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Mini review

Proline-rich AKT substrate of 40-kDa (PRAS40) in the pathophysiology of cancer

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ABSTRACT

Dysregulation of PI3K-AKT-mTOR pathway has been reported in various pathologies, such as cancer and insulin resistance. The proline-rich AKT substrate of 40-kDa (PRAS40), also known as AKT substrate 1 (AKT1S1), lies at the crossroads of these cascades and inhibits the activity of the mTOR complex 1 (mTORC1) kinase. This review discusses the role of PRAS40 and possible feedback mechanisms, and alterations in AKT/PRAS40/mTOR signaling that have been implicated in the pathogenesis of tumor progression. Additionally, we probed new datasets extracted from Oncomine, a cancer microarray database containing datasets derived from patient samples, to further understand the role of PRAS40 (AKT1S1). These data strongly supports the hypothesis that PRAS40 may serve as a potential therapeutic target for various cancers.

1. Introduction

The phosphatidylinositol 3-kinase (PI3K) signaling pathway, when activated by various growth factors and hormones, results in the phosphorylation of downstream effectors like the serine/threonine protein kinase Akt (i.e. protein kinase B) [1]. mTOR is a serine kinase downstream of Akt and it exists in two forms: mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2) [2]. mTORC1 is a composed of Raptor, GβL and PRAS40, whereas mTORC2 consists of mTOR, mLST8, Rictor, and mSIN1 [3]. PRAS40, a direct substrate of AKT, is also a component and substrate of mTORC1 [4,5]. PRAS40 negatively regulates mTORC1 and upon the removal of inhibitory stress, mTORC1 enhances cell survival and growth by increasing mRNA translation via activation of p-70S6K and 4-EBP1 [6,7]. Akt phosphorylation occurs at Thr308 by phosphoinositide-dependent kinase 1 (PDK-1) [8], whereas mTORC2 activates Akt by phosphorylation of S473 [9]. PRAS40 was first identified in total lysates of insulin-incubated rat hepatoma cells after elution from 14-3-3 affinity columns as a 40 kDa molecule. It was then purified, sequenced, and identified as a protein "rich in proline", and thus designated "proline-rich Akt substrate of 40 kDa molecular weight" PRAS40 [7]. Since both AKT and mTOR are key regulators of basic cell processes, including cell proliferation, apoptosis and glucose metabolism, dysregulation of these pathways has been well implicated in cancer and diabetes [8]. PRAS40 is phosphorylated in both precancerous and malignant cancer cell lines, suggesting a role in tumor development congruent with Akt [10]. Although it has been 12 years since PRAS40 was first reported, its physiological function and role in various diseases are not yet fully understood. This mini-review briefly addresses recent developments related to PRAS40 in various forms of human cancer and the delineation of its role in the Akt/mTOR cascades, based on feedback mechanisms.

2. Role of PRAS40 in diabetes and other metabolic disorders

PRAS40 is altered in various disease states such as stroke [11], cerebral ischemia [12], Alzheimer's disease [13], diabetic cardiomyopathy [14], diabetic neuropathy [15], diabetes [[16,17], Malla et al., unpublished work], and cancer [18–20]. PRAS40/mTOR activity is deregulated in insulin-resistant rodent models [14,21]. Völkers et al. found that short-term mTOR inhibition by PRAS40 overexpression prevents diabetic cardiomyopathy and improves

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insulin sensitivity in the livers of high fat diet-fed mice [14]. Moreover, recent data from our lab on PRAS40 knockout mice shows improvement in glucose homeostasis, resulting from increased AKT-mTOR signaling [unpublished]. The role of PRAS40 in diabetes and cancer has recently gained interest as these disease states share common risk factors. It has been reported that diabetic treatments correlate with increased cancer risk, but the literature linking the two pathologies is equivocal. Although PRAS40 has been identified as a predictive protein biomarker for diverse cancers [22,23], its use as a dual prognostic marker in the pathophysiological prognosis of both cancer and diabetes remains to be determined. Such a dual biomarker would be quite valuable, as patients suffering from diabetes also have a higher risk of cancer [24,25].

Besides its role in glucose metabolism, insulin is a known growth factor that transduces signals through the IRS-1/AKT/mTOR cascade, and can potentially amplify the proliferation of premalignant and malignant cancer cells [21]. Similarly, insulin resistance in diabetes causes hyperinsulinemia, and thus can induce a significant increase in mitogenesis. Schematic diagram in Fig. 1 illustrates how altered AKT/PRAS40/mTORC1 signaling could contribute to various diseases. Since there is limited information regarding the involvement of PRAS40 in various diseases, this review primarily focuses on its possible role in cancer progression.

3. Role of PRAS40 in cancer

The most common PRAS40 alteration found in tumors is its overexpression and increased Akt signaling [26–28]. PRAS40 is overexpressed in both breast and lung cancers, as compared to their corresponding normal tissues [29]. Andersen et al. reported PRAS40 as a new biomarker for PI3K/Akt activation demonstrating that phospho-PRAS40Thr246 (p-PRAS40Thr246) expression is positively correlated with PI3K pathway activation in a PTEN-deficient mouse prostate tumor model and in triple-negative breast tumor tissues [30]. To determine and summarize PRAS40 (AKT1S1) overexpression in various carcinomas, we used Oncomine, a bioinformatics database and tool that collects, standardizes, analyzes, and provides cancer transcriptome data [31]. Based on this analysis, we determined that in studies comparing cancer to normal tissue, PRAS40 is highly overexpressed in datasets from breast, melanoma, colon, prostate, liver and lung cancers, but not pancreatic cancer (Fig. 2).

3.1. PRAS40 in colon cancer

Colorectal cancer is defined as uncontrolled cell proliferation in the colon and rectum, and it is frequently associated with small tumors or polyps on the intestinal wall [32]. In the U.S., colorectal cancer is the third most common malignancy among men and women, causing approximately 50,000 deaths annually. In a recent study by Baricevic et al., the incubation of five colon cancer cell lines (HCT 116, HT-29, C32, CaCo2, LoVo) with insulin significantly increased phosphorylation of AKT, PRAS40 and p-70S6K association with chemotherapy resistance [33]. These data suggests that the AKT/mTORC1 pathway could be a therapeutic target in colon cancer studies. In another report, genetic ablation of eIF3D (eukaryotic translation initiation factor 3 subunit D), a subunit of eIF3 and a downstream effector of mTORC1, suppressed colon cancer cell growth, suggesting that colon cancer

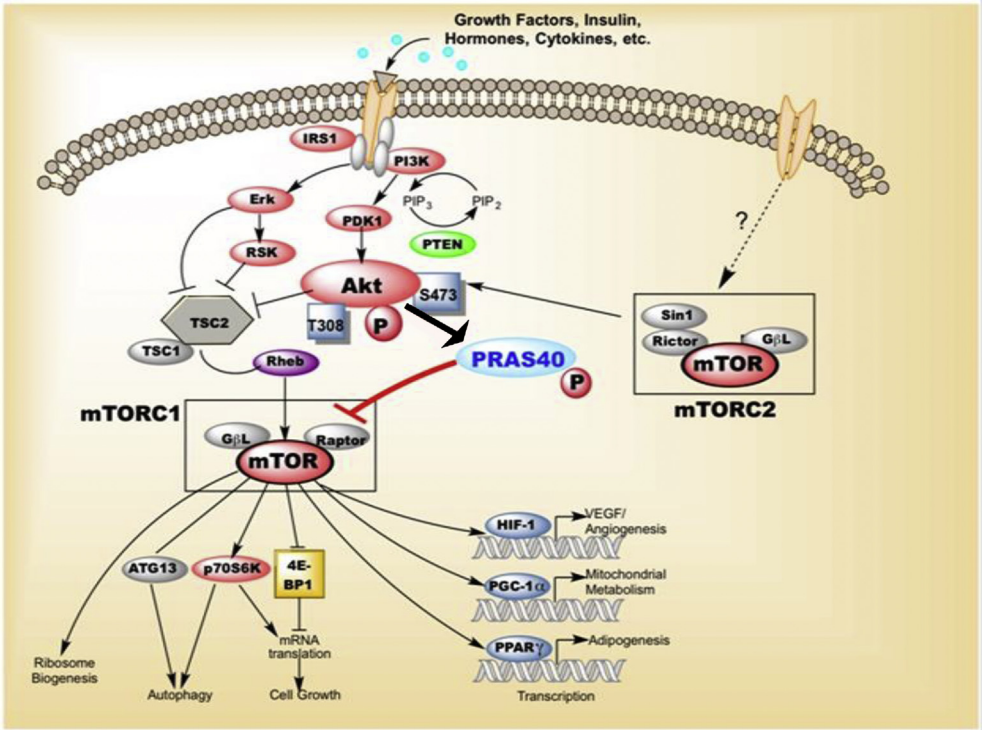

Fig. 1. Signaling events involved in PRAS40/mTORC1/AKT-dependent regulation of growth, metabolism and angiogenesis with both mTOR complexes being implicated in the etiology of cancer and other disorders. Growth factors, such as insulin, activate mTORC1 through phosphoinositide 3 kinase (PI3K)/AKT. mTORC1 consists of the regulatory-associated protein mTOR (Raptor), the proline rich Akt substrate 40 kDa (PRAS40), and GβL including others. mTORC2 contains Rictor, mTOR, mLST8, Deptor, the mammalian stress-activated protein kinase interacting protein (mSIN1), and protein observed with Rictor-1 (Protor-1). Upon activation of phosphoinositide dependent kinase 1 (PDK1), it recruits AKT to the plasma membrane. The phosphorylation of AKT occurs at Thr308 and Ser473. p-AKT (308), selectively targets mTORC1/S6, whereas mTORC2 activation causes phosphorylation of AKT selectively at Ser473. TSC1 and TSC2 are a pair of tumor suppressor genes that inhibit mTORC1 activity. HIF1α, PGC-1 α and PPAR-γ are involved in VEGF/Angiogenesis, Mitochondrial Metabolism and Adipogenesis, respectively. ChemBioDraw was used to create this graphical representation of AKT/PRAS40/mTOR signaling cascade.

progression likely occurs via PRAS40/mTORC1-related signaling proteins [34].

3.2. PRAS40 in liver cancer

Liver cancer, or hepatocellular carcinoma (HCC), is ranked as the sixth most common type of cancer in the world [35]. Approximately 630,000 people are diagnosed each year in the US, and the etiology of this cancer type is linked to oncogenic viruses such as hepatitis C virus and hepatitis B virus (HBV). Additionally, the underlying cause of more than 70% of HCC cases is liver cirrhosis [35]. A reduction in PRAS40 expression has been shown to inhibit the proliferation of HCC in a study by Ma et al., where lentiviral-mediated shRNA ablation of myosin VI (MYO6) in HepG2 and SMMC-7721 HCC cell lines inactivated PRAS40, increasing the activation of p38-MAPK-dependent signaling [36].

3.3. PRAS40 in lung cancer

In non-small cell lung carcinoma (NSCLC), Akt (T308) phosphorylation was positively correlated with phosphorylation of three AKT substrates: PRAS40, TSC2 and TBC1D4 (or AS160), but not with AKT (S473) autophosphorylation [37]. In order to target mTORC1/AKT in NSCLC, a dual inhibitor of these two pathways, Fisetin, a dietary flavonoid (5–20 μM), has been shown to significantly decrease phosphorylation of p-70S6K, a PI3K downstream serine/threonine kinase, in addition to PRAS40 [38]. Also, the daily administration of 600 mg of the oral pan-class I PI3K inhibitor SAR245408 (XL147) to patients with advanced solid tumors (Phase I clinical trial) significantly reduced phosphorylation of Akt PRAS40, 4EBP1, and S6, was well-tolerated, and resulted in one partial response and 6/69 patients with progression-free survival of >6 months [39].

3.4. PRAS40 in prostate cancer

PIM1, a serine/threonine kinase involved in prostate carcinomas, facilitates cell proliferation by positively regulating 4EBP1, an mTORC1 substrate [18,40]. Zhang et al. similarly reported that PIM1 overexpression in prostate cancer stimulates mTORC1 activity by PRAS40 phosphorylation [40]. Furthermore, incubation of FDCP1 murine bone marrow cells with PIM1 inhibitors significantly reduced both PRAS40 and 4EBP1 phosphorylation, and free (unphosphorylated) PRAS40 (i.e. unbound to TORC1) inhibited FDCP1 apoptosis [40]. Similarly, p-PRAS40, along with SMAD4, CCND1, SPP1 and pS6, represents a recently developed novel 5-marker protein signature for predicting prostate cancer-specific death, suggesting that quantifying the phosphorylated states of these markers in intact tissue is a viable option [22].

3.5. PRAS40 in breast cancer

The oncogenic Akt/PRAS40/mTOR cascade is an attractive target in cancer therapeutics. However, it is still unknown whether this pathway can be clinically targeted using Akt inhibitors. However, the promise of Akt inhibitors was demonstrated by Rhodes et al. reported that GSK690693, a novel ATP-competitive pan-AKT kinase inhibitor, significantly reduced tumor volume by day 21 in immunocompromised mice bearing HCC-1954 and BT474 breast carcinoma xenografts [41]. In addition, GSK690693 significantly decreased the level of GSK3-beta, PRAS40, and several forkhead family of transcription factors, which are downstream substrates for Akt [41]. Moreover, in trastuzumab-resistant HER2-positive patients, approximately 40% of the tumors were identified as being p-PRAS40Thr246-positive, indicating that PRAS40 may be associated with increased activation of the PI3K pathway, and an increased risk of tumor progression [20].

3.6. PRAS40 in melanoma

Melanoma is the most invasive and lethal form of skin cancer, with limited effective treatment regimens [27,42]. However, Madhunapantula et al., reported that siRNA knockdown of Akt3 or PRAS40 increased melanoma cell apoptotic sensitivity and inhibited tumor development in mice. In addition, p-PRAS40 levels were decreased upon silencing AKT3 expression via siRNA, but nor via Akt2 silencing, suggesting that PRAS40 is selectively regulated by Akt3 in melanomas [42]. Additionally, when PRAS40 was targeted using small molecule inhibitors, its phosphorylation was reduced following administration of with PI3K inhibitors such as wortmannin and LY-294002, but not by the MEK inhibitor (PD098059), suggesting that PRAS40 is downstream of PI3K but not MAPK [7,43].

3.7. PRAS40 in pancreatic cancer

PI3K mutations are the most frequent kinases mutations in human cancers. Approximately 11% of all pancreatic cancers associated with alterations in PI3K signaling [44]. However, there was no significant difference in PRAS40 expression between pancreatic exocrine-insufficient and normal pancreas, suggesting that PRAS40 might be activated by Akt-independent mechanisms [45]. Currently, there is only limited information about downstream Akt/mTOR signaling in pancreatic cancer, leukemia, gliomas and cervical cancer, thereby warranting future studies of this oncogenic pathway and PRAS40.

4. PRAS40 as a therapeutic target

The aberrant activation of PI3K/Akt/mTOR pathway leads to tumor proliferation in many cancer types, including breast cancer [20,30,41]. However, it is not yet fully established whether tumor growth inhibition is clinically sustained when this cascade is therapeutically targeted, although many reports have confirmed the findings of pharmacodynamic studies using ATP-competitive and selective AKT1/2/3 inhibitors. For example, AZD5363, a novel pyrrolopyrimidine-derived compound, inhibits the activity of all Akt isoforms, as demonstrated by dose- and time-dependent reduction of PRAS40, GSK3β, and S6 phosphorylation in BT474c Her2-positive breast cancer cell xenografts [46]. Similarly, the light/darkness-regulated hormone melatonin has been shown to inhibit breast cancer proliferation by inhibiting the phosphorylation of PI3K/Akt-regulated proteins suggesting a possible influence of circadian rhythms on this oncogenic pathway [47].

5. Summary

Gene-based silencing of the Akt/mTOR pathway has led to the identification of new biomarkers and proteins in cancer and insulin signaling. Some of these proteins that mediate mTOR signaling such as Raptor, Rictor, PRAS40, mSin1, FKBP38, and IRS-1 may represent therapeutic targets affected by the recent development of small molecule mTOR inhibitors. For example, mTOR complexes- TOR1 and TOR2 have independent inputs and substrates, which leads to a complex network of upstream regulators, downstream effectors and negative feed-back mechanisms. Over-activation of mTOR signaling accelerates tumorigenesis and enhances inflammatory responses that may eventually increase the likelihood of disease progression.

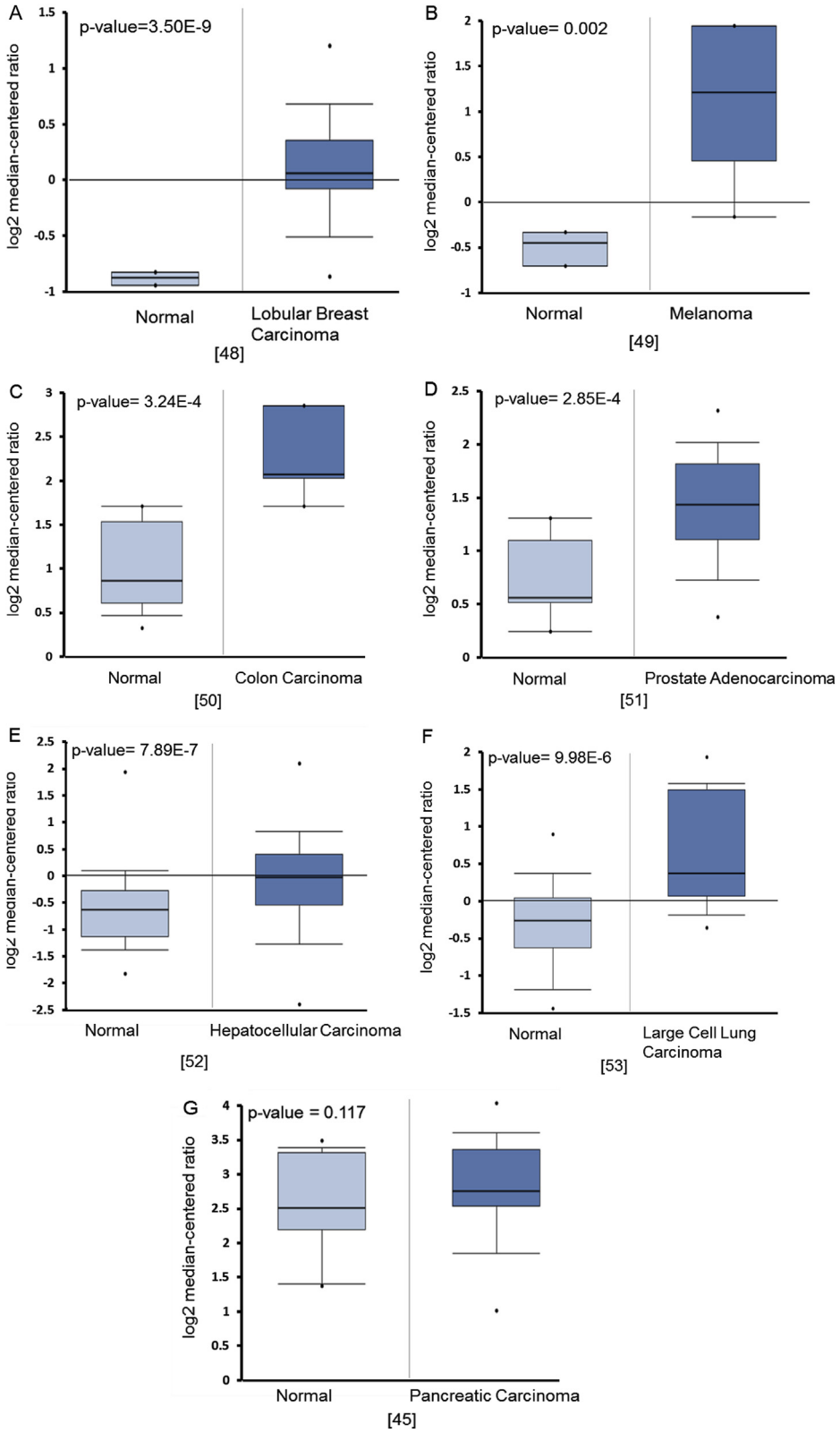

Fig. 2. Oncomine analysis of PRAS40 expression in breast, melanoma, lung, liver, colon, prostate and pancreatic cancers. PRAS40 (AKT1S1) gene expression profiles and pathology data from cancer patients were obtained from publicly available microarray datasets. PRAS40 is significantly overexpressed in breast, melanoma, lung, liver, colon, prostate cancer and pancreatic datasets within the Oncomine datasets. p-value < 0.05 was considered to be statistically significant. Student's t-test was used for all datasets. A. PRAS40 is significantly overexpressed in lobular breast carcinoma versus normal breast tissue, p-value = 3.50E-9 and n = 64 [48]. B. PRAS40 is significantly overexpressed in melanoma versus normal skin tissue, p-value = 0.002 and n = 37 [49]. C shows that PRAS40 is overexpressed in colon carcinoma versus normal tissue, p-value = 3.24E-4 and n = 40 [50]. D. PRAS40 is significantly overexpressed in prostate adenocarcinoma versus normal prostate tissue, p-value = 2.85E-4 and n = 40 [51]. E. PRAS40 is significantly overexpressed in hepatocellular carcinoma versus normal liver, p-value = 7.89E-7 and n = 197 [52]. F. PRAS40 is significantly overexpressed in large cell lung carcinoma versus normal lung tissue, p-value = 9.98E-6 and n = 40 [53]. G. PRAS40 is not significantly different in pancreatic carcinoma versus normal tissue, p-value = 0.117 and n = 52 [45].

Over the past few years, novel insights have been reported regarding PRAS40 function and its modulation. However, many questions still need to be answered, due to conflicting results in different reports along with addressing the void that limits PRAS40 research. For example, it is unknown whether proline-rich domains could regulate the interaction of PRAS40 with other subdomain moieties in AKT, MAPK and TSC [1/2]. It also remains to be determined which AKT phosphorylation site plays a more active role in altering PRAS40 levels in human cancers and it would be interesting to determine which AKT isoform contributes to the PRAS40 regulation of cellular apoptosis and senescence in cancer. As addressed in this review, it is clear that alterations in PRAS40/AKT/mTORC1 are involved in central oncogenic pathways and thus, targeting PRAS40 offers a potential therapeutic treatment for diverse cancers.

Conflict of interest

We declare no conflict of interest between the authors or with any institution in relation to the contents of this article.

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