[CANCER RESEARCH 52, 5416–5420, October 1, 1992]

Advances in Brief

An Ovarian Tumor Marker with Homology to Vaccinia Virus Contains an IgV-like Region and Multiple Transmembrane Domains

Ian G. Campbell,[1] Paul S. Freemont, William Foulkes, and John Trowsdale

Human Immunogenetics Laboratory [I. G. C., W. F., J. T.] and Protein Structure Laboratory [P. S. F.], Imperial Cancer Research Fund, P. O. Box 123, Lincoln's Inn Fields, London WC2A 3PX, United Kingdom

Abstract

The monoclonal antibody OVTL3 has a highly restricted reactivity with ovarian carcinomas and defines a surface glycoprotein, OA3, which has been used for immunotargeting. To understand why OA3 is found on ovarian tumors we isolated a complementary DNA by expression cloning. The clone encodes a 323-amino acid protein with 5 putative membrane spanning domains, reminiscent of a membrane receptor or channel, but of a new type with little or no sequence similarity with these families of proteins. Interestingly, the OA3 sequence is highly related to a vaccinia virus encoded protein (VA38) and its extracellular domain is a member of the immunoglobulin V region superfamily.

Introduction

It is now clear that tumors are unlikely to express unique cell surface molecules. However, there are a number of cases where normal cellular products are expressed with altered epitopes (1), or in inappropriate tissues (2), or at elevated levels (3). An understanding of the molecular basis for these markers may aid in diagnosis and therapy. About 90% of ovarian carcinomas, including mucinous carcinomas, express an antigenic surface determinant (OA3) which is rarely found on normal tissues (4) and the monoclonal antibody recognizing this epitope, OVTL3, is approved for human use (5, 6). While OVTL3 has been studied both *in vitro* and *in vivo* (7–10) nothing is known about the antigen due to the lability of the epitope. To optimize the effectiveness of OVTL3 based therapies and diagnosis an understanding of the precise nature of OA3 is essential. As a first step in gaining such insight we describe here the cloning of OA3 and its identification as a novel multimembrane spanning protein.

Materials and Methods

Cell Culture. Cell lines and growth conditions have been described (3). The ovarian carcinoma cell line OVM1 was supplied by Professor W. H. Stimson, University of Strathclyde, Scotland.

Monoclonal Antibodies and FACS. MAb OVTL3 was supplied by Dr. V. Zurawski (Centocor, Malvern, Philadelphia, PA). FACS[2] analysis was performed as described previously (3).

cDNA Libraries and Cloning. A cDNA library of approximately 2×10^7 recombinants was prepared from ovarian carcinoma line PE/01 in the expression vector CDM8 (11) using the *Bst* XI cloning site. The SKOV3 ovarian cDNA library has been described (3). The remaining cDNA libraries used in this study, also in CDM8, were supplied by David Simmonds (Imperial Cancer Research Fund, Oxford, United Kingdom). Plasmid DNA was introduced into COS-7 cells by electroporation as described for WOP cells (3) except that a voltage of 250 V was used. Recovery of OVTL3 positive transfectants by magnetic bead separation was carried out as described by Campbell *et al.* (3).

RNA and DNA Blot Analyses. Poly(A)$^+$ RNA was isolated from the cells lines using a commercial kit (Fast Track; Invitrogen) and 0.5 µg of poly(A)$^+$ loaded per track. Poly(A)$^+$ RNA from 8 different human tissues was probed using a commercially available blot (MTN; Clonetech). Hybridization was carried out in 5 × SSC, 0.2% sodium dodecyl sulfate, and 10% dextran sulfate with random primed [^{32}P]-dCTP labeled DNA probes. The membranes were washed under high stringency conditions unless stated otherwise (0.1 × SSC-0.1% sodium dodecyl sulfate at 65°C; 1 × SSC = 0.15 M NaCl, 0.015 M sodium citrate).

PCR. PCRs were performed in 100 µl containing 1 µg of the appropriate cDNA library and 1 µg of each primer. Reactions were run for 15–35 cycles at 98°C, 2 min; 55–60°C, 2 min; 72°C, 3 min.

Glycosylation Inhibition. Cells (1×10^6) were seeded into 10-cm dishes and grown in E4 + 10% fetal calf serum containing either tunicamicin at 2 µg/ml or benzyl-2-acetamido-2-deoxy-α-D-galactopyranoside (Sigma) at 640 µg/ml. Controls with no inhibitor were included. After 24 and 48 h aliquots of cells were treated with OVTL3 and analyzed by FACS.

Results and Discussion

Cloning of the OA3 Antigen. OA3 cDNAs were isolated from PE/01 (ovarian carcinoma line) and U937 (macrophage/monocyte line) cDNA libraries by expression cloning using transiently transfected COS-7 cells (3). Both cDNAs encoded a protein, OA3-323, of 323 amino acids (Fig. 1*A*). Sequence analysis of the longer clone, cOA3-4, showed a 969-base pair open reading frame flanked by 5′ and 3′ noncoding regions of 106 and 210 base pairs, respectively. The first ATG is preceded by an in-frame termination codon, and although it conforms only poorly to the eukaryotic translation initiation consensus, it is rare for the first methionine not to be the initiating one (12). This assignment was confirmed with a cOA3-323 construct lacking the sequence 5′ of the *Eco* RI site (position 84) which failed to express an OVTL3 reactive antigen in either COS-7 or WOP cells (mouse NIH 3T3 derivatives). Additionally the extended NH_2 terminus has some characteristics of a hydrophobic signal sequence (13).

Splice Variants of OA3. Three additional cDNAs were isolated by hybridization from a PE/01 cDNA library which lacked all or part of the 3′ coding region and encoded proteins consisting of 293, 305, or 312 amino acids, probably derived by differential splicing. OA3-312, OA3-305, and OA3-293 lack 33, 58, and 90 base pairs, respectively, upstream of the G in the last codon. OA3-293 and OA3-312 retain the terminal glutamic acid residue and utilize the same stop codon as OA3-323. The 58-base pair deletion of OA3-305 results in a frame shift, extending the open reading frame by two codons which predicts a

Received 7/30/92; accepted 8/27/92.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] To whom requests for reprints should be addressed.

[2] The abbreviations used are: FACS, fluorescence activated cell sorter; PCR, polymerase chain reaction; cDNA, complementary DNA; poly(A), polyadenylate; SSC, standard saline-citrate (1 × SSC is 0.15 M NaCl-0.015 M sodium citrate; TM, transmembrane.

Downloaded from http://aacrjournals.org/cancerres/article-pdf/52/19/5416/2448414/cr0520195416.pdf by guest on 13 April 2024

A

```
                                                                                          -106
                                                                                          GGGCTG CCTGTGACGC  -91
                              *
GCGGCGCGGT CGGTCCTGCC TGTAACGGCG GCGGCGGCTG CTGCTCCAGA CACCTGCGGC GGCGGCGGCG ACCCCGCGGC GGGCGCGGAG  -1

ATG TGG CCC CTG GTA GCG GCG CTG TTG CTG GGC TCG GCG TGC TGC GGA TCA GCT CAG CTA CTA TTT AAT AAA ACA   75
Met Trp Pro Leu Val Ala Ala Leu Leu Leu Gly Ser Ala Cys Cys Gly Ser Ala Gln Leu Leu Phe Asn Lys Thr   25

AAA TCT GTA GAA TTC ACG TTT TGT AAT GAC ACT GTC GTC ATT CCA TGC TTT GTT ACT AAT ATG GAG GCA CAA AAC  150
Lys Ser Val Glu Phe Thr Phe Cys Asn Asp Thr Val Val Ile Pro Cys Phe Val Thr Asn Met Glu Ala Gln Asn   50

ACT ACT GAA GTA TAC GTA AAG TGG AAA TTT AAA GGA AGA GAT ATT TAC ACC TTT GAT GGA GCT CTA AAC AAG TCC  225
Thr Thr Glu Val Tyr Val Lys Trp Lys Phe Lys Gly Arg Asp Ile Tyr Thr Phe Asp Gly Ala Leu Asn Lys Ser   75

ACT GTC CCC ACT GAC TTT AGT AGT GCA AAA ATT GAA GTC TCA CAA TTA CTA AAA GGA GAT GCC TCT TTG AAG ATG  300
Thr Val Pro Thr Asp Phe Ser Ser Ala Lys Ile Glu Val Ser Gln Leu Leu Lys Gly Asp Ala Ser Leu Lys Met  100

GAT AAG AGT GAT GCT GTC TCA CAC ACA GGA AAC TAC ACT TGT GAA GTA ACA GAA TTA ACC AGA GAA GGT GAA ACG  375
Asp Lys Ser Asp Ala Val Ser His Thr Gly Asn Tyr Thr Cys Glu Val Thr Glu Leu Thr Arg Glu Gly Glu Thr  125

ATC ATC GAG CTA AAA TAT CGT GTT GTT TCA TGG TTT TCT CCA AAT GAA AAT ATT CTT ATT GTT ATT TTC CCA ATT  450
Ile Ile Glu Leu Lys Tyr Arg Val Val Ser Trp Phe Ser Pro Asn Glu Asn Ile Leu Ile Val Ile Phe Pro Ile  150

TTT GCT ATA CTC CTG TTC TGG GGA CAG TTT GGT ATT AAA ACA CTT AAA TAT AGA TCC GGT GGT ATG GAT GAG AAA  525
Phe Ala Ile Leu Leu Phe Trp Gly Gln Phe Gly Ile Lys Thr Leu Lys Tyr Arg Ser Gly Gly Met Asp Glu Lys  175

ACA ATT GCT TTA CTT GTT GCT GGA CTA GTG ATC ACT GTC ATT GTC ATT GTT GGA GCC ATT CTT TTC GTC CCA GGT  600
Thr Ile Ala Leu Leu Val Ala Gly Leu Val Ile Thr Val Ile Val Ile Val Gly Ala Ile Leu Phe Val Pro Gly  200

GAA TAT TCA TTA AAG AAT GCT ACT GGC CTT GGT TTA ATT GTG ACT TCT ACA GGG ATA TTA ATA TTA CTT CAC TAC  675
Glu Tyr Ser Leu Lys Asn Ala Thr Gly Leu Gly Leu Ile Val Thr Ser Thr Gly Ile Leu Ile Leu Leu His Tyr  225

TAT GTG TTT AGT ACA GCG ATT GGA TTA ACC TCC TTC GTC ATT GCC ATA TTG GTT ATT CAG GTG ATA GCC TAT ATC  750
Tyr Val Phe Ser Thr Ala Ile Gly Leu Thr Ser Phe Val Ile Ala Ile Leu Val Ile Gln Val Ile Ala Tyr Ile  250

CTC GCT GTG GTT GGA CTG AGT CTC TGT ATT GCG GCG TGT ATA CCA ATG CAT GGC CCT CTT CTG ATT TCA GGT TTG  825
Leu Ala Val Val Gly Leu Ser Leu Cys Ile Ala Ala Cys Ile Pro Met His Gly Pro Leu Leu Ile Ser Gly Leu  275
                                                                     293
AGT ATC TTA GCT CTA GCA CAA TTA CTT GGA CTA GTT TAT ATG AAA TTT GTG GCT TCC AAT CAG AAG ACT ATA CAA  900
Ser Ile Leu Ala Leu Ala Gln Leu Leu Gly Leu Val Tyr Met Lys Phe Val Ala Ser Asn Gln Lys Thr Ile Gln  300
            305                         312                                             |  *    **
CCT CCT AGG AAA GCT GTA GAG GAA CCC CTT AAT GCA TTC AAA GAA TCA AAA GGA ATG ATG AAT GAT GAA TAA CTG A 976
Pro Pro Arg Lys Ala Val Glu Glu Pro Leu Asn Ala Phe Lys Glu Ser Lys Gly Met Met Asn Asp Glu          323
           ---------------------------------------------------------------------------Asn Asn

AGTGAAGTGA TGGACTCCGA TTTGGAGAGT AGTAAGACGT GAAAGGAATA CACTTCTGTT TAAGCACCAT GGCCTTGATG ATTCACTGTT   1066
GGGGAGAAGA AACAAGAAAA GTAACTGGTT GTCACCTATG AGACCCTTAC GTGATTGTTA GTTAAGTTTT TATTCAAAGC AGCTGTAATT   1156
TAGTTAATAA AATAATTATG ATC  1179
```

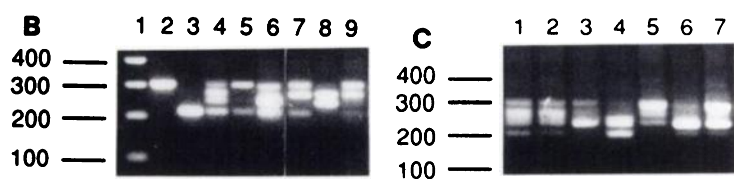

Fig. 1. (*A*) Nucleotide and deduced amino acid sequence of the OA3 cDNA. *Underlined sequences*, putative transmembrane domains predicted by regional hydropathicity analysis (see Fig. 3*B*). A potential NH_2-terminal hydrophobic signal sequence is *dashed underlined* and a AATAAA poly(A) addition sequence is *boxed*. Consensus *N*-glycosylation sites are marked by a *dot*. The extended reading frame predicts a protein terminating with Asn Asn as indicated below the main sequence. Splice variants OA3-312, OA3-305, and OA3-293 lack the sequence indicated by the appropriate *arrow* up to and including the G in the last codon (*vertical line*). (*B* and *C*). Agarose gel (2%) electrophoresis of PCR amplified DNA across the alternate splice region of OA3. PCRs were performed in 100 μl containing 500 ng of the indicated cDNA library (*B*; *C*, *Track 1*) or 10 ng of OA3-323 or OA3-293 cDNA (*B*, *Tracks 2* and *3*) or first strand cDNA from cell lines indicated (*C*). (*B*) *Track 1*, 100-base pair ladder (sizes indicated on the *left*); *Track 2*, cOA3-323; *Track 3*, cOA3-293; *Track 4*, PE/01; *Track 5*, SKOV3; *Track 6*, U937; *Track 7*, colon carcinoma; *Track 8*, placenta; *Track 9*, HT29. (*C*) *Track 1*, PE/01 (cDNA library); *Track 2*, PE/01; *Track 3*, SKOV3; *Track 4*, U937; *Track 5*, OVCA432; *Track 6*, MANN; *Track 7*, MOLT4.

protein terminating with *Asn Asn*. All three cDNAs expressed an OVTL3 reactive antigen when transfected into either COS-7 or WOP cells.

The distribution of differentially spliced mRNAs was assessed by PCR amplification of cDNA libraries (Fig. 1 *B*) and first strand cDNA (Fig. 1 *C*). Amplification of the 3′ end of OA3 cDNAs were performed with the following primers: Primer 1 (sense) 5′-GTTGGACTGAGTCTCTGTATTGCG-GCCTGT-3′ (corresponding to nucleotide position 760 to 789); and primer 2 (antisense) 5′-CCCAACAGTGAATCATCAAG-GCCATGGTGC-3′ (corresponding to nucleotide positions 1040 to 1069). At least two of the alternate products were evident in cDNA libraries from the cell lines (Fig. 1 *B*) while it was possible to detect more when using first strand cDNA (Fig. 1 *C*). Cycle frequencies of 20 or 30 made no appreciable difference to the relative amounts of each splice product within the same sample. In some cases (*e.g.*, SKOV3) the cDNA libraries were not representative showing fewer bands than obtained with first strand cDNA. There was no correlation between cell type or surface expression of OA3 with the predominance of a particular splice product.

Expression of OA3 among Cell Lines. To confirm that our cDNA encoded the *in vivo* target of OVTL3 we compared the cell surface reactivity of OVTL3 with cOA3 RNA levels.

Downloaded from http://aacrjournals.org/cancerres/article-pdf/52/19/5416/2448414/cr0520195416.pdf by guest on 13 April 2024

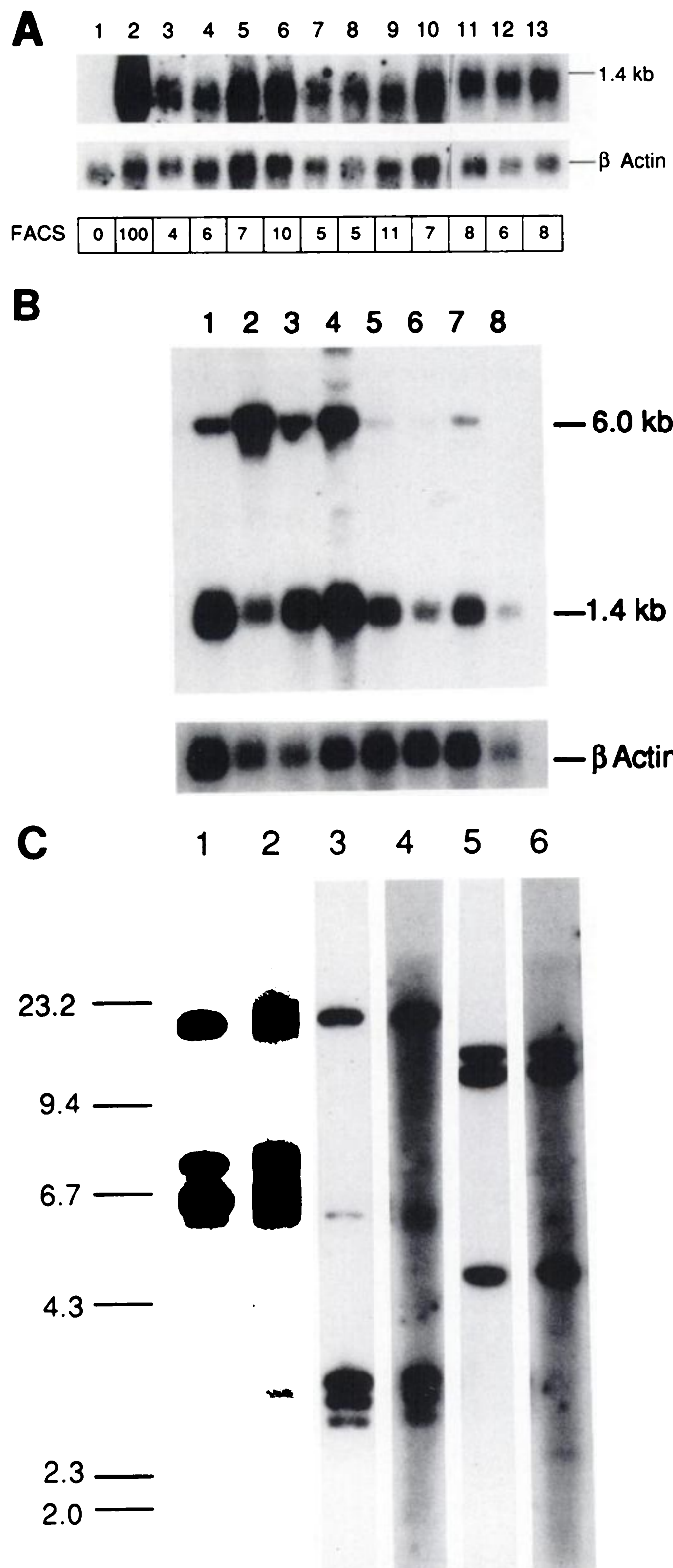

Fig. 2. (*A*) Expression of OA3 mRNA on Northern blots and surface reactivity of cell lines with MAb OVTL3. The 3' *Eco* RI fragment of the cOA3-4 cDNA was used as probe (*top*). *kb*, kilobases. The membrane was washed under stringent conditions (0.1 × SSC, 65°C). *Bottom*, result with a β-actin probe as a loading control. Poly(A)$^+$ RNA (0.5 μg) was isolated by standard procedures from ovarian carcinoma cell lines: ovM1 (*Track 1*); OVCA432 (*Track 2*); OVCA433 (*Track 3*); SKOV3 (*Track 4*); PE/01 (*Track 5*); JAMA (*Track 6*); breast carcinoma cell line SKBR3 (*Track 7*); epitheloid carcinoma line HeLa (*Track 8*); neuroblastoma cell line NB100 (*Track 9*); fibrosarcoma cell line HT1080 (*Track 10*); B-cell line Mann (*Track 11*); lymphoblastic leukemia cell line Molt4 (*Track 12*); macrophage/monocyte cell line U937 (*Track 13*). The relative surface expression of OA3 using MAb OVTL3 as assessed by FACS analysis is indicated at the bottom of each lane in arbitrary units. (*B*) Expression of OA3 on Northern blots of normal human tissue. The blot was probed and washed under stringent conditions with the 3' *Eco* RI cOA3-4 probe. The membrane was exposed for 24 h at −70°C with an intensifying screen. Each track contains approximately 2 μg of poly(A)$^+$ RNA from either heart (*Track 1*), brain (*Track 2*), placenta (*Track 3*), lung (*Track 4*), liver (*Track 5*), skeletal muscle (*Track 6*), kidney (*Track 7*), or pancreas (*Track 8*). Bottom, result with a β-actin probe. (*C*) Southern blot using the 3' *Eco* RI cOA3-4 probe hybridized to normal human DNA under stringent (0.1 × SSC, 65°C) conditions (*Tracks 1, 3,* and *5*) or nonstringent (0.6 × SSC, 55°C) conditions (*Tracks 2, 4,* and *6*). Tracks contain 10 μg of DNA digested with either *Bam* HI (*Tracks 1* and *2*), *Eco* RI (*Tracks 3* and *4*), or *Pst* I (*Tracks 5* and *6*). The membrane was exposed for 24 h at −70°C with an intensifying screen. *Left*, sizes in kilobases (*kb*) of λ *Hin* dIII fragments.

Surface reactivity with OVTL3 was detected with all but one (the ovarian carcinoma cell line OVM1) of over 25 cell lines tested. Northern blots from a selection of these (Fig. 2*A*) showed a band at 1.4 kilobases, the intensity of which reflected the OVTL3 cell surface reactivity as measured by FACS. In particular, OVCA432 showed particularly strong reactivity with OVTL3 and a correspondingly intense signal in the Northern blot. With OVM1, neither surface reactivity with OVTL3 nor cOA3 RNA was detected, supporting the identity of our cDNA product as the *bona fide* target of OVTL3. Additionally we have mapped the *OA3* gene to chromosome 3 using human/rodent somatic hybrids and only those containing the *OA3* gene showed surface reactivity with OVTL3 (data not shown).

Expression of OA3 in Normal Tissue. We extended the Northern analysis to normal human tissues and here too expression of the 1.4-kilobase OA3 mRNA was ubiquitous, although differences in levels were observed (Fig. 2 *B*). Paradoxically, the *in vivo* reactivity of OVTL3 is highly restricted to ovarian carcinoma although the epithelia of normal endometrium, fallopian tube, endocervix, and lymphocytes have been shown to weakly express OA3 (4, 14). This suggests that the epitope on OA3, recognized by OVTL3, is revealed in the tumor cells either after posttranslational modification or possibly as a result of association with other membrane proteins. However, glycosylation differences do not appear to be responsible inasmuch as treatment of cells with tunicamicin or benzyl-2-acetamido-2-deoxy-α-D-galactopyranoside did not significantly affect OVTL3 binding to either SKOV3, PE/10, or OVCA432 (not shown).

In addition to the 1.4-kilobase transcript a 6-kilobase band of variable intensity was also visible and since the signal was not diminished even after extended stringent washing it is probably derived from the same gene. It is not known if this is a mature transcript or if it represents unprocessed cOA3 mRNA.

Southern Analysis. The complexity of the *OA3* locus was determined by Southern hybridization under both stringent and nonstringent conditions, respectively (Fig. 2 *C*). The probe consisted of the 3' *Eco* RI fragment of cOA3-4 because of the presence of a repeat at the 5' end of the cDNA. Under stringent hybridization conditions at least three bands are evident. Nonstringent conditions revealed in each digest only one additional very weakly hybridizing band of 3.2 kilobases (*Bam* HI), 5.6 kilobases (*Eco* RI), or 2.4 kilobases (*Pst* I). However, prolonged exposure of the stringently hybridized filter (4 days) revealed the same bands indicating that they are most likely located at the extreme end of the *OA3* gene and contain a short stretch of homology with the cOA3 probe. These results suggest that OA3 is a single copy gene, a finding in contrast to the families of genes normally associated with proteins of this general architecture (15). We have also examined by hybridization matched

Downloaded from http://aacrjournals.org/cancerres/article-pdf/52/19/5416/2448414/cr0520195416.pdf by guest on 13 April 2024

tumor and normal DNA from over 20 ovarian carcinoma patients and found no obvious amplifications, deletions, or rearrangements of this gene.

Sequence Searches and Predicted Protein Structure. A protein sequence data bank search found 28% identity of OA3 with VA38 (Fig. 3 *A*) a protein from two stains of vaccinia virus (16, 17). The sequence was analyzed in terms of the propensity of a residue to be inside or just outside transmembrane regions, according to the algorithm of Rao and Argos (18) as implemented in a program written by M. Sternberg. A similar analysis performed for the VA38 sequence shows a striking resemblance to the plot for OA3-323 (Fig. 3 *B*). From this analysis we propose a speculative model for the OA3 structure showing 5 TM domains (Fig. 3 *C*). In OA3, TM IV is long and contains two peaks but we believe that only one TM region is likely by comparison with the corresponding region of VA38. This raises the possibility that TM IV is preceded by a hydrophobic cytoplasmic domain (*shaded circle*). In VA38, TMV has two peaks but neither is long enough to span the membrane, and only one TM domain is predicted in OA3. This model places the NH_2 terminus of the protein on the extracellular side of the membrane on the assumption that the first 19 amino acids constitute a signal peptide. This is supported by the presence of 5 potential extracellular *N*-linked glycosylation sites at positions 23, 34, 50, 73, and 110 present on the first extracellular domain . Alternatively if a hydrophobic signal is not removed, then six transmembrane domains are predicted with the NH_2 terminus on the cytoplasmic side. In either case the *N*-glycosylation sites remain on the extracellular side. According to this model sequence differences found in the splice variants are restricted to the COOH-terminal cytoplasmic domain. Interestingly, there is no counterpart to this region in vaccinia VA38 protein. A further search of the protein database with the putative extracellular domain revealed a significant homology to V regions of the immunoglobulin gene superfamily, particularly to those IgV-related sequences from cartilage link proteins (19) and poliovirus receptor (20) (Fig. 3 *D*).

OA3 is one of the few tumor antigens that have been cloned. Although its function is unknown, the multimembrane spanning domain structure is suggestive of a role in membrane transport and/or signal transduction. It has been suggested that the vaccinia virus homologue of OA3 may be involved in membrane permeability changes induced following virus infection (17). What role this gene has in ovarian carcinoma and indeed

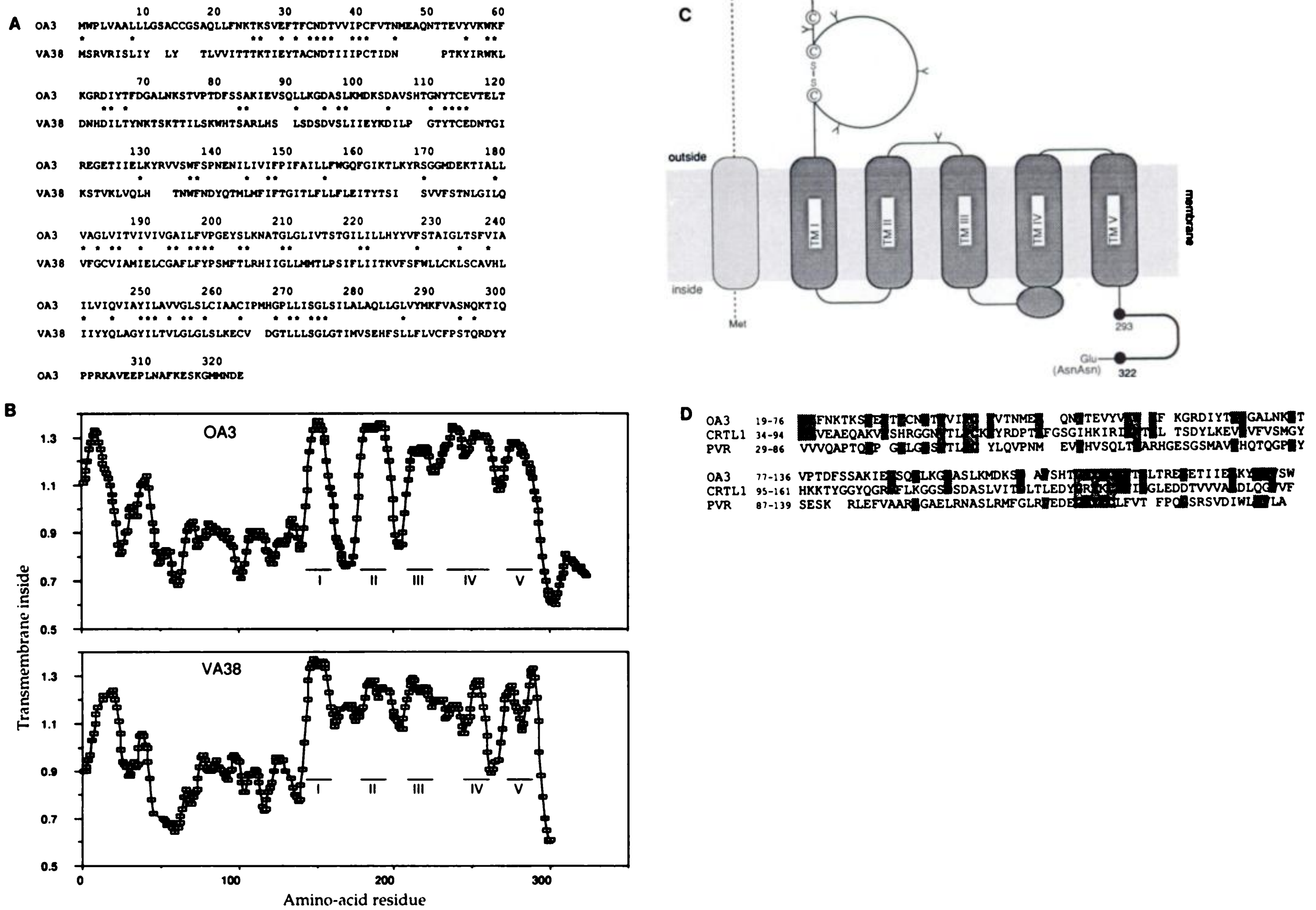

Fig. 3. (*A*) Sequence alignment of OA3-323 with VA38. *, identical residues. (*B*) Transmembrane prediction. □, proposed TM regions of OA3 and VA38. The calculation for residues inside transmembrane regions used a window length of 7 with 2 smoothing cycles and residues with scores greater than 1.1 were deemed as potential transmembrane residues. (*C*) Structural model for OA3. Cylinders (*dark shading*) indicate transmembrane domains TM I–V and a possible NH_2 terminus TM region is indicated (*light shading*). Putative sites of *N*-glycosylation (*Y*) and extracellular cysteine residues (©) are indicated. A cysteine pair is shown (S-S) based on homology with the immunoglobulin superfamily. The domain is most likely of the V-type based on the longer distance (74 amino acids) between cysteine residues and homology with human cartilage link protein and poliovirus receptor (see *D*). *Thick line*, COOH terminus region deleted in the shorter variants of OA3. (*D*) Amino acid homology of OA3 with immunoglobulin superfamily (V set) members. *CRTL1*, human cartilage link protein (19); *PVR*, poliovirus receptor (20). Amino acids identical with OA3 are *shaded* and those conserved in all three are boldface.

Downloaded from http://aacrjournals.org/cancerres/article-pdf/52/19/5416/2448414/cr0520195416.pdf by guest on 13 April 2024

in normal cells is unknown but its presence on vaccinia virus should facilitate elucidation of its function. OA3 is the first example of an immunoglobulin related domain associated with multiple transmembrane regions characteristic of a pore. Many members of the immunoglobulin superfamily participate in recognition and the identification of the ligand of OA3 could have important therapeutic implications.

Acknowledgments

We thank David Simmonds for generously providing cDNA libraries. We are also grateful to Lambert Poels, Vincent Zurawski, and Leslie Coney for helpful discussion.

References

1. Gendler, S. L. Burchell, J. M., Duhig, T., Lamport, D., White, R., Parker, M., and Taylor-Papadimitriou, J. Cloning of partial cDNA encoding differentiation and tumor-associated mucin glycoproteins expressed by human mammary epithelium. Proc. Natl. Acad. Sci. USA, *84:* 6060–6064, 1987.
2. Nouwen, E. J., Pollet, D. E., Schelstraete, J. B., Eerdekens, M. W., Hansch, C., van de Voorde, A., and De Broe, M. E. Human placental alkaline phosphatase in benign and malignant ovarian neoplasia. Cancer Res., *45:* 892–902, 1985.
3. Campbell, I. G., Jones, T. A., Foulkes, W. D., and Trowsdale, J. Folate-binding protein is a marker for ovarian cancer. Cancer Res., *51:* 5329–5338, 1991.
4. Poels, L. G., Peters, D., Van Megen, Y., Vooijs, G. P., Verheyen, R. N. M., Willemen, A., van Niekerk, C. C., Jap, P. H. K., Mungyer, G., and Kenemans, P. Monoclonal antibody against human ovarian tumor associated antigens. J. Natl. Cancer Inst., *76:* 781–791, 1986.
5. Kenemans, P. CA125 and OA3 as target antigens for immunodiagnosis and immunotherapy in ovarian cancer. Eur. J. Obstet. Gynecol. Reprod. Biol., *36:* 221–238, 1990.
6. Buist, M. R., Kenemans, P., Vermorken, J. B., Golding, R. P., Burger, C. W., Denhollanders, W., Vankamp, G. J., Vanlingens, A., Teules, G. J. J., Baak, J. P. A., and Rooss, J. C. Radioimmunotargetting in ovarian carcinoma patients with indium-111 labeled monoclonal antibody OV-TL3 F(ab')$_2$: pharmacokinetics, tissue distribution, and tumor imaging. Int. J. Gynecol. Cancer, *2:* 23–34, 1992.
7. Henzen-Logmans, S. C., Schipper, N. W., Poels, L. G., Kenemans, P., and Meyer, C. J. L. M. Use of statistical evaluation of antigen profiles in differential diagnosis between colonic and ovarian adenocarcinomas. J. Clin. Pathol., *41:* 644–649, 1988.
8. Kuhnel, R., Rao, R. B., Poels, L. G. T., Delemarre, J. F. M., Kenemans, P., and Stolk, J. G. Multiple parameter analyses of human ovarian cancer: morphology, immunohistochemistry, steroid hormone receptors and aromatase. Anticancer Res., *8:* 281–286, 1988.
9. Boerman, O. C., Massuger, L., Makkink, K., Thomas, C., Kenemans, P., and Poels, L. Comparative *in vitro* binding characteristics and biodistribution in tumor-bearing athymic mice of anti-ovarian carcinoma monoclonal antibodies. Anticancer Res., *10:* 1289–1296, 1990.
10. Boerman, O. C., van Niekerk, C. C., Makkink, K., Hanselaar, T. G. J. M., Kenemans, P., and Poels, L. Comparative Immunohistochemical study of four monoclonal antibodies directed against ovarian carcinoma-associated antigens. Int. J. Gynecol. Pathol., *10:* 15–25, 1991.
11. Seed, B. An LFA-3 cDNA encodes a phospholipid membrane protein homologous to its receptor CD2. Nature (Lond.), *329:* 840–842, 1987.
12. Kozac, M. Analysis of 5'-noncoding sequences from 699 vertebrate messenger RNAs. Nucleic Acids Res., *15:* 8125–8148, 1988.
13. van Heijne, G. A new method for predicting signal sequence cleavage sites. Nucleic Acids Res., *14:* 4683–4690, 1986.
14. Massuger, L. F. A. G., Kenemans, P., Claessens, R. A. M. J., Verheijen, R. H. M., and Corstens, F. H. M. Detection and localization of ovarian cancer with radiolabeled monoclonal antibodies. Eur. J. Obstet. Gynecol. Reprod. Biol., *41:* 47–63, 1991.
15. Montal, M. The molecular anatomy and molecular design of channel proteins. FASEB J., *9:* 2623–2635, 1990.
16. Goebel, S. J., Johnson, G. P., Perkus, M. E., Davis, S. W., Winslow, J. P., and Paoletti, E. The complete DNA sequence of vaccinia virus. Virology, *179:* 247–266, 1990.
17. Smith, G. L., Chan, Y. S. and Howard, S. T. Nucleotide sequence of 42 kbp of vaccinia virus strain WR from near the right inverted terminal repeat. J. Gen. Virol., *72:* 1349–1376, 1991.
18. Rao, J., and Argos, P. A. A conformational preference parameter to predict helices in integral membrane proteins. Biochim. Biophys. Acta, *869:* 197–214, 1986.
19. Osborne-Lawrence, S. L., Sinclair, A. K., Hicks, R. C., Lacey, S. W., Eddy, R. L., Jr., Byers, M. G., Shows, T. B., and Duby, A. D. Complete amino acid sequence of human cartilage link protein (CRTL1) deduced from cDNA clones and chromosomal assignment of the gene. Genomics, *8:* 562–567, 1990.
20. Mendelsohn, C. L., Wimmer, E., and Racaniello, V. C. Cellular receptor for poliovirus: molecular cloning, nucleotide sequence, and expression of a new member of the immunoglobulin superfamily. Cell, *56:* 855–865, 1989.

Downloaded from http://aacrjournals.org/cancerres/article-pdf/52/19/5416/2448414/cr0520195416.pdf by guest on 13 April 2024