The dual function of KSR1: a pseudokinase and beyond

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Abstract
Protein kinases play a pivotal role in regulating many aspects of biological processes, including development, differentiation and cell death. Within the kinome, 48 kinases (~10%) are classified as pseudokinases owing to the fact that they lack at least one conserved catalytic residue in their kinase domain. However, emerging evidence suggest that some pseudokinases, even without the ability to phosphorylate substrates, are regulators of multiple cellular signalling pathways. Among these is KSR1 (kinase suppressor of Ras 1), which was initially identified as a novel kinase in the Ras/Raf pathway. Subsequent studies showed that KSR1 mainly functions as a platform to assemble different cellular components thereby facilitating signal transduction. In the present article, we discuss recent findings regarding KSR1, indicating that it has dual activity as an active kinase as well as a pseudokinase/scaffolding protein. Moreover, the biological functions of KSR1 in human disorders, notably in malignancies, are also reviewed.

Introduction
KSR (kinase suppressor of Ras) 1 was originally discovered more than 15 years ago as a novel protein kinase evolutionarily conserved in Drosophila and Caenorhabditis elegans functioning between Ras and Raf in the Ras signalling pathway [1,2]. However, mammalian KSR1 has been extensively referred to as a pseudokinase, because of the mutation in the lysine residue in the catalytic domain, which is required for its kinase activity. Subsequently, the role of KSR1 as a scaffolding protein was revealed. Murine KSR1 was first reported to co-operate with activated Ras to facilitate MEK [MAPK (mitogen-activated protein kinase)/ERK (extracellular-signal-regulated kinase) kinase] and MAPK activation, thereby promoting Xenopus oocyte maturation and cellular transformation [3]. In addition, KSR1 was observed to translocate from the cytoplasm to the plasma membrane in the presence of activated Ras, where it forms a complex involving Raf-1, MEK1 and 14-3-3 protein. This in turn led to the activation of Raf-1, which is independent of its enzymatic activity, highlighting its role as a scaffold in the MAPK pathway [4,5]. Meanwhile, the notion of KSR1 as an active kinase was described from the finding that TNFα (tumour necrosis factor α) and ceramide were shown to significantly increase KSR1 autophosphorylation and its activity as an active kinase as well as a pseudokinase/scaffolding protein. Moreover, the biological functions of KSR1 in human disorders, notably in malignancies, are also reviewed.

Structure of KSR1
The KSR family members, namely KSR1 and KSR2, are conserved from invertebrates to mammals. From sequence comparison, the similarity of amino acids between KSR1 and KSR2 is approximately 61%. KSR1 is closely related to the Raf kinase family containing five conserved areas named CA1–CA5. The CA1 domain is located in the N-terminus encompassing 40 amino acids which are exclusive to KSR1, but absent from KSR2; CA2 is a proline-rich domain with undetermined function; CA3 is a cysteine-rich atypical C1 motif mediating its membrane recruitment with phospholipids [5]; CA4 is a serine/threonine-rich region with an FXFP (Phe-Xaa-Phe–Pro) motif that interacts with ERK [3,7]; and CA5 is a putative kinase domain in which the conserved lysine residue required for phosphorylation is lacking [7]. One recent study identified another domain composed of a CC (coiled coil) and a SAM (sterile α-motif) in KSR1. In fact, by binding directly to micelles and bicelles, the CC–SAM domain guided KSR1 to certain sites at the plasma membrane upon growth factor stimulus. Furthermore, NMR spectroscopy and in vitro assays demonstrated that the helix α of the CC motif is essential for modulation of membrane binding, indicating that, combined with the atypical C1 domain, the CC–SAM domain is indispensable for KSR1 cellular translocation [8].

KSR1 as a scaffold protein
The scaffolding function of KSR1 has been well described in numerous cellular contexts in various conditions, although different binding partners or even contradictory downstream
KSR1 regulation in the canonical Ras/Raf/MAPK pathway

In non-stimulated cells, KSR1 is sequestered in the cytosol through 14-3-3 protein binding after phosphorylation by C-TAK1 at Ser297 and Ser392. Meanwhile, KSR1 constitutively interacts with MEK and ERK. Upon growth factor stimulation, activated Ras triggers the dephosphorylation of KSR1 at Ser392 by PP2A, leading to the release of 14-3-3 protein from its binding sites. This in turn allows KSR1 to translocate to the cell membrane, where KSR1 forms a complex with Raf, MEK and ERK. KSR1 thus potentially enhances the phosphorylation of Raf, MEK and ERK, facilitating the upstream signalling transduction as well as regulating multiple cellular functions by activation of various substrates.

Recent advances in KSR1 structure demonstrated further its involvement in multiple scaffold complexes. Upon growth factor stimulation, a functional CA1 region of KSR1 was reported to be necessary for the assembly of a ternary complex with B-Raf and MEK, resulting in activation of MEK and ERK. This in turn allows ERK to phosphorylate KSR1 and B-Raf on several feedback serine/threonine phosphorylation sites, which leads to the dissociation of KSR1 from the plasma membrane [18]. Rajakulendran et al. [19] showed that, by forming side-to-side KSR1–Raf heterodimers, KSR1 can regulate Raf activation directly, despite its lack of catalytic function. Moreover, mutations in the dimer interface of KSR1 suppressed the activity of Raf [19]. The case for the scaffolding role of KSR1 is supported further by the observation that Raf inhibitors can trigger KSR1–B-Raf complex formation, dependent on conserved dimer interface residues in each partner [20]. In addition, this study demonstrates that KSR1 competes with C-Raf for inhibitor-induced dimerization to B-Raf, thereby modulating downstream ERK signalling [20].
KSR1 as an active kinase

Mounting evidence supports the concept of KSR1 as an active kinase, despite suggestions of an incomplete catalytic domain. Initial in vitro kinase assays reported that different concentrations of natural ceramide induced KSR1 autophosphorylation and transactivated Raf-1 at Thr269 [6]. Afterwards, EGF was shown to be able to stimulate the kinase activity of KSR1 in a two-stage kinase assay. In addition, only full-length KSR1 was capable of signalling c-Raf-1-dependent activity, but not kinase-inactive and C- and N-terminal deletion mutants [21]. The same group proposed further that phosphorylation of c-Raf-1 on Thr269 by KSR1 is required for optimal activation in response to EGF stimulation. The kinase activity of KSR1 appears to act independently of KSR1-binding to MEK [22,23]. Direct phosphorylation of Raf-1 by KSR1 is essential for TNFα-induced ERK1/2 activation in intestinal epithelial cells, revealing a protective role of KSR1 in inflammation process, which requires its regulatory kinase activity [24,25].

New biochemical techniques such as protein purification and molecular modelling have enabled further aspects of KSR1 function to be probed, generating new perspectives on its catalytic functions. By using a specific monoclonal antibody against phospho-Raf-1 (Thr269), the purified KSR1 was shown to be capable of phosphorylating BSA-conjugated Raf-1 peptide [26]. Consistently, recombinant wild-type KSR1, but not kinase-inactive KSR1, can autophosphorylate its serine residues and directly activate MEK1 through phosphorylation, regulating cell survival in response to TNFα [27]. Furthermore, in order to distinguish between the scaffold and kinase function of KSR1, Hu et al. [28] generated a KSR1 mutant construct (A587F) by adding a bulky phenylalanine residue at the ATP-binding pocket of KSR1 to impair ATP binding. However, this mutant can still maintain a closed active conformation, but is unable to interact with ATP. Indeed, the KSR1 mutant was not able to phosphorylate MEK, although it can constitutively bind to MEK as a scaffold. On the other hand, the wild-type KSR1 was shown to phosphorylate MEK induced by c-Raf, indicating a requirement of an active kinase activity in this process [28]. Crystal structure studies of the kinase domain of KSR2 illustrated that the side-to-side interaction between KSR2 and MEK1 is through their respective activation segments and C-lobe αG helices. Upon ATP binding to its catalytic site, KSR2 was shown to phosphorylate MEK1 by in vitro kinase assays and chemical genetics [29].

Taken together, these findings support dual function of KSR1 as a scaffolding protein and active kinase in orchestrating the Ras/Raf/MAPK signalling cascade.

The roles of KSR1 in various biological processes

KSR1 in cancers: a therapeutic target?

As KSR1 plays an essential role in the Ras/Raf/MAPK module, which is one of the well-known oncogenic pathways, studies are beginning to explicate its biological characteristics in different cancers. In a v-Ha-Ras-mediated skin cancer mouse model, KSR1 was shown to contribute to tumorigenesis through the Raf-1/MAPK cascade [30]. Subsequent work from the same group reported that targeting KSR1 by continuous infusion of phosphorothioate antisense ODNs (oligodeoxynucleotides) reduced tumour growth of K-Ras-dependent human PANC-1 pancreatic and A549 non-small-cell lung carcinoma xenografts in nude mice, suggesting inhibition of KSR1 as a potential therapeutic target in Ras-dependent malignancies [31]. Similarly, the introduction of KSR1 into Ksr1−/− MEFs (mouse embryonic fibroblasts) induced cell proliferative and oncogenic potential, and removal of KSR1 inhibited Ras(V12)-dependent transformation [13]. In addition, KSR1 was involved in cell sensitivity to cisplatin-induced apoptosis. Specifically, in comparison with wild-type MEFs, KSR1 depletion in MEFs correlated with reduced ERK activation by cisplatin and elevated resistance to cisplatin-stimulated apoptosis. Moreover, transduction of KSR1 into Ksr1−/− MEFS and MCF7 cells increased ERK activation and sensitivity to cisplatin [32]. A further screen to characterize KSR1 expression on drug sensitivity using a collection of cancer cell lines and the NC166 anticancer drugs suggested an important role for KSR1 in defining cellular sensitivity [33]. In human acute myeloid leukaemia cells, KSR1 was revealed to be down-regulated by the oncoprotein Cot1, thus providing extended information of its involvement in non-solid tumours [34]. Metastasis suppressor Nm23-H1 can bind directly to KSR1 and phosphorylate KSR1 at Ser992, and can therefore facilitate its degradation as a result of decreased ERK activation [35,36]. KSR1 was also required for cell-cycle reinitiation in response to DNA-damage agents such as mitomycin C [37]. Additionally, through modulation of PGClα [PPARγ (peroxisome-proliferator-activated receptor γ) co-activator 1α] and ERKα (oestrogen-related receptor α), KSR1 was demonstrated to induce oncogenic Ras-dependent anchorage-independent growth [38].

The inactivation of KSR1 in Myc (v-Myc myelocytomatosis viral oncogene homologue)-overexpressing mice led to an enhanced in B-cell apoptosis as well as an impediment in the onset of B-cell tumorigenesis, suggesting further that KSR1 modulates the co-operation of Ras/MAPK signalling pathway and Myc to drive oncogenic transformation [39]. Of note, a recent study has revealed that KSR2 controls tumour metabolism and cell growth in an AMPK (AMP-activated protein kinase)-dependent manner [40]. The forced expression of KSR2 in Ksr1-null MEFs resulted in augmented proliferation and induction of anchorage-independent growth, whereas the introduction of AMPK restores the transformed phenotype and tumour metabolic activities upon KSR2 depletion. Interestingly, our kinase screen using RNAi on identifying novel regulators of ERα (oestrogen receptor α) revealed a potential contribution of KSR1 to ERα-dependent breast cancer [41]. These results indicate a significant role of KSR1 in various cancers and it might be a potential therapeutic target in Ras-dependent cancers.
KSR1 in immune regulation

KSR1 is an important regulator of immune function. Ksr1-null mice displayed impairment in MEK and ERK activities, thus resulting in a marked reduction in T-cell proliferation [42]. A recent study has established a relationship between MAPK and mTOR (mammalian target of rapamycin) pathways through the involvement of KSR1 in T-cells. Although KSR1 modulates mTORC1 (mTOR complex 1) activity, KSR1 deficiency has no obvious effects in mTOR-dependent T-cell differentiation [43]. As for NK (natural killer) cells, it was shown that the loss of KSR1 in NK cells perturbed NK cell cytolytic capacity. Upon T-cell activation, KSR1 was recruited to NK lytic synapse that in turn regulated the localization of active ERK to the synapse [44]. Several studies have documented the protective role of KSR1 against cytokine-induced apoptosis such as TNFα in intestinal inflammatory conditions [25,45]. Ksr1−/−Il10−/− mice were shown to be susceptible to an early manifestation of colitis [46]. Additionally, elevated expression of IFNγ (interferon γ) in T-cells was detected in the double-knockout mice and the inhibition of IFNγ was able to reduce the extent of severity in colitis. KSR1 has also been shown to be a protective factor against bacterial infection. In fact, Ksr1-deficient mice were highly vulnerable to pulmonary Pseudomonas aeruginosa infection, compared with wild-type mice. Upon infection, KSR1 was capable of iNOS (inducible nitric oxide synthase) and Hsp90 (heat-shock protein 90) recruitment resulting in increased iNOS activity and NO release to eliminate bacteria [47]. These findings underscore an important role of KSR1 in immune responses.

KSR1/2 in metabolism

Both KSR members are involved in the maintenance of cellular metabolism in which metabolic abnormalities were evident in Ksr1- and Ksr2-deficient mice. Phenotypically, Ksr1−/− mice exhibited adipocyte hypertrophy, whereas Ksr2−/− counterparts were obese and glucose-intolerant [14,48]. The initiation of adipocyte differentiation relied indirectly on suitable amounts of KSR1 that orchestrated the temporal co-ordination of Raf/MEK/ERK and RSK (ribosomal protein S6 kinase) signalling, and, in this manner, controlled the activities of adipogenic transcription factors such as C/EBPβ (CCAAT/enhancer binding protein β) and PPARγ [14]. A key study by Costanzo-Garvey et al. [48] has also revealed a direct interaction between the two KSR isoforms and AMPK, a prominent energy sensor for metabolic processes. The disruption of this interaction prevented AMPK activation and phosphorylation that is imperative to induce fatty acid oxidation and regulate glucose uptake. Apart from the role of KSR2 in insulin homeostasis, KSR1 has also been recently implicated in regulating glucose tolerance and insulin sensitivity via the interplay between KSR1 and MARK2 (microtubule affinity-regulating kinase 2) [49]. The mode of action in which MARK2 acts as a negative modulator in insulin sensitivity relies on the direct phosphorylation on Ser592 of KSR1 [49]. Collectively, these studies have underlined the significance of KSR1/2 in regulating cellular metabolism and in energy homeostasis, and KSR1/2 could be potential therapeutic targets in metabolic disorders, such as obesity.

Closing remarks

Comprehensive insights regarding the structural and functional studies of KSR1 have started to redefine our previous knowledge, as it is becoming obvious now that the original classification of KSR1 as a pseudokinase may not be representative of its exact biological behaviour. Advances made in the last few years have changed our perspectives in the functions of KSR1, whereas a more complicated conception of KSR1 as a scaffold protein and an active kinase is established. Nevertheless, numerous gaps remain to be filled in order to understand the complexity of KSR1 biological actions. First of all, physiological substrates of KSR1 and its catalytic activity need to be identified further and validated in differential cellular contexts, as well as its subcellular localization where it performs certain functions. Moreover, as a scaffold protein, it is crucial to determine its binding partners in dynamic scenarios since the subsequent interaction will eventually contribute to various biological outcomes. Finally, the inquiry as to whether KSR1 is an attractive therapeutic target in malignancies that are confined to be Ras-dependent tumours requires further clarification.

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