Technology Summary: Dansyl Molecular Probe

Opportunity Statement

Cell apoptosis refers to the active death process of cells controlled by genes for multi-cellular organisms to regulate development and maintain homeostasis. It is one of the main means of senescence and natural death of cells, and is necessary for organisms to maintain normal physiological processes. Interest in apoptosis has increased due to the recognition that many diseases involve too much apoptosis (e.g., Parkinson's and Alzheimer's disease) or too little apoptosis (e.g., cancer). Therefore, research in cell apoptosis is very helpful in the study, diagnosis and treatment of these diseases.

Optical imaging is an emerging technology used to study cell apoptosis. It is a non-invasive method that uses light to interrogate cellular and molecular function in the living body. Contrast is derived either from the use of exogenous agents that provide signals or from endogenous molecules with optical signatures. Optical imaging technologies are capable of resolving across a range of spatial scales from tens of nanometers to centimeters. Compared to other conventional imaging methods like MRI or PET, optical imaging has the advantages of being non-radioactive and requiring less costly equipment.

Problem

Annexin V is currently the most widely used probe in optical imaging, and is considered the gold standard for detecting cell apoptosis. Annexin V is a Ca2+ dependent phospholipid-binding protein, and can bind with the phosphatidylserine (PS) rotated out of the cell membrane in the early stages of cell apoptosis.

Although Annexin V is a valuable tool for the study of apoptosis, it has the following drawbacks:

1. Annexin V is a biological molecule with a molecular weight of 35.8 KDa, which makes it unstable and difficult to handle. It requires very specific buffer and pH conditions for storage and activity.
2. Since it is a protein, it can have non-specific interactions with parts of normal cells and produces high background signal.
3. It has slow metabolism in living organisms and after binding to its target, its clearance from the body takes a long time.
4. Annexin V binds to the PS, which is not a unique characteristic of apoptosis. PS also occurs in necrosis; hence, assays with Annexin V cannot distinguish cells that die from necrosis from cells that die from apoptosis.

Therefore, there is a need for a technology that can overcome the shortcomings of current Annexin V used to study apoptosis in optical imaging.

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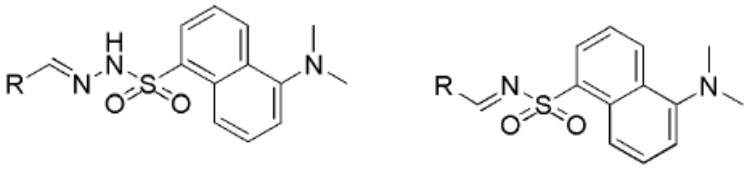
360ip Partner's Solution

360ip Partner's invention relates to the synthesis and application of novel dansyl molecular probes used for detecting apoptosis. Generally, dansyls are fluorophores that are used as markers and are conjugated to larger molecular probes. However, 360ip's Partner has designed novel dansyl molecules that have the ability to act as selective and specific probes for apoptosis. The dansyl probes can be used directly to bind to apoptotic cells, thereby serving the function of a probe and a marker.



Structure of dansyl molecular probes developed by 360ip's Partner

The probes have the following benefits:

- Are organic micro-molecules with relatively simple structures as compared to the probes in current use.Can be synthesized with ease using easily available raw materials. High derivatization can be achieved by changing different functional groups to regulate the biochemical characteristics of the molecular probes.
- Have a fast metabolism in vivo, and thus will clear from the body soon after administration.
- Are simple molecules that are not proteins. This makes them less sticky, and thus non-specific interactions occur less often.
- Selectively bind to the apoptotic cells.
- Are intrinsically fluorescent and there is no need to label them.
- Localize in the cell cytoplasm as compared to the cell membrane in Annexin V.

Annexes A and B illustrate the experimental results demonstrating the efficacy of the invention.

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Patents

360ip's partner has filed one patent on this technology and plans to seek protection in multiple jurisdictions.

360ip is seeking interested parties for the licensing, further development and commercialization of this technology-based product.

For additional information, contact:
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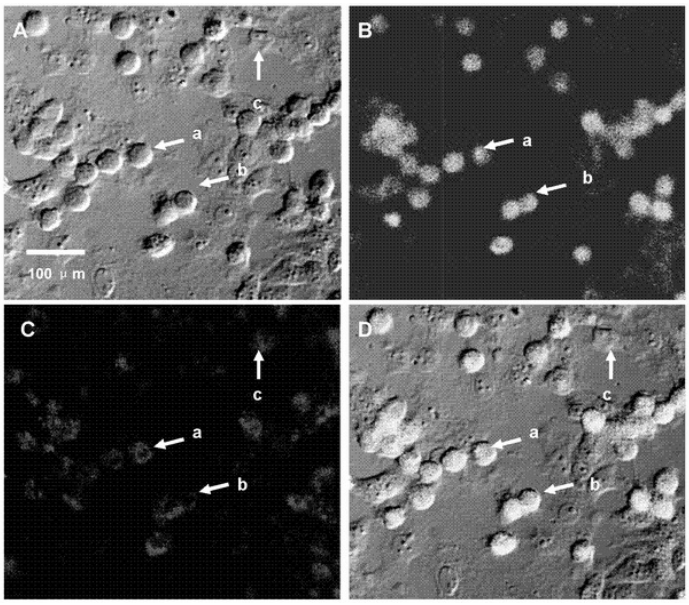
Annex A Comparison of optical imaging result between invention and Annexin V-FITC

Arrows a and b show the apoptotic breast cancer cells, and c shows the normal cells. **Figure A** is the apoptosis image of human breast cancer cells (MCF-7) treated by Taxol. **Figure B** is the fluorescent image of the dansyl molecular probe on human breast cancer cells (MCF-7) in Figure A. **Figure C** is the fluorescent image of Annexin V-FITC on human breast cancer cells (MCF-7) in Figure A. **Figure D** is the laser confocal image of human breast cancer cells (MCF-7) in Figure A. *Figure D indicates that the dyed apoptotic cells in Figure B and Figure C are basically consistent, but the dansyl molecular probe has more specificity since the normal cells shown by arrow c is dyed by Annexin V-FITC but not with dansyl.*	

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Annex B Comparison of high-resolution optical imaging result (high-resolution ratio of 20 mu m)

Figure A is the apoptosis image of human breast cancer cells (MCF-7) treated by Taxol. **Figure B** is the fluorescent image of the dansyl molecular probe on human breast cancer cells (MCF-7) of Figure A. **Figure C** is the fluorescent image of Annexin V-FITC on human breast cancer cells (MCF-7) of Figure A, **Figure D** is the laser confocal image of human breast cancer cells (MCF-7) of Figure A with Annexin V-FITC and dansyl molecular probe. *From the high-resolution image of the cell in Figure D, it can be seen that Annexin V-FITC lies in the membrane while the novel dansyl probe localizes in the cell cytoplasm.*