Journal of Cellular Biochemistry 91:504–512 (2004)

Par-4 Inducible Apoptosis in Prostate Cancer Cells

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Abstract Prostate cancer is associated with the inability of prostatic epithelial cells to undergo apoptosis rather than with increased cell proliferation. Prostate apoptosis response-4 (Par-4) is a unique pro-apoptotic molecule that is capable of selectively inducing apoptosis in cancer cells when over-expressed, sensitizing the cells to diverse apoptotic stimuli and causing regression of tumors in animal models. This review discusses the salient functions of Par-4 that can be harnessed to prostate cancer therapy. J. Cell. Biochem. 91: 504–512, 2004. © 2004 Wiley-Liss, Inc.

Key words: Par-4; prostate; apoptosis

Prostate cancer is the most prevalent form of cancer among men after lung cancer in the United States. The natural progression of prostate cancer follows a pattern starting from a localized disease that is androgen-dependent to a more advanced, invasive, and metastatic disease, which is often associated with loss of androgen-dependence. Cells of the normal adult prostate or those of a primary prostate cancer consist of a majority of cells that are dependent on androgen for survival and proliferation. Upon withdrawal of androgen, the rate of apoptosis exceeds the rate of cell proliferation, resulting in the involution of the normal prostate as well as regression of the tumor. This observation forms the basis of androgen ablation, the mainstay form of treatment for prostate cancer. As androgen-ablation treatment targets the androgen-dependent cells, the heterogeneous presence of androgen-independent cancer cells present within the tumor often leads to development of a more aggressive androgen-independent tumor. The development of androgen-independent prostate cancer is a consequence of lack of an apoptotic response to androgen-ablation owing to mechanisms that allow survival of the cells within primary or metastatic tumors. Although androgen-independent cells contain intact cell death programs, they fail to initiate or execute these programs in response to conventional modes of treatment, thereby tilting the balance in favor of cell proliferation. The search is on to identify effective therapeutic molecules which have the potential to induce apoptosis by over-riding the roadblocks created by the anti-apoptotic mechanisms in androgen-independent or metastatic cells. One such promising molecule that can circumvent the anti-apoptotic mechanisms in prostate cancer cells is prostate apoptosis response-4 (Par-4). This review highlights the salient features of Par-4 including induction of apoptosis, inhibition of transformation and regression of tumors, and emphasizes the untapped potential of Par-4 in combination strategies against prostate cancer.

IDENTIFICATION AND CHARACTERIZATION OF PAR-4

Androgen-ablation causes apoptosis of the androgen-dependent prostate cancer cells resulting in rapid involution of the tumor and is the mainstay of prostate cancer treatment. The effect of androgen-ablation is emulated in the rat ventral prostate gland, which serves as an excellent model to study apoptosis and prostate involution, by withdrawal of androgen. Several

Grant sponsor: NIH/NCI (to V.M.R.); Grant numbers: CA60872, CA84511.

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Received 12 November 2003; Accepted 14 November 2003
DOI 10.1002/jcb.20000

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novel transcripts and proteins are induced post-castration in the regressing prostate and inhibitors of protein and RNA synthesis inhibit regression of the prostate, indicating that the gene products induced by androgen ablation are necessary for apoptosis. Importantly, the calcium channel inhibitor nifedipine causes inhibition of both apoptosis and prostate involution after castration, indicating a role for intracellular calcium elevation in apoptosis of the prostate. In a differential screen for genes induced during apoptosis of prostate cancer cells, we first identified the rat prostate apoptosis response-4 (*Par-4*) gene [Sells et al., 1994]. Par-4 was up-regulated when androgen-independent prostate cancer cells were induced to undergo apoptosis upon treatment with a calcium ionophore ionomycin, mimicking the increase in intracellular calcium induced by androgen ablation. Par-4 was also induced in androgen-dependent luminal/secretory cell compartment of the rat ventral prostate after castration. Interestingly, Par-4 was not induced in other organs, such as the liver or kidneys, which express androgen receptors but do not undergo apoptosis and involution after castration. Moreover, the *Par-4* gene is not induced by growth stimulation, growth arrest or necrosis in cell culture paradigms. Subsequent studies identified the human *Par-4* gene, which shared a high degree of nucleotide and amino acid similarity with the rat *Par-4* gene [Diaz-Meco et al., 1996; Johnstone et al., 1996].

Par-4 is a 38-kDa protein that maps to human chromosome 12q21, a region that is unstable and often deleted in pancreatic and gastric cancer [Kimura et al., 1998; Schneider et al., 2003]. The Par-4 sequence encodes a 332 amino acid protein, consisting of two putative nuclear localization sequences in the N-terminal region and a leucine zipper domain and a nuclear export sequence in the C-terminal portion of the protein. These domains are 100% conserved in human, rat and mouse homologs of Par-4, suggesting that the function, localization and regulation of Par-4 is similar in mammalian systems. In addition, Par-4 protein is conserved during evolution in vertebrates [Boghaert et al., 1997]. Consistent with its pro-apoptotic function, Par-4 is highly expressed in involuting tadpole tail. The fact that the structure and function of Par-4 are evolutionarily conserved indicates the global significance of the apoptotic function of Par-4.

In mammals, Par-4 is widely expressed in mammalian tissues. In the normal prostate gland, Par-4 is expressed in the mesenchyme surrounding the ventral part of the prostate and the basal glandular epithelial cells but is absent in adjacent differentiated ductal cells. A similar pattern is seen in epithelial cells of mammary gland and the retina where Par-4 is undetectable in terminally differentiated cells implying that Par-4 expression is down-regulated during differentiation. Interestingly, withdrawal of androgen results in massive apoptosis of the ductal cells and this apoptotic process is mediated by Par-4 that is induced during androgen ablation. Upon testosterone ablation caused by castration, ductal cells undergo apoptosis, which peaks at day 3. Par-4 levels increase in ductal cells of the rat prostate on day 1 and day 3 post castration and diminish by day 5, suggesting that Par-4 induction is an early and transient event in apoptosis of prostate ductal cells [Sells et al., 1994]. Interestingly, the anti-apoptotic protein Bcl-2 is normally well expressed in the ductal cells and androgen-withdrawal results in significant decrease in Bcl-2 expression. Consistently, an inverse correlation between Par-4 and Bcl-2 expression is noticed in human prostate tumors [Qiu et al., 1999a]. Par-4 but not Bcl-2 is detected in primary and metastatic prostate tumors and in xenografts of human androgen-dependent CWR22 tumors, whereas the androgen-independent CWR22R tumors show pockets that stain for Bcl-2 but not Par-4. A similar inverse pattern of expression between Par-4 and Bcl-2 was also observed in lymphocytes from acute lymphoblastic leukemia patients [Boehrer et al., 2002].

APOPTOSIS BY PAR-4 IN CANCER CELLS

Par-4 over-expression is sufficient to induce apoptosis in most cancer cells but normal primary or immortalized cells are resistant to its apoptotic action in the absence of a second apoptotic signal. Par-4 very efficiently induces apoptosis in all hormone independent cancer cell lines tested including lung, pancreatic, and head and neck cancers as well as transformed cells such as Ras transformed NIH 3T3 fibroblasts [Nalca et al., 1999; Chakraborty et al., 2001; El-Guendy et al., 2003]. Interestingly, Par-4 is able to override potential anti-apoptotic mechanisms such as high NF-κB activity, elevated expression of Bcl-xL or Bcl-2 present

in these cell lines. The apoptotic function of Par-4 is also independent of the status of tumor suppressors such as p53 or PTEN. Interestingly, in cancers that arise in organs such as the prostate or the breast regulated by hormones such as estrogen or androgen a unique dichotomy of Par-4 function is noted: Par-4 efficiently kills the more advanced hormone independent cancer cells whereas the hormone dependent cancer cells are resistant to the action of Par-4. Par-4 induces apoptosis in androgen independent prostate cancer cells such as PC-3 and DU-145 but not in androgen dependent prostate cancer cells such as LNCaP, LAPC-4, MDA PCa 2a and 2b [Chakraborty et al., 2001]. Similarly in breast cancer cells, Par-4 induces apoptosis in estrogen independent cells such as MDA MB-231, MDA MB-435 but is unable to do so in the estrogen dependent cells such as MCF-7 and T47D [El-Guendy et al., 2003].

The mechanism of apoptosis by Par-4 involves a unique co-parallel activation of a pro-death pathway together with the inhibition of a pro-survival pathway. Par-4 activates the pro-death FasL-Fas-FADD-caspase-8 pathway by translocation of Fas and FasL to the cell membrane, and in parallel it inhibits the key pro-survival transcriptional activity of NF-κB [Chakraborty et al., 2001]. Par-4 does not inhibit the ability of NF-κB to bind to DNA but abrogates the transcriptional activity of NF-κB, thereby preventing the expression of NF-κB-regulated anti-apoptotic genes. As a corollary, it is interesting to note that, MEFs derived from Par-4−/− mice have elevated levels of NF-κB activity compared to matched Par-4+/+ MEFs [Garcia-Cao et al., 2003]. In addition to inhibition of NF-κB transcription Par-4, in parallel, activates the pro-death FasL-Fas-FADD-caspase-8 pathway by effecting the golgi dependent translocation of Fas and FasL to the cell membrane. The trafficking of Fas and FasL by Par-4 triggers the interaction of death receptor Fas and FADD that is essential for the formation of a death-inducing signaling complex (DISC) [Chakraborty et al., 2001]. Certain cancer cells, such as the acute myeloid leukemia cells, acquire resistance to Fas-induced apoptosis by phosphorylating FADD and thereby inhibiting DISC formation. It was recently found that Par-4 inhibits phosphorylation of FADD by inhibiting atypical PKC and promotes DISC formation [de Thonel et al., 2001]. Activation of the Fas-FADD pathway is crucial for apoptosis by Par-4, as dominant negative or dominant interferring mutants of Fas, FADD, or caspase-8-blocked apoptosis by Par-4. In prostate cancer cells, the inhibition of NF-κB alone with IκB or the activation of the Fas pathway alone is not sufficient to induce apoptosis; but when the IκB and Fas are used together they mimic Par-4 induced apoptosis. This implies that each pathway is essential but not sufficient to accomplish apoptosis of the prostate cancer cells and that Par-4 alone regulates both these pathways [Chakraborty et al., 2001].

STRUCTURE, FUNCTION AND LOCALIZATION ANALYSIS: IDENTIFICATION OF SELECTIVE FOR APOPTOSIS INDUCTION IN CANCER CELLS (SAC) DOMAIN

Par-4 is strongly localized in the nucleus in most cancer cell lines. On the other hand, in all the normal cells tested, over-expressed Par-4 in the absence of another apoptotic signal is predominantly localized in the cytoplasm and does not induce apoptosis. Concordantly, the ΔNLS2 mutant of Par-4 in which both NLS1 and NLS2 are deleted, is purely cytoplasmic in its localization and is unable to induce apoptosis [El-Guendy et al., 2003]. Intriguingly, in hormone-dependent breast cancer cells MCF-7 or prostate cancer cells LNCaP, Par-4 is purely cytoplasmic in localization and does not induce apoptosis. However, derivatives of these cell lines known to be hormone independent such as MCF-7 stably expressing oncogenic Ras (MCF-7 Ras) or LNCAP stably expressing IL-6 (LNCaP-IL-6) show nuclear translocation of Par-4 and are susceptible to apoptosis by Par-4 [El-Guendy et al., 2003]. All these findings taken together suggest that nuclear entry is essential for direct apoptosis by Par-4. The mechanism by which Par-4 is excluded from the nucleus of normal cells, estrogen-dependent breast cancer cell lines or androgen-dependent prostate cancer cell lines is not known. Nuclear exclusion is possibly a highly efficient mechanism to protect healthy cells from the apoptotic action of Par-4, and it is conceivable that the one of the outcomes of tumor progression is the diminished stringency of the nuclear transport machinery in more advanced cancers relative to the normal cells leading to increased nuclear presence of Par-4. Our ongoing studies will verify whether nuclear entry is a regulatory mechanism for apoptosis by Par-4 and identify the molecular

basis for the differences underlying the nucleocytoplasmic trafficking of Par-4 in normal and cancer cells.

A deletion mutant of Par-4 that contains amino acids 137-195, which includes intact NLS2, constitutively enters the nucleus in all normal and cancer cell lines. The 137–195 mutant has an expanded range in inducing apoptotic killing both hormone-dependent and hormone-independent cancer cell lines. In fact, amino acids 137–195, represents a unique core domain of Par-4 that is essential and sufficient for Fas/FasL translocation to the cell membrane, inhibition of NF-κB activity and induction of apoptosis in cancer cells. Interestingly, although this core domain localizes to the nucleus, it does not induce apoptosis in normal cells and was therefore designated as the domain for Selective Apoptosis-induction in Cancer cells (SAC domain) [El-Guendy et al., 2003]. This core domain is 100% conserved in rat, mouse, and human Par-4. These findings indicate that nuclear entry is essential but not sufficient for apoptosis by Par-4, and that Par-4 may require additional activation events; for example, posttranslational changes in the SAC domain; that occur selectively in cancer cells leading to induction of apoptosis. The SAC domain resembles neither the death domains nor the death effector domains of other pro-apoptotic proteins, nor does it show significant homology with other known proteins in GenBank; and unlike the previously characterized death domains and death effector domains, the SAC domain specifically induces apoptosis in cancer cells and not normal cells. These findings suggest that the leucine zipper domain of Par-4 at aa 290–332 is not required for the direct induction of apoptosis by Par-4.

PAR-4 SENSITIZES NORMAL CELLS TO VARIOUS APOPTOTIC STIMULI

Although Par-4 over-expression does not induce apoptosis in normal cells, Par-4 expression sensitizes cells to apoptosis by a wide variety of pro-apoptotic stimuli such as growth factor withdrawal, agents that elevate intracellular Ca^{2+}, TNF, UV, X-ray and gamma irradiation, or IFNγ. Ablation of endogenous Par-4 protein levels by treatment with antisense or siRNA against Par-4 or functional interruption of Par-4 using a dominant negative mutant of Par-4 abrogates apoptosis by these apoptotic agents. Various reports have indicated that Par-4 plays a sensitizing function in various cell types including epithelial cells of the prostate, endothelial cells, transformed fibroblasts and a variety of carcinomas. In its apoptosis-sensitizing role in these diverse cell backgrounds, Par-4 inhibits several pro-survival pathways, activates pro-apoptotic proteins and binds to several different partners proteins of varying function. The leucine zipper of Par-4 is the functional domain responsible for sensitization to apoptotic stimuli. Various studies have shown that deletion of the leucine zipper domain abrogates this function of Par-4. Interestingly, the leucine zipper domain alone acts as a dominant negative mutant of Par-4; it inhibits endogenous Par-4 function by binding to the leucine zipper domain of intact Par-4. The leucine zipper domain of Par-4 is required for Par-4 to interact with a wide variety of proteins such as WT-1, atypical PKCs, p62, DLK/ZIP kinase, and THAP1.

Par-4 down-regulates the anti-apoptotic protein Bcl-2. The *bcl-2* gene was first identified in the t(14:18) translocation breakpoint from human follicular B cell lymphoma [Reed et al., 1996; Yang and Korsmeyer, 1996]. Bcl-2 has been found to be over-expressed in androgen independent prostate cancer. Over-expression of Bcl-2 enables prostate cancer cells to resist apoptosis induced by androgen-withdrawal, physiological death inducers such as TRAIL, or chemotherapeutic agents [reviewed in Bruckheimer et al., 1999]. Qiu et al. [1999a] demonstrated that in immortalized mouse fibroblasts NIH 3T3 and prostate cancer cells PC-3 that over-expressed Par-4, there was a significant down-modulation of bcl-2 both at the promoter level and the protein level and this down-regulation of bcl-2 was essential for Par-4-mediated apoptosis. Indeed, co-transfection of bcl-2 rescued the cells from apoptosis induced by growth factor withdrawal. Par-4 exerts a pro-apoptotic role, augmenting chemosensitivity by down-regulating Bcl-2, promoting disruption of mitochondrial membrane potential and enforcing caspase-activation. It is interesting to note that in prostate cells, *bcl-2* is down-regulated by androgen withdrawal, the same signal that up-regulates Par-4, suggesting that Par-4 is an important molecular component involved in Bcl-2 down-modulation and induction of apoptosis [Qiu et al., 1999a]. A better understanding of the mechanism of repression of *bcl-2* by Par-4

is now possible with recent findings by Cheema et al. [2003] that Par-4 utilizes WT1 to bind to the *bcl-2* promoter to transcriptionally down-regulate Bcl-2 expression. WT-1 binds to, and up-regulates the expression of, the bcl-2 promoter in the androgen independent cell line LNCaP/LN3. Co-expression of WT-1 and Par-4, leads to leucine zipper domain mediated interaction of Par-4 with WT-1 at the bcl-2 promoter followed by repression of the bcl-2 promoter.

WT-1 is a tumor suppressor protein frequently mutated in Wilm's tumors, a rare form of kidney tumor. WT-1 mediates growth arrest mediated by several cytokines such as IL-1 and abrogates thapsigargin-induced apoptosis. Expression of Par-4 was able to override the WT-1-mediated abrogation of apoptosis in prostate cancer and melanoma cells [Johnstone et al., 1996; Sells et al., 1997]. Although WT-1 is mainly expressed in the organs of the urinogenital tract, less is known about the status of WT-1 protein in prostate cancer. As WT-1 can differentially regulate bcl-2 depending on the availability of Par-4 and as bcl-2 is often over-expressed in advanced prostate tumors, it is pertinent to further study the role of the interplay between WT-1 and Par-4 in the development of prostate cancer.

Par-4 inhibits the pro-survival pathway activated by atypical PKCs, ζPKC and λ/Iι PKC, by binding and inhibiting their kinase activities [Diaz-Meco et al., 1996]. aPKCs are serine/threonine kinases that positively regulate cell proliferation and cell survival by activating transcription factors AP-1 and NF-κB, respectively. aPKCs also block apoptosis by phosphorylating pro-apoptotic protein FADD and preventing DISC formation. The interaction between Par-4 and ζPKC, mediated through the leucine zipper domain of Par-4 and the zinc finger in the regulatory domain of ζPKC, induces a conformational change leading to inhibition of the catalytic activity of ζPKC. Exposure to apoptotic stimuli such as UV irradiation, ceramide or TNF triggers the interaction between endogenous Par-4 and endogenous ζPKC leading to a dramatic reduction of ζPKC enzymatic activity and an increase in apoptosis. Concordantly, replenishment of ζPKC levels by over-expression inhibits apoptosis by these agents [Diaz-Meco et al., 1996, 1999]. aPKCs activate NF-κB by functioning as IKKβ kinases. Par-4 potentiates TNF-induced apoptosis by inhibiting NF-κB through the blockade of the aPKC-IKK signaling cascade. It was recently identified that aPKC and Par-4 are part of a ternary complex with an adapter protein, p62 [Chang et al., 2002]. p62 has been suggested to play a critical role in ζPKC-mediated NF-κB activation in a way that p62 recruits ζPKC to the TNF signaling complex when the TNF receptor is activated by TNF, IL-1, or other ligands. p62 and Par-4 do not compete to bind to ζPKC but interact directly with each other and form a ternary complex with ζPKC. Binding of p62 to the ζPKC-Par-4 complex blocks Par-4 mediated inhibition of ζPKC kinase activity. This shows that the functions of endogenous Par-4 and ζPKC are very tightly controlled. However, little is known about the role of p62 in regulating the activity of ζPKC that is not recruited to the ternary signaling complex or about the role of p62 in the complex in the presence of diverse apoptotic signals.

Not surprisingly, Par-4 also activates several pro-apoptotic molecules. Recently, the idea that Par-4 mediates apoptotic events from PML bodies or PODs in the nucleus has emerged. In 1999, studies by Page et al. [1999] suggested that DLK/ZIP kinase (ZIPK) bound and phosphorylated Par-4, and that this function was essential for ZIPK-mediated apoptosis. These findings were corroborated recently by John Reed's group when they found that in response to several apoptotic stimuli such as IFNγ or As_2O_3, ZIPK associated with pro-apoptotic protein DAAX and that the interaction of ZIPK and Par-4 enhanced this association [Kawai et al., 2003]. Their work suggested that in response to IFNγ or As_2O_3, the ZIPK-DAAX complex is recruited to the PODs where they activate apoptosis. siRNA-mediated knockdown of Par-4 resulted in suppression of apoptosis-induced by IFNγ and As_2O_3. In primary endothelial cells and human fibroblasts endogenous Par-4 colocalizes with ectopic THAP1 within PODs [Roussigne et al., 2003]. In addition, Par-4 is a component of PML NBs in blood vessels, a major site of PML expression in vivo. THAP1 is a novel nuclear pro-apoptotic factor associated with PML NBs, which interacts with the pro-apoptotic protein Par-4 and potentiates both serum withdrawal- and TNF-induced apoptosis. Par-4 is recruited to PODs in endothelial cells where PML plays a critical role. Thus, Par-4 is a global sensitizing agent, whose functions/partners are modified in a context-specific manner.

INHIBITION OF TRANSFORMATION

Apart from induction of apoptosis, Par-4 has also been shown to be involved in suppression of transformation. Evasion of apoptosis is one of the key requirements of oncogenic transformation [Hanahan and Weinberg, 2000]. Oncogenic *ras*, a potent oncogene involved in 40% of epithelial tumors, has been shown to down-regulate the expression of Par-4 at the mRNA and protein level via the Raf-MEK-ERK pathway [Barradas et al., 1999; Qiu et al., 1999b]. The expression of oncogenic Ras promotes a potent reduction of Par-4 protein and mRNA levels through a MEK-dependent pathway. Treatment of the cells with MEK-inhibitor PD98059 restored the levels of Par-4. In addition, the ectopic expression of constitutively active mutants of Raf-1 or MEK, but not phosphatidylinositol 3-kinase (PI3-kinase) was sufficient to decrease Par-4 levels, suggesting that other oncogenic pathways that utilize these molecules as intermediates can potentially repress Par-4 to promote cellular transformation. Restoration of Par-4 levels by ectopic expression results in reduced expression of ERK1 and ERK2 MAP kinases [Qiu et al., 1999b]. Importantly, restoration of Par-4 levels in Ras-transformed cells by stable expression severely impairs colony formation in soft agar [Barradas et al., 1999; Qiu et al., 1999b]. This indicates that the down-regulation of Par-4 by oncogenic Ras is a critical event in tumor progression. Interestingly, it appears that the mechanism by which Par-4 suppresses Ras-mediated cellular transformation is independent of the mechanism by which it induces apoptosis in these cells. Consistently, inhibition of NF-κB is essential for Par-4 to induce apoptosis [Diaz-Meco et al., 1999; Nalca et al., 1999], and the Ras/Par-4 stably expressing cells do not show any difference in NF-κB activity when compared to the Ras/vector control cells.

Commensurate with the anti-transformation function, Par-4 is found to be down-regulated in several cancers. Expression of Par-4 is diminished in a majority of renal cell carcinoma (RCC) specimens or RCC cell lines relative to normal proximal renal tubular (PRT) cells within tumors or PRT cell lines [Cook et al., 1999]. Par-4 expression is also decreased in neuroblastoma cells [Kogel et al., 2001] and in cells of patients of acute lymphatic leukemia and chronic lymphocytic leukemia [Boehrer et al., 2001]. Interestingly, the immunological profile of Par-4−/− knockout mice shows an increased proliferative response of peripheral T cells together with increased cell cycle entry and inhibition of apoptosis, elevated NF-κB activity and decreased JNK activity [Garcia-Cao et al., 2003; Lafuente et al., 2003]. These findings suggest that Par-4 loss or down-regulation contributes to the pathogenesis of lymphatic malignancies. It remains to be seen whether the loss of Par-4 in the Par-4−/− mice contributes to the development of other malignancies. Further characterization of the tumor profile of these mice shed more light on the role of Par-4 in tumor development, progression and metastasis.

TUMOR REGRESSION

Consistent with its pro-apoptotic and anti-transformation role in cell culture paradigms, Par-4 over-expression in mouse tumor models results in tumor regression. Subcutaneous tumors were generated in nude mice with PC-3 cell implants and a single injection of adenovirus over-expressing Par-4 caused a dramatic reduction in tumor volume in <3 weeks compared to tumors injected with an adenovirus-producing GFP [Chakraborty et al., 2001]. Stable over-expression of Par-4 decreased the development of tumors resulting from xeno-transplanted A375-C6 melanoma cells in SCID mice and this diminished tumor growth correlated with increased tumor cell apoptosis [Lucas et al., 2001]. In addition to nude mice, Par-4 also reduced orthotopic tumors in immuno-competent mice indicating that this function of Par-4 is independent of the immuno-deficient status of the nude mice. Intra-prostatic tumors were generated in C57BL/6 mice by injecting syngenic prostate cancer cells RM-1 into the prostate gland of the mice. Similar to the subcutaneous tumors, a single injection of adenovirus expressing Par-4 caused significant reduction in tumor volume compared to the GFP expressing control virus demonstrating that tumor regression is a potent function of Par-4 [Herman et al., 2001]. The mechanism by which Par-4 causes reduction in tumor size involves induction of apoptosis as assessed by TUNEL staining of the tumors. Similar to the mechanism of apoptosis in cancer cell cultures, regression of tumors by Par-4 is dependent on

the activation of the Fas-FADD death receptor pathway and inhibition of NF-κB pathway. Tumors derived from cells stably expressing dn-FADD or RelA failed to show regression [Chakraborty et al., 2001]. Together, these findings suggest that Par-4 is an ideal candidate for therapy of tumors. However, not much is known about the role of Par-4 in two critical events of tumorigenesis including angiogenesis and local tissue invasion leading to metastasis of tumors. In a promising development, an in vitro study with melanoma cells suggested that Par-4 decreases migration in these cells. Over-expression of Par-4 in mouse melanoma B16 F1 cells decreases their migration ability in a ζPKC-dependent manner. The mechanism by which the migration was affected was not elucidated but in the presence of low levels of ζPKC, Par-4 did not affect cell migration [Sanz-Navares et al., 2001]. It was hypothesized that in non-metastatic cells, the ζPKC-Par-4 complex provides a brake on migration and events that prevent Par-4-mediated down-regulation of ζPKC activity in melanoma cells may promote metastasis.

FUTURE DIRECTIONS

Deregulated proliferation and reduced cell death are the two most common traits of diverse heterogeneous cancers. The key reason why Par-4 holds promise in the treatment of human cancers is that it targets these common traits by inhibiting proliferation and activating a cell death pathway leading to the collapse of the cancer platform. The cancer selective apoptotic ability of SAC is an especially advantageous trait compared to the contemporary chemotherapeutic agents, which often affect normal cells in addition to cancer cells and give rise to serious side effects. However, further levels of testing are essential before Par-4 can be put to clinical trials. Regulatable transgenic mouse models of oncogenesis are required to study anti-tumorigenesis by Par-4 and SAC, their effect on angiogenesis, tumor invasion, and metastasis. Also, most studies done so far have used mouse models and it is not known if the rapid reversal of the tumor seen in the mouse models translates similarly in human tumors. Identification of chemical agents that up-regulate Par-4 in cancer cells leading to apoptosis will provide comparatively easy delivery options compared to adenoviral therapy. Various COX-2 inhibitors such as NS-398, SC-58125, and nimesulide are currently being used as chemotherapy for colon and other cancers [Kalgutkar and Zhao, 2001] and considerable optimism is being generated in this area with the discovery that COX-2 inhibitors and polyethylene glycol (PEG) can up-regulate Par-4 in colon cancer cells [Corpet et al., 2000; Zhang and DuBois, 2000].

Although Par-4 over-expression results in significant regression of tumors in mouse models, it often does not lead to complete regression of the tumor. Taking this into consideration, it is essential to take a multi-pronged approach by combining other successful therapeutic strategies along with Par-4 so as to shut down the anti-apoptotic mechanisms and, in parallel, activate apoptotic pathways. For example, the traditional androgen ablation therapy used in prostate cancer leads to the loss of only the androgen dependent prostate cancer cells and results in the selection of a more invasive tumor with mainly androgen-independent cells. The efficacy of androgen therapy can be vastly improved if used in combination with over-expression of the SAC domain, since the SAC domain effectively induces apoptosis in androgen-independent prostate cancer cells and the combination should eliminate both hormone-dependent and -independent prostate cancer cells. Studies of the potential use of combination of Par-4 along with traditional forms of cancer therapy such as radiation therapy and chemotherapy are currently underway in various laboratories.

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