231, MDA-MB-361, MDA-MB-435, MDA-MB-436, MDA-MB-453, MCF-7, Hs578T, BT-20, BT483, BT-549, T47-D, ZR-75-1, ZR-75-30, CAMA-1, SK-BR-3, UACC-812 and Du4475, all obtained from R. Zeillinger, Department of Obstetrics and Gynecology, University of Vienna, Austria. Human pancreatic tissues were obtained from patients with histologically verified adenocarcinoma of the pancreas (n=15) or chronic pancreatitis (n=3); normal pancreas controls (n=7) were derived from resection margins or organ donor tissue (Department of Internal Medicine I of the University of Ulm, Germany). All cell lines were free of contamination with mycoplasm.

Affymetrix GeneChip® Profiling. Transcriptional profiling according to Affymetrix protocols was performed as described (8).

Cloning of UKW cDNA. Based on the 590 bp fragment corresponding to EST Acc.-Nr. AA044830, a 280 bp PCR fragment was generated for screening and the isolation of the full length cDNA of UKW. A human uterus 5'-stretch cDNA library was used (Clontech, Palo Alto, CA, USA). The cDNA library was cloned into a λgt11 vector. The isolated cDNA clones were sequenced and compared to the subcloned PCR fragment. For isolation of full-length cDNA, a 5'-probe of the cDNA was prepared and used for rescreening of the library. This procedure was repeated three times until a full-length cDNA was identified. Based on five overlapping cDNA fragments, isolated by cDNA library screening, the nt sequence data of 5.1 kb was analyzed with respect to homology to known genes in the Genbank and EMBL DNA data bases using BLAST (Basic Local Alignment Search Tool). Specific primers for the ORF (forward primer 5'GATCGGGATGTCCCTCCT3'; reverse primer 5'CTGGTGACTTGAGCTCCAA3') were generated and the ORF of the UKW cDNA was amplified by RT-PCR. The resulting 1209 bp fragment was subcloned into the PCR 2.1-TOPO vector using the TOPO TA cloning system (Invitrogen, San Diego, CA, USA).

Northern blot analysis. Northern blotting was performed as described (8) and the blot was hybridized to a RT-PCR product derived from UKW cDNA labelled with α-[^{32}P]dATP of a specific activity of 2 x 10^9 cpm/μg. Equal loading and transfer of mRNA to the membrane was assessed by rehybridizing the blot with α-[^{32}P]dATP-labelled GAPDH cDNA probe.

Taqman®-PCR. Real-time quantitative PCR of the UKW cDNA was performed with the TaqMan® technology and the ABI PRISM 7700 apparatus (Applied Biosystems, Foster City, CA, USA). Ten μg total RNA isolated from frozen pancreatic adenocarcinomas, chronic pancreatitis-related and control pancreas tissues were used for reverse transcriptase reactions in a volume of 20 μl. The PCRs were then carried out by mixing 200 ng cDNA with 4μl of 10x SYBR-Green buffer, 3 mM $MgCl_2$, 1 mM dNTDs, 0.2 units Uracil-N Glycosylase, 1 unit AmpliTaq Gold, 4μl primer mix (300 nM each primer: forward 5'TTCTCTTTGACAGGTTCTGGGC3'; reverse 5'GGTTGGAACCAGTAGGGCCTC3') in a final volume of 40 μl. PCR primers were designed to generate a DNA fragment of 50 bp using the Primer Express Software (PE Biosystems, CA, USA). The amplification cycles were as follows: 2 min at 50˚C followed by 10 min at 95˚C and 40 amplification cycles (95˚C for 15 sec and 60˚C for 60 sec). The relative quantity of UKW mRNA was calculated by subtraction of the UKW gene and housekeeping gene xs13 expression for each sample. Each value was divided by the averaged steady-state level of UKW mRNA of three healthy tissues. Ratios were squared and a reciprocal value was formed. (xs13for: 5'AGATCCGCATGTCCCTTC3'; xs13rev: 5'CCTT GCGCATCATGGTGTT3').

LightCycler™-PCR. LightCyclerTM–PCR was performed with a LightCycler™ (Roche Molecular Biochemicals, Mannheim, Germany) in LightCycler™ capillaries using a commercially available master mix containing Taq DNA polymerase, SYBR-Green I, deoxyribonucleoside triphosphates (LightCyclerTM DNA master SYBR-Green I, Roche Molecular Biochemicals). After addition of primers (forward 5'CCCCAGGAGTTTATGCTTGG3'; reverse 5'GCCTGGATACCACACTACCAG3'; final concentration: 0.5 μM), 3 mM $MgCl_2$ and template DNA to the master mix, 37 cycles of denaturation (95˚C for 1 sec), annealing (58˚C for 5 sec) and extension (72˚C for 8 sec) were performed. All temperature transition rates were set to 20˚C per sec. After completion of PCR amplification, melting curve analysis was performed. For this procedure, PCR products were denatured at 95˚C, annealed at 65˚C and gradually heated to 95˚C. SYBR-Green I fluorescence was monitored stepwise every 0.1˚C. A control without template DNA (water control) was included in each experiment to identify primer dimer formation. A small peak was usually visible at 78˚C, which could be discriminated from the specific amplification UKW peak at 85-88˚C. The results were calculated as the ratio of gene UKW and calnexin expression and were normalized to the average expression in invasive mammary tumor cell lines or invasive pancreatic tumor cell lines, respectively. Calnexin forward primer 5'ATTGTCAGATCGTTCATTGC3'; reverse primer 5'ATGGA ACAGGTAACCAGCAT3'.

Human multiple tissue expression array (MTETM). This array (Clontech) contains normalized loadings of poly A+-RNA from 76 different tissues as well as control RNAs and DNAs as displayed in Figure 6. The blot was hybridized with an α-[^{32}P]dATP-labelled probe derived from UKW cDNA according to the instructions of the manufacturer and exposed to X-ray film at -70˚C for 62 h.

Antibodies and Western blotting. Rabbit polyclonal antibody anti-UKW was generated by Eurogentec S.A. (Herstal, Belgium). The synthetic peptide STANSASRSQRTLSTC, corresponding to residues 306-320 of UKW, was coupled to KLH (keyhole limpet hemocyanin) and injected into rabbits. Immunoreactive sera were affinity-purified against the synthetic peptide.

For Western blotting, proteins were extracted in RIPA buffer (50 mM Tris-HCl pH 7.5, 150 mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS) containing a protease inhibitor mixture (Complete EDTA-free protease inhibitors, Roche Diagnostics, Mannheim, Germany). Polyacrylamide gel electrophoresis was performed under reducing conditions using NuPAGE 12% Bis-Tris gel (Invitrogen, Carlsbad, USA) and NuPAGE (MES SDS running buffer (Invitrogen)). Gels were semi-dry blotted to a nitrocellulose membrane and blocked in TBS buffer (100 mM Tris-HCl pH 7.5, 150 mM NaCl) plus 5% non-fat dry milk (Merck, Darmstadt, Germany). After incubation with the rabbit anti-UKW as primary antibody, membranes were washed in TBS buffer with 0.1% Tween20, incubated with horseradish peroxidase-conjugated anti-rabbit (Roche Diagnostics) as secondary antibody and washed

again. Antibody detection was performed using enhanced chemiluminescence (Lumi-lightPlus Western blotting substrate, Roche Diagnostics).

Transient and stable transfectants. To generate a UKW-GFP fusion construct, UKW cDNA was amplified by PCR with appropriate oligonucleotide primers and cloned into the pEGFP-N3 vector (Clontech) *via* SalI/BamHI restriction sites. The accuracy of the fusion constructs with GFP attached to the 3'end of UKW was confirmed by DNA-sequencing. 1.5 x 10^5 SUIT- 2-028 cells were plated onto 17mm WillCo-dish™ (Willco Wells BV, Amsterdam, The Netherlands) and propagated in DMEM containing 10% fetal calf serum and 1mM L-glutamine. Cells were cultivated in a humidified incubator at 37˚C and 5% CO_2. Twenty-four h after plating, cells were transfected using FuGENE 6 Transfection Reagent (Roche Molecular Biochemicals). In short, 100 μl serum-free medium and 3 μl FuGENE 6 Transfection Reagent were gently mixed and 2 μg pEGFP-N3-UKW DNA solution was added. The dilution was incubated for 15 min at room temperature. The complex mixture was added dropwise to the cells followed by another 24-h incubation prior to fluorescence microscopy. Transfection efficiency was determined with a by flow cytometry (Beckman-Coulter XL flow cytometer). For confocal microscopy, a Zeiss Axiovert 135 with appropriate filters was used and images were processed using MetaMorph software.

Transfection of S2-028 pancreatic cancer cells with the pBIG2i (9) vector was performed with the Lipofectamin2000 reagent (Invitrogen), according to the manufacturer's instructions. In the pBIG2i vector system, gene expression can be induced with doxycycline. 2 x 10^6 cells were plated in T75-flasks and cultured for 24 h. Subsequently, the medium was removed and 3.8 ml of transfection mixture containing 7.5 μg of vector DNA and 15.2 μl Lipofectamin2000 reagent were added. Cells were then cultured for 6 h at 37˚C. After the addition of 7.6 ml of complete culture medium, the cells were cultured for another 24 h. Transfected clones were selected in complete medium containing 300 μg/ml Hygromycin for 2 weeks. Subsequently, Hygromycin-resistant clones were isolated with cloning cylinders and cultured in complete medium containing 300 μg/ml Hygromycin. MOCK-transfection was performed simultaneously with the empty pBIG2i plasmid. Expression of UKW was induced with 2μg/ml doxycycline and the cells were lysed after 24h for Western blotting.

Bioinformatic tools. The TMHMM (v. 2.0) computer program of the Center for Biological Sequence Analysis, BioCentrum-DTU (The Technical University of Denmark) was used for the prediction of the transmembrane domain and its orientation in the membrane. PeptideStructure and PlotStructure in GCG (Genetics Computer Group, Inc.; Pharmacopeia Inc., San Diego, CA, USA) generated the model for the topology of the protein. Translation of the nucleotide sequence into protein sequence was performed with the algorithm Translate. Signal anchor prediction was performed with the algorithm SignalP-HMM. NetNGlyc and NetOGlyc programs were used to predict potential N-glycosylation and O-glycosylation sites. BLAST (http://www.ncbi.nlm.nih.gov/blast/) was used for homology searches against nucleotide and protein databases. The alignment of homologous proteins was generated with Pileup of a multiple sequence file in GCG and displayed with Prettybox in GCG. The Ensembl Genome Browser software of The Wellcome Trust Sanger Institute and EMBL-EBI was used for the prediction of the genomic localization of the UKW gene and the SIM4 computer program was applied for the exon-intron-analysis.

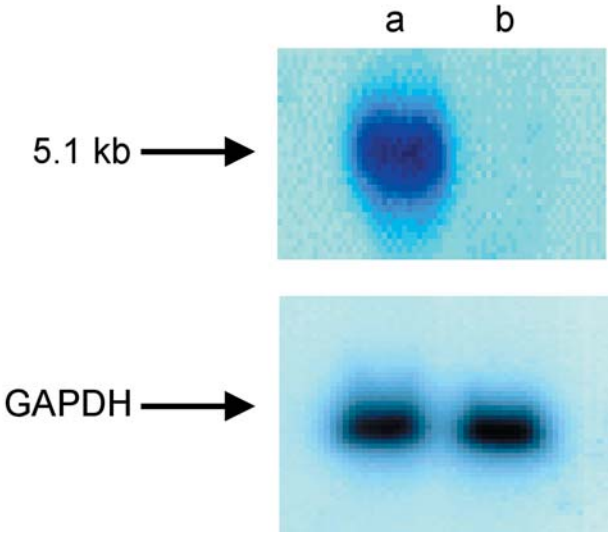


Figure 1. *Northern blot analysis for UKW mRNA in pancreatic tumor cell lines S2-007 and S2-028. Lanes a and b display RNA from cell lines S2-007 and S2-028, respectively. GAPDH cDNA probe was used as an internal reference.*

Immunohistochemistry. Briefly, paraffin wax sections were dewaxed in xylene and rehydrated in a graded ethanol series. The slides were pre-incubated in a steamer with 1 x Reveal (Biocarta) (20 min) and with 3% hydrogen peroxide in PBS (5 min) to block endogenous peroxidase activity. The sections were incubated with 10% goat serum for 20 min (ICH-Block EnvisionTM, DAKO) to block non-specific binding. Polyclonal antibody against UKW was diluted 1:30 with antibody diluent (DAKO). The sections were allowed to react with the primary antibody overnight and, after washing with PBS, sections were processed further using Envision™ anti-rabbit IgG (10 min). The reaction products were developed with a mixture of 3,3'-diaminobenzine-4 HCl (DAB) and H_2O_2.

Results

Identification of UKW, a novel transmembrane glycoprotein, member of the immunoglobulin supergene family. We investigated differential gene expression of the metastatic S2-007 and the non-metastatic S2-028 cell lines using Affymetrix GeneChip® EST arrays (to be published elsewhere). We focussed on an EST (EMBL accession number AA044830) exhibiting an increased signal in the S2-007 cells. Differential expression was confirmed by Northern blotting. As shown in Figure 1, the corresponding mRNA of 5.1 kb was 100-fold overexpressed in the metastasizing cell line. The cDNA was identified by isolation of overlapping clones from a uterus cDNA library. The cDNA consists of 365 bp 5'UTR, 1122 bp ORF and 3633 bp 3'UTR. In Figure 2, the 5'UTR, the coding region and part of the 3'UTR are shown. The ORF encodes

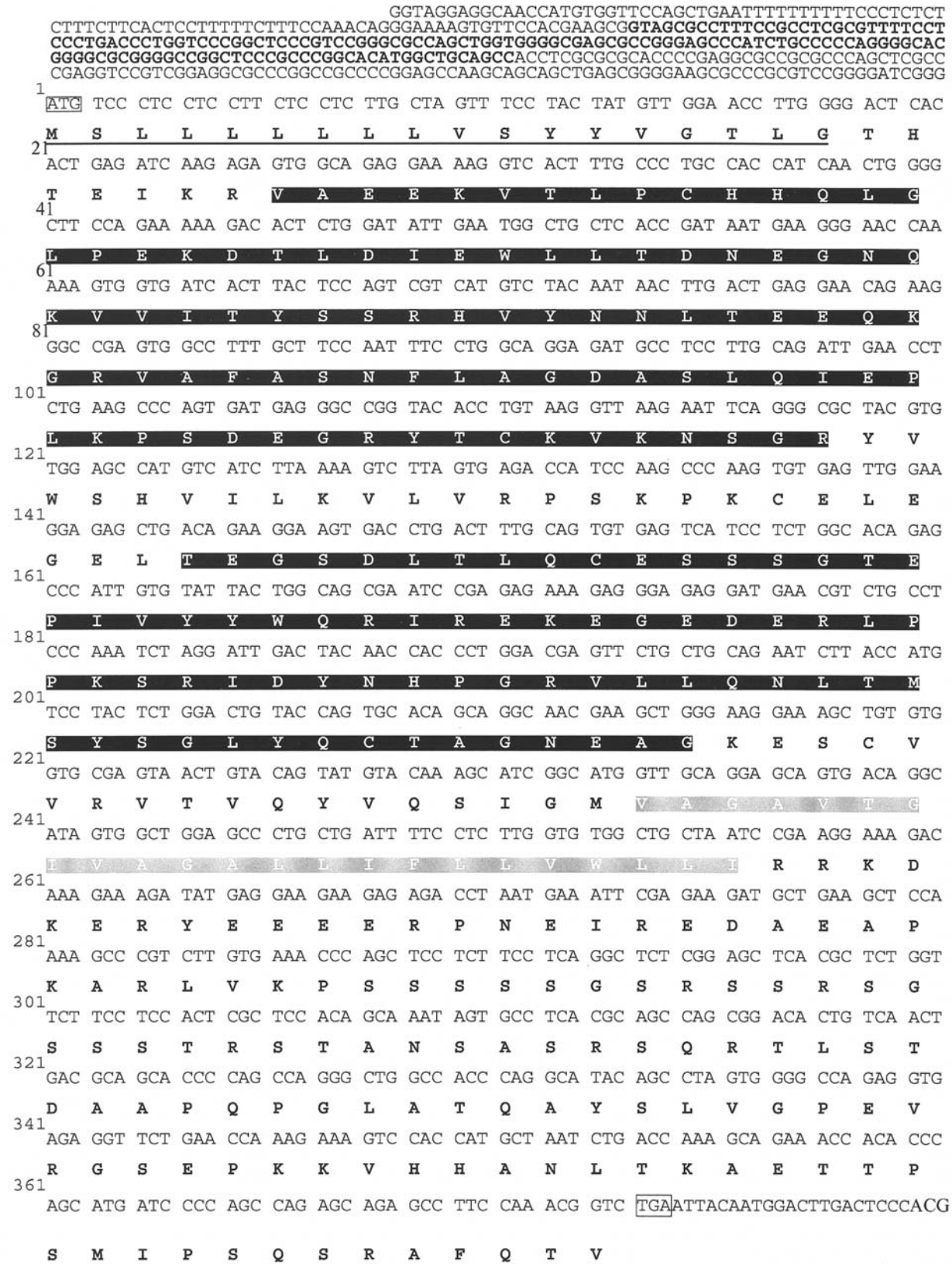


Figure 2. *Nucleotide and amino acid sequence of the UKW cDNA. The two immunoglobulin C-2 type domains are labelled dark and the putative transmembrane region is tinged with grey. The putative signal peptide sequence at the amino terminus is underlined.*

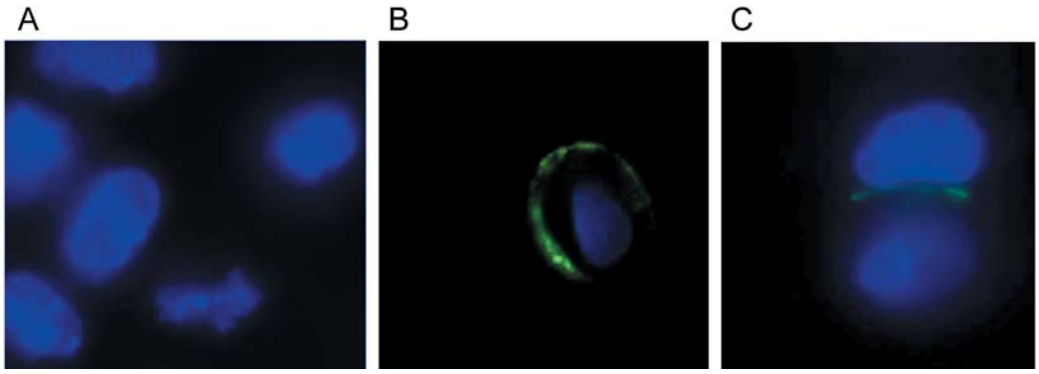


Figure 3. *Cellular localization of UKW. Cells were transfected with an expression vector for UKW-GFP fusion protein. The pictures display green fluorescence and DNA staining. S2-028 cells were transfected as described in the 'Materials and Methods' section. Nuclei are stained in blue, UKW-GFP fusion protein gives rise to a green fluorescence signal.*
A: untransfected cells; B and C: transfected cells.

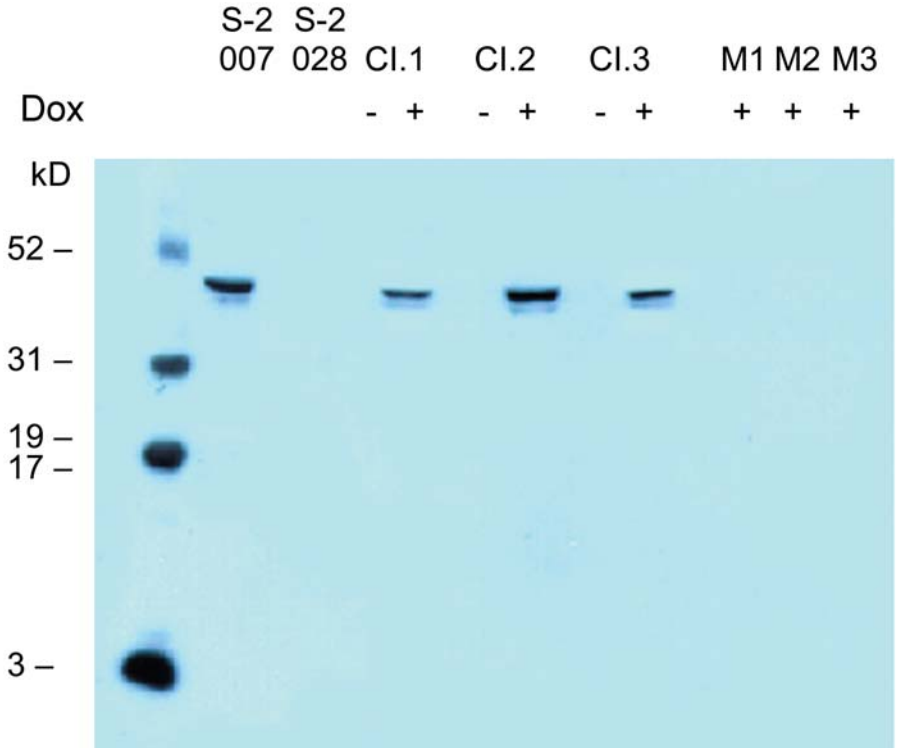
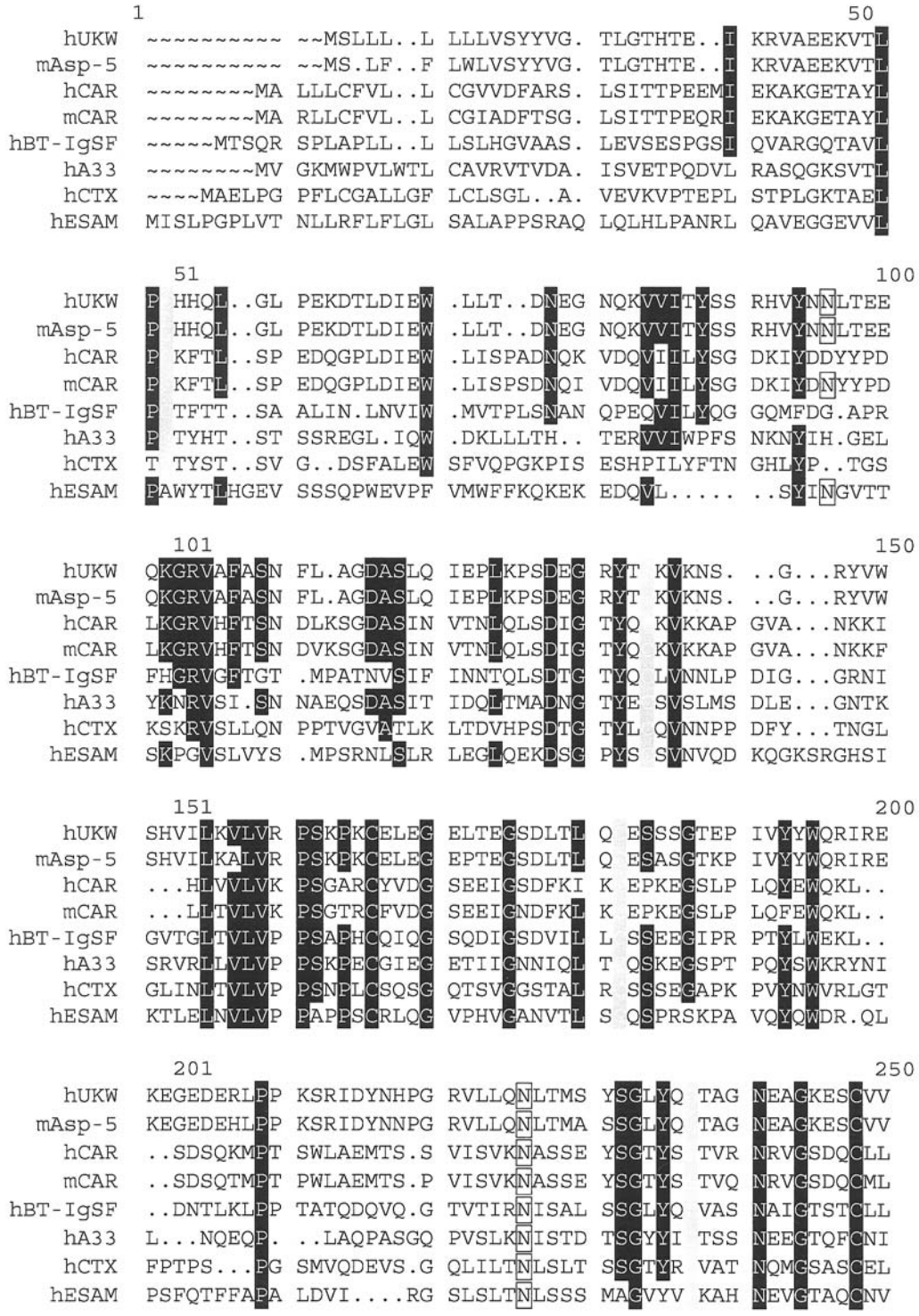


Figure 4. *Expression of UKW in inducible transfectants. Stable transfectants of cell line S2-028 were established as described in the 'Materials and Methods' section. Cells were grown in the presence and absence of 2 µg/ml doxycycline, lysed after 24h and Western blotting was performed with a polyclonal serum directed against UKW raised in rabbits, all performed as described in the 'Materials and Methods' section. Results are shown for three transfectants and three mock-transfectants.*

for a protein of 374 aa with a putative signal sequence of 18 aa. According to the predicted topology of UKW, the protein consists of an extracellular domain of 215 aa, a transmembrane domain of 23 aa and a highly acidic cytoplasmic domain of 117 aa. A serine-rich region was identified in the cytoplasmic domain (data not shown), but no consensus signaling-related motives were identified. The extracellular domain contains two immunoglobulin C2-type folds composed of 93 aa and 72 aa and two putative N-glycosylation sites (aa 74 and aa 197).

Based on the cDNA sequence of UKW, we were able to derive the genomic organization of the gene. Making use of bioinformatic tools, we found that UKW is composed of seven exons (393 bp, 158 bp, 202 bp, 168 bp, 123 bp, 142 bp

Figure 5



and 3934 bp) separated by six introns (96962 bp, 13082 bp, 665 bp, 473 bp, 8242 bp and 928 bp). The coding region is located on exons 1 – 7. The gene is located on chromosome 11q24.1. Transient transfection of an expression vector for a UKW-GFP fusion protein into S2-028 cells revealed localization of UKW in the plasma membrane and

Figure 5 continued

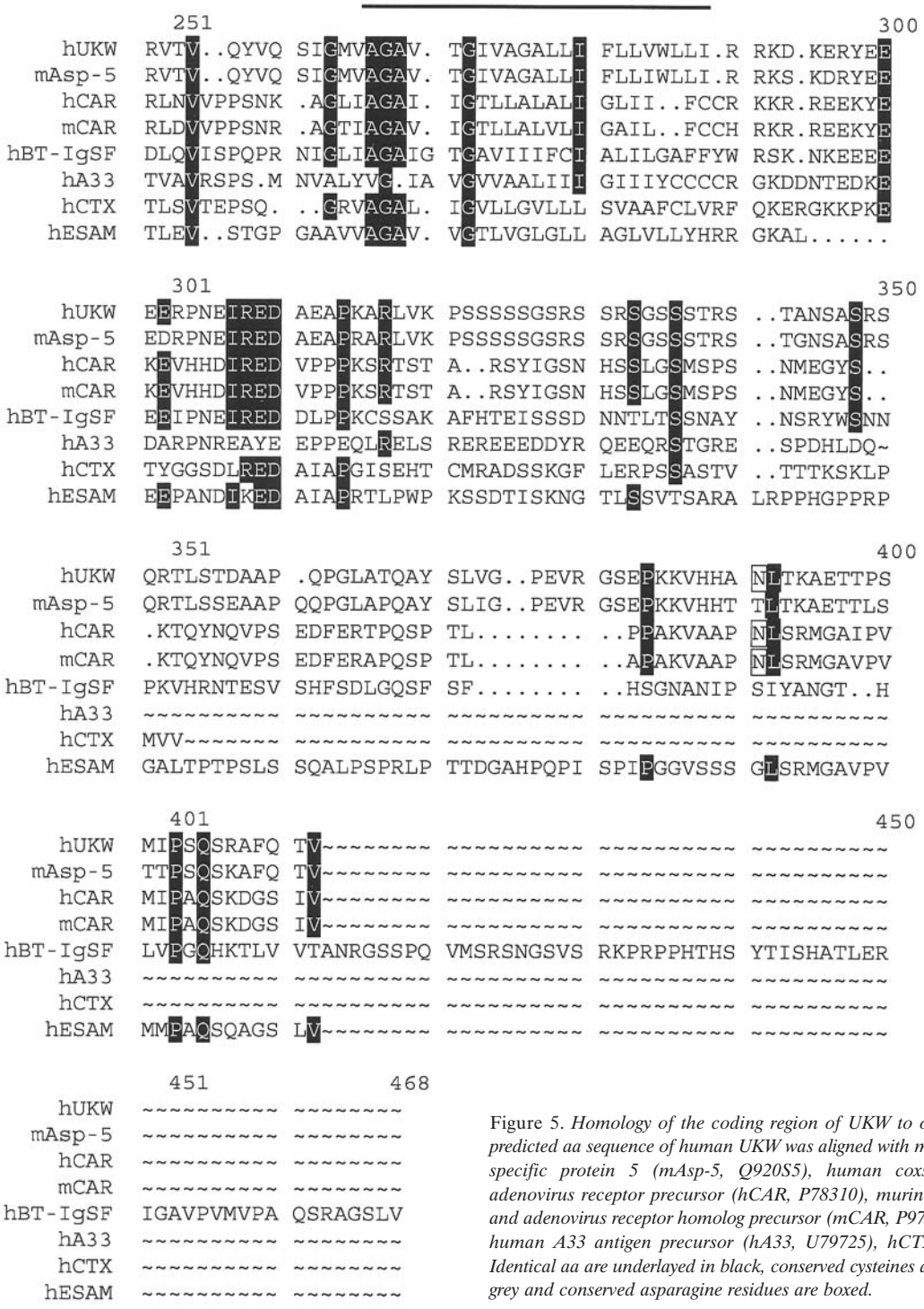
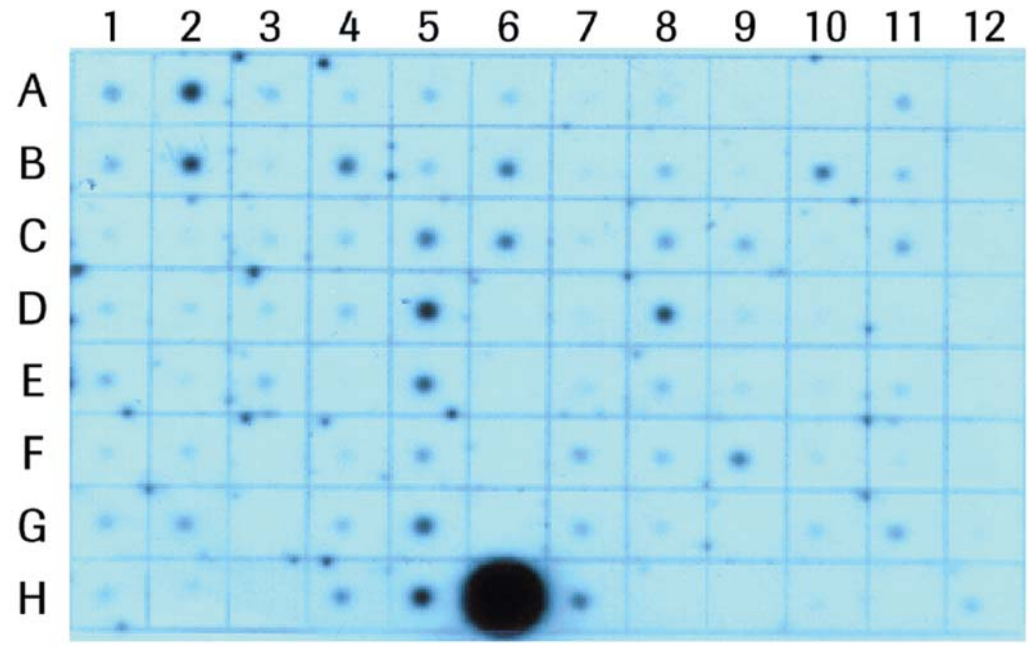


Figure 5. *Homology of the coding region of UKW to other genes. The predicted aa sequence of human UKW was aligned with murine adipocyte-specific protein 5 (mAsp-5, Q920S5), human coxsackievirus and adenovirus receptor precursor (hCAR, P78310), murine coxsackievirus and adenovirus receptor homolog precursor (mCAR, P97792), hBT-IgSF, human A33 antigen precursor (hA33, U79725), hCTX and hESAM. Identical aa are underlayed in black, conserved cysteines are highlighted in grey and conserved asparagine residues are boxed.*

especially at sites of intercellular contacts (Figure 3). Stable transfectants of cell line S2-028 were established, in which expression of UKW could be induced by doxycycline. As shown in Figure 4, a 41 kD protein could be induced by doxycycline in three independent isolates in contrast to transfectants transformed with the insert-free vector.



	1	2	3	4	5	6	7	8	9	10	11	12
A	whole brain	cerebellum, left	substantia nigra	heart	esophagus	colon, transverse	kidney	lung	liver	leukemia, HL-60	fetal brain	yeast total RNA
B	cerebral cortex	cerebellum, right	accumbens nucleus	aorta	stomach	colon, desending	skeletal muscle	placenta	pancreas	HeLa S3	fetal heart	yeast tRNA
C	frontal lobe	corpus callosum	thalamus	atrium, left	duodenum	rectum	spleen	bladder	adrenal gland	leukemia, K-562	fetal kidney	*E. coli* rRNA
D	parietal lobe	amygdala	pituitary gland	atrium, right	jejunum		thymus	uterus	thyroid gland	leukemia, MOLT-4	fetal liver	*E. coli* DNA
E	occipital lobe	caudate nucleus	spinal cord	ventricle, left	ileum		peripheral blood leukocyte	prostate	salivary gland	Burkitt's lymphoma, Raji	fetal spleen	Poly r(A)
F	temporal lobe	hippo-campus		ventricle, right	ilocecum		lymph node	testis	mammary gland	Burkitt's lymphoma, Daudi	fetal thymus	human C_0t-1 DNA
G	p. g.* of cerebral cortex	medulla oblongata		inter-ventricular septum	appendix		bone marrow	ovary		colorectal adeno-carcinoma SW480	fetal lung	human DNA 100 ng
H	pons	putamen		apex of the heart	colon, ascending		trachea			lung carcinoma, A549		human DNA 500 ng

* paracentral gyrus

Figure 6. *Expression analysis of the UKW mRNA in human tissues and tumor cell lines. The upper and lower parts correspond to the hybridized blot and the corresponding code, respectively.*

Homology of UKW to other proteins. As shown in Figure 5, we aligned the sequence of UKW with other membrane proteins containing two Ig-folds, such as the human and murine coxsackievirus and adenovirus receptor (hCAR and mCAR) (11-13) and human A33 antigen (14), resulting in homologies of 35%, 33% and 32%, respectively. UKW also exhibits aa sequence homology with endothelial cell-selective adhesion molecule (ESAM), brain- and testis-specific immunoglobulin superfamily gene (BT-IgSF) and *Xenopus* thymocyte receptor (CTX) of 32%, 32% and 28%,

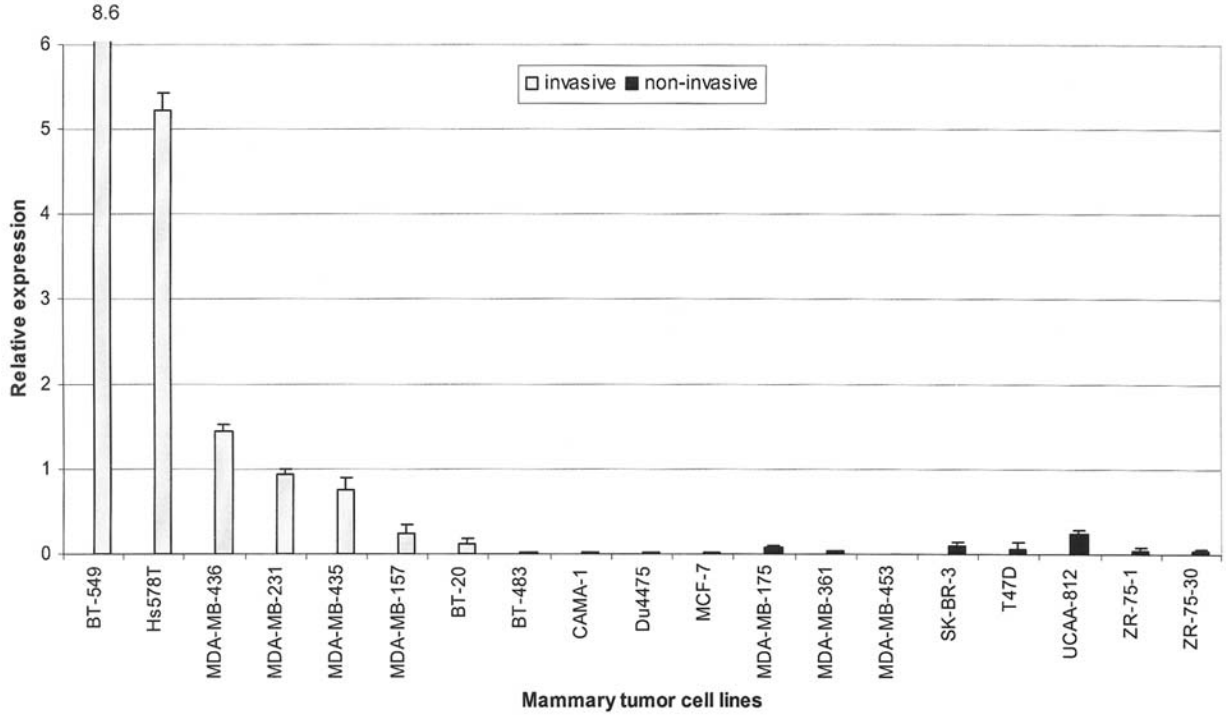


Figure 7. *Relative expression of UKW mRNA in mammary tumor cell lines. Mammary tumor cell lines with invasive and non-invasive potential were analyzed. Relative levels of UKW mRNA were determined as described in the 'Materials and Methods' section.*

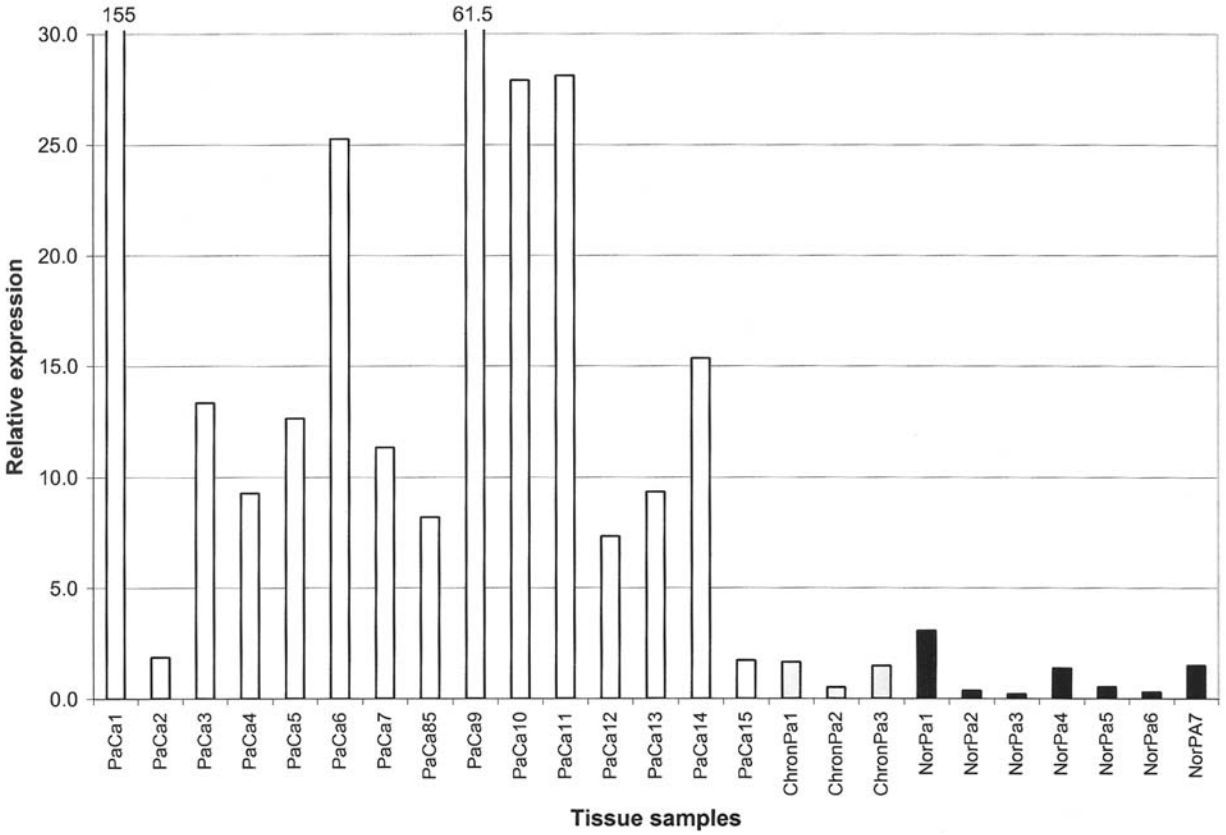


Figure 8. *Relative expression of UKW mRNA in pancreatic adenocarcinomas, chronic pancreatitis and pancreatic control tissues. Results are displayed as normalized UKW mRNA expression levels determined as described in the 'Materials and Methods' section.*

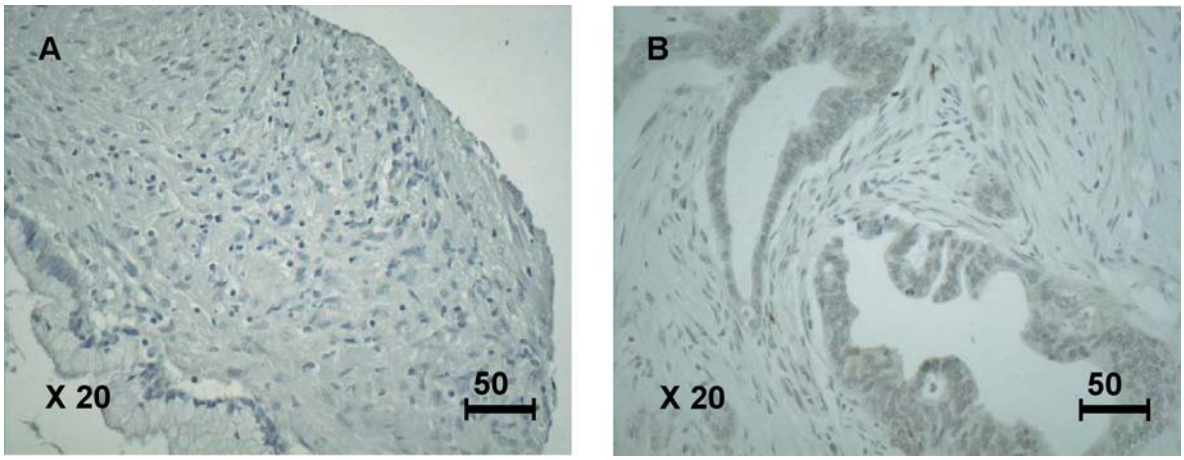


Figure 9. *Immunohistochemistry of UKW expression in normal pancreatic tissue and pancreatic carcinoma. Staining was performed as described in the 'Materials and Methods' section. Magnification: 20-fold. A: normal pancreatic tissue; B: pancreatic carcinoma*

respectively (15-17). The data reveal the closest homology of UKW to mAsp-5 (93% homology), suggesting that UKW might be the human orthologue of mAsp-5 (10).

All of these proteins, except hCAR, are composed of 7 exons and hCTX and hESAM are located, like UKW, on chromosome 11q24. Conservation of several residues, as well as a similar organisation of the genes encoding UKW, Asp-5, CAR, A33, ESAM and BT-IgSF, indicate that these genes are members of a subfamily of the immunoglobulin superfamily. Several residues, in addition to those conserved in Ig-like domains, were invariable among UKW, mAsp5, hCAR, mCAR, hA33 and hCTX, as indicated in Figure 5.

UKW mRNA expression in human normal tissues and tumor cell lines. We investigated the expression of UKW at the RNA level in more detail using normal human tissues and a few selected tumor cell lines by MTE-blotting (Figure 6). The strongest signals were observed in gastrointestinal tissues (duodenum, jejunum, ileum, appendix, colon and rectum), brain (cerebellum), aorta and uterus. In the panel of tumor cells investigated, only HeLa S3 scored positive, whereas the leukemia cell lines HL-60, K-562, MOLT-4 and the Burkitt's lymphoma cell lines Raji and Daudi, the colorectal adenocarcinoma cell line SW480 and the lung carcinoma cell line A540 scored negative for UKW mRNA.

UKW mRNA expression and correlation with the invasion potential in mammary carcinoma cell lines. We explored whether there is a correlation between the steady-state mRNA level of gene UKW and the invasive status of several mammary carcinoma cell lines. The invasiveness of these cell lines was evaluated by their ability to penetrate into a collagen-fibroblast matrix (18-20). It was shown that cells with estrogen receptor (ER), progesteron receptor (PR) and pS2 expression, but no plasminogen activator inhibitor 1 (PAI-1) expression, showed a non-invasive phenotype, while cells expressing PAI-1 mRNA, but no ER mRNA, were scored as invasive (19, 20). Based on the analysis of the invasive and non-invasive human mammary carcinoma cell lines, we noted a 29-fold higher steady-state level of UKW mRNA in the invasive cells by comparing averaged mRNA levels of the invasive *versus* the non-invasive cell lines (Figure 7).

UKW expression in human pancreatic cancer tissues. The relative UKW mRNA expression was analyzed in tissues derived from fifteen patients with pancreatic carcinoma, three patients with chronic pancreatitis and seven pancreatic control tissues. Compared to normal pancreas and tissues from chronic pancreatitis, a significant increase in mRNA steady-state levels was detected in tissues derived from pancreatic carcinoma (Figure 8). UKW expression was analyzed by immunohistochemistry. Ten pancreatic carcinomas and the corresponding normal tissues were stained with a polyclonal serum directed against UKW. An example is shown in Figure 9, highlighting the expression of UKW in the tumor, but not in the corresponding normal tissue. However, only one out of ten pancreatic carcinomas gave rise to a positive staining, indicating that we have to prepare antibodies with improved affinity to address the issue of protein expression of UKW in pancreatic tumors.

Discussion

A novel transmembrane protein, referred to as UKW, was identified by transcriptional profiling of the metastatic human pancreatic carcinoma cell line S2-007 *versus* the non-

metastatic cell line S2-028 (Figure 1). Expression could be detected only in the metastatic cell line (Figure 1). UKW is composed of a putative signal sequence of 18 aa, an extracellular domain of 215 aa containing two Ig-folds, a 23 aa transmembrane region and a cytoplasmic region covering 117 aa. Sequence alignments with other receptors containing two Ig-folds revealed highest homology (93%) to murine adipose-specific protein Asp-5 (10). This suggests that UKW might represent the human orthologue of Asp-5. A function for Asp-5 has not been described yet. We have identified the homology of UKW to other receptors like human and murine coxsackievirus and adenovirus receptor (CAR) (11-13), human A33 antigen (14), human brain- and testis-specific immunoglobulin superfamily protein (BT-IgSF) (16), endothelial cell-selective adhesion molecule (ESAM) (14) and *Xenopus* thymocyte receptor (hCTX) (17) (93%, 35%, 33%, 32%, 32%, 32% and 28%). An adhesion function for murine CAR has been identified by Honda *et al.* (21). mCAR transfectants were shown to aggregate *in vitro* mediated by homophilic interactions. Furthermore, mCAR-mediated adhesion functions seem to be involved in brain development through involvement in neuro-network formation (11, 22). hCAR might also function as a cell adhesion molecule, because it has been demonstrated that the most distal extracellular Ig loop of hCAR facilitates homotypic interactions leading to aggregation of cells expressing hCAR (23, 24). Furthermore, a role of mCAR and hCAR as receptors for adenovirus and coxsackievirus has been demonstrated (11, 12). mA33 was identified as a marker for basolateral intestinal epithelial cells in the mouse (25). BT-IgSF might play a role in the development and function of the central nervous system (16). ESAM is selectively expressed in endothelial cells and mediates cell-cell adhesion through homotypic interactions (15). Our studies revealed the localization of UKW in the plasma membrane and a pronounced concentration of UKW at intercellular contact sites (Figure 3), in agreement with the functions of other members of this receptor family. Western blotting revealed expression of a 41 kD protein in S2-028 cells stably transfected with an expression vector for UKW (Figure 4).

We assessed UKW mRNA levels in a panel of invasive *versus* non-invasive mammary carcinoma cell lines. These cell lines were characterized by their ability or inability to penetrate into a collagen-fibroblast matrix (19). Figure 7 shows that steady-state levels of UKW mRNA correlate with the invasive phenotype. This indicates that UKW represents a marker for the invasive status of breast cancer cell lines.

We further analyzed the expression of UKW mRNA in a panel of 15 pancreatic ductal adenocarcinomas, 3 tissues derived from patients with chronic pancreatitis and 7 normal pancreatic tissues. Compared to the median levels of UKW mRNA in normal pancreatic tissues and patients with chronic pancreatitis, we found a medium 8.5-fold increase of UKW mRNA in pancreatic adenocarcinomas (Figure 8). An immunhisto-chemical analysis of expression of UKW in normal pancreatic tissues and a papillary pancreatic carcinoma is shown in Figure 9. For a more detailed analysis, however, we have to derive antibodies with higher affinity.

The functional contribution of UKW will be addressed by the evaluation of non-metastasizing S2-028 cells stably transfected with an inducible expression vector UKW in several *in vitro* systems and, finally, in murine xenograft models in nude mice with respect to growth of the primary tumor and metastasis. Preliminary experiments (data not shown) indicate that the proliferation status and invasiveness in a matrigel invasion assay are not affected by the expression of UKW. Adhesion of transfectants to components of the extracellular matrix such as collagen I and collagen IV, vitronectin, fibronectin and laminin, seem to be affected only marginally (data not shown). However, in agreement with the localization of UKW at cell-cell junctions (Figure 3), and the documented function of other receptors with closest homology to UKW, we noticed an increased cell aggregation in the transfectants (data not shown). Also, the investigation of de-regulation of expression of UKW in several types of human cancer warrants further investigation as soon as improved antibodies become available.

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