A SHORT RESEARCH REVIEW ARTICLE

Novel Function of DAP3 as A Tumor Suppressor Gene

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ABSTRACT

DAP3 is a cell death mediator of the signaling pathways induced by interferon-γ, Fas ligand, tumor necrosis factor (TNF)-α, and TNF-related apoptosis-inducing ligand (TRAIL). DAP3 is also critical for the mitochondrial homeostasis during cell death and the anoikis induction. Anoikis is induced by detachment of adherent epithelial cells from the extracellular matrix. The deregulation of anoikis induction leads to the survival of cancer cells to promote the metastasis formation. Since the metastasis causes 90% of human cancer deaths, DAP3 might be a critical target for the effective cancer therapy. In fact, in the case of human breast cancer patients, lower DAP3 expression levels were significantly associated with local recurrence, high tumor grade, distant metastasis, and high mortality. Therefore, DAP3 could be used as a prognostic marker of the cancer patients and represents a potential therapeutic target as a novel tumor suppressor gene for the cancer treatment. Osteosarcoma is the primary bone tumor, and the sensitivity of cancer cells to TRAIL-induced apoptosis is lower than that of other type tumor cells. We found that DAP3 was interacted with LKB1, a serine/threonine kinase, expressed in bone and soft tissue sarcoma cells, and DAP3 and LKB1 cooperatively induce apoptosis by TRAIL stimulation. Therefore, new therapeutic modalities targeting these molecules are required to be developed for the improvement of the therapy. Furthermore, we identified a novel DAP3-binding protein termed death ligand signal enhancer (DELE). Stable expression of DELE in human non-small-cell lung cancer cell line A549 enhanced the sensitivity to apoptosis induction by TRAIL stimulation. In addition, knockdown of DELE protected the HeLa cells from apoptosis induction and significantly inhibited the activation of caspase-3, 8, and 9. Since DELE mRNA is ubiquitously expressed in a variety of organs, further studies are required to develop a novel therapeutic approach for many types of cancer.

Key words: Anoikis, apoptosis, DAP3, death ligand signal enhancer, Fas, interferon-γ, LKB1, tumor necrosis factor-related apoptosis-inducing ligand

INTRODUCTION

DAP3 was identified as a cell death mediator of interferon-γ by a functional gene cloning using an antisense cDNA expression library.[1] Downregulation of DAP3 expression by the antisense RNA inhibited interferon-γ-mediated cell death induction. DAP3 gene is shown to be ubiquitously expressed in different tissues and codes for a 46-kDa protein. In the nematode *Caenorhabditis elegans*, a potential homolog of DAP3 shows 35% identity and 64% similarity to the human protein. Overexpression of the nematode DAP3 cDNA in mammalian cells induced cell death, indicating that the protein is conserved at the structural and functional levels in nematodes and mammals. Intact full-length protein of DAP3 is required to induce apoptosis by the overexpression; on the other hand, the expression of the N-terminal 230 amino acids exhibited a dominant-negative effect on the apoptosis induction. In addition, inhibition of DAP3 expression by antisense RNA and also expression of the dominant-interfering form of DAP3 protected cells from

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apoptosis induction mediated by Fas and tumor necrosis factor (TNF)-α receptors.[2] Furthermore, we have shown that DAP3 is required for apoptosis induction by TNF-related apoptosis-inducing ligand (TRAIL).[3,4] The N-terminal sequence targets DAP3 to mitochondria as mitochondrial ribosomal proteins[5-8] and DAP3 is critical for the mitochondrial homeostasis and the process of mitochondrial fragmentation during cell death.[4,9] It was also demonstrated that DAP3 is important for the anoikis induction.[10-12] Anoikis is induced by detachment of adherent epithelial cells from the extracellular matrix (ECM) and integrin stimulation is shown to protect cells from anoikis induction by Akt activation to inhibit DAP3 function.[10] The deregulation of anoikis induction leads to the survival of cancer cells leaving the primary tumor site without the adhesion to ECM as a critical step in metastasis formation. Since the metastasis is a primary cause of cancer mortality, the regulation of the expression and function of DAP3 is prospected to bring about the effective cancer therapy in the future.

TARGET MOLECULE FOR THERAPY

Recently, it was shown that DAP3 expression is regulated by LTRs of one of the endogenous retrovirus families, human endogenous retroviruses (HERV)-K (HML-10).[13] HERVs constitute 8% of the human genome and generate RNAs that modulate host gene expression. HML-10 is shown to be enriched within introns of human genes. LTRs of HML-10 have variable promoter activity in human cancer cell lines. Surprisingly, HML-10 LTR-primed RNA was found to be in opposite orientation to DAP3 gene.[13] It was demonstrated that HML-10 LTR-primed transcripts negatively regulate DAP3 expression and inhibit DAP3-mediated apoptosis induction in human cervical cancer HeLa cells. Since the expression of HML-10 RNA is upregulated in various tumor cell lines and primary tumor cells, it might contribute to inhibition of apoptosis induction in cancer cells. Therefore, it suggests that DAP3 might be involved in the tumorigenesis of cancers caused by the endogenous retroviruses. Furthermore, it is demonstrated that knockdown of DAP3 expression in human breast cancer cells, MCF7, and MDA-MB231 significantly increased the adhesiveness, compared to the control cells.[14] Furthermore, the ability of invasion and migration was significantly increased by the DAP3 knockdown in MDA-MB-231 cells. It suggests that low expression of DAP3 contributes to breast carcinogenesis by increasing the ability of cell adhesion, migration, and invasion. In fact, it had been reported that lower DAP3 expression levels were significantly associated with local recurrence, distant metastasis, and mortality in human breast cancer patients by the clinical study over a 10-year follow-up period.[15] This study demonstrates the relevance of DAP3 mRNA expression levels to the tumor stage and clinical outcome in breast cancer. Therefore, DAP3 could be used as a marker for prognosis of the cancer patients and represents a potential therapeutic target as a novel tumor suppressor gene for the cancer treatment. In addition, as aforementioned, DAP3 plays a critical role in TRAIL-mediated apoptosis by activation of procaspase-8.[3,4] Since TRAIL has a function as a selective apoptosis inducer in most tumor cells, but not in normal cells, it is one of the targets for cancer therapy. However, in the case of osteosarcoma, the sensitivity of cells to TRAIL-induced apoptosis is lower than that of other type tumor cells. We found that DAP3 was interacted with LKB1, a serine/threonine kinase, expressed in bone and soft tissue sarcoma cells, through LIP1, LKB1-binding protein.[16] We also demonstrated that expression of DAP3-induced apoptosis in osteosarcoma cells. Furthermore, expression of LKB1-induced apoptosis by activation of caspases and coexpression of DAP3 with LKB1 strongly induced apoptosis in osteosarcoma cells. In addition, DAP3-induced apoptosis was inhibited by the expression of LKB1K78M, LKB1 kinase-dead mutant in these cells. These results suggest that DAP3 and LKB1 are critical for TRAIL-induced apoptosis induction, in cooperation with each other in osteosarcoma cells. In spite of improvements in chemotherapy and surgery for the therapy of osteosarcoma, satisfactory treatments are still difficult to achieve. Therefore, new therapeutic modalities are required to be developed for the improvement of these treatments. In the future, these molecules could be critical target molecules for the therapy of osteosarcomas. Importantly, we found a novel DAP3-binding protein termed death ligand signal enhancer (DELE).[17] DELE protein is identified to interact with DAP3 by yeast two-hybrid screening, and the subcellular localization is in mitochondria. It is shown to contain a mitochondrial targeting sequence and two predicted tetratricopeptide repeats motifs, responsible for protein-protein interaction. In addition, by the amino acid sequence analysis using PEST find, DELE is demonstrated to carry the PEST sequence in the N-terminus mitochondrial-targeting sequence. The PEST sequence is responsible for protein destabilization, and the protein level of exogenously expressed DELE is increased by the treatment of MG132, an inhibitor of the ubiquitin-proteasome pathway. The expression level of DELE regulated by ubiquitin-proteasome might be one of the mechanisms for the induction of apoptosis by DELE. We demonstrate that DELE binds to DAP3 by coimmunoprecipitation assay in mammalian cells. Overexpression of DELE-induced apoptosis in HeLa cells, and human non-small-cell lung cancer cell line A549, stably expressing DELE was susceptible to apoptosis induction by TNF-α and TRAIL stimulation.[17] In addition, knockdown of DELE expression by siRNA transfection protected the HeLa cells from apoptosis induction by these ligands stimulation. The knockdown of DELE expression significantly inhibited the activation of caspase-3, 8, and 9 induced by TNF-α, anti-Fas, or TRAIL stimulation. These results indicate the significance of DELE for apoptosis induction by these death receptors. The expression level profile in the HUGE protein database demonstrated that DELE mRNA is ubiquitously

expressed in a variety of organs. These data suggest that DELE might be involved in apoptosis induction of the cells in various organs. To develop a novel therapeutic approach, further investigations are required to clarify the physiological importance of DELE and the functional relationship between DAP3 and DELE for the regulation of apoptosis and anoikis in the tumorigenesis and metastasis.

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