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Biochemistry

Characterization of ROS1 cDNA from a human glioblastoma cell line

(tyrosine kinases/oncogenes/sevenless/receptors)

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Contributed by Michael Wigler, March 29, 1990

ABSTRACT **We have isolated and characterized a human ROS1 cDNA from the glioblastoma cell line SW-1088. The cDNA, 8.3 kilobases long, has the potential to encode a transmembrane tyrosine-specific protein kinase with a predicted molecular mass of 259 kDa. The putative extracellular domain of ROS1 is homologous to the extracellular domain of the sevenless gene product from *Drosophila*. No comparable similarities in the extracellular domains were found between ROS1 and other receptor-type tyrosine kinases. Together, *ROS1* and sevenless gene products define a distinct subclass of transmembrane tyrosine kinases.**

Oncogenes are defined as genetic elements that are able to induce malignant transformation. Many oncogenes are mutated or activated analogues of cellular genes that normally function in signal-transduction pathways. We have previously reported the isolation and characterization of the activated human *ROS1* gene, which we call *MCF3* (1). This oncogene, which was isolated by a transfection–tumorigenicity assay, encodes a transmembrane protein with a sequence typical of tyrosine kinases (2). *MCF3* was activated by a rearrangement in which all but eight amino acids of the *ROS1*-specific extracellular domain were replaced with sequences of unknown origin. Structurally, *MCF3* is very similar to the *erbB*, *fms*, *neu*, *trk*, and *kit* oncogenes (3–7). The normal cellular analogues of *erbB* and *fms* encode receptors for the epidermal growth factor and colony-stimulating factor, respectively (8, 9). Hence, we assume that *ROS1* also encodes a cellular receptor.

ROS1 is not a ubiquitously expressed gene. In a survey of 40 different human tumor cell lines, *ROS1* was found to be expressed frequently in cell lines established from one particular type of human tumor, glioblastomas. *ROS1* transcripts were not found in a normal glial cell line or in adult brain tissue (10). Most glioblastoma cell lines express a *ROS1* transcript of identical length, 8.3 kilobases (kb), with the exception of one particular line, U-118 MG. This line expresses a 4-kb transcript and has a rearranged *ROS1* locus (10). The characterization of this mutant *ROS1* gene has been reported elsewhere (11).

We report here the isolation and sequence[§] of a ROS1 cDNA from the SW-1088 glioblastoma cell line. This cell line expresses an 8.3-kb transcript. The cDNA can encode a protein of 259,000 daltons with a large extracellular domain, a transmembrane domain, and an intracellular domain with the characteristic sequence of a tyrosine protein kinase. It was previously noted that the products of the *Drosophila* gene sevenless and of *ROS1* have extensive homologies in their cytoplasmic domains (12). Sevenless is a gene required for normal eye development in the fruit fly and also encodes a transmembrane tyrosine-specific protein kinase (13, 14).

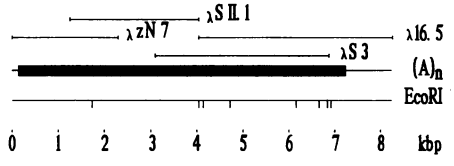

FIG. 1. Schematic representation of the ROS1 cDNA from SW-1088 cells. Overlapping phage λ clones used for sequence determination and a diagram of the composite cDNA structure are shown. Untranslated sequence and translated sequence are indicated by a line and a box, respectively. The *Eco*RI restriction map and the nucleotide coordinates are indicated below.

We find that the extracellular domains of the *ROS1* and the sevenless gene products share similarities in size and sequence as well. The distribution of cysteine residues in the extracellular domains of these gene products do not fit the patterns of previously described classes of transmembrane protein kinases. Thus, *ROS1* and *Drosophila* sevenless gene encode a new structural class of transmembrane protein kinases.

MATERIAL AND METHODS

cDNA Library. RNA from the human glioblastoma cell line SW-1088 was prepared by the guanidinium/CsCl method and purified on oligo(dT)-cellulose (15). Two cDNA libraries, one in phage λ gt10 and one in λ ZAP (Stratagene), were constructed by standard techniques (16). cDNA for the λ gt10 library was primed with oligo(dT). cDNA for the λ ZAP library was primed with a synthetic oligonucleotide of the sequence 5′-GGTTCACTAGCTGGCACCAGGGTAGTA-3′, the antisense sequence of positions 2204–2230 of ROS1 cDNA, and was cloned via *Not* I linkers into λ ZAP (17). cDNA fragments were used as probes to screen the libraries. Their coordinates were 6183–6649, 3160–4019, and 1207–1785. Phages containing ROS1 cDNA were identified by plaque hybridization (15) and characterized by restriction mapping.

Sequence and Analysis. Nucleotide sequence determination was performed after subcloning into pUC118 (18) by using the dideoxynucleotide chain-termination method (19) and Sequenase (United States Biochemical). All of the coding sequence was determined in both orientations. The hydropathic index was computed by the method of Kyte and Doolittle (20) with the PC/Gene program "SOAP" (IntelliGenetics). For the sequence comparison, the programs "COMPARE" and "DOTPLOT" from the University of Wisconsin, Genetics Computer Group (UWGCG), were used (21).

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‡To whom reprint requests should be addressed.

§The sequence reported in this paper has been deposited in the GenBank data base (accession no. M34353).

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   1                                                          CCGCATTCAAGCTTTCAAGCATTCAAAGGTCTAAATGAAAAAGGCTAAGTATTATTTCAAAAGGCAAGTAT     71

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 207 ATGAAGAACATTTACTGTCTTATTCCGAAGCTTGTCAATTTTGCAACTCTTGGCTGCCTATGGATTTCTGTGGTGCAGTGTACAGTTTTAAATAGCTGCCTAAAGTCGTGTGTAACTAATCTGGGCCAGCAGCTT   341
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 342 GACCTTGGCACACCACATAATCTGAGTGAACCGTGTATCCAAGGATGTCACTTTTGGAACTCTGTAGATCAGAAAAACTGTGCTTTAAAGTGTCGGGAGTCGTGTGAGGTTGGCTGTAGCAGCGCGGAAGGTGCA   476
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 136 AlaGlnLeuLeuGlySerTrpThrTyrThrLysThrValSerArgProSerTyrValValLysProLeuHisProPheThrGluTyrIlePheArgValValTrpIlePheThrAlaGlnLeuGlnLeuTyrSer   180

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 316 LeuArgLeuAspAlaIleTyrHisAsnIleThrGlyIleSerValAspValHisGlnGlnIleValTyrPheSerGluGlyThrLeuIleTrpAlaLysLysAlaAlaAsnMetSerAspValSerAspLeuArg   360

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1422 CCCTCTATTAGTGCACCTCAAAAAATTGTGGCTGATTCATACAATGGGTATGTCTTTTACCTCCTGAGAGATGGCATTTATAGAGCAGACCTTCCTGTACCATCTGGCCGGTGTGCAGAAGCTGTGCGTATTGTG   1556
 406 ProSerIleSerAlaProGlnLysIleValAlaAspSerTyrAsnGlyTyrValPheTyrLeuLeuArgAspGlyIleTyrArgAlaAspLeuProValProSerGlyArgCysAlaGluAlaValArgIleVal   450

1557 GAGAGTTGCACGTTAAAGGACTTTGCAATCAAGCCACAAGCCAAGCGAATCATTTACTTCAATGACACTGCCCAAGTCTTCATGTCAACATTTCTGGATGGCTCTGCTTCCCATCTCATCCTACCTCGCATCCCC   1691
 451 GluSerCysThrLeuLysAspPheAlaIleLysProGlnAlaLysArgIleIleTyrPheAsnAspThrAlaGlnValPheMetSerThrPheLeuAspGlySerAlaSerHisLeuIleLeuProArgIlePro   495

1692 TTTGCTGATGTGAAAAGTTTTGCTTGTGAAAACAATGACTTTCTTGTCACAGATGGCAAGGTCATTTTCCAACAGGATGCTTTGTCTTTTAATGAATTCATCGTGGGATGTGACCTGAGTCACATAGAAGAATTT   1826
 496 PheAlaAspValLysSerPheAlaCysGluAsnAsnAspPheLeuValThrAspGlyLysValIlePheGlnGlnAspAlaLeuSerPheAsnGluPheIleValGlyCysAspLeuSerHisIleGluGluPhe   540

1827 GGGTTTGGTAACTTGGTCATCTTTGGCTCATCCTCCCAGCTGCACCCTCTGCCAGGCCGCCCGCAGGAGCTTTCGGTGCTGTTTGGCTCTCACCAGGCTCTTGTTCAATGGAAGCCTCCTGCCCTTGCCATAGGA   1961
 541 GlyPheGlyAsnLeuValIlePheGlySerSerSerGlnLeuHisProLeuProGlyArgProGlnGluLeuSerValLeuPheGlySerHisGlnAlaLeuValGlnTrpLysProProAlaLeuAlaIleGly   585

1962 GCCAATGTCATCCTGATCAGTGATATTATTGAACTCTTTGAATTAGGCCCTTCTGCCTGGCAGAACTGGACCTATGAGGTGAAAGTATCCACCCAAGACCCTCCTGAAGTCACTCATATTTTCTTGAACATAAGT   2096
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2502 ACATATGTGATACAAAGGCAGTCTGTGTTGACGGGACACACAGACATTGTTACCCACGTGAAGCTATTGGTGAATGACATGGTGGTGGATTCAGTTGGTGGATATCTCTACTGGACCACACTCTATTCAGTGGAA   2636
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2637 AGCACCAGACTAAATGGGGAAAGTTCCCTTGTACTACAGACACAGCCTTGGTTTTCTGGGAAAAAGGTAATTGCTCTAACTTTAGACCTCAGTGATGGGCTCCTGTATTGGTTGGTTCAAGACAGTCAATGTATT   2771
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2907 TTTAGGATTATCACAACTCAAGAAATAGGTCAGAAAACCAGTGTCTCTGTTTTGGAACCAGCCAGATTTAATCAGTTCACAATTATTCAGACATCCCTTAAGCCCCTGCCAGGGAACTTTTCCTTTACCCCTAAG   3041
 901 PheArgIleIleThrThrGlnGluIleGlyGlnLysThrSerValSerValLeuGluProAlaArgPheAsnGlnPheThrIleIleGlnThrSerLeuLysProLeuProGlyAsnPheSerPheThrProLys   945

3042 GTTATTCCAGATTCTGTTCAAGAGTCTTCATTTAGGATTGAAGGAAATGCTTCAAGTTTTCAAATCCTGTGGAATGGTCCCCCTGCGGTAGACTGGGGTGTAGTTTTCTACAGTGTAGAATTTAGTGCTCATTCT   3176
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3177 AAGTTCTTGGCTAGTGAACAACACTCTTTACCTGTATTTACTGTGGAAGGACTGGAACCTTATGCCTTATTTAATCTTTCTGTCACTCCTTATACCTACTGGGGAAAGGGCCCCAAAACATCTCTGTCACTTCGA   3311
 991 LysPheLeuAlaSerGluGlnHisSerLeuProValPheThrValGluGlyLeuGluProTyrAlaLeuPheAsnLeuSerValThrProTyrThrTyrTrpGlyLysGlyProLysThrSerLeuSerLeuArg   1035

3312 GCACCTGAAACAGTTCCATCAGCACCAGAGAACCCCAGAATATTTATATTACCAAGTGGAAAATGCTGCAACAAGAATGAAGTTGTGGTGGAATTTAGGTGGAACAAACCTAAGCATGAAAATGGGGTGTTAACA   3446
1036 AlaProGluThrValProSerAlaProGluAsnProArgIlePheIleLeuProSerGlyLysCysCysAsnLysAsnGluValValValGluPheArgTrpAsnLysProLysHisGluAsnGlyValLeuThr   1080

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1126 PheGlnValArgAlaPheThrSerLysGlyProGlyProTyrAlaAspValValLysSerThrThrSerGluIleAsnProPheProHisLeuIleThrLeuLeuGlyAsnLysIleValPheLeuAspMetAsp   1170

3717 CAAAATCAAGTTGTGTGGACGTTTTCAGCAGAAAGAGTTATCAGTGCCGTTTGCTACACAGCTGATAATGAGATGGGATATTATGCTGAAGGGGACTCACTCTTTCTTCTGCACTTGCACAATCGCTCTAGCTCT   3851
1171 GlnAsnGlnValValTrpThrPheSerAlaGluArgValIleSerAlaValCysTyrThrAlaAspAsnGluMetGlyTyrTyrAlaGluGlyAspSerLeuPheLeuLeuHisLeuHisAsnArgSerSerSer   1215

3852 GAGCTTTTCCAAGATTCACTGGTTTTTGATATCACAGTTATTACAATTGACTGGATTTCAAGGCACCTCTACTTTGCACTGAAAGAATCACAAAATGGAATGCAAGTATTTGATGTTGATCTTGAACACAAGGTG   3986
1216 GluLeuPheGlnAspSerLeuValPheAspIleThrValIleThrIleAspTrpIleSerArgHisLeuTyrPheAlaLeuLysGluSerGlnAsnGlyMetGlnValPheAspValAspLeuGluHisLysVal   1260

3987 AAATATCCCAGAGAGGTGAAGATTCACAATAGGAATTCAACAATAATTTCTTTTTCTGTATATCCTCTTTTAAGTCGCTTGTATTGGACAGAAGTTTCCAATTTTGGCTACCAGATGTTCTACTACAGTATTATC   4121
1261 LysTyrProArgGluValLysIleHisAsnArgAsnSerThrIleIleSerPheSerValTyrProLeuLeuSerArgLeuTyrTrpThrGluValSerAsnPheGlyTyrGlnMetPheTyrTyrSerIleIle   1305

4122 AGTCACACCTTGCACCGAATTCTGCAACCCACAGCTACAAACCAACAAAACAAAAGGAATCAATGTTCTTGTAATGTGACTGAATTTGAGTTAAGTGGAGCAATGGCTATTGATACCTCTAACCTAGAGAAACCA   4256
1306 SerHisThrLeuHisArgIleLeuGlnProThrAlaThrAsnGlnGlnAsnLysArgAsnGlnCysSerCysAsnValThrGluPheGluLeuSerGlyAlaMetAlaIleAspThrSerAsnLeuGluLysPro   1350
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FIG. 2. (*Figure continues on the opposite page.*)

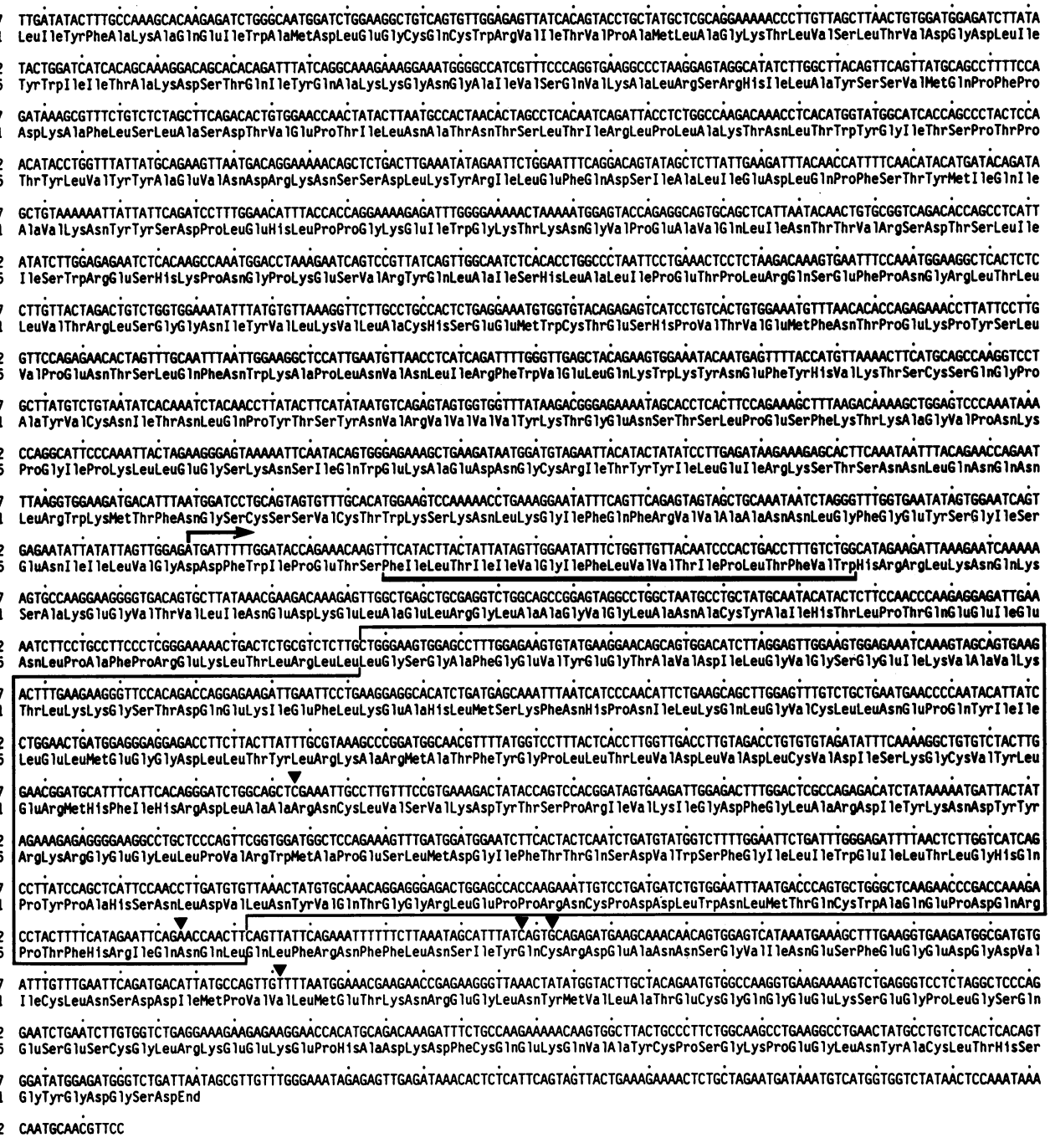

FIG. 2. Nucleotide sequence of ROS1 cDNA from SW-1088 cells and deduced amino acid sequence. The putative signal sequence is underlined. The start of the sequence that is also present in *MCF3* is indicated by an arrow. The putative transmembrane domain is underlined, and the tyrosine kinase domain is boxed. The positions where the ROS1 cDNA of SW-1088 differs from the other ROS1 coding published sequences are marked by arrowheads. Nucleotide and amino acid coordinates are indicated in the margin.

RESULTS

The ROS1 cDNA was isolated in several steps from two libraries that were prepared from poly(A)$^+$ RNA of the glioblastoma cell line SW-1088. An oligo(dT)-primed library was first screened by hybridization with a *ROS1*-specific probe derived from the previously isolated cDNA of the *MCF3* gene (2). In the subsequent steps, we used 5′ sequences from new cDNA isolates as probes and finally screened a second library prepared with an internal *ROS1*-specific primer (for details see *Material and Methods*). Four overlapping cDNA clones, which together span 8.3 kilobase pairs (kbp), were chosen for further sequence analysis (Fig. 1).

The sequence of the composite ROS1 cDNA (Fig. 2) has one large open reading frame, which starts at position 207 and ends at position 7247 with two consecutive termination codons. Approximately 1 kbp of 3′ untranslated sequence follows, which was not fully sequenced. We have assigned the ATG at positions 207–209 as the initiating codon because upstream termination codons exist in all three reading frames and because the nucleotide sequence flanking this ATG fulfills Kozak's criteria for an authentic initiation codon (22). Moreover, the downstream stretch of 36 amino acids has all the features of a signal sequence (23), which includes a hydrophobic stretch of 21 amino acids, clearly identifiable on a hydropathic profile (Fig. 3). By analogy with other signal-peptide cleavage sites (24), the amino terminus of the mature *ROS1* gene product would be Cys-37 (Fig. 2). The predicted molecular weight of the *ROS1* gene product, without post-

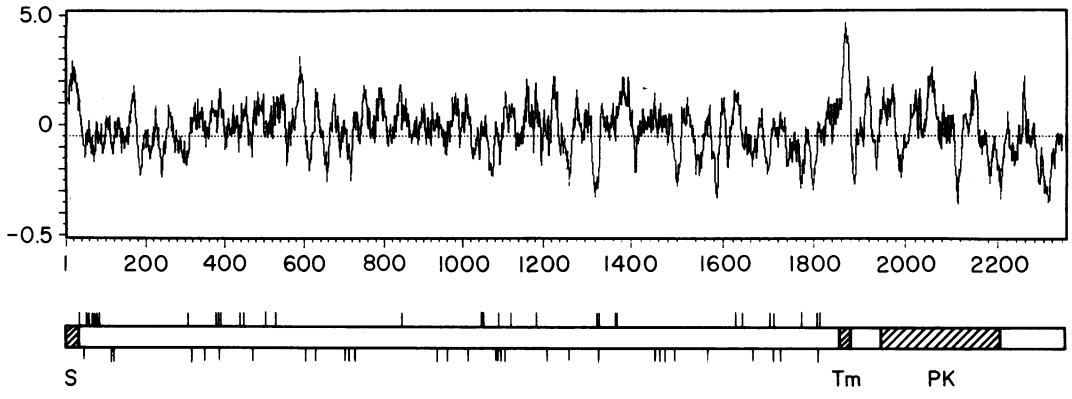

FIG. 3. Hydropathicity of the predicted *ROS1*-encoded protein. (*Upper*) The hydropathicity index (21) was determined with a window setting of 15 amino acids. Coordinates refer to amino acid positions of Fig. 2. (*Lower*) Hatched boxes represent the putative signal sequence (S), the transmembrane domain (Tm), and the tyrosine protein kinase (PK) domain. Vertical lines mark the positions of cysteine residues in the extracellular domain (above) and potential glycosylation sequences (below).

translational modification and after cleavage of the signal peptide, would thus be 259,000.

The amino acid sequence of the *ROS1* gene product from position 37 to position 1861 constitutes the putative extracellular domain and includes 31 potential N-linked glycosylation sites (Fig. 3). This sequence does not show similarities to the extracellular domains of previously described mammalian receptor-type tyrosine kinases. When compared to the epidermal growth factor or insulin receptor classes of tyrosine kinases (25), the ROS1 protein is not rich in cysteine residues except for one cluster of 11 cysteines at the very amino terminus. The spacing of cysteine residues is also not similar to the spacing found in the ligand binding domain of the platelet-derived growth factor receptor, the prototype of the third class of transmembrane tyrosine kinases (25). The consecutive stretch of 21 hydrophobic amino acids (residues 1862–1882) constitutes the putative transmembrane domain, which is followed by four closely spaced arginine and lysine residues (Fig. 3). The carboxyl-terminal 464 amino acids constitute the cytoplasmic domain and include sequences typical for tyrosine-specific protein kinases.

Two partial human *ROS1* sequences have been reported previously. One, the cDNA sequence of the activated human *ROS1* gene, *MCF3*, encodes a truncated protein missing all but eight amino acids of the extracellular domain (2). It corresponds to nucleotides 5764–7375, which encode amino acids 1854–2347, of the sequence of the full-size ROS1 cDNA. The other *ROS1* sequence was determined from a placental genomic DNA clone isolated by virtue of its homology to the chicken v-*ros* gene (26). It corresponds to nucleotides 5573–6941, which encode amino acids 1790–2245, of ROS1 cDNA from SW-1088 cells. There are no differences between the coding sequences of the *MCF3* gene and the human placental *ROS1* gene in the region of overlap between the two. There are, however, five differences between these sequences and the sequence of the ROS1 cDNA from SW-1088 cells (indicated in Fig. 2). The difference at position 6453 is silent. It changes the AGA codon (arginine) to CGA (Arg-2083). The differences at positions 6843, 6888, 6892, and 6991 change GAC (aspartic acid) to AAC (Asn-2213), AAG (lysine) to CAG (Gln-2228), TCC (serine) to TGC (Cys-2229), and GCT (alanine) to GTT (Val-2262), respectively. Apparently, the *ROS1* gene from the glioblastoma cell line SW-1088 has accumulated several mutations. The effect of these alterations on the physiological function of the gene product is presently unknown.

Previous investigators noted that the tyrosine kinase domains of the *ROS1* and the *Drosophila* sevenless gene products are closely related (12). In addition, we show here that both genes have also the potential to encode proteins of similar overall structures—i.e., transmembrane tyrosine kinases with unusually large extracellular domains. Further comparison by DOTPLOT analysis (Fig. 4) demonstrates that the sequences of the *ROS1* and sevenless gene products can be aligned over more than 2200 amino acids and that homologies exist in the extracellular domains as well. The extent of similarity differs in distinct parts of the proteins. Extensive similarities are present in the intracellular domain, and patches of homologies are found over 900 amino acids located in the amino-terminal half of the extracellular domain. In addition, the sevenless gene product contains a cluster of cysteine residues (13, 14) that occurs at a position that is roughly comparable to the location of the cysteine cluster of the ROS1 protein. The very amino-terminal 200 amino acids of the sevenless protein, which constitute a second potential transmembrane domain, have no equivalent in the ROS1 protein. No comparable alignment could be found when the extracellular domains of the ROS1 protein and other receptor-type tyrosine kinases (insulin, epidermal growth factor receptor, platelet-derived growth factor receptor, c-*kit*, and c-*fms*) were analyzed under the same stringency.

DISCUSSION

We have described the sequence of an 8.3-kb transcript of the *ROS1* gene from the human glioblastoma cell line SW-1088.

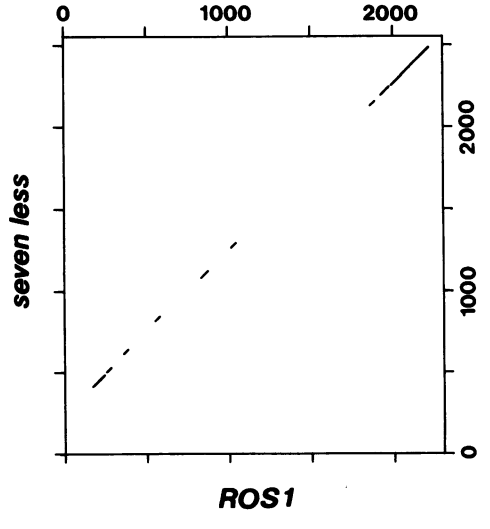

FIG. 4. DOTPLOT comparison of the amino acid sequences of *ROS1* and sevenless gene products. The predicted amino acid sequences of *ROS1* and sevenless gene products were analyzed for similarities at a window length of 30 and a stringency setting of 21 using the COMPARE/ALL option of the UWGCG package.

This transcript has the potential to encode a protein with an intracellular domain typical of tyrosine protein kinases, a transmembrane domain, a very large extracellular domain, and a putative amino-terminal signal peptide. Thus, *ROS1* resembles several protooncogenes that encode transmembrane tyrosine kinases that function as receptors. The extracellular domains of these proteins can be categorized by size and the distribution of cysteine residues. The amino acid sequence of the extracellular domain of the ROS1 product resembles none of the known mammalian transmembrane tyrosine kinases. However, it does resemble the sequence of the extracellular domain of the *Drosophila* sevenless gene product in size, amino acid sequence, and distribution of cysteine residues. *ROS1* and sevenless genes thus encode members of a distinct subfamily of transmembrane tyrosine kinases. Other similarities in structure and function may therefore exist between these proteins.

An 8.3-kb transcript of the *ROS1* gene is found frequently in human glioblastoma cell lines but not in a primary glial cell line or in adult brain tissue. Rearrangement of the *ROS1* gene and expression of a truncated transcript has been observed in one particular glioblastoma cell line, U-118 MG (10). In the sequence from the glioblastoma cell line SW-1088, we found four amino acids changes carboxyl-terminal of the tyrosine kinase domain, a part of the protein implicated in regulation of enzyme activity. Point mutations in this region have been observed to activate the oncogenic potential of the c-*src* and the c-*erbB* genes (25, 27). Thus, even in the absence of chromosomal rearrangements, such mutations might alter the physiological function or activate the oncogenic potential of the *ROS1* gene product and contribute to the malignancy of glioblastomas. In primary human tumors of glial origin, frequent chromosomal abnormalities have been observed on chromosome 17 and chromosome 10 (28). *ROS1* is located on chromosome 6 (29). It should be interesting to examine whether a particular chromosomal abnormality or a specific stage in tumor progression correlates with expression of the *ROS1* gene in primary tumors. Since the sequence of the ROS1 cDNA predicts a transmembrane protein accessible from the outside of the cell, the ROS1 protein might provide a specific target for antibody-based diagnosis or therapy.

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