Annexin B12 Cys101,Cys260-N,N'-dimethyl-N-(iodoacetyl)-N'-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)ethylenediamine

pSIVA$_m$

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Chemical name:	Annexin B12 Cys101,Cys260-N,N'-dimethyl-N-(iodoacetyl)-N'-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)ethylenediamine	
Abbreviated name:	pSIVA$_m$	
Synonym:	AnxB12 Cys101,Cys260-IANBD	
Agent Category:	Proteins	
Target:	Phosphatidylserine	
Target Category:	Lipids	
Method of detection:	Optical imaging	
Source of signal / contrast:	N,N'-dimethyl-N-(iodoacetyl)-N'-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)ethylenediamine (IANBD)	
Activation:	Yes	
Studies:	• *In vitro* • Rodents	No structure is available.

Background

[PubMed]

Apoptosis, or programmed cell death, presents specific biochemical changes at its different stages (1-4). By targeting changes such as exposed phosphatidylserine (PS), dysfunctional mitochondria, activated caspases, fragmented DNA, and disrupted membrane integrity, various apoptosis-detecting methods have been developed and

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provide diagnostic and prognostic value for human diseases, most notably for cancer, myocardial dysfunction, and neurodegenerative disorders (2-5). For several reasons, exposed PS has been used as a single and direct target for apoptosis detection (2, 6-10) although it is not specific for apoptosis. First, PS is normally limited to the inner leaflet of the plasma membrane bilayer, whereas it is externalized to the outer leaflet of the membrane in the presence of calcium ions during apoptosis. Second, cell surface exposure of PS has been considered to be an indicator of early apoptotic processes in the absence of necrosis. Therefore, monitoring PS exposure provides a way to observe the initiation of the apoptotic pathway before other changes are present. Third, PS exposure provides extracellular binding targets that can be detected without the need to penetrate the cells.

Annexins are cellular proteins that are widely used to design PS-targeted, apoptosis-detecting probes (1, 7, 9). Annexins are characterized by their calcium-dependent ability to bind to negatively charged PS. All annexins are composed of two distinct regions: a C-terminal core and an amino N-terminal “head” region. The annexin core is highly conserved across the annexin family, and the N-terminus varies greatly. Radiolabeled annexin derivatives have already been used for clinical imaging in many diseases (2, 8, 10). However, annexin-based probes suffer from several drawbacks, such as slow delivery to the site of interest, slow clearance from tissue leading to high effective doses, and low specificity. Annexin-based probes do not appear to distinguish apoptosis from necrosis. Furthermore, PS externalization is associated with inflammation and platelet activation, causing further challenges with annexin-based probe specificity.

To address these problems, Kim et al. developed a class of polarity-sensitive annexin-based probes (pSIVAs) with built-in "on" (membrane-bound) and "off" (in solution) fluorescent states (1). The probes were generated by placing polarity-sensitive thiol-reactive fluorophores in the loop regions of annexin B12 (anxB12, also known as annexin XII) or annexin A5 (anx5, also known as annexin V), taking advantage of loop region mediation of Ca^{2+}-dependent membrane interactions, transitioning from a polar (aqueous solution) to a nonpolar (lipid membrane) environment upon membrane binding. Two thiol-reactive fluorophores, N,N'-dimethyl-N-(iodoacetyl)-N'-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)ethylenediamine (IANBD) and 6-bromoacetyl-2-dimethylaminonaphthalene (BADAN), were tested, and the resulting probes were evaluated for apoptosis detection. The results showed that probe binding with PS on the apoptotic cells resulted in a measurable increase in fluorescence emission intensity. $pSIVA_m$ (also known as anxB12 Cys101,Cys260-IANBD) is one of the promising probes for apoptosis detection (1).

Related Resource Links:

- Chapters of annexin-based probes in MICAD
- Clinical trials of annexin-based probes in ClinicalTrials.gov
- Gene information for human annexins
- Annexin information in OMIM
- Annexin-related substances in PubChem

- Apoptosis-related bioassay in PubChem

Synthesis

[PubMed]

Kim et al. screened for polarity-sensitive molecules that emit increased fluorescence intensity in nonpolar environments, and the investigators selected two thiol-reactive fluorophores, IANBD and BADAN, as reporters (detailed data not shown) (1). AnxB12 and anxA5 proteins with cysteine mutations were then generated in cysteine-less variants of anxA5 (C316A) and anxB12 (C113A, C302A) plasmids with site-directed mutagenesis. The mutant protein products were expressed, purified, and reacted with a ten-fold molar excess of IANBD or BADAN at the introduced cysteine sites. The generated probes included IANBD- or BADAN-labeled anxA5 Cys262, anxB12 Cys4, anxB12 Cys101, and anxB12 Cys101,Cys260. The probe pSIVA$_m$ was referred to the anxB12 labeled at both residue positions 101 and 260 with IANBD (anxB12 Cys101,Cys260-IANBD). Kim et al. also generated two negative control probes (anxB12 Cys4-IANBD and anxB12 Cys4-BADAN) by labeling residue 4 in the N-terminal tail of anxB12, which were expected to stay fully exposed to the aqueous environment in both the solution and membrane-bound states (1). The purity, labeling efficiency, and yield of the probes were not described in detail.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

Kim et al. evaluated the differences in the fluorescence emission of the probes between the solution and membrane-bound states with *in vitro* binding assay (1). The fluorescence intensity was negligible for all probes free in solution and for the negative control molecules in both in-solution and membrane-bound states. Considerable increases in fluorescence intensity were measured for all probes in their membrane-bound states, suggesting binding to membrane PS. The membrane-bound pSIVA$_m$ exhibited fluorescence ~45-fold greater than that found in the in-solution state, and the membrane-bound pSIVA$_m$ was brighter than other membrane-bound probes. Slightly lower fluorescence intensities were detected for BADAN-labeled probes than for corresponding IANBD-labeled probes. There was no detectable decrease in membrane binding or loss of specificity to PS for all probes. These results indicate that attachment of polarity-sensitive IANBD and BADAN to cysteine residues in the membrane-binding loops provides an effective way to generate annexin derivatives with built-in "on" and "off" fluorescent states.

To test the application of pSIVA$_m$ to image live cells undergoing apoptosis, Kim et al. added pSIVA$_m$ directly to cultured COS-7 cells that had been induced to undergo apoptosis by etoposide (1). As expected, the investigators observed bright pSIVA$_m$ staining of the COS-7 cells at the early stages of apoptosis and a gradual increase in staining concurrent with progression into late-stage cell death. In comparison, no staining was observed for cells grown under normal conditions, suggesting that pSIVA$_m$ binding

and fluorescence emission were specific to apoptotic cells. The background fluorescence from the in-solution state was undetectable. $pSIVA_m$ did not influence the cell growth (data not shown).

Kim et al. next determined whether $pSIVA_m$ could be used to study axonal degeneration using purified primary dorsal root ganglion (DRG) sensory neurons (1). DRG neurons have a single axon without dendrites, and under various conditions axonal degeneration and death occur at different times and independently from each other. In addition, DRG neurons are dependent on trophic factor support for survival. The investigators observed $pSIVA_m$ staining in both axons and cell bodies of nerve growth factor (NGF)-deprived neurons, whereas $pSIVA_m$ staining was largely absent in neurons grown in the presence of NGF. The fluorescence intensity increased gradually in the NGF-deprived neurons, corresponding to both a gradual increase in the amount of PS exposure in an individual neuron and the number of degenerating neurons present over longer periods of NGF deprivation. $pSIVA_m$ binding occurred in a specific spatiotemporal order, which indicates that PS exposure occurred successively, originating from a particular location in the axon and progressing toward the cell body. The dynamic, sequential, punctate staining pattern observed in the axons was characteristic of binding to the PS exposed on the outer leaflet of the plasma membrane rather than binding to the PS from the intracellular side, where PS is more abundant and uniformly distributed.

Axonal degeneration is a highly regulated dynamic process that does not necessarily result in cell death. Understanding the temporal dynamics of whether and when rescue is possible in degenerating neurons will provide an indication of the severity of the cellular response and the time window in which neuronal survival mechanisms can still be effective (1-3). Therefore, Kim et al. first initiated degeneration in DRG neurons by NGF deprivation and then added back NGF once they detected PS exposure on the axons (at 7, 10, and 15 h after initial NGF removal) (1). Complete cell death was blocked with re-administration of NGF in some cells but not all. Concurrent with neuronal survival after replacing NGF, the investigators observed a decrease in $pSIVA_m$ staining fluorescence, indicating that binding decreased as PS was restored to the inner leaflet of the plasma membrane. For neurons that were deprived of NGF for 15 h before replenishing NGF, maximum fluorescence intensity was reached at 20–24 h, followed by a decrease to the end of the 40-h time course. On the other hand, when the neurons were continuously deprived of NGF, $pSIVA_m$ fluorescence increased and was maintained to the end of the experiment. Therefore, Kim et al. observed rescue from degeneration in neurons with $pSIVA_m$-stained axons but not in neurons with $pSIVA_m$-stained cell bodies, which was indicative of later stages of cell death. Some axons retained $pSIVA_m$ staining after the re-administration of NGF, indicating that rescue was not possible in intermediate stages. These results indicate that there is a critical period after the initiation of apoptotic mechanisms in which rescue is still possible (after PS exposure in the axon but before PS exposure in the cell body) (1).

Animal Studies

Rodents

[PubMed]

To test the utility of pSIVAs for in vivo applications, Kim et al. administered pSIVA$_m$ by intramuscular injection along the rat sciatic nerve 3 days after nerve transection and then imaged the degenerating neurons (1). pSIVA$_m$ exclusively stained axons in the sciatic nerve distal to the site of injury, whereas staining in the contralateral control (uninjured) sciatic nerve was undetectable. A dynamic punctate staining pattern in the sciatic nerve axons was observed, which was similar to the findings in degenerating axons of DRG neurons in vitro.

Other Non-Primate Mammals

[PubMed]

No references are currently available.

Non-Human Primates

[PubMed]

No references are currently available.

Human Studies

[PubMed]

No references are currently available.

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