कैंसर चिकित्सा हेतु प्रकाश-सुग्राहीकारक

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एक नोवेल-बोडिपी -संयुग्मित डाई द्वारा एंडोप्लाज़्मिक रेटिकुलम और लिपिड बूंदों की सटीक प्रकाश-गतिकी चिकित्सा हेतु दोहरा प्रतिबिंबन

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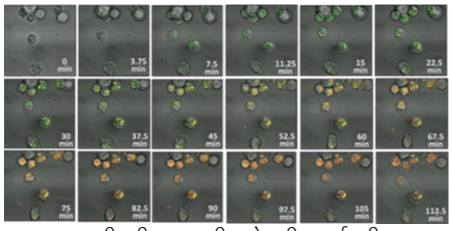

एन. बी. बी., एक बी. ओ. डी. आई. पी. वाई.-नैफ्थोलिमिन-बी. एफ. 2 डायड के कोशिकीय ग्रहण की गतिविज्ञान

सारांश

प्रकाश गतिकी चिकित्सा का अगला उत्पादन प्रकाश-सुग्राहीकता का विकसित करने पर केंद्रित है, जो संवेदनशील अंगों में कैंसर को प्रभावी ढंग से लक्षित कर सकता है। चूंकि, अग्नाशय के कैंसर हार्मोन संश्लेषण के लिए एंडोप्लाज्मिक रेटिकुलम पर बहुत अधिक निर्भर होते हैं, इसलिए अग्नाशय के कैंसर में एंडोप्लाज़्मिक रेटिकुलम (ईआर) का प्रतिबिंबन और सटीक लक्ष्यीकरण विकसित करना अग्नाशय के कैंसर के उपचार की असाध्य चुनौती को साध कर सकता है। इस संबंध में, हमने एक नए PS, एक BODIPY-नेफ्थोलिमाइन-BF2 डायड (NbB) के संश्लेषण को इंजीनियर किया है, जो एकल उत्तेजना के साथ एक साथ ER और लिपिड बूंदों (LDs) की बिंब बना सकता है और अग्नाशय के कई कैंसर कोशिकाओं के मज़बूत प्रकाश-सुग्राहीकता को प्रेरित कर सकता है। यांत्रिक रूप से, NbB कोशिकाओं में सिंगलेट ऑक्सीजन के उत्पादन और ग्लूटाथियोन के खंडन के माध्यम से गंभीर ER तनाव का कारण बनता है।

Photosensitizers for Cancer Therapy

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Precision Photodynamic Therapy Dual Imaging of Endoplasmic Reticulum and Lipid Droplets by a Novel BODIPY-conjugate Dye

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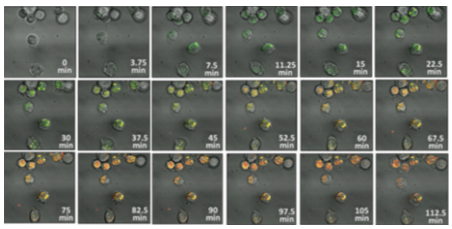

Kinetics of cellular uptake of NbB, a BODIPY-naphtholimine-BF2 dyad

ABSTRACT

Next generation photodynamic therapy is focused on developing photosensitizers (PS), which can effectively target sensitive organelles in cancer. Since, pancreatic cancers are heavily dependent on Endoplasmic Reticulum (ER) for hormone synthesis, developing imaging and precision targeting agents for ER in pancreatic cancers may address the unmet challenge of pancreatic cancer treatment. In this regard, we have engineered synthesis of a novel PS, a BODIPY-naphtholamine-BF2 dyad (NbB), which can image ER and lipid droplets (LDs) simultaneously with single excitation wavelength and induces robust photosensitization of multiple pancreatic cancer cells. Mechanistically, NbB causes severe ER stress through generation of singlet oxygen and depletion of glutathione in the cells.

KEYWORDS: *Dual imaging, Endoplasmic reticulum, Lipid droplets, Pancreatic cancer, Precision photodynamic therapy*

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Introduction

Pancreatic cancer, especially pancreatic ductal carcinoma (PDAC) is one of the most difficult to treat cancers. It remains an incurable malignancy for half a century due to no or limited therapeutic options[1]. Recently, photodynamic therapy (PDT) has emerged as a promising approach for the treatment of pancreatic cancers[2]. PDT agents like meso-Tetra (hydroxyphenyl)chlorin (mTHPC) and verteporfin showed some positive therapeutic outcomes for the treatment of PDAC patients in the clinic[3]. In order to enhance the therapeutic efficacy, next-generation PDT agents are designed to target cancer cells. Since pancreatic cancer cells heavily rely on endoplasmic reticulum (ER) for the synthesis of inherent requirements of hormones, ER is considered as an attractive target for the development of precision medicine for the treatment of PDAC[4,5]. ER is closely associated with lipid droplets, the latter is known to be positively correlated with advanced clinical staging, metastasis, and poor survival[6]. Considering the importance of ER and LDs in PDAC, there is an urgent need for the development of a precision photomedicine or photosensitizer (PS), which can (1) specifically accumulate to ER and LD, (2) image ER and LDs and (3) photosensitize PDAC cells through ER and LD stress. To the best of our knowledge, only one report shows dual imaging of ER and LDs by single probe at single excited wavelength[7]. In order to have all three properties (ER targeting, dual fluorescence imaging of ER and LD and photosensitization) in one molecule, we have chosen BODIPY based skeleton to fuse with an ER-targeting scaffold, like naphthalene moiety, for appropriate functional tuning. In this regard, we have rationally designed and synthesized a new class of bis-chromophoric fluorescent probes by fusing naphtholamine-BF2 derivative at *meso*-linked BODIPY (N*m*B) and *β*-linked BODIPY (N*b*B) (Fig.1A). Rational designing is based on the fact that position of the naphthalene moiety in N*m*B and N*b*B may influence (1) the efficient generation of triplet excited state and singlet oxygen for PDT, (2) dual fluorescence in ER and LD in polar and non-polar milieu, respectively and (3) photodamage to ER and LDs in PDAC cells.

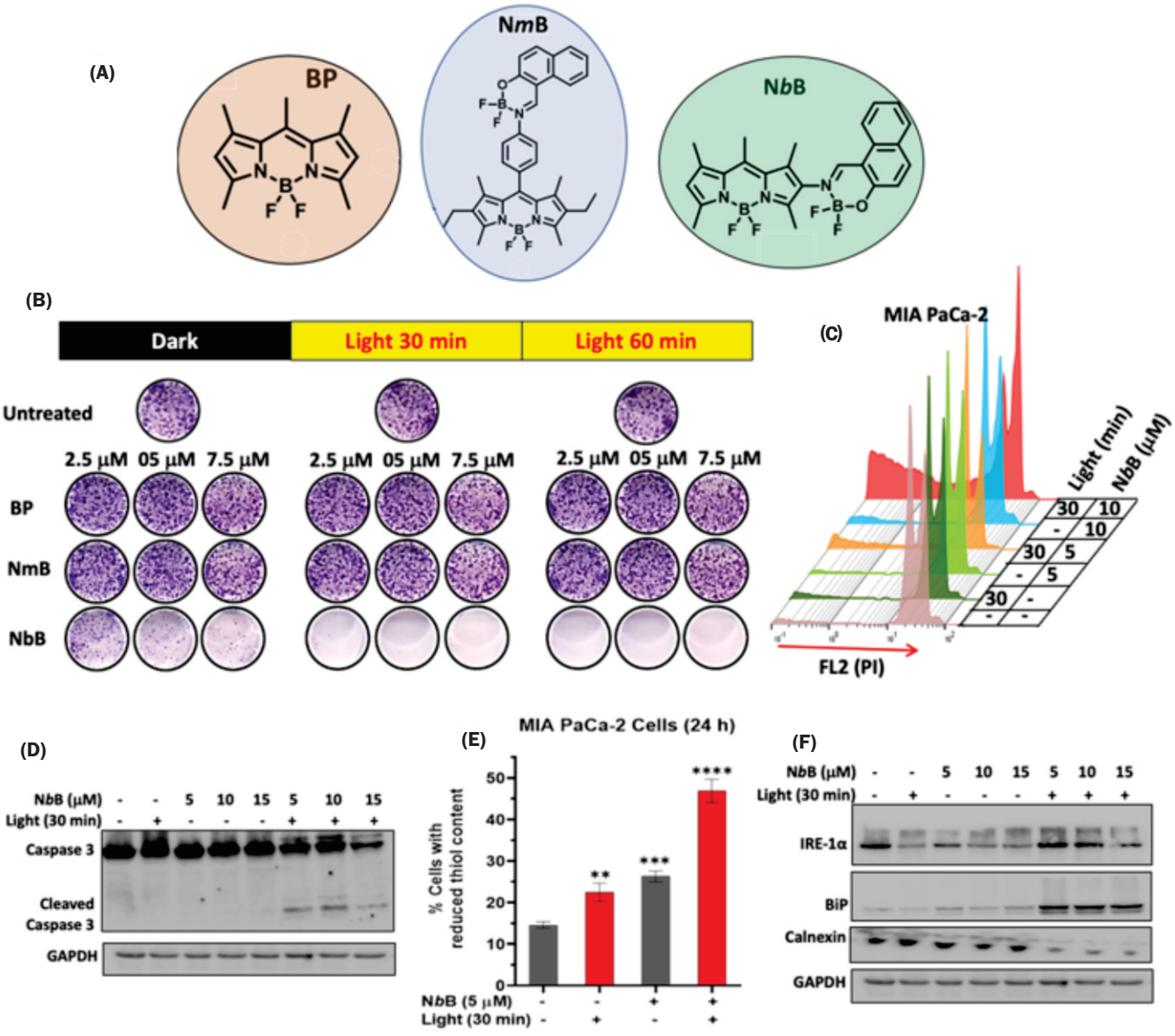

Fig.1: (A) Structure of BP, NmB and NbB. (B) Photosensitization effects of BP, NmB and NbB in MIA-PaCa-2 PDAC cells. (C, D) Sub-G1 and Caspase 3 activation analysis in response to photosensitizing effects of NbB in MIA-PaCa-2 PDAC cells. (E) Flowcytometry analysis of GSH (monobromobimane) content in cells in response to photosensitizing effects of NbB, (F) NbB mediated induction of ER stress in MIA-PaCa-2 PDAC cells in the presence/absence of light. (Adopted from our previous report[8]).

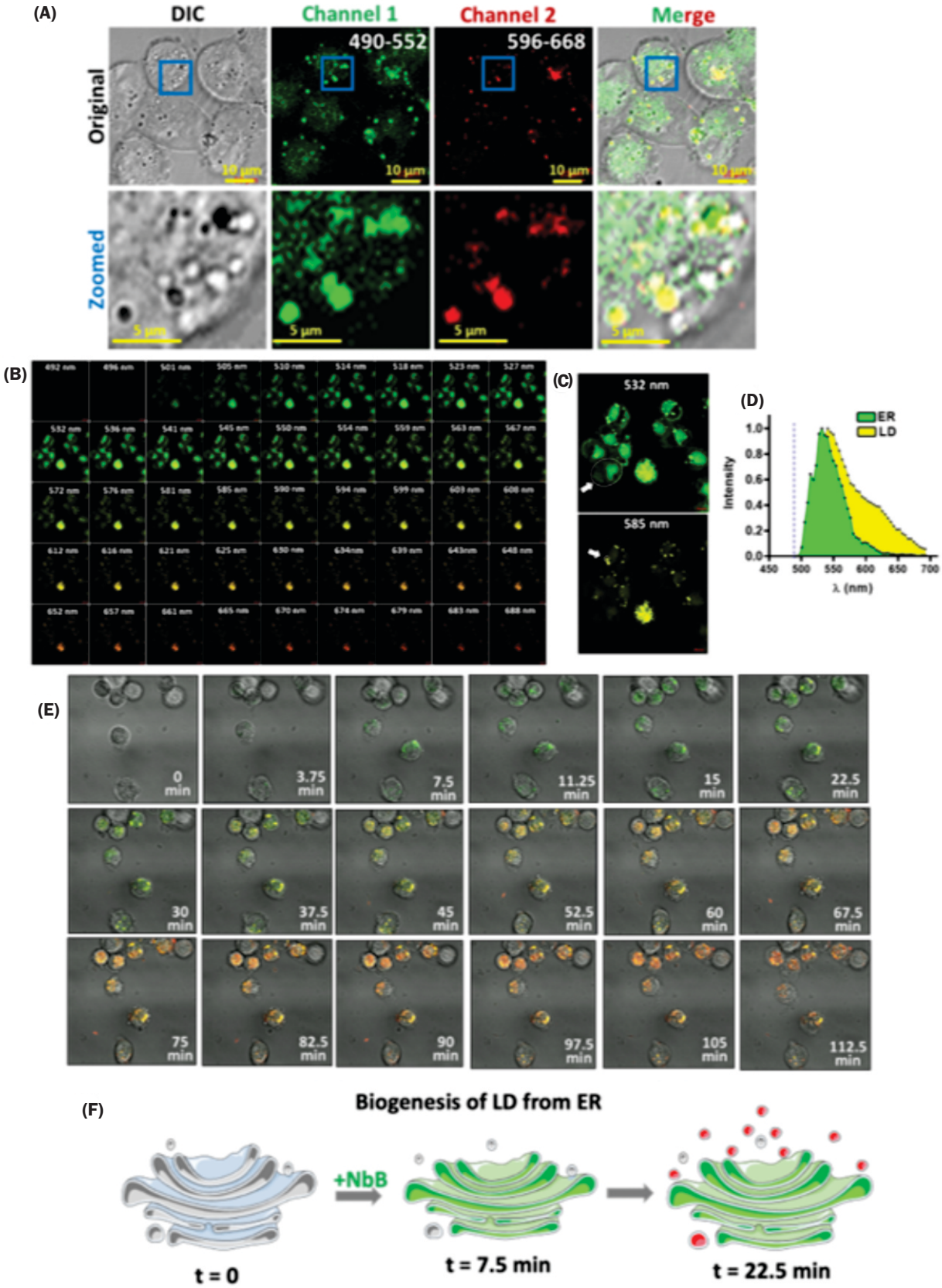

Fig.2: (A) Confocal microscopic analysis of the distribution of NbB in MIA-PaCa-2 cells, acquired in DIC, green and red channel. (B) Lambda mode spectral scanning of NbB emission in MIA-PaCa-2 cells in the range of 492-688 nm, upon excitation with 488 nm laser. (C, D) Representative images of above experiment at 532 nm and 585 nm were shown in C. ER and LD specific ROIs were marked and indicated with an arrow. Emission spectra of NbB in ER and LD specific ROIs, respectively, were shown. (E, F) Kinetics of cellular uptake of NbB and its distribution in ER and LDs in MIA-PaCa-2 cells. (Adopted from our previous report[8]).

Results and Discussion

In order to assess the photosensitization effects, PDAC (MIA-PaCa-2) cells were incubated with BP, N*m*B and N*b*B for 30 min and exposed to visible light for different time periods (Fig.1B). Our results revealed that BP and N*m*B were not effective in killing MIA-PaCa-2 cells in the dark or upon light exposure. Interestingly, N*b*B induced robust photokilling of the PDAC cells in a concentration and light dose dependent manner (Fig.1B). Dark toxicity was apparent only at higher concentrations of N*b*B. Further, Combenefit software-based

analysis showed a strong synergistic interaction of N*b*B and light for the photokilling of PDAC cells. Together, our result confirms the photosensitizing ability of N*b*B while BP and N*m*B do not have any PDT properties. Further, N*b*B found to induce robust apoptosis in MIA-PaCa-2 in the presence of light, as assessed for induction of sub-G1 by flow cytometry (Fig.1C). Similar photosensitization effects were also observed with other PDAC (PANC-1) cells. In corroboration with sub-G1 data, NbB treatment induced photoactivation of caspase-3 through its cleavage (Fig.1D). Mechanistically, NbB induced oxidative stress through lowering of cellular glutathione level and damaged ER, leading to ER stress (Fig.1E). Upon light exposure, NbB elicited the level of IRE-1α and BiP and reduced calnexin level significantly, indicating the induction of ER stress in PDAC cells by N*b*B (Fig. 1F).

Considering the impressive photosensitization properties of N*b*B, we ventured to explore its cellular distribution and photoimaging properties of N*b*B. Confocal microscopy analysis revealed a rapid cellular uptake of N*b*B, where N*b*B is localized to ER and LDs. Interestingly, upon excitation at single wavelength (488 nm), N*b*B fluoresces green in ER while it appeared both green and red in LD particles, due to polar and non-polar milieu in these two organelles, respectively (Fig. 2A).

To further confirm the dual emission fluorescence of N*b*B in ER and LDs, the fluorescence emission of the spectrum of the dye in ER and LDs was assessed by Lambda Scanning Mode (LSM) in confocal microscopy. Our data showed fluorescence emission of N*b*B in the region of 505-565 nm while fluorescence emission drops sharply at >570 nm in ER-specific ROI (region of interest), when 488 nm excitation wavelength was used (Fig.2B-D). In contrast, fluorescence emission of N*b*B was significantly retained in both green (505-565 nm) and red channels (570-638 nm) in LD specific ROI, upon excitation with 488 nm laser (Fig.2B-D). These results showed that, both ER and LD can be simultaneously visualized with one probe i.e., N*b*B at single excitation wavelength. Next, colocalization studies with commercially available dyes, ER (ER Tracker Red/Blue), mitochondria (Mito Tracker Red) and lysosomes (Lyso Tracker Red), showed that N*b*B is specifically localized to ER and LD with no apparent accumulation in lysosomes and mitochondria. In order to assess the kinetics of cellular distribution of N*b*B, time-lapse confocal imaging was employed. Our results showed a rapid uptake of N*b*B into ER (green fluorescence only; t = <10 min) initially, while during lipid droplet biogenesis, N*b*B was distributed to LDs and appeared as yellow punctate (due to emission of green and red fluorescence; t = >12 min) (Fig.2E-F). Of note, the above properties were observed with multiple cell lines with excellent photoimaging properties at concentrations as low as 0.5 μM.

Conclusion

Current investigation showed a first case of design of a novel single bis-chromophoric molecule with three biological functions: specific targeting of the dye/photomedicine to ER and LDs, simultaneous dual imaging of ER and LDs, robust photosensitization of pancreatic cancers, especially PDAC cells. A detailed investigation on the synthesis, photophysical/photochemical and biological properties and its effect on PDAC cells are reported elsewhere[8].

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