A role of actin-to-CD95 connection in CD-95 apoptotic pathway

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Historic aspects and features of apoptosis

Balance between cell division and cell death is of utmost importance for the development and maintenance of multicellular organisms. Disorders of either process have pathologic consequences and can lead to disturbed embryogenesis, neuro-degenerative diseases, or the development of cancer (Alderson et al., 1993).

Historically, cell death has been divided into two broad categories: programmed cell death (PCD), in which the cell plays an active role; and necrotic cell death. Whereas the former is an inherent, controlled cellular death program, the latter results from circumstances outside the cell, and is characterised by cellular edema and disruption of the plasma membrane, leading to release of cellular components and subsequent inflammatory tissue response (Hirsch et al., 1997).

However, the real difference between the two is that necrosis is a primary process that is not preceded by early steps, while PCD may be characterised by a secondary necrosis but preceded by some intracellular events that differ between the various apoptotic pathways. Moreover, although facets of the apoptotic machinery are activated in many pathologic conditions, a host of physiologic and environmental factors can influence the segregation of downstream cellular events so that apoptotic or necrotic morphology prevails.

Heterogenous toxic, ischemic, degenerative, and immunologic stimuli that are typically associated with necrosis can induce the apoptotic phenotype (Elmore, 2007). As far as PCD is concerned, it is further classified into three physiological cell death pathways: type I (nuclear or apoptotic); type II (autophagic); and type III (cytoplasmic) (Clarke, 1990). Despite the numerous models proposed to categorize PCD, exclusive definitions are difficult to make and are probably artificial due to the overlap and shared signaling pathways between the different death programs. Furthermore, as already mentioned for necrosis, the same cell type may die in one way or the other, depending on the noxious stimulus applied (Chi et al., 1999). Although all the above listed types of programmed cell deaths and the recently classified ones such as paraptosis, oncosis, calcium mediated and AIF/PARP (Bredesen, 2007) capture attention and deserve further elucidations due to the novel in depth mechanistic taxonomy attempted, we chose not to highlight them since they represent a field in itself. We will therefore focus on reviewing current literature on crosstalks between CD95 mediated apoptosis and actin cytoskeleton. PCD most frequent phenotype is apoptosis. This evolutionary conserved, genetically controlled process has a role in a variety of physiological settings, as development, homeostasis of tissues and maintenance of the organism integrity (Wyllie et al., 1997). In both mammalian and yeast cells, apoptosis is morphologically characterized by mitochondrial depolarization, generation of high levels of reactive oxygen species (ROS), DNA fragmentation, and phosphoserine exposure at the plasma membrane (Hengartner, 2000). The apoptotic cascade can be initiated via two major pathways, involving either the activation of cell surface death receptors in response to ligand binding (extrinsic pathway or type I cell death pathway) or mitochondrial membrane perturbations promoted by physical or chemical stress agents (intrinsic pathway or type II cell death), which culminate in cytochrome c release in the cytoplasm. Upon triggering of either pathways, a
specific family of aspartate directed cysteine proteases, known as caspases, is activated to execute the cell’s fate in a programmed fashion, leading to the typical morphologic changes described herein (Earnshaw et al., 1999). Ongoing research on physiological programmed cell death, which was described as early as in 1842 by C. Vogt, has highlighted the existence of unexpected and more sophisticated cell death pathways that may evolve also in the absence of caspases involvement (Bröker et al., 2005) or without a mitochondrion dependent step (Bratosin et al., 2001). When deranged by impaired regulation or inappropriate activation apoptosis contributes to the pathogenesis of diseases as autoimmunity, cancer, restenosis, ischaemia, heart failure and neurodegenerative disorders (MacFarlane et al., 2004).

**Apoptosis from membrane to cytoplasm: CD95 a death receptor and beyond**

CD95 (Fas/Apo-1) is the most common and also best characterized member of the tumor necrosis factor (TNF) superfamily of receptors. It is a type I transmembrane glycoprotein broadly expressed as preassociated trimers on the surface of a variety of cell types and tissues. It mediates rapid apoptosis when triggered in cells receptive to its apoptotic signaling. In the immune system, it regulates the persistence and resolution of immune responses (Zeuner et al., 2000).

In depth analysis of CD95 functions has highlighted that besides its best known function of death transducer, this receptor is highly pleiotropic and is also involved in induction of T cell activation, proliferation (Wajant, 2003), differentiation (Rescigno et al., 2000), migration (Ottonello et al., 1999), neurite outgrowth (Desbarats et al., 2003), integrin expression (Jarad et al., 2002), hepatic regeneration (Desbarats and Newell, 2000) and inflammatory angiogenesis (Biancone et al., 1997). As far as apoptotic death induction is concerned, upon triggering of CD95 either by its ligand (FasL) or by an anti CD95 antibody, the Fas associated death domain protein (FADD) and caspase-8 are recruited to the intracellular part of the receptor, forming the death inducing signaling complex (DISC). Following caspase-8 enrollment to the DISC, it undergoes autoproteolytic cleavage and subsequent activation, which in turn leads to the cascade of events characteristic of the apoptotic pathway (Fig. 1).

![Figure 1. Ezrin-mediated CD95 linkage to actin cytoskeleton. Ezrin linkage of CD95 to the actin cytoskeleton determinates CD95 polarization, drives the actin-dependent DISC formation and confers susceptibility to programmed cell death.](image-url)
In type I cells, apoptosis follows the extrinsic pathway. A high quantity of DISC is rapidly formed upon stimulation and apoptosis is rapidly induced. Conversely in type II cells, which follow the intrinsic pathway, DISC formation is limited and the apoptotic signal is amplified and executed through the mitochondrion. Activation of CD95 receptor signaling has been described in the absence of deliberate CD95L mediated stimulation. Ultraviolet light (UV) for example, causes CD95L independent activation of the DISC and induces apoptosis (Aragane et al., 1998). An additional case of CD95 DISC mediated apoptosis in the apparent absence of demonstrable CD95L triggering, was observed in germinal center B cells (Hennino et al., 2001). The latter examples of CD95 pleiotropic behaviour and capacity of inducing apoptotic features also in the absence of CD95L, depicts the existence of an alternative pathway that allows such receptor to transduce death signals bypassing interactions with its ligand.

The involvement of the apoptotic mediator CD95 in both proliferative signaling and apoptosis depicts the existence of a tightly regulated mechanism that switches the cell response towards one faith or the other in accordance to the best choice for the maintenance of cellular homeostasis. Recent findings have begun to shed light on the early events induced by CD95 triggering that in turn precedes death receptor clustering. Receptor clustering resembles a kind of polarizing phenomenon requiring asymmetric organization of the plasma membrane. The capacity of a cell to polarize is directly related to an interaction between the membrane and the actin cytoskeleton. Accordingly, interactions between the plasma membranes and the cytoskeleton play a crucial role in various cell functions and this also applies for death receptor mediated apoptosis.

Actin cytoskeleton-CD95 interactions and cellular fate: the perfect balance between transduction of life supporting signals and induction of altruistic suicide. Actin is the major constituent of the actin cytoskeleton in eukaryotic cells. It exists either in a monomeric form, G-actin, or in a filamentous form, F-actin. The interchange between different types of actin by polymerization and depolymerization is referred to as actin filamental turnover or actin dynamics. The physiological significance of F-actin in non muscle cells is to organize the actin cytoskeleton, which is utilized for cell locomotion, adhesion, and cell proliferation.

The dynamic assembly and disassembly of F-actin and its organization in ordered arrays are coordina-ted by various accessory proteins (Tseng et al., 2002). More than 60 actin binding proteins have already been identified in mammalian cells (Uribe et al., 2007). Despite the functional redundancy of actin binding proteins, remodeling of actin cytoskeleton which mediates cell shape change and polarization of membrane receptors is mainly regulated by ERM (Ezrin, Radixin and Moesin) proteins, a family of closely related molecules that link integral membrane proteins to the cortical actin cyto-skeleton and localized beneath the plasma membrane. The FERM amino terminal domain of ERMs binds to integral membrane proteins such as CD44, CD43, ICAM2, P-glycoprotein and L-selectin (Tsukita et al., 1997; Luciani et al., 2002). ERMs function as general crosslinkers between the plasma membrane and the actin cytoskeleton, mediating signal transduction of extracellular signals to the latter. Interaction of ERMs to actin occurs through their carboxy terminal halves. Normally ERMs are in a dormant/quiescent/closed inactivated state in resting cells. The inactive form is in fact due to a particular conformation of these globular proteins through which the amino terminal and carboxy terminal halves bind to one another through head to tail interactions (Tsukita et al., 1997). However, this dormant conformation is profoundly altered when the cells go from the resting to the activated state, thus allowing linkage and interaction between membrane and actin. This also occurs in T lymphocytes during antigenic or mitogenic activation (Parlato et al., 2000; Luciani et al., 2004). Molecular biology studies have elucidated the functions of the various components of the cytoskeleton system and enabled the characteri-zation of protein-protein interactions involved in their assembly. It has become clear that the ezrin family, through its ability to transfer signals from the extracellular matrix to the interior of the cell, critically modulates cytoskeleton organization and contributes to engagement of cell proliferation, differentiation or apoptosis (Kondo et al., 1997; Fais et al., 2003; Fais et al., 2005).

A recent breakthrough in the elucidation of the apoptotic process was the demonstration that actin cytoskeleton participates to CD95 mediated apo-totic signaling through its ezrin-mediated actin linkage (Fais et al., 2005). Evidence has accumulated that in both mammalian and non-mammalian systems actin deregulation can be detrimental for cell survival and lead to apoptotic cell death (Odaka et al., 2000), while CD95/actin cytoskeleton linkage is related to a functional apoptotic cell pathway. As far as the former point is concerned, in yeast it has been demonstrated that alterations in actin dynamics correlate to perturbations in ROS cellular levels. Moreover, in such system, the actin cytoskeleton acts as a regulator of ROS release from the mito-chondrion (Gourlay et al., 2004). This unicellular organism provides further evidence of the direct association between the actin cytoskeleton and apoptotic signal transduction. Data concerning mammalian cells generated clear results supporting links between actin and apoptotic pathways through the use of drugs that affect actin turn over (Fig. 2). Jasplakinolide induces very robust actin stabiliza-tion and rapidly leads to accumulation of large F-actin aggregates. Concomitant with this reduction in actin...
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Turn over is the induction of a cell death pathway (Odaka et al., 2000). Actin deregulation proofs to be detrimental for cell survival and may lead to apoptotic cell death. Conversely, correct signaling in CD95 induced apoptotic cell death depends on the correct linkage to the actin cytoskeleton. It has been recently demonstrated that the actin cytoskeleton participates in both CD95 signaling pathway and in CD95 induced apoptosis (Parlato et al., 2000; Lozupone et al., 2004). An ezrin mediated CD95 connection to actin has a leading role in predisposing T lymphocytes to CD95 mediated cell death and such connection to actin is crucial for the occurrence of the early events mediated by CD95 signaling. The polarized state of a cell is determined and maintained by the actin cytoskeleton. Membrane proteins that com-monly polarize in the cells, such as the P-glycoprotein, are frequently associated to the actin cytoskeleton through the ERM family proteins (Kusumi et al., 1996; Fais et al., 2003; Luciani et al., 2002).

Regardless of the high degree of sequence homo-logy between ERM proteins, ezrin is mandatory in conferring CD95 linkage to actin and not inter-changeable with the other two ERM proteins in exploiting such function. In fact, moesin does not colocalize with CD95 and radixin is a weak linker poorly expressed in the majority of normaly cells, particularly in lymphocytes (Parlato et al., 2000; Lozupone et al., 2004). CD95 binding domain on ezrin has been identified on the N-terminal FERM domain (Lozupone et al., 2004), while the hypo-thetical ezrin binding domain on CD95 is supposed to be located in the iuxtamembrane region of CD95. The ezrin-mediated CD95 linkage to actin has a role not only in conferring cell susceptibility to CD95 triggering but also in driving the actin-dependent DISC formation and CD95 internaliza-tion (Algeciras-Schimnich et al., 2002) (Fig.1). After caspase activation ezrin translocates from the plasma membrane to the cytoplasm concomitant with its dephosphorylation, resulting in gross dissociation of actin-based cytoskeleton from plasma membranes (Kondo et al., 1995).

Lymphocytes don’t need a full mitogenic activation to become CD95 sensitive through ezrin linkage (Luciani et al., 2004). Following such recruitment, a morphological cell shape change occurs from a round to a pear-shaped (polarized) form. This is what happens in some infectious diseases such as AIDS, where lymphocyte polarization may be induced by both HIV-1 cell-to-cell infection (Fais et al., 1995) and gp120 stimulation without infection (Luciani et al., 2004), but also following stimulation with HIV-1-induced cytokines, such as IL-7 (Fluur et al., 2007).

As previously mentioned, it is very hard to distinctly and sharply draw boundaries between the various forms of programmed cell death that cells utilise during the variety of challenges to which they are constantly exposed such as: embryonic development, neurodegeneration, AIDS, carcino-genesis and chemotherapeutic drugs (Santucci et al., 2006). As far as apoptosis is concerned though, mounting evidence, culminating in in vivo beyond doubt results, highlights clear cut differences in the death programs undertaken by type I and type II cells (Lacronique et al., 1996).

The main diversity between type I and type II cells, which is the complete and abundant assembly of the DISC obtained in the former, is ascribed to actin participation to the phenomenon. In type I cells, CD95 triggering leads to CD95 microaggregates at the cell surface. Subsequently FADD recruits to the DISC through an actin driven mechanism. Finally, large CD95 clusters generate and activated CD95 is then internalised through an endosomal pathway (Algeciras-Schimnich et al., 2002). Microaggre-gation of CD95 is subsequent to its stimulation and disruption of actin filaments causes impairmen in the formation of the DISC. It is therefore assumable that not only CD95 is linked through ezrin, to actin cytoskeleton, but also some component of the DISC links CD95 to actin.

Figure 2. Natural actin inhibitors

Cytochalasyn D Latrunculin A Jasplakinolide

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The importance of cytoskeletal actin in ligand induced internalization of a plethora of receptors has been thoroughly demonstrated (Qualmann et al., 2000) so it is not at all surprising that the same principle applies for CD95. Evidence supporting Parlato S and colleagues studies on actin cytoskeleton involvement in CD95 induced apoptosis, paralleled advances in cellular biology and biochemistry. Latrunculin A (ltnA), a toxin isolate from a Red sea sponge, causes disruption of microfilament organization by binding to mono-meric G-actin in a 1:1 complex at submicromolar concentrations (Spector et al., 1983). Treatment of a wide spectrum of cells with ltnA inhibited CD95 mediated apoptosis, confirming Parlato S data on actin involvement in signalling through CD95 (Subauste et al., 2000).

CD95 involvement in life determining decisions is greatly dependent on cellular contest since as already mentioned, apart from its well documented role as a death inducer, it may also protect. T helper lymphocytes have been classified by immunologists in Th1 and Th2 subtypes on the basis of their representative cytokine profile (Interferon alpha and interleukin 4 respectively). Th1 cells are mainly responsible for phagocyte-mediated host defence and these cells are the principal effectors of cell-mediated immunity and delayed-type hypersensitivity reactions.

Th2 lymphocytes are responsible for the immune defence not mediated by phago-ocytes, the recruitment of eosinophils and allergic reactions (Mosman et al., 1986). These two populations of cells serve different purposes and one of the ways in which such aim is full filled is through CD95 -actin linkage. In fact, Th1 and Th2 response to CD3 ligation differs since the former become sensitive to Fas mediated apoptosis while the latter develop resistance. (Vardhachary et al., 2001).

Treatment of Th2 cells with the cell permeable mycotoxin, Cytochalasin D which disrupts actin filaments and inhibits actin polymerization, sensitises apoptosis resistant Th2 cells to CD95 mediated death. Th2 cells are thought to be protected from CD95 mediated cell death through inhibition of CD95 aggregates and subsequent caspase 8 activation. Cells response to cytochalasin D demonstrated that inhibition of CD95 aggregate formation is mediated by changes in the actin cytoskeleton, which in turn inhibit CD95 lateral diffusion.

**CD95 and actin in diseases**

Under physiological conditions, the major role exerted by the CD95 pathway is that of terminating immune responses by causing peripheral deletion of activated mature T lymphocytes. Moreover, it is also involved in preventing inflammation in “immune privileged” sites, such as eyes, brain and testes, where FasL is highly expressed. Another important function is that of eliminating virus infected or transformed cells (Nagata, 1997). Apoptosis constitutively occurs in senescent or damaged cells, it is therefore consequential that impairment or over exploitation in signalling of CD95 and modifications in its connection to actin cytoskeleton, one of its main executioners, reflects in cellular pathology.

As previously mentioned, Luciani F and colleagues (2004) and Parlato S and colleagues (2000) revealed both that ezrin-mediated CD95/actin association plays a crucial role in rendering human CD4+T lymphocytes susceptible to CD95-mediated apoptosis and that CD95-mediated apoptosis of bystander uninfected T cells exerts a major role in the HIV-1-mediated CD4+ T-cell depletion. In the latter, gp120-mediated CD4 engagement induces susceptibility of primary human T lymphocytes to CD95-mediated apoptosis through ezrin phospho-rylation and ezrin-to-CD95 association. These studies were pioneer to highlighting the involvement of the association CD95-actin cytoskeleton and human disease. Subsequently, data accumulated in confirmation to such results.

Recently, strong evidence has shown that deregulation of Fas expression and/or signaling contributes to the pathogenesis of tissue destructive diseases such as graft-versus-host disease, toxic epidermal necrolysis, stroke and multiple sclerosis (MS) (French et al., 2003). As far as the latter is concerned, mounting evidence demonstrates that oligodendrocyte (OL) of patients affected by this disease, which is an inflammatory demyelinating pathology of the central nervous system, displayed intense immunoreactivity to CD95. Intriguingly, In contrast to the activity of CD95 in other cellular systems, dying OLs did not exhibit evidence of apoptosis, assessed morphologically and by terminal transferase–mediated d-uridine triphosphate-biotin nick-end-labeling staining for DNA fragmentation (D'Souza et al., 1996). This result suggests that in MS CD95 might dual with other tasks rather than mediate apoptosis. Another interesting common end point in MS patients is the overexpression of a subtype of heat shock proteins (hsp): the 27 kDa small hsp (hsp27). The latter protein is overexpressed in MS patients (Acquino et al., 1997). One of the known functions of this chaperone molecule is that of binding cytoskeletal actin and it has been demonstrated that its over expression inhibits actin polymerization in vivo, therefore altering cellular interactions (Schneider et al., 1998). It is plausible to suppose, on the basis of the information gathered herein and on existing literature on the topic, that during the inflammatory reaction typical of MS; hsp27 mediated rearrangement of actin cytoskeleton restricts CD95 in non functional aggregates or at least in a conformation that does not trigger programmed cell death of OL but rather mediates some other function.

It is now widely accepted that in tumour cells many anticancer agents act as apoptosis inducers, and an important determinant of drug resistance is the inability of drugs to trigger apoptosis. The involvement of the
CD95/FasL system in cancer chemotherapy is an old story, first postulated in the mid 1990s by Friesen et al., (Friesen et al., 1996). Evidence arose in the past decade, supporting the fact that many antitumour drugs function by clustering CD95 and all the downstream signaling molecules previously mentioned into membrane microdomains (lipid rafts) highly enriched in cholesterol and sphingolipids with an estimate size between 50-70 nm (Gajate et al., 2005).

CD95 recruitment to lipid rafts is a consequence of CD95 linkage to the actin cytoskeleton through ezrin (Gajate et al., 2005). In fact, cell treatment with jasplakinolide, not only disrupts CD95 accumulation in lipid rafts but also prevents apoptosis induced by the chemotherapeutic Aplidin. Although the primary intracellular targets of action of chemotherapeutic agents are distinct, it has become evident that induced cytotoxicity ultimately converges on a common pathway that induces apoptosis (Petak et al., 2002). Cells treated with cytotoxic agents can show the typical characteristics of apoptosis analogous to apoptosis induced by “physiologic” stimuli such as death receptor activation. Most importantly, drug-induced cell death is frequently mediated by caspase-dependent apoptosis. Some reports indicate that certain DNA damaging agents may induce apoptosis via CD95 signaling (Kasibhatla et al., 1998).

Given the close linkage between the actin cytoskeleton and CD95 signaling, it is not surprising that not only CD95 aberrations but also cytoskeletal protein ones, are the underlying reason for many pathological phenotypes (Ramaekers et al., 2004). Moreover, cancer metastasis and as mentioned above, chemoresistance, are most probably caused by pathological cytoskeletal rearrangements. It is therefore worth while to keep in mind the linkage between the actin cytoskeleton and CD95 responses during pharmacological studies, not only by examining in deep detail CD95 expression and signalling pathway but also by monitoring the status of the former.

Despite the intense scientific endeavor, functional studies aimed at shedding light on the mechanisms that correlate signal transduction networks to both the actin cytoskeleton and CD95 mediated programmed cell death, should continue to be carried out intensively due to the mounting evidence that such field leads to the discovery of new potential targets for molecular therapies.

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