Immunocytochemical staining of endogenous nuclear proteins with the HIS-1 anti-poly-histidine monoclonal antibody: a potential source of error in His-tagged protein detection

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Abstract

Histidine-tagged proteins are widely used in biochemical studies and frequently detected with antibodies specific for the histidine tag. Immunocytochemistry is widely used in studies with overexpressed proteins to determine cellular localization and in the case of histidine-tagged proteins can be carried out with anti-polyhistidine antibodies. Recent studies have suggested that polyhistidine sequences are present within a small number of human proteins and may direct expression to the nucleus and nuclear speckles compartments of the cell. In this study immunocytochemical staining of human SH-SY5Y neuroblastoma cell lines with the HIS-1 anti-polyhistidine monoclonal antibody were determined. Results showed that the HIS-1 anti-polyhistidine monoclonal antibody stained endogenous nuclear proteins in SH-SY5Y cells. The stained proteins were contained within the nuclear membrane, but were not directly linked to DNA. In a histidine-tagged catalase overexpressing cell line the HIS-1 anti-polyhistidine monoclonal antibody showed nuclear staining, whilst staining with the CAT-505 anti-catalase monoclonal antibody showed primarily cytoplasmic staining. These results suggest that anti-polyhistidine antibody staining shows significant cross-reactivity with endogenous nuclear proteins in SH-SY5Y neuroblastoma cells and may not be suitable for localization studies of histidine-tagged proteins. Immunocytochemical studies with anti-polyhistidine antibodies and localization of histidine-tagged proteins must be confirmed with protein specific antibodies or other methodology.

Keywords: Immunocytochemistry, SH-SY5Y neuroblastoma, Anti-polyhistidine, transfection, His-tagged proteins, Catalase overexpression

Introduction

The human SH-SY5Y neuroblastoma cell line is frequently used for neuroscience research including its use as a model for Parkinson's or Alzheimer's disease (Chang et al., 1993; Zadina et al., 1993; Ida et al., 1996; Bennett et al., 1999; Moussa et al., 2004; Milton et al., 2012; Ashabi et al., 2013; Chilumuri et al., 2013). There are many reports using the cell line and as such a considerable body of knowledge has accumulated to aid interpretation of novel results (Chang et al., 1993; Zadina et al., 1993; Ida et al., 1996; Beckman and Iverfeldt, 1997; Bennett et al., 1999; Delobel et al., 2003; Marx et al., 2003; Celsi et al., 2004; Moussa et al., 2004; Milton et al., 2012; Skommer and Brittain, 2012; Ashabi et al., 2013; Chilumuri et al., 2013). The cell line is easy to maintain, can be chemically induced to differentiate into a neuronal phenotype and can readily be transfected with vectors to modify protein expression (Beckman and Iverfeldt, 1997; Ashabi et al., 2013). Transfecting cells with vectors containing the protein of interest alongside an antibiotic selection gene have created many protein overexpressing cell lines. The SH-SY5Y parent cell line is susceptible to the antibiotics used in such systems allowing the selection of stable cell lines that overexpress the protein of choice (Delobel et al., 2003; Marx et al., 2003; Celsi et al., 2004; Milton et al., 2012; Skommer and Brittain, 2012; Chilumuri et al., 2013). This has allowed studies to be conducted to understand the biological actions of a given protein in a neuronal setting and also to determine the effects of proteins on neuronal function (Delobel et al., 2003; Marx et al., 2003; Celsi et al., 2004; Skommer and Brittain, 2012). This methodology has allowed researchers to assess the potential role of proteins in Parkinson's or Alzheimer's disease and to identify neuroprotective proteins that may be targets for further development as therapies for these diseases (Delobel et al., 2003; Marx et al., 2003; Celsi et al., 2004; Milton et al., 2012; Skommer and Brittain, 2012; Chilumuri et al., 2013).

The use of histidine (His) repeats as protein tags is a popular methodology in the production of recombinant proteins, recombinant protein purification, protein interaction studies and also to identify overexpressed proteins in Western blotting plus immunocytochemical studies (Motin et al., 1996; Franco et al., 1997; Fu et al., 2000; Kawabe et al., 2000; Racine et al., 2004; Mirzaei et al., 2006; Ulmer et al., 2006; Zou and Sun, 2006; Davison et al., 2011; Ma et al., 2013). The choice of the His-tag has been influenced by its ability to bind metals such as nickel that can be used in affinity chromatography methods to purify recombinant protein (Katayama et al., 1999; Ostrow et al., 2003; Racine et al., 2004; Yang et al., 2006; Deshpande et al., 2012). Antibodies against the His-tag are widely available and are frequently used in Western blotting and immunocytochemical studies (Marshall et al., 2002; Howell et al., 2005; Hussack et al., 2011; Land, 2012). The use of 6x-His-tags is frequent and allows both metal affinity chromatography plus detection with anti-poly-His antibodies (Marshall et al., 2002; Ostrow et al., 2003; Mirzaei et al., 2006). Many commercially available protein expression vectors are available that insert a 6x-His-tag at either the C or N terminus of the protein whose gene is cloned into them. This allows the use of anti-poly-His antibodies in the detection of expressed proteins, which is particularly beneficial where antibodies to the expressed protein are unavailable or where the protein of interest is endogenously expressed and there is a need to differentiate endogenous for overexpressed protein (Marshall et al., 2002; Mirzaei et al., 2006).

Endogenous proteins with poly-His sequences have been identified and their cellular localization determined (Oma et al., 2004). Genome analysis has revealed poly-His containing proteins in both eukaryotes and prokaryotes (Faux et al., 2005). Recent studies have suggested that the poly-His sequence may direct the protein expression to the nucleus and the nuclear speckles compartment (Paraguison et al., 2005; Salichs et al., 2009). This raises the possibility that anti-poly-His antibodies used in the detection of His-tagged proteins may also cross-react

with endogenous proteins. The relatively high expression levels of these endogenous poly-His containing proteins in the brain also suggests that they may be present in neuronal cells such as the SH-SY5Y line (Salichs et al., 2009). Cross-reactivity with endogenous poly-His proteins is particularly relevant to immunocytochemical studies where the detection is based purely on antibody binding to a cellular protein (Fritschy, 2008; Daneshtalab et al., 2010; Burry, 2011; Skoog and Tani, 2011). The present study was conducted to determine if the commercially available HIS-1 anti-poly-His monoclonal antibody would show cross reactivity with endogenous poly-His containing proteins in SH-SY5Y neuroblastoma cells (Kawabe et al., 2000; Marshall et al., 2002; Di Bonito et al., 2006). For comparison two SH-SY5Y cell lines stably expressing the pcDNA4/TO/myc–His expression vector (PVect) and the C-terminally His-tagged catalase (PCat) were compared using immunocytochemical staining with both the HIS-1 monoclonal and the CAT-505 monoclonal anti-catalase antibody (Chilumuri et al., 2013).

Materials and methods

Materials

Goat anti-mouse IgG Alexa-fluor 568 was purchased from Chemicon, UK. The pcDNA4/TO/myc–His expression vector and TO-PRO®-3 Iodide (642⁄661) were obtained from Invitrogen, UK. The HIS-1 mouse anti-poly-His monoclonal antibody, CAT-505 mouse anti-catalase monoclonal antibody and all other chemicals were purchased from Sigma-Aldrich, UK.

Cell cultures

Human SH-SY5Y neuroblastoma cells were routinely grown in a 5% CO_2 humidified incubator at 37°C in a 1:1 mixture of Dulbecco's modified Eagle's medium and HAM's F12

with Glutamax (Invitrogen) supplemented with 10% fetal calf serum (FCS), 1% non-essential amino acids, penicillin (100 units/ml) and streptomycin (100 mg/ml) (Milton et al. 2012; Chilumuri et al., 2013).

Transfection with pcDNA4/TO/myc–His expression vector

SH-SY5Y cells were transfected with pcDNA4/TO/myc–His expression vector (PVect) using lipofectamine (Invitrogen), and stably expressing clones were selected by culturing in 100 µg/ml Zeocin (Invitrogen) (Milton et al., 2012; Chilumuri et al., 2013).

Transfection with pcDNA4/TO/myc–His expression vector containing human catalase

The human catalase cDNA clone (NM_001752.3) was obtained from Origene and PCR cloned into the pcDNA4/TO/myc–His expression vector using forward (5'-AAGCTTATGGCTGACAGCCGGGAT-3') and reverse (5'-GCGGCCGCCAGATTTGCCTTCTCCCTTGC-3') oligonucleotides to create the PCat expression vector. SH-SY5Y cells were transfected with PCat using lipofectamine (Invitrogen), and stably expressing clones were selected by culturing in 100 µg/ml Zeocin (Invitrogen). RT PCR was used to confirm catalase overexpression (Chilumuri et al., 2013).

Immunocytochemistry of catalase expression

Naïve (untransfected), PVect and PCat SH-SY5Y neuroblastoma cells were washed with PBS, fixed with 4% paraformaldehyde for 15 min, and permeabilized in ice cold methanol for 30 min. Cells were incubated in block solution (10% bovine serum albumin in PBS) for 15 min, followed by a 1 h incubation with either primary CAT-505 mouse anti-catalase antibody (1:1,000; 1 µg/ml final concentration) or primary HIS-1 monoclonal anti-poly-His antibody (1:1,000; 1 µg/ml final concentration) in block solution. Primary antibody was removed followed by 3×5 min washes in PBS, prior to incubation with goat anti-mouse IgG-Alexa-

fluor 568 secondary (Abcam PLC, Cambridge; 1:500) in block solution for 45 min. Secondary antibody was removed and cells were washed 3 times in PBS. Cells were incubated with 100 µg/ml RNase A for 20 min at 37ºC, followed by 3x5 min washes and incubation with 1 µM TO-PRO®-3 Iodide (642⁄661; Invitrogen) for 20 min. Cells were washed 3 times in PBS and fluorescence was visualized by sequential scanning using a Leica TCS SP2 confocal system (Leica Microsystems, Milton Keynes, UK) (Milton et al. 2012; Chilumuri et al. 2013).

Cellular catalase and His-tagged catalase determination by ELISA

NUNC Immunoplates were coated with anti-catalase antiserum (Calbiochem) and blocked with 5% marvel. Purified catalase from human erythrocytes or recombinant His-tagged catalase was used as a standard. Cell extracts or catalase standards were applied in PBS containing 0.1% BSA plus 0.05% Tween 20. Either anti-catalase monoclonal antibody CAT-505, for catalase determination, or HIS-1 anti-poly-His, for His-tagged catalase determination, was added and incubated for 2 hrs. After washing to remove unbound material ir-catalase or ir-His-tagged catalase was detected using an anti-mouse IgG-HRP conjugate and TMB substrate.

Western-blot analysis of catalase

PVect and PCat SH-SY5Y neuroblastoma cells were lysed on ice in 20 mM HEPES buffer containing 1% Nonindet P-40, 1 mM EDTA (EDTA), 150 mM sodium chloride (NaCl), 0.25% sodium deoxycholate, plus protease inhibitors. Cell lysates were incubated for 1 h in lysis buffer and centrifuged at 12,000 g for 10 min at 4ºC. Total protein was measured by using the BCA assay. Supernatants were diluted to 1 mg/ml total protein and resuspended in sample buffer before boiling for 5 min and separation of samples using a 15% SDS-PAGE gel. Proteins were then transferred to a nitrocellulose membrane and membranes blocked with

3% nonfat dried milk powder in PBS containing 0.1% Tween 20 (1 h at room temperature). Membranes were incubated overnight at 4ºC with either CAT-505 mouse anti-catalase antibody, HIS-1 monoclonal anti-poly-His antibody or rabbit anti-actin antibody. Unbound antibody was rinsed from the membranes before incubation with horseradish peroxidase-conjugated goat anti-mouse secondary antibody or horseradish peroxidase-conjugated goat anti-rabbit secondary antibody. Immunoreactivity was detected using an enhanced chemiluminescence substrate and UVP Bio Imaging system (Chilumuri et al., 2013). Bound antibody was stripped from membranes using 200 mM glycine, 3.4 mM SDS, 0.001% Tween-20 buffer pH 2.2 to allow reprobing with a different primary antibody.

Results

Immunocytochemistry of SH-SY5Y neuroblasoma cells with anti-polyhistidine

Immunocytochemistry of SH-SY5Y neuroblastoma cells was carried out using double labeling with HIS-1 monoclonal anti-poly-His antibody, to stain immunoreactive (ir) poly-His proteins, plus TO-PRO®-3 Iodide to stain DNA and localize the nucleus. The immunocytochemistry of SH-SY5Y neuroblasoma cells using the HIS-1 anti-poly-His monoclonal antibody showed significant labeling in all cells (Fig. 1a). Staining with TO-PRO®-3 Iodide showed labeling in all cells (Fig. 1b). The anti-poly-His labeling co-localized with the TO-PRO®-3 Iodide staining (Fig. 1c), suggesting that the ir-poly-His positive proteins were primarily within the nucleus. Staining of a dividing cell, at the anaphase stage of mitosis, at higher magnification showed significant labeling with anti-poly-His throughout the cell (Fig. 1d). The TO-PRO®-3 Iodide showed labeling of the DNA in two sets of condensed chromosomes at the poles of the cell (Fig. 1e). The anti-poly-His labeling and TO-PRO®-3 Iodide staining did not co-localize (Fig. 1f), suggesting that the poly-His proteins are not directly associated with the DNA components of the nucleus but are localized within the

nuclear membrane, which had disintegrated during cell division and is yet to reform at the anaphase stage observed.

Anti-polyhistidine and anti-catalase immunocytochemistry of SH-SY5Y neuroblasoma cells transfected with pcDNA4/TO/myc–His expression vector

To determine whether transfection with a His-tag expression vector plus the selection of stable cell lines altered HIS-1 monoclonal antibody staining of endogenous poly-His containing proteins a cell line generated via this process with a blank vector not containing a protein sequence for overexpression was used. The SH-SY5Y neuroblastoma cells were transfected with the pcDNA4/TO/myc–His expression vector and cells stably expressing the vector (PVect) selected using zeocin (Milton et al., 2012; Chilumuri and Milton, 2013; Chilumuri et al., 2013). Immunocytochemistry of PVect SH-SY5Y neuroblastoma cells was carried out using double labeling with either HIS-1 monoclonal anti-poly-His antibody, to stain poly-His positive proteins, or CAT-505 monoclonal anti-catalase antibody, as a control, plus TO-PRO®-3 Iodide to stain DNA and localize the nucleus. The immunocytochemistry of the PVect SH-SY5Y neuroblasoma cells using the HIS-1 anti-poly-His monoclonal antibody showed significant labeling in all cells (Fig. 2a). Staining with TO-PRO®-3 Iodide showed labeling in all cells (Fig. 2b). The anti-poly-His labeling co-localized with the TO-PRO®-3 Iodide staining (Fig. 2c), suggesting that the poly-His positive proteins were again primarily within the nucleus. The immunocytochemistry of the PVect SH-SY5Y neuroblasoma cells using the CAT-505 anti-catalase monoclonal antibody showed very little labeling of cells (Fig. 2d). Staining with TO-PRO®-3 Iodide showed labeling in all cells (Fig. 2e). The anti-catalase labeling was not co-localized with the TO-PRO®-3 Iodide staining (Fig. 2f). The catalase staining in the PVect cells is minimal and is due to staining of endogenous enzyme within these cells in agreement with a previous study (Chilumuri et al., 2013).

Anti-polyhistidine and anti-catalase immunocytochemistry of SH-SY5Y neuroblasoma cells transfected with the human catalase gene

To confirm if the cross-reactivity of the HIS-1 monoclonal antibody with endogenous nuclear proteins would contribute to the observations with a cell line overexpressing a His-tagged protein the immunocytochemical study was carried out using the PCat cell line which overexpresses human catalase (Chilumuri et al., 2013). The SH-SY5Y neuroblastoma cells were transfected with the pcDNA4/TO/myc–His expression vector in which the human catalase gene had been inserted in-frame with the C-terminal myc and His tags. Cells stably overexpressing the human catalase (PCat) were selected using zeocin. Immunocytochemistry of catalase overexpressing PCat SH-SY5Y neuroblastoma cells was carried out using double labeling with either HIS-1 monoclonal anti-poly-His antibody, to stain poly-His positive proteins, or CAT-505 monoclonal anti-catalase antibody, to detect overexpressed His-tagged catalase, plus TO-PRO®-3 Iodide to stain DNA and localize the nucleus. The immunocytochemistry of the catalase overexpressing PCat SH-SY5Y neuroblastoma cells using the HIS-1 anti-poly-His monoclonal antibody showed significant labeling in all cells (Fig. 3a). Staining with TO-PRO®-3 Iodide showed labeling in all cells (Fig. 3b). The anti-poly-His labeling co-localized with the TO-PRO®-3 Iodide staining (Fig. 3c), suggesting that the poly-His positive proteins, including His-tagged catalase, were primarily within the nucleus. Significant staining of catalase overexpressing PCat SH-SY5Y neuroblasoma cells using the CAT-505 anti-catalase monoclonal antibody labeling was observed (Fig. 3d). Staining with TO-PRO®-3 Iodide also showed labeling (Fig. 3e). The anti-catalase labeling did not co-localize with the TO-PRO®-3 Iodide staining (Fig. 3f). The catalase staining in the catalase overexpressing PCat cells was substantial and primarily within the cytoplasm in agreement with a previous study (Chilumuri et al., 2013).

ELISA and Western blotting analysis of cellular catalase using anti-polyhistidine and anti-catalase antibodies

Extracts of catalase overexpressing cells were analyzed by ELISA and Western blotting to determine if the HIS-1 monoclonal antibody detected the His-tagged catalase from the expression vector. Using a two-site ELISA with a polyclonal anti-catalase antibody plus the CAT-505 anti-catalase monoclonal antibody the cellular level of catalase was found to be 0.26 ± 0.04 µg/mg protein (n=6) in PVect cell extracts and 2.01 ± 0.04 µg/mg protein (n=6) in PCat cell extracts. The two-site ELISA using polyclonal anti-catalase antibody plus the HIS-1 anti-poly-His monoclonal antibody failed to detect catalase in both PVect and PCat cell extracts, suggesting that the HIS-1 antibody does not recognise the His-tagged catalase. Western blotting of PVect and PCat cell extracts confirmed that the CAT-505 anti-catalase monoclonal antibody detected a band with a molecular weight of 60 kDa (Fig. 4a), in agreement with a previous study (Chilumuri et al., 2013). The HIS-1 anti-poly-His monoclonal antibody failed to detect any protein bands in the extracts (Fig. 4b).

Discussion

These results suggest that the HIS-1 monoclonal antibody cross-reacts with endogenous poly-His containing proteins in the nucleus of SH-SY5Y neuroblastoma cells (Paraguison et al., 2005; Salichs et al., 2009). The study by Salichs et al. (2009) identified twenty-two proteins with poly-His sequences that were found in the nucleus, which include neuronal proteins and transcription factors that could be found in the SH-SY5Y neuroblastoma cells. It is unknown which protein or proteins are being detected by the HIS-1 antibody within the SH-SY5Y nucleus. To specifically identify the proteins detected would require either mass spectrometry or amino acid sequencing of the material. The results also suggest that use of this antibody in immunocytochemical studies to detect His-tagged proteins requires appropriate control

experiments to check for endogenous protein staining by the HIS-1 monoclonal and that the results of such studies should be compared to staining with other antibodies specific for the protein itself rather than the His-tag (Kawabe et al., 2000; Marshall et al., 2002; Di Bonito et al., 2006).

Protein expression vectors that contain His-tags combined with an antibiotic selection sequence are routinely used to generate stable cell lines overexpressing the protein of choice with a His-tag (Marshall et al., 2002; Howell et al., 2005; Hussack et al., 2011; Land, 2012). The stable cell line selection process involves 4-6 weeks exposure to toxic antibiotics to eliminate cells not containing the expression vector. As such there may also be changes in the endogenous protein expression in cell lines selected in this manner. These results suggest that the anti-poly-His labeling is specific for the HIS-1 monoclonal antibody and not an artifact due to the secondary antibody used binding to endogenous nuclear proteins. The results confirm that the HIS-1 staining of nuclear proteins is not altered by the stable cell line selection procedure used to create the PVect cell line (Milton et al., 2012; Chilumuri and Milton, 2013; Chilumuri et al., 2013). The results also suggest that HIS-1 staining of endogenous nuclear proteins in SH-SY5Y cells transfected with vectors containing proteins with a His-tag may contribute to results obtained (Marshall et al., 2002; Howell et al., 2005; Hussack et al., 2011; Land, 2012).

These results suggest that the anti-poly-His labeling did not pick up the His-tagged catalase in the cytoplasm, but instead stained endogenous poly-His containing proteins within the nucleus. The cross-reactivity observed and the failure of the HIS-1 antibody to stain the overexpressed His-tagged catalase or detect the His-tagged catalase in Western blotting and ELISA assays suggests that this antibody is not suitable to discriminate between the endogenous catalase and His-tagged catalase in the SH-SY5Y neuroblastoma cell line. The pcDNA4/TO/myc–His expression vector allows tetracycline inducible expression of proteins

and has been used to show inducible expression of catalase protection against hydrogen peroxide toxicity (Chilumuri et al., 2013). When expressed in the pcDNA4/TO/myc–His expression vector the His tag is located on the C-terminus of catalase and this region contains a peroxisome targeting sequence (Purdue et al., 1996). The HIS-1 monoclonal antibody preferentially binds to N-terminal His tagged proteins and may not be suitable for detection of the C-terminally tagged catalase, as indicated by its failure to detect the catalase by immunocytochemistry, western blotting or ELISA. This also suggests that different anti-poly-His antibodies may recognize different endogenous poly-His containing proteins dependent on the localization and accessibility of the poly-His sequence within the proteins. Thus the observations for the HIS-1 monoclonal antibody may not hold for all anti-poly-His antibodies and there is a need for each anti-poly-His antibody to be tested in the individual cell or tissue type that is being studied.

The results also confirm the need for appropriate controls in such studies (Marshall et al., 2002; Howell et al., 2005; Hussack et al., 2011; Land, 2012). Had the immunocytochemistry staining of catalase overexpressing cells been only with the anti-His antibody, the localization of overexpressed catalase may have been misinterpreted and suggested to be in the nucleus rather than the cytoplasm (Chilumuri et al., 2013). The suggestion that poly-His sequences specifically direct proteins to the nucleus should also be considered, and may contribute to misinterpretation of results from His-tagged proteins (Paraguison et al., 2005; Salichs et al., 2009). This is particularly relevant to studies where cellular localization influences protein function (Schriner et al., 2005) or the localization has been specifically targeted (Schriner et al., 2000; Schriner et al., 2005).

In conclusion, the HIS-1 anti-poly-His monoclonal antibody shows significant immunocytochemical staining of endogenous proteins in the nucleus of SH-SY5Y neuroblastoma cells. This cross-reactivity limits the use of the antibody in the detection of

His-tag labeled proteins by immunocytochemistry and suggests that such studies require the use of antibodies specific for the protein of interest rather than the His-tag. The study also suggests that immunocytochemical studies using poly-His antibodies include comparison with appropriate controls to insure that the results are not misinterpreted.

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References

Ashabi G, Ahmadiani A, Abdi A, Abraki SB, Khodagholi F. Time course study of Aβ formation and neurite outgrowth disruption in differentiated human neuroblastoma cells exposed to H2O2: protective role of autophagy. Toxicol In Vitro 2013;27:1780-8.

Beckman M, Iverfeldt K. Increased gene expression of beta-amyloid precursor protein and its homologues APLP1 and APLP2 in human neuroblastoma cells in response to retinoic acid. Neurosci Lett 1997;221:73-6.

Bennett MC, Bishop JF, Leng Y, Chock PB, Chase TN, Mouradian MM. Degradation of alpha-synuclein by proteasome. J Biol Chem 1999;274:33855-8.

Burry RW. Controls for immunocytochemistry: an update. J Histochem Cytochem 2011;59:6-12.

Celsi F, Ferri A, Casciati A, D'Ambrosi N, Rotilio G, Costa A, et al. Overexpression of superoxide dismutase 1 protects against beta-amyloid peptide toxicity: effect of estrogen and copper chelators. Neurochem Int 2004;44:25-33.

Chang SL, Zadina JE, Spriggs L, Squinto SP. Prolonged activation of c-fos and optimal activation of pro-opiomelanocortin mRNA after repeated morphine exposure in SH-SY5Y Cells. Mol Cell Neurosci 1993;4:25-9.

Chilumuri A, Milton NGN. The role of neurotransmitters in protection against amyloid-β toxicity by KiSS-1 overexpression in SH-SY5Y neurons. ISRN Neuroscience 2013;2013:253210.

Chilumuri A, Odell M, Milton NGN. Benzothiazole aniline tetra(ethylene glycol) and 3-amino-1,2,4-triazole inhibit neuroprotection against amyloid peptides by catalase overexpression in vitro. ACS Chem Neurosci 2013;4:1501-12.

Daneshtalab N, Doré JJE, Smeda JS. Troubleshooting tissue specificity and antibody selection: Procedures in immunohistochemical studies. J Pharmacol Toxicol Methods 2010;61:127-35.

Davison LJ, Herrtage ME, Catchpole B. Autoantibodies to recombinant canine proinsulin in canine diabetic patients. Res Vet Sci 2011;91:58-63.

Delobel P, Mailliot C, Hamdane M, Sambo A-V, Bégard S, Violleau A, et al. Stable-tau overexpression in human neuroblastoma cells: an open door for explaining neuronal death in tauopathies. Ann N Y Acad Sci 2003;1010:623-34.

Deshpande AG, Darton NJ, Yunus K, Fisher AC, Slater NKH. In situ fabrication of a microfluidic device for immobilised metal affinity sensing. N Biotechnol 2012;29:494-

501.

Di Bonito P, Grasso F, Mochi S, Accardi L, Donà MG, Branca M, et al. Serum antibody response to Human papillomavirus (HPV) infections detected by a novel ELISA technique based on denatured recombinant HPV16 L1, L2, E4, E6 and E7 proteins. Infect Agents Cancer 2006;1:6.

Faux NG, Bottomley SP, Lesk AM, Irving JA, Morrison JR, la Banda de MG, et al. Functional insights from the distribution and role of homopeptide repeat-containing proteins. Genome Res. 2005;15:537-51.

Franco P, Iaccarino C, Chiaradonna F, Brandazza A, Iavarone C, Mastronicola MR, et al. Phosphorylation of human pro-urokinase on Ser138/303 impairs its receptor-dependent ability to promote myelomonocytic adherence and motility. J Cell Biol 1997;137:779-91.

Fritschy J-M. Is my antibody-staining specific? How to deal with pitfalls of immunohistochemistry. Eur J Neurosci 2008;28:2365-70.

Fu Y, Liu HW, Forsythe SM, Kogut P, McConville JF, Halayko AJ, et al. Mutagenesis analysis of human SM22: characterization of actin binding. J Appl Physiol 2000;89:1985-90.

Howell M, Williams T, Hazlewood SA. Herpesvirus pan encodes a functional homologue of BHRF1, the Epstein-Barr virus v-Bcl-2. BMC Microbiol 2005;5:6.

Hussack G, Arbabi-Ghahroudi M, van Faassen H, Songer JG, Ng KKS, MacKenzie R, et al. Neutralization of Clostridium difficile toxin A with single-domain antibodies targeting the cell receptor binding domain. J Biol Chem 2011;286:8961-76.

Ida N, Masters CL, Beyreuther K. Rapid cellular uptake of Alzheimer amyloid betaA4

peptide by cultured human neuroblastoma cells. FEBS Lett 1996;394:174-8.

Katayama K, Ueda N, Katoh I, Yamamoto S. Equilibrium in the hydrolysis and synthesis of cannabimimetic anandamide demonstrated by a purified enzyme. Biochim Biophys Acta 1999;1440:205-14.

Kawabe T, Fujihira E, Yamaguchi A. Molecular construction of a multidrug exporter system, AcrAB: molecular interaction between AcrA and AcrB, and cleavage of the N-terminal signal sequence of AcrA. J Biochem 2000;128:195-200.

Land SC. Inhibition of cellular and systemic inflammation cues in human bronchial epithelial cells by melanocortin-related peptides: mechanism of KPV action and a role for MC3R agonists. Int J Physiol Pathophysiol Pharmacol. 2012;4:59-73.

Ma Y, Kubicek J, Labahn J. Expression and purification of functional human mu opioid receptor from E.coli. PLoS ONE 2013;8:e56500.

Marshall KR, Rowley KV, Rinaldi A, Nicholson IP, Ishov AM, Maul GG, et al. Activity and intracellular localization of the human cytomegalovirus protein pp71. J Gen Virol 2002;83:1601-12.

Marx FP, Holzmann C, Strauss KM, Li L, Eberhardt O, Gerhardt E, et al. Identification and functional characterization of a novel R621C mutation in the synphilin-1 gene in Parkinson's disease. Hum Mol Genet 2003;12:1223-31.

Milton NGN, Chilumuri A, Rocha-Ferreira E, Nercessian AN, Ashioti M. Kisspeptin Prevention of Amyloid-β Peptide Neurotoxicity in Vitro. ACS Chem Neurosci 2012;3:706-19.

Mirzaei H, Schieler JL, Rochet J-C, Regnier F. Identification of rotenone-induced

modifications in alpha-synuclein using affinity pull-down and tandem mass spectrometry. Anal Chem. 2006;78:2422-31.

Motin VL, Nedialkov YA, Brubaker RR. V antigen-polyhistidine fusion peptide: binding to LcrH and active immunity against plague. Infect Immun. 1996;64:4313-8.

Moussa CE-H, Wersinger C, Rusnak M, Tomita Y, Sidhu A. Abnormal migration of human wild-type alpha-synuclein upon gel electrophoresis. Neurosci Lett 2004;371:239-43.

Oma Y, Kino Y, Sasagawa N, Ishiura S. Intracellular localization of homopolymeric amino acid-containing proteins expressed in mammalian cells. J Biol Chem 2004;279:21217-22.

Ostrow KL, Mammoser A, Suchyna T, Sachs F, Oswald R, Kubo S, et al. cDNA sequence and in vitro folding of GsMTx4, a specific peptide inhibitor of mechanosensitive channels. Toxicon 2003;42:263-74.

Paraguison RC, Higaki K, Sakamoto Y, Hashimoto O, Miyake N, Matsumoto H, et al. Polyhistidine tract expansions in HOXA1 result in intranuclear aggregation and increased cell death. Biochem Biophys Res Commun 2005;336:1033-9.

Purdue PE, Castro SM, Protopopov V, Lazarow PB. Targeting of human catalase to peroxisomes is dependent upon a novel C-terminal peroxisomal targeting sequence. Ann N Y Acad Sci 1996;804:775-6.

Racine S, Kheyar A, Gagnon CA, Charbonneau B, Dea S. Eucaryotic expression of the nucleocapsid protein gene of porcine circovirus type 2 and use of the protein in an indirect immunofluorescence assay for serological diagnosis of postweaning multisystemic wasting syndrome in pigs. Clin Diagn Lab Immunol 2004;11:736-41.

Salichs E, Ledda A, Mularoni L, Albà MM, la Luna de S. Genome-wide analysis of histidine

repeats reveals their role in the localization of human proteins to the nuclear speckles compartment. PLoS Genet 2009;5:e1000397.

Schriner SE, Smith AC, Dang NH, Fukuchi K, Martin GM. Overexpression of wild-type and nuclear-targeted catalase modulates resistance to oxidative stress but does not alter spontaneous mutant frequencies at APRT. Mutat Res 2000;449:21-31.

Schriner SE, Linford NJ, Martin GM, Treuting P, Ogburn CE, Emond M, et al. Extension of murine life span by overexpression of catalase targeted to mitochondria. Science 2005;308:1909-11.

Skommer J, Brittain T. Extended survival of SH-SY5Y cells following overexpression of Lys67Glu neuroglobin is associated with stabilization of ΔψM. Cytometry A 2012;81:602-10.

Skoog L, Tani E. Immunocytochemistry: an indispensable technique in routine cytology. Cytopathology. 2011;22:215-29.

Ulmer TA, Keeler V, Loh L, Chibbar R, Torlakovic E, André S, et al. Tumor-associated antigen 90K/Mac-2-binding protein: possible role in colon cancer. J Cell Biochem 2006;98:1351-66.

Yang C-Y, Kuo T-H, Ting L-P. Human hepatitis B viral e antigen interacts with cellular interleukin-1 receptor accessory protein and triggers interleukin-1 response. J Biol Chem 2006;281:34525-36.

Zadina JE, Chang SL, Ge LJ, Kastin AJ. Mu opiate receptor down-regulation by morphine and up-regulation by naloxone in SH-SY5Y human neuroblastoma cells. J Pharmacol Exp Ther 1993;265:254-62.

Zou Z, Sun PD. An improved recombinant mammalian cell expression system for human transforming growth factor-beta2 and -beta3 preparations. Protein Expr Purif 2006;50:9-17.

Figure Legends

Figure 1. Double-labeling immunofluorescence demonstrating co-localization of HIS-1 monoclonal anti-polyhistidine staining and TO-PRO®-3 Iodide DNA staining in SH-SY5Y neuroblastoma cells. The HIS-1 monoclonal anti-polyhistidine staining appears red (a), (c), (d) and (f). The TO-PRO®-3 Iodide DNA staining appears blue (b), (c), (e) and (f). The overlap of polyhistidine proteins and DNA appears purple (c). Scale bars = 10 µm (a-c) and 2 µm (d-f).

Figure 2. Double-labeling immunofluorescence demonstrating co-localization of HIS-1 monoclonal anti-polyhistidine staining and TO-PRO®-3 Iodide DNA staining (a), (b) and (c) in PVect SH-SY5Y neuroblastoma cells stably expressing the pcDNA4/TO/myc–His expression vector. The HIS-1 monoclonal anti-polyhistidine staining appears red (a) and (c). The TO-PRO®-3 Iodide DNA staining appears blue (b) and (c). The overlap of polyhistidine proteins and DNA appears purple (c). Scale bars = 5 µm. Double-labeling immunofluorescence of CAT-505 monoclonal anti-catalase staining and TO-PRO®-3 Iodide DNA staining (d), (e) and (f) in PVect SH-SY5Y neuroblastoma cells stably expressing the pcDNA4/TO/myc–His expression vector. The CAT-505 monoclonal anti-catalase staining staining appears red (d) and (f). The TO-PRO®-3 Iodide DNA staining appears blue (e) and (f). Scale bars = 5 µm.

Figure 3. Double-labeling immunofluorescence demonstrating co-localization of HIS-1 monoclonal anti-polyhistidine staining and TO-PRO®-3 Iodide DNA staining (a), (b) and (c) in PCat SH-SY5Y neuroblastoma cells stably expressing His-tagged human catalase in the pcDNA4/TO/myc–His expression vector. The HIS-1 monoclonal anti-polyhistidine staining appears red (a) and (c). The TO-PRO®-3 Iodide DNA staining appears blue (b) and (c). The overlap of polyhistidine proteins and DNA appears purple (c). Scale bars = 5 µm. Double-

labeling immunofluorescence of CAT-505 monoclonal anti-catalase staining and TO-PRO®-3 Iodide DNA staining (d), (e) and (f) in PCat SH-SY5Y neuroblastoma cells stably expressing His-tagged human catalase in the pcDNA4/TO/myc–His expression vector. The CAT-505 monoclonal anti-catalase staining appears red (d) and (f). The TO-PRO®-3 Iodide DNA staining appears blue (e) and (f). Scale bars = 5 µm.

Figure 4. Western blotting staining of cell extracts from PVect and PCat SH-SY5Y neuroblastoma cells with CAT-505 monoclonal anti-catalase and HIS-1 monoclonal anti-polyhistidine antibodies. Cell extracts from PVect and PCat cells were separated by SDS-PAGE and then Western blots stained with either (a) CAT-505 monoclonal anti-catalase antibody or (b) HIS-1 monoclonal anti-polyhistidine antibody. The anti-ß actin staining shows equal loading of protein of the PVect and PCat extracts.

Figure 1

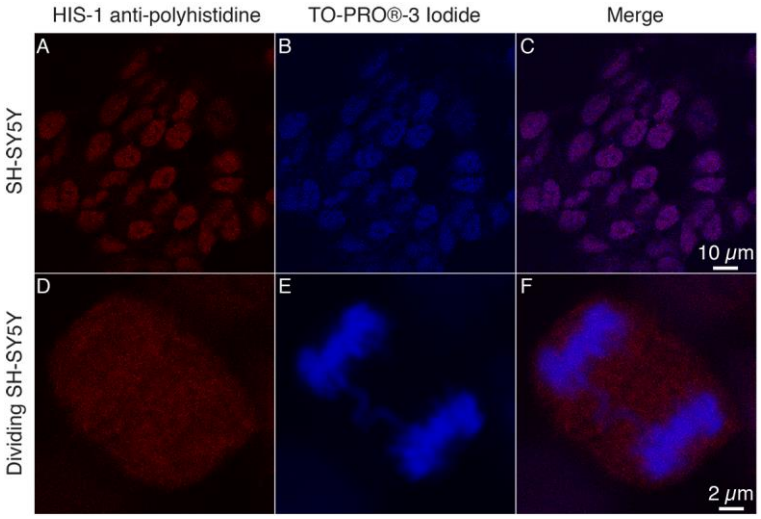

Figure 2

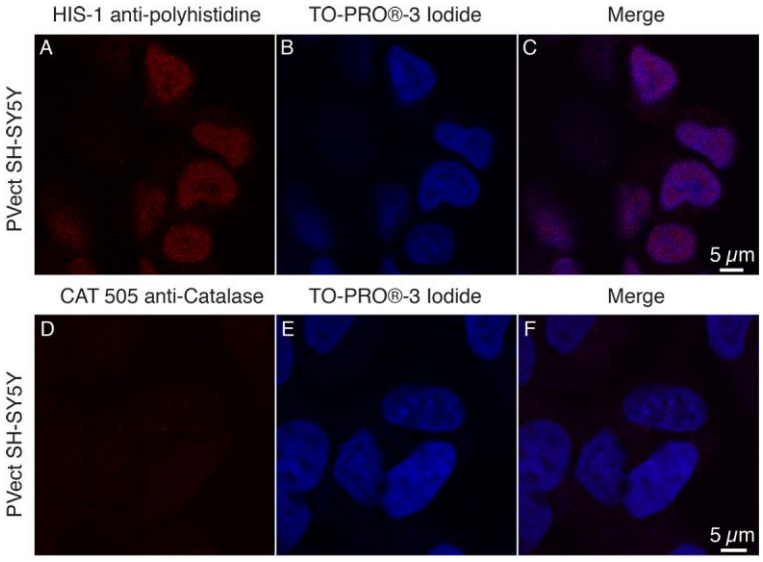

Figure 3

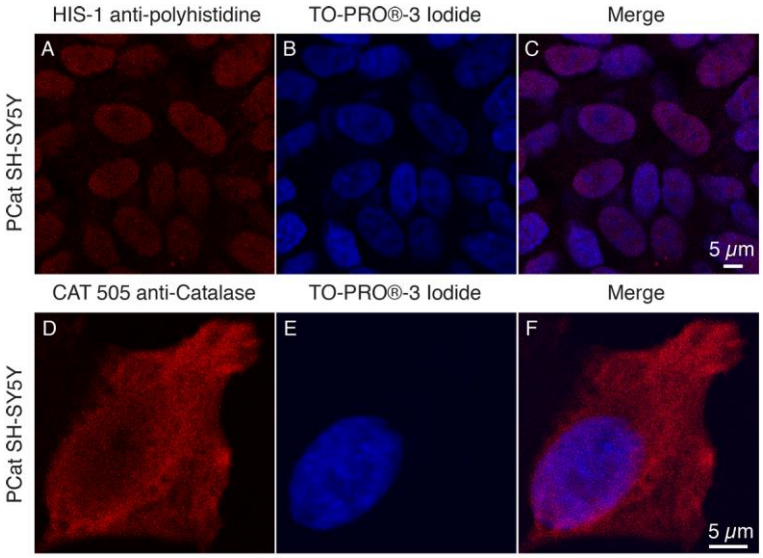

Figure 4