Journal of Cell Science & Therapy
ISSN: 2157-7013
Boldicke, J Cell Sci Ther 2015, 6:4
DOI: 10.4172/2157-7013.1000214
Letter to Editor
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ER Intrabodies: Potent Molecules for Specific Knockdown of Proteins Passing the ER

Thomas Boldicke*

Helmholtz Centre for Infection Research, Department Structural and Functional Protein Research, Germany

***Corresponding author:** Thomas Böldicke, PhD, Helmholtz Centre for Infection Research, Department Structural and Functional Protein Research, Inhoffenstr. 7, 38124 Braunschweig, Germany, Tel: 0531-6181-5050; E-mail: Thomas.Boeldicke@helmholtz-hzi.de

Rec Date: June 22, 2015; **Acc Date:** July 19, 2015; **Pub Date:** July 22, 2015

Letter to Editor

Today the function of proteins can be analyzed by nucleotide based functional genomics for example siRNA [1] or protein knockdown approaches. Knockdown approaches based on direct interference of the target protein with the inhibitor results in detailed insights of protein functions not obtainable with genetic methods.

A very promising protein knockdown technique is based on recombinant antibody fragments expressed inside the ER (ER intrabodies). ER intrabodies mediate inhibition of the function of proteins passing the ER very efficiently and specifically. Many ER intrabodies against a large range of attractive targets have been described [2,3]. Targets include oncogenic receptors, virus proteins (to prevent virus assembly), cellular virus receptors (to block virus entry), proteins of the immune system and the nervous system [4-8]. Even ER intrabodies with therapeutic potential have been generated [9-15].

Intrabodies are recombinant antibody fragments, mainly constructed in the scFv or Fab format. They are produced inside target cells expressing the corresponding antigens. Intrabodies can be targeted to the nucleus, ER or mitochondrium by fusion of an appropriate signal sequence to the N-terminus of the antibody coding sequence or expressed without any signal sequence inside the cytoplasm [16].

ER intrabodies retain proteins passing the ER via a sequence encoding the short retention peptide KDEL which is fused to the C-terminus of the intrabody gene [17]. The inhibition of the translocation of the antigen from the ER to the cell compartment where it normally acts leads to inhibition of antigen function. The method is suitable to retain the transport of secretory molecules, cell surface proteins or proteins which are active in the Golgi or endosomal compartment [2,13,18]. Even an intrabody without retention sequence inactivated the ribosomal P proteins of Trypanosoma cruzi [19].

Due to the high specificity of antibodies, this technology is an attractive alternative to RNAi/miRNA based methods and small molecule inhibitor molecules such as receptor tyrosine kinases inhibitors, which often show off target effects [20-23].

An ER intrabody just requires efficient binding to its antigen to retain it inside the ER. In contrast, a cytosolic antibody must inactivate its target, or interfere with the binding of the target protein to its corresponding binding partner. In contrast to ER intrabodies which are correctly folded in the ER, cytosolic intrabodies are often not correctly folded due to the fact that disulfide bridges are not formed in this environment. Consequently, a considerable effort is required to select stable cytosolic intrabodies [24]. Today the most used methods for selection of cytosolic intrabodies are the Intracellular Antibody Capture Technology based on an antigen-dependent two hybrid system [25] and construction of single domain antibodies derived from camels [26] or shark which are very stable expressed in the cytoplasm [27-29].

The antigen specific scFv antibody fragment for ER intrabody generation can be selected from (1) a hybridoma clone [30] or (2) from in vitro display systems such as phage display or yeast display antibody repertoires [31,32] (Figure 1).

The advantages of ER intrabodies are

- Very high specificity to the antigen
- In vitro display techniques open the door for constructing ER intrabodies with a single cloning step.
- Splice variants can be targeted
- Post translational modifications can be analyzed
- Isoforms of a protein can be inactivated by one intrabody
- Transgenic intrabody mice can be generated

The number of ER intrabodies will grow much more faster in the future compared to the last twenty years, as (1) thousands of new scFv antibody fragments have been generated by phage display and yeast display and the corresponding V region genes are available for construction of new ER intrabodies, and (2) optimized consensus primer mixes, RACE and adapter-ligated RT-PCR enables reliable isolation of antibody variable domains from hybridoma clones today.

Until now most ER intrabodies have been tested in vitro. In addition some ER intrabodies have been applied in xenograft tumor mouse models [9,33], in an Alzheimer's disease mouse model [34] and in an human papillomavirus (HPV)-associated tumor mouse model [35]. Recently two transgenic intrabody mice has been generated showing significant knock down of VCAM and gesolin in vivo [36,37]. The VCAM intrabody mouse expressed the intrabody as scFv fragment and the gesolin intrabody mouse as VHH antibody. Interestingly, the transgenic VCAM intrabody mouse was viable in contrast to the lethal knock out counterpart [38]. In addition an anti-EVH1 intrabody has been investigated in vivo in a transgenic intrabody mouse [39]. However this report has been questioned because the intrabody was targeted to the secretory pathway but interacted with a cytosolic protein [40].

The results obtained from the VCAM and gesolin intrabody transgenic intrabody mice pave the way to new fascinating possibilities: for example the possibility to establish transgenic intrabody mice with inducible or tissue specific ER intrabody expression which can further be regulated by different exogenous promoters.

Very interesting would be therapeutic clinical approaches of ER intrabodies [15,16]. Problems not yet solved in gene therapeutic appraches is the need of safety viral or efficient non-viral transfection systems. Construction of retrovirus with low safety risk, of transductional and transcriptional targeting vectors for cell-specific gene transfer and the use of mRNA might be promising tools for successful gene therapy in the future [41-43].

In summary, ER intrabodies are very potent and specific molecules to study the function of proteins in vitro and in vivo. Particularly the availability of variable antibody domains selected from phage display libraries simplifies the generation of new ER intrabodies for a more broad application in functional analysis of proteins.

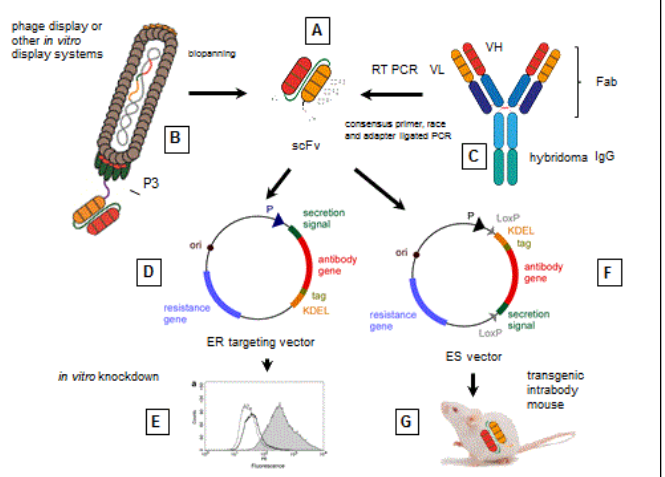

Figure 1: Scheme of ER intrabody construction and establishment of transgenic intrabody mouse.

(A) Shows a scFv fragment, which will be converted into an ER intrabody by cloning it in one step into ER targeting vector (D) for in vitro characterization of ER intrabody or into Embronic Stem Cell vector for generation of transgenic mice (E). The scFv comprises the variable domain of the heavy chain (VH) and light chain (VL) linked together by a synthetic 15 (Gly4Ser)3 amino acid linker. CDR1, CDR2, CDR3, complementarity determing regions.

(B) scFv's can be selected using universal antibody phage display libraries which comprises the complete genes of a man's antibody repertoire. Seen is a typically filamentous M13 antibody phage with the antibody gene inside the phage and the corresponding protein fused to P3 on the surface of the phage. In a process called biopanning the recombinant antibody phages are incubated with antigen and after washing, specific phages can be eluted and the antibody genes further amplified in E.coli.

(C) scFv's can also be constructed from the variable domains of a hybridoma clone. Therefore the variable domains of the heavy and light chain will be amplified from the cDNA by PCR. In general the variable genes are amplified with consensus primer. If it is not possible to amplify less common non-consensus antibody sequences, two other methods are available: Rapid amplification of cDNA ends (RACE, [44] and adapter ligated PCR [45]. Assembly of the variable domains with the synthetic linker by PCR will result in the scFv.

(D) The gene of the scFv will be cloned into ER targeting vector providing a secretion signal at the N-terminus and a peptide affinity tag and the retention sequence KDEL at the C-terminus of the antibody coding sequence.

(E) After cloning the retention of the corresponding cell surface antigen inside the ER can be estimated by FACS. Shown is as an example of the inhibition of Neural Cell Adhesion Molecule (NCAM) cell surface expression by anti-NCAM ER intrabodies. TE671cells (NCAM+, shaded area) and TE671 cells expressing anti-NCAM intrabodies (bold line) incubated with anti-NCAM antibodies and secondary anti-mouse phycoerythrin conjugated antibodies. TE671 cells expressing ER intrabodies against NCAM stained with secondary antibody only (thin lane).

(F) To generate transgenic mice the coding sequence of the antibody will be cloned into an Embryonic stem cell (ES) vector and the vector transfected into ES cells. Then recombinant ES cells will be used for the generation of transgenic intrabody mice. The intrabody gene can be oriented in reverse orientation and flanked by reverse LoxP sites. To express the intrabody gene, the established intrabody mouse will be crossed with a mouse expressing the cre recombinase (Cre mouse [46]). The cre recombinase inverts the intrabody gene at the two inverse DNA recognition sites (LoxP sites), thereby the intrabody gene is activated.

(G) Transgenic intrabody mice can be established with constitutive intrabody expression or inducible or tissues specific intrabody expression [46].

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