

# Chapter 9

## Intrabody Expression in Mammalian Cells

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**Abstract** The intracellular expression of antibodies or antibody fragments (intrabodies) in different compartments of mammalian cells allows to block or modulate the function of endogenous molecules. Intrabodies can alter protein folding, protein-protein, protein-DNA, protein-RNA interactions and protein modification. They can induce a phenotypic knockout and work as neutralizing agents by direct binding to the target antigen, by diverting its intracellular traffic or by inhibiting its association with binding partners. They have been largely employed as research tools and are emerging as therapeutic molecules for the treatment of human diseases as viral pathologies, cancer and misfolding diseases. The fast growing bio-market of recombinant antibodies provides intrabodies with enhanced binding specificity, stability and solubility, together with lower immunogenicity, for their use in therapy. This chapter describes the crucial aspects required to express intrabodies in different intracellular compartments of mammalian cells, their various modes of action and gives an update on the applications of intrabodies in human diseases.

### 9.1 Introduction

The proof of principle that antibodies could be efficiently expressed and targeted to different intracellular compartments in mammalian cells dates to 1990 (Biocca et al. 1990) and led to the concept of exploiting recombinant antibodies to block or modulate the function of target antigens for intercellular and intracellular immunization (Biocca and Cattaneo 1995; Cattaneo and Biocca 1997). Intrabodies have unique advantages comparing to other knockout gene techniques or RNA interference. First, they can target the antigen in different intracellular compartments including extracellular milieu. Secondly, they are highly specific reagents and are very stable in mammalian cells, especially when expressed in the secretory compartment. In addition, intrabodies can hit a variety of possible targets: (i) specific protein domains, (ii) specific protein-protein interaction sites, (iii) post-translational modifications, (iv) multiple conformational isoforms (oligomers, fibrils etc) and (v) even non protein antigens.

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In order to specifically target intrabodies to the physiological site of the antigen or to new intracellular localisations, dominant and autonomous targeting sequences should be grafted onto antibody chains. Recombinant antibody domains, in particular single-chain Fv (scFv) fragments have been expressed in the cytoplasm (Biocca et al. 1994), the nucleus (Duan et al. 1994; Mhashilkar et al. 1995) and the secretory pathway of mammalian cells (Marasco et al. 1993) and successfully used to inhibit the function of several intracellular antigens. New suitable antibody formats for their intracellular use are now available and functional intrabodies are employed as research tools to define the mechanisms of human pathologies at the molecular level and for a variety of therapeutic applications. For instance, intrabodies have been designed to inhibit single or simultaneously multiple signal transduction pathways (Lener et al. 2000; Tanaka et al. 2007; Jendreyko et al. 2005), inhibition of HIV viral proteins (Lo et al. 2008), inhibition of oncogene products (Williams and Zhu 2006; Griffin et al. 2006), misfolding-prone proteins (Cardinale and Biocca 2008a), receptors of the immune system (Kirschning et al. 2010) and also applied in post-transplantation surgery (Zdoroveac et al. 2008).

## 9.2 Targeting Intrabodies to Different Intracellular Locations

Antibody fragments can be directed to specific target antigens present in the cytosol, nucleus, endoplasmic reticulum (ER), plasma membrane (PM), mitochondria, peroxisomes and trans-Golgi network (TGN) through in frame fusion with intracellular trafficking sequences (Cardinale et al. 2004). The following is a list of targeting signals successfully used for intracellular expression of antibodies.

The way to target intrabodies throughout the secretory pathway, as secreted proteins, is by exploiting the leader sequence for secretion of the immunoglobulin (Ig) at the N terminus. ER-retained intrabodies are designed with a leader sequence at the N terminus and a retention peptide, KDEL, at the C terminus (Biocca et al. 1995). Intrabodies in the ER behave as intracellular anchors and can be used either to prevent the appearance of receptor proteins on the plasma membrane or to inhibit the secretion of a protein. Similar protein retention in the trans-Golgi has been achieved with a trans-Golgi retention signal (Zhou et al. 1998). Targeting to the plasma membrane has been obtained by fusing a scFv with a receptor transmembrane domain (Chesnut et al. 1996).

Removal of the leader sequence of variable heavy (VH) and variable light (VL) domains which target antibody fragments to the lumen of the endoplasmic reticulum allows the cytoplasmic expression of intrabodies (leader-less). Nuclear targeting can be achieved by adding one or more nuclear localisation sequences (NLS) to the leader-less antibody fragments, such as the PKKKRKV sequence of the large T antigen of SV40, either at the N- and C-terminus (Biocca et al. 1995). Intrabodies have been also targeted to mitochondria. N-terminal presequences are present in most of the nuclear-encoded mitochondrial proteins. These sequences are removed once the protein is translocated through the mitochondrial membrane. The N-terminal

presequence of the subunit VIII of human cytochrome oxidase (COX8.21), covering the cleavage junction, can be fused to the scFv fragment. The resulting molecule correctly localizes to mitochondria (Biocca et al. 1995).

To facilitate the expression of scFv fragments as secreted or intracellular proteins, a set of general vectors have been designed. The integrated system of scFvexpress vectors (Persic et al. 1997a) derives from the VHexpress vector, a vector used to produce secretory immunoglobulin heavy chains from cloned IgH regions (Persic et al. 1997b). All the scFvexpress plasmids contain an N- or C-terminal localisation signal that allows the targeting of the antibody fragments to different compartments, including the endoplasmic reticulum (scFvex-ER), the cytoplasm (scFvex-cyt), the nucleus (scFvex-nuc) and the mitochondria (scFvex-mit). The scFvexpress-cyt has no targeting signal (leader-less) and directs the expression of scFv in the cytoplasm, with an N-terminal methionine instead of the leader sequence for secretion. All other targeting vectors are derivatives of the scFvexpress-cyt and were obtained by the insertion of well characterized targeting signals (Biocca et al. 1995) either N- or C-terminal to the scFv, as appropriate.

Besides the specific targeting signals, these vectors contain cassettes that encode for strong promoters and sequences for the resistance to selection antibiotics and a C-terminal myc-tag in frame with the scFv allowing its detection with the monoclonal antibody 9E10 (Persic et al. 1997a).

These vectors can be used for transient or stable transfection of mammalian cells.

### 9.3 Intrabody Formats

Innovative DNA recombinant technologies have allowed the reformatting of antibody molecules in new smaller fragments with improved properties for their intracellular expression. So far the single chain variable fragment (scFv) has been the recombinant antibody format more widely used for intrabodies. It contains the complete antigen binding site and it consists of the variable domains of the immunoglobulin heavy (VH) and light (VL) chains linked with a flexible polypeptide which prevents dissociation. The resulting molecule is a monovalent antibody fragment, with a molecular weight of about 30 kDa compared to the 150 kDa of the full-length antibody. Other formats that have been successfully expressed inside cells are recombinant bispecific and tetravalent antibody fragments made of two scFvs linked through the second and third heavy chain constant domain, named intradiabodies. These bispecific intrabodies have been designed for simultaneous trapping of two endothelial transmembrane receptors in the same compartment (Jendreyko et al. 2005).

ScFv is not the minimal size for functional antibody fragments. Other fragments made of one variable domain such the single VL and VH domains are the smallest functional fragments derived from immunoglobulin light and heavy chains. The so-called VHH single domain antibody fragments, derived from naturally occurring heavy-chain antibodies devoid of light chain present in the immune system

of camelids, retain the antigen specificity of the whole antibody and have excellent properties of solubility, stability and expression in mammalian cells, aside from the absence of intra-domain disulfide bonds (Hamers-Casterman et al. 1993; Wesolowski et al. 2009). They are easily produced as recombinant antibodies, much smaller in size and can be forged into new multispecific and multivalent reagents with enhanced therapeutic efficacy. Due to their smaller size, they possess a great capacity to form long finger-like extensions that can potentially target cryptic epitopes that are difficult for intact antibodies or scFv fragments to reach.

## 9.4 Selection Strategies to Improve Intrabody Stability and Solubility

Antibody fragments can be potentially targeted to any subcellular compartment, but their folding performance and stability can be limited by the micro-environmental intracellular conditions of the compartment where they are directed to. Thus, a major issue raised in the initial phase of work on intrabodies was the limited half life of antibody and antibody domains and their tendency to aggregate when expressed in the cytoplasm of mammalian cells. Studies on targeting of scFv fragments showed, in fact, that the expression levels of the retargeted antibody domains may vary and the cytoplasm may be considered the worst case (Cattaneo and Biocca 1999). In general, intrabodies expressed in the secretory compartment are more stable than those expressed in the cytoplasm. This is due to the fact that intradomain disulfide bonds, which contribute 4–6 Kcal mol<sup>-1</sup> to the stability of the antibody domain, do not form in the reducing environment of the cytoplasm (Biocca et al. 1995). As a consequence of the lower stability, some scFvs tend to misfold and aggregate as insoluble proteins. Notwithstanding this fact, it is worth noting that in many cases cytoplasmic-targeted intrabodies bind the antigens and maintain their *in vivo* functional activity (Cardinale et al. 2001).

A recent comparative study between the cytoplasmic expression in mammalian cells of well characterized single-chain variable fragments and camelid VHHs, selected from antibody libraries based on similar scaffolds, have outlined the physico-chemical determinants that correlate with enhanced intracellular solubility. Soluble expression in the cytoplasm appears to be influenced by the complementary determining regions (CDRs) content and by the overall charge and hydrophobicity of the intrabody sequence (Kvam et al. 2010).

This issue is very important and during the last years many selection strategies have been developed to improve stability, solubility and functional properties of antibody fragments under conditions of intracellular expression. Two different strategies have been followed: the knowledge-based and the selection-based approaches. The former relies on the introduction of educated mutations that stabilize pre-existing antibody fragments in order to obtain a “super-framework” (Jung and Plückthun 1997; Wörn et al. 2000; Monsellier and Bedouelle 2006). The second approach exploits the availability of different antibody libraries for selection of the

best functional molecules for intracellular expression (Hudson and Souriau 2003). For the expression in the cytoplasm, new human phage antibody libraries have been generated based on single framework optimized for intracellular expression (Philibert et al. 2007). On the other hand, highly stable ribosome-display libraries, based on *Escherichia coli* SecM translation arrest mechanism, have been used for isolating scFvs that are stable under reducing conditions (Contreras-Martínez and DeLisa 2007). Moreover, antibody-antigen intracellular selection methods have been also developed for the isolation of intrabodies able to efficiently interact with the antigen *in vivo*. To this aim an *in vivo* yeast two-hybrid system has been realized, described as intracellular antibody capture (IAC) technology (Visintin et al. 1999). This approach combines a first round of *in vitro* selection of scFvs with a second round of *in vivo* screening of selected intrabodies and allows the isolation of antibody domains with no need to use purified antigens. IAC technology has recently allowed the direct *in vivo* intracellular selection of conformation-sensitive anti-oligomeric scFvs against the Alzheimer's amyloid  $\beta$  peptide (Meli et al. 2009). Another procedure for direct *in vivo* selection of antigen-specific intrabodies, which utilises a single domain antibody format and is based on a predefined intrabody consensus framework has been described (IAC<sup>2</sup>) (Tanaka and Rabbitts 2003) and further developed (IAC<sup>3</sup>) (Tanaka and Rabbitts 2010). This new protocol allows the isolation of functional VH and VL domains from different libraries in four steps, including confirmation of functional intrabodies in mammalian cells.

For clinical applications of intrabodies, generation of humanized and/or human-derived antibody domains offers obvious potential advantages. Modern emerging strategies have improved *in vitro* selection of fully humanized recombinant antibodies directly from human antibody-display libraries, through the creation of large natural or synthetic repertoires of antibody fragments (Hudson and Souriau 2003; Hoogenboom 2005). A direct selection of human phage antibody libraries on tumor cells has been described (Goenaga et al. 2007).

## 9.5 The Mode of Action of Intrabodies

Intrabodies can mediate their effect inside the cells by neutralizing the target protein through direct binding to the functional domain (Biocca et al. 1994; Cohen et al. 1998), blocking protein-protein interaction (Griffin et al. 2006; Tanaka et al. 2007; Van den Abbeele et al. 2010) or by relocating the antigen to a different intracellular location (Lener et al. 2000; Cardinale et al. 2005; Böldicke 2007). In this last case also non-neutralizing antibodies, according to *in vitro* biochemical criteria, can be effective when expressed *in vivo*. This can be obtained by adding to the intrabody a specific targeting signal, as the ER retention signal KDEL, which confers to the antigen-antibody complex the retention in the endoplasmic reticulum. In some described cases, intrabodies divert the antigen to the proteasome degradation pathway (Cardinale et al. 2003; Filesi et al. 2007). As mentioned before, intrabodies have different folding performance and stability particularly when they are expressed in

the cytoplasm. In this compartment, intradomain disulfide bonds do not form and intrabodies complexed to their corresponding antigen tend to misfold and aggregate. This has been demonstrated for cytoplasmic, nuclear and secretory intrabodies. In the first case they aggregate and form aggresomes in a perinuclear location, whereas in the secretory compartments the antigen-antibody complexes are retrotranslocated from the ER, ubiquitinated and finally proteasome degraded (Filesi et al. 2007).

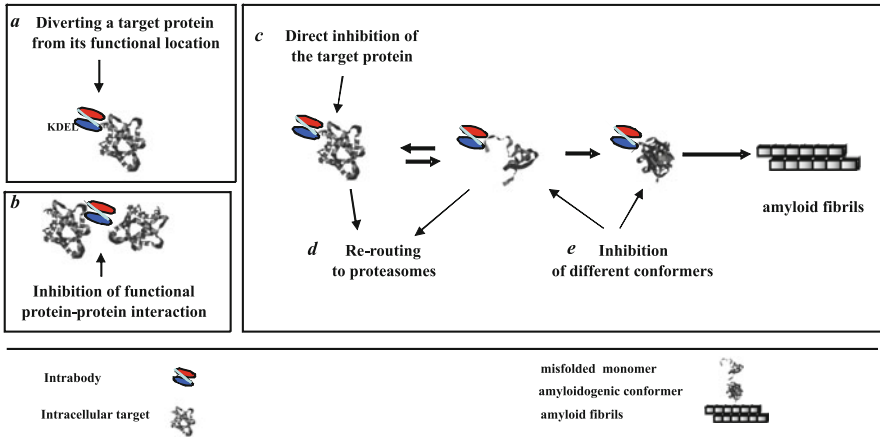
In order to define the mode of action of new intrabodies, the solubility properties, the intracellular distribution and the resulting phenotypes should be routinely analysed with ad hoc assays. Protocols for studying the expression, solubility, stability properties, intracellular localisation and for the analysis of the antigen-intrabody complex *in vivo* have been described (Cardinale et al. 2004 and Cardinale and Biocca 2010).

In summary, intrabodies can be used for preventing or treating human diseases by exploiting their ability to (a) divert the antigen from its functional location, (b) inhibit functional protein-protein interaction, (c) inhibit the functional site of the target antigen, (d) re-route the antigen to the degradation pathway and (e) inhibit different stages of the aggregation process. This latter mode of action can be achieved through stabilization of the native state isoform, inhibition of oligomerization, inhibition of fibril formation and disruption and clearance of preformed aggregates as demonstrated with scFv directed against misfolded prone proteins (Cardinale and Biocca 2008b).

Figure 9.1 summarises the different modes of action of intrabodies observed *in vivo*. These activities have a great potential in medicine and represent a viable option for different pathologies, including neurodegenerative diseases, infectious diseases and cancer.

## 9.6 Intrabodies Against Misfolding Diseases

A hallmark of misfolding diseases is the accumulation of amyloid or amyloid-like aggregates deriving from the fibrillization process of a native protein. During the aggregation process of an amyloidogenic protein many different conformers, including misfolded monomers, oligomers and fibrils are generated (Chiti and Dobson 2006). In the neurodegenerative diseases the aggregation occurs in the brain and is accompanied by cognitive decline. The available therapeutic treatments to slow or prevent these devastating disorders are still not effective, so the development of new molecular therapies that target the pathogenic proteins are urgently needed. In particular, there is great interest in searching new molecules able to prevent unfolding and aggregation either by stabilizing the native state of the amyloidogenic precursors or by disassembling amyloid fibrils. Conformational specific antibodies are very promising agents against neurodegenerative disorders, because they can be raised against different structural isoforms of an amyloidogenic antigen, act at the protein level and can be used in an intrabody approach (Fig. 9.1). Interestingly, although there are no obvious structural or sequence identities among proteins that



**Fig. 9.1** Schematic model for different modes of action of intrabodies. (a) Re-routing the antigen from its functional location, in this case by adding the ER-retention signal KDEL at the C-terminus of the intrabody. The retention sequence KDEL is specifically recognized by the ERD2 receptor inside the cis-Golgi. The complex formed by the KDEL-tagged intrabody and the target protein binds to the ERD2 receptors, is transported through the Golgi back to the ER, where it is released. (b) Inhibition of functional protein-protein interaction. (c) Direct inhibition of the native target protein. (d) Re-routing the antigen antibody complex or the prone-misfolded antigen to proteasome degradation. (e) Inhibition of different conformers. Intrabodies that distinguish native and misfolded conformations can be generated and used either to re-route the misfolding-prone protein from its site of aggregation and, eventually, divert it to the proteasome pathway or to inhibit different stages of the aggregation process. This latter mode of action can be achieved through (i) stabilization of the monomeric isoform, (ii) inhibition of oligomerization, (iii) neutralization of potentially toxic oligomeric species, (iv) inhibition of fibril formation and (v) clearing of preformed aggregates

cause neurodegenerative diseases, it has been shown that conformation-dependent antibodies, raised against a molecular mimic of A $\beta$  oligomers, react with oligomers derived from different kinds of amyloidogenic proteins, such as  $\alpha$ -synuclein, polyglutamine, prions, etc. inhibiting the toxicity associated to these aggregates (Kayed et al. 2003). This finding indicates that the conformational structure rather than the amino acid sequence of these misfolded isoforms is probably the key factor for their neurotoxicity.

To generate conformation-specific intrabodies for therapeutic purposes it can be used either the IAC technology, that does not need the purified antigen (Meli et al. 2009) or it is crucial to isolate specific misfolded isoforms. In this case, atomic force microscopy (AFM) and electron microscopy are methods that allow to visualise any different isoforms even if they are small oligomers. The combination of phage display technology and screening by AFM has been used as a new approach to select morphology-specific intrabodies against different conformers, as reported for A $\beta$  fibrils and  $\alpha$ -synuclein oligomers (Emadi et al. 2007; Marcus et al. 2008; Zameer et al. 2008).

Many recombinant intrabodies against proteins involved in the pathogenesis of Alzheimer's, Prion, Huntington's and Parkinson's diseases have been generated and successfully expressed in cellular and animal models (Cardinale and Biocca 2008a).

For Alzheimer's disease (AD), the most common form of dementia characterized by extracellular deposits and intracellular accumulation of amyloid beta ( $A\beta$ ) peptide and hyperphosphorylated tau protein (Blennow et al. 2006), either peripheral and central nervous system (CNS) targeted antibodies have been largely used and proved to be effective to reduce  $A\beta$  plaque burden and memory impairment (Steinitz 2009). Paganetti et al. generated intrabodies directed to the EFRH peptide adjacent to the  $\beta$ -secretase cleavage site of human amyloid precursor protein (APP) (scFv- $\beta$ 1). Expression of scFv- $\beta$ 1 along the secretory pathway shields the  $\beta$ -secretase cleavage site and inhibits the formation of toxic  $A\beta$ . The KDEL version of the same intrabody is more effective, since it anchors APP in the ER preventing its appearance on the plasma membrane (Paganetti et al. 2005). In another study, the expression of anti-nicastrin intrabodies disrupts the proper folding and glycosylation of the endogenous nicastrin. This protein is required for the stability of the  $\gamma$ -secretase complex. As a result, the anti-nicastrin intrabody suppresses the  $\gamma$ -secretase enzymatic activity in vivo (Hayashi et al. 2009).

Prion diseases are fatal transmissible spongiform encephalopathies affecting humans and animals (Prusiner 1998; Aguzzi et al. 2008). So far, intrabody applications against prion disorders have been targeted to the endogenous prion protein (PrP<sup>C</sup>). Anti-prion KDEL-8H4 scFv fragments were generated and stably expressed in a neuronal cell line susceptible to scrapie infection. Its intracellular expression causes a marked impairment of prion maturation and translocation towards the membrane compartment, with a strong reduction of the PrP<sup>C</sup> membrane fraction. As a consequence, the pathogenic scrapie isoform (PrP<sup>Sc</sup>) does not form and accumulate in infected cells (Cardinale et al. 2005). Moreover, mice, intracerebrally injected with a lysate derived from KDEL-8H4 expressing cells infected with scrapie, neither develop scrapie clinical sign nor brain damage, demonstrating effective treatment (Vetruigno et al. 2005). The secretory version of the same intrabody (Sec-8H4), able to recognize PrP<sup>C</sup> in the secretory pathway, strongly inhibits PrP<sup>Sc</sup> accumulation in 139A scrapie strain infected cells. By analysing its mode of action, it was found that PrP<sup>C</sup> total level is markedly reduced due to a selective re-routing of PrP<sup>C</sup> to the proteasome pathway. Moreover, Sec-8H4 intrabody impairs the secretion of endogenous prion molecules associated to exosomes-like vesicles, a potential spreading route for prion infectivity (Filesi et al. 2007). A drastic reduction of PrP<sup>Sc</sup> accumulation was also obtained by co-culturing cells secreting anti-prion scFv fragments with chronically scrapie infected neuroblastoma cells (Donofrio et al. 2005).

Huntington's disease (HD) is a genetic disorder associated with a progressive neurodegeneration in the brain areas of cortex and striatum (Walker 2007). It is caused by the aggregation of mutated forms of huntingtin (htt) protein which present abnormally long polyglutamine (polyQ) sequences at the N-terminal. Cytoplasmic intrabodies directed against the poly-proline region, flanking the polyglutamine



region at the C-terminal side of htt, inhibit cell death and aggregation in transiently transfected HEK293 cells, while intrabodies to the polyQ region are cytotoxic and ineffective, because they accelerate aggregate formation and apoptotic cell death (Khoshnan et al. 2002). A phage-derived C4 scFv against the N-terminal portion of htt reduces aggregation and toxicity in cell cultures, in neuronal organotypic slice cultures and in a *Drosophila* model of HD (Murphy and Messer 2004; Wolfgang et al. 2005; McLear et al. 2008). Another potent intrabody against mutant htt, when expressed in the striatum of HD mice via adenoviral infection, reduces neuropil aggregate formation and ameliorates neurological symptoms (Wang et al. 2008). Interaction of this intrabody with mutant huntingtin increases the ubiquitination of cytoplasmic htt and its degradation, indicating that intrabody-mediated re-routing of htt to the proteasome pathway is the mechanism underlying the protective activity (Fig. 9.1). Furthermore, an intrabody against the N-terminal htt, V<sub>L</sub>12.3 and another, Happ1, which recognizes the proline rich domain of htt, both prevents htt aggregation and inhibits toxicity in an immortalized striatal cell model of HD by different mechanisms (Southwell et al. 2008). Tested both in brains of five mouse models of HD, V<sub>L</sub>12.3 increases severity of phenotype and mortality in two models, while Happ1 treatment ameliorates motor, cognitive and neuropathological symptoms (Southwell et al. 2009).

Parkinson's disease (PD) is the second most prevalent neurodegenerative illness clinically characterized by motor and cognitive dysfunction. The main neuropathological feature is the presence of intracytoplasmic, proteinaceous inclusions termed as Lewy bodies (LB) (Wood-Kaczmar et al. 2006). The protein  $\alpha$ -synuclein is a major structural component of LB. Cytoplasmic expression of an anti-monomeric  $\alpha$ -synuclein single-chain intrabody rescues the cell adhesion, stabilizes the monomeric isoform and inhibits the formation of high molecular weight insoluble species in a cell model (Zhou et al. 2004). More recently, a novel intrabody against the nonamyloid component of  $\alpha$ -synuclein, selected from a yeast surface display library, shows highly significant reduction of aggregation in stably transfected cellular models (Lynch et al. 2008). Anti-oligomeric scFv fragments block fibril formation in vitro (Emadi et al. 2007) and alleviate toxicity when intracellularly expressed in mammalian cells (Yuan and Sierks 2009).

Oculopharyngeal muscular dystrophy (OPMD) is an autosomal dominant dystrophy clinically characterized by progressive weakening of specific muscles. It is a protein aggregation disorder caused by short expansions of the N-terminal polyalanine tract in the nuclear poly(A)-binding protein 1 (PABPN1). Mutant PABPN1 aggregates in intranuclear inclusions in OPMD patient muscles. In order to inhibit the aggregation of the PABPN1 mutant, a panel of specific single domain antibody fragments (VH) was expressed in cellular and animal OPMD models. One of them was effective in reducing aggregate formation and clearing pre-existing aggregates in cells and was a strong suppressor of muscle degeneration in a characterized *Drosophila* OPMD model, restoring muscle gene expression (Verheesen et al. 2006; Chartier et al. 2009).

## 9.7 Intrabodies for Treatment of Infection Diseases

The development of intrabodies for the inhibition of virus structural, regulatory, enzymatic, envelope proteins and receptors on the surface of host cells has been also largely explored, demonstrating their efficacy either at the early and late events of the viral life cycle. In the beginning, they have been used to target proteins of the human immunodeficiency virus 1 (HIV-1) (Lo et al. 2008). Intrabodies equipped with a ER retention signal were employed against the viral coat proteins gp120 and gp41. They caused inhibition of virus replication and syncytial formation (Marasco et al. 1993; Zhou et al. 1998). Inhibition of early and late events of HIV-1 life cycle was also obtained by expression of cytoplasmic intrabodies directed against the matrix protein p17 (Levin et al. 1997) and against proteins involved in replication (reverse transcriptase and Vif). Camelized single-domain intrabodies efficiently interact with HIV-1 Vif protein and neutralize Vif-mediated proviral integration in non permissive cells (Aires da Silva et al. 2004). Moreover, intrabodies that block regulatory proteins, such as Tat and Rev have been used to inhibit HIV-1 replication. A cytosolic anti-Tat scFv fragment modified with a C-terminal human C kappa domain to increase cytoplasmic stability inhibits Tat-mediated long terminal repeat (LTR) transactivation and HIV-1 infection in transformed lymphocytes (Mhashilkar et al. 1995; Bai et al. 2003) and a cytosolic anti-Rev scFv fragment inhibits HIV-1 replication in HeLa-T4 cells (Duan et al. 1994). Recently, a llama derived VH domain, selected against the N-terminal  $\alpha$ -helical multimerization domain of Rev, was used to block Rev multimerization and prevent HIV replication (Vercruyssen et al. 2010).

Intrabodies have been described for treatment of various other viral infections, such as Hepatitis B (HBV) and Hepatitis C virus (HCV) infections, which represent a global health problem. ER-retained single-domain intrabodies (VHHs) targeting the envelope protein of HBV induce more than two log reduction in virion secretion in a HBV mouse model (Serruys et al. 2009) and, in a still preliminary study, cytosolic and nuclear VHH intrabodies targeting the core antigen (HBcAg) were produced and tested in infected cells (Serruys et al. 2010). Intrabodies against the NS3 serine protease, which is necessary for viral replication and innate immune evasion, inhibit HCV replication when expressed in hepatoma cells (Gal-Tanamy et al. 2010).

Intrabodies inhibit Papilloma virus protein function in cervical cancer cells (Griffin et al. 2006), decrease Kaposi sarcoma-associated herpes viral persistence in lymphoma cells (Corte-Real et al. 2005) and reduce transcription and replication of influenza A virus (Mukhtar et al. 2009).

Interestingly, novel disulfide-free proteins that target severe acute respiratory syndrome (SARS) N protein with high affinity and selectivity have been generated by using mRNA display selection and directed evolution. Although these molecules are structurally very different from antibodies as they utilise a discontinuous binding surface, they may represent interesting alternatives to intrabodies. At least for the molecules tested in this study, seven block SARS replication with different efficiency and do not disrupt mammalian cell function (Liao et al. 2009).

## 9.8 Intrabodies in Cancer

Many tumors are characterized by the presence of mutated or aberrantly expressed proteins involved in the control of vital processes. Most oncogenic proteins are located inside cells and are not available to be targeted by standard antibody-mediated anti-tumor therapies. Intrabodies have clear clinical potential in cancer therapy and have been successfully used as effectors of intracellular cancer targets and pathways associated with tumor cell proliferation, differentiation and invasion. In particular intrabodies have been extensively studied as inhibitors of growth factors receptors or other oncogenic antigens in the secretory compartment, in the cytoplasm and in the nuclei.

Aberrant expression of members of the epidermal growth factor receptor (EGFR) family, such as EGFR and erbB2, has been observed in a variety of human tumors, including breast and ovary carcinoma. Many reports describe the successful phenotypic knockout of the epidermal growth factor receptor (EGFR) and erbB2 achieved by retention of them by ER-retained scFv intrabodies (Graus-Porta et al. 1995; Jannot et al. 1996; Deshane et al. 1997). Down regulation of surface-exposed erbB2 receptors results in induction of apoptosis, cytotoxicity and inhibition of tumor cell proliferation *in vitro* and *in vivo*. However, a phase 1 clinical trial using an anti-erbB-2 scFv-encoding adenovirus, carried out to treat erbB-2-overexpressing ovarian cancer, highlighted the need for more efficient gene delivery systems (Alvarez et al. 2000). Reversion of transformed phenotype in ovarian cancer cells was also obtained by the intracellular expression of ER-retained anti- $\alpha$  folate receptor intrabodies (Figini et al. 2003).

To identify and functionally characterize tumor specific markers, a human scFv phage display library was used to select intrabodies able to bind and rapidly internalize into human breast cancers. One of these, the 3GA5, has been proved to knock down the surface display of the CD9 partner 1, a tumor specific receptor antigen, when expressed as an ER-retained intrabody inside cells (Goenaga et al. 2007).

RAS is a guanine nucleotide binding protein which plays a crucial role in the regulation of cell proliferation, oncogenic transformation and differentiation located at the inner surface of the plasma membrane. A panel of non neutralizing anti-RAS scFvs were proved to inhibit cell proliferation by sequestering the antigen and diverting it in cytoplasmic aggresomes (Lener et al. 2000). Intrabody-mediated RAS aggregation led to proteasome dysfunction and apoptosis (Cardinale et al. 2003). In another study, neutralization of RAS promoted apoptosis in human cancer cells and led to tumor regression of a colon carcinoma tumour model in nude mice (Cochet et al. 1998). More recently, a soluble single VH domain that specifically binds to activated GTP-bound RAS inhibited RAS-effector protein interactions with RAS, preventing tumorigenesis in a mouse model (Tanaka et al. 2007).

The tumor suppressor gene p53 is mutated in almost half of human tumors. Restoration of its transcriptional activity may trigger massive apoptosis of cancer cells. Intracellular expression of nuclear targeted anti-p53 scFv fragments in human tumor cells leads to restoration of the p53 mutant deficient transcriptional activity (Caron de Fromentel et al. 1999) and induces tumor regression in an animal model

of mice carrying human xenografts, with no apparent deleterious side effects (Orgad et al. 2010).

Functional apoptotic pathways are crucial for cell homeostasis and for the elimination of damaged or transformed cells. A dysregulation of apoptosis is implicated in many pathologies, including neurodegenerative diseases and cancer. Two camelid-derived single VH domains, selected against recombinant Caspase-3 and expressed as intrabodies in a neuroblastoma cell line, show different *in vivo* apoptotic-modulating effects. Notably, while one of them is an antagonist towards Caspase 3 and protects cells from oxidative-stress-induced apoptosis, the other is able to induce cell death (McGonigal et al. 2009).

## 9.9 Against Toxins

Other very recent applications are the use of intrabodies as anti-toxin agents. Toxins from microbial and other sources continue to cause substantial human and veterinary pathologies and represent serious biosecurity threats. Currently there is no antidote that can reverse symptoms of the botulinum neurotoxin. Camelid single domain intrabodies (VHHs) specifically selected against *Clostridium botulinum* neurotoxin (BoNT) protect neuronal cell synaptosomal-associated protein 25 (SNAP25) protein from cleavage, demonstrating its potential as a component of therapeutic agents against botulism intoxication (Tremblay et al. 2010). Some ADP-ribosylation toxins, such as *Salmonella* SpvB toxin, are secreted directly from the *Salmonella*-containing vacuole into the cytosol of target cells and, thus, are inaccessible to conventional antibodies. A single-domain antibody expressed as a cytosolic intrabody blocks the actin ADP-ribosylating toxin of *Salmonella typhimurium* (Alzogaray et al. 2010).

## 9.10 Concluding Remarks

Targeting antigens not accessible by circulating antibody molecules is the powerful of the intrabody technology. This approach has greatly improved by recent advances in scaffold design, repertoire construction and ad hoc selection methods to improve the stability and solubility of intrabodies inside cells. The camelid derived single domain antibodies, which are the smallest fragments intracellularly expressed, represent the new generation of intrabodies for their capacity to enter small cavities on antigens, their high solubility and good tissue penetration.

There are still major problems to be solved before intrabodies will be clinically approved therapeutic agents. These include, in particular, development of appropriate, efficient and safe delivery strategies, in terms of transduction systems and routes of delivery. Adeno-associated (AAV) viral vectors still represent the best choice of delivery in mammalian tissues although a novel generation of non viral delivery systems (nanoparticles, protein transduction domain peptides or modified liposomes)

characterized by low toxicity and immunogenicity are now emerging. The general interest in this issue is so diffuse that promises to bring new improvements in the next future.

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## References

- Aguzzi A, Baumann F, Bremer J (2008) The prion's elusive reason for being. *Annu Rev Neurosci* 31:439–77
- Aires da Silva F, Santa-Marta M, Freitas-Vieira A, Mascarenhas P, Barahona I, Moniz-Pereira J, Gabuzda D, Goncalves J (2004) Camelized rabbit-derived VH single-domain intrabodies against Vif strongly neutralize HIV-1 infectivity. *J Mol Biol* 340:525–42
- Alvarez RD, Barnes MN, Gomez-Navarro J, Wang M, Strong TV, Arafat W, Arani RB, Johnson MR, Roberts BL, Siegal GP, Curiel DT (2000) A cancer gene therapy approach utilizing an anti-erbB-2 single-chain antibody-encoding adenovirus (AD21): a phase I trial. *Clin Cancer Res* 6:3081–7
- Alzogaray V, Danquah W, Aguirre A, Urrutia M, Berguer P, García Véscovi E, Haag F, Koch-Nolte F, Goldbaum FA (2010) Single-domain llama antibodies as specific intracellular inhibitors of SpvB, the actin ADP-ribosylating toxin of *Salmonella typhimurium*. *FASEB J* 25:526–34
- Bai J, Sui J, Zhu RY, Tallarico AS, Gennari F, Zhang D, Marasco WA (2003) Inhibition of Tat-mediated transactivation and HIV-1 replication by human anti-hCyclinT1 intrabodies. *J Biol Chem* 278:1433–42
- Biocca S, Cattaneo A (1995) Intracellular immunization: antibody targeting to subcellular compartments. *Trends Cell Biol* 5:248–52
- Biocca S, Neuberger MS, Cattaneo A (1990) Expression and targeting of intracellular antibodies in mammalian cells. *EMBO J* 9:101–8
- Biocca S, Pierandrei-Amaldi P, Campioni N, Cattaneo A (1994) Intracellular immunization with cytosolic recombinant antibodies. *Biotechnology (NY)* 12:396–9
- Biocca S, Ruberti F, Tafani M, Pierandrei-Amaldi P, Cattaneo A (1995) Redox state of single chain Fv fragments targeted to the endoplasmic reticulum, cytosol and mitochondria. *Bio/Technology* 13:1110–15
- Blennow K, de Leon MJ, Zetterberg H (2006) Alzheimer's disease. *Lancet* 368:387–403
- Böldicke T (2007) Blocking translocation of cell surface molecules from the ER to the cell surface by intracellular antibodies targeted to the ER. *J Cell Mol Med* 11:54–70
- Cardinale A, Biocca S (2008a) The potential of intracellular antibodies for therapeutic targeting of protein-misfolding diseases. *Trends Mol Med* 14:373–80
- Cardinale A, Biocca S (2008b) Combating protein misfolding and aggregation by intracellular antibodies. *Curr Mol Med* 8(1):2–11
- Cardinale A, Biocca S (2010) Expressing intrabodies in mammalian cells. In *Antibody Engineering Vol 2*. Springer Protocols 161–172
- Cardinale A, Filesi I, Biocca S (2001) Aggresome formation by anti-Ras intracellular scFv fragments. The fate of the antigen-antibody complex. *Eur J Biochem* 268:268–77
- Cardinale A, Filesi I, Mattei S, Biocca S (2003) Evidence for proteasome dysfunction in cytotoxicity mediated by anti-Ras intracellular antibodies. *Eur J Biochem* 270:3389–97
- Cardinale A, Filesi I, Mattei S, Biocca S (2004) Intracellular targeting and functional analysis of single-chain Fv fragments in mammalian cells. *Methods* 34:171–8
- Cardinale A, Filesi I, Vetrugno V, Pocchiari M, Sy MS, Biocca S (2005) Trapping prion protein in the endoplasmic reticulum impairs PrP<sup>C</sup> maturation and prevents PrP<sup>Sc</sup> accumulation. *J Biol Chem* 280:685–94

- Caron de Fromentel C, Gruel N, Venot C, Debussche L, Conseiller E, Dureuil C, Teillaud JL, Tocque B, Bracco L (1999) Restoration of transcriptional activity of p53 mutants in human tumour cells by intracellular expression of anti-p53 single chain Fv fragments. *Oncogene* 18:551–7
- Cattaneo A, Biocca S (1997) *Intracellular Antibodies: Development and Applications*. Springer, Berlin, Heidelberg
- Cattaneo A, Biocca S (1999) The selection of intracellular antibodies. *Trends Biotech* 17:115–21
- Chartier A, Raz V, Sterrenburg E, Verrips CT, van der Maarel SM, Simonelig M (2009) Prevention of oculopharyngeal muscular dystrophy by muscular expression of Llama single-chain intrabodies in vivo. *Hum Mol Genet* 18:1849–59
- Chesnut JD, Baytan AR, Russell M, Chang MP, Bernard A, Maxwell IH, Hoeffler JP (1996) Selective isolation of transiently transfected cells from a mammalian cell population with vectors expressing a membrane anchored single-chain antibody. *J Immunol Methods* 193:17–27
- Chiti F, Dobson CM (2006) Protein misfolding, functional amyloid, and human disease. *Annu Rev Biochem* 75:333–66
- Cochet O, Kenigsberg M, Delumeau I, Virone-Oddos A, Multon MC, Fridman WH, Schweighoffer F, Teillaud JL, Tocqué B (1998) Intracellular expression of an antibody fragment-neutralizing p21 ras promotes tumor regression. *Cancer Res* 58:1170–6
- Cohen PA, Mani JC, Lane DP (1998) Characterization of a new intrabody directed against the N-terminal region of human p53. *Oncogene* 17:2445–56
- Contreras-Martínez LM, DeLisa MP (2007) Intracellular ribosome display via SecM translation arrest as a selection for antibodies with enhanced cytosolic stability. *J Mol Biol* 372:513–24
- Corte-Real S, Collins C, Aires da Silva F, Simas JP, Barbas CF 3rd, Chang Y, Moore P, Goncalves J (2005) Intrabodies targeting the Kaposi sarcoma-associated herpesvirus latency antigen inhibit viral persistence in lymphoma cells. *Blood* 106:3797–802
- Deshane J, Siegal GP, Wang M, Wright M, Bucy RP, Alvarez RD, Curiel DT (1997) Transductional efficacy and safety of an intraperitoneally delivered adenovirus encoding an anti-erbB-2 intracellular single-chain antibody for ovarian cancer gene therapy. *Gynecol Oncol* 64:378–85
- Donofrio G, Heppner FL, Polymenidou M, Musahl C, Aguzzi A (2005) Paracrine inhibition of prion propagation by anti-PrP single-chain Fv miniantibodies. *J Virol* 79:8330–8
- Duan L, Bagasra O, Laughlin MA, Oakes JW, Pomerantz RJ (1994) Potent inhibition of human immunodeficiency virus type 1 replication by an intracellular anti-Rev single-chain antibody. *Proc Natl Acad Sci USA* 91:5075–9
- Emadi S, Barkhordarian H, Wang MS, Schulz P, Sierks MR (2007) Isolation of a human single chain antibody fragment against oligomeric  $\alpha$ -synuclein that inhibits aggregation and prevents  $\alpha$ -synuclein-induced toxicity. *J Mol Biol* 368:1132–44
- Figini M, Ferri R, Mezzanzanica D, Bagnoli M, Luison E, Miotti S, Canevari S (2003) Reversion of transformed phenotype in ovarian cancer cells by intracellular expression of anti folate receptor antibodies. *Gene Ther* 10:1018–25
- Filesi I, Cardinale A, Mattei S, Biocca S (2007) Selective re-routing of prion protein to proteasomes and alteration of its vesicular secretion prevent PrP(Sc) formation. *J Neurochem* 101:1516–26
- Gal-Tanamy M, Zemel R, Bachmatov L, Jangra RK, Shapira A, Villanueva RA, Yi M, Lemon SM, Benhar I, Tur-Kaspa R (2010) Inhibition of protease-inhibitor-resistant hepatitis C virus replicons and infectious virus by intracellular intrabodies. *Antiviral Res* 88(1):95–106
- Goenaga AL, Zhou Y, Legay C, Bougherara H, Huang L, Liu B, Drummond DC, Kirpotin DB, Auclair C, Marks JD, Poul MA (2007) Identification and characterization of tumor antigens by using antibody phage display and intrabody strategies. *Mol Immunol* 44:3777–88
- Graus-Porta D, Beerli RR, Hynes NE (1995) Single-chain antibody-mediated intracellular retention of ErbB-2 impairs Neu differentiation factor and epidermal growth factor signaling. *Mol Cell Biol* 15:1182–91
- Griffin H, Elston R, Jackson D, Ansell K, Coleman M, Winter G, Doorbar J (2006) Inhibition of papillomavirus protein function in cervical cancer cells by intrabody targeting. *J Mol Biol* 355:360–78

- Hamers-Casterman C, Atarhouch T, Muyldermans S, Robinson G, Hamers C, Songa EB, Bendahman N, Hamers R (1993) Naturally occurring antibodies devoid of light chains. *Nature* 363(6428):446–8
- Hayashi I, Takatori S, Urano Y, Iwanari H, Isoo N, Osawa S, Fukuda MA, Kodama T, Hamakubo T, Li T, Wong PC, Tomita T, Iwatsubo T (2009) Single chain variable fragment against nicastrin inhibits the gamma-secretase activity. *J Biol Chem* 284:27838–47
- Hoogenboom HR (2005) Selecting and screening recombinant antibody libraries. *Nat Biotechnol* 23:1105–16
- Hudson PJ, Souriau C (2003) Engineered antibodies. *Nat Med* 9:129–34
- Jannot CB, Beerli RR, Mason S, Gullick WJ, Hynes NE (1996) Intracellular expression of a single-chain antibody directed to the EGFR leads to growth inhibition of tumor cells. *Oncogene* 13:275–82
- Jendreyko N, Popkov M, Rader C, Barbas CF 3rd (2005) Phenotypic knockout of VEGF-R2 and Tie-2 with an intradiabody reduces tumor growth and angiogenesis in vivo. *Proc Natl Acad Sci USA* 102:8293–8
- Jung S, Plückthun A (1997) Improving in vivo folding and stability of a single-chain Fv antibody fragment by loop grafting. *Protein Eng* 10:959–66
- Kayed R, Head E, Thompson JL, McIntire TM, Milton SC, Cotman CW, Glabe CG (2003) Common structure of soluble amyloid oligomers implies common mechanism of pathogenesis. *Science* 300:486–9
- Khosnhan A, Ko J, Patterson PH (2002) Effects of intracellular expression of antihuntingtin antibodies of various specificities on mutant huntingtin aggregation and toxicity. *Proc Natl Acad Sci USA* 99:1002–7
- Kirschning CJ, Dreher S, Maass B, Fichte S, Schade J, Köster M, Noack A, Lindenmaier W, Wagner H, Böldicke T (2010) Generation of anti-TLR2 intrabody mediating inhibition of macrophage surface TLR2 expression and TLR2-driven cell activation. *BMC Biotechnol* 10:31
- Kvam E, Sierks MR, Shoemaker CB, Messer A (2010) Physico-chemical determinants of soluble intrabody expression in mammalian cell cytoplasm. *Protein Eng Des Sel* 23:489–98
- Lener M, Horn IR, Cardinale A, Messina S, Nielsen UB, Rybak SM, Hoogenboom HR, Cattaneo A, Biocca S (2000) Diverting a protein from its cellular location by intracellular antibodies. The case of p21Ras. *Eur J Biochem* 267:1196–205
- Levin R, Mhashilkar AM, Dorfman T, Bukovsky A, Zani C, Bagley J, Hinkula J, Niedrig M, Albert J, Wahren B, Göttlinger HG, Marasco WA (1997) Inhibition of early and late events of the HIV-1 replication cycle by cytoplasmic Fab intrabodies against the matrix protein, p17. *Mol Med* 3:96–110
- Liao HI, Olson CA, Hwang S, Deng H, Wong E, Baric RS, Roberts RW, Sun R (2009) mRNA display design of fibronectin-based intrabodies that detect and inhibit severe acute respiratory syndrome coronavirus nucleocapsid protein. *J Biol Chem* 284:17512–20
- Lo AS, Zhu Q, Marasco WA (2008) Intracellular antibodies (intrabodies) and their therapeutic potential. *Handb Exp Pharmacol* 181:343–73
- Lynch SM, Zhou C, Messer A (2008) An scFv intrabody against the nonamyloid component of alpha-synuclein reduces intracellular aggregation and toxicity. *J Mol Biol* 377:136–47
- Marasco WA, Haseltine WA, Chen SY (1993) Design, intracellular expression, and activity of a human anti-human immunodeficiency virus type 1 gp120 single-chain antibody. *Proc Natl Acad Sci USA* 90:7889–93
- Marcus WD, Wang H, Lindsay SM, Sierks MR (2008) Characterization of an antibody scFv that recognizes fibrillar insulin and  $\beta$ -amyloid using atomic force microscopy. *Nanomedicine* 4:1–7
- McGonigal K, Tanha J, Palazov E, Li S, Gueorguieva-Owens D, Pandey S (2009) Isolation and functional characterization of single domain antibody modulators of Caspase-3 and apoptosis. *Appl Biochem Biotechnol* 157:226–36
- McLear JA, Lebrecht D, Messer A, Wolfgang WJ (2008) Combinational approach of intrabody with enhanced Hsp70 expression addresses multiple pathologies in a fly model of Huntington's disease. *FASEB J* 22:2003–11

- Meli G, Visintin M, Cannistraci I, Cattaneo A (2009) Direct in vivo intracellular selection of conformation-sensitive antibody domains targeting Alzheimer's amyloid-beta oligomers. *J Mol Biol* 387:584–606
- Mhashilkar AM, Bagley J, Chen SY, Szilvay AM, Helland DG, Marasco WA (1995) Inhibition of HIV-1 Tat-mediated LTR transactivation and HIV-1 infection by anti-Tat single chain intrabodies. *EMBO J* 14:1542–51
- Monsellier E, Bedouelle H (2006) Improving the stability of an antibody variable fragment by a combination of knowledge-based approaches: validation and mechanisms. *J Mol Biol* 362: 580–93
- Mukhtar MM, Li S, Li W, Wan T, Mu Y, Wei W, Kang L, Rasool ST, Xiao Y, Zhu Y, Wu J (2009) Single-chain intracellular antibodies inhibit influenza virus replication by disrupting interaction of proteins involved in viral replication and transcription. *Int J Biochem Cell Biol* 41:554–60
- Murphy RC, Messer A (2004) A single-chain Fv intrabody provides functional protection against the effects of mutant protein in an organotypic slice culture model of Huntington's disease. *Brain Res Mol Brain Res* 121:141–5
- Orgad S, Dimant H, Dor-On E, Azriel-Rosenfeld R, Benhar I, Solomon B (2010) TAR1, a human anti-p53 single-chain antibody, restores tumor suppressor function to mutant p53 variants. *J Immunother* 33:146–54
- Paganetti P, Calanca V, Galli C, Stefani M, Molinari M (2005)  $\beta$ -site specific intrabodies to decrease and prevent generation of Alzheimer's A $\beta$  peptide. *J Cell Biol* 168:863–8
- Persic L, Righi M, Roberts A, Hoogenboom HR, Cattaneo A, Bradbury A (1997a) Targeting vectors for intracellular immunization. *Gene* 187:1–8
- Persic L, Righi M, Roberts A, Hoogenboom HR, Cattaneo A, Bradbury A (1997b) An integrated vector system for the eukaryotic expression of antibodies or their fragments after selection from phage display libraries. *Gene* 187:9–18
- Philibert P, Stoessel A, Wang W, Sibling AP, Bec N, Larroque C, Saven JG, Court ete J, Weiss E, Martineau P (2007) A focused antibody library for selecting scFvs expressed at high levels in the cytoplasm. *BMC Biotechnol* 7:81
- Prusiner SB (1998) Prions. *Proc Natl Acad Sci USA* 95:13363–83
- Serruys B, Van Houtte F, Farhoudi-Moghadam A, Leroux-Roels G, Vanlandschoot P (2010) Production, characterization and in vitro testing of HBcAg-specific VHH intrabodies. *J Gen Virol* 91:643–52
- Serruys B, Van Houtte F, Verbrugge P, Leroux-Roels G, Vanlandschoot P (2009) Llama-derived single-domain intrabodies inhibit secretion of hepatitis B virions in mice. *Hepatology* 49:39–49
- Southwell AL, Khoshnan A, Dunn DE, Bugg CW, Lo DC, Patterson PH (2008) Intrabodies binding the proline-rich domains of mutant huntingtin increase its turnover and reduce neurotoxicity. *J Neurosci* 28:9013–20
- Southwell AL, Ko J, Patterson PH (2009) Intrabody gene therapy ameliorates motor, cognitive, and neuropathological symptoms in multiple mouse models of Huntington's disease. *J Neurosci* 29:13589–602
- Steinitz M (2009) Immunotherapy for Alzheimer's disease. *Immunotherapy* 1:461–9
- Tanaka T, Rabbitts TH (2003) Intrabodies based on intracellular capture frameworks that bind the RAS protein with high affinity and impair oncogenic transformation. *EMBO J* 22:1025–35
- Tanaka T, Rabbitts TH (2010) Protocol for the selection of single-domain antibody fragments by third generation intracellular antibody capture. *Nat Protoc* 5:67–92
- Tanaka T, Williams RL, Rabbitts TH (2007) Tumour prevention by a single antibody domain targeting the interaction of signal transduction proteins with RAS. *EMBO J* 26:3250–9
- Tremblay JM, Kuo CL, Abeijon C, Sepulveda J, Oyler G, Hu X, Jin MM, Shoemaker CB (2010) Camelid single domain antibodies (VHHs) as neuronal cell intrabody binding agents and inhibitors of Clostridium botulinum neurotoxin (BoNT) proteases. *Toxicon* 56:990–8
- Van den Abbeele A, De Clercq S, De Ganck A, De Corte V, Van Loo B, Soror SH, Srinivasan V, Steyaert J, Vandekerckhove J, Gettemans J (2010) A llama-derived gelsolin single-domain antibody blocks gelsolin-G-actin interaction. *Cell Mol Life Sci* 67:1519–35



- Vercruyse T, Pardon E, Vanstreels E, Steyaert J, Daelemans D (2010) An intrabody based on a llama single-domain antibody targeting the N-terminal alpha-helical multimerization domain of HIV-1 rev prevents viral production. *J Biol Chem* 285:21768–80
- Verheesen P, de Kluijver A, van Koningsbruggen S, de Brij M, de Haard HJ, van Ommen GJ, van der Maarel SM, Verrips CT (2006) Prevention of oculopharyngeal muscular dystrophy-associated aggregation of nuclear polyA-binding protein with a single-domain intracellular antibody. *Hum Mol Genet* 15:105–11
- Vetruigno V, Cardinale A, Filesi I, Mattei S, Sy MS, Pocchiari M, Biocca S (2005) KDEL-tagged anti-prion intrabodies impair PrP lysosomal degradation and inhibit scrapie infectivity. *Biochem Biophys Res Commun* 338:1791–7
- Visintin M et al. (1999) Selection of antibodies for intracellular function using a two-hybrid in vivo system. *Proc Natl Acad Sci USA* 96:11723–8
- Walker FO (2007) Huntington's disease. *Lancet* 369:218–28
- Wang CE, Zhou H, McGuire JR, Cerullo V, Lee B, Li SH, Li XJ (2008) Suppression of neuropil aggregates and neurological symptoms by an intracellular antibody implicates the cytoplasmic toxicity of mutant huntingtin. *J Cell Biol* 181:803–16
- Wesolowski J, Alzogaray V, Reyelt J, Unger M, Juarez K, Urrutia M, Cauherhff A, Danquah W, Rissiek B, Scheuplein F, Schwarz N, Adriouch S, Boyer O, Seman M, Licea A, Serreze DV, Goldbaum FA, Haag F, Koch-Nolte F (2009) Single domain antibodies: promising experimental and therapeutic tools in infection and immunity. *Med Microbiol Immunol* 198:157–74
- Williams BR, Zhu Z (2006) Intrabody-based approaches to cancer therapy: status and prospects. *Curr Med Chem* 13:1473–80
- Wolfgang WJ, Miller TW, Webster JM, Huston JS, Thompson LM, Marsh JL, Messer A (2005) Suppression of Huntington's disease pathology in *Drosophila* by human single-chain Fv antibodies. *Proc Natl Acad Sci USA* 102:11563–8
- Wood-Kaczmar A, Gandhi S, Wood NW (2006) Understanding the molecular causes of Parkinson's disease. *Trends Mol Med* 12:521–8
- Wörn A, Auf der Maur A, Escher D, Honegger A, Barberis A, Plückthun A (2000) Correlation between in vitro stability and in vivo performance of anti-GCN4 intrabodies as cytoplasmic inhibitors. *J Biol Chem* 275:2795–803
- Yuan B, Sierks MR (2009) Intracellular targeting and clearance of oligomeric alpha-synuclein alleviates toxicity in mammalian cells. *Neurosci Lett* 459(1):16–18
- Zameer A, Kasturirangan S, Emadi S, Nimmagadda SV, Sierks MR (2008) Anti-oligomeric Abeta single-chain variable domain antibody blocks Abeta-induced toxicity against human neuroblastoma cells. *J Mol Biol* 384:917–28
- Zdoroveac A, Doebis C, Laube H, Brösel S, Schmitt-Knosalla I, Volk HD, Seifert M (2008) Modulation of graft arteriosclerosis in a rat carotid transplantation model. *J Surg Res* 145:161–9
- Zhou C, Emadi S, Sierks MR, Messer A (2004) A human single-chain Fv intrabody blocks aberrant cellular effects of overexpressed alpha-synuclein. *Mol Ther* 10:1023–31
- Zhou P, Goldstein S, Devadas K, Tewari D, Notkins AL (1998) Cells transfected with a non-neutralizing antibody gene are resistant to HIV infection: targeting the endoplasmic reticulum and trans-Golgi network. *J Immunol* 160:1489–96