Review/ Derleme

Evidences for the presence of caspase-like activities in plants

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Abstract

Organizing the activation of cell division and cell death in animals and plants may lead a variety of developmental processes for instance determining of cells, tissues and organs. But, cell death also is used in a number of other processes such as control of cell populations and defense against invading microbes. Beyond the similarities between plant and animal programmed cell death (PCD), the molecular mechanisms underlying the cellular changes in plant remain unclear. In particular there is no indication in plant genomes for genes orthologous to caspases. Proteases not connected to caspases are thought to be related in PCD in plant cells in recent years there are numerous studies showing that caspase inhibitors can suppress cell death in plants. The aim of this review is to focus on recent advances in PCD molecular mechanisms in plants.

Keywords: PCD, plant, caspases

Kaspaz benzeri aktivitelerin bitkilerdeki varlığına ilişkin kanıtlar

Özet

Hayvanlarda ve bitkilerde hücre bölünmesi ve ölümü olayları ile ilgili aktivitelerin organizasyonu hücrelerin, dokuların ve organların belirlenmesi gibi farklı olaylara neden olabilir. Fakat hücre ölümü aynı zamanda hücre sayısının kontrolü ve mikropların saldırısından korunma gibi daha birçok olaylarda kullanılmaktadır. Bitki ve hayvanlarda programlı hücre ölümünde benzerlikler olmasına rağmen bitkilerde meydana gelen hücresel değişikliklerin moleküler mekanizması hala açıklık kazanmamıştır. Özellikle bitki genomunda kaspazlarla ortoloji gösteren genler belirlenememiştir. Bitkilerdeki programlı hücre ölümü ile ilgili olduğu düşünülen proteazlar kaspazlarla ilişkili değildir. Birçok araştırma kaspaz ket vurucularının bitkilerde programlı hücre ölümünü baskıladığını ortaya koymuştur. Bu makalenin amacı bitkilerde meydana gelen programlı hücre ölümünün moleküler mekanizmasına ilişkin son gelişmeleri derleyebilmektir.

Anahtar kelimeler: Programlı hücre ölümü, bitkiler, kaspazlar

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Introduction

Characteristic apoptotic properties have not been determined in all the cases of plant programmed cell death (PCD), the studies do indicate the existence of apoptotic machinery in plant cells. In mammalian cells, typical hallmarks of apoptosis are assigned to caspase-mediated processing of specific target molecules such as the activation of a caspase-activated DNase [1]. It is tempting to speculate that like caspase mediated proteolytic events reason the apoptotic phenotype observed in dying plant cells. In this review we are going to concentrate on the recent findings in relation to the involvement caspase-like specificity in PCD.

Programmed Cell Death

The cells of multicellular organisms are members of highly organized community. Controlling the rate of cell division and of cell death precisely regulates the number of cells in this community strictly. If cells are no more needed, they die by activating intracellular death program, for this reason this process named as PCD and regularly apoptosis. The term apoptosis comes from plant kingdom from old Greek apoptosis that originally means the loss of petals or leaves. Unpredictably, despite the evident role of cell death in plants the concept of PCD is developed and established in animal and medical sciences.

Apoptosis is characterized by certain features like cell shrinkage, blebbing of the plasma membrane, condensation and fragmentation of the nucleus in animal cells, and internucleosomal cleavage of DNA (Table 1). The final stage of apoptosis is the fragmentation of the cell into cellular debris-containing vesicles called "apoptotic bodies" that are being phagocytosed by other cells.

Table l. Apoptotic parameters (Adapted from Studzinski, [2]).

Apoptosis detection parameters	Specifity
Morphological features	Membrane blebbing Shrinking cytoplasm Nucleus condensation Apoptotic body formation
DNA fragmentation	DNA strands break Apoptotic DNA fragments (DNA laddering)
Proteases activation	Caspases
Cell membrane alterations	Phosphatidylserine translocation
Mitochondrial changes	Alteration of mitochondria membrane Release of Cytochrome c and apoptosis inducing factor

The level of apoptosis that happens in developing vertebrate nervous system and adult animal system is astonishing. In the developing vertebrate nervous system half or more of the nerve cells typically die soon after the formation. In healthy adult human, every hour billions of cells die in the bone marrow and intestine. What is the purpose of this massive cell death?

A molecular mechanism for removing unnecessary cells is basic for successful development and growth of complex multicellular organisms. In addition to regulating the rate of cell division, animals and plants contain a biochemical pathway to control cell death. By coordinating the activation of cell division and cell death, animals and plants may direct a variety of developmental processes such as shaping of cells, tissues and organs. However, cell death also be used in a number of other processes such as control of cell populations and defense against invading microbes [3, 4, 5, 6, 7].

Cells that die in consequence of damage, typically swell and burst and they spill their content all over the neighbors. This process named as necrosis and it reasons inflammatory response in animals. By contrast, a cell that undergoes apoptosis dies without damaging neighbors. The cell shrinks and condenses. The cytoskeleton collapses, nuclear envelope disassembles and nuclear DNA breaks up into fragments. Apoptotic bodies that are formed during apoptosis are engulfed and recycled by neighboring cells or specific macrophages; therefore complete elimination of the cell occurs.

Caspases: The enzymes of death

The caspases are a distinctive family of cysteinyl proteases, which cleave proteins next to an aspartate residue. In addition to a fundamental role of caspases in the initiation and execution phases of apoptosis, the enzymes have some other non-apoptotic roles in living cells. During apoptosis, upon activation, caspases cleave specific substrates and in that way mediate many of the typical biochemical and morphological changes in apoptotic cells. Therefore determination of activated caspases can be used as a biochemical marker for apoptosis produced by different stimuli in various types of cells [8].

This family named caspases (cisteinyl aspartate-specific proteases) in fact has been shown to play an essential function in apoptosis. The significance of caspases in apoptosis was documented as follows:

1. Over-expression of caspases kills cells
2. Inhibitors of caspases inhibit apoptosis induced by various stimuli
3. Knock-out animals lacking certain caspases show defects in apoptosis.

The caspase family proteases consist of at least 14 mammalian members. Phylogenic analysis revealed that gene family is composed of two major subfamilies, which are related to either caspase-1 (ICE) or the mammalian homologues of CED-3 [9, 10]. CED-3 subfamily appears to be involved mainly in cell death. According to the sequence analysis, CED-3 family

subdivided into two groups, namely the caspase-3 and caspase-2 subfamilies. Caspases are constitutively expressed in the majority of cells as inactive pro-enzymes (zymogens) that become proteolytically processed and activated in response to variety pro-apoptotic stimuli. The pro-caspases contain several domains: an N-terminal pro-domain, a large subunit (17-21 kDa) and a small subunit (10-13 kDa); some proteins have a short linker between these subunits [11].

PCD in Plants

There are various examples of cell death during plant development that correspond to the general description of PCD for example cell death during xylogenesis, aerenchyma formation, plant reproductive processes, leaf and petal senescence, and endosperm development [12]. Additionally, cell death is reaction to the pathogen attack and in response to a variety of abiotic factors such as ozone and UV radiation also agrees with the definition of PCD. A number of morphological relationships were found between animal cells undergoing apoptosis and dying plant cells, including shrinkage of the cytoplasm and nucleus, DNA and nuclear fragmentation, and the formation of DNA-containing bodies [13].

PCD in plants can be compared with apoptosis and with autophagic apoptosis in animals [14]. These types of apoptosis in animals can be caspase-dependent or caspase independent. A number of studies have shown that protease inhibitors can suppress PCD in various plant species and plant tissues. This is especially true of caspase inhibitors that can block PCD and its marker DNA laddering. There are up to six different caspase-like activities that can be measured in plant extracts, the most prominent being caspase1-like and caspase3- like. This represents a striking apparent similarity with animal PCD. Because there are no caspase orthologue in plant genomes, a major challenge is to identify these proteases [15].

Plant cells have walls that may effect as physical barriers preventing the recycling of cellular material from dead cells by apoptotic bodies; this is dissimilar to animal cells. For this reason, recycling of cellular content from dead cells may occur by degradation of cell fragments to compounds with low molecular weight and neighbor cells receive them. This kind of process releasing cellular debris into the intercellular space would have reasoned an inflammatory response in animals, but plants are different from animals, because they have no immune response. Morphogenesis in plants is mainly established by cell division and cell death but no cell migration contrasting animal morphogenesis. Another feature of plant life that involves PCD is the relations interaction of plants with their environment. Thus the defense of plants against biotic and abiotic stresses often involves activation of apoptosis. Consequently function of cell death is similar but mechanisms involved are very different and specific for particular organisms.

Beyond the similarities between plant and animal PCD, the molecular mechanisms underlying the cellular changes in plant remain unclear [16]. Especially, there is no indication in plant genomes for genes orthologous to caspase or Bcl-2 proteins [17].

Proteases not connected to caspases are thought to be related in PCD in plant cells [18]. In recent years there are numerous studies showing that caspase inhibitors can suppress cell death in plants.

Plant caspases

There are numerous reports that associate protease activity with the regulation of plant PCD. Proteasome inhibitors can block tracheary element differentiation in *Zinnia* cells when added at the time of culture induction, whereas proteasome inhibition after commitment to differentiation results in a delay. This finding reveals that proteasome function is necessary for induction of tracheary element differentiation [19].

Moreover the secretion of protease is matched with secondary cell wall synthesis and cell death during tracheary element differentiation. Protease activity and cell death are both prevent by soybean trypsin inhibitor, while exogenous treatment of serine protease activate cell death. These findings make possible the suggestion that extracellular proteolysis causes cell death [20]. Some studies show that inhibitors involve with serine proteases in signal transduction during elicitin-induced hypersensitive response (HR) cell death [21].

Also PCD activating oxidative stress stimulates a set of cysteine proteases in soybean cells. Inhibition of the induced cysteine protease activity by ectopic expression of cystatin, a cysteine protease inhibitor gene, can prevent PCD triggered either by an avirulent pathogen or by reactive oxygen species (ROS) [22]. These findings indicate that the interaction between proteases and endogenous protease inhibitors is a manner for plants to regulate cell death. So far, indication for the presence of caspase-like proteins (CLPs) in plants is still indirect and mainly based on the inhibitory effects of caspase-specific inhibitors in plant cells.

Caspase-like activities in plants

Caspase-like activities have been identified in plants using synthetic tetrapeptide substrates using consensus of members of the mammalian caspase family. These substrates are not precisely 'caspase-specific' and have overlapping specificities with several caspases and other proteases [23]. Consequently it is difficult to understand caspase-like activity profiles in whole plant extracts. YVADase (an enzyme that recognized the YVAD (Tyr-Val-Ala-Asp) sequence is referred to sometimes also as YVADase) and DEVDase (An enzyme that recognized the DEVD (Asp-Glu-Val-Asp) sequence is referred to sometimes also as DEVDase) have been the most studied, and both activities were established in the majority of PCD positions, showing their ubiquity. Recently, variety of substrates like IETDase (an enzyme that recognized the Ile-Glu-Thr-Asp sequence is referred to sometimes also as IETDase) has become commercially presented.

Recently, Bozhkov et al. [24] focused on the question of whether caspase-like proteolytic activity is involved in the regulation of plant developmental cell death using Norway spruce (*Picea abies*) somatic embryogenesis as the model system. They showed that VEIDase is a

major caspase-like activity implicated in plant embryogenesis. This activity is increased at the early stages of embryo development, and is directly involved in the final differentiation and death of the embryo suspensor. A variety of protease inhibitors such as calpain, inhibitor I, aprotinin, leupeptin, PMSF and TLCK, pepstatin and lactacystine had no inhibitory effect. Same researchers also showed that in *Arabidopsis*, the VEIDase is a different protease from IETDase. A caspase-like proteolytic activity (TATDase) was established through the N gene-mediated hypersensitive response (HR) in tobacco plants infected with TMV [25]. In addition Belenghi et al [26] was detected the caspase like activity in the course of plant development during petal senescence and endosperm PCD. They also applied caspase or cysteine protease inhibitors into epicotyl tissue and suppressed the death of secondary shoots; therefore they concluded that shoot selection in pea seedlings is controlled by PCD through the caspase like proteases. In summary, all these findings indicate that there are at least six different caspase-like activities in plants. Most of these activities have been established in a variety of plants and in different tissues or cell types (Table 2). In addition, chemical-induced PCD in tomato suspension cells can be inhibited by caspase-specific inhibitors [27]. Caspase-like activity has also been determined in barley cell extracts and could only be inhibited by a specific caspase-3 inhibitor, not by cysteine protease inhibitors [28].

Proteolytic activity in plant cells in PCD has also been searched using poly(ADP-ribose) polymerase (PARP), a well-known substrate for human caspase-3. Cleavage of endogenous PARP form during menadione-induced PCD in tobacco protoplasts [29] and in heat-shock-applied tobacco suspension cells [30]. Exogenous PARP is endoproteolytically cleaved in extracts of fungus-infected cowpea plants, and cleavage can be blocked by caspase-3 inhibitor. But, cleavage of exogenous PARP in cowpea extracts results in fragments that are dissimilar to the fragments that keep on after cleavage by an animal caspase [31].

Table 2: Caspase-like activities in plants (Adapted by Rotari et al., [15]).

Activity	Plant and Tissue
YVADase **Caspase-1-like**	Tobacco leaf tissue Barley embryonic suspension cells *Arabidopsis thaliana* seedlings Germination of white spruce seeds Tobacco suspension cells
DEVDase **Caspase-2-like**	Barley embryonic suspension cells *Arabidopsis thaliana* seedlings Germination of white spruce (*Picea glauca*) seeds Tobacco suspension cells *Avena sativa* leaves Tobacco suspension cells Embryogenic cell line of Norway spruce *Papaver* pollen
VEIDase **Caspase-6-like**	*Arabidopsis thaliana* seedlings Embryogenic cell line of Norway spruce

IETDase	*Arabidopsis thaliana* seedlings
Caspase-8-like (saspase)	*Avena sativa* leaves
VKMDase (saspase)	*Avena sativa* leaves
TATDase	Tobacco Xanthi, leaves

IAP protein family which is suppressing apoptosis has been assumed to play its regulating role by inhibiting caspases in animals. It has been established Hansen [32] that *Agrobacterium*-induced PCD in maize cells can be repressed by ectopic expression of an the inhibitors of apoptotic proteins (IAP) from baculovirus. These data point out the existence of plant proteases that are able to recognize caspase specific inhibitors, and their involvement in cell death.

Metacaspases

Genome analysis revealed the deficiency of caspase orthologous sequences in the *Arabidopsis* genome. Early determinations with cell extracts indicated that metacaspases could match with the caspase-like activities in plants, yeast, and fungi [33, 34]. But researchers recently revealed that plant metacaspases are not capable to cleave caspase substrates [35, 36, 37]. According to the recent findings, recombinant metacaspases do not cleave caspase substrates and their enzymatic activity is not inhibited by caspase inhibitors [38, 35]. Additionally extracts of *Arabidopsis* plants overexpressing metacaspase-8 have stimulated FRase activities and do not have enhanced caspase-like activities [37].

Although metacaspases do not have caspase-like activities, several publications support a role for metacaspases in PCD. Uren et al., [39] established two families of expected proteases that are more closely related to animal caspases than to other proteases; these were designated as paracaspases and metacaspases. Paracaspases and caspases seem to be specific to animal, but metacaspases are present in other eukaryotes including plants. In brief, a main effect for metacaspases in plant PCD is considered probably [40]. It is now important to determine the role of metacaspase as a caspase like activities. Some studies support the role of metacaspases in PCD [41, 42].

It has been also established that the only metacaspase existing in *Saccharomyces cerevisiae* shows a caspase-like proteolytic activity that is activated when yeast is stimulated by H_2O_2 to have apoptosis [33]. In the same way Suarez et al. [34] determined that the down-regulation of a type II metacaspase suppresses PCD in the suspensor cells of an embryogenic culture of *Picea abies*. In addition to all these findings, Hoeberichts et al. [16] reported the type II metacaspase increment upon infection of leaves with fungal pathogen *Botrytis cinerea* that stimulates cell death in *Lycopersicon esculentum*. Watanabe and Lam [36] also reported the up-regulation of type I metacaspases in *Arabidopsis thaliana* leaves after infection with bacterial pathogens, on the other hand, they also found that type II metacaspases are not

significantly affected. Recent studies revealed the indirect indication that metacaspases are proteases with a caspase-like activity [33, 34].

Legumains

Another group of caspase-related proteases are legumains, cysteine endopeptidases first detected in plants. Legumains are distributed in all major kingdoms and play an critical function in seed maturation and protein breakdown during germination [43]. It also has been estimated that a-, and g-vacuolar processing enzymes (VPE), legumains from Arabidopsis, are stimulated in vegetative organs during senescence, a kind of PCD, and under different stress conditions [44].

This stimulation was coupled with the increment of vacuolar proteins revealing that legumains regulate the activation of some proteins in the lytic vacuoles, including inducible cysteine proteases. Besides, the role of legumains in plant PCD was supported by the findings that a g-VPE precursor accumulates in endoplasmic reticulum in *Arabidopsis,* which may help for cell death under stress conditions [45]. Recently it was shown that VPE has a caspase-1 activity and is needed for the achievement of cell death during TMV HR in tobacco [46].

Saspase

Saspase is belonging to the subtilisin which is a large family in plants [47]. The oat saspase is not the only instance of a serine protease exhibiting caspase-like cleavage. Coffeen and Wolpert, [48] demonstrated the saspase inhibition by caspase inhibitors, and its involvement indirectly in victorin-induced cleavage of Rubisco during PCD in *Avena sativa.*

Papain-like Cysteine Protease

Generally plant papain-like cysteine proteases are found in vacuoles [49 that may carry out a lysosomal-like function [50] and autophagy appear to engage vacuolar proteolysis [51]. Certain cysteine proteases are stimulated during oxidative stress-induced PCD in soybean cells [22] and this stimulation is required for PCD. This activity could cleave other substrates of papain-like proteases but no activity has been determined with a caspase-1 substrate. A similar activity was proposed recently in *Arabidopsis* cystatin-1 (AtCYS1). The overexpression of AtCYS1 prevents cell death induced by oxidative and nitrosative stress in *Arabidopsis* cells suspension and in transgenic tobacco plants.

In conclusion, there is indirect indication of papain-like protease connection in developmental PCD during germination of castor bean (*Ricinus communis*), PCD in endosperm is correlated with the accumulation of a 45-kDa papain-like pro-peptidase in ricinosome [52]. Additionally, Wan et al. [53] revealed the papain-like cysteine protease relation with PCD of the inner integument of seed coat during early stages of seed development in *Brassica napus* .

Function of mitochondria

Alterations in mitochondrial membrane permeability, following release of cytochrome c and the formation of the apoptosome play an important role in apoptosis in animal systems. BCL2-like proteins can act as regulators of apoptosis both by interference with caspase activation or during their effect on mitochondrial membrane integrity. In a variety of plant systems, the release of cytochrome c from mitochondria into the cytosol precedes PCD [29, 32, 54]. In addition, HR-induced PCD is correlated with the disruption of mitochondrial functions [55]. Cytochrome c is not released during petal cell death in pollinated *Petunia* flowers [56] showing in any case one form of plant PCD in which cytochrome c release is not necessary (Figure 1). But, the release of cytochrome c from plant mitochondria as reasoned by ROS, increased calcium levels, or inhibition of electronransport has been assumed to be a means for integrating cellular stress and activating plant PCD [57]. Data for a role of Bcl-2 like proteins in plant PCD is increasing. Indication for cell death processes in plants [58, 59] is now also revealed by the isolation of homologues of of human Bax inhibitor-1 from *Arabidopsis thaliana* and rice. Bax is a proapoptotic of Bcl-2 family member.

In summary, it has been shown that proteases with caspase like activity play a determinative role in the development of plants. Since the sequencing projects of model plant genomes have not been identified true caspases, it is difficult to attribute the cleavage of the various caspase specific substrates to specific enzymes.
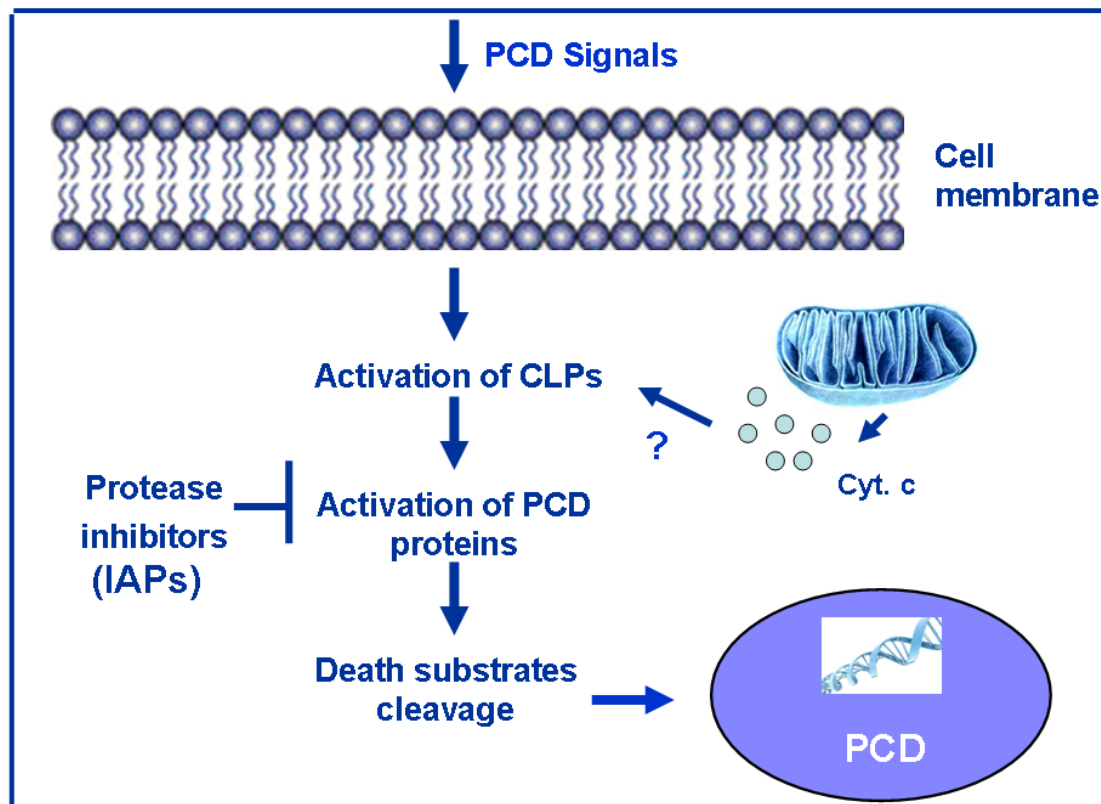

Figure 1: Possible connections of caspase like proteases with PCD in plants. CLPs: Caspase-like proteases; PCD: PCD; IAPs: Inhibitors of apoptosis.

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