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Perspective

The ARTS Connection

Role of ARTS in Apoptosis and Cancer

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ABSTRACT

ARTS is an unusual septin that is localized to mitochondria in living cells and promotes apoptosis by antagonizing IAPs. ARTS functions as a tumor suppressor in Acute Lymphoblatic Leukemia (ALL) and is lost in more than 70% of leukemic patients. The loss of ARTS is specific as levels of H5, a closely related non-apoptotic septin protein derived from the same gene, were unaffected. Thus, ARTS, a new member in the mitochondrial pro-apoptotic arsenal, provides a link between mitoohondria, apoptosis and cancer.

ARTS belongs to the septins, a family of proteins that were originally studied for their role in cytokineses and cell motility.[1-3] The ARTS protein is derived as a splice variant from the H5/PNUTl2 human septin gene.[4] Although it exhibits a high degree of sequence similarity with other family members, ARTS has several unique features. While other septins are usually localized mainly to the cytoskeleton, ARTS localizes to the mitochondria in living cells and translocates to the nucleus upon pro-apoptotic stimuli.[4,5] The basis for the differences in cellular localization appears to be the N' terminus of ARTS, where ARTS lacks 20 amino acids found in most other septins. This change exposes a putative mitochondrial import sequence (KRFLED) which is presumably responsible for mitochondrial location of ARTS. Interestingly, another septin (M-septin) was recently reported to localize to mitochondria as well.[6] However, M-septin contains the additional 20 amino acids at its N-terminus, suggesting that its localization in mitochondria may involve a different import mechanism then that employed by ARTS.

Evidence that ARTS plays a role in apoptosis has come both from gain and loss-of-function studies. In certain cells, high level overexpression of ARTS is sufficient to induce apoptosis (see ref. 5 and our unpublished results). More generally, expression of ARTS can promote apoptosis in response to a variety of pro-apoptotic stimuli such as, Fas, TGF-β, ara-C, etoposide, and staurosporine (STS).[4,5,7] Conversely, downregulation of endogenous ARTS by anti-sense expression was shown to protect cells against TGF-β-induced apoptosis.[4] In all these cases, ARTS-mediated cell killing leads to caspase activation (see below). Because ARTS is implicated in a wide variety of apoptotic paradigms, it seems to function at a central apoptotic junction where different upstream apoptotic inputs converge to mediate caspase activation.

ARTS is a mitochondrial protein that translocates from mitochondria to the nucleus during apoptosis.[4,5] At this point, little is known about the underlying molecular mechanism. The release of ARTS from mitochondria does not require caspase activity, though this activity is required for efficient nuclear entry.[5] The translocation of ARTS to the nucleus may not simply be the consequence of apoptosis since ARTS contains a putative nuclear import sequence (Lotan R, Larisch S, unpublished observation). Furthermore, during apoptosis ARTS becomes localized in a highly specific pattern within the nucleus, in close association with PML-NBs (promyelocytic leukemia nuclear bodies) (Fig. 1A; Lotan R, Larisch S, unpublished results). The PML-NBs are protein complexes associated with the nuclear matrix. PML constitutes the scaffold component of NBs and recruits a variety of proteins onto these domains, many of which are involved in apoptosis.[8,9] However, the nature and reason of the association of ARTS with PML-NBs is not yet clear.

ARTS induces apoptosis through activation of caspases. In addition to activation of caspase-3, ARTS-induced-apoptosis is associated with caspase-9 activation (Fig. 1B). Whether the activation of caspase-9 by ARTS is the result of a direct effect on the apoptosome, or whether the effect is indirect remains an open question.

The main mechanism by which ARTS exerts is apoptotic activity appears to be through direct binding and inhibition of IAPs.[5] Upon induction of apoptosis, ARTS is released from mitochondria and co-localizes with XIAP in the cytosol. Binding of ARTS to XIAP

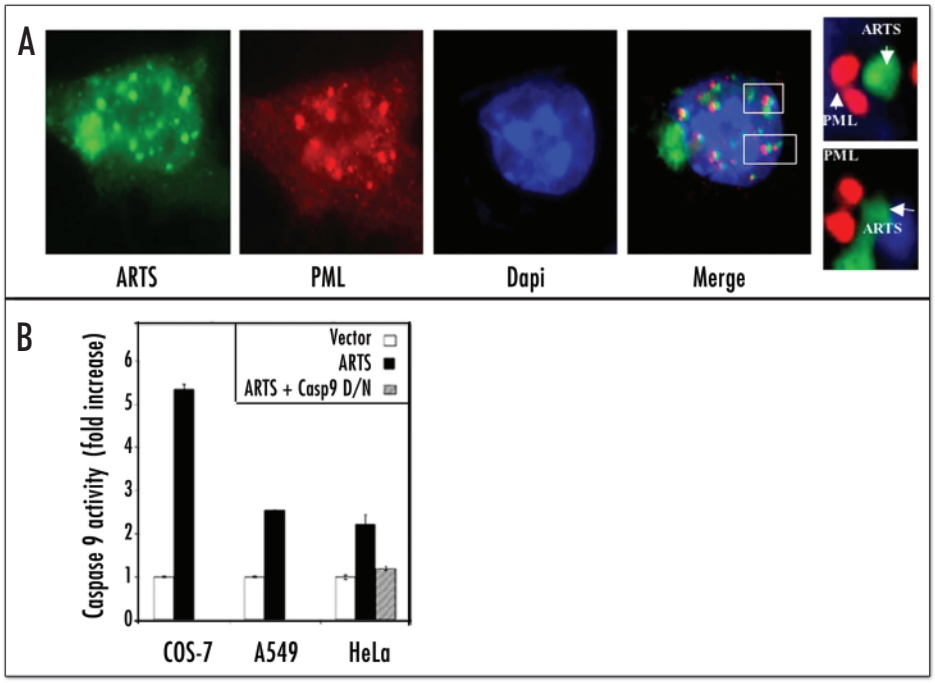

Figure 1. ARTS nuclear localization is associated with PML NBs. (A) COS-7 cells over-expressing ARTS were treated with staurosporine and subsequently stained with anti-ARTS (green) anti-PML (red) and with Dapi (blue) for nuclear localization. The merged picture shows an association of ARTS with PML NBs. Insets show an enlargement of areas in the merged picture. (B) ARTS-induced apoptosis leads to caspase-9 activation. COS-7, A549 and HeLa cell lines were transiently transfected with AU5-ARTS or AU5 empty vector. HeLa cells were also co-transfected with AU5-ARTS and caspase-9 Dominant Negative (D/N) (Cas9 D/N). Activation of caspase-9 was measured with Caspase-9 activity assay kit (R&D). The results are presented as fold increase relative to results obtained in cells transfected with control vector, which are calculated as 1. Overexpression of ARTS induces activation of caspase-9 in these cell lines, and co-transfection of ARTS and caspase-9 D/N resulted in inhibition of caspase-9 activation.

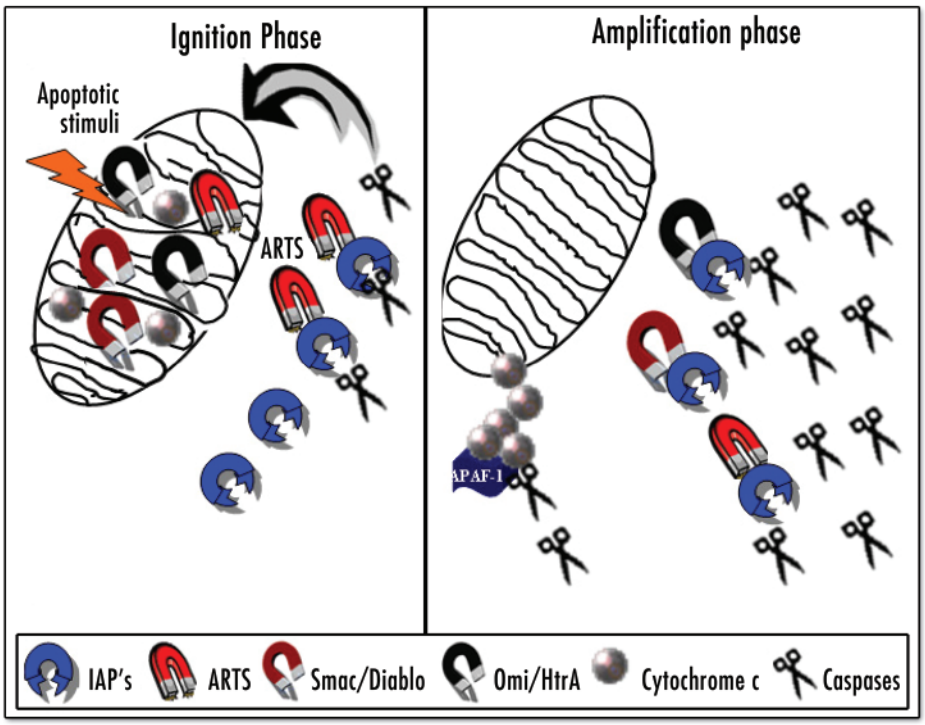

Figure 2. Two phase model for mitochondrial induced caspase activation. Proposed hypothetical model for mitochondrial induced caspase activation. The first step is an "ignition phase" in which ARTS, and possibly other caspase–independent factors, exit the mitochondria, bind to IAPs and reduce their activities. Thereby, a first sub-lethal (and possibly reversible) wave of caspase activation may be initiated. If pro-apoptotic stimuli are sufficiently strong and/or persist, this first wave of caspase activity will promote the release of cytochrome c and Smac/Diablo from mitochondria into the cytosol. As a result, an "amplification phase" will be initiated which leads to full-blown activation of caspases, cleavage and degradation of IAPs and the execution of apoptotic cell death.

is direct, as recombinant ARTS and XIAP proteins can bind to each other in vitro. ARTS binding to XIAP is specific and related to its pro-apoptotic function, as mutant forms of ARTS (and other related but non-apoptotic septins) that fail bind XIAP fail to induce apoptosis. Binding of ARTS to XIAP causes a significant reduction in XIAP levels and leads to caspase activation and cell death.[5] Other mitochondrial pro-apoptotic proteins, such as Smac/Diablo and Omi-HtrA2, are known to act through antagonizing IAPs.[10-13] How does ARTS fit within these paradigms? Does it compete with the other mitochondrial proteins for binding to IAPs or, does it complement their function?

Several unique properties of ARTS suggest the following hypothetical model for how ARTS may function in apoptosis:

1. ARTS antagonizes IAPs by a novel mecanism. ARTS lacks any recognizable IBM (IAP-Binding-Motif), a short sequence that is necessary and sufficient for IAP-binding and inhibition, and that has been conserved amongst all other known IAP-antagonists.[14-17] Therefore, ARTS appears to bind IAPs using a novel mechanism. It is possible that binding of ARTS to XIAP requires the unique C-terminus of ARTS, a stretch of 27 amino acids not found in H5 or other septins.[4,5] Consistent with this idea, deletion of the C-terminus of ARTS results in loss of XIAP-binding.[5]

2. ARTS appears to act upstream of Smac/Diablo and cytochrome c. The release of both Smac/Diablo and cytochrome c has been reported to require caspase activity.[18-22] In contrast, the release of ARTS from mitochondria and its ability to bind and antagonize XIAP are not blocked by caspase inhibitors.[5]

In addition, upon apoptotic induction, ARTS levels in the cytosol peak before the release of cytochrome c.[5] Therefore, it seems that

ARTS exits mitochondria prior to the release of both Smac/Diablo and cytochrome c.

Although both postulates await direct experimental confirmation, the available data suggest a two-phase model of caspase activation by mitochondrial factors (Fig. 2). The first step is an "ignition phase" in which ARTS, and possibly other caspase-independent factors, exit the mitochondria, bind to IAPs and reduce their activities. Thereby, a first sub-lethal (and possibly reversible) wave of caspase activation may be initiated. If pro-apoptotic stimuli are sufficiently strong and/or persist, this first wave of caspase activity will promote the release of cytochrome c and Smac/Diablo from mitochondria into the cytosol (for example, through cleavage of Bid).[18] As a result, an "amplification phase" will be initiated which leads to full-blown activation of caspases, cleavage and degradation of IAPs,[23-25] and the execution of apoptosis. The advantage of such a two-phase mechanism may be that it provides additional safety and better integration of diverse signaling pathways controlling apoptosis.

The loss of pro-apoptotic genes is one of the known mechanisms by which transformed cells obtain selective advantage through reduced susceptibility to apoptotic signals.[26-28] If ARTS expression is important for the cells ability to undergo apoptosis, than we would expect cancer cells to lose ARTS expression in order to gain selective advantage. Indeed, ARTS expression is lost in lymphoblasts of more than 70% of leukemic patients.[7] In contrast, levels of the related non-apoptotic H5 septin were unaffected in ALL patients, indicating specific selection against the pro-apoptotic function encoded by the H5/PNUTl2 gene. Interestingly, two other septins, CDCrel-1 and MSF have been found as fusion proteins with MLL (ALL1, Htrx, HRX) in de novo leukemias in children and therapy-related acute leukemia patients.[29,30] Moreover, MSF is located on chromosome 17q25 in the vicinity of ARTS, which maps to chromosomal location 17q23.[30,4] This region is deleted in some breast and ovarian tumors, and Kalikin et al. hypothesized that this region contains a tumor suppressor gene.[31] Further investigation should indicate whether ARTS is this proposed tumor suppressor which contributes to the malignant transformation of hematopoietic cells, and possibly other cell types as well.

ARTS levels may critically influence IAP protein levels, both in normal and malignant cells. IAPs are over-expressed in a variety of tumors, which is thought to promote cancer cell survival,[27,32] but the underlying molecular mechanism is still unknown. Furthermore, upregulation of IAPs is associated with resistance of tumor cells to apoptosis.[33,34] It is possible that elevated IAPs are, at least in some tumors, the consequence of loss of ARTS. Thus, the absence of ARTS may contribute to the resistance of cancer cells to apoptosis through its direct effect on increased IAP levels.

Altogether, ARTS appears to be a central mitochondrial pro-apoptotic protein with a preferred loss in certain cancers. Therefore, strategies aimed at restoring ARTS function may provide promising new therapeutic opportunities.

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