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Review

Targeting Raf/MEK/ERK pathway in pituitary adenomas

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ABSTRACT

Pituitary adenomas are the common neoplasms that cause mass effect and/or endocrine dysfunction. Studies in the pathogenesis and functional regulation of pituitary adenomas are mainly focused on the following two topics: (a) the origin of pituitary adenomas and abnormal physical adjustment due to the activation of oncogenes and loss of function for tumour-suppressor genes; and (b) the mechanistic anomalies of the intracellular signal transduction. Among which, the Raf/MEK/ERK signalling has been considered to be one of the major and central pathways in disease aetiology. Raf/MEK/ERK signalling is evolutionarily conserved that controls cellular growth, differentiation and survival. Altered functionality of this signalling pathway has been found to be involved in the development of several types of cancers in humans including pituitary adenomas. This review summarises the roles of Raf/MEK/ERK signalling pathway in pituitary tumourigenesis and highlights the clinical potential of this signalling pathways to be a therapeutic target for intervention and treatment of pituitary adenomas.

1. Introduction

Pituitary adenomas are common neoplasms in the central nervous system, which constitute around 15–20% of intracranial tumours. These adenomas are mostly benign associated with inappropriate secretion of pituitary hormones, while they usually cause significant morbidity through mass effects on adjacent structures and/or endocrine dysfunction. Despite the advancement in laboratory evaluation, the molecular mechanisms underlying tumourigenesis and functional regulation of pituitary adenomas largely remain enigmatic.[1]

Interestingly, several genetic syndromes have been noted associated with pituitary adenomas, which once caught the attention of researchers. Very disappointedly, no pathogenic mutation has been found in these candidate genes in sporadic adenomas.[2–5] More recently, there is considerable advance in elucidating the involvement of signalling pathways in regulating and controlling cell growth. Given the fact that the Raf/MEK (mitogen activated protein kinase/ERK kinase)/ERK (extracellular signal regulated kinase) signalling is a major pathway in the regulation of cell growth, proliferation and survival, and that it is an evolutionarily conserved signal transduction module, its importance in tumourigenesis has been highlighted. Indeed, Raf/MEK/ERK pathway plays a central role in the signalling networks that govern the regulation of cell proliferation, differentiation, apoptosis and cell cycle arrest and the induction of drug resistance. As a result, it has been noted to be involved in the pathogenesis of several

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types of human cancers including pituitary adenomas.[6–9] Other than the Raf/MEK/ERK pathway, the PI3K (phosphatidylinositol 3-kinase)/Akt signalling has also been found implicated in the pathogenesis of pituitary adenomas. It is noteworthy that crosstalk and synergic action between Raf/MEK/ERK and PI3K/Akt signalling might occur at multiple levels.[10]

In this review, we intend to selectively dissect the role of Raf/MEK/ERK signalling pathway in pituitary tumourigenesis and evaluate its therapeutic potential as a target for intervention and treatment of pituitary adenomas.

2. Raf/MEK/ERK signalling pathway

2.1. The MAPK cascade

Mitogen-activated protein kinase (MAPK) cascade consists of serine–threonine kinases, which convert extracellular molecules such as growth factors, hormones and cytokines, into intracellular signals for control of cell proliferation, differentiation and survival. Previous studies have consistently demonstrated that aberrant regulation of MAPK cascade and its upstream activators predispose to the development of numerous tumours and other diseases in humans.[11–21] MAPK cascade is a big protein family which contains ERK1 and ERK2, c-Jun N-terminal kinases (JNK1, JNK2, JNK3), p38s (p38α, β, γ, δ) and ERK5 in humans.[22,23]. Among which, ERK1 and ERK2 (ERK1/2) are the best known, and have been suggested to play a key role in the RAF–MEK–ERK signalling cascade.[24–26] Thus far, more than a dozen of Raf/MEK/ERK1/2 substrates have been identified which range from cytoskeletal proteins to other kinases, phosphatases, enzymes and transcription factors.[6,27–29]

2.2. The initiating factors for activating Raf/MEK/ERK

Extracellular signals such as a wide variety of hormones, growth factors and differentiation factors, as well as tumour-promoting substances, employ the Raf/MEK/ERK pathway to execute their functional effect. In general, most of these stimuli arrive at the cell surface and activate receptor tyrosine kinase (RTK) through their binding properties. Upon the activation of RTK-Grb2-SOS signalling axis, it activates Ras small GTPase, a critical oncogene that is commonly mutated in approximately 20% or more human tumours. Ras small GTPase then serves as a key signal transduction component to activate MAPK cascade.[30–32] Once activated, Ras functions as an adapter by binding to Raf kinase with high affinity. As a consequence, it activates Raf by inducing its membrane translocation.[33] Raf kinases [A-Raf, B-Raf, and C-Raf (also termed Raf-1)] are a family of three protein serine/threonine kinases.[19] They possess restricted substrate specificity and participate in a sequential cascade of phosphorylation events to activate MEK1/2, which in turn activates ERK1/2. Therefore, MEK1/2 has been found to be co-expressed with ERK1/2, a downstream component of MAPK cascade[34,35] (Fig. 1).

2.3. Molecules to enhance Raf/MEK/ERK activity

GTP-bound Ras binding to Raf is prerequisite but not sufficient for the stable activation of Raf.[36] In many cases, there

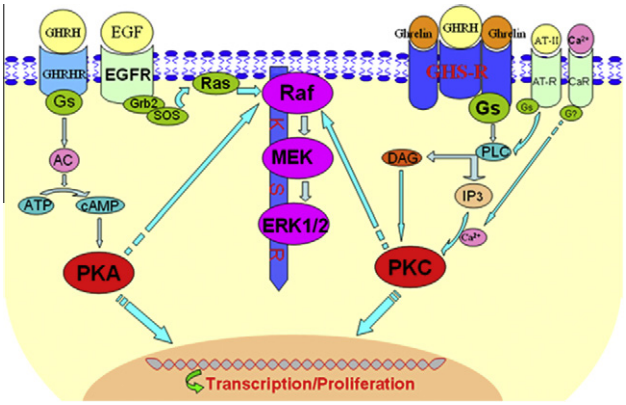

Fig. 1 – The Raf/MEK/ERK cascade in the signalling network of pituitary adenomas. Extracellular stimuli are combined with their receptors and in turn activate PKA, PKC and Raf–MEK–ERK1/2 pathways, respectively. Finally, they cause the response of the nuclei. GHRH; growth hormone releasing hormone; GHRHR: growth hormone releasing hormone receptor; Gs: guanine nucleotide binding protein; AC: adenylate cyclase; PKA: protein kinase A; EGF: epidermal growth factor; EGFR: epidermal growth factor receptor; Grb2: growth factor receptor binding protein 2; SOS: mammalian son-of-sevenless; PKC: protein kinase C; PLC: phospholipase C; DAG: diacylglycerol; IP3: inositol triphosphate; AT-II: angiotensin II.

are several other molecules involved in the activation of RAF–MEK–ERK signalling pathway cascade. Of which, 14-3-3 is an abundant, ubiquitously expressed and evolutionarily highly conserved protein family that regulates cell-cycle checkpoints, proliferation, differentiation and apoptosis.[37,38] The ability of 14-3-3 to enhance Raf activity is dependent on the phosphorylation of serine residues in Raf and the integrity of 14-3-3 dimer.[36,39] Although the functional consequence is yet to be fully elucidated, Raf-1 is the first among all signalling proteins discovered to be associated with 14-3-3. It is believed that 14-3-3 regulates target protein activation by changing its protein associations or subcellular localisation.[40–42]

KSR (kinase suppressor of Ras) is firstly named and identified genetically as a general and evolutionarily conserved component for the RAS signalling pathway.[43] KSR is another candidate to induce Raf catalytic function. It is believed that KSR only binds to Raf together with MEK and ERK in a core signalling module in the cell membrane. KSR does not solely act as a putative scaffolding protein for the Raf/MEK/ERK module, but facilitates Ras-mediated Raf activation through a kinase-independent mechanism.[44,45] In addition, KSR also participates in forming side-to-side heterodimers with Raf. Given that dimerisation of Raf proteins is critical for their activation and the catalytic function of Raf is regulated by a specific mode of dimerisation of its kinase domain,[46–48] it is assumed that KSR is important to drive Raf activation.

3. Raf/MEK/ERK pathway in the pathogenesis of pituitary adenomas

The pathogenesis of pituitary adenomas has been intensively studied and abnormalities in cell signalling pathways are

frequently seen, but the causative mechanism largely remains obscure.[27,49]

Raf/MEK/ERK cascade is a key signalling pathway involved in pituitary tumourigenesis, as well as PI3K/AKT pathway. However, it is not clear whether the Raf/MEK/ERK pathway plays the most critical and central role in all cell and tissue types of pituitary adenomas.[49,50] The B-Raf is found overexpressed in human pituitary adenomas, particularly in non-functional pituitary adenomas (NFPAs), suggesting that altered activity for the B-Raf/MAK/ERK pathway may play a pivotal role in the pathogenesis of these tumours.[6,51] However, a study revealed that mutations in B-RAF mutations are a rare event in pituitary adenomas, in which only one V600E mutation (the strongest activating mutation of B-Raf) in a NFPA sample has been identified among 50 human pituitary adenomas which include 25 NFPAs and 25 secreting adenomas.[52]

Extensive studies in a rat somatotroph adenoma cell line, GH3 cells, demonstrate that Raf/MEK/ERK signalling pathway is apparently related to cell growth and GH expression. Studies have shown that MAPK is an intracellular mediator for CXCL12/CXCR4 in GH3 cell proliferation, and it also plays an important role in GH production and secretion.[53] Another study suggests that the baseline and serum-induced prolactin mRNA levels are enhanced through EGF/EGFR pathway and this effect could be suppressed by Gefitinib, an EGFR antagonist (add reference). There is evidence that the downstream Gefitinib response occurs through ERK signalling.[54] Of note, IL-6 has also been demonstrated to stimulate GH expression in GH3 cells, and this stimulating effect can be completely abolished by the MEK inhibitor PD98059 and p38 MAPK inhibitor SB203580.[55]

The G protein-coupled receptors (GPCRs) are a large family of proteins that contain seven transmembrane domains involved in two principal signalling pathways: the cAMP signalling pathway and the phosphatidylinositol signalling pathway.[56] The activation of GPCRs has been demonstrated to affect the ERK1/2 cascade in different cell lines such as in pituitary adenoma cells.[57–62] In line with this result, mutations for G proteins and GPCRs have been identified in various endocrine diseases.[63–66] Similarly, the activity for ERK1/2 in GH adenomas is increased by GHRH which can be almost completely abolished by blockades for protein kinase C (PKC). Studies have shown that blockade of ERK cascade completely prevents the increase of GHRH-induced cyclin D1 expression.[59] Consistently, over activation of ERK is associated with enhanced cyclin D1 expression in pituitary adenomas, which then promotes cell cycle progression.[67,68]

Previously, we have demonstrated that staurosporine, a PKC inhibitor, possesses the capacity to suppress GH secretion, although this inhibitory effect cannot be observed in all tumours examined.[69] Nevertheless, the role of phosphatidylinositol–PKC cascade in controlling GH secretion in pituitary somatotrophinomas indeed attracted a lot of research focuses, and follow up studies further confirmed that PKC activators (e.g. PMA) enhance GH expression, while other PKC inhibitors (e.g. Gö6983 and rottlerin) or knockdown of PKCσ inhibit GH expression. Mechanistic studies revealed that Ca^{2+} is essential for the downstream of PKC to activate MAPK.[70] Based on these data, it has been assumed that GHRP-6 activates PKC, which then phosphorylates cAMP-responsive element-binding protein (CREB) to mediate growth hormone (GH) secretion.[71] In support of this notion, CREB has been recognised to play an important role in the regulation of pituitary cell proliferation,[71,72] and recent studies further demonstrate that the phosphorylation CREB acts as a marker for hypoxia and is likely associated with the invasiveness of tumour cells in pituitary adenomas.[73]

It has been well known that the growth hormone secretagogue receptor (GHSR) controls GH release in normal pituitary cells,[74] while Ghrelin, originally characterised in rat and human stomachs, acts as a natural endogenous ligand for GHSR.[75,76] Therefore, the Ghrelin/GHSR axis, has been recognised to be the third independent pathway in regulating GH release[77,78] implicated in the pathogenesis of pituitary adenomas.[79–81] It is assumed that Ghrelin, growth hormone-releasing peptides (GHRP) and so on serve as ligands for GHSR which then mediates the synthesis of phospholipase C (PLC) and inositol 1,4,5-trisphosphate (IP3), followed by the release of diacylglycerol (DAG) and the activation of protein kinase C (PKC) along with active cAMP signalling.[71,74,82–84] Indeed, PKC pathway activated by DAG and IP3-induced intracellular Ca^{2+} release has been demonstrated to play a central role in GH release and GHRH secretion,[85] in which Ghrelin enhances the MAPK activity (mainly ERK1/2) through activation of PKC.[86–88] However, the functional relevance between Raf and PKC remains controversial. Several studies suggest that PKC directly phosphorylates and activates Raf-1,[89,90] while conflict results have also been reported.[48]

The cAMP/PKA and IP_3/PKC cascades are two well-recognised signalling systems in normal pituitary cells and pituitary adenoma cells. cAMP is produced through the action of adenylate cyclase (AC) and is an activator for PKA. Upon activation, PKA phosphorylates selected substrates such as RAF, MEK and G protein coupled receptor kinases.[91] Furthermore, receptor-mediated enzymatic cleavage like Ca^{2+} and angiotensin II (AT II) promotes the formation of two important second messengers, IP3 and diacylglycerol (DAG), through phospholipase C (PLC). IP3 increases intracellular Ca^{2+}, while DAG activates PKC.[92–95] Kawabe and colleagues suggest that PKC could directly and potently regulate AC activity in an isoenzyme-specific manner which then regulates cAMP-dependent pathways.[96] However, Garcia et al. demonstrate that stimulation of the cAMP/PKA pathway might modulate PKC catalytic activity.[97] They find that the AC/cAMP/PKA cascade is essential for GH release through the stimulation of GHSR. Upon activation, PKA activates PKC, which in turn induces the activation of CREB through phosphorylation of ERK.[97] Together, these studies demonstrate a crosstalk between PKA and PKC in coordinating the activity of the two principal signal transduction pathways. Yet, contradictory debate relevant to the role of this crosstalk between the two pathways has also been disclosed.[98–100]

Taken together, although the Raf/MEK/ERK pathway plays a pivotal role in the complex signalling network along with many interactions, further research is urgently needed to clarify the exact functions and the underlying mechanisms of this signalling pathway in the pathogenesis of pituitary adenomas in humans.

4. Targeting Raf/MEK/ERK pathway

Given the role of the Raf/MEK/ERK pathway played in the regulation of cell proliferation, differentiation and survival and the high frequency for alteration of its functionality observed in human tumours, it has become an attractive target for drug development. In clinical settings, therapeutic approaches with selectively targeting activated kinases of this signalling pathway have been realised to be valuable for the treatment of a variety of diseases.[8,28,32,101–104] Other than this, strategies aimed at targeting components of this cascade have also been suggested to be effective.[40,44,48,105,106]

4.1. *The kinase inhibitors*

The laboratorial and clinical achievements of selective kinase inhibitors as therapeutic agents for several human cancers have prompted substantial interest in further development and clinical testing of such inhibitors for a wide variety of human malignancies. Among these kinases, the Ras, Raf and MEK kinases have received substantial attention, owing largely to the relatively high frequency of activating mutations characterised in the Raf/MEK/ERK cascade.[107,108] However, these inhibitors such as Ras inhibitors, Raf inhibitors and MEK inhibitors, are currently used for treating malignant tumours targeting the Raf/MEK/ERK cascade, and comparable and comprehensive data for their specificity and effect on the common benign pituitary adenomas are currently lacking. As a result, developing and testing novel kinase inhibitors in clinical settings with pituitary adenomas would be a promising and challenging task in the future research.

Similarly, inhibition of Raf kinase dimerisation may be another potential therapeutic point of research and treatment options for intervention of Raf-dependent tumourigenesis in the future.[48] However, further investigations are necessary to draw firm conclusions in terms of the significance of C-Raf/B-Raf dimerisation and its relevance to pituitary tumourigenesis.[6,13,52,109–113].

4.2. *Targeting scaffold and activator*

Specific scaffolding proteins such as kinase suppressor of RAS (KSR) and connector enhancer of KSR (CNK) scaffolds may serve as another array of potential therapeutical targets. KSR and CNK not only play important roles as a scaffold, but also act as an activator for directly inducing Raf catalytic function.[44,48,114,115] Furthermore, KSR is reported to have a role in the determination of cellular sensitivity to anticancer agents.[116] The 14-3-3 protein, which regulates Raf/MEK/ERK cascade at several points,[38,40] may be another interesting approach for antagonising this cascade. Various 14-3-3 antagonists have been developed for potential therapeutic interventions against diseases involving 14-3-3 malfunction, and therefore, 14-3-3 has been considered as a potential molecular target for anticancer therapeutic development.[117,118] However, conclusive data on the effectiveness of targeting 14-3-3 in pituitary adenomas are lacking, further investigations are needed.

4.3. *Other therapeutics*

Clinically widely used drugs such as somatostatin analogues (SSAs) have been proved to exert their anti-proliferative and antisecretory effects through MAPK pathways. Hubina and colleagues evaluated the mechanistic action of SSAs, and found that administration of SSAs (Octreotide and Pasireotide) upregulates p27Kip1 in pituitary adenoma samples, which is associated with inhibition of ERK1/2 activation both in human pituitary cells and GH3 cells.[119]

Given the difficulties to predict which of the various RAF/MEK/ERK cascade components would constitute the most useful therapeutic target, together with the existing crosstalks among the complex signalling network, it is plausible to consider targeting the upstream or downstream of the RAF/MEK/ERK cascade. In this regards, the Ghrelin/GHSR axis, the cAMP/PKA and IP3/PKC signalling would be quite attractive for the purpose of searching alternative therapeutic targets in the RAF/MEK/ERK signalling pathway.

5. Conclusion

Although the Raf/MEK/ERK signalling pathway plays a central role in the regulation of cell proliferation, differentiation and survival, its exact functional relevance in the settings of complex signalling network and pituitary tumourigenesis are far from being fully defined. Notably and particularly, current data relevant to its role in the signalling of complex pathways are not consistent in all studies and are not always applicable to all pituitary cells and tissue types. Therefore, further research in the future needs to dissect both the mechanisms of these signalling pathways and their pituitary cell specific features to clearly enlighten their roles in the pathogenesis of pituitary adenomas.

Conflict of interest statement

None declared.

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