Cy5-Tat-Glu-Pro-Asp-acyloxymethyl ketone

tAB50-Cy5

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Chemical name:	Cy5-Tat-Glu-Pro-Asp-acyloxymethyl ketone	
Abbreviated name:	tAB50-Cy5	
Synonym:		
Agent Category:	Small molecule	
Target:	Caspase-3, caspase-7	
Target Category:	Enzyme	
Method of detection:	Optical, near-infrared fluorescence imaging	
Source of signal / contrast:	Cy5	
Activation:	No	
Studies:	• *In vitro* • Rodents	No structure is available.

Background

[PubMed]

Caspases are a group of cysteine proteases, which exist within cells as inactive zymogens, and are cleaved to form active enzymes after induction of apoptosis (1-3). There are two major pathways through which caspases are activated. One is the death signal–induced, death receptor–mediated extrinsic pathway, and the other is the stress-induced, mitochondria-directed intrinsic pathway (3, 4). Caspases convey the apoptotic signal in a proteolytic cascade, with caspases cleaving and activating other caspases. Caspase-8 and -9 act as initiators at the upper stream of this cascade, while caspase-3, -6, and -7 work as downstream executioners (1, 3, 4). Although physiological apoptosis takes place during embryonic development and in adult homeostasis, excessive apoptosis has been observed in some human diseases such as myocardial infarction, rheumatoid arthritis, ischemia,

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transplant rejection, and neurodegenerative disorders (Parkinson's and Alzheimer's) (5, 6). On the contrary, evading apoptosis is a hallmark of human cancer (7, 8).

Many different molecular probes have been developed for imaging apoptosis both at the cellular level and *in vivo* (2, 5, 7, 9, 10). Of them, a large number of the probes are annexin V–based. Annexin V is a 36-kDa protein that binds to externalized phosphatidylserine residues in the presence of calcium ions on the surface of apoptotic cells (5). Radiolabeled annexin V derivatives have already been used for clinical imaging in many diseases, most notably for imaging myocardial dysfunction. A drawback of annexin V–based probes is their low specificity to apoptotic cells. Annexin V cannot distinguish apoptosis from necrosis (5). Phosphatidylserine externalization is also associated with inflammation and platelet activation, causing further challenges with annexin V specificity. During recent years, much work has focused on the development of probes for caspase-3 and -7. Thornberry et al. have observed that most caspases prefer a tetrapeptide motif with an aspartyl residue at P4 (11). Asp-Glu-Val-Asp (DEVD) is the peptide sequence optimal for caspase-3 and -7, while the Val-Glu-His-Asp (VEHD) sequence is preferred by caspase-6 (5). These sequence-based probes demonstrate high caspase specificity; however, most probes suffer from poor cellular uptake in the intact cell, a requirement for early detection.

Edgington et al. developed a class of probes based on the acyloxymethyl ketone (AOMK) and the optimal sequence of caspase-3 and -7 (2). AB50-Cy5 is one of the probes, which contains a Glu-Pro-Asp-AOMK sequence labeled with the Cy5 fluorophore. This probe showed labeling of caspase-3 and legumain with no detectable cathepsin B labeling. To enhance the cell permeability of AB50-Cy5, the investigators synthesized the probe tAB50-Cy5, a version of the probe containing a tat peptide. For this probe, the tat peptide was used to increase the cell uptake of the probe through multiple positively charged amino acids, while AOMK was used to label caspases. This chapter summarized the synthesis and comparative analysis of tAB50-Cy5 and AB50-Cy5.

Related Resource Links:

- Chapter of AB50-Cy5 (or Cy5-AB50) in MICAD
- Apoptosis imaging chapters in MICAD
- Apoptosis related information in OMIM
- Apoptosis imaging clinical trials in ClinicalTrials.gov

Synthesis

[PubMed]

The detailed synthesis of AB50-Cy5 and tAB50-Cy5 was described by Edgington et al. and can also be found in the MICAD chapter for AB50-Cy5 (2). The tat version, tAB50-Cy5, differed from AB50-Cy5 in that the Cy5 fluorochrome was moved to a lysine side chain, and the tat peptide was coupled through a cysteine residue to a maleimide group at the amino terminus of the probe. The tat peptide (Arg-Lys-Lys-Arg-Arg-Orn-Arg-Arg-Arg-

Cys, all D-amino acids, except for the cysteine) was custom synthesized. Edgington et al. also generated the control version of tAB50-Cy5 (tAB50-Ctrl) that contains an amide in place of the reactive AOMK functional group. The purity and identity of all probes were assessed with liquid chromatography-mass spectrometry analysis (detailed data not shown).

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

Edgington et al. used SDS-PAGE gel analysis to evaluate the probes' ability to label caspases (2). As expected, the active probes AB50-Cy5 and tAB50-Cy5 efficiently labeled recombinant caspase-3, whereas control probes did not. In intact Jurkat cells, when the cells were first treated with anti-Fas antibody to activate apoptosis and then labeled with probes, both AB50-Cy5 and tAB50-Cy5 labeled caspases. When the cells were pretreated with the probes, followed by washout before apoptosis activation with anti-Fas antibody, only tAB50-Cy5 showed labeling of caspases. These results indicated that the tat peptide increased the penetrating ability of the probes into intact cells. However, addition of the tat peptide also resulted in a substantial increase in the labeling of legumain (MICAD chapter for AB50-Cy5). This increase in labeling of a lysosome-resident protease is probably due to uptake of the probes *via* the endo-lysosomal route before release into the cytosol.

Animal Studies

Rodents

[PubMed]

To test the utility of tAB50-Cy5 for imaging apoptosis, Edgington et al. monitored caspase activation in mice treated with dexamethasone for 6, 12, or 24 h (n = 3 mice for each time point) (2). Probes were injected intravenously 2 h before removal of thymi for imaging and SDS-PAGE analysis. The results showed that AB50-Cy5 and tAB50-Cy5 had same trend in fluorescent signals in the intact thymi and the same pattern in labeling caspases and legumain (labeled both caspase-3 and legumain), but the overall signal intensity increased for tAB50-Cy5. The amount of labeled caspases directly correlated with the imaging signal intensity. A similar increase in tAB50-Cy5 signal was not observed in the samples treated with vehicle, suggesting increased uptake of the probe only into apoptotic cells. Quantitative analysis revealed that legumain activity was low and remained largely unchanged in the first 12 h after dexamethasone treatment. Different from legumain, caspase-3 activity was observable at 6 h after treatment, peaking at 12 h and then sharply dropping to background levels at 24 h after injection. These data agreed with previous studies that showed a peak in $TUNEL^+$ thymocyte staining at 16 h after dexamethasone treatment followed by a sharp decrease at 18 h and 24 h. Flow cytometry further confirmed that tAB50-Cy5 accumulated only in dying cells, and the probe-positive cells

were also positive for annexin V. These results suggested that caspases are likely to be activated at early time points and, therefore, may serve as effective markers of the early stages of apoptosis. The fluorescence observed in whole organs can be used as a direct readout of total probe-labeled proteases.

Edgington et al. further monitored caspase activation in xenografted human tumor tissues that had been induced to undergo apoptosis by treatment with the monoclonal antibody Apomab (2). Apomab induces the extrinsic apoptosis pathway by binding death receptor-5 and is highly distinct from the dexamethasone-induced intrinsic apoptosis in $CD4^+CD8^+$ thymocytes. In agreement with the results obtained for the dexamethasone model, total fluorescent signals in the tumors closely mirrored levels of labeled caspase-3 and -7. The maximum caspase activity occurred 12 h after Apomab treatment, while the overall levels of legumain labeling were constant throughout the time course. tAB50-Cy5 showed substantially brighter signals but had slow clearance from all tissues, resulting in a low signal/background ratio at the early time points with optimal contrast (4.5-fold) at 5 h after probe injection. AB50-Cy5, in contrast, cleared rapidly and showed a good signal/background ratio even at the early time points (3.2-fold contrast at 50 min). Notably, tAB50-Ctrl showed nearly identical accumulation in Apomab-treated tumors as tAB50-Cy5 but also produced higher background fluorescence in vehicle-treated tumors. Thus, although the control probes had some accumulation in apoptotic cells, only the active probes showed a marked contrast between apoptotic and nonapoptotic tumors. The *ex vivo* images closely matched the images obtained in live mice. Specific labeling of caspases was observed only in Apomab-treated samples. Specific labeling of apoptotic cells was further confirmed with histology of tumor tissues.

The investigators concluded that caspase-specific probes can be used to noninvasively monitor apoptosis in tumors treated with chemotherapy agents (2). The non–tat-labeled probes provide the best direct readout of caspase activity, but the tat-labeled probes boost overall specific signal and may therefore be useful for carrying other contrast agents into apoptotic cells. The cross-reactivity with legumain does not appear to hinder the probes' ability to monitor dynamic changes in caspase activity because of the constant low activity of legumain during apoptosis.

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.

References

1. Fan T.J., Han L.H., Cong R.S., Liang J. *Caspase family proteases and apoptosis.* Acta Biochim Biophys Sin (Shanghai). 2005;37(11):719–27. PubMed PMID: 16270150.
2. Edgington L.E., Berger A.B., Blum G., Albrow V.E., Paulick M.G., Lineberry N., Bogyo M. *Noninvasive optical imaging of apoptosis by caspase-targeted activity-based probes.* Nat Med. 2009;15(8):967–73. PubMed PMID: 19597506.
3. Salvesen G.S., Riedl S.J. *Caspase mechanisms.* Adv Exp Med Biol. 2008;615:13–23. PubMed PMID: 18437889.
4. Boatright K.M., Salvesen G.S. *Mechanisms of caspase activation.* Curr Opin Cell Biol. 2003;15(6):725–31. PubMed PMID: 14644197.
5. Smith G., Nguyen Q.D., Aboagye E.O. *Translational imaging of apoptosis.* Anticancer Agents Med Chem. 2009;9(9):958–67. PubMed PMID: 19663785.
6. Shirvan A., Reshef A., Yogev-Falach M., Ziv I. *Molecular imaging of neurodegeneration by a novel cross-disease biomarker.* Exp Neurol. 2009;219(1):274–83. PubMed PMID: 19500576.
7. Nguyen Q.D., Smith G., Glaser M., Perumal M., Arstad E., Aboagye E.O. *Positron emission tomography imaging of drug-induced tumor apoptosis with a caspase-3/7 specific [18F]-labeled isatin sulfonamide.* Proc Natl Acad Sci U S A. 2009;106(38): 16375–80. PubMed PMID: 19805307.
8. Ledgerwood E.C., Morison I.M. *Targeting the apoptosome for cancer therapy.* Clin Cancer Res. 2009;15(2):420–4. PubMed PMID: 19147745.
9. Blum G., von Degenfeld G., Merchant M.J., Blau H.M., Bogyo M. *Noninvasive optical imaging of cysteine protease activity using fluorescently quenched activity-based probes.* Nat Chem Biol. 2007;3(10):668–77. PubMed PMID: 17828252.
10. Paulick M.G., Bogyo M. *Application of activity-based probes to the study of enzymes involved in cancer progression.* Curr Opin Genet Dev. 2008;18(1):97–106. PubMed PMID: 18294838.
11. Thornberry N.A., Rano T.A., Peterson E.P., Rasper D.M., Timkey T., Garcia-Calvo M., Houtzager V.M., Nordstrom P.A., Roy S., Vaillancourt J.P., Chapman K.T., Nicholson D.W. *A combinatorial approach defines specificities of members of the caspase family and granzyme B. Functional relationships established for key mediators of apoptosis.* J Biol Chem. 1997;272(29):17907–11. PubMed PMID: 9218414.