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RTN3 and RTN4: Candidate Modulators in Vascular Cell Apoptosis and Atherosclerosis

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ABSTRACT

The vascular cell apoptosis may play an important role in the development of atherosclerosis. Reticulons, the only molecular so far to participate in all three apoptosis signaling pathways, may be a novel player in the progress of AS. We presumes that reticulons may belong to the principle node of apoptosis pathway and be the candidate factor linking apoptosis and AS. J. Cell. Biochem. 111: 797–800, 2010.

KEY WORDS: RETICULONS; APOPTOSIS; ATHEROSCLEROSIS

Atherosclerosis (AS) is the leading cause of mortality and morbidity over the world. Apoptosis plays an important role in the development of AS. Reticulons (RTNs), shaping the tubular networks of the endoplasmic reticulum (ER), is a novel eukaryotic gene family with broad expression and special topological patterns. Among them, Reticulon3 (RTN3) and Reticulon4 (RTN4) have been proved to not only participate in apoptosis signaling pathways [Tagami et al., 2000; Watari and Yutsudo, 2003; Liu et al., 2009], but also can interact with each other to regulate cell apoptosis [Bing et al., 2001; Qi et al., 2003; Qingzhen et al., 2003], suggesting a potential effect on the cell apoptosis/survival and apoptosis related diseases. A lot of studies suggested RTN4 involved in vascular remodeling and seemed to be a novel player in the progress of AS [Acevedo et al., 2004; Rodriguez-Feo et al., 2007; Harrison et al., 2009]. Our research group recently found plasma RTN3 levels in patients with coronary artery disease were significantly elevated and were correlated with the severity of coronary artery disease, suggesting circulating levels of sRTN3 (soluble RTN3) may serve as a biologic marker of coronary artery disease. So we presume that reticulons may be a candidate factor linking apoptosis and AS.

AS AND APOPTOSIS

The mechanistic development of atherosclerosis is evolutionary and intricate in which cell apoptosis and proliferation at different phases have been proved to play an all-important role. The earlier progression of AS is defined by the endothelial dysfunction, which may be due to endothelial over-apoptosis, and inflammation with prominent lipid retention [Kockx et al., 1998]. In advanced lesions, extensive apoptosis of lipid-laden macrophages and smooth muscle cells (SMCs) may lead to plaque destabilization, rupture, and thrombosis [Soldani et al., 2005], which may trigger subsequent clinical presentation of the lesion such as acute coronary syndromes, cerebrovascular events, even cardiac death. Thereby, functional involvement of apoptosis in the lesion development of atherosclerosis mainly targets three cell types: a preferential pro-apoptotic stimulation for endothelial cells (ECs) and macrophages, an anti-apoptotic and proliferative stimulation for SMCs.

The molecular apoptotic signaling mechanisms in vascular cells and inflammatory cells of AS have been a subject of intensive studies for the past few decades. Three main pathways with overlapping components have been identified (Fig. 1): (1) An extrinsic pathway which involves direct initiator cascades triggered by cell surface death receptor such as Fas, TNFR or DR3 with its ligand; (2) An intrinsic pathway which involves mitochondria and intracellular death signals regulated by Bcl-2 family proteins; (3) Endoplasmic reticulum (ER)-stress pathway induced by accumulation of unfolded protein aggregates (unfolded protein response, UPR) or by excessive protein traffic (ER overload response, EOR).

However, the apoptosis pathways, instead of working separately, effect on each other in a network and activation of caspases-2, -3, -8, and -9 has been shown to be involved in this cross talking, which

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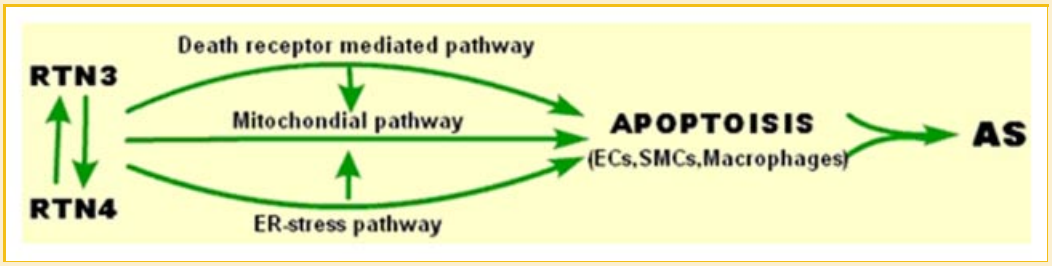

Fig. 1. Reticulons as the only molecular so far to participate in all three apoptosis signaling pathways, regulate apoptosis process of vascular cells (ECs, SMCs) and inflammatory cells (macrophages) in AS. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

has made it more difficult to develop definite targets for treatment of apoptosis-related disease, including AS. A key node of apoptosis pathway is expected to be found to regulate the network in a multi-channel and multi-ground way, and finally could be newly and efficiently anti-atherosclerotic target. RTN3, together with RTN4 has been proved to be the only molecular so far to participate in all three apoptosis signaling pathways [Tagami et al., 2000; Qu et al., 2002; Watari and Yutsudo, 2003; Kuang et al., 2005; Xiang et al., 2006; Qingwen et al., 2007; Zhu et al., 2007; Lee et al., 2009; Liu et al., 2009]. We presume that the brother of RTN family: RTN3 and RTN4 may belong to this principle node of apoptosis pathway in AS.

RTN3/RTN4 AND APOPTOSIS

RTN3 AND RTN4 REGULATING APOPTOSIS IN HOMODIMER/HETERODIMER MANNER

RTN3 and RTN4 both located on endoplasmic reticulum membrane. Several researches have found out the interaction between RTN3 and RTN4, which proves that RTN3/RTN4 proteins choose to form homodimer/heterodimer, and then promote/suppress apoptosis in those pathological areas [Bing et al., 2001; Qi et al., 2003; Qingzhen et al., 2003]. Apoptosis-regulating activity of RTN3 and RTN4 in normal cell lines is adjusted by their binding manners. The homodimer induces apoptosis while the heterodimer inhibits apoptosis, and the key point is the ratio of homodimer and heterodimer of RTN proteins. Herein, we further postulate that RTN3/RTN4, as the juncture of the apoptotic pathways, regulates progress of AS by protein–protein interactions including their homodimerization or RTN3/RTN4 heterodimerization. The homodimer/heterodimer formed by RTN3/RTN4B brings its apoptosis-inducing ability/apoptosis suppression ability into full play during the development of AS.

RTN3 AND RTN4 MODULATING APOPTOSIS BY INTERACTION WITH APOPTOTIC PROTEINS

The modulatory effect of RTN3/RTN4 in apoptotic pathways involved in the atherosclerotic lesion progression has been studied. Bcl-2, mainly acting on the mitochondria, can alternately interact with RTN3 on the ER. When the HeLa cells stably expressing Bcl-2 were treated with tunicamycin, endogenous RTN3 increased in the cell microsomal fraction. This change increased the Bcl-2 in microsomal fractions and also in the mitochondrial fractions where the anti-apoptotic activity of Bcl-2 mainly acts [Qingwen et al., 2007; Zhu et al., 2007]. Furthermore, it has been reported that RTN4 associates with the anti-apoptotic proteins Bcl-2 and Bcl-XL, changes their localization to the ER, and reduces their anti-apoptotic activity [Tagami et al., 2000]. These results suggest that the overexpression of RTN3 and RTN4 protein can induce apoptosis by disturbing the anti-apoptotic mechanism of the Bcl-2 family proteins in the ER.

Fas-associating death domain/mediator of receptor-induced toxicity (FADD/MORT1) is a cytoplasmic adaptor molecule that participates in the initiation of apoptosis signal transduction induced by engagements of ligands to the death receptors. An emerging model for the regulation of cell apoptosis has established that ER bound RTN3 protein recruited endogenous FADD to the ER membrane and subsequently regulated and integrated death signals [Xiang et al., 2006; Liu et al., 2009]. Tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) is one member of type II membrane protein of the TNF family. More recently, the role of RTN3 in TRAIL-induced apoptosis by up-regulating the levels of the TRAIL receptor DR5 has been demonstrated [Lee et al., 2009]. Thus, RTN3 may play a critical role in the membrane bound death-inducing signaling complexes (DISC).

On endoplasmic reticulum (ER), either the mutant proteins not folding correctly or an excessive accumulation of proteins will lead to perturbation of ER function, which is termed as ER overload response (EOR). Recent research characterized RTN3 as a novel EOR-induced protein, eliciting ER-specific apoptosis with activation of caspase-12 and mitochondrial dysfunction through ER Ca^{2+} depletion and the sustained elevation of cytosolic Ca^{2+} [Qu et al., 2002; Kuang et al., 2005]. These results support that the overexpressed RTN3 induce apoptosis by release of ER Ca^{2+}stores, to trigger the initial signal-transducing pathways for EOR.

As RTN3 play its regulatory role in modulating and integrating upstream death signals, it seems reasonable to speculate RTN3 may also modulate the apoptosis of vascular cells and inflammatory cells in AS by these three apoptosis pathway.

RTN3 AND RTN4 ADJUSTING APOPTOSIS BY SUBCELLULAR LOCALIZATION ALTERATION

RTN3 and RTN4, as ER location protein though, may have a life outside of the ER. Previous studies showed that that RTN3 protein

with EGFP at its N-terminal located on ER while RTN3 protein with EGFP at its C-terminal suffused in the whole cell [Qi-lan et al., 2004]. Moreover, apparent apoptosis characteristics exhibited in ER-locating RTN3 overexpressing but not in suffused RTN3 over-expressing [Qi-lan et al., 2004], demonstrating that the apoptosis regulating function of RTN3 is ER dependent. Recent results suggested that CRELD1, a RTN3 binding protein, could partly change the localization of RTN3 from the endoplasmic reticulum to the plasma membrane and modulate the apoptotic activity of RTN3 [Xiang and Zhao, 2009].

These findings raise the intriguing possibility that the apoptosis-regulating activity of RTN3 and RTN4 in vascular cell line is adjusted by their subcellular localization alteration. Different subcellular location of RTN3 and RTN4 may change the apoptosis-modulating activity.

RETICULONS AND AS

Studies in vitro indicate that RTN4 expresses in human endothelial cells (ECs), smooth muscle cells (SMCs) as well as mouse fibroblasts. Furthermore, research findings on atherosclerotic plaque characteristics show that local expression of RTN4B reduced in atherosclerotic tissue and this might contribute to plaque formation and/or instability triggering luminal narrowing [Rodriguez-Feo et al., 2007].But plasmatic levels of Nogo-B (soluble Nogo-B) did not differ between atherosclerotic subjects and risk-factor matched controls [Rodriguez-Feo et al., 2007]. Our research group studied the different process of atherosclerotic foam cell formation, finding that the expression level varied and subcellular localization of RTN3 altered. The preliminary data suggest the importance of these proteins in vascular cell apoptosis and AS. Present knowledge shows that RTN3/RTN4 act as important regulators of extrinsic and intrinsic apoptosis signaling pathways and RTN3 is proved to be the only factors so far to mediate the cell apoptotic process in response to the all three well-established apoptosis pathways either in a provocative or an inhibitory mode of action, which support our hypothesis about the feasible role of RTN3/RTN4 in AS.

CONCLUSION

The balance between pro- and anti-apoptotic signaling pathways determines the fate of a cell in response to the external or internal stimuli, and the amount of cell apoptosis and proliferation determines the progress of AS. It has been well-established that all the three classical apoptotic pathways participate in the apoptosis of vascular cells and inflammatory cells in AS which perform a vital role in the development of atherosclerotic lesion throughout. Recent researches revealed that RTN3/RTN4 not only involves in but also can moderate the three apoptotic pathways yet discovered in mammalian cells. It is a very interesting to speculate that the balance of apoptosis-inducing/apoptosis-reducing activity of RTN3/RTN4 in vascular cells including ECs, SMCs, and inflammatory cells such as macrophages, may have effect on the pathogenesis of AS at various stages (Fig. 1).

The molecular and cellular mechanisms that regulate apoptosis in different vascular cells in the particular phases of atherosclerotic lesion development are complex. RTNs may potentially act as the juncture of these mechanisms although the available data on the role of RTN family members in vascular apoptosis in AS is so far limited. Therefore, further functional explorations of RTNs in normal cell line and at different subcellular locations will contribute to a better understanding of this widely distributed reticulon family. Identification of these pivotal decision points and crosstalk is a critical issue in terms of finding new biomarkers and developing novel therapeutic approaches for AS.

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