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Protein expression and intracellular localization of prostate apoptosis response-4 (Par-4) are associated with apoptosis induction in nasopharyngeal carcinoma cell lines

Jeng-Woei Lee [a,c,*], Kuei-Fang Lee [a], Hsue-Yin Hsu [a], Lee-Ping Hsu [b], Wen-Ling Shih [a], Yi-Chih Chu [c], Wei-Ting Hsiao [c], Po-Fan Liu [a]

[a] *Department and Institute of Life Science, College of Life Science, Tzu-Chi University, 701, Sec. 3, Chung-Yang Road, Hualien 97071, Taiwan*
[b] *Department of Otolaryngology, Buddhist Tzu-Chi General Hospital, Hualien, Taiwan*
[c] *Graduate Institute of Molecular & Cellular Biology, College of Life Science, Tzu-Chi University, 701, Sec. 3, Chung-Yang Road, Hualien 97071, Taiwan*

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Abstract

Prostate apoptosis response-4 (Par-4) is a proapoptotic gene that selectively induces cell death in most cancer cells. In addition to the increased percentage of apoptotic cells, caspase-3 activity, and poly (ADP-ribose) polymerase (PARP) cleavage, we demonstrate that elevated expression of Par-4 and nuclear entry resulted in apoptosis of nasopharyngeal carcinoma (NPC) cell lines either in serum deprivation or by ectopic overexpression of Par-4. Moreover, disassociation from the Par-4/Akt complex was correlated with the induced proapoptotic ability of Par-4. Therefore, our data suggest that the cytoplasmic localization and expression level of endogenous Par-4 in NPC cells are not sufficient to augment apoptosis.

Keywords: Par-4; Apoptosis; Nasopharyngeal carcinoma

1. Introduction

Nasopharyngeal carcinoma (NPC) is an endemic cancer with a very high incidence in South China and Southeast Asia regions, such as Hong Kong and Taiwan. Epstein–Barr virus (EBV), genetic factors, certain dietary (nitrosamine, herbal medicine), and environmental conditions (smoking, occupational exposures) have been associated with NPC [1], but the molecular mechanisms by which NPC develops remain largely unknown. Various tools such as cytogenetic analysis, loss of heterozygosity (LOH), comparative genomic hybridization (CGH), and microsatellite polymorphic markers have been employed to investigate the candidate genes associated with NPC. These studies offered a genome-wide investigation based upon a comprehensive pattern of DNA sequence copy number changes. To understand the putative order of genetic alteration in NPC tumorigenesis, evolution-

* Corresponding author. Tel.: +886 3 8565301x7547; fax: +886 3 8572526.
E-mail address: jwlee@mail.tcu.edu.tw (J.-W. Lee).

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ary tree models were used to analyze CGH data and from them it was determined that the loss of chromosome 3p and the amplification of chromosome 12 are important early events in NPC progression [2]. Additional studies have confirmed these findings [3,4]. Therefore, the data seem to suggest the presence of NPC-associated candidate genes on chromosome 12. Among them, the proapoptotic gene, prostate apoptosis response-4 (Par-4), plays an important role in the induction of apoptosis [5].

Par-4 is a proapoptotic gene that was originally identified in androgen-independent prostate cancer cells undergoing apoptosis [6]. Its molecular weight is 38 kDa and it has been conserved during evolution in vertebrates [7]. Par-4 protein, which contains a leucine zipper domain at the carboxy-terminus and two putative nuclear localization sequence (NLS) signals at the N-terminus, shares 100% homology in the human, rat, and mouse, thereby suggesting that the function and regulation of Par-4 is similar in mammalian systems [8]. Most research suggests that Par-4 overexpression is sufficient to induce apoptosis in most but not all cancer cells. However, in the absence of a second apoptotic signal, normal or immortalized cells are resistant to its apoptotic action. Functional and localization analysis suggest that Par-4 localizes in the nucleus in most cancer cell lines and that nuclear entry is essential for direct apoptosis. Evidence also provides a selective apoptosis induction in cancer cells (SAC domain) with an intact NLS2 region that has been identified as an essential process for induction of apoptosis, whereas, this core domain does not induce apoptosis in normal cells [9]. Various studies have shown that through the leucine zipper domain, Par-4 interacts with a variety of proteins such as WT1 [10], PKC ζ [11], p62 [12], DLK/ZIP (DAP-like kinase) [13], Amida [14], F-actin [15], or Akt [16] to inhibit several prosurvival pathways and to activate apoptosis. The molecular mechanism of apoptosis by Par-4 involves the inactivated transcriptional activity of NF-κB and the down-regulation of *bcl-2* transcription [8]. Conversely, the genetic inactivation of Par-4 also results in the hyperactivation of NF-κB and the impairment of JNK and p38 [17]. In addition, Par-4 expression also recruits Fas and FasL to the cell membrane, activates FasL-Fas-FADD-caspase eight pathways [18], and/or subsequently facilitates intrinsic and extrinsic caspase pathways to induce apoptosis [19]. Par-4 possesses a number of conserved consensus sites for phosphorylation by protein kinase A (PKA) and protein kinase C (PKC). Studies show that phosphorylation of Par-4 at Thr^{155} by PKA or at Ser^{249} by Akt is critical for apoptotic activity of Par-4. However, phosphorylation by PKA is required for apoptotic functioning. In contrast, modification by Akt antagonizes the proapoptotic activity of Par-4 [16,20]. Collectively, the nuclear entry and phosphorylation are two important but distinct processes for Par-4 proapoptotic actions.

Apart from inducing apoptosis, Par-4 also has been shown to inhibit cell transformation. Oncogenic Ras down-regulates the expression of Par-4 via the Raf-MAPK-ERK pathway. On the other hand, the restoration of Par-4 expression in Ras-transformed cells severely impairs cell transformation [21–23]. Consistent with the anti-transformation activity of Par-4, *Par-4*-null mice are prone to develop tumors, both spontaneously and on carcinogen treatment [24]. Evidence also demonstrates suppressed migration of mouse melanoma cells by the Par-4/PKC ζ complex [25]. Interestingly, expression of Par-4 is diminished in renal cell carcinoma [26] and other tumors [27]. Given the role of Par-4 in tumorigenesis and metastasis, it should be considered a cancer-selective therapeutic target [24,28].

To date, no research has evaluated the tumor suppressive effects of Par-4 and the pathophysiology of NPC. In this study, we demonstrated that elevated expression and translocalization of Par-4 causes its proapoptotic activity. Furthermore, we also provide a possible tumor therapeutic way via regulated intracellular positioning of Par-4.

2. Materials and methods

2.1. Cell culture

Human nasopharyngeal carcinoma cell lines, HONE-1 (a gift from Dr. Ching-Hwa Tsai, National Taiwan University, Taipei, Taiwan), and NPC-TW01 (a gift from Dr. Chin-Tarng Lin, National Taiwan University Hospital, Taipei, Taiwan) were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 0.1 mM nonessential amino acid, 50 μg/mL streptomycin, 50 U/mL penicillin and 10% fetal bovine serum at 37 °C in the 5% CO_2 incubator.

2.2. RNA isolation and RT-PCR

Total RNA was isolated with TRI™ Reagent (Sigma, St. Louis, MO) following the manufacturer's instructions. For RT-PCR analysis, one microgram of RNA was reverse transcribed by Superscript™III reverse transcriptase