Molecular Pathways: P-Rex in Cancer

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Abstract

P-Rex proteins are Rho/Rac guanine nucleotide exchange factors that participate in the regulation of several cancer-related cellular functions such as proliferation, motility, and invasion. Expectedly, a significant portion of these actions of P-Rex proteins must be related to their Rac regulatory properties. In addition, P-Rex proteins control signaling by the phosphoinositide 3-kinase (PI3K) route by interacting with PTEN and mTOR. The interaction with PTEN inhibits its phosphatase activity, leading to AKT activation. The interaction with mTOR may be important in nutrient-stimulated Rac activation and migration. In humans, several studies have implicated P-Rex proteins in the pathophysiology of various neoplasias. Thus, overexpression of P-Rex proteins has been linked to poor patient outcome in breast cancer and may facilitate metastatic dissemination of prostate cancer cells. In addition, whole-genome sequencing described P-Rex2 as a significantly mutated gene in melanoma. Furthermore, expression in melanocytes of mutated forms of P-Rex2 found in patients with melanoma showed the protumorigenic role of these P-Rex mutations in melanoma genesis. These findings open interesting opportunities for P-Rex targeting in cancer. Moreover, the implication of P-Rex partner proteins such as Rac, mTOR, or PTEN in cancer has opened the possibility of acting on P-Rex to restrict protumorigenic signaling through these pathways. Clin Cancer Res; 19(17); 1–6. ©2013 AACR.

Background

P-Rex proteins have recently been shown to be involved in the pathophysiology of several neoplasias, opening the possibility of their targeting with therapeutic purposes (1). P-Rex proteins belong to the large family of guanine nucleotide exchange factors (GEF), which act on the Rho/Rac family of GTPases (2, 3). Rho/Rac proteins play important roles in several physiologic processes, and have also been implicated in neoplastic transformation and metastatic dissemination (4). Expectedly, many of the roles of P-Rex proteins in cellular physiology must be due to their Rac regulatory properties. In addition, P-Rex proteins may also control signaling by the phosphoinositide 3-kinase (PI3K) route (5, 6), and carry out Rac-independent actions (7). In this article we review the functions of P-Rex proteins and the potential relevance of their targeting in cancer.

P-Rex genes and protein isoforms

The first member of the P-Rex family, P-Rex1, was identified in a search for phosphatidylinositol-(3,4,5)-trisphosphate (PIP3)-activated Rac GEFs (8). The isolated protein was named P-Rex1 for PIP3-dependent Rac exchanger. Up to six different P-Rex proteins were derived from two genes, PREX1 and PREX2 (9). Transcription from the PREX1 gene may generate three different P-Rex1 protein isoforms of 186 (isoform 1), 110 (isoform 2), and 175 kDa (isoform 3; Fig. 1A). The PREX2 gene generates three isoforms of 182 (also known as P-Rex2a), 171, and 111 kDa (also known as P-Rex2b; ref. 9).

P-Rex proteins contain an N-terminal Dbl-homology (DH) domain, the typical catalytic domain of the GEFs that act on the Rho/Rac family of GTPases (Fig. 1A). P-Rex proteins also include a pleckstrin homology (PH) domain, as well as two pairs of DEP and PDZ domains. The C-terminal region includes a domain homologous to inositol polyphosphate 4-phosphatase (IPP4P; 8, 10), which is absent in P-Rex2b (11).

Physiologic relevance of P-Rex proteins

P-Rex1 is mainly expressed in peripheral blood leukocytes and in the brain and is also expressed in several other tissues (8, 11–14). P-Rex2 is widely expressed and is strongly present in skeletal muscle, the small intestine, heart, lung, and placenta, being absent from peripheral blood leukocytes (10, 11, 13).

P-Rex1−/− mice are viable and apparently healthy but have mild neutrophilia (15). In the neutrophils of these mice, the G protein–coupled receptor (GPCR)-dependent Rac activation and recruitment to inflammatory sites were impaired (15, 16). In leukocytes, P-Rex1 has been involved in integrin-mediated rolling and intravascular crawling (17). In lung microvascular endothelial cells, P-Rex1 participated in TNF-α-stimulated Rac activation and ROS
production (18). Removal of P-Rex1 significantly reduced neutrophilic transendothelial migration and leukocyte sequestration in TNF-α–challenged mouse lungs. The P-Rex1 knockout mice were also refractory to lung vascular hyperpermeability and edema in a lipopolysaccharide-induced sepsis model. P-Rex2b also seems to be relevant.
in the regulation of Rac1 activation and cell migration of endothelial cells (19).

P-Rex proteins are widely expressed in the nervous system (13) and have been involved in neuronal migration (14), differentiation (7), and memory-related functions (20). Transfection of P-Rex1 inhibited neurite sprouting in PC12 cells (7). Moreover, expression of an N-terminal truncated form of P-Rex had the same action as full-length P-Rex1, suggesting that the neurite-regulating functions of P-Rex1 may be partially independent of its Rac regulatory properties (7). On the other hand, expression of a mutant lacking the DH domain, which acts as a dominant-negative form with respect to the GEF activity of P-Rex1, decreased neuronal migration induced by neurotrophic factors (14). With respect to P-Rex2, mice lacking the PREX2 gene are viable and fertile but develop a mild motor coordination defect and have alterations of the Purkinje cell dendrite morphology (13).

Together, these studies show the nonredundant functions of P-Rex proteins in animal physiology. This is relevant if one considers the large complement of Rac GEF factors reported that are also widely expressed (21).

**Regulation of P-Rex proteins**

The activation of P-Rex is directly and synergistically produced by the binding ofPIP3 to the PH domain and the Gβγ subunits to the DH domain (8, 22, 23; Fig. 1B). These bindings favor translocation of P-Rex from the cytosol to the plasma membrane, where P-Rex GEF activity is higher (8, 23). Deletion studies indicated that the DH/PH domains are sufficient to activate P-Rex by PIP3 (22). The other domains (2 DEP, 2 PDZ, and IP4P) keep the catalytic activity of full-length P-Rex1 low (22), suggesting that interactions between the DH/PH domains and the other domains may autoinhibit P-Rex activity.

Phosphorylation of P-Rex represents an important mechanism of regulation of its function. Several reports have indicated that P-Rex1 is phosphorylated at multiple sites (9, 24, 25), and the phosphorylation of these sites regulates P-Rex1 activity in an opposite fashion. In breast cancer cells, phosphorylation of Ser<sup>605</sup> and Ser<sup>1169</sup>, located in the PH domain, inhibits P-Rex1 activity under resting conditions, and mutation to nonphosphorylatable residues augments Rac activity (9). Stimulation of ErbB receptors, which increases Rac activity in those cells, was accompanied by dephosphorylation of Ser<sup>113</sup> and Ser<sup>319</sup> (9). Interestingly, β-adrenergic receptor activation increases the phosphorylation of P-Rex1, likely through PKA, and such a phosphorylation diminishes P-Rex1 GEF activity (24). Moreover, dephosphorylation of P-Rex1 by λ-phosphatase (24) or protein phosphatase 1α (PP1α; ref. 25) increases the activity of P-Rex1. In the aggregate, these reports support the concept that phosphorylation of P-Rex1 at certain residues inhibits its GEF activity toward Rac.

Phosphorylation of P-Rex1 at other sites activates its Rac GEF function. In breast cancer cells, stimulation of ErbB receptors caused phosphorylation of Ser<sup>605</sup> and Ser<sup>1169</sup> (9). Mutagenesis experiments showed that phosphorylation of Ser<sup>1169</sup> facilitated P-Rex1 GEF function. Therefore, stimulation of ErbB receptors activates a phosphorylation/dephosphorylation cycle of P-Rex1, which results in dephosphorylation of inhibitory residues (Ser<sup>113</sup>, Ser<sup>319</sup>) and phosphorylation of stimulatory residues (Ser<sup>605</sup>, Ser<sup>1169</sup>) to control the GEF activity of P-Rex1.

**The PI3K/mTOR route and P-Rex proteins**

Of particular relevance is the interplay between the PI3K route and P-Rex proteins. P-Rex1 was initially described as a Rac GEF upmodulated by the βγ subunits of heterotrimeric G proteins and by PIP3, which is generated by PI3K (8). P-Rex proteins interact with mTOR and PTEN, two important players in PI3K signal transduction (5, 6). The interaction of P-Rex1 and P-Rex2 with mTOR occurs through the DEP domains of the P-Rex proteins and regulates nutrient-stimulated Rac activation and cell migration (6).

P-Rex may participate in the upregulation of the activity of the PI3K pathway by antagonizing PTEN (5). This latter protein dephosphorylates PIP3, an important lipid second messenger implicated in the regulation of cellular proliferation and survival (26). Inactivation of PTEN leads to increased levels of PIP3 and therefore promotes cellular responses, such as augmented cell proliferation or decreased cell death, which are hallmarks of oncogenic progression. In breast cancer cells, Fine and colleagues (5) found that PTEN was able to bind P-Rex2a. Such interaction decreased the phosphatase activity of PTEN and resulted in augmented phosphorylation of AKT at activating residues. Interestingly, this action on PTEN was independent of the GEF activity of P-Rex2a. In MCF10A cells that express endogenous active PTEN, forced expression of P-Rex2a augmented AKT phosphorylation at Ser<sup>473</sup> and favored the formation of multinuclear structures (5), a feature that in these cells has been associated to a pre tumorigenic status (27). In ovarian cancer cells, expression of P-Rex1 has been shown to promote activation of AKT1 and P-Rex1 knockdown inhibited AKT1 (28).

**Clinical–Translational Advances**

Several circumstances indicate that P-Rex proteins may play a role in the pathophysiology of various neoplastic diseases. On the one hand, P-Rex expression levels have been shown to correlate with patient outcomes in several neoplasias. Moreover, molecular alterations of P-Rex have been described in melanoma, and some of these alterations promote tumorigenesis. On the other hand, P-Rex proteins have been shown to participate in the regulation of several prooncogenic properties, such as migration, cell proliferation, or invasion. These clinicopathologic and biologic findings open the possibility of targeting P-Rex proteins with therapeutic purposes. Moreover, the implication of P-Rex partner proteins such as Rac, mTOR, or PTEN in cancer offers the possibility of acting on P-Rex to restrict protumorigenic signaling through these pathways.
Expression and molecular alterations of P-Rex proteins in cancer

Studies in breast (9, 29) and prostate cancer (30) have shown a link between P-Rex expression and patient outcome. Sosa and colleagues found that more than 50% of breast tumors stained for P-Rex1 (29). Normal breast tissue and the stroma did not stain for P-Rex1. P-Rex1 staining was higher in patients with metastatic disease, suggesting a role of P-Rex1 in metastatic dissemination. Montero and colleagues analyzed P-Rex expression in breast tumors by quantitative Western blotting (9). Expression of P-Rex was found to be variable among different patient samples. Kaplan–Meier analyses confirmed that patients whose breast tumors expressed high levels of P-Rex1 had a shorter disease-free survival.

PREX1 is located on chromosome 20q13.13, a region commonly amplified in breast tumors (31) and some breast cancer cell lines (32). The PREX2 gene is located on chromosome 8q13, a region frequently amplified in breast, prostate, or colorectal cancer (33–35). Analysis of the COSMIC database indicates that mutations in PREX2 are present in 3% of tumors, a frequency slightly higher than that of mutations in PREX1 (2% of tumors). Mutations in PREX1 and PREX2 have a tendency to cluster in the GEF exchange region. Additional mutations in PREX1 accumulate in the IP4P region. Skin and lung cancers represent the tumors that most frequently bear PREX mutations. Around 10% of lung cancers contain PREX1 or PREX2 mutations. Mutations of PREX1 (0.99%) or PREX2 (1.17%) in breast cancer are scarce and are absent in hematopoietic cancers.

In skin cancer, 23% of tumors carry PREX2 mutations, and 3.6% of tumors bear PREX1 mutations. Massive sequencing of melanoma genomes identified PREX2 as a significantly mutated gene, accounting for a 14% frequency in a cohort of 107 human melanomas (36). These mutations were distributed along the entire length of P-Rex2 and included nonsynonymous PREX2 mutations as well as truncations (36). Biologic experiments in which some of the PREX2 mutations found in patients were reproduced in TERT-immortalized human melanocytes showed the role of this GEF in melanoma genesis and showed that PREX2 mutations generate altered proteins with oncogenic activity (36).

Prometastatic properties of P-Rex

In prostate cancer cell lines and human samples, expression of P-Rex1 strongly correlated with the metastatic phenotype (30). Qin and colleagues (30) reported that P-Rex1 acted through Rac to facilitate migration and invasion of prostate cancer cells upon activation of the EGF receptor. Moreover, overexpression of P-Rex1 in a nonmetastatic cell line increased its motility and invasion properties. In their prostate cancer models, P-Rex1 did not affect prostate tumor growth but caused spontaneous lymph node metastases. Similarly, in ovarian cancer cells, knockdown of P-Rex1 inhibited insulin-like growth factor-I (IGF-I)–induced activation of cell migration or invasion (28). In breast cancer cells, activation of ErbB or chemokine receptors facilitated cellular migration in a P-Rex–dependent manner (9, 29).

In the skin, Lindsay and colleagues showed that P-Rex1 was required for efficient melanoblast migration and melanoma dissemination (37). Using an animal model of melanoma, which included mutant N-Ras as well as deletion of the INK4a tumor suppressor, the authors described decreased melanoma metastases when these mice were crossed with P-Rex1−/− mice (37). Interestingly, no differences in tumor latency or burden in the primary melanomas were observed when P-Rex1+/+ mice and P-Rex1−/− mice were compared. In line with these data, Campbell and colleagues (38) reported that P-Rex1 favored the invasiveness of WM852 melanoma cells. Therefore, targeting of P-Rex1 in prostate, ovarian, breast, or skin cancer may be beneficial in fighting disease dissemination.

Role of P-Rex proteins in proliferation

Although the above reports support a prometastatic role of P-Rex proteins, their intermediate action in cell proliferation is less clear. In breast cancer cells, knockdown of P-Rex1 reduced cell proliferation both under resting conditions and after ligand-induced stimulation of ErbB receptors (9, 29). However, the lack of effect of P-Rex knockdown on the proliferation of prostate cancer cells (30) or tumor burden in melanoma (37) contrasts with the data obtained in breast cancer and indicates intertumoral differences in the protumorigenic actions of P-Rex proteins.

P-Rex proteins in pro-oncogenic signaling

The role of P-Rex proteins as mediators of signaling by the cell surface receptors, especially those involved in the pathophysiology of several neoplasias, merits consideration. Initial work suggested a potential role of P-Rex1 in signaling by GPCRs. This idea was confirmed by showing the intermediate action of P-Rex1 in migration upon activation of the chemokine receptor CXCR4 (29).

In addition to the intermediate role of P-Rex in GPCR signaling, evidence is accumulating suggesting that P-Rex proteins may play a relevant role in signaling by different classes of cell surface receptors. This idea is supported by the fact that the receptor tyrosine kinases (RTK) ErbB (9), platelet-derived growth factor receptor β (38), TrkA (7), and IGF-I receptor (IGF-IR; ref. 39) use P-Rex. That P-Rex proteins may act as general intermediates in signaling by RTKs is a relevant concept, especially because of the important roles of these receptors in oncology and physiology. It will be necessary to analyze whether RTKs other than those already reported use P-Rex as part of their signal transduction machinery. These studies may uncover novel roles of P-Rex proteins in mediating physiologic actions of RTKs that may be relevant in tumor growth. An example of the latter is the role of P-Rex proteins in glucose metabolism. P-Rex1 has been reported to regulate the membrane exposure of the glucose transporter GLUT4 in adipocytes in response to IGF-IR activation (39). This action indicates that P-Rex proteins may control supply of metabolic energy to cells by upregulating glucose transport into their cytosol. Obviously, this
effect of P-Rex could be of relevance for the fitting of the tumoral tissues, but such physiologic action may even outweigh its oncological relevance in case P-Rex proteins are shown to play important roles in insulin receptor-mediated glucose transport.

Mechanistically, it will be important to define whether RTKs and other cell surface receptors activate P-Rex by the same mechanism used by the ErbB receptors, i.e., by activating the P-Rex phosphorylation/dephosphorylation cycle. It will also be relevant to identify the kinases and phosphatases involved in controlling that cycle, and the signaling cascades that mediate activation of those kinases and phosphatases. Finally, elucidation of additional P-Rex-interacting proteins could help in understanding the protein networks in which P-Rex proteins participate.

Targeting P-Rex in cancer

Given the ample number of Rac GEFs (21), we may consider it unreasonable to target P-Rex proteins. However, clinicopathologic as well as preclinical data support roles of P-Rex proteins in human cancer, opening therapeutic options for the targeting of P-Rex proteins in cancer. In fact, P-Rex expression has been correlated with patient outcome in breast and prostate cancers, and reduction of P-Rex expression has been shown to restrict proliferation and/or metastatic dissemination (9, 29, 30). Therefore, targeting P-Rex proteins may be therapeutically relevant in neoplasias in which those proteins play an important role in regulating their growth or dissemination.

Of particular relevance is the role of P-Rex proteins in melanoma pathophysiology. The fact that mutated forms of P-Rex2 have been detected in melanoma and are tumorigenic (36) raises the possibility of targeting P-Rex2 in melanomas bearing such mutations. Moreover, Lindsay and colleagues showed that P-Rex1 was required for efficient melanoblast migration and melanoma dissemination (37). Interestingly, expression of P-Rex1 in melanoma cell lines has been reported to be controlled by the extracellular signal-regulated kinase (ERK) route (40). This finding is relevant given the high frequency of activation of the ERK pathway in melanomas due to upstream mutations in BRAF (36). It is possible that BRAF inhibitors used in the clinic, such as vemurafenib (41) or other BRAF/ERK pathway inhibitors, may reduce P-Rex1 levels and therefore contribute to preventing P-Rex1–facilitated melanoma dissemination.

Besides controlling P-Rex proteins by downregulating their cellular levels, another way of acting on these proteins may be the use of strategies that affect their functionality. Such strategies may include inhibition of the Rac-GEF activity of P-Rex proteins or their interaction with mTOR or PTEN. Inhibition of the Rac-GEF activity of P-Rex proteins could be possible by using compounds that prevent interaction of the PH domain of P-Rex proteins with PIP3, or that directly inhibit the GEF activity of the DH domain. Interference with the GEF activity should reduce the Rac-mediated actions of P-Rex proteins, especially those related to cellular motility and invasion. Reduction of the interaction of P-Rex proteins with mTOR and PTEN could be used to restrict P-Rex–dependent PI3K signaling. Compounds in development that may inhibit interaction of the DEP domain of P-Rex proteins with mTOR are expected to reduce P-Rex/mTOR-dependent Rac activation and migration. Similarly, preventing the binding of the PH domain of P-Rex proteins to PTEN may allow the latter to inhibit PI3K signaling.

In addition to the above-mentioned strategies that directly target P-Rex proteins, indirect ways to reduce P-Rex signaling may be contemplated. These strategies rely on the use of drugs that act on signaling proteins upstream or downstream of P-Rex, such as inhibitors of RTKs, GPCRs, Rac, or dual PI3K/mTOR inhibitors. It is expected that increasing the knowledge about the regulatory mechanisms responsible for controlling P-Rex functioning may allow the development of additional strategies. As an example, augmenting the phosphorylation of P-Rex1 at inhibitory residues may also be exploited to reduce its functionality. This would require unveiling the kinases and phosphatases responsible for maintaining P-Rex in its "off" state (Fig. 1B).

In conclusion, the ample range of physiologic cancer-related functions of P-Rex, together with the accumulating evidence that links P-Rex proteins to several types of neoplasias, opens possibilities for the targeting of P-Rex proteins with therapeutic purposes.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: A. Pandiella, J.C. Montero
Writing, review, and/or revision of the manuscript: A. Pandiella, J.C. Montero
Study supervision: A. Pandiella

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