

Review

The Ig CAM CAR is Implicated in Cardiac Development and Modulates Electrical Conduction in the Mature Heart

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Abstract: The coxsackievirus and adenovirus receptor (CAR, *CXADR*) is a multi-functional cell adhesion molecule which forms with CLMP, BT-IgSF, ESAM and CTX a structural subgroup within the Ig superfamily. These proteins share an overall domain organization with two extracellular Ig domains, a transmembrane region and a cytoplasmic tail which includes a PDZ binding motif. CAR is strongly expressed in brain and heart during embryonic development and becomes down-regulated in early postnatal stages. Cell adhesion experiments, binding studies and as well as crystallographic investigations on the extracellular domain reveal a flexible ectodomain for CAR that mediates homophilic and heterophilic binding. Several animal models showed an essential role for CAR during embryonic heart development and for electrical conduction between neighboring cardiomyocytes at mature stages. CAR gets re-expressed in diseased or damaged cardiac tissue, probably to induce regeneration and remodeling of the cardiac muscle.

Keywords: CAR; cell adhesion; IgCAM; cardiac development; electrical conduction

1. The CAR Subgroup of IgCAMs

The coxsackie and adenovirus receptor (CAR, *CXADR*) was initially identified in human and mouse as a common receptor for coxsackie B viruses and adenoviruses of the groups A, C, D, E and F and has been recognized for its involvement in virus-mediated myocarditis [1–5]. It is a 46 kD type I transmembrane protein which functions as homophilic and heterophilic cell adhesion protein. CAR represents a prototype of a structurally related subgroup of Ig-like proteins consisting, besides of CAR itself, of CLMP (CAR-like membrane protein), BT-IgSF (brain- and testis-specific immunoglobulin superfamily, also termed IgSF11), ESAM (endothelial cell-selective adhesion molecule) [6–10] and CTX (marker for cortical thymocytes of *Xenopus*) [11,12]. These proteins are composed of two extracellular Ig domains, a short transmembrane region and a cytoplasmic tail (Figure 1A). Two forms of mouse CAR consisting of either 365 or 352 amino acid residues are known that differ in their most C-terminal stretches of the cytoplasmic tail. The membrane-distal V-type domain (D1) and the membrane-proximal C2-type domain (D2) are separated by a short junction which creates flexibility in the extracellular part of CAR. By adhesion and binding assays [13–16] and further by crystallographic studies (Figure 1B,C) using recombinantly expressed human or mouse CAR it was shown that CAR promotes homophilic binding between neighboring cells which is mediated by D1-D1 domain or antiparallel D1-D2 domain interactions [15,17]. Furthermore, heterophilic binding of chick CAR to extracellular matrix glycoproteins such as fibronectin, agrin or tenascin-R [15] and of mouse CAR to other IgCAMs such as mouse JAML and JAM-C has been described [18–23].

Although the intracellular signalling of CAR is not well understood multiple cytoplasmic interaction partners were identified that bind to the PDZ class I binding motif of human or murine CAR including the tight junction protein ZO-1 (Zona occludens 1), MUPP-1 (Multi-PDZ domain protein-1), MAGI-1b (Membrane associated guanylate kinase, WW and PDZ domain containing 1b), PICK-1 (Protein interacting with C kinase 1), the synaptic scaffolding protein PSD-95 (postsynaptic density protein 95) and LNX (Ligand-of-Numb protein-X) and LNX2 [16,24–29].

The *CXADR* gene is located on human chromosome 21q21.1 or on the syntenic region of murine chromosome 16 and consists of 8 exons whereby exons 1–5 encode the signal peptide and the extracellular Ig domains and exon 6 encodes the transmembrane region [27,30,31]. The cytoplasmic tail is generated by alternative splicing variants (CAR1/CAR2) of exon 7 and 8 [32].

2. Localization of CAR in the Heart

CAR is mainly expressed in the developing heart and brain but it is also detected in lower amounts in pancreas, prostate and testis, liver, kidney and intestine [4,15,33]. On epithelial cells it is associated with tight junctions proteins like ZO-1 [24]. Interestingly, CAR expression is highly regulated during development. At embryonic stages the protein level of CAR increases but becomes dramatically downregulated shortly after birth [13,34–37]. In adult hearts low amounts of CAR protein are still detected at the myocardial intercalated disc in contrast to widely diffuse expression of CAR on cardiomyocyte surface at birth (Figure 2) [37,38]. Further investigations on the developing heart showed that only myocardium and pericardium express CAR, while the endocardium and endothelial cells from blood vessels lack CAR expression [25,35,37,39]. This characteristic developing expression patterns distinguishes CAR from other IgCAMs.

Figure 1. (A) Scheme of the members of the CAR subgroup. Ig domains are indicated by loops. The junction (J) between both Ig domains of CAR and the linker (L) are indicated by a small box or ellipse, respectively, which creates flexibility in the extracellular region. It is currently not known whether other members of the subgroup contain such a junction or linker since crystallographic data are lacking. BT-IgSF, brain- and testis-specific immunoglobulin superfamily; CAR, coxsackievirus and adenovirus receptor; CTX, marker for cortical thymocytes of *Xenopus*; C2, Ig-like domain of the C2 subtype; CLMP, CAR-like membrane protein; ESAM, endothelial cell-selective adhesion molecule; PM, plasma membrane; V, Ig-like domain of the V subtype; S-S, disulfide bond. (B) The crystal structure of the extracellular regions of two CAR molecules reveals a U-shaped dimer [15,18]. D1 domains are colored in red or brown and D2 in green. (C) Detailed view of the amino acid residues implicated in D1-D1 dimer formation (adopted from Patzke *et al.* [15]). The D1-D1 binding interface has a size of 684 Å. The single letter code for amino acids is used.

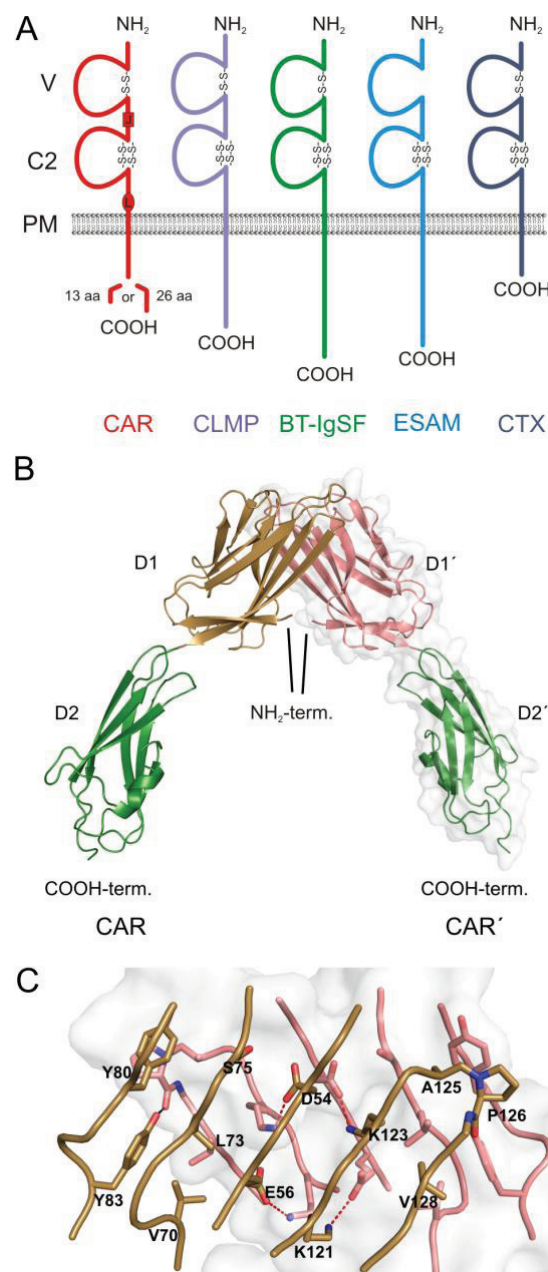
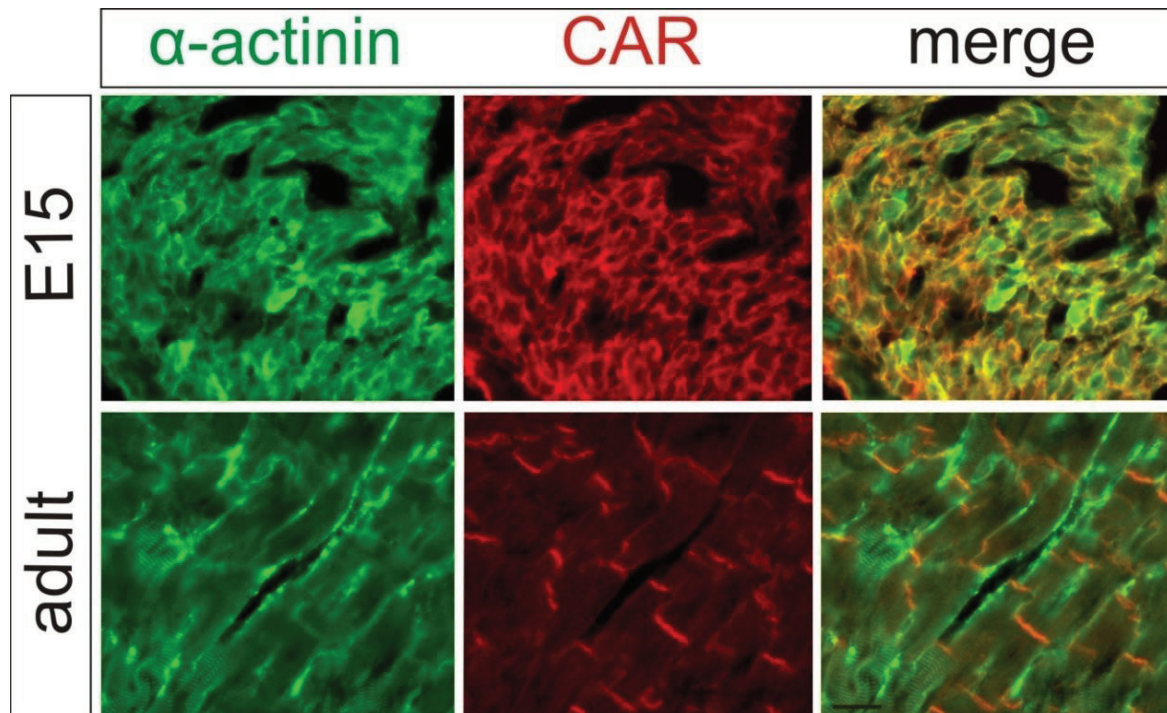


Figure 2. Location of CAR in the embryonic (E15) and mature murine heart (adult). At embryonic stages CAR is uniformly localized on the plasma membrane of cardiomyocytes. In contrast, in the mature heart CAR is primarily detected on intercalated discs (see [37,38] for details). Bar, 50 μ m.



3. CAR Re-Expression in Diseased Cardiac Tissue

In adult cardiac tissue CAR expression is strongly reduced and restricted only to intercalated discs [37,40,41]. Remarkably, a strong expression of the CAR protein was observed in the intercalated discs and sarcolemma in human dilated cardiomyopathy (DCM) in comparison to non-failing hearts, and in addition an up-regulation of CAR mRNA in DCM, in ischemic cardiomyopathy (ICM) [42,43], in valve-failure associated heart disease [40] and in animal models of cardiac inflammation and myocardial infarction was measured [34,41]. Surprisingly, postnatal overexpression of murine CAR in cardiomyocytes resulted in inflammatory cardiomyopathy associated with MAPK activation, increased proinflammatory cytokine expression and dysregulation of the cadherin-catenin complex [44,45]. Ito *et al.* (2000) [41] and Noutsias *et al.* (2001) [42] proposed that CAR up-regulation might be required for regeneration of damaged myocardium. Therefore, CAR might have a dual function in the pathogenesis of myocarditis: as viral receptor and in addition induction of signals that activate components characteristic for tissue remodeling. It is currently not known whether the high CAR mRNA expression is of relevance for the development of DCM or ICM as a virus receptor or whether it is simply a consequence of these diseases. The mechanism responsible for the induction of CAR in these diseases is also not known but might include inflammatory mediators [44]. Furthermore, it would be of interest to study point mutations in the human CAR gene which might influence virus pathogenesis followed by myocarditis or DCM [43,46,47].

4. CAR is Essential for Embryonic Heart Development

In addition to studies on CAR and its involvement in viral infections the physiological role of CAR has been investigated by gene deletion in mice [35,48,49]. Absence of CAR results in a malformation of the heart and death between embryonic day 11.5 and 12.5—a period of intense organogenesis when the heart starts to transform from a single looped tube to a chambered structure. Some differences in the detailed description of the malformation exist between these three studies which, however, might be explained by structural variability in the development of the heart in the absence of CAR. CAR^{-/-} embryos revealed dilation of cardiac veins and enlarged pericards due to edema formation which might be taken as a sign of insufficient heart function. Sections of embryonic hearts of CAR mutants showed smaller lumens of the ventricles and enlarged endocardial cushions at this developmental stage [35]. Furthermore, CAR-deficient hearts contain atypical sinuatrial valves and atrioventricular canals [35,49]. At the ultrastructural level CAR deficient cardiomyocytes revealed a reduced number of myofibrils with a decreased diameter and which were shorter and contained a smaller number of sarcomers [35,49]. Furthermore, CAR mutant cardiomyocytes contained enlarged mitochondria and an accumulation of glycogen granule content [35]. These observations on mitochondrial morphology and in glycogen granule content are reminiscent of similar changes in cardiomyocytes of infarcted areas of the adult heart. The observation that CAR-deficient myocardial cells die by an apoptotic pathway [48] or that CAR-deficient hearts contain thinner ventricular walls has not been confirmed by the two other studies [35,49] (see also [50]). In summary, the delay in the correct morphological development may lead to insufficient heart function which then causes embryonic death. Interestingly, CAR might be important only at a specific developmental window since Chen *et al.* (2006) [49] observed that heart-specific CAR deletion after E11 is not lethal.

5. CAR is Essential for Electrical Conduction in the Mature Heart

To understand the physiological function of CAR in the adult heart when CAR is predominantly localized at the intercalated disc conditional knockout mice were generated [51,52]. Inactivation of CAR at mature stages was obtained either by a cardiac-specific Cre driver line (α -myosin heavy chain) or by a heart-specific tamoxifen-inducible CAR knockout. In the first approach inactivation occurs from about E12.5 on [51] while in the second mouse line inactivation is occurring after tamoxifen injection to activate a cardiac specific Cre recombinase [52]. Both strategies resulted in a marked decrease of CAR protein at the intercalated disc in mature mice. Electrocardiograms demonstrated a first degree or complete block of the atrioventricular conduction in these animals reflected by a prolonged PR interval while the QRS morphology and the pattern of atrial depolarization at the area of the sinoatrial node appeared normal. The impaired electrical conduction between atrium and ventricle was associated with molecular changes at the AV node and the intercalated discs including altered expression of gap junction proteins connexin 45 and 43, β -catenin and ZO-1 (zonula occludens-1) and an aberrant dye spreading via gap junctions. These observations suggested a molecular cross talk between the cell adhesion molecule CAR and gap junctions and might indicate that CAR is implicated in the organization of substructures at the AV node. Connexins have also previously been implicated in AV conduction problems [53].

In summary, although further detailed studies are required these two reports on conditional CAR knockouts clearly indicate that in addition to its developmental role CAR is essential for cardiac function. Consistently, an increased susceptibility to ventricular arrhythmia was recently observed in mice heterozygous for CAR [54] and its absence in mice is also associated with a myocardial fibrosis [51].

6. Outlook

Taken together, CAR is a multifunctional adhesion protein important for cell-cell communication, virus uptake, cardiac development and electrical conduction in the mature heart at the AV node and probably other parts of the heart as well [54]. Although CAR has been shown to be complexed with intracellular components, a deeper understanding of its function in the developing as well as mature heart clearly requires the characterization of its intracellular signaling function. The intracellular segment of CAR has several phosphorylation sites which might allow interactions with signal transduction pathways. Such studies might clarify whether the function of CAR during embryonic development of the heart is distinct from or similar to that at mature stages or at regenerating phases.

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Author Contributions

All authors contributed to the writing of this manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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