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REVIEW ARTICLE

MG132, a proteasome inhibitor, induces apoptosis in tumor cells

Na GUO and Zhilan PENG

Department of Obstetrics and Gynecology, West China Second University Hospital, Sichuan University, Chengdu, China

Abstract

The balance between cell proliferation and apoptosis is critical for normal development and for the maintenance of homeostasis in adult organisms. Disruption of this balance has been implicated in a large number of disease processes, ranging from autoimmunity and neurodegenerative disorders to cancer. The ubiquitin–proteasome pathway, responsible for mediating the majority of intracellular proteolysis, plays a crucial role in the regulation of many normal cellular processes, including the cell cycle, differentiation and apoptosis. Apoptosis in cancer cells is closely connected with the activity of ubiquitin–proteasome pathway. The peptide-aldehyde proteasome inhibitor MG132 (carbobenzoxyl-L-leucyl-L-leucyl-L-leucine) induces the apoptosis of cells by a different intermediary pathway. Although the pathway of induction of apoptosis is different, it plays a crucial role in anti-tumor treatment. There are many cancer-related molecules in which the protein levels present in cells are regulated by a proteasomal pathway; for example, tumor inhibitors (P53, E2A, c-Myc, c-Jun, c-Fos), transcription factors (transcription factor nuclear factor-kappa B, IκBα, HIFI, YYI, ICER), cell cycle proteins (cyclin A and B, P27, P21, IAP1/3), MG132 induces cell apoptosis through formation of reactive oxygen species or the upregulation and downregulation of these factors, which is ultimately dependent upon the activation of the caspase family of cysteine proteases. In this article we review the mechanism of the induction of apoptosis in order to provide information required for research.

Key words: anti-tumor, apoptosis, MG132, proteasome inhibitor, tumor cells.

INTRODUCTION

The balance between cell proliferation and apoptosis is critical for normal development and for the maintenance of homeostasis in adult organisms. The disruption of this balance has been implicated in a large number of disease processes, ranging from autoimmunity and neurodegenerative disorders to cancer. Although apoptosis can be triggered by many diverse intracellular and extracellular stimuli, once initiated a conserved set of molecules are recruited for the execution of the cell death program, which is ultimately dependent upon the activation of the caspase family of cysteine proteases.[1]

Correspondence: Dr Zhilan Peng MD, Department of Obstetrics and Gynecology, West China Second University Hospital, Sichuan University, Chengdu 610041, China. Email: guona507@yahoo.cn

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The ubiquitin–proteasome pathway, responsible for mediating most intracellular proteolysis, plays a crucial role in the regulation of many normal cellular processes, including the cell cycle, differentiation and apoptosis. There are many cancer-related molecules in which the protein levels present in the cells are regulated by proteasomal pathway, for example, tumor inhibitors (P53, E2A, c-Myc, c-Jun, c-Fos), transcription factors (nuclear factor-kappa B [NF-κB], IκBα, HIFI, YYI, ICER), cell cycle proteins (cyclin A and B, P27, P21, IAP1/3). Apoptosis in cancer cells is closely connected with the activity of ubiquitin–proteasome pathway. The ubiquitin–proteasome pathway is a highly specific extralysosomal system for the selective degradation of short-lived proteins. The proteasome is present in the nucleus and cytoplasm of all eukaryotic cells as a highly conserved multi-catalytic enzyme complex. Proteins that are degraded by the ubiquitin–proteasome mechanism are first conjugated to ubiquitin, a 76 amino acid, highly

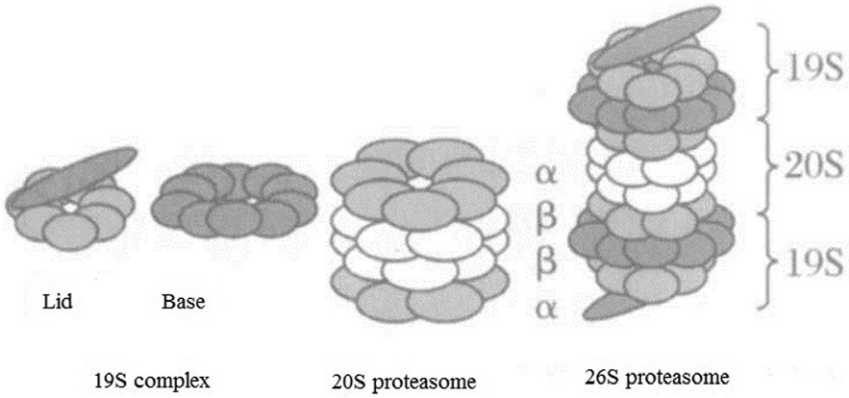

Figure 1 Schematic diagram of proteasome structure.

conserved residue. Ubiquitinated proteins are recognized by the 26S proteasome, which hydrolyzes and degrades ubiquitinated protein substrates, including transcription factors, cell cycle regulators and apoptotic proteins. The 26S proteasome is composed of a 20S core protease, in which proteins are digested to short peptides, and one or two 19S regulatory particles, which confer adinosine triphosphate (ATP) dependence and substrate specificity (Fig. 1).[2] The 20S proteasome, the large core unit with a molecular mass of approximately 700 kDa is made up of multiple subunits and performs several peptidolytic functions to maintain cellular homeostasis by acting as an important disposal mechanism.[3] The 20S proteasome is a cylindrical particle composed of four stacked rings, making it look like a barrel. The rings form a tunnel in which the target proteins are hydrolyzed, after which ubiquitin is released, to be reused in the proteolytic pathway. The 19S recognizes and binds to polyubiquitylated proteins, releases the attached ubiquitinated proteins as free monomers, unfolds the protein substrates and directs the unfolded proteins into the 20S lumen for degradation. In this way, ubiquitinated proteins conjugation, the proteasome architecture and their linkage to ATP hydrolysis ensure that only unwanted proteins are selectively degraded.[4]

The peptide-aldehyde proteasome inhibitor MG132 (carbobenzoxyl-L-leucyl-L-leucyl-L-leucinal) is a natural triterpene proteasome inhibitor derived from a Chinese medicinal plant and is able to suppress the growth of human prostate cancer in nude mice. It is a peptide aldehyde that inhibits 20S proteasome activity by covalently binding to the active site of the beta subunits and effectively blocks the proteolytic activity of the 26S proteasome complex (Fig. 2). MG132 inhibits the growth of tumor cells by inducing the cell cycle arrest as

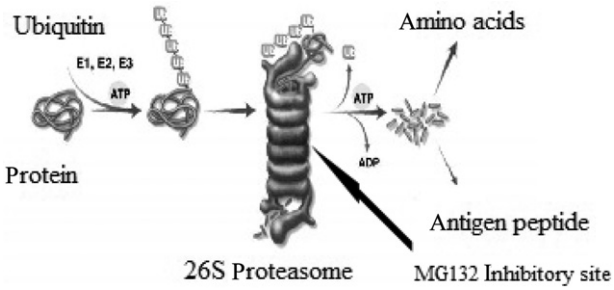

Figure 2 The inhibitory pathway of MG132. ADP, adenosine diphosphate; ATP, adinosine triphosphate.

well as triggering apoptosis.[5] The pathway of induction of apoptosis can be divided, as discussed below.

MG132 INDUCES APOPTOSIS THROUGH FORMATION OF REACTIVE OXYGEN SPECIES (ROS)

MG132 has been shown to induce apoptotic cell death through the formation of ROS. ROS formation and glutathione (GSH) depletion due to proteasome inhibitors may cause mitochondrial dysfunction and subsequent cytochrome release, which leads to cell viability loss.[6] Enhanced oxidative stress and defects in mitochondrial function are involved in the induction of apoptotic cell death. The membrane permeability transition of mitochondria is recognized as a central event in the course of toxic and oxidative forms of cell injury. The opening of the mitochondrial permeability transition pore causes a depolarization of the transmembrane potential, the release of Ca2t and cytochrome c and the loss of oxidative phosphorylation, which results in the loss of cell viability[7] (Fig. 3).

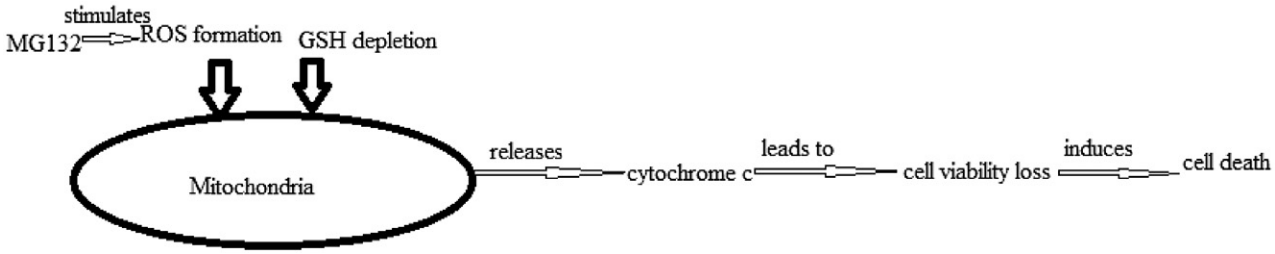

Figure 3 Schematic diagram of MG132-induced cell apoptosis through formation of reactive oxygen species (ROS). GSH, glutathione.

ROS include hydrogen peroxide (H_2O_2), superoxide anion and hydroxyl radical.[8] These molecules have recently been implicated in regulating many important cellular events including transcription factor activation, gene expression, differentiation and cell proliferation.[9] GSH is the main non-protein antioxidant in the cell and provides electrons for enzymes such as glutathione peroxidase, which reduce H_2O_2 to H_2O. GSH has been shown to be crucial for cell proliferation, cell cycle progression and apoptosis and is known to protect cells from toxic insult by detoxifying toxic metabolites of drugs. Although cells possess antioxidant systems to control their redox state, which is important for their survival, the excessive production of ROS can be induced and gives rise to the activation of events that lead to death or survival in different cell types.[10] ROS formation and glutathione depletion due to proteasome inhibitors may cause mitochondrial dysfunction and subsequent cytochrome c release, which leads to cell viability loss.[11]

The mitogen-activated protein kinases (MAPK) are a large family of serine/threonine kinases, which are major components of signaling pathways in cell proliferation, differentiation and cell death.[12] There are currently four known MAPK: the extracellular signal-regulated kinase (ERK1/2), the c-Jun N-terminal kinase (JNK) stress-activated protein kinase and the p38. Each MAPK pathway has relatively different upstream activators and specific substrates. ROS are known to induce ERK phosphorylation and activate the ERK pathway. There is evidence that JNK and p38 are strongly activated by ROS or by a mild oxidative shift of the intracellular thiol–disulfide redox state, leading to apoptosis. In relation to MAPK activities in MG132-treated cells, MG132 increases the activities of ERK and p38 but does not affect JNK activity. The signaling pathways of MAPK are involved in the inhibition of growth but are not closely related to cell death by MG132, because MG132 dose not activate JNK in cells. The enhancement of growth inhibition of MG132-treated cells by JNK inhibitor probably results from the downregulation of basal JNK activity and induces apoptosis.[13]

MG132 INDUCES APOPTOSIS THROUGH COOPERATION WITH APO2L OR TUMOR NECROSIS FACTOR (TNF)-RELATED APOPTOSIS INDUCING LIGAND (TRAIL)

TRAIL is a novel cytokine that belongs to the TNF family of ligands and is a potentially important anticancer agent awaiting clinical trials. Unfortunately, some cancer cells exhibit resistance to TRAIL, which could limit the use of this potentially promising anticancer agent. TRAIL has attracted considerable attention as a novel anticancer agent since it appears to selectively induce apoptosis in cancer cells but not in normal cells of various tissues types.[14]

TRAIL is expressed as a type 2 membrane protein and also exists as a soluble form after proteolytic cleavage of its extracellular domain. TRAIL mediates its apoptotic effects by binding to its membrane death receptors including death receptor 4 (DR4) and DR5 (also known as TRAIL-R1 and TRAIL-R2, respectively).[15] TRAIL also binds to two other receptors named TRAIL-R3 and TRAIL-R4. These receptors are called anti-apoptotic decoy receptors since TRAIL-R3 is a glycosylphosphatidylinositol -linked molecule lacking the death domain, whereas TRAIL-R4 carries a deleted version of the death domain and accordingly, neither can transduce apoptotic signals.[16]

By upregulating the TRAIL death receptor DR5, MG132 cooperates with TRAIL to induce apoptosis in general and in TRAIL-resistant human cancer cells in particular. The combination of MG132 and TRAIL induce the activation of caspase-8 and caspase-3, and

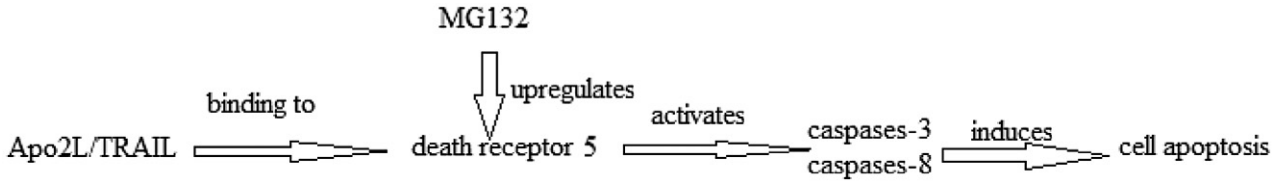

Figure 4 Schematic diagram of MG132-induced cell apoptosis through cooperation with Apo2L/tumor necrosis factor-related apoptosis inducing ligand (TRAIL).

that of the intrinsic pathway (including cytochrome c and Smac release and caspase-9 activation). The combination of both agents also promotes caspase-8 and caspase-3 activation. The combination of MG132 and TRAIL, by engaging DR5, that is the extrinsic pathway, appears to overcome the Bax deficiency-induced defects in the intrinsic pathway and induce apoptosis in tumor cells[14] (Fig. 4).

MG132 INDUCES APOPTOSIS THROUGH TRIMERIZATION OF HSF1

Heat shock proteins, in addition to their role in protecting cells from thermal stress, have been shown to play multiple roles in cell cycle progression, cell development and cell differentiation, signal transduction and apoptosis. Heat shock factors (HSF) are a multi-gene family of transcription factors involved in the transcriptional regulation of heat shock genes.[17] HSF is essential for the induction of heat shock proteins and for the acquisition of thermotolerance in mammalian cells.[18]

Among the various members of HSF gene family, HSF1 is specifically involved in the transactivation of stress-inducible *hsp* genes and is the key factor in the regulation of hsp gene expression. Upon heat shock, HSF1 trimerizes, acquires DNA-binding ability and becomes hyperphosphorylated.[19]

In the presence of MG132 the activity of protein kinase C, casein kinase II and P38 MAPK targeting HSF1 is upregulated through the ubiquitin–proteasome dependent pathway.[20] Furthermore, kinases targeting HSF1 upon heat shock and kinases targeting HSF1 during MG132 treatment appear to be distinct, reconfirming that activation of heat shock response is under the control of multiple complex signaling pathways. HSF1 is capable of efficiently activating transcription only after heat shock. The increase in transcriptional activity is correlated with an increase in the phosphorylation of HSF1, and it has been proposed that this hyperphosphorylation is responsible for the increase in HSF's activation potential, although no causal link has been established. MG132 has been confirmed that the activity of HSF1 is highly regulated both at the level of DNA binding and at the level of transcriptional activation.[20]

MG132 induces hyperphosphorylation and trimerization of HSF1, and transactivates heat shock genes at 37°C. The upregulated protein kinase(s) may efficiently phosphorylate HSF1, or facilitate HSF1 to remain in its hyperphosphorylated state, and prevent the recovery of hyperphosphorylated HSF1 to the pre-heat-shocked, dephosphorylated state. Alternatively, MG132 treatment may lead to the phosphorylation of HSF1 at amino acid residues that were different from those induced by heat shock, and a different form of phosphorylated HSF1 may have a different impact on its transactivation activity.

MG132 INDUCES APOPTOSIS THROUGH NF-KB INHIBITION

The transcription factor nuclear factor-kappa B (NF-κB) plays an important regulatory role in the control of inflammation, oncogenic transformation, tumor progression and the acquisition of resistance to anticancer drugs. The NF-κB family comprises p50/p105, p52/p100, p65, c-Rel and RelB proteins that exist in an inactive state as homodimers or heterodimers bound to inhibitory NF-κB proteins in the cytoplasm.[21] Diverse stimuli, including TNF alpha and oxidants, activate the IκB kinase complex, which phosphorylates and triggers the proteasome-dependent degradation of IκB-alpha, the predominant NF-κB inhibitory molecule. NF-κB then translocates to the nucleus and modulates transcription by binding to specific DNA sequences in target promoters.[22]

There are multiple mechanisms by which NF-κB promotes cell survival. Target genes induced by NF-κB that are important for the control of cell survival include the anti-apoptotic bcl-xL, cFLIP, cIAP1/2 and Bcl-2, and

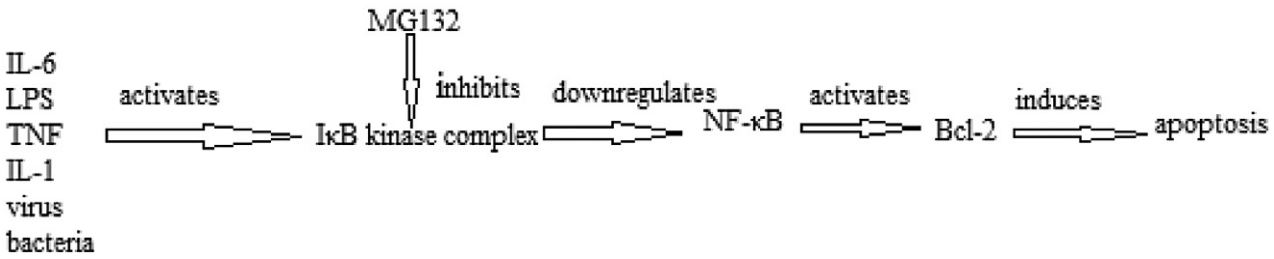

Figure 5 Schematic diagram of MG132-induced cell apoptosis through nuclear factor-kappa B (NF-κB) inhibition. Il, interleukin; LPS, lipopolysaccharide; NIK, NF-κB-inducible kinase; TNF, tumor necrosis factor.

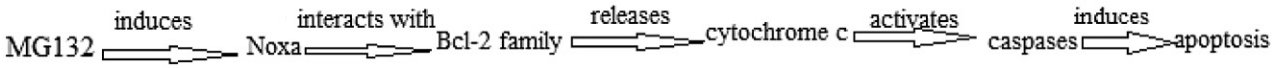

Figure 6 Schematic diagram of MG132-induced cell apoptosis through p53-independent pathway.

the antioxidants superoxide dismutase[22] and ferritin heavy chain,[23] which act to prevent the pro-apoptotic machinery activation. NF-κB overstimulation has been associated with cellular resistance to classical chemotherapeutic agents such as doxorubicin, etoposide and imatinib, and it has been correlated with chemotherapy resistance and therapy failure *in vivo*.

MG132 acts as a powerful apoptotic agent in several tumor cell lines, and much of the MG132 activity has been attributed to NF-κB inhibition through the inhibition of IκB degradation.[24] Since the degradation of inhibitors of κB proteins (IκBα and IκBβ) is essential for the canonical pathway of NF-κB activation and the aberrant expression of anti-apoptotic protein Bcl-2 is regulated in part by IκB, the inhibition of proteasome function has emerged as a useful strategy to manipulate programmed cell death[25] (Fig. 5).

MG132 INDUCES APOPTOSIS THROUGH P53-INDEPENDENT PATHWAY

The tumor suppressor protein p53 is known to mediate apoptosis induced by the DNA tumor virus oncoproteins, adenovirus E1A and SV40 T antigen,[26] it activates cellular death programs through multiple pathways. Because the high frequency of p53 mutations in human tumors is believed to contribute to resistance to commonly used chemotherapeutic agents, it is important to identify drugs that induce p53-independent cell death and to define the mechanisms of action of such drugs.[27]

Noxa is a pro-apoptotic BH3-only member of the Bcl-2 family of proteins that is upregulated at a transcriptional level by the nuclear protein p53 in response to cellular stresses such as DNA damage or growth factor deprivation. Noxa is able to interact with anti-apoptotic members of the Bcl-2 family and causes the release of cytochrome c into the cytosol, leading to the activation of caspases and the induction of apoptosis.[28] MG132 inhibits the growth of several tumor cells independently of p53 status and it induces the pro-apoptotic protein Noxa via a p53-independent mechanism that leads to caspase-dependent apoptosis[29] (Fig. 6).

In summary, the proteasome inhibitor MG132 induces the apoptosis of cells, although the pathway of induction of apoptosis is different, it plays a crucial role in anti-tumor treatment. Here, we review the mechanism of the induction of apoptosis, in order to provide information for research.

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