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A REVIEW ON A NEW TETRASPANIN, TSPAN-11

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ABSTRACT

The tetraspanins are a superfamily of four-span membrane proteins that are widely expressed in mammalian cells. Although they have been implicated in important cell processes and some diseases, it has been difficult to characterise their exact functions. Tspan-11 is a recently identified member of the tetraspanin family. Little is known about Tspan-11; however sequence evidence indicates that Tspan-11 most closely resembles tetraspanin CD151. Recently, Tspan-11 GFP transfected CHO cells were used to immunize mice for generation of monoclonal antibodies (mAbs) and found that these mAbs bound to the surface of Tspan-11 GFP transfected CHO cells, indicating that they are able to recognize native forms of the tetraspanin. Surprisingly, one of cancer cell lines tested, the A549 lung adenocarcinoma cell line, was strongly positive for Tspan-11 mAbs, showing both intracellular and plasma membrane staining. Some preliminary investigations have been carried out on normal cells/tissues; these indicate that Tspan-11 is only expressed weakly, if at all on white blood cells, and may be present on epithelial cells in the colon. Taken together, these findings suggest that Tspan-11 has low, or restricted expression, or that the mAbs characterised so far recognize only some forms of the protein.

KEYWORDS – Tetraspanins, Tetraspanin 11, Tspan-11, CD151

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INTRODUCTION

The tetraspanin family was first recognized in 1990, when sequences from Cluster of Differentiation (CD) antigens CD9, CD37, CD53 and CD81 (TAPA1) were compared to the tumour-associated genes ME491 (later CD63), CO-029 and the *Schistosoma mansoni* antigen Sm23 [1]. Tetraspanins constitute an important membrane protein family that is involved in roles such as the immunological response [2,3], sperm-egg fusion [4,5] and vision [6]. Also, tetraspanins have been shown to be implicated in various diseases, including cancer [7], hepatitis C virus infection [8] and retinitis pigmentosa [9]. The first tetraspanin to be discovered was the melanoma-associated protein, ME491 [10]. This superfamily now comprises a wide range of members with at least 33 in mammals,[1], 36 members in Drosophila and 20 members in *Caenrhabditis elegans* [11]. Tetraspanins have also been identified in fungi (but not yeast) [12]and plants [13].

1. Tetraspanin Members

Thirty-three of human tetraspanins have been identified, nearly half of those resulting from follow-up of EST (expressed sequence tag) databases. The expression of several tetraspanins in invertebrates indicates that these molecules appeared early during evolution [14,15]. The tetraspanin genes in human are located on different chromosomes, several are located on chromosome 11 (CD81, CD82, CD151, NAG-2 and Rom-1) and chromosome 12 (CD9, CD63, Co-029, SAS and NET-5). The conservation of gene structure strongly suggests that these molecules derive from a common ancestor. The tetraspanins include leukocyte differentiation antigens CD9, CD37, CD53, CD63, CD82 and CD151, TALLA-1, Co-029 or SAS. Moreover, the other proteins are the uroplakins UP1a, UP1b and proteins encoded by the retinal dystrophy syndrome genes are RDS/peripherin and Rom-1 [16].

2. Tetraspanin distribution

Most cells seem to express tetraspanins, [17-19], with CD151 present even on erythrocytes [20]. Some tetraspanins are found in several cell types (e.g. CD9, CD63, CD81, CD82, CD151), whereas other tetraspanins have a very restricted distribution (e.g. peripherin/rds and ROM-1 on photoreceptor rod cells [21]. Studies of tetraspanin distribution show that they are membrane proteins, which are usually located at the cell surface but some of them are expressed within the cells on intracellular membranes [16,22]. Many cells express multiple different tetraspanins, which have a propensity to form homo- and heterodimers on the cell surface, giving rise to membrane microdomains termed “tetraspanin-enriched microdomains” or TEMs [1]. Individual tetraspanins can also interact with non-tetraspanin “partner” proteins such as integrins.

3. Tetraspanin structure

Tetraspanins have four conserved transmembrane domains (TM1-4) (Figure 1). The proteins are typically of only 200-300 amino acids, usually have a molecular weight of 20- 30 kD (protein core) and the four transmembrane domains are linked by 2 extracellular loops. The small extracellular loop (EC1 or SEL) contains 20-28 amino acids and the large extracellular loop (EC2 or LEL) contains 76-131 amino acids [16,23].

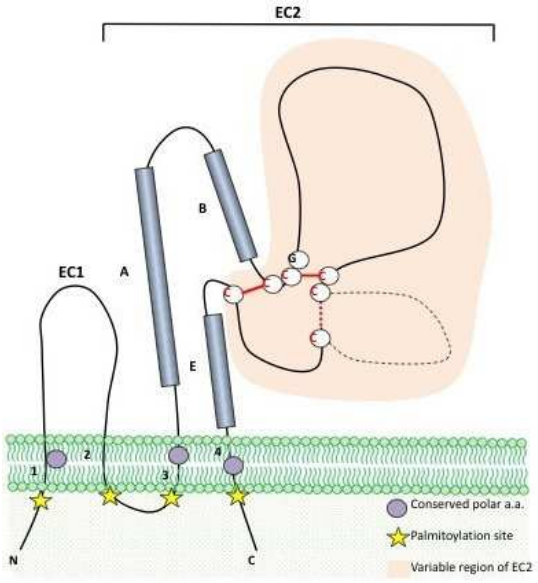

Figure 1

The diagram depicts a generic tetraspanin protein. Tetraspanin comprised of four transmembrane helices (1-4), a large (EC2) and a small (EC1) extracellular domain, short cytoplasmic N and C termini and a small putative intracellular loop between transmembrane helices 2 and 3. The EC2 is divided into a constant region, containing A, B and E, α helices, and a variable region that contains the signature CCG motif, two conserved disulphide bonds and a third loop and disulphide bond (dashed) that is present in some tetraspanins ***Adapted from 24***.

4. Tetraspanin functions

Tetraspanins have been reported to interact with a wide range of proteins. However, their interactions with integrins have probably attracted most attention [16,25]. The role of tetraspanins in integrin complexes may be linked with their ability to recruit signaling enzymes [26]. Early evidence for roles for tetraspanins in the immune response was obtained using antibodies that inhibited or promoted cell functions. There have been several examples of tetraspanins playing roles in viral life cycles [24,27,28]. Tetraspanins have been implicated in tumor cell adhesion, migration, invasion and metastasis [29] [29] although different tetraspanins appear to have different roles. For example, whereas tetraspanins CD9 and CD82 are potential metastasis suppressors [7,30-32], tetraspanin CD151 has been linked to enhanced metastasis of colon, prostate and lung cancer [33-35]. Overall, the relationship of tetraspanin expression with cancer varies between tumors of different origin [36]. Moreover, It is also clear that tetraspanins can play a role in cell fusion processes. CD9 was first investigated as a potential sperm receptor because of its associations with integrin α6β1 [37]. The strongest evidence for the involvement of a tetraspanin in fusion comes from studies on CD9 knockout mice, which have shown that CD9, and to some extent CD81, are required for sperm-egg fusion, since oocytes from CD9-/- or CD9- /-, CD81-/- mice are unable to fuse with sperm, resulting in partial or complete infertility, respectively [38-41].

5. Tetraspanin 11 (Tspan-11)

Tspan-11 is a recently identified member of the tetraspanin family (Figure 2). Little is known about Tspan-11; however sequence evidence indicates that Tspan-11 most closely resembles tetraspanin CD151 (Figure 3). The Tspan-11 gene of Homo sapiens is located at chromosome 12 (12p11.21) while *Mus musculus* (house mouse) is located at chromosome 6 (6F3). The human Tspan-11 gene consists of 762 bp (7 introns), 253 amino acid and the molecular weight is predicted to be 28 kD approximately (NCBI database). The human Tspan-11 gene

appears to consist of 8 exons, 6 of which code for protein (ENSEMBL database). As shown in Figure 3, the human CD151 and Tspan-11 proteins are the same length, ~47% identical and ~65% similar. Like CD151, Tspan-11 has a putative N-linked glycosylation site in the EC2 domain, it also has membrane proximal cysteines at positions 241 and 242, which in CD151 are known to be palmitoylated (Figure 4) [42]. The cysteines at positions 11 and 15 that are palmitoylated in CD151 are not present, but there are membrane proximal cysteines at positions 25, 26, 79 and 80 that could serve as palmitoylation sites. Like CD151, Tspan-11 has 6 cysteines in the EC2 region that are thought to be involved in disulfide bond formation [43]. However, the Tspan-11 protein does not have the putative tyrosine-based protein-sorting motif that is present at the C-terminus of CD151. There are also some significant amino acid changes in the region of the EC2, which in CD151 is involved in interaction with α3β1 integrin [44].

The Tspan-11 gene appears to be conserved in chimp, mouse, rat, dog, cow and zebrafish (ENSEMBL database). In mouse, rat and cow, the gene is predicted to code for 253 amino acid proteins that are 86-87% identical and 92-93% similar. The zebrafish protein is predicted to be 254 amino acids long and be 71% identical (83% similar) to the human protein.

Garcia-España, Chung et al. described the evolution of the large tetraspanin superfamily (Figure 5) suggesting that they can be classified into four families; the CD family, the CD63 family, the uroplakin family and the RDS family. Additionally, the close similarity between Tspan-11 and CD151, indicates function relatedness between them [15].

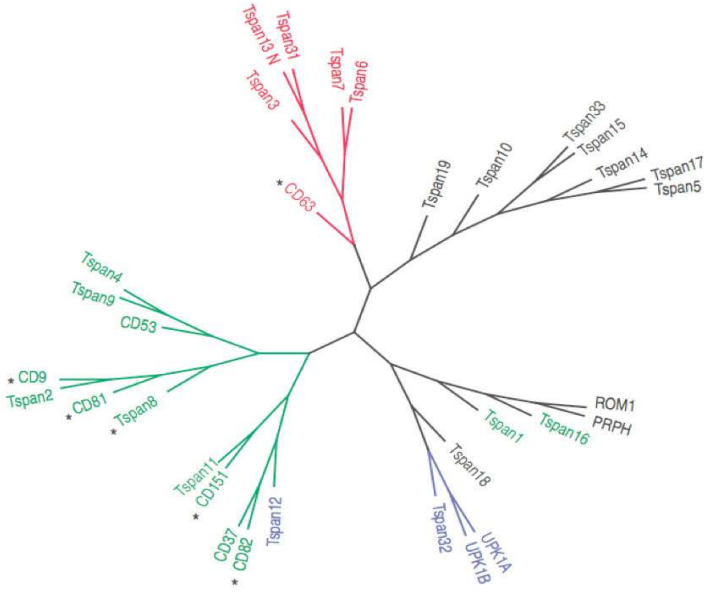

Figure 2

Evolutionary tree of the tetraspanin superfamily drawn as an unrooted tree. Phylogenetic analysis of the 33 human tetraspanins was performed using Clustal alignment and maximum likelihood phylogenetic analysis. The superfamily can be subdivided into four major monophyletic subfamilies (the CD family (green), CD63 family (red), uroplakin family (blue), and RDS family (black). The six tetraspanins that functionally contribute to cancer are indicated by asterisk (*) Adapted from 29.

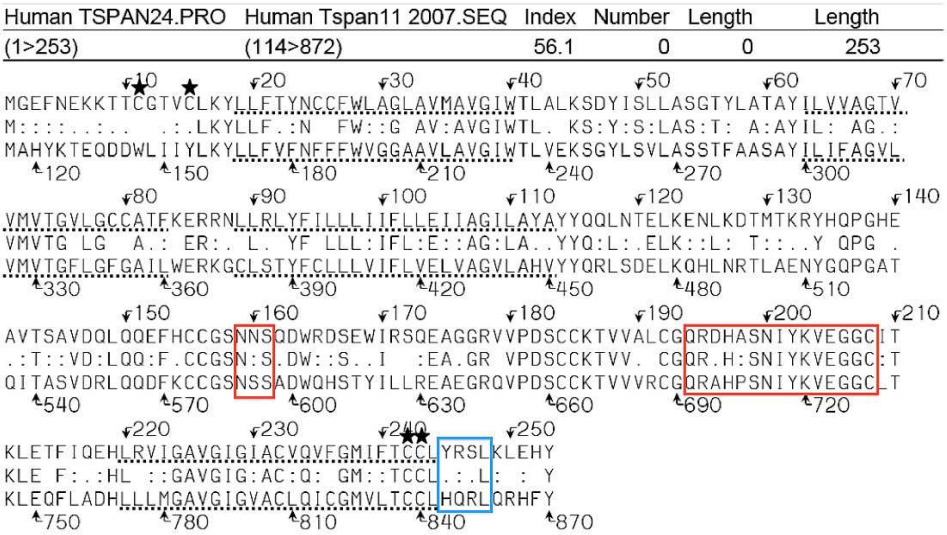

Figure 3

The amino acid alignment between human CD151 (top) and Tspan- 11 (bottom), which shows that there is have 46.8% identity and 64.4% similarity (EMBOSS sequence alignment program). The putative position of the transmembrane domains are shown underlined, based on data for CD151 (Neil Barclay 1997). Known pamitoylation sites in CD151 are marked with stars. The red marker indicates a single putative N linked glycosylation site on Tspan-11 EC2, which is also present in CD151. The interaction site of the α3β1 integrin on the EC2 of CD151 [44] and the internalisation motif present at the C-terminus of CD151 [22] are also shown with red and in blue markers, respectively.

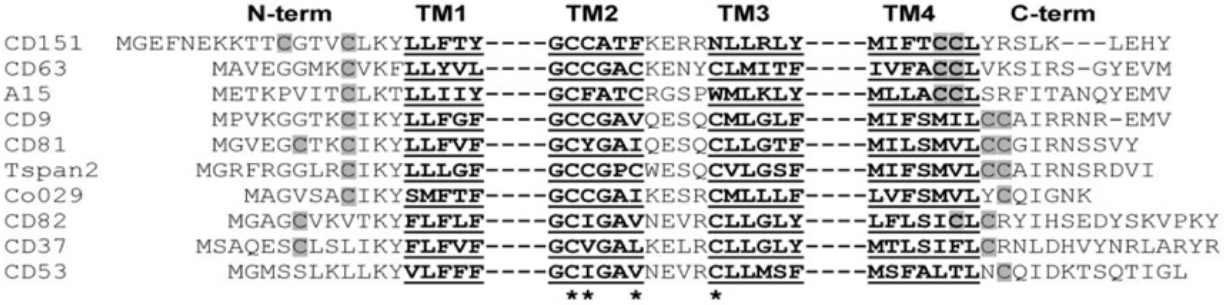

Figure 4

Potential tetraspanin palmitoylation sites. Cysteine residues in CD151 (C11, C15, C241, C242) used as palmitoylation sites and proximal to TM1 and TM4 are compared with cysteine alignments in other tetraspanin proteins. Positions of additional membrane proximal cysteines that could be palmitoylated are marked with asterisks. Only portions of TM1, TM2, TM3, and TM4 are shown [Adapted from 42].

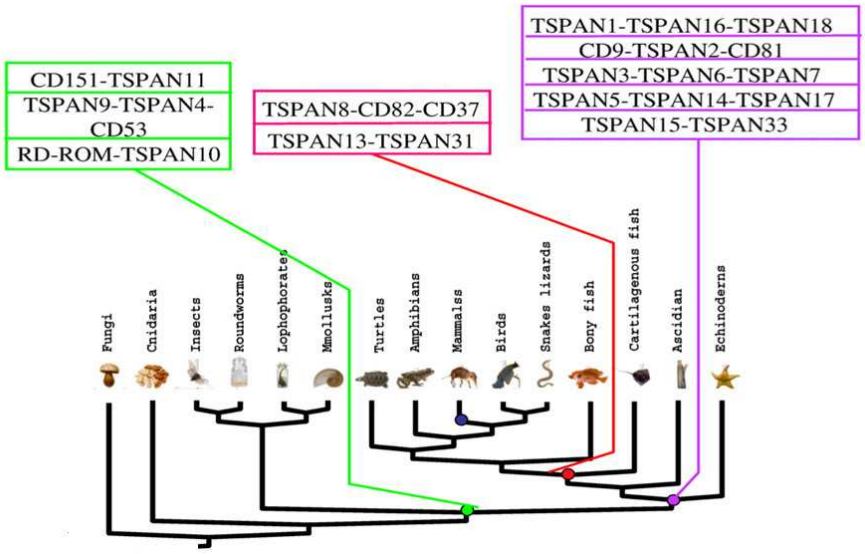

Figure 5
phylogenetic tree showing the point of ancestral origin for clusters of tetraspanin groups. Three points of origin are shown-vertebrate, chordate, and deuterostome [Adapted from 15].

6. Studies on Tspan-11 antibodies

Monoclonal antibodies (mAbs) have been used extensively to identify novel cell surface proteins and to investigate their functions. Specific antibodies to native tetraspanins are valuable tools. There are many strategies for generating and characterising tetraspanin antibodies, for instance, murine IgG1 mAb, 14A2.H1, was raised against acute myeloid leukaemia cells and bound to a 27 kD glycoprotein present in human platelet and endothelial cell membranes named PETA-3 (Platelet-endothelial cell tetraspan antigen-3), which we now know as CD151 [45]. To identify proteins that might associate with integrins, α3β1-containing complexes were used to immunize mice [46]. Of the antibodies raised, one included a new anti- CD151 antibody, 5C11. Immunizing Balb/c mice with HeLa cells generated three new IgG1 anti-CD151 antibodies and based on the recognition of CD151 transfected CHO cells, it was found that CD151 mAb TS151r was only able to recognize CD151 when it was not associated with integrins i.e. mAb TS151r was actually directed against a discrete epitope of CD151 molecules normally masked by integrins [47]. All anti-CD151 antibodies that have been produced previously [18,48]were screened and shown to bind specifically to murine FDC-P1 cells transfected with CD151 cDNA but not to untransfected cells [49]. Differential reactivity of monoclonal antibodies with CD antigen-transfected versus untransfected cells has long been a mainstay of the Human Leukocyte Typing Workshops [50].

In order to make antibody recognizing native Tspan-11, Balb/c mice were injected five times with 1×10^7 Tspan-11 GFP transfected cells for making hybridoma cells. Hybridoma culture supernatants were harvested and tested against Tspan-11 GFP transfected and GFP transfected CHO cells. In immunofluorescence staining, Tspan-11 proteins were clearly detected in permeabilised A549 cells. The stained images demonstrated that Tspan-11 was localized to intracellular structures, possibly endoplasmic reticulum, endosomes, and Golgi apparatus. Notably, by using FACS, some of the monoclonal antibodies, reacted with A549 cells even without cell permeabilisation, suggesting that they reacted with the extracellular domain of Tspan-11 on these cells, as well as on Tspan-11 GFP cells these Tspan-11 GFP transfected CHO cells were used to immunize mice for generation of monoclonal antibodies (mAbs). All mAbs bound to the

surface of Tspan-11 GFP transfected CHO cells, indicating that they are able to recognize native forms of the tetraspanin. Surprisingly, only one of a panel of cell lines tested, the A549 lung adenocarcinoma cell line, was strongly positive for the mAbs, showing both intracellular and plasma membrane staining. Some preliminary investigations have been carried out on normal cells/tissues; these indicate that Tspan-11 is only expressed weakly, if at all on white blood cells, and may be present on epithelial cells in the colon. Taken together, these findings suggest that Tspan-11 has low, or restricted expression, or that the mAbs characterised so far recognize only some forms of the protein [51].

CONCLUSION

Tetraspanins are small integral membrane proteins that belong to a superfamily encompassing 33 members in human and were first identified in mammals as cell specific antigens (Hemler 2005). Tetraspanins are present in different combinations in almost all types of cell and tissue and have been implicated in diverse cellular functions and human disease. Tetraspanins associates with each other and with other transmembrane proteins, for example, integrins and immunoglobulin superfamily proteins, forming multimolecular membrane microdomains, often referred to as a “tetraspanin web” or “tetraspanin enriched microdomain”. However, antibodies which are important for characterising the expression of tetraspanin proteins, would be useful in functional studies have been generated to only ~ one third of the proteins. In the future Tspan-11 mAbs would be extremely useful in determining its protein expression pattern and for functional studies in different cell and tissue types.

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