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PAK4 signaling in health and disease: defining the PAK4–CREB axis

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Abstract

p21-Activated kinase 4 (PAK4), a member of the PAK family, regulates a wide range of cellular functions, including cell adhesion, migration, proliferation, and survival. Dysregulation of its expression and activity thus contributes to the development of diverse pathological conditions. PAK4 plays a pivotal role in cancer progression by accelerating the epithelial–mesenchymal transition, invasion, and metastasis. Therefore, PAK4 is regarded as an attractive therapeutic target in diverse types of cancers, prompting the development of PAK4-specific inhibitors as anticancer drugs; however, these drugs have not yet been successful. PAK4 is essential for embryonic brain development and has a neuroprotective function. A long list of PAK4 effectors has been reported. Recently, the transcription factor CREB has emerged as a novel effector of PAK4. This finding has broad implications for the role of PAK4 in health and disease because CREB-mediated transcriptional reprogramming involves a wide range of genes. In this article, we review the PAK4 signaling pathways involved in prostate cancer, Parkinson's disease, and melanogenesis, focusing in particular on the PAK4-CREB axis.

Introduction

p21-Activated kinase (PAK) was initially identified as an effector of Rho GTPases that play a central role in reorganization of the cytoskeleton[1]. Early studies on this kinase thus focused on its signaling pathways that control cellular morphology, adhesion, and migration[2,3]. Later, its known roles expanded to a wide range of cellular functions, including cell proliferation and survival. The number of PAK family members has increased to six, and they are classified into group I (PAK1–3) and group II (PAK4–6) based on their structures and functions[4].

In general, PAKs are composed of an N-terminal regulatory region and a C-terminal catalytic region (Fig. 1). Group I PAKs contain a p21-binding domain (PBD) and an autoinhibitory domain (AID) in the N-terminus, while group II PAKs contain a PBD and an AID or a pseudosubstrate domain (PSD), depending on the protein. The kinase domain of all PAK family members is located at the C-terminus. In the inactive state, group I PAKs are homodimers, and group II PAKs are monomers. The AID plays a key role in inhibiting kinase activity when group I PAKs are in the dimeric form. Upon binding of Rac/Cdc42 Rho GTPase to the PBD, AID-mediated inhibition is relieved, dissociating the dimer into monomers and thereby activating the kinase. However, controversy exists regarding whether the PBD in group II PAKs plays a similar role (Fig. 1). Group II PAKs show a binding preference for Cdc42 over Rac1. Binding of Cdc42 to the PBD of group II PAKs alters their intracellular location; for example, it can induce their translocation to the plasma membrane[5]. Moreover, a recent study revealed unexpected contact between Cdc42 and the polybasic region (PBR) and C-terminal lobe of PAK4 in addition to PBD[6] (Fig. 1). These additional interactions were shown to suppress PAK4 kinase activity in vitro. Notably, PAK4 and PAK6 possess a PSD (Fig. 1), which blocks the entry of their substrates into the catalytic site; removal of this blockade by phosphorylation of S474 (human PAK4)/S602 (human PAK6) in the activation loop may represent

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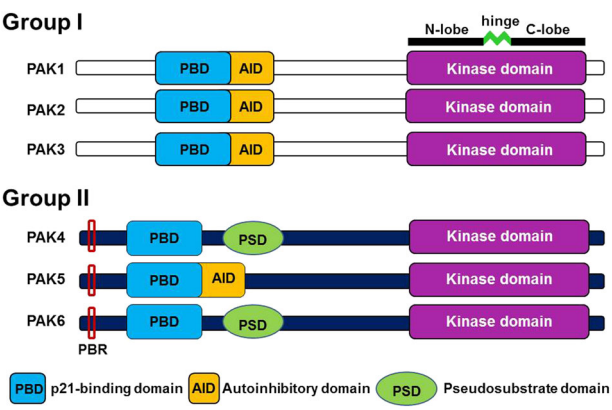

Fig. 1 Domain architecture of PAK family kinases. Group I PAKs contain an overlapping PBD and AID in their N-terminal regions. Among the group II PAKs, PAK5 also contains a PBD and an AID. In contrast, PAK4 and PAK6 lack the AID but contain the PBD and PSD. Group II PAKs all contain a polybasic region (PBR), but its role has only been defined for PAK4 (see the main text for detail). N-lobe N-terminal lobe, C-lobe C-terminal lobe

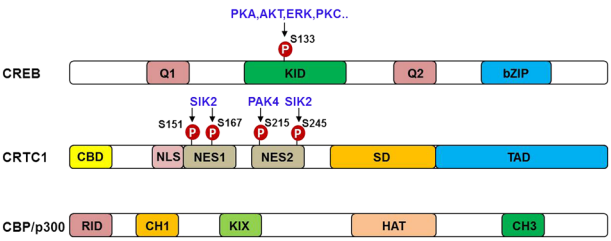

Fig. 2 Domain architecture of CREB and its coactivators. The functional domains and major phosphorylation sites of CREB and its coactivators are shown. Kinases responsible for phosphorylating each amino acid residue are also depicted. KID kinase-inducible domain, CBD CREB-binding domain, NLS nuclear localization signal, NES nuclear export signal, SD splicing domain, TAD transactivation domain, RID nuclear receptor interaction domain, CH Cys- and His-rich region, KIX KID-interacting domain, HAT histone acetyltransferase

an activation mechanism. Together with PSD-mediated inhibition, the extended Cdc42-PAK4 interactions may contribute to the full suppression of PAK4 kinase activity[6].

cAMP response element-binding protein (CREB) is a transcription factor that regulates the expression of a number of genes in diverse types of cells. Many signaling pathways converge on this factor, whose dysregulation subsequently leads to various pathological states, including carcinogenesis, abnormal metabolism, and neurodegeneration. Diverse posttranslational modifications contribute to regulation of the transcriptional activity of CREB. Phosphorylation of CREB has been extensively studied. Multiple kinases have been shown to directly phosphorylate CREB (Fig. 2): protein kinase A (PKA), protein kinase B (PKB/AKT), p42/44 mitogen-activated kinase (MAPK), and 90 kD ribosomal S6 kinase[7–10]. PKA is a heterotetramer composed of two regulatory subunits and two catalytic subunits. Four molecules of cAMP bind to the two regulatory subunits, resulting in the release of the catalytic subunits. Active free forms of the catalytic subunits phosphorylate CREB on S133, which induces its translocation to the nucleus and subsequent binding to CRE sites in the promoters of its target genes.

For its full activity, CREB requires cofactors such as CREB-binding proteins (CBPs) and CREB-regulated transcriptional cofactors (CRTCs) (Fig. 2). CREB phosphorylation induces the recruitment of CBPs, which enhance CREB activity; multiple phosphorylation sites in the kinase-inducible domain (KID) of CREB and the kinases that phosphorylate these sites have been documented[11]. In contrast, CREB phosphorylation is not required for its binding to CRTCs. Instead, CRTC dephosphorylation controls CREB activity[12].

Recently, PAK4 has emerged as a novel regulator of CREB in health and disease. In this article, we will review PAK4 signaling, particularly in the PAK4–CREB axis, and discuss its clinical implications.

PAK4 signaling pathways in cancer

Deregulated proliferation, apoptosis, migration, adhesion, and invasion are hallmarks of cancer cells[13]. PAKs play important roles in these events; thus, unsurprisingly, their overexpression, gene amplification, and hyperactivation (although mutations leading to hyperactivation are rare) contribute to the development and progression of many cancers. A number of studies have revealed PAKs as hub molecules linking major signaling pathways, including the Ras–ERK, Wnt/β-catenin, and androgen receptor/estrogen receptor (AR/ER)-dependent pathways[14–18] (Fig. 3). Because of this strategic role, PAKs, particularly PAK1 and PAK4, have emerged as attractive targets in the field of cancer therapy.

PAK4 is overexpressed in breast, gastric, prostate, lung, gallbladder, and ovarian cancers[19–24]. Amplification of the PAK4 gene, which is located on chromosome 19 (19q13.2)[25], is frequently detected in ovarian, breast, and pancreatic cancer, as well as in squamous cell carcinoma[25–28]. PAK4 controls cell proliferation, survival, invasion, metastasis, the epithelial–mesenchymal transition (EMT), and drug resistance in vitro and in vivo (Fig. 3), thereby promoting overall cancer progression[14,21,22,29,30]. Notably, PAK4, but not the other PAK isoforms, is sufficient to transform normal cells[31].

PAK4 is activated through diverse signaling pathways in cancer (Fig. 3). Oncogenic forms of Ras (mostly K-Ras) prevail in many human cancers, including pancreatic, colorectal, and lung adenocarcinomas[32–34]. Numerous approaches targeting mutant Ras have not yet been successful. Based on accumulating evidence, PAK4 is an

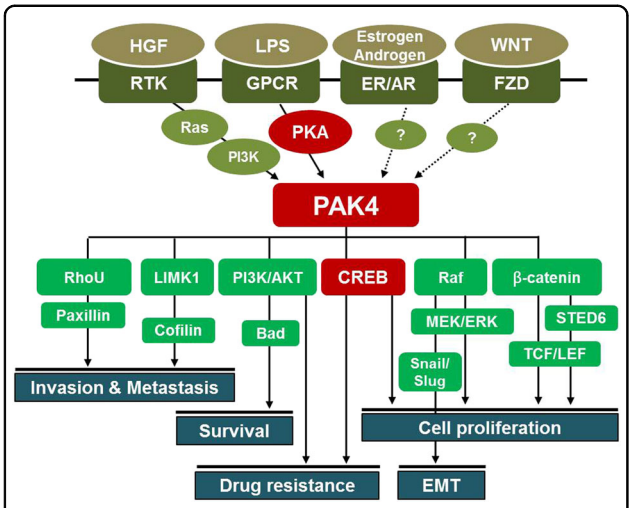

Fig. 3 PAK4-mediated signaling pathways in cancer. PAK4 is activated by various extracellular signals through receptor tyrosine kinases (RTKs), G protein-coupled receptors (GPCRs), ERs, ARs, and WNT-FZD receptors. Activated PAK4 regulates cell proliferation, survival, invasion, metastasis, the epithelial-mesenchymal transition (EMT), and drug resistance in cancers (see the main text for a detailed description). In GPCR and RTK signaling, PKA and Ras, respectively, function upstream of PAK4. The upstream regulators of PAK4 in ER/AR and WNT signaling remain to be determined. HGF hepatocyte growth factor, LPS lipopolysaccharide

alternative target in mutant K-Ras-driven cancers. PAK4 knockdown inhibits the proliferation of HCT116 colon cancer cells harboring mutant K-Ras[14]. This inhibition is independent of Raf/MEK/ERK or PI3K/AKT signaling, suggesting the involvement of unidentified PAK4 effectors. PAK4 activation mediates hepatocyte growth factor (HGF)-induced changes in the cytoskeleton and in cell adhesion downstream of PI3K;[15] these changes require a physical interaction between PAK4 and the p85 alpha subunit of PI3K[35]. This interaction may also explain HGF-stimulated invasion in pancreatic ductal adenocarcinoma[35], which frequently shows amplification of the PAK4 gene[25]. In contrast to the role of PAK4 as an effector of PI3K, PAK4 may act upstream of PI3K in promoting cisplatin resistance in gastric and cervical cancer cells[16,36]. A feedback loop between Ras/PI3K and PAK4 may explain these apparently incompatible findings. Development of specific inhibitors dissociating PI3K from PAK4 may represent a novel therapeutic modality in diverse types of cancers in which PI3K and PAK4 play a central role in progression[37].

Aberrant activation of the canonical Wnt pathway is a hallmark of many human cancers, such as colorectal and hepatocellular carcinomas. PAK4 regulates the Wnt/β-catenin pathway through several mechanisms. PAK4 phosphorylates β-catenin at serine 675[38], which is also phosphorylated by PAK1[17]. Phosphorylation at this site prevents β-catenin ubiquitination and subsequent proteasomal degradation in the cytoplasm. PAK4 is also involved in the nuclear transport of β-catenin[38]. A recent study revealed a mechanism underlying PAK4-mediated stabilization of β-catenin in the nucleus[38]. SETD6, a member of the family of protein lysine methyltransferases, methylates PAK4 bound to chromatin in cells[18]. This methylation induces close interaction between PAK4 and β-catenin, stabilization of β-catenin, and nuclear localization of β-catenin, resulting in increased β-catenin transcriptional activity[18]. Based on this evidence, PAK4 is a key regulator of the Wnt/β-catenin signaling pathway and thereby contributes to cancer progression. Researchers have not yet determined which Wnt ligands activate PAK4 and which activators function upstream of PAK4.

Early studies on PAK4 revealed its central role in actin cytoskeletal reorganization, which is similar to that of PAK1. HGF-activated PAK4 phosphorylates LIM kinase 1 (LIMK1)[39], which phosphorylates and inactivates cofilin in migrating cells[40], reducing the ability of cofilin to depolymerize F-actin. HGF is a potent agonist of tumor progression and invasiveness, and PAK4 is required for HGF-induced progression and invasion of human prostate cancer cells. The PAK4 gene is amplified in ~20% of patients with pancreatic cancer, and pancreatic tumors display increased PAK4 kinase activity[29]. The PAK4–LIMK1 pathway may contribute to the metastasis of pancreatic cancer. PAK4 is overexpressed in human non-small cell lung cancer (NSCLC), and its overexpression is associated with metastasis, decreased survival, and an advanced stage of NSCLC. PAK4 expression in NSCLC correlates with LIMK1 phosphorylation[22]. Overall, because of its importance in the reorganization of the actin cytoskeleton, the PAK4–LIMK1 pathway appears indispensable for the progression, including the invasion and metastasis, of prostate, lung, and pancreatic cancers.

PAK4 exerts its biological functions through both kinase-dependent and kinase-independent mechanisms[41,42]. Despite the presence of a PBD, PAK4 kinase activity appears to be independent of Rac/Cdc42 binding. What is the role of this PBD? Binding of Cdc42 to the PBD of PAK4 changes the subcellular localization of PAK4; for example, this binding can induce translocation of PAK4 to the plasma membrane[5]. Therefore, PAK4 may regulate cell adhesion and migration. PAK4 is expressed at high levels in breast cancer, with the highest levels detected in carcinomas with high grades and high invasiveness. PAK4 regulates the migration and adhesion turnover of these cells[42]. However, this regulatory pathway is independent of both its kinase activity and Cdc42 binding; surprisingly, binding of PAK4 to a nonconventional Rho GTPase, RhoU, regulates adhesion turnover[42]. This interaction stabilizes RhoU and prevents its

ubiquitin-mediated destruction. Because of these kinase-dependent and kinase-independent modes of action, which are shared by other PAK isoforms[43,44], inhibitors targeting the ATP-binding pockets of PAKs would be less effective therapeutic agents than similar inhibitors of other kinases.

The PAK4–CREB axis in prostate cancer

The cAMP–PKA signaling pathway is a key regulator of tumorigenesis, tumor progression, chemotherapy resistance, and survival in patients with cancer; it is also a key regulator of survival, growth, and differentiation in normal cells[45]. CREB plays a central role in the cAMP signaling pathway by upregulating the expression of genes such as Bcl-2, Cyclin D1, and Egr-1[46]. CREB is overexpressed or hyperactivated in various human cancers, such as prostate cancer, NSCLC, brain tumors (glioblastoma), melanoma, acute leukemia, and breast cancer. Huang et al. identified CREB as a critical effector in prostate cancer bone metastasis[47]. In a recent study, we discovered that PAK4 regulates the transcriptional activity of CREB and thereby promotes prostate cancer progression through such mechanisms as the emergence of drug resistance and neuroendocrine differentiation[21]. The identification of GRK3 as a direct target of CREB supports a role for CREB in neuroendocrine differentiation[48]. PAK4-mediated CREB activation is independent of its phosphorylation on S133. In another study by our group, PAK4 was shown to activate CREB by phosphorylating CRTC1, a coactivator of CREB[49]. However, whether this mechanism is involved in tumorigenesis remains to be ascertained. Considering its critical role in cancer, many researchers have attempted to target CREB but have achieved limited success[45,50]. Strategies targeting the PAK4–CREB axis may represent an alternative therapeutic approach.

PAK signaling in Parkinson's disease

Overview of Parkinson's disease

Parkinson's disease (PD) is a chronic, slowly progressing neurological disease[51]. PD is defined by the degeneration of dopaminergic neurons in the substantia nigra and the formation of Lewy body inclusions containing aggregated alpha-synuclein (α-Syn)[52]. The resulting dopamine deficiency in the basal ganglia leads to a movement disorder that is clinically characterized by parkinsonian motor symptoms such as bradykinesia, rest tremor, rigidity, postural instability, and gait impairment[53]. The etiology of PD remains unclear, but the disease may be caused by a combination of genetic and environmental factors[54,55].

α-Syn has emerged as a critical protein in PD pathogenesis because its accumulation and aggregation have been mechanistically linked to PD pathogenesis[56,57]. According to accumulating evidence, aggregated α-Syn is the major toxic species that promotes cell death. Danzer et al. discovered that α-Syn oligomers, but not monomers, inhibit PAK4 kinase activity, as assessed by its autophosphorylation levels in vitro[58]. Furthermore, phosphorylation of the PAK4 substrate LIMK1 is reduced in brainstem extracts from α-Syn (A30P) transgenic mice[58]. Mutations in leucine-rich repeat kinase 2 (LRRK2) are the most common cause of monogenic PD[59]. LRRK2 mutations result in the accumulation of α-Syn aggregates and ubiquitin-positive inclusions in the brains of subjects with PD. Mitochondrial impairment results in ROS production in patients with PD[60,61]. Numerous studies have reported increases in the levels of several markers for oxidative damage in the substantia nigra of patients with PD[62], indicating a contribution of impaired mitochondrial function to PD pathogenesis.

PAK signaling pathways in Parkinson's disease

Deregulation of PAK1 and PAK4 activity has been observed in subjects with PD[49,63]. Downregulation of PAK1 is involved in the loss of mesencephalic dopaminergic neurons[63]. Expression of a dominant-negative form of PAK1 (PAK1-DN) decreases neuronal cell viability and increases cell death induced by oxidative stress. PAK1-DN expression decreases levels of the Bcl-2 protein through a ubiquitin/proteasome-dependent mechanism[63]. Total levels of PAK4 and levels of active phosphorylated PAK4 are markedly reduced in patients with PD compared with age-matched controls[49]. Notably, PAK4 deficiency or inhibition renders dopamine neurons more vulnerable to 6-hydroxydopamine (6-OHDA)-mediated neurotoxicity in vivo[49]. Thus, deregulation of PAK1 and PAK4 is involved in PD pathogenesis and may provide potential therapeutic targets for the treatment of other neurodegenerative diseases.

The PAK4–CREB axis in Parkinson's disease

The expression of constitutively active PAK4 (caPAK4) protects dopaminergic neurons in both 6-OHDA and α-Syn rat models of PD and preserves motor function[49]. This neuroprotective effect of caPAK4 is mediated by the CREB transcription pathway (Fig. 4). CREB is well known to promote neuronal survival[64]. In the SH-SY5Y neuronal cell line, expression of caPAK4 increases CRE reporter activity and prevents 6-OHDA-induced suppression of the expression of the CREB target proteins PGC-1α, BDNF, and Bcl-2. CREB phosphorylation at S133 has been shown to be essential but not sufficient for CRE-driven gene transcription, suggesting the involvement of other mechanisms. The molecular mechanism by which PAK4 regulates CREB transcription does not require CREB phosphorylation (Fig. 4)[49]. A new family of CREB-specific coactivators has been identified, CRTC, which stands for "CREB-regulated transcription coactivator"[65]. The

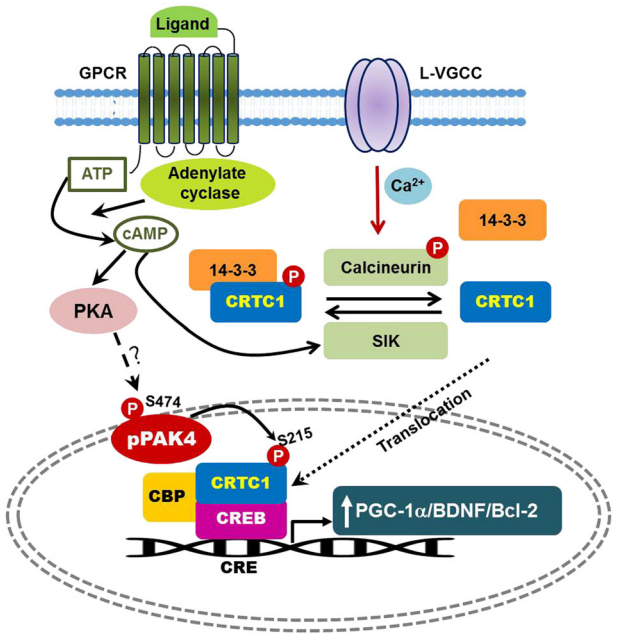

Fig. 4 PAK4-mediated signaling pathways in PD. In the cytosol, CRTC1 is phosphorylated at S151 by the cAMP-SIK pathway and is inactivated through interactions with 14–3–3 proteins[12]. Ca^{2+} influx through L-type VGCCs stimulates calcineurin-dependent CRTC1 dephosphorylation[12]. CRTC1 is activated by calcineurin-dependent dephosphorylation[12]. Dephosphorylated CRTC1 is translocated to the nucleus, where it is likely phosphorylated at S215 by PAK4, which triggers CREB-mediated transcription. In PD, oxidative stress and aggregated α-Syn decrease PAK4 activity and reduce $pCRTC1^{S215}$ levels, which subsequently decrease the expression of CREB target genes such as BDNF, Bcl-2, and PGC-1α, leading to dopaminergic neuron death. CRE, cAMP response element; CREB CRE-binding protein, CRTC1 CREB-regulated transcription coactivator 1, CBP CREB-binding protein, SIK1 salt-inducible kinase 1, VGCC voltage-gated calcium channel, BDNF brain-derived neurotrophic factor, Bcl-2 B-cell lymphoma 2, PGC-1α peroxisome proliferator-activated receptor gamma coactivator 1 alpha, TH tyrosine hydroxylase

CRTC1 isoform is mainly expressed in the nuclei of dopaminergic neurons in the human and rat brain[49]. Cyclic AMP, salt-inducible kinase (SIK) and calcineurin antagonistically regulate CRTC-1-CREB signaling (Fig. 4). CRTC1 is inactive in the cytoplasm; cAMP and SIK phosphorylate CRTC1 and sequester it through interactions with 14–3–3 proteins. For CRTC1 activation, calcium influx through VGCCs stimulates calcineurin-dependent dephosphorylation of CRTC1, which induces its nuclear translocation (Fig. 4). Nuclear CRTC1 binds CREB, which induces the transcription of CREB target genes such as the PGC-1α and BDNF genes and protects cells from ischemia[66]. Deregulation of CRTC1-dependent CREB transcriptional activity is implicated in Alzheimer's disease, Huntington's disease, ischemia and disturbances in circadian clock activity[67]. Moreover, CRTC1-deficient mice show depression-related behaviors[68]. These mice display decreased levels of dopamine metabolites, suggesting that CRTC1 regulates dopamine metabolism in subjects with PD[68]. PAK4 directly interacts with CRTC1 and phosphorylates it at S215 (Fig. 4)[49]. Knockdown of CRTC1 in dopaminergic neurons compromises the ability of caPAK4 to protect these neurons from 6-OHDA toxicity in a rat model of PD. The nonphosphorylated form, $CRTC1^{S215A}$, compromises the ability of caPAK4 to induce the expression of the CREB target proteins Bcl-2, BDNF, and PGC-1α[49]. Thus, phosphorylation of CRTC1 at S215 is essential for PAK4-mediated CREB activation and neuroprotection. Most neuromelanin-positive dopaminergic neurons in the normal aged human brain contain high levels of nuclear $pCRTC1^{S215}$. In contrast, $pCRTC1^{S215}$ levels in dopaminergic neurons are significantly lower in postmortem brain tissues from patients with PD. Based on these findings, the PAK4–$CRTC1^{S215}$–CREB pathway is impaired in subjects with PD.

PAK4 signaling in melanogenesis

Overview of melanogenesis

Melanogenesis (synthesis of the pigment melanin) occurs in melanocytes, which are derived from the neural crest[69]. Two types of melanin, dark brown/black eumelanin and light red/yellow pheomelanin, are synthesized in the melanosomes of melanocytes. Although melanin has diverse functions, its protective role in the skin has been extensively studied; it minimizes the hazardous effects of UV radiation by absorbing UV and converting it into heat[70]. Excessive or defective melanogenesis not only causes diverse types of skin pigmentation disorders but also produces cosmetic issues. Melanogenesis is thus tightly regulated through multiple signaling pathways in epidermal and hair follicle melanocytes.

Human skin is easily exposed to UV irradiation, which induces DNA damage in keratinocytes. In response to DNA damage, the transcription factor p53 is stabilized and induces the transcription of multiple target genes required for melanogenesis, including the gene encoding proopiomelanocortin (POMC), the precursor of the pigmenting hormones α-melanocyte-stimulating hormone (α-MSH) and adrenocorticotropic hormone (ACTH)[71]. α-MSH is secreted from keratinocytes and binds to its receptor, melanocortin-1 receptor (MC1R), which is expressed on the surface of neighboring melanocytes. α-MSH-stimulated MC1R initiates a signaling cascade that includes the cAMP–PKA pathway, which activates CREB[72]. CREB stimulates the transcription of the microphthalmia-associated transcription factor (MITF), a master regulator of melanogenesis, which induces the expression of the melanogenic enzymes tyrosinase, TRP-1 and TRP-2[73]. Heterozygous mutations in MITF lead to Waardenburg syndrome IIA[74,75], which manifests as

abnormal pigmentation and deafness. In addition to pigmentation, MITF also regulates the proliferation and survival of melanocytes;[73] thus, its deregulation is closely linked to melanomagenesis.

The Wnt/β-catenin signaling pathway is essential for skin development through processes including the expansion of neural crest cells, the generation of melanoblasts, and the differentiation of melanoblasts into melanocytes[76,77]. Wnt/β-catenin signaling is also important for hair growth and wound healing[78,79]. Therefore, this signaling pathway has been the focus of extensive research in both epidermal and follicular stem cells[80,81]. Wnt signaling also plays a role in cutaneous pigmentation[81–84]. In the absence of Wnt ligands, β-catenin is subjected to ubiquitination-dependent proteolysis, and transcription of its downstream target MITF is subsequently inhibited. In the presence of Wnt ligands, binding to Frizzled and LRP5/6 coreceptors stabilizes β-catenin by disrupting the APC/Axin/GSK3β destruction complex, which increases MITF levels and stimulates melanogenesis. β-Catenin phosphorylation at S675 by PAK4 is another mechanism of β-catenin stabilization[38]. α-MSH induces the phosphorylation of β-catenin at S675[81], but the mechanism has remained elusive. Our study revealed PAK4 as a downstream mediator of the effects of α-MSH[85]. We therefore postulate that α-MSH stabilizes β-catenin through phosphorylation at S675 by PAK4, which increases the coactivator activity of β-catenin and initiates TCF/LEF-dependent transcription of target genes, including MITF. Thus, PAK4 might enhance melanogenesis through crosstalk with the Wnt/β-catenin pathway. Overall, PAK4 is a central regulator of melanogenesis because it provides a signaling hub linking two major melanogenic pathways, the cAMP–PKA pathway and the Wnt/β-catenin pathway (Fig. 5).

Stem cell factor (SCF)/c-Kit (a tyrosine kinase receptor) signaling plays multiple roles in melanocytes, including roles in proliferation, differentiation, and hair shaft pigmentation. SCF was initially identified as a growth factor for melanocytes and mast cells[86]. Diverse types of skin cells, including keratinocytes, fibroblasts, and endothelial cells, secrete this factor. Similar to α-MSH, in response to UVB radiation, SCF is released from keratinocytes in a p53-dependent manner[87]. Mutations in the c-Kit gene are responsible for human piebaldism[88], a rare autosomal pigmentation disorder caused by abnormal melanocyte development, which supports the importance of SCF/c-Kit signaling in melanocytes. SCF/c-Kit signaling activates the Ras/MAP kinase pathway, which leads to MITF phosphorylation[89] and increases MITF transcriptional activity through recruitment of p300/CBP coactivators[90]. Upon stimulation by SCF, c-Kit is activated and recruits the guanine nucleotide exchange factor SOS and the adapter protein Grb2, which in turn activate Ras by converting Ras-GDP to Ras-GTP[91]. Active Ras then sequentially activates the RAF/MEK/ERK pathway. Interestingly, persistent activation of the BRAF/MEK/ERK MAP kinase (MAPK) pathway induces proteolytic degradation of MITF[92], thereby inhibiting melanogenesis. This proteolytic step may involve ubiquitin-conjugating enzymes such as hUBC9[93]. MAP kinase increases CREB activity by phosphorylating CREB on S133; thus, SCF/c-Kit signaling upregulates melanogenesis via the $pCREB^{S133}$–MITF pathway in addition to its direct effect on MITF phosphorylation. Furthermore, considering the role of PAK4 as a downstream effector of oncogenic Ras, we predict that PAK4 also functions downstream of Ras in SCF-stimulated melanocytes. These findings provide evidence for crosstalk between the α-MSH and SCF/c-Kit signaling pathways. Thus, melanogenesis is regulated by a complex array of signaling networks that involve the three major pathways described above and other pathways that are not reviewed here[94].

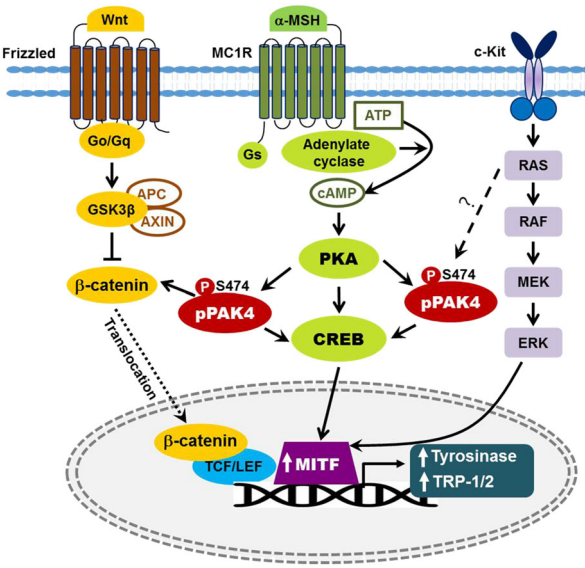

Fig. 5 PAK4-mediated signaling pathways in melanogenesis. PAK4 is activated downstream of PKA in the α-MSH signaling pathway and activates the CREB–MITF pathway. PAK4 phosphorylates β-catenin and activates TCF/LEF-dependent transcription, but the Wnt ligands that activate PAK4 remain unclear. PAK4 also functions downstream of Ras, but the exact mechanism is unknown. MITF microphthalmia-associated transcription factor

The PAK4–CREB axis in melanogenesis

We recently identified PAK4 as a downstream effector of PKA; PKA directly binds PAK4 and activates it by inducing the phosphorylation of S474[21]. This observation suggests a potential mechanism by which PAK4 regulates CREB activity globally in diverse cell types. Indeed, PAK4 increases the transcriptional activity of CREB in

melanocytes[85]. As MITF is a downstream target of CREB, PAK4 activation enhances melanogenesis through the CREB–MITF pathway[85]. Initially, the mechanism by which PAK4 activates CREB was unclear because PAK4 does not directly phosphorylate CREB[21]. Dephosphorylation of CRTC was recently shown to facilitate its nuclear translocation and CREB activation, which occurs in a CREB phosphorylation-independent manner through recruitment of the transcriptional machinery[65,95]. SIK2 is a serine/threonine kinase that phosphorylates CRTC and thus inhibits its nuclear translocation and subsequent CREB activation. Indeed, depletion of SIK2, the predominant SIK isoform expressed in melanocytes, upregulates melanogenesis in mice[96]. A further study showing that small-molecule SIK inhibitors upregulate MITF and induce melanogenesis supports a critical role for CRTC in the regulation of the CREB–MITF pathway[97]. Interestingly, CRTC functions downstream of AMPK in adiponectin signaling to suppress UV radiation- and α-MSH-induced melanogenesis[98]. In contrast to a role for CRTC dephosphorylation as an activation mechanism, PAK4-mediated CRTC1 phosphorylation on S215 increased CREB activity through an unknown mechanism in our previous study[49]. According to a recent study, melanocytes express CRTC2 and CRTC3[98]. Consensus residues similar to those surrounding S215 in human CRTC1 are present in these isoforms (S244 in CRTC2 and S243 in CRTC3); thus, PAK4 likely regulates the CREB–MITF pathway by phosphorylating CRTC2 and CRTC3 in melanocytes.

Regarding the possible roles of other PAK isoforms in melanogenesis, we also detected PAK2 in melanocytes, but its knockdown did not affect melanogenesis[85], suggesting that PAK2 is dispensable for this process. PAK1 is expressed in melanocytes at very low levels, but forced expression increases forskolin- and α-MSH-induced melanogenesis[99]. However, its function in the skin remains to be determined. PAK1 has been implicated in the development of melanoma[100]. PAK4 has been well documented as a tumor progression factor in a number of tumor types[101], but its role in melanoma has not been studied. Deregulation of pigmentation genes is linked to the development of melanoma[102,103]. Because PAK4 plays a central role in melanogenesis, investigations into the mechanism by which deregulation of PAK4 contributes to the development of melanoma would be fruitful.

Perspectives

In the current review, we have highlighted the role of PAK4 signaling in many biological events, including prostate cancer progression, neuroprotection in Parkinson's disease, and the promotion of melanogenesis. Because CREB is a key transcription factor involved in diverse pathophysiologies and because PAK4 controls CREB activity, we postulate that PAK4 regulates many unidentified biological functions. For instance, the roles of CREB and CRTC2 in diabetes mellitus have been extensively studied[104,105], suggesting the involvement of the PAK4-CRTC-CREB pathway in the pathogenesis of diabetes mellitus.

Regarding potential translational implications, the PAK4–CREB axis may represent a therapeutic target. Because of its frequent mutations in human cancers, numerous researchers have attempted to target mutated K-Ras[106] but have not yet achieved success. Based on accumulating evidence, PAK4 represents an alternative target in mutant K-Ras-driven cancers. Recently, Karyopharm Therapeutics (Newton, MA, USA) has made notable progress by identifying PAK4 allosteric modulators and has provided proof of concept for the treatment of pancreatic cancer in animal models[107]. One of the identified compounds, KPT-9274, is currently being investigated in a phase I clinical trial.

Some questions remain unanswered. PKA regulates CREB activity by directly phosphorylating it on S133. Is the PAK4–CREB axis a safeguard for the biological functions of the cAMP signaling pathway? Our recent study revealed the Slug transcription factor as a direct target of PAK4 in the TGF-β signaling pathway[30], suggesting a distinct role for PAK4 as a transcriptional regulator. In this regard, we are tempted to speculate that PAK4 activation downstream of PKA might partially explain the wide range of cellular functions of PKA reported in previous studies. Another interesting question is whether CRTC isoforms other than CRTC1 are regulated by PAK4 through similar mechanisms. Finally, researchers have not determined whether PAK5 or PAK6 functions downstream of PKA and then activates CREB.

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Conflict of interest

The authors declare that they have no conflict of interest.

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