Lysosome: as a proposed target for rose bengal in inducing cell death in melanoma cells

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Received: 29 Feb 2008
Accepted: 5 May 2008
Published: 11 May 2008
Iran J Med Hypotheses Ideas, 2008, 2:12


Abstract

We have previously shown that rose bengal (RB) itself and not as a photosensitiser could induces dual modes of cell death in melanoma cells and has clinical activity against melanoma. But the mechanisms of RB-induced cell death are unclear. Recently lysosome was reported to initiate different kinds of cell death including necrosis, caspase-dependent and -independent apoptosis. The hypothesis of this study is to investigate the role of lysosome in mediating RB-induced cell death in melanoma cells focusing on lysosomal protease cathepsin B (CB). We present two lines of evidence indicating a central role for the CB in mediating cell death. First, inhibition of CB would result in a strong protection against drug-induced cell death and apoptosis in melanoma cells. Simultaneous inhibition of caspases would determine that CB acts upstream or downstream the caspase cascade. Second, we show RB triggers disruption of lysosomes leading to release and activation of CB using an engineered yellow fluorescent protein-tagged CB.

It is hypothesized that RB-induced cell death in melanoma cells is mediated through lysosomal CB in this novel cell death pathway. This idea points to a new tumor-suppressive role for lysosomes which may be less affected by chemotherapy-induced resistance mechanisms.

Keywords
Lysosome, Cathepsin B, Melanoma, Rose Bengal

Introduction

Over the past decade, it has become known that many therapeutic agents kill cancer cells by inducing apoptosis or necrosis (1,2). Apoptosis is a programmed cell death is characterized by distinct morphological features including: chromatin condensation, cell and nuclear shrinkage, membrane blebbing and oligonucleosomal DNA fragmentation. Three major apoptotic pathways originating from separate subcellular compartments have been identified the death receptor, mitochondrial and the endoplasmic reticulum pathway (3,4,5). Although each pathway is initially
mediated by different mechanisms, they share a common final phase of apoptosis, consisting of activation of the executioner caspases and dismantling of substrates critical for cell survival (6,7).

However, apoptosis signaling mechanisms induced by many agents are impaired in tumor cells, leading to resistance against therapy. Lysosomes and the endoplasmic reticulum (ER) hold promise as drug targets and mediators of apoptosis signaling which may be less affected by intrinsic or chemotherapy-induced resistance mechanisms (8,9). Recently lysosome was reported to initiate different kinds of cell death including necrosis, caspase-dependent and -independent apoptosis. Lysosomes proteases such as cathepsins may account for these alternative types of PCD leading to a new tumor-suppressive role for lysosomes which may be less affected by resistance mechanisms (10,11,12).

Hypotheses

Rose Bengal (RB) has been used as a systemic diagnostic of hepatic function, ophthalmic diagnostic and photosensitiser in photodynamic treatment. We have previously found that RB itself and not as photosensitiser could induces dual modes of cell death (apoptotic and non-apoptotic cell death) in melanoma cells and has clinical activity against melanoma (13,14,15). RB-induced apoptosis was both caspase-dependent and -independent. This toxicity was also independent of Reactive Oxygen Species (ROS) production. Taking together the mechanisms of RB-induced toxicity are unclear (14,15).

Lysosome could be considered as a one of the proposed targets for the selective RB-induced toxicity in melanoma cells. Rupture of lysosomes, leading to the release of their cathepsins content, has long been recognized as potentially harmful to the cell (16). It has been shown that lysosomal cathepsins including B, D, and L translocate from the lysosomal lumen to the cytosol in response to a variety of signals such as TNF receptor ligation, p53 activation, oxidative stress and also by lysosomotropic agents such as cyprophloxacin and hydroxychloroquine (11). In this study lysosome is considered as a target for RB-induced toxicity in melanoma cells focusing on CB. We present two lines of evidence indicating a central role for the CB in mediating cell death. First, inhibition of CB would result in a strong protection against drug-induced cell death and apoptosis in melanoma cells. Simultaneous inhibition of caspases would determine that CB acts upstream or downstream the caspase cascade. Second, we show RB triggers disruption of lysosomes leading to release and activation of CB using an engineered yellow fluorescent protein-tagged CB.

Evaluation of the hypotheses

Human melanoma cell line, SK-Mel-28, described previously is cultured in DMEM containing 5% FCS. (13,14). Cells are seeded overnight, and then incubated with RB in the dark. SK 28 cells are treated with pan-caspase inhibitor z-VAD-fmk, or CA-074 Me as a selective inhibitor of CB, 1 h before adding RB (200 µM) for another 24 h.

Cell viability is measured by MTT cell proliferation assay according to the literature. (13,14) The extent of apoptosis is determined by flow cytometry, using propidium iodide (Sigma) staining of hypodiploid DNA-content, as previously described (12,13,17).

Lysosomal integrity assays are performed with the lysosomotropic probe LysoTracker Red according to the literature (18). For studying of CB release, a yellow fluorescent protein-tagged CB is cloned into the expression vector pEFYFPC1, to allow easy detection by fluorescence microscopy. First full length human CB cDNA is generated by reverse transcription-PCR (5'-ATC TGT TCT CGA GCT ATG TGG CAG CTC TTA GAT CTT TTC GCT ATG TGG CAG CTC TTA GAT CTT TTC GCA GTA CTG-3'). Then it is cloned into the pEFYFPC1 vector. Cells are seeded onto glass coverslips in a 6-well tray and are transfected with 1.5 µg of plasmid DNA using the FuGene6 transfection reagent (Roche Molecular Biochemicals), according to the manufacturer’s protocol (19).

CB activity is determined fluorimetrically using the methyl-coumarylamide substrate z-Arg-Arg-NHMec at pH 6.0, as described in literature (20). Fluorescence is measured with an excitation wavelength of 360 nm and emission wavelength of 460 nm. One unit of enzyme activity is defined as the release of 1 µmol of product/min.

Experimental data

Effect of pan-caspase and CB inhibitor on RB-induced cell death

To elucidate pathways mediating cell death induced by RB, the contribution of CB to the cytotoxic potential of RB is evaluated. For this purpose, RB-induced cell death and apoptosis are studied in presence of pan-caspase inhibitor z-VAD-fmk or CA-074 Me as a selective inhibitor of CB. CA-074 Me is a highly specific inhibitor of CB in vitro and easily penetrates into cells (21). The percentage of apoptosis in the presence of z-VAD-fmk, is considered as caspase-independent apoptosis.

Effect of RB on lysosome integrity, CB release and activity

To assess whether treatment with RB results in a lysosomal membrane permeabilization, lysosomal integrity assays is performed using the
lysosomotropic fluorescence probe Lysotracker Red. In cells treated with RB, increased number of cells with a weak fluorescence (so-called “pale cells”) is detected, a phenomenon reported to be due to leakage of the probe into the cytoplasm, indicating lysosomal rupture. Next we would show whether the disruption of the lysosomal integrity is consistent with release of CB using an expression plasmid encoding yellow fluorescent protein-tagged CB. If there is a diffuse staining pattern of the fusion protein throughout the cells after treatment, indicates CB is released from the lysosomes of melanoma cells on incubation with RB. To examine the activation of CB after its release into the cytoplasm, CB activity is performed. Increased CB activity after treatment with RB indicates role of CB in RB-induced cell death.

Discussion

Cross-resistance of cells to cytotoxic effects of natural and synthetic anticancer drugs is the well-known phenomenon called multidrug resistance (MDR). The sensitisation of MDR-resistant cells to anticancer drugs by lysosomotropic compounds recommends scrutinizing the potential of lysosomotropic drugs in cancer therapy (22).

In regard to melanoma there has been little progress in the medical treatment of metastatic melanoma because of its resistance to current chemotherapeutic agents (23). We have recently reported that RB triggers pronounced cell death in melanoma cells via apoptotic- and non-apoptotic routes by unknown mechanism. In this study, we proposed lysosome as a target for RB in melanoma cells. If inhibition of CB strongly protects against cell death, indicates that CB is the mediator of RB-induced cell death in melanoma cells. If treatment with RB, results in disruption of lysosomal membrane but not activation of CB, role of other cathepsins such as D, and L, should be explored. We have shown that both caspase-dependent and -independent pathways were induced by RB in melanoma cells (14,15). If CB inhibitor completely inhibits RB-induced apoptosis, it indicates CB triggers both caspase-dependent and caspase-independent pathways in melanoma cells and caspase activation is downstream to CB activation (Figure 1). CB has been reported to contribute to apoptosis via induction of mitochondrial membrane permeabilization, possibly via cleavage of Bid, in some systems, thereby acting upstream of the caspase cascade (24, 25, 26). If inhibition of CB, only blocks the caspase-independent apoptosis, in this respect it could be conclude that RB initiate only caspase-independent apoptosis pathways which has been shown by other studies (27,28).

Strong evidence is now accumulating for the involvement of alternative proteases, such as CB, in PCD (29), but the molecular identity of the mediators and the necessity of activation of the apoptotic pathways remain to be elucidated in most cases and may vary on the type of cells and the applied death stimulus (30).

Taken together, if this idea is verified, it emphasizes using of lysosomotropic drugs in cancer therapy. It is concluded that RB-induced cell death in melanoma cells is mediated through lysosomal CB in the novel cell death pathway. This hypothesis points to a new tumor-suppressive role for lysosomes which may be less affected by chemotherapy-induced resistance mechanisms.

Acknowledgements

The authors would like to thank Dr Peter Heresy (Professor of Immunology and Oncology, Newcastle, Australia) for supervising the previous studies.
Fig. 1. Proposed model for RB-induced different kind of cell death in melanoma cells: Role of cathepsin B

t-VAD-fmk: pan-caspase inhibitor
CA-074 Me: selective cathepsin B inhibitor
AIF: apoptosis inducing factors

References


