The Phosphoinositide 3-Kinase/Akt1/Par-4 Axis: A Cancer-Selective Therapeutic Target

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Abstract

Activation of the phosphoinositide 3-kinase (PI3K)/Akt1 pathway in many cancer cells makes it an appealing target for therapeutic development. However, because this pathway also has an important role in the survival of normal cells, tactics to achieve cancer selectivity may prove important. We recently showed that the cancer-selective proapoptotic protein Par-4 is a key target for inactivation by PI3K/Akt signaling. Additionally, we found that Par-4 participates in mediating apoptosis by PTEN, the tumor suppressor responsible for blocking PI3K/Akt signaling. As a central player in cancer cell survival, Par-4 may provide a useful focus for the development of cancer-selective therapeutics.

The process of tumor growth and progression is linked primarily to the suppression of apoptotic pathways that normally safeguard against uncontrolled cell proliferation (1–3). Mutations and deletions of tumor suppressor genes, such as p53 or PTEN (phosphatase and tensin homologue deleted on chromosome 10), are two of the several events underlying the loss of apoptotic pathways (4). Owing to the permanent loss of their functions, such mutated or deleted genes cannot be recruited in treatment strategies that rely on apoptotic end points. The identification of proapoptotic, tumor suppressor genes that are functionally silenced rather than mutated or deleted offers the prospect of reactivating and using the function of such genes for cancer treatment. The interaction of the cell survival kinase, Akt1 (5), with the prostate apoptosis response-4 protein, Par-4 (6), results in phosphorylation and silencing of Par-4 (7) and thus offers an intervention target for the selective induction of apoptosis in cancer cells.

Akt1 is a key cell survival protein functionally involved in antiapoptosis in various cancers (8, 9). Akt1 activity is elevated in cancer due to the loss of PTEN tumor suppressor function, activation of upstream lipid kinase phosphoinositide 3-kinase (PI3K) signaling following growth and survival factor stimulation, and activation of oncogenes (Fig. 1A). The antiapoptotic role of Akt1 accounts for its transforming potential and for the resistance of cancer cells to the action of chemotherapeutic agents and ionizing radiation (8–10). Akt1 protects cells from apoptosis by phosphorylating proapoptotic substrates; these substrates are subsequently sequestered by the chaperone 14-3-3, which locates them away from their target sites of action (9). Some of the key substrates of Akt1 are depicted in Fig. 1A.

Par-4 is a leucine zipper domain protein first identified in prostate cancer cells undergoing apoptosis in response to an exogenous insult (11). Par-4 is ubiquitously expressed in normal tissues and cell types and is found primarily in the cytoplasm (12–15). In contrast, Par-4 localizes both to the cytoplasm and the nucleus in many but not all cancer cells and clinical specimens (6, 13–17). Endogenous Par-4 expressed in normal and cancer cells does not, by itself, cause apoptosis. However, inhibition of endogenous Par-4 with antisense oligodeoxynucleotides, a dominant-negative leucine zipper domain, or RNA interference precludes apoptosis by exogenously applied agents (such as tumor necrosis factor–related apoptosis-inducing ligand, tumor necrosis factor, growth factor withdrawal, chemotherapeutic agents, or ionizing radiation), thus indicating Par-4 function is essential for apoptosis via diverse cell death pathways (6, 15). Consistent with this observation that endogenous Par-4 has apoptotic potential, Par-4 knockout mice spontaneously develop tumors of the liver, lung, and endometrium, exhibit prostatic intraepithelial neoplasia (PIN), and show an increased frequency of estrogen-inducible tumors in the endometrium and BBN-inducible tumors in the bladder (18). Interestingly, ectopic Par-4 overexpression is sufficient to induce apoptosis in most cancer cells, but not in normal or immortalized cells, and this action of Par-4 does not require the leucine zipper domain (14, 15). Apoptosis by ectopic Par-4 involves activation of the Fas death receptor signaling pathway and concurrent nuclear factor-κB (NF-κB) inhibition, which withdraws the antiapoptotic roadblocks and allows the caspase cascade to proceed uninterrupted (19). Neither p53 nor PTEN are required for apoptosis by ectopic Par-4, and Par-4 action is not inhibited by Bcl-2 or BclxL overexpression (19). Importantly, overexpression of Par-4 in prostate cancer xenografts by i.t. injections of adenoviral Par-4, either in s.c. tumors in nude mice or in orthotopic tumors in the prostatic milieu (6, 19), results in apoptosis and tumor growth inhibition, therefore implying that Par-4 has therapeutic potential.

Nuclear translocation of Par-4 is essential for apoptosis (14, 15). Endogenous Par-4 is present in cancer cells primarily in the cytoplasm; however, in certain cancer cells, it is also expressed in the nucleus, although at relatively lower levels (13–17). Thus, it is apparent this endogenous Par-4 is in a nonfunctional state and unable to effect apoptosis in these cancer cells. The Par-4 gene has been mapped to chromosome 12q21, and mutations in the Par-4 gene have not been reported in cancer cells. Accordingly, endogenous Par-4 must be bound and functionally inactivated by an antiapoptotic protein, which prevents Par-4 from executing its proapoptotic functions. Indeed, our recent studies reveal that binding of Akt1 to Par-4 results in both Par-4 phosphorylation and inactivation of its proapoptotic potential (7).

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Because tumor cell survival pathways, which are activated by growth factors, oncogenes, or antiapoptotic proteins, can actively inhibit the proapoptotic potential of tumor suppressor proteins (20), we tested the hypothesis that endogenous Par-4 is inactivated in cancer cell lines by one or more cell survival proteins. As part of this effort, two broad approaches were undertaken to identify potential Par-4 inhibitory proteins in prostate cancer cells: (a) identification of cell survival proteins
that communoprecipitated with Par-4 using anti-Par-4 antibodies and (b) identification of proteins that bound to GST-Par-4 in pull-down assays. Both approaches led to the identification of the cell survival kinase, Akt1, as a Par-4–interacting protein in prostate cancer cell lines (7). It became apparent from further experiments that this interaction was not limited to prostate cancer cell lines, and that Akt1 bound to Par-4 in diverse established cell lines originating from human cervical, lung, and breast cancers, benign prostatic hyperplasia, and normal and immortalized fibroblasts (7). As Akt exists in three different isoforms (Akt1, Akt2, and Akt3), the specificity of the interaction between Par-4 and Akt1 was confirmed by using mouse embryo fibroblasts (MEF) from Akt1−/− mice and wild-type MEFs. Par-4 failed to interact with Akt in Akt1−/− cells, implying specific interaction between Akt1 and Par-4, and this observation was confirmed by cotransfection studies using the different isoforms of Akt (7). The interaction between Akt1 and Par-4 was direct, as mixing purified Akt1 and Par-4 proteins led to the phosphorylation of Par-4 in vitro, hence identifying Par-4 as a new substrate of Akt1 (7). Importantly, the kinase active form of Akt1 but not the inactive form binds to Par-4 (7). Because activated Akt1 but not Akt2 or Akt3 is primarily associated with cell survival, these findings justified further characterization of the interaction between Akt1 and Par-4 to uncover cancer cell survival mechanisms.

Nuclear translocation of Par-4 is essential for induction of NF-κB–dependent transcription activity; binding to Par-4 partner proteins WT1, ZIPK/DAXX, and THAP; and induction of apoptosis (6, 14). Akt1 binding and phosphorylation of Par-4 results in 14-3-3–mediated cytoplasmic retention of Par-4 and abrogation of apoptosis (7). Par-4 deletion analysis indicates that the Par-4 leucine zipper domain, which is involved in binding to all of the previously identified partners of Par-4, is also essential for binding to Akt1 (7). Consequently, Par-4 mutants lacking either the leucine zipper domain or the Akt1 phosphorylation site can induce apoptosis in cancer cells, despite high intracellular levels of activated Akt1 (7). Thus, these Par-4 mutants are not phosphorylated by Akt1, fail to bind to the 14-3-3 proteins, and are not retained in the cytoplasm (7).

PTEN inactivation, and concurrent constitutive activation of Akt, is a well-defined genetic lesion in the initiation and progression of tumors (8–10). The importance of PTEN, and correspondingly Akt, in cancer is underscored by the fact that the PTEN gene lies in a chromosomal hotspot often deleted in many human cancers, including >60% of advanced prostate cancers. Germ line mutations in PTEN have been detected in human autosomal dominant disorders involving the increased susceptibility to benign and malignant tumors. Furthermore, heterozygous disruption of the PTEN gene results in spontaneous development of tumors in several tissues and intraepithelial neoplasia lesions in the prostate (references cited in ref. 7). In our studies (7), PTEN induced apoptosis in prostate cancer cells via a mechanism that was inhibited by blocking endogenous Par-4 expression with RNA interference. The Par-4–dependent apoptotic action of PTEN in prostate cancer cells was duplicated by the pharmacologic inhibition of PI3K and by the inhibition of Akt1 expression or function. The essential role of endogenous Par-4 in PTEN-induced apoptosis was also rigorously verified by using isogenic PTEN+/− and PTEN−/− MEFs. The relationship between PTEN and Par-4 is supported by the observation in Par-4 knockout mice that, similar to PTEN disruption, haploinsufficiency of Par-4 is associated with PIN and spontaneous tumors (18). Thus, Par-4 is a key mediator of the apoptotic effect of PTEN.

The biological significance of this interaction between Akt1 and Par-4 is underscored by the binding of endogenous Akt1 to Par-4 in human prostate cancer specimens. Interestingly, although phosphorylation of Par-4 at the T155 residue by PKA activates the Par-4 apoptotic potential in cancer cells (15), T155 phosphorylation does not induce apoptosis in the presence of Par-4 phosphorylation by Akt1 and cytoplasmic retention by 14-3-3 proteins (7). Akt1 confers cell survival by phosphorylating proapoptotic proteins, which in turn are bound by 14-3-3 proteins and sequestered from their cellular sites of action. Despite such multiple target interactions of Akt1, parallel coactivation of Akt1 and Par-4 expression by RNA interference resulted in the inhibition of apoptosis, indicating the failure of the other Akt1 substrates to induce apoptosis in the absence of Par-4 expression or function. Moreover, coinhibition of Akt1 and other Akt1 substrates did not inhibit apoptosis, implying that the observed apoptosis upon Akt1 inhibition can be ascribed primarily to Par-4. These observations highlight both the significance of Akt1 inhibition of endogenous Par-4 to promote cancer cell survival and the Par-4–dependent nature of apoptosis upon inhibition of Akt1 (Fig. 1B).

Several anticancer therapies target, albeit indirectly, the PI3K/Akt1 axis and cause inhibition of Akt1 phosphorylation and induction of apoptosis. Examples include hereceptin, which inhibits ErbB2 in breast cancer cells; cyclooxygenase-2 (COX-2) inhibitors, which inhibit COX-2 and PDK1 in colon and prostate cancer; gefinitib (Iressa), which targets mutant epidermal growth factor receptor in lung cancer cells; and imatinib mesylate (Gleevec, STI-571), which targets bcr-abl in leukemia. Conversely, inhibition of anokisin, which contributes to metastasis by trkB or TWIST, involves Akt activation. Given that Akt1 binds to Par-4 in diverse cell types, the action of these therapeutic agents may involve regulation of Par-4 function via Akt1.

As the inhibition of Akt1 leads to the release of Par-4 and apoptosis, this interaction can be exploited in the design of targeting strategies for specific cancer cells depending on their sensitivity/resistance response toward chemotherapeutic agents and ionizing radiation. Moreover, beyond causing apoptosis on its own, Par-4 sensitizes cells to the apoptotic action of other anticancer agents. Such targeting strategies could be judiciously combined with chemotherapeutic agents and ionizing radiation to bring about maximal tumor regression, particularly in patients whose tumors are refractory to conventional treatment. In view of the fact that Akt1 also binds to Par-4 in normal cells, and that Par-4 does not cause apoptosis of normal cells on its own (14, 15), an ideal approach may be to use small chemical molecules that can prevent the inactivation of Par-4 by Akt1, rather than inhibit Akt1 per se, as the latter strategy may prove toxic to normal cells. Such studies may provide the basis for innovative and targeted chemotherapeutic strategies for the activation of Par-4 in cancer and afford essential insight into the cellular mechanisms of sensitivity and resistance to apoptosis in cancer cells.

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