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tANCHOR: a novel mammalian cell surface peptide display system

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ABSTRACT

A novel tool for the presentation of peptides and small proteins on the surface of human cells has been developed. Our tANCHOR system utilizes tetraspanin anchors containing heterologous amino acid sequences inserted instead of the large extracellular loop. This technology allows a highly effective extracellular display of epitopes for antibody binding studies and many other potential applications.

METHOD SUMMARY

We demonstrate that tetraspanins are able to present heterologous antigens instead of their large extracellular loop on the cell surface by employing confocal laser scanning microscopy. Cell-based ELISA techniques were used to identify the most efficient tetraspanin anchors for displaying of peptides on the surface of human cells. Extracellular accessibility of antigens by antibodies was confirmed by scanning electron microscopy. Our approach was further used to study tetraspanin-anchored 2F5 antigen binding of a broadly HIV-1 neutralizing antibody.

KEYWORDS:

2F5 ● antibodies ● CD20 ● display system ● large extracellular loop ● LEL ● PD-1 ● plasma membrane ● tetraspanin anchors

Protein display methods have advanced over the past few decades in order to identify novel drug targets or to investigate various cellular mechanisms. Among those approaches, *Escherichia coli*-based phage display is one of the most frequently used methods but it is not suitable for the study of all kinds of human protein–protein interactions. Proteins expressed on the surface of *E. coli* might be properly presented but will lack posttranslational modifications, such as glycosylation sites [1]. Expression in yeast cells may provide glycosylation modifications, but the glycosylation patterns are different from those found in mammalian cells [2–4]. To address the need for human-like glycosylation patterns, experimental approaches allowing the presentation of peptides on the surface of human cells have relied so far mostly on the PDGFR transmembrane region in combination with an N-terminal secretion peptide [5]. Highly efficient presentation of recombinant proteins and peptides on the surface of human cells is one important feature of novel display systems. Screening of antibodies against membrane proteins such as ion channels or GPCRs is sometimes extremely challenging because they may be expressed at low abundance [6–8]. Here we describe a novel, highly efficient, alternative display system for peptides and small proteins in the context of an extracellular loop of a transmembrane protein. Our developed display system (tANCHOR) is based on tetraspanin anchors. The main characteristic of tetraspanin proteins is the presence of a small extracellular loop and a large extracellular loop (LEL), which are flanked by four transmembrane domains [9,10]. Members of the superfamily of tetraspanins are localized in multivesicular bodies and are generally organized in tetraspanin-enriched microdomains on the cell surface [11–13]. We show that the tANCHOR system [14] allows efficient and reliable presentation of heterologous peptides and proteins on the surface of human cells for antibody binding studies and other applications.

Materials & methods

Molecular cloning

DNA sequences, deduced amino acid (aa) sequences and primers used in molecular cloning steps are listed in Supplementary Tables 1 & 2. The tANCHOR vector constructs are based on the pCMV-Tag2B vector (Stratagene, Heidelberg, Germany). The vector pCMV-CD63ΔLEL-mCherry was cloned in two steps. First, the mCherry reporter sequence was amplified from pmCherry-N1 (Clontech, Heidelberg, Germany) using the primers 01/02, and cloned using the restriction sites *Xho*I/*Apa*I in the vector pCMV-CD63-YFP [15]. Second, the CD63 transmembrane domain (TM) 1–3 (aa 1–110; GenBank accession number KF998086) was amplified from the template vector pPR3-N-CD63 [16] using the primer 03/04 and inserted in the vector pCMV-Tag2B through the restriction sites *BamH*I/*Pst*I; the sequence TM4 (aa 201–238; GenBank accession number KF998086) of CD63 was amplified with primers 05/06 and cloned in the restriction sites *EcoR*V/*Hind*III. Cyan fluorescent protein (CFP) was inserted in the vector pCMV-CD63ΔLEL-mCherry from the vector pSCFP3A-C1 [17]

using restriction sites *EcoR*I/*EcoR*V and primers 07/08. Other vectors containing full-length CD9, CD81, CD82, CD151 and CLDN1 gene sequences were synthesized by ATG:biosynthetics (Merzhausen, Germany) and cloned in the vector pCMV-CD63-YFP [16] using restriction sites *Not*I/*Xho*I. Similarly, the LEL-deleted tetraspanin construct (ΔLEL) and the extracellular loop (EC)-deleted Claudin-1 constructs (ΔEC1, ΔEC2) fused to the linker V5-6xHis were generated in the vector pCMV-CD63ΔLEL-mCherry. The vector pCMV-CD63ΔLEL-V5-6xHis-mCherry was generated using the tANCHOR vector pCMV-CD63ΔLEL-mCherry by cloning the *EcoR*I-V5-6xHis-*EcoR*V fragment from pCMV-CD9ΔLEL-V5-6xHis-mCherry. The gp41 linker sequence (aa 646–678; GenBank accession number AAT67507.1), containing 2F5-4E10 core epitopes ELDKWAS and NWFNITNWLW, was amplified using the template vector pNL4-3 (obtained from the AIDS reagent program of the NIH, MD, USA) and primers 12/13 and cloned in the vector pCMV-CD82ΔLEL-V5-6xHis-mCherry using restriction sites *EcoR*I/*EcoR*V. The vector pCMV-CD82ΔLEL-mCherry-mCherry was cloned using primers 14/15 and the template vector pmCherry-N1 in a similar manner. The inserts for the vector containing CD82-anchored PD-1 epitopes (aa 62–86; GenBank accession number EF064716.1) and CD20 (aa 162–191; GenBank accession number FN555175.1) were synthesized and cloned in the vector pCMV-CD82ΔLEL-V5-6xHis-mCherry using restriction sites *EcoR*I/*EcoR*V. In the same way, the vector containing the PDGFR transmembrane domain for insertion of V5 and 2F5 epitopes was generated by using synthesized gene construct (IgK-leader-FLAG-V5-6xHis-PDGFR) and the restriction sites *Sac*I/*Xho*I. The control vector pcDNA4-Rev-V5-CFP was generated by amplification of the sequence encoding Rev protein (aa 1–116) from the vector pcDNA3.1-coRev-V5 that contains a synthetic *rev* sequence fused with V5-tag sequence (Supplementary Table 2) in the vector pcDNA3.1+ (Invitrogen, Darmstadt, Germany) using the primers 16/17. This amplified *rev* gene fragment was then cloned in the vector pcDNA-CFP [16] using *Hind*III/*Xho*I restriction sites. The tANCHOR construct pCMV-CD63ΔLEL-gp41$_{ecto}$-mCherry containing the ectodomain of gp41 (aa 535–672; GenBank accession number AAT67507.1) was generated using the primers 18/19 and the template pNL4-3. Vectors flexMAM-CD82ΔLEL-V5-6xHis and flexMAM-CD82ΔLEL-V5-6xHis-mCherry, used for testing of epitope surface delivery, were produced by ATG:biosynthetics and are commercially available [18].

Cell culture & transfection

Human embryonic kidney (HEK 293T) and HeLa cells were grown in Dulbecco's modified Eagle medium supplemented with 10% fetal bovine serum (Biochrom AG, Berlin, Germany), 100 IU/ml penicillin, 100 μg/ml streptomycin (PAA Laboratories, Cölbe, Germany) and 2mM L-glutamine (Biochrom AG). Cells were transfected using Metafectene®PRO (Biontex, Munich, Germany) according to the manufacturer's information. Cells were cultured at 37°C with 95% relative humidity and 5% CO_2.

Confocal laser scanning microscopy & epifluorescence microscopy

HEK 293T or HeLa cells (1×10^4/well) were seeded into glass-bottomed eight-well IBIDI μ-slides (Ibidi, Munich, Germany) and after 24 h were transiently transfected with 0.3 μg (HEK 293T) or 0.8 μg (HeLa) plasmid DNA of each corresponding vector. At 24 h post-transfection, cells were washed once with 1 × phosphate-buffered saline (PBS) and fixed with 200 μl 2% paraformaldehyde in 1 × PBS for 20 min. Cells were washed once with 1 × PBS and left in 200 μl 1 × PBS in the presence of 20 μl Duolink mounting medium (Sigma-Aldrich, Darmstadt, Germany). All images were acquired using an inverted confocal laser scanning microscope (LSM 780; Carl Zeiss Microscopy GmbH, Oberkochen, Germany) and a Plan-Apochromat oil immersion objective (63 ×, numerical aperture 1.4; Carl Zeiss Microscopy GmbH). Fluorescence signals were detected with the Zeiss ZEN smart setup settings for CFP, yellow fluorescent protein, mCherry and DAPI dyes.

Cell-based ELISA techniques

HeLa or HEK 293T cells (2×10^4) were seeded in 96-well Advanced TC™ plates (BioGreinerOne, Frickenhausen, Germany) and transfected at confluency of 80% with 0.6 μg plasmid DNA added to 2 μl MetafectenePRO per well, following the manufacturer's instructions. Each well from triplicates was transfected with a different plasmid preparation in order to exclude possible errors during plasmid preparations affecting plasmid quality and purity. After 24 h, HeLa cells were fixed with 2% paraformaldehyde in 1 × PBS for 20 min and washed twice with 300 μl of 1 × PBS. Cells were blocked for 2 h with 200 μl blocking buffer (3% bovine serum albumin in 1 × PBS) and epitopes were detected by incubation with 100 μl of primary antibodies or conjugated antibodies diluted in blocking buffer for 2 h, anti-V5-HRP (1 μg/ml, Invitrogen), anti-FLAG-HRP (1 μg/ml, Invitrogen), human anti-2F5 (0.5 μg/ml AIDS reagent program, NIH) rabbit anti-DsRed (Living Colors® 632496, Clontech) 1 μg/ml; experimental serial dilutions of antibodies were made using blocking buffer. After three washing steps with 300 μl of 0.05% Tween®/PBS, cells were incubated with 100 μl of diluted secondary antibody if needed: 0.1 μg/ml anti-human-HRP (DAKO, Hamburg, Germany), 0.1 μg/ml anti-mouse-HRP (DAKO) and 0.1 μg/ml anti-rabbit-HRP (DAKO). Cells were carefully washed five times with 300 μl of 0.05% Tween/1 × PBS. For the peptide-based ELISA, 96-well plates (Nuc MaxiSorp, Thermo Fisher Scientific, Langenselbold, Germany) were coated with 100 μl of 1μM Enfuvirtid peptide (Fuzeon®, Roche, Grenzach-Wyhlen, Germany) resuspended in carbonate buffer (15mM Na_2CO_3, 35mM $NaHCO_3$, 0.2 g/l NaN_3, pH 9.6) overnight at 4°C. Wells were washed three times with 300 μl wash buffer (1 × PBS containing 0.05% Tween 20) and blocked for 2 h with 200 μl blocking buffer. The blocking buffer was replaced by 100 μl of anti-2F5 antibody diluted in blocking buffer and incubated for 1 h, followed by five successive washes with 300 μl of 0.05% Tween/1 × PBS. The diluted primary antibody (100 μl of 0.1 μg/ml anti-human-HRP, DAKO) was added and incubated for 30 min. and washed five times with 1 × PBS. Bound secondary antibodies were detected with 100 μl of TMB (3,3′,5,5′-tetramethylbenzidine) substrate (Pierce TMB substrate kit, Thermo Fisher Scientific) by incubation at room temperature for 15–30 min and addition of 100 μl stop

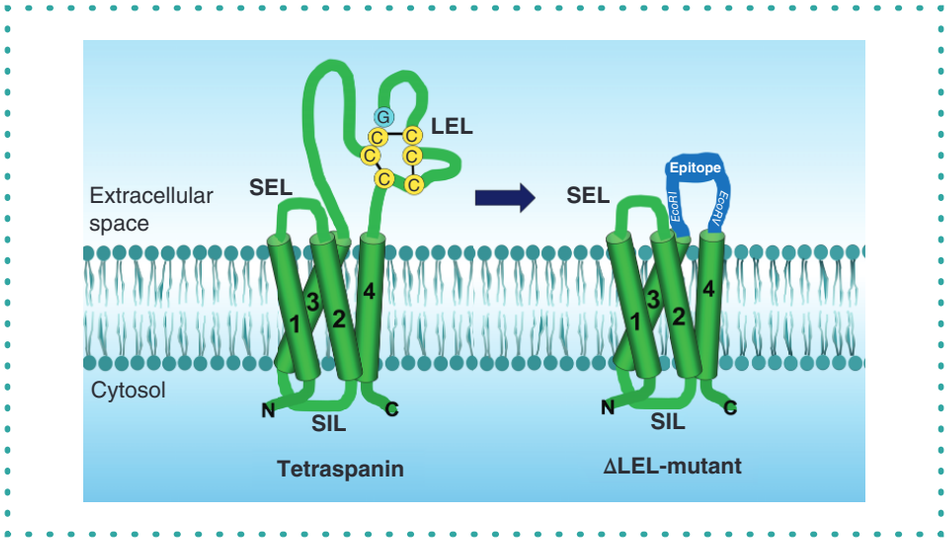

Figure 1. Schematic representation of the tetraspanin anchored (tANCHOR) display system. Tetraspanins containing four transmembrane domains (TM1–4) connected by a small extracellular loop, large extracellular loop and intracellular loops. Tetraspanin ΔLEL mutants are anchors of the 4TM bundle, to which peptides or proteins are fused, replacing parts of the large extracellular loop.
LEL: Large extracellular loop; SEL: Small extracellular loop

solution (2M H_2SO_4). Absorbance values were measured at 450 nm with a microplate reader (Multiscan™GO, Thermo Fisher Scientific). Cutoff was calculated using the mean + 3 standard deviations derived from negative controls [19].

Western blot analysis

HeLa or HEK 293T cells (5×10^5 cells/well) were seeded in six-well plates and transfected with the corresponding vector (6 μg/well) after cells reached 80% confluency. At 24 h posttransfection, cells were lysed in 100 μl 2 × Laemmli buffer, chromosomal DNA was degraded with 25 U of benzonase (Novagen, CA, USA), proteins were reduced with 5 μl β-mercaptoethanol and cell lysates were run on SDS-PAGE using 10 or 12% TGX™ gels (BioRad, Munich, Germany). Migrated samples were then transferred onto an Immobilon-P polyvinylidene difluoride membrane with 0.45-μm pore size (Millipore, Schwalbach, Germany). Membranes were blocked in 10% low-fat milk powder (Carl Roth, Karlsruhe, Germany) for 2 h. Proteins were probed with 0.5 μg/ml mouse anti-DsRed (Living Colors 632496, Clontech), 1.0 μg/ml mouse anti-β-actin (AM00194PU-N, Acris, Herford, Germany), 0.5 μg/ml goat anti-FLAG (NB600-344, NovusBio, CO, USA) and 0.1 μg/ml human anti-2F5 (NIH). Primary antibodies were detected with 0.1 μg/ml of HRP-conjugated (DAKO) rabbit anti-goat IgG, rabbit anti-human IgG and goat anti-mouse IgG, and visualized with Pierce ECL Western blotting substrate (Thermo Fisher Scientific). Western blot images were acquired using a Chemocam device (INTAS, Göttingen, Germany) with implemented contrast correction.

Immunogold scanning electron microscopy analysis

HEK 293T cells were grown on small circular coverslips (12 mm Ø) in a six-well plate and transfected with 6 μg plasmid DNA (pCMV-CD63ΔLEL-CFP-mCherry). At 24 h posttransfection, cells were fixed with 2% paraformaldehyde in 1 × PBS for 20 min, washed with 1 × PBS, and incubated in 3% bovine serum albumin/1 × PBS for 2 h. Samples were next incubated with rabbit anti-GFP (0.5 μg/ml, ab6556, Abcam, Cambridge, UK) and goat anti-rabbit conjugated with 10 nm gold particles for 1 h. After five washes with 1 × PBS, cells were fixed with glutaraldehyde (2.5% in HEPES 0.05M) dehydrated in alcohol (stepwise 30–100%) and finally air-dried using hexamethyldisilazane as the final medium to avoid shrinkage. After carbon coating, samples were analyzed with a field-emission scanning electron microscope (Leo 1530 Gemini; Carl Zeiss Microscopy GmbH). For detection of surface topography and gold particles, the in-lens secondary electron detector and the back scattered electron detector (Centaurus; Deben UK Ltd, Bury St. Edmunds, UK) were used simultaneously in mixed mode (50/50%) at 5 kV acceleration voltage.

Results & discussion

We reasoned that the LEL of tetraspanins (type III integral membrane proteins) might bear the potential for deletion of amino acid stretches and/or insertion and efficient presentation of peptides and small proteins (Figure 1). In order to test this assumption, we used CMV promoter-driven expression constructs for the tetraspanins CD9, CD63, CD81, CD82 and CD151 with an N-terminal FLAG-tag and C-terminal fused sequences coding for the red (mCherry) or the yellow fluorescent protein (Figure 2A). In the mCherry versions, large parts of the LEL (Supplementary Table 1) have been deleted and substituted for a V5-6xHis sequence. In addition to the mentioned tetraspanins, analogous expression constructs were generated for the topologically related transmembrane protein CLDN1 in which we substituted parts of the large extracellular loop (EC1) or the smaller loop (EC2) for the same V5-6xHis-tag. These constructs were then

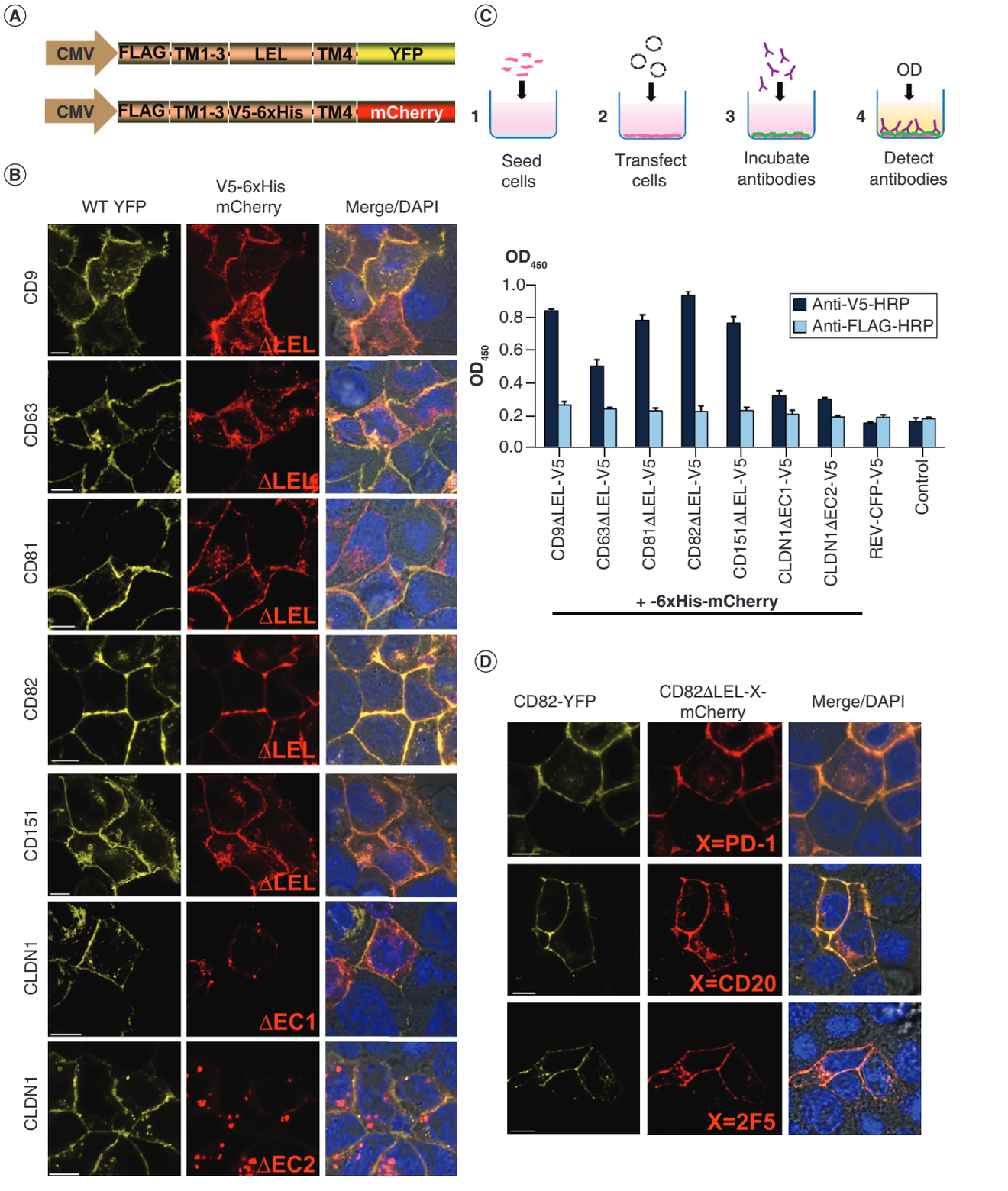

Figure 2. Tetraspanin anchored peptide presentation on the surface of human cells. **(A)** Schematic representation of expression cassettes constructed for tANCHOR vectors (not to scale). **(B)** Confocal images of transfected HeLa cells demonstrating cell localization of expressed proteins. Wild-type proteins are fused with yellow fluorescent protein and deletions of extracellular domains are fused with mCherry. **(C)** Quantification cell surface presentation of a peptide by antibody binding studies. A cartoon illustrating the experimental steps for antibody binding studies is shown in the upper panel. The lower panel shows ELISA results of bound anti-V5-HRP and control antibody (anti-FLAG-HRP) on transfected (nonpermeabilized) HeLa cells. Optical density (OD_{450}) values were measured in triplicate; control refers to untransfected cells. **(D)** Localization of epitopes derived from PD-1, CD20 or 2F5 fused with the CD82 anchor to the cell surface. All scale bars = 10 μm.

used for the transfection of HeLa cells. As shown in Figure 2B, all of the tetraspanins were efficiently expressed and the substitution in the LELs did not have any apparent effect on expression efficiency and subcellular localization of the proteins. In contrast, the substitutions in the EC1 or EC2 of CLDN1 had a significant effect on the expression level and largely (EC1) or completely (EC2) prevented the regular targeting of the protein to the plasma membrane of the cell (Figure 2B).

We next investigated the efficiency of diverse tetraspanins in relation to their capacity to expose foreign peptides and small proteins on the surface of HeLa cells, using antibodies specific for the V5-tag and the FLAG-tag. The V5-tag is supposed to be exposed on the cell surface while the FLAG-tag is expected to be cytoplasmic and therefore inaccessible for antibodies. For this experiment, cells were transfected with the respective expression plasmid and binding of tag-specific antibodies was analyzed on nonpermeabilized cells (Figure 2C). Specific anti-V5 binding, measured by the optical density at 450 nm (OD_{450}) value, was two- to four-times higher with cells expressing tetraspanins compared with nontransfected cells. Moreover, binding of V5 antibody to the CLDN1 EC1 and EC2 mutants carrying the V5-6x-His-tag was only slightly above the background determined with nontransfected cells. No specific binding was measured to the FLAG-tag (Figure 2C). These results indicate that unlike the N-terminal FLAG-tag, the V5-tag positioned in the LEL is exposed to the extracellular space and is accessible by antibodies. An expressed HIV-1 Rev-CFP-V5 protein that localizes in the nucleus (Supplementary Figure 1A) served as a control for demonstrating that anti-V5-HRP antibodies are only able to bind to extracellular epitopes. We observed that the binding efficiency of anti-V5 antibodies on HeLa cells was the highest for the CD82-anchored V5-tag when compared with the other tested tetraspanin anchors, and the lowest for CD63. A relatively low localization of CD63 at the cell surface was previously demonstrated for full-length CD63 as well [20]. In order to confirm the results in another cell line, we repeated the experiments using HEK 293T cells and poly-L-lysine-coated plates to increase adherence. Very similar results were obtained in these cells, although the CD63 anchor showed slightly higher efficiency in V5 epitope presentation compared with HeLa cells (Supplementary Figure 1B). We therefore decided to focus on CD82 as the most promising tetraspanin-based display variant for most of the subsequent analysis steps.

In this respect, we first tested whether the CD82-based tANCHOR is able to accommodate and display relevant epitopes for human cancer immunotherapy derived from PD-1 (aa 62–86) or CD20 (aa 162–191) – which harbor binding sites for nivolumab and rituximab, respectively – and the 2F5 epitope present in the gp41 (aa 660–678) subunit of the HIV-1 glycoprotein. All three constructs bearing the heterologous peptides in the LEL were efficiently expressed and showed the same subcellular localization as the variant with the wild-type LEL (Figure 2D), indicating efficient presentation of the heterologous peptides on the cell surface.

We next sought to determine whether the CD82-based tANCHOR allows the displaying of larger proteins. For this we introduced the sequence coding for the 27-kDa mCherry protein into the LEL and performed a cell-based ELISA on transfected HeLa cells. In this experiment, the mCherry-specific antibodies bound only to cells transfected with mCherry positioned in the extracellular loop of CD82; no binding was detected in cells transfected with a CD82-version with a C-terminal mCherry (Figure 3A). Protein expression was confirmed by Western blot analyses (Figure 3B). The results are in agreement with the expected topology.

To demonstrate efficient display and glycosylation in HEK 293T cells of a heterologous protein anchored with CD63, we used a vector expressing the ectodomain of HIV-1 known to contain four canonical N-glycosylation sites [21]. Significant N-glycosylation leads to smeared protein bands in a Western blot [22–25]. As illustrated in Figure 3C, expression of the 60 kDa (calculated molecular weight of the protein) CD63ΔLEL-$gp41_{ecto}$-mCherry resulted in such a smeared band. A smear band was also observed for the 54 kDa CD63-mCherry protein with the wild-type LEL that contains three N-glycosylation sites [26–28]. In contrast, expression of CD63ΔLEL-CFP-mCherry (72 kDa) and CD63ΔLEL-V5-6xHis-mCherry (44 kDa) proteins, which do not have N-glycosylation sites, resulted in sharp and well-defined protein bands in the same Western blot. These results strongly indicate that heterologous peptides and proteins substituting the LEL of a tetraspanin can be glycosylated if N-glycosylation sites are present.

Next we analyzed the display of CFP in the LEL of CD63 on the cell surface of another transfected cell type, HEK 293T, by confocal laser scanning microscopy and immunogold scanning electron microscopy using anti-GFP antibodies. We observed specific fluorescence signals for CFP and mCherry in the same compartments when visualized by confocal laser scanning microscopy (Figure 3D). This colocalization of CFP and mCherry signals provided confirmation that two fluorescent proteins were transported to the cell surface. Cells expressing CD63-anchored CFP showed a dense labeling with gold particles in scanning electron microscopy, while untransfected (control) cells did not show any labeling (Figure 3E), indicating that CD63-anchored CFP was exposed extracellularly.

To validate our tANCHOR system further, and to compare the display efficiency with that of a previously introduced display technology that takes advantage of a single spanning membrane domain of PDGFR [29–31], we focused on the V5-6xHis and the 2F5 epitope. Figure 3F demonstrates specific binding of the V5 and 2F5 antibodies to cells expressing their displayed epitopes in the context of both, the CD82-based tANCHOR and PDGFR. Moreover, both V5 and 2F5 epitopes were more efficiently recognized on cells transfected with plasmids coding for the modified tetraspanin than for the modified PDGFR. Notably, when mCherry was fused at the C-terminal position of the CD82 anchor, epitope presentation on the cell surface was slightly improved (Figure 3G). In addition, we compared the use of our tANCHOR system for cell-based ELISAs with a common ELISA technique using wells simply coated with the peptides (Enfuvirtid) containing the epitope. In these experiments, we found that the binding of 2F5 antibodies was higher in wells coated with peptides, but only at very high concentrations of the antibody (Figure 3H). At physiologically more relevant concentrations and conditions, binding to the CD82-anchored peptide was even more efficient, especially when considering the low available amount of CD82-anchored 2F5 in the wells (Figure 3I). Although 2F5 targets linear epitopes, the ELDKWA (aa 662–667) core amino acid stretch (core epitope) contains a hydrophobic surface and is part of the membrane proximal region of the gp41 protein [32–34]. The topology of the modified LEL carrying

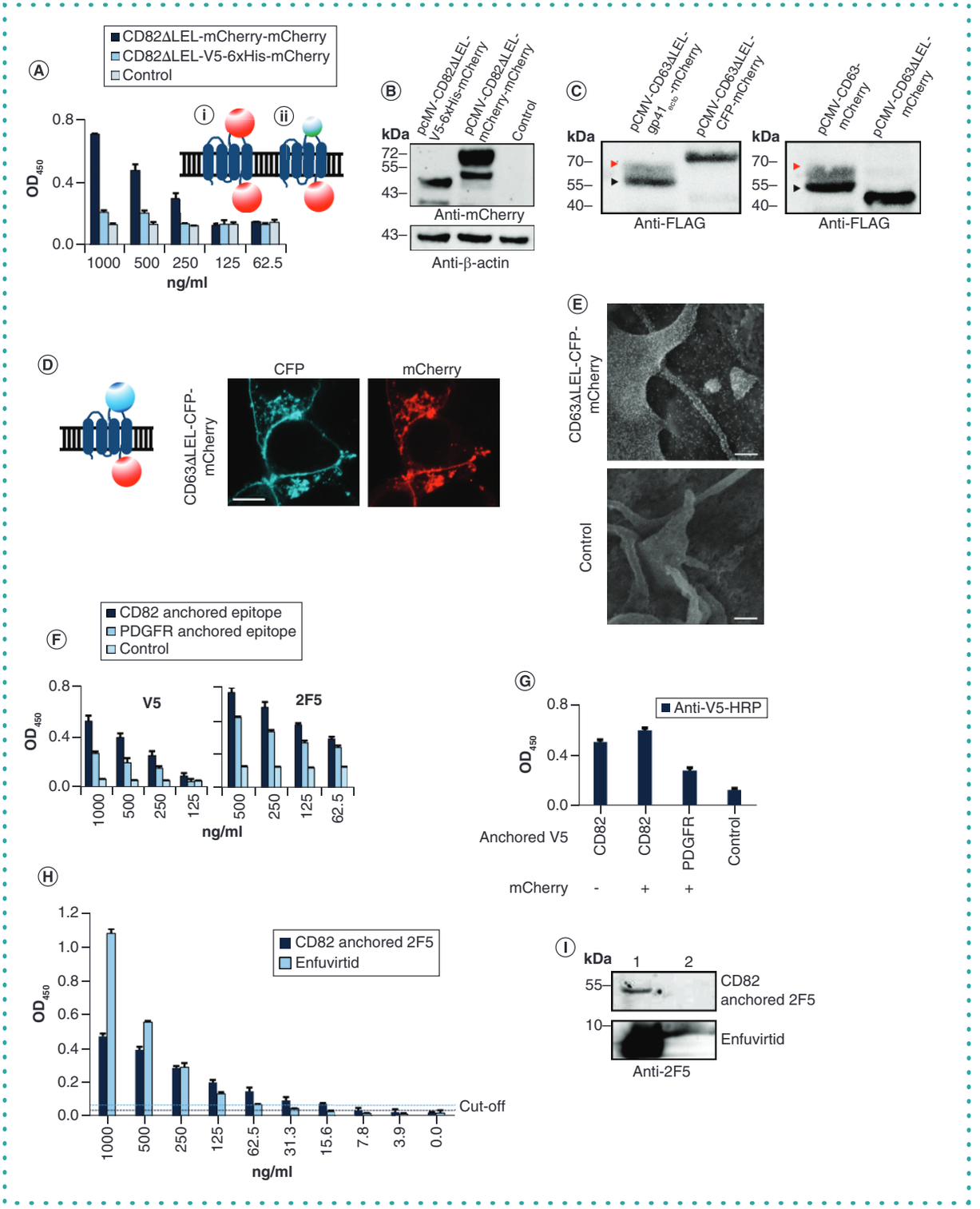

Figure 3. Validation of the tANCHOR system. **(A)** Binding assay using anti-mCherry antibodies. The CD82 anchor version **(I)** contains mCherry in the LEL and at the C-terminus while version (II) contains mCherry at the C-terminus only. **(B & C)** Western blot analysis of cells transfected with indicated vectors; black arrow indicates the unglycosylated and the red the highest glycosylated protein form. **(D)** Confocal images of cells expressing CD63ΔLEL-CFP-mCherry and schematic illustration of protein topology. **(E)** Scanning immunoelectron microscopy of HEK 293T cells expressing CD63-anchored CFP (including mCherry tag) labelled with anti-GFP antibody and a secondary antibody coupled to 10 nm gold colloids. Scale bars = 300 nm. **(F)** Anti-V5 antibody binding assay for comparison between CD82-anchored and PDGFR-anchored V5 or 2F5 epitopes. **(G)** The influence of the C-terminally fused mCherry reporter on epitope presentation at the cell surface was tested using an anti-V5-HRP binding analysis on mCherry+ or mCherry− cells. **(H)** Comparison between Enfuvirtid peptide-based ELISA and CD82-anchored 2F5 epitope fused to mCherry, OD background was subtracted. **(I)** Western blot analysis of lysates collected from wells used in experiment (H) probed with anti-2F5. Lane 1 in the upper blot was loaded with lysates of cells transfected with pCMV-CD82ΔLEL-mCherry and in lane 1 in the lower blot material from wells coated with Enfuvirtid was loaded. Lanes 2 are loaded with lysates of untransfected cells (upper blot) or lysates from wells without Enfuvirtid coating (lower blot).
CFP: Cyan fluorescent protein; GFP: Green fluorescent protein; LEL: Large extracellular loop; OD: Optical density.

the 2F5 epitope is not known; it is tempting to speculate that a close proximity to the cellular membrane might contribute to the enhanced binding of the 2F5 antibody to the epitope in the context of the tetraspanin anchor.

The current study did not investigate potential adverse effects for the cell by ectopic expression of tetraspanins; however, in transiently transfected cells we did not see any such effects. Tetraspanins play an important role in the modulation of multiple biological processes, such as cell adhesion, signal transduction, immunoregulation, tumorgenesis and differentiation [13,35,36]. Potential negative effects caused by overexpression of tetraspanin or tetraspanin with deleted LEL should be addressed in the future.

In summary, we present a novel tool for displaying peptides and small proteins on the surface of human cells. The developed tetraspanin-based tANCHOR system allows presentation of reasonable levels of antigen on cell surfaces for antibody binding studies and other applications. However, it remains to be established to what extent conformational epitopes are preserved and displayed. The tANCHOR-based ELISA formats have the potential to improve the efficiency of antibody screenings, which are in close proximity to membranes and other cellular components on the surface of mammalian cells. Along these lines, tetraspanin-anchored antigens might also be beneficial for vaccination strategies aimed at developing potent neutralizing antibody responses against viruses and other pathogens.

Future perspective

Display technologies have opened up the possibility of generating highly specific antibodies that are used for a plethora of applications, including oncological therapies. The tANCHOR system is a new alternative in the molecular toolbox of options for the display of peptides on the surface of living cells. A key property of the tANCHOR system is its flexibility, which allows fusions of short and longer protein sequences to be presented on the cell surface. Exposed epitopes can be used as baits for identifying specific antibodies *in vitro* in formats where wells are coated with a monolayer of cells and are then incubated with sample material. Moreover, the tANCHOR system should enable tetraspanin-anchored antigen platforms for the rapid development of vaccines.

Supplementary data

To view the supplementary data that accompany this paper please visit the journal website at: www.future-science.com/doi/suppl/10.2144/btn-2020-0073

Author contributions

D Ivanusic developed the tANCHOR concept, designed and performed the experiments and analyzed the data together with N Bannert. D Ivanusic and K Madela acquired confocal laser scanning microscopy images. G Holland performed the scanning electron microscopy preparation and analysis. H Burghard helped in generating expression vectors. The manuscript was written by D Ivanusic, M Laue and N Bannert. All authors read and approved the manuscript.

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Financial & competing interests disclosure

This work was supported by the Peter und Traudl Engelhorn Foundation, Germany. The non-profit organization Peter und Traudl Engelhorn Foundation holds a patent application for the tANCHOR system where D Ivanusic is listed as an inventor. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

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