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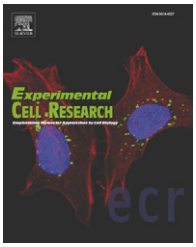

Review

Trafficking and function of the tetraspanin CD63

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ABSTRACT

Tetraspanins comprise a large superfamily of cell surface-associated membrane proteins characterized by four transmembrane domains. They participate in a variety of cellular processes, like cell activation, adhesion, differentiation and tumour invasion. At the cell surface, tetraspanins form networks with a wide diversity of proteins called tetraspanin-enriched microdomains (TEMs). CD63 was the first characterized tetraspanin. In addition to its presence in TEMs, CD63 is also abundantly present in late endosomes and lysosomes. CD63 at the cell surface is endocytosed via a clathrin-dependent pathway, although recent studies suggest the involvement of other pathways as well and we here present evidence for a role of caveolae in CD63 endocytosis. In late endosomes, CD63 is enriched on the intraluminal vesicles, which by specialized cells are secreted as exosomes through fusion of endosomes with the plasma membrane. The complex localization pattern of CD63 suggests that its intracellular trafficking and distribution must be tightly regulated. In this review we discuss the latest insights in CD63 trafficking and its emerging function as a transport regulator of its interaction partners. Finally, the involvement of CD63 in cancer will be discussed.

Contents

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The tetraspanin family

The tetraspanin family was first recognized in 1990, when sequences from Cluster of Differentiation (CD) 37 and CD81 were compared to the tumour-associated gene CD63 [1]. This revealed sequence homology and a conserved predicted structure of 4 hydrophobic transmembrane domains, a small and a large extracellular loop and 2 short intracellular amino- and carboxyl tails. A typical tetraspanin consists of 200–300 amino acids and contains 4–8 conserved extracellular cysteines of which 2 are present in a CCG motif located 28–47 residues after the third transmembrane domain. In addition, tetraspanins contain 3 conserved polar residues within transmembrane domains 1, 3 and 4 [1].

The tetraspanin superfamily consists of 4 subfamilies; the CD-, CD63-, uroplakin- and RDS families. The CD family of tetraspanins is the largest and contains all CD tetraspanins except for CD63. CD63 constitutes its own subfamily as it has a more ancient origin than the other CD tetraspanins [2]. The human tetraspanin family has 33 members. For an increasing number of tetraspanins it is found that they are post-translationally modified by the addition of palmitate to the membrane-proximal cysteine residues. This modification results in their ability to organize into the "tetraspanin-enriched microdomains" (TEMs), or "tetraspanin web", at the cell-surface, which consists of several tetraspanins and associated components [3–5]. These components are e.g. (co-) receptors, integrins and cholesterol [3]. Since most cell types express a specific tetraspanin subset, TEMs are also cell-type specific [4].

Many tetraspanins exert their function through interaction with integrins, often within the context of a TEM. These interactions are important for integrin-mediated cell adhesion to the extracellular matrix (ECM) [6], a process that is often affected in cancer. In addition, tetraspanins can play a role in intracellular transport (see below). Although the specific functions of most tetraspanins still need to be unravelled, it becomes increasingly clear that mutations in tetraspanin genes can cause severe disease phenotypes [1].

Tetraspanin trafficking

Like most transmembrane proteins, tetraspanins are synthesized in the endoplasmic reticulum (ER). The transmembrane regions of tetraspanins are important for ER exit, since deletion of one or more transmembrane domains of CD9, CD151, CD82 and uroplakin Ib resulted in ER retention, even when their extracellular domain was properly folded [7–10]. Many tetraspanins are palmitoylated, which occurs in the Golgi complex [11]. After palmitoylation, tetraspanins often form homodimers, which are subsequently transported to the cell surface to function as building blocks for TEMs [12]. In addition to palmitoylation, tetraspanins are post-translationally modified with several N-glycans [13].

Besides the exocytotic pathway and cell surface, tetraspanins are also found in the endosomal system (Fig. 1). In general, endocytosis starts with the budding of endocytic vesicles from the plasma membrane that fuse with an early endosome. From early endosomes, proteins either recycle to the cell surface or become incorporated into intraluminal vesicles (ILVs) that bud into the endosomal lumen. During their maturation, endosomes acquire increasing numbers of ILVs, which is why late endosomes are also called multivesicular bodies (MVB). Late endosomes/MVBs can mature into- or fuse with lysosomes [14,15]. Within the endosomal system tetraspanins are predominantly found within MVBs and lysosomes. The significant levels of tetraspanins in these organelles suggest that tetraspanins are relatively protected from lysosomal proteolysis.

Tetraspanins are also present in so-called lysosome-related organelles or secretory lysosomes. These include the dense granules and α-granules in platelets, melanosomes in melanocytes, cytotoxic granules in T-cells, Weibel–Palade bodies in endothelial cells and Major Histocompatibility Complex II (MHCII) compartments in dendritic cells [16–19]. Upon stimulation, these lysosome-related compartments can fuse with the cell surface, releasing their content into the extracellular environment. Furthermore, in many cell types, late endosomes/MVBs can be triggered to fuse with the cell surface and release their ILVs. The released ILVs are then indicated as 'exosomes' [20,21] (Fig. 1). The tetraspanins CD37, CD53, CD63, CD81 and CD82 are highly enriched on exosomes [16]. Exosomes may function in discarding proteins from cells, as was shown for the transferrin receptor in maturing reticulocytes [22], but can also transfer lipids and proteins from one cell to another. For example, exosomes can provide antigen-presenting cells with new antigens or co-stimulatory molecules [20,23] and elicit T-cell responses [24]. Recently, exosomes were also shown to shuttle mRNA or messenger RNA from one cell to another, which could be translated by the receiving cell [25].

Since exosomes are secreted by many types of cancer cells, they have been a main subject in the cancer field. Tumour cell derived exosomes might have great potential in eliciting an anti-cancer immune response, since they can deliver specific tumour antigens to APCs [26]. In mice, tumour-peptide-pulsed dendritic cells secrete exosomes that elicit anti-tumour immune responses and reduce the size of established tumours in vivo [26]. Recent evidence, however, suggests that tumour derived exosomes could also have immune-suppressing features and bear proteins that are involved in angiogenesis promotion and chemo resistance, hence promoting tumour progression [27]. The function of tetraspanins in exosomes is currently not known, although they have been implicated in the adhesion of exosomes to target cells [16].

The intrinsic distribution pattern of tetraspanins indicates that their transport to the various cellular locations must be tightly