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Mini Review

Antibodies inside of a cell can change its outside: Can intrabodies provide a new therapeutic paradigm?

Andrea L.J. Marschall [a], Stefan Dübel [b,*]

[a] Department of Systems Immunology and Braunschweig Integrated Centre of Systems Biology, Helmholtz Centre for Infection Research, Braunschweig, Germany
[b] Institute of Biochemistry, Biotechnology and Bioinformatics, Technische Universität Braunschweig, Spielmannstr.7, 38106 Braunschweig, Germany

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ABSTRACT

Challenges posed by complex diseases such as cancer, chronic viral infections, neurodegenerative disorders and many others have forced researchers to think beyond classic small molecule drugs, exploring new therapeutic strategies such as therapy with RNAi, CRISPR/Cas9 or antibody therapies as single or as combination therapies with existing drugs. While classic antibody therapies based on parenteral application can only reach extracellular targets, intracellular application of antibodies could provide specific advantages but is so far little recognized in translational research. Intrabodies allow high specificity and targeting of splice variants or post translational modifications. At the same time off target effects can be minimized by thorough biochemical characterization. Knockdown of cellular proteins by intrabodies has been reported for a significant number of disease-relevant targets, including ErbB-2, EGFR, VEGFR-2, Metalloproteinase MMP2 and MMP9, β-amyloid protein, α-synuclein, HIV gp120, HCV core and many others. This review outlines the recent advances in ER intrabody technology and their potential use in therapy.

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1. Utilizing the specificity of antibodies inside of living cells

Intrabodies are antibodies expressed intracellularly to block cellular functions. In contrast to the naturally expressed antibodies which are secreted and directed towards extracellular targets, intracellularly expressed antibodies, are directed towards targets inside the cell. This allows utilizing the very high specificity of antibody/antigen binding for the functional analysis of proteins in living cells or even living organisms.

The use of antibodies in living cells started in the 1980s when they were found to be sufficiently stable after microinjection into the cytoplasm, and they were shown to be able to interfere with the function of their intracellular antigen. For instance, intermediate filaments were found to collapse after blocking their assembly with microinjected antibodies [8]. However, microinjection is laborious and allows only small cell numbers to be manipulated, which limited a widespread application of the technique. Hence, a number of approaches using reagents or peptides for protein delivery have been tried to introduce antibodies into living cells [33]. While the so called "cell penetrating peptides" (CPPs) gained considerable attention at early times after their discovery, their initially proposed mechanism of uptake and the general efficacy as transduction modules for macromolecules has

* Corresponding author.
E-mail address: s.duebel@tu-bs.de (S. Dübel).

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meanwhile been questioned [33,35,39]. In contrast to initial assumptions, CPPs are now believed to be internalized by endocytosis if linked to macromolecules and the majority remains in endosomes, which may result in very low efficiency of cytosolic delivery [18,33,39]. Because inhibition of antigen function by the binding of antibodies to their antigen usually requires an at least 1:1 M ratio of the latter, this low efficiency of cytosolic delivery can substantially limit its applications for functional interference. Protein transfection (profection), which is based on reagents that are believed to possess properties which can enhance or trigger endosomal release [5], therefore has been suggested as a promising alternative [52]. However, similar to the initial difficulties to detect the true cytosolic release of cargo-molecules in research on CPPs, the efficiency of profection has recently been found to be largely overestimated too, due to the common usage of artifact-prone detection methods [35].

Despite the numerous attempts to deliver antibodies to the cytosol by using peptides or by means of profection, delivery into larger cell populations of amounts of antibodies comparable to the early microinjection experiments of the 1980s was only recently achieved by electroporation [15,20,35] and demonstrated that scFv-Fc antibodies (which are similar to the microinjected whole IgG but rely on the structural integrity of a scFv moiety) are functional for at least 96 h after electroporation into cells [35].

The limitations of protein delivery motivated attempts to express the genes of antibodies in cells early on [49]. As intrabody approaches work well with just the antigen binding fragments of an IgG, typically single chain Fv fragments (Fig. 1) or even single domain antibodies/nanobodies, they do not require assembly from two protein chains like the original antibody, eliminating the need for bicistronic vectors and the associated problems to achieve the correct heavy chain/light chain ratio upon expression. However, cytosolic expression of intrabodies did not always result in functional antibodies as many antibodies tend to misfold in the cytosol. This can be attributed to the reducing milieu preventing formation of disulfide bonds [6,44] and lack of endoplasmic reticulum (ER) chaperones. In contrast, because antibodies are naturally secreted, their folding is optimal in the ER, which prompted the introduction of a different type of intrabodies: ER retained intrabodies. Functional knockdowns of membrane or secreted proteins can be achieved by means of ER retained intrabodies by providing a target specific antibody together with an ER retention signal, the amino acid sequence "KDEL" [29]. In this way, the intrabody is kept within the ER together with its target antigen. Trapping individual

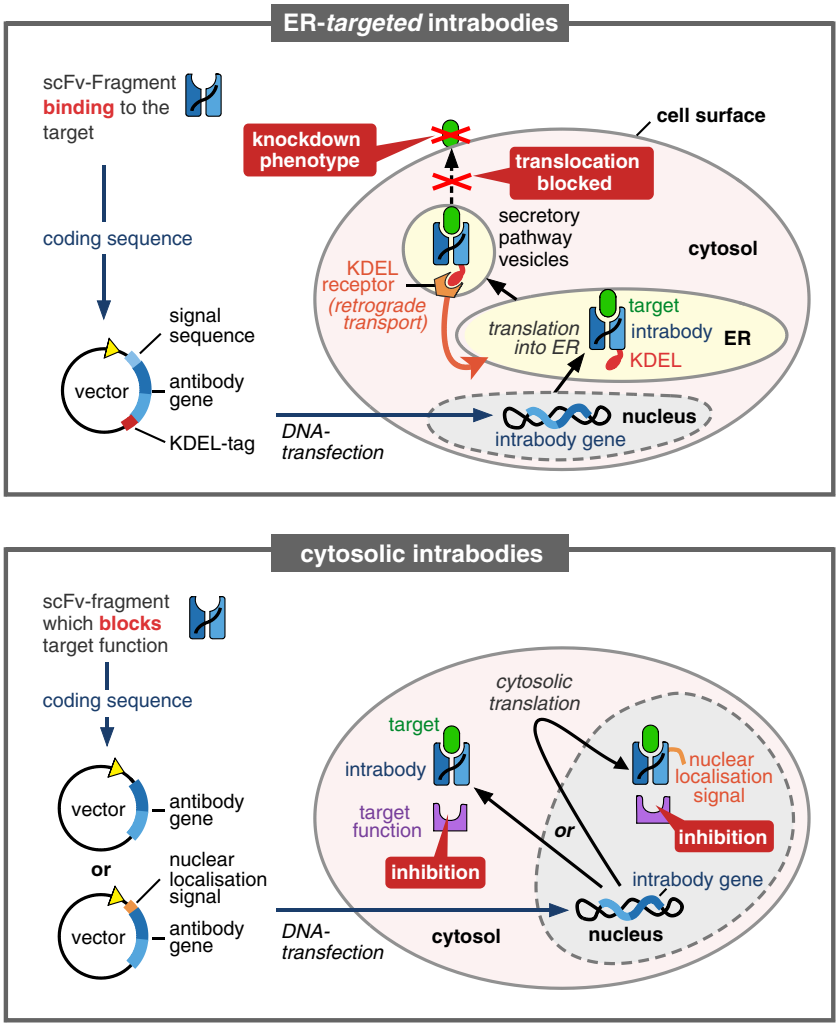

Fig. 1. Properties and modes of action of intrabodies targeting antigens in the ER (top) or in the cytoplasm (bottom). ER targeted intrabodies require a signal sequence guiding them to be produced into the ER. There they cause a functional knockdown by binding to their target protein (antigen) inside of the ER and retaining it there, thus preventing it from reaching the cell surface or from being secreted. In order to achieve ER retention of a target protein, the intrabody itself needs to be retained in the ER, which is achieved by adding the ER retention signal peptide "KDEL". Specific binding of an ER intrabody to its target is usually sufficient to provide the knockdown, while cytosolic intrabodies are usually required to additionally be inhibitory or blocking. Consequently, cytosolic intrabodies usually need to target a particular epitope. A second difference to ER intrabodies is that cytosolic intrabodies are translated in the cytosol, where the protein folding conditions for antibodies are less favorable than in the ER. Cytosolic intrabodies may also be targeted to the nucleus if provided with a nuclear localization sequence.

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