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ORIGINAL PAPER

Isolation of a novel human voltage-dependent anion channel gene

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The voltage-dependent anion channel of the mitochondrial outer membrane (VDAC) is a small, abundant pore-forming protein found in the outer membranes of all eukaryotic mitochondria. The VDAC protein is believed to control the movement of adenine nucleotides through the outer membrane and to be the mitochondrial binding site for hexokinase and glycerol kinase. Two human VDAC cDNAs (HVDAC1 and HVDAC2) have been previously isolated and mapped on chromosome X and 21, respectively. Here, we report the isolation of a novel third human VDAC cDNA, corresponding to the mouse *MVDAC*3 gene. The expression of this gene in various tissues and its chromosomal localization by polymerase chain reaction (PCR) using a human/rodent somatic cell mapping panel and by fluorescence *in situ* hybridization is also presented.

Keywords: VDAC; fluorescence *in situ* hybridization; gene mapping; chromosome 8

Introduction

Voltage-dependent channels (VDACs), also known as mitochondrial porin, are a small (30-35 Kda), abundant pore-forming protein found in the outer membranes of mitochondria from cells of all eukaryotic organisms.[1] VDACs have been examined in great detail by electrophysiological methods and are a model for voltage regulation of membrane protein structure. Physiologically, VDAC is thought to function as the primary pathway for the movement of adenine nucleotides and other metabolites through the mitochondrial outer membrane, thus controlling the traffic of these essential compounds to and from this organelle as well as the entry of other substrates into a variety of metabolic pathways. VDAC has also been shown to be the site for binding of hexokinase and glycerol kinase to the mitochondrial outer membrane.[2,3] It has been proposed that binding to the mitochondrial outer membrane allows these enzymes preferential access to mitochondrial ATP, thereby linking cytoplasmic metabolism[4] to mitochondrial respiration and oxidative phosphorylation. Furthermore, VDAC can be part of a complex forming the mitochondrial benzodiazepine receptor,[5] a distinct receptor that is similar to the central receptor in its affinity for diazepam but differs in its affinity for other drugs.

Because of the wide variety of apparent functions, it has been suggested that there is more than one VDAC isoform.[2] The cDNAs of two forms of human VDAC (HVDAC1 and HVDAC2) have been cloned and found

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to be expressed in various tissues.[6,7] More recently, the isolation of a third novel murine VDAC cDNA (MVDAC3) has been reported.[8] In this study, we report the isolation of a novel human VDAC3 cDNA corresponding to the third mouse MVDAC3 cDNA. In addition, we present the tissue expression patterns and the chromosomal localization by PCR using a panel of human/rodent hybrid cell lines and by fluorescence *in situ* hybridization (FISH) analysis.

Materials and Methods and Results

In the process of studying the interaction of the HBX protein, encoded by the hepatitis B virus genome, with cellular proteins, using the yeast two-hybrid method[9] we have isolated one positive clone from a Hela Matchmaker library (Clontech, Palo Alto, Cal., USA) (Unpublished results). The DNA sequence of this clone showed a strong homology to the *VDAC* gene family. Primers derived from this partial sequence were designed and used by PCR on a human liver marathon cDNA library (Clontech) to isolate the entire cDNA. The human *VDAC*3 sequence contains an open reading frame of 283 residues (Figure 1) and the protein sequence is 67% identical to HVDAC1 and 73% identical to HVDAC2. The amino acid sequence of this gene displays a greater that 98% homology to a recently cloned mouse *VDAC*3 gene[8] and therefore may represent a human equivalent of the mouse gene. The majority of the amino acid differences between HVDAC1, HVDAC2 and HVDAC3 are conservative substitutions.

Northern blot analysis of various human tissues (Clontech) shows a 1.4 Kb transcript that is expressed in all tissues examined, with a high expression in testis (Figure 2). This expression pattern is comparable to the expression pattern of the *MVDAC*3 gene.[8]

Oligonucleotides primers (5'-GTAACACACCAACG-TACTG-3' and 5'-GTTGAAGACATCCTTAGCAGC-3') specific for this new sequence were used to amplify DNAs from a monochromosomal somatic cell hybrid panel (UK HGMP Resource Center, Cambridge) and a human/rodent somatic cell mapping panel from Biosys Laboratories (Compiégne, France). A band of the expected size (53 BP) was obtained in the human control DNA but not in the mouse or the CHO DNA and was also present in the hybrids 803, 811 and 909 (Biosys Laboratories panel) and C4a (UK HGMP panel). These hybrids all contain the human chromosome 8 (data not shown). This localization is consistent with the localization of the *MVDAC*3 gene on the proximal region of the mouse chromosome 8[8] since there is a region of synteny between the mouse chromosome 8 and the human chromosome 8p. Regional mapping of this new gene was done by isolating three genomic clones from a PAC library (RessourcenZentrum/PrimärDatenbank, Berlin, Germany) using the human VDAC3 cDNA as a probe. The three PAC clones were labelled with biotin 14-dATP by nick translation (Gibco BRL Bionick labelling system) and used on metaphasic chromosomes as described.[10] Two PACs clones showed a specific hybridization signal on chromosome 8p11.2 (Figure 3). No other hybridization site could be detected. However with PAC 458, another signal on chromosome 14q32.3 was observed (data not shown). This signal could result from a cross-hybridization of the probe to another unknown *VDAC* gene or to a pseudogene. The 3 PAC clones were digested with EcoRI, run out on a 0.8% agarose gel and transferred to

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HVDAC2                                                AVPPTYADLGKSARDVFTKGYGFGLIKLDLKTK
HVDAC1          MSWCNELRLPALKQHSIGRGLESHITMCIPPSYADLGKAARDIFNKGFGFGLVKLDVKTK
HVDAC3                                              MCNTPTYCDLGKAAKDVFNKGYGFGMVKIDLKTK

HVDAC2          SENGLEFTSSGSANTETTKVTGSLETKYRWTEYGLTFTEKWNTDNTLGTEITVEDQLARG
HVDAC1          SCSGVEFSTSGSSNTDTGKVTGTLETKYKWCEYGLTFTEKWNTDNTLGTEIAIEDQICQG
HVDAC3          SCSGVEFSTSGHAYTDTGKASGNLETKYKVCNYGLTFTQKWNTDNTLGTEISWENKLAEG

HVDAC2          LKLTFDSSFSPNTGKKNAKIKTGYKREHINLGCDMDFDIAGPSIRGALVLGYEGWLAGYQ
HVDAC1          LKLTFDTTFSPNTGKKSGKIKSSYKRECINLGCDVDFDFAGPAIHGSAVFGYEGWLAGYQ
HVDAC3          LKLTLDTIFVPNTGKKSGKLKASYKRDCFSVGSNVDIDFSGPTIYGWAVLAFEGWLAGYQ

HVDAC2          MNFETAKSRVTQSNFAVGYKTDEFQLHTNVNDGTEFGGSIYQKVNKKLETAVNLAWTAGN
HVDAC1          MTFDSAKSKLTRNNFAVGYRTGDFQLHTNVNDGTEFGGSIYQKVCEDLDTSVNLAWTSGT
HVDAC3          MSFDTAKSKLSQNNFALGYKAADFQLHTHVNDGTEFGGSIYQKVNEKIETSINLAWTAGS

HVDAC2          SNTRFGIAAKYQIDPDACFSAKVNNSSLIGLGYTQTLKPGIKLTLSALLDGKNVNAGGHK
HVDAC1          NCTRFGIAAKYQLDPTASISAKVNNSSLIGVGYTQTLRPGVKLTLSALVDGKSINAGGHK
HVDAC3          NNTRFGIAAKYMLDCRTSLSAKVNNASLIGLGYTQTLRPGVKLTLSALIDGKNFSAGGHK

HVDAC2          LGLGLEFQA-----------------------------------
HVDAC1          VGSPWSWRLNPAERNLWEWISEDLALIYFHCDQQQAFFPPEDDQNKG
HVDAC3          VGLGFELEA-----------------------------------
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Figure 1 *Alignment of human voltage-dependent anion channel protein sequences generated with the Clustal program. Amino acid identities are represented in bold.*

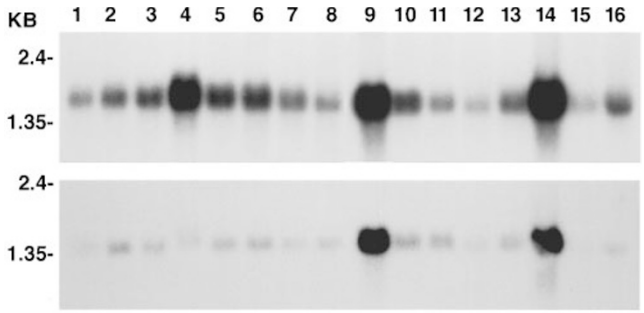

Figure 2 *Northern blot analysis of the expression of the HVDAC cDNA. Poly A + RNA (2 μg/lane) from the indicated human tissues (multiple tissue northern blot from Clontech) was hybridized with the entire human VDAC3 (top) or human glycerolaldehyde-3-phosphate-dehydrogenase, GAPDH, (bottom) cDNAs labelled with d-CTP (α ^{32}P) by random priming method. 1 : spleen, 2 : thymus, 3 : prostate, 4 : testis, 5 : ovary, 6 : small intestine, 7 : colon, 8 : peripheral blood leucocytes, 9 : heart, 10 : brain, 11 : placenta, 12 : lung, 13 : liver, 14 : skeletal muscle, 15 : kidney, 16 : pancreas. Size of molecular weight markers is indicated.*

a Zetabind membrane (Biorad Laboratories). Hybridization of the membrane with the human VDAC3 cDNA probe showed only a signal on the PAC clones 1 and 2 (data not shown). Therefore the signal observed with the PAC clone 3 (PAC 458) on the chromosome 14q32.3 could result from a cross-hybridization to another isoform. Since Craigen *et al* showed that the mouse genome contains three *MVDAC*3 genes, an expressed gene, a processed pseudogene and an intronless MVDAC3-like sequence with only 8 nucleotides

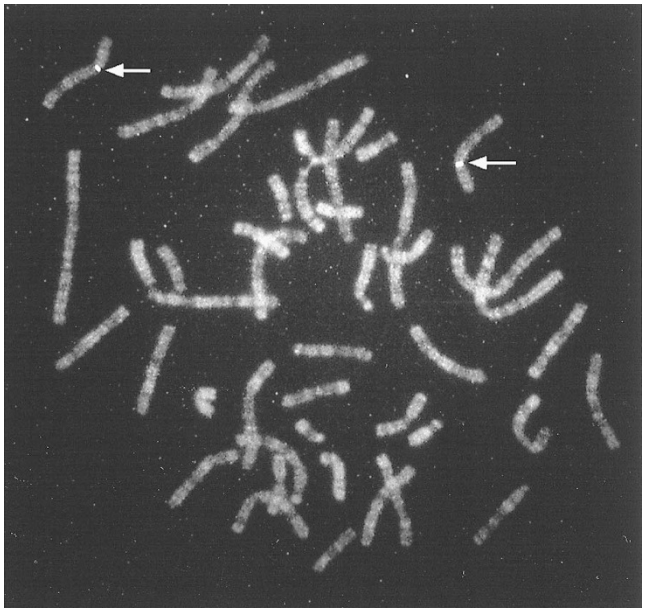

Figure 3 *FISH analysis of the human* VDAC*3 gene. Human metaphase showing the hybridization of the PAC LLNLP704F09304Q13. Chromosomes are counterstained with propidium iodide. Arrowheads indicate positive signals on chromosome 8p11.2.*

differences from the expressed gene,[11] we subcloned the human VDAC3 cDNA into an eukaryotic expression vector containing an HA tag at the N-terminus of the gene and transfected it into COS-1 cells which were then metabolically labelled with ^{35}S methionine in order to see if this human VDAC3 cDNA was functional. Immunoprecipitation of the ^{35}S methionine-labelled cellular protein extract with a monoclonal antibody to the HA epitope showed a single band at 30 Kda (Huh KW, 1997, unpublished results). Therefore this result confirms that this HVDAC3 cDNA can be expressed into eukaryotic cells.

Discussion

In this communication, we reported the isolation, characterization and chromosome localization of a third human *VDAC* gene. Based on the remarkable amino acid homology (98%), we suggest that this gene may be referred to as HVDAC3 form. HVDAC3 was shown to map to chromosome 8p11.2. However, Blachly-Dyson *et al* recently reported the cytogenetic localization of HVDAC3 to chromosome 12 and HVDAC4 to chromosome 1.[7] A plausible explanation for this inconsistency is that the two human VDAC map positions were determined using PCR of somatic cell hybrid DNA and fluorescence *in situ* hybridization which would thus preferably detect human VDAC pseudogenes.

Although there is some evidence of controversy,[12] subcellular distribution of VDACs in compartments other than mitochondria has been previously described.[13–19] These observations are consistent with the existence of multiple genes encoding VDAC isoforms in mammals with various cellular functions. In particular, the association of the mitochondrial receptor for benzodiazepines (mBzR) with the voltage-dependent anion channel (VDAC) has been reported[20] and indicates a possible auxiliary role for VDAC as a putative drug binding protein. HVDAC3 is highly expressed in testis and therefore may be involved in this complex and may play a role in the metabolism of cholesterol for steroidogenesis. Craigen *et al* have shown that the mouse *VDAC3* gene expressed in a yeast strain lacking the endogenous *VDAC* gene was only able to partially complement the mutant phenotype, and suggest that there is an alternate physiological function for VDAC3 protein.[11] Therefore further studies will be needed to examine the various functions of the VDAC protein family and to see if the interaction between the HbX and HVDAC3 proteins has some biological significance or not.

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