Review

Cancer Research

The p90 RSK Family Members: Common Functions and Isoform Specificity

Romain Lara, Michael J. Seckl, and Olivier E. Pardo

Abstract

The p90 ribosomal S6 kinases (RSK) are implicated in various cellular processes, including cell proliferation, survival, migration, and invasion. In cancer, RSKs modulate cell transformation, tumorigenesis, and metastasis. Indeed, changes in the expression of *RSK* isoforms have been reported in several malignancies, including breast, prostate, and lung cancers. Four RSK isoforms have been identified in humans on the basis of their high degree of sequence homology. Although this similarity suggests some functional redundancy between these proteins, an increasing body of evidence supports the existence of isoform-based specificity among RSKs in mediating particular cellular processes. This review briefly presents the similarities between RSK family members before focusing on the specific function of each of the isoforms and their involvement in cancer progression. *Cancer Res; 73(17); 5301–8. ©2013 AACR.*

Introduction

The extracellular signal–regulated kinase (ERK)1/2 pathway is involved in key cellular processes, including cell proliferation, differentiation, survival, metabolism, and migration. More than 30% of all human cancers harbor mutations within this pathway, mostly resulting in gain of function and consequent ERK hyperactivation (1). Among ERK substrates are the serine/threonine kinases, 90 kDa-ribosomal S6 kinases (RSK), and their structural homologs, the mitogen- and stress-activated kinases (MSK1 and 2; ref. 2). In humans, four RSK isoforms (RSK1 to 4) have been identified on the basis of sequence homology. Although a degree of functional redundancy between these proteins is expected, evidence exists for specificity among RSK isoforms in mediating particular cellular processes (3–5). These findings are consistent with similar observations made for other proteins in the ERK1/2 pathway, including the RAS (HRAS, KRAS, and NRAS), RAF (RAF-1, B-RAF, and A-RAF), and ERK kinases (ERK1 and 2). Although recent reviews have presented the generic roles of RSKs (2), we focus here on the differences between the isoforms and their involvement in cancer.

The RSK Family

RSK genes and associated pathologic features

Originally identified on the basis of their kinase activity toward the 40S ribosomal subunit protein S6 (RPS6), *RSK*s were subsequently cloned throughout the Metazoan kingdom (2). The genomic analysis of several cancer types suggests that these genes are not frequently amplified or mutated, with some notable exceptions (e.g., in the case of hepatocellular carcinoma; ref. 6). Table 1 summarizes reported genetic changes in *RSK* genes. Although *RSK* genes do not appear to be hotspots for mutation or amplification in malignant lesions, larger studies are required to highlight their potential role in cancer progression.

The Coffin–Lowry syndrome (CLS) is the only case in which a direct link has been established between a disease and mutations in an RSK gene: *RSK2*. CLS is characterized by severe mental retardation, facial dimorphisms, tapering fingers, heart defects, and skeletal abnormalities (7). Although the observed mutations in RSK2 are heterogeneous, they all result in loss of kinase activity (7). Moreover, knockout of *Rsk2* in the mouse leads to a phenocopy of the human disease (8), and our own unpublished findings suggest that morpholino-mediated downregulation of RSK2 in the developing zebrafish embryo also reproduces some aspects of the human disease. These aspects include spinal deformity, reminiscent of the scoliosis observed in humans, often combined with tail shortening as well as cardiac hypertrophy. Therefore, the role of RSK2 in CLS may extend across the vertebrate world.

RSK expression patterns

The expression of *RSK1-3* has been detected in all human tissues tested, although the relative levels of each isoform vary widely between tissues (2). *RSK1* transcripts are more abundant in the lung bone marrow and T cells, whereas *RSK2* mRNAs are predominantly found in T cells, lymph nodes, and the prostate. *RSK3* transcripts are mainly expressed in the lung, brain, spinal cord, and retina. Interestingly, *RSK4* mRNA expression is much lower than that of the other three isoforms, and its levels vary far less between tissues. However, Sun and colleagues showed the existence of novel RSK4 splice variants as well as of various RSK4 protein variants of molecular weights ranging from 33 to

Authors' Affiliation: Division of Oncology, Oncology Imperial College London, London, United Kingdom

Corresponding Authors: Michael J. Seckl, Imperial College London, Hammersmith Hospital Campus, Du Cane Road, London W12 ONN, United Kingdom. Phone: 44-20-3311-1421; Fax: 44-20-3313-5577; E-mail: m.seckl@imperial.ac.uk; and Olivier E. Prado, E-mail: o.pardo@imperial.ac.uk

doi: 10.1158/0008-5472.CAN-12-4448

Table 1. Mutations reported for RSK isoforms and their frequency

				Mutation	
Prot	**Gene**	**Locus**	**Freq**	**DNA**	**Prot**
RSK1	RPS6KA1	1 p36-p35	1/225(L)	1591G>T	V531F
RSK2	RPS6KA3	Xp22.13	1/238 (Br)	1246A>G	1416V
			4/498 (CNS)	18220T	L608F
				8980A	R300R
				5500T	L184L
				1142T>A	L381H
			1/359 (L)	329G>A	R110Q
			1/85 (Ov)	1011 A>G	R337R
			1/34 (St)	1448A>G	Y483C
RSK3	RPS6KA2	6q27	1/477 (CNS)	18540T	T618T
			1/42 (LI)	2195G>A	R732Q
			1/359 (L)	12800G	S427*
			2/85 (Ov)	1581+1G>A (intron)	?
				2054G>T	R685L
			3/8 (Sk)	931 G>A	E311K
				1849G>A	D617N
				1339G>T	D447Y
			1/20 (UAT)	7100T	T237M
RSK4	RPS6KA6	Xq21	1/53 (CNS)	790-1 (del intron)	?
			4/275 (L)	367G>T	E123*
				13900T	P464S
				772T>A	S258T
				419A>G	Y140C
			1/7 (Sk)	16240T	R542C
			1/34 (St)	833G>T	R278I
			2/21 (UAT)	2087G>T	R696I
				11030A	T368K

Abbreviations: Br, breast; CNS, central nervous system; Freq, frequency; L, lung; LI, liver; Ov, ovarian; Prot, protein; Sk, skeletal; St, stomach.

135 kDa from a single cDNA (9), complicating our understanding of previously assumed RSK4 expression patterns.

Structural features and mechanism of RSK activation

The structure of RSKs is characterized by the presence of two functional kinase domains separated by a linker region and flanked by N- and C-terminal regions (2). RSKs display high homology (73–80% protein sequence identity) especially in the two kinase domains (78–90%; Fig. 1). The carboxyl-terminal kinase domain (CTKD) is closely related to the kinase domains found in the calcium/calmodulin-dependent protein kinase (CAMK) family. In contrast, the amino-terminal kinase domain (NTKD) is homologous to that of AGC kinases. Finally, the C-terminal region contains an ERK1/2 docking site also known as the D-domain.

Although no complete crystal structure of RSKs has been reported, several crystal structures exist for isolated kinase domains (PDB IDs: 3RNY, 2Z7S, 2Z7R, and 2Z7Q for RSK1, and 2QR8, 2QR7, 4D9T, 4D9U, and 3G51 for RSK2). Helpfully, the RSK2 NTKD was crystallized in an active conformation. This was associated with a spatial arrangement of the βB-sheet, stabilizing the kinase domain while the RSK2 CTKD's structure revealed a C-term autoinhibitory αL-helix that prevents access of both peptide substrate and ATP to the kinase pocket.

The activation of RSKs can be initiated by phosphorylation of tyrosine residues by receptor tyrosine kinases (RTK; ref. 2). Phosphorylation of RSK2 on Tyr-529 by fibroblast growth factor receptor 3 (FGFR3) and/or Src family kinases, SRC and FYN, promotes the recruitment of ERK to RSK2 (Fig. 1). Further phosphorylation of Tyr-707 by FGFR3 contributes to activation of RSK2 through displacement of the αL-helix and activation of the CTKD. Although RSK1 does not appear to be phosphorylated by FGFR3, it interacts with FGFR1 and FGFR2 IIIb to mediate some of their functions. Therefore, it is conceivable that different RTKs specifically activate individual RSK isoforms. Other upstream signaling events, independent of RTKs but feeding into the Ras/ERK pathway, also result in RSK activation. Interestingly, ERK is recruited in its inactive form to the D-domain of RSKs (10–13), where a highly conserved sequence required for interaction (^{722}LAQRRVRKL^{730} of RSK1) has been identified (14).

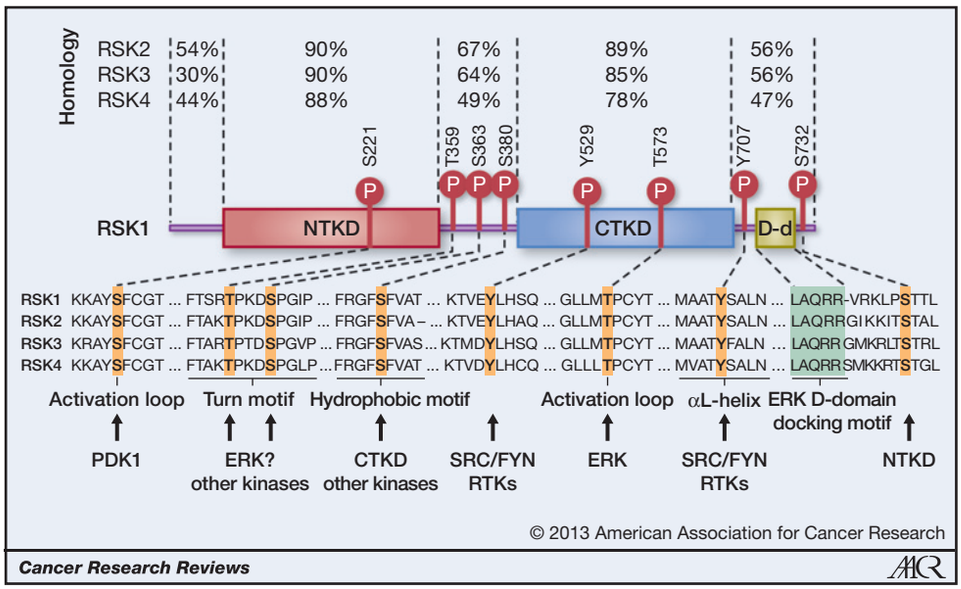

Figure 1. Structural features of RSKs, homology between members and phosphorylating kinases. Top, percentage of amino-acid identity across domains of isoforms as compared with RSK1. Middle, schematic of RSK structure. N-terminal kinase domain (NTKD); C-terminal kinase domain (CTKD); D-domain sequence (D-d). Amino-acid numbering refers to human RSK1. Bottom, conserved phosphorylation sites required for RSK activation and their phosphorylating kinases.

Upon activation, ERK phosphorylates RSK on the Thr-573 and subsequently dissociates (except in the case of RSK3; ref. 15) to translocate to the nucleus (Fig. 1), suggesting that RSKs may act as scaffolds regulating ERK localization (2). Phosphorylation of Thr-573 leads to a fully active CTKD that autophosphorylates RSK on Ser-380. Further phosphorylation of Thr-359 and Ser-363, probably by ERK, recruits the phosphatidylinositide-dependent protein kinase 1 (PDK1) to the hydrophobic motif. PDK1 then phosphorylates Ser-221 in the NTKD, leading to a fully active RSK. Similarly to the CTKD, the NTKD can autophosphorylate Ser-741 located next to the ERK docking site, reducing the affinity of ERK for RSK and providing a negative feedback loop for RSK activation. Finally, dephosphorylation of RSKs by protein phosphatases is thought to mediate their inactivation (4).

Subcellular localization of RSKs

In quiescent cells, RSKs are mainly cytoplasmic (2). Following growth factor stimulation, RSK1 undergoes a transient translocation to the plasma membrane that precedes maximal kinase activity and subsequent relocalization to the nucleus (16). This suggests that membrane translocation may contribute to RSK activation. Similarly, mitogen stimulation triggers RSK2 relocalization to the nucleus, but, upon oxidative stress, RSK2 localizes to stress granules to promote cell survival (17). The ability of RSKs to enter the nucleus is thought to be associated with a putative conserved nuclear localization signal (NLS; ref. 18). Indeed, an RSK2 mutant lacking the C-terminal part of the protein where this putative NLS is positioned is incapable of entering the nucleus, suggesting that this region is indispensible for nuclear translocation (17).

Cellular Processes Regulated by RSK Isoforms

Analysis of RSK1 substrates, together with the use of synthetic peptide libraries, has identified a minimum consensus sequence, Arg/Lys-Xaa-Arg-Xaa-Xaa-pSer/Thr or Arg-Arg-Xaa-pSer/Thr, where RSK1 preferentially phosphorylates serine residues (Table 2; ref. 18). This consensus sequence is also shared by other AGC kinases, suggesting a potential degree of redundancy between these proteins. However, our own data suggest that RSKs are capable of targeting sequences *in vivo* that differ from this consensus motif (3).

Regulation of proliferation by RSKs

RSKs have been involved in the regulation of cell proliferation in various malignancies through indirect (e.g., modulation of transcription factors) or direct effects on the cell-cycle machinery.

Indirect control of cell cycle: transcription factors and RSKs. In addition to regulating the expression of many regulators of transcription, including JUN (19), RSKs posttranslationally target various transcription factors known to regulate the cell cycle. For instance, RSK phosphorylates serum response factor (SRF) to promote the transcription of c-FOS, itself stabilized through phosphorylation by ERK and RSK (20). This promotes cell-cycle progression. Similarly, RSK1 and ERK activation downstream of cKIT signaling in melanoma cells promotes the phosphorylation of microphthalmia-associated transcription factor (MITF), a protein involved in the development of malignant melanoma. Whereas phosphorylation by ERK alone promotes MITF activation, concomitant phosphorylation by ERK and RSK1 induces the ubiquitin-dependent proteolysis of this factor (21). Thus, ERK and RSK1 cooperate to fine-tune the activity of this transcription factor.

In addition, RSK collaborates with the phosphoinositide 3-kinase (PI3K)/AKT pathway in regulating MAD1, a proposed tumor suppressor member of the MYC/MAD/MAX family. Phosphorylation of MAD1 by RSK1 and S6K1 promotes its proteasomal degradation (22), thereby promoting MYC-dependent cell proliferation. In addition, RSK1 targets ETS variant gene 1 (*ETV1*; ref. 23), a transcription factor that participates in the progression of several malignancies. Its phosphorylation by

Table 2. Known substrates of RSKs

Protein	Sp	Site	Sequence
5-HT(2A)	R	S314	AGRRTMQSISNEQKA
AS160	H	S318	EFRSRCSSVTGVQRR
AS160	H	S588	RMRGRLGSVDSFERS
AS160	H	S341	QPRRRHASAPSHVQP
AS160	H	S751	EGRKRTSSTCSNESL
AS160	H	T568	NKAKRSLTSSLENIF
ATF-4	H	S245	TRGSPNRSLPSPGVL
ATF-4	M	S251	NLPSPGGSRGSPRPK
BAD	H	S75	EIRSRHSSYPAGTED
BAD	H	S99	PFRGRSRSAPPNLWA
BAD	M	S112	EIRSRHSSYPAGTEE
BAD	M	S136	PFRGRSRSAPPNLWA
BAD	M	S155	GRELRRMSDEFEGSF
BUB1	X		
C/Ebpβ	R	S105	AKPSKKPSDYGYVSL
C/Ebpβ	M	T217	AKAKAKKTVDKLSDE
C/Ebpβ	R	S273	QKKVEQLSRELSTLR
Capicúa	H	S173	PGKRRTQSLSALPKE
CCTβ	H	S260	GSRVRVDSTAKVAEI
C-FOS	H	S362	AAAHRKGSSSNEPSS
cMyBP-C	R	S288	AGTGRRTSDSHEDAG
CREB	H	S133	EILSRRPSYRKILND
CRHSP24	H	S52	TRRTRTFSATVRASQ
CTNL	M	S22	PAPVRRRSSANYRAY
CTNL	M	S23	APVRRRSSANYRAYA
DAPK1	H	S289	QALSRKASAVNMEKF
DLC1	R	S322	VTRTRSLSTCN KRVG
eEF2K	H	S366	SPRVRTLSGSRPPLL
eIF2A	H	S52	MILLSELSRRRIRSI
eIF4B	H	S422	RERSRTGSESSQTGT
ER-α	H	S167	GGRERLASTNDKGSM
ERP1	X	S335	LDRCRRLSTLRERGS
ERP1	X	T336	DRCRRLSTLRERGSQ
ETV1	H	S191	HRFRRQLSEPCNSFP
ETV1	H	S216	PMYQRQMSEPNIPFP
ETV1	H	S334	PTYQRRGSLQLWQFL
Filamin A	H	S2152	TRRRRAPSVANVGSk
GMF-beta	H	T27	KFRFRKETNNAAIIM
GSK3A	H	S21	SGRARTSSFAEPGGG
GSK3B	H	S9	SGRPRTTSFAESCKP
Hist H3	H	S11	TKQTARKSTGGKAPR
HSP27	H	S78	PAYSRALSRQLSSGV
HSP27	H	S82	RALSRQLSSGVSEIR
Integ β4	H	S1364	PSGSQRPSVSDDTGC
iKBα	H	S32	LLDDRHDSGLDSMKD
iKBα	R	S36	RHDSGLDSMKDEDYE
IkBβ	R	S19	DADEWCDSGLGSLGP
IkBβ	R	S23	WCDSGLGSLGPDAAA
KCNK3	H	S393	GLMKRRSSV
Kv4.3	R	S535	YPSTRSPSLSSHSGL
Kv4.3	R	S569	LPATRLRSMQELSTI

(Continued in the following column)

Table 2. Known substrates of RSKs (Cont'd)

Protein	Sp	Site	Sequence
L1CAM	R	S1154	RSKGGKYSVKDKEDT
LKB1	M	S431	SNKIRRLSACKQQ
MAD1	H	S145	IERIRMDSIGSTVSS
MEF2C	R	S192	ATLHRNVSPGAPQRP
METTL 1	H	S27	YYRQRAHSNPMADHT
MITF	H	S409	KTSSRRSSMSMEETE
MRLC	H	S19	KRPQRATSNVFAMFD
MYT1	X		
NDRG2	H	S332	LSRSRTASLTSAASV
NDRG2	H	S350	RSRSRTLSQSSESGT
NFAT3	H	S281	SGTPSSASPALSRRG
NFAT3	H	S285	SSASPALSRRGSLGE
NFAT3	H	S289	PALSRRGSLGEEGSE
NFAT3	H	S344	QAVALPRSEEPASCN
NFAT3	H	S676	SNGRRKRSPTQSFRF
NHE-1	H	S703	MSRARIGSDPLAYEP
NNOS	R	S847	SYKVRFNSVSSYSDS
NOR1	M	S377	GRRGRLPSKPKSPLQ
NUR77	M	S354	GRRGRLPSKPKQPPD
NURR1	M	S347	GRRGRLPSKPKSPQD
$p27^{Kip1}$	H	S10	NVRVSNGSPSLERMD
$p27^{Kip1}$	H	T198	PGLRRRQT
p65	H	S536	SGDEDFSSIADMDFS
PKR	H	T451	KRTRSKGTLRYMSPE
PLD1	R	T147	PIPTKRHTFRRQNVK
PPP1R3A	H	S46	PQPSRRGSDSSEDIY
PPP1R3A	H	S65	SSGTRRVSFADSFGF
RANBP3	H	S126	VKRERTSSLTQFPPS
RAPTOR	H	S719	PCTPRLRSVSSYGNI
RAPTOR	H	S721	TPRLRSVSSYGNIRA
RAPTOR	H	S722	PRLRSVSSYGNIRAV
RPS6	H	S235	IAKRRRLSSLRASTS
RPS6	H	S236	AKRRRLSSLRASTSK
SRF	H	S103	RGLKRSLSEMEIGMV
TCP-1	H	S260	GSRVRVDSTAKVAEI
TH	H	S71	RFIGRRQSLIEDARK
TIF-1A	H	S649	PVLYMQPSPL
TSC2	H	S1798	GQRKRLISSVEDFTE
VASP	H	T278	LARRRKATQVGEKTP
YB-1	H	S102	NPRKYLRSVGDGETV

NOTE: The targeted sequences are shown, and modified serine or threonine residues are highlighted in red.
Abbreviations: H, human; M, mouse; R, rat; Sp, species; X, *Xenopus*.

PKA alone inhibits its function, whereas phosphorylation by ERK and RSK1 promotes ETV1-mediated transcription. Thus, the balance of activity between the ERK/RSK and PKA pathways regulates the function of ETV1.

Finally, RSK has been reported to phosphorylate cAMP response element-binding protein (CREB; ref. 24). Studies conducted in fibroblasts derived from patients with CLS show that the EGF-induced phosphorylation of CREB and

transcription of c-FOS was impaired in these cells (25). However, both platelet-derived growth factor (PDGF) and insulin-like growth factor (IGF-I) could promote CREB phosphorylation and c-FOS transcription in RSK2 knockout cells, suggesting that other kinases can substitute for RSK2 depending on the signaling context. Indeed, MSK1 and 2 can also phosphorylate CREB downstream of mitogens and stress (18).

Direct effects of RSK on the cell-cycle machinery. In the breast cancer cell line MCF-7, inhibition of RSKs using the pan-inhibitor SL-0101 arrests the cell cycle in G_1 by preventing expression of Cyclin D1 (26). Indeed, expression of Cyclin D1, as well as that of p21, has been shown to be part of the transcriptional program of RSKs (19). With the use of RNA interference, it was further shown that the growth of MCF-7 cells depended on RSK2. Phosphorylation of $p27^{Kip1}$ by RSK1 and RSK2 has also been reported to promote G_1 progression (27), as phosphorylated $p27^{Kip1}$ interacts with the scaffolding protein 14-3-3 in the cytosol, preventing the $p27^{Kip1}$-dependent inactivation of the Cyclin E/A–CDK2 complexes in the nucleus.

In contrast, several reports suggest that RSK4 behaves as a tumor suppressor the expression of which is downregulated in human tumors (28). Indeed, in breast cancer cells, overexpression of RSK4 leads to G_0/G_1 cell-cycle arrest whereas inhibition of RSK4 results in a bypass of stress- and oncogene-induced senescence (28). However, in the lung, our own data suggest that, while undetectable in normal epithelium, RSK4 is overexpressed in more than 50% of primary malignant lesions (3), suggesting that RSK4 may provide tumor cells with a growth advantage. It is interesting to note that RSK4 differs from other RSKs in that its basal activity in cells is high (29), which may provide it with significant biologic activity in the absence of upstream signaling.

Regulation of mRNA translation by RSK

The control of mRNA translation in response to environmental cues heavily involves the mTOR that participates in at least two distinct multiprotein complexes, mTORC1 and mTORC2. The phosphorylation of tuberous sclerosis complex 2 (TSC2) by RSK releases its inhibitory effect on the RAS homolog enriched in the brain (RHEB) to promote mTORC1-mediated translation (30). Furthermore, both RSK1 and RSK2 are required for the phosphorylation of the regulatory associated protein of mTOR (RAPTOR), a constituent of the mTORC1 complex. This results in increased mTORC1 activity (31) and subsequent activation of S6K1 that phosphorylates the eukaryotic initiation factor 4B (eIF4B) to promote translation. Moreover, in activated-mutant BRAF-driven melanoma, RSK and ERK have been shown to phosphorylate LKB1 on sites that impair its interaction with and activation of AMPK, an inhibitor of mTOR signaling, resulting in uncontrolled cell proliferation (32). In addition, RSK1 and RSK2 can directly phosphorylate elF4B, circumventing mTOR to induce a transient cell-growth response that contrasts with the more sustained signaling obtained downstream of S6K1 (33).

Regulation of cell survival by RSK

In addition to its involvement in cell proliferation, phosphorylation of CREB by RSK1 and RSK2 promotes cell survival in neurons by increasing the transcription of prosurvival genes such as members of the B-cell lymphoma protein-2 (BCL2) family (*BCL2*, *BCL-X_L*, and *MCL1*; ref. 34). Moreover, RSKs directly modulate the activity of the BCL2 family members. Indeed, RSK1 and RSK2 phosphorylate the proapoptotic protein, BAD (34), preventing its heterodimerization with, and inhibition of, the prosurvival proteins BCL-X_L and BCL2 while enhancing its binding to 14-3-3. BIM-EL was also shown to be sequentially phosphorylated by ERK and RSK1 or RSK2 in non–small cell lung carcinoma (NSCLC) cells with EGFR-activating mutations (35). This leads to the proteosomal degradation of BIM-EL and increased cell survival. In addition, RSK1 directly inhibits caspase activity: In hepatic stellate cells, the phosphorylation of the CCAAT/enhancer binding protein β (C/EBPβ) by RSK1 creates a functional XEXD caspase substrate/inhibitor box that binds and inhibits caspase 1 and 8, leading to increased cell survival (36). Another family of proteins that have been involved in the regulation of apoptosis are the death-associated protein kinases (DAPK). Following mitogen stimulation, both ERK and RSK phosphorylate DAPK1 on distinct residues. ERK-mediated phosphorylation increases the catalytic activity of DAPK1 while that by RSK1 and RSK2 prevents DAPK1-mediated cell death (37).

Regulation of invasion and metastasis by RSK

RSKs have been extensively involved in cell migration, invasion, and metastasis in a variety of cell systems both *in vitro* and *in vivo* (3, 19, 38–42). In particular RSKs have been shown to be the convergence point in the promigratory effects of various pathways (38). However, the function of individual RSK isoforms appears to vary depending on the cell system studied. Although RSK2 was repeatedly found to mediate the prometastatic functions of ERK (19, 40), the roles of RSK1 and RSK4 are more controversial. Indeed, silencing of RSK1 alone in head and neck carcinoma did not affect cell invasiveness (40), but overexpression of RSK1 in two melanoma cell lines was found to increase cell motility (41). This finding was attributed to the phosphorylation-dependent stabilization of $p27^{Kip1}$ by RSK1 and promoting its cytosolic interaction with, and inhibition of, RHOA. In contrast, using RNA interference against individual RSK isoforms, our laboratory found that silencing RSK1 increased, whereas downregulating RSK2 and RSK4 decreased, lung cancer cell motility (3). Furthermore, RSK1 silencing promoted cell invasion *in vitro* and metastasis *in vivo* (3). Conversely, overexpression of wild-type RSK1 inhibited lung cancer cell migration (3). The fact that a constitutively active mutant of RSK1 further inhibited the ability of lung cancer cells to migrate suggested that these effects were dependent on kinase activity. These discrepancies among cell systems render the assessment of the therapeutic potential of RSK small-molecule inhibitors difficult. Indeed, whereas Doehn and colleagues showed that pan-RSK kinase inhibitors impaired invasiveness in several cell systems (19), our own unpublished data suggest that exposure to a pan-RSK inhibitor, or the combined downregulation of RSK1 and RSK4 using siRNAs, increases lung cancer cell invasion and migration,

respectively, thereby phenocopying the effects of RSK1 silencing. In addition, differences in the potency profiles of the pan-RSK inhibitors, SL-0101 and BI-D1870, for RSK isoforms (43) modified the outcome of RSK inhibition in lung cancer cell invasion and migration, suggesting that the varied degree of RSK isoform inhibition by these compounds may dictate their biologic efficacy (our unpublished data).

One explanation for the discrepancies observed in the role of RSK isoforms between biologic systems could reside in the nature of the upstream signals leading to RSK activation. For instance, RSK1 silencing impairs the motility of wild-type MCF10A cells but accelerates the migration of MCF10A cells overexpressing the receptor tyrosine kinase ERBB2 (44).

Among the substrates of RSKs regulating cell motility and invasiveness is the transcription factor FRA1, which promotes the expression of several prometastatic genes upon phosphorylation by RSKs and ERK2 (19). In addition, RSKs target VASP, and phosphorylation of this protein on T278 by RSK1 reduces its ability to mediate actin polymerization (3). Finally, RSK1 and RSK2 can reduce cell adhesion by phosphorylating integrin β4 to promote cell migration (45).

Involvement of RSKs in signaling feedback loops

The involvement of RSKs in feedback loops was first shown with the RSK2-mediated phosphorylation of the guanine exchange factor for RAS, son of sevenless (SOS; ref. 46), interrupting RAS signaling. This was consistent with findings in *Drosophila melanogaster*, where *Rsk* knockout led to abnormalities typical of an increase in ERK-dependent differentiation. Overexpression of RSK in this model reversed the phenotype by sequestering ERK in the cytoplasm, thereby preventing its transcriptional program (47). Similarly, skeletal muscle cells from *Rsk2* knockout mice exhibit enhanced ERK activation and increased expression of *c-FOS* mRNA in response to insulin as compared with their wild-type counterparts. Interestingly, phosphorylation of AKT was also increased in *Rsk2* knockout cells, suggesting that this feedback may involve a cross-talk between the mitogen-activated protein kinase (MAPK) and AKT pathways.

RSK Inhibitors

Several pan-RSK small-molecule inhibitors exist. These include two competitive inhibitors that target the NTKD (SL-0101 and BI-D1870) and an irreversible inhibitor of the CTKD, fmk. Despite the obvious therapeutic implications of the divergent biologic outputs reported for individual RSK proteins, no RSK isoform-selective inhibitors have been described to date. The existing inhibitors are briefly described in the following section.

NTKD inhibitors

SL-0101 is an acetylated flavonol glycoside from the tropical plant *Forsteronia refracta*. When tested against a panel of 70 kinases, it was shown to target RSK1 and RSK2 in the nanomolar range (IC_{50} for RSK2, 90 nmol/L at 10 μmol/L ATP; ref. 48) while having no significant activity against other tested AGC kinases (19). However, SL-0101 has shown a much higher EC_{50} *in vivo*, a fact that may be of concern to specificity. When used in culture, this compound impaired the growth of MCF-7 breast cancer cells without effect on normal MCF-10A breast epithelial cells, suggesting that it may specifically target cancer cells that rely on RSK for their growth (48). However, we have found that this inhibitor promotes the invasive behavior of NSCLC cells (unpublished data).

The dihydropteridinone BI-D1870 was found to inhibit RSK1 and RSK2 *in vitro* with IC_{50}s ranging from 15 to 30 nmol/L at 100 μmol/L ATP. However, BI-D1870 also significantly inhibits the activity of PLK1, Aurora B, MELK, PIM3, MST2, and GSK3β (49). Furthermore, while the activity of all RSK isoforms is completely abrogated at BI-D1870 concentrations of 10 μmol/L, reduced concentrations of this inhibitor (0.1 μmol/L and 10 nmol/L) showed two-fold lower potency toward RSK3 and RSK4 as toward RSK1 and RSK2 (43). This suggests that structural divergence between the NTKD of RSK isoforms may modulate the activity of this molecule and could, therefore, be exploited for the design of isoform-selective compounds.

CTKD inhibitor

The pyrrolopyrimidine fmk was synthetized as a nanomolar irreversible inhibitor of the CTKD of RSKs (50). Currently, no published *in vitro* specificity profile for this compound against a kinase panel is available. The potency of fmk was later improved using click chemistry to produce fmk-pa. When used on cells, fmk inhibits RSK autophosphorylation (Ser-386) and downstream signaling in response to phorbol ester stimulation. However, it showed no effect on lipopolysaccharide-mediated RSK activation, suggesting that other kinases can substitute for the CTKD and activate RSKs. Indeed, in dendritic cells p38 downstream of MAPKAPK2 has also been shown to activate RSK by substituting for the need for the CTKD (51). This context-dependent requirement for the CTKD may be exploitable therapeutically to limit the scope of RSK inhibition to specific targeted pathways.

Conclusions

RSK proteins show a high degree of sequence homology. Nevertheless, individual RSK isoforms can harbor specific biologic functions. This characteristic is likely due to selective expression patterns, distinct upstream signaling or activation states, differing subcellular localization, and subtle isoform-specific preferences for individual substrates, although most studies have failed to address the latter satisfactorily due to their single isoform-based approach. Although it has been initially informative to assess the common functions of RSKs by generalizing conclusions based on work conducted with single isoforms (commonly RSK1 or RSK2), studying how these proteins differ may highlight novel functions for RSKs and enable the development of more targeted, safer therapeutic strategies. Indeed, our data obtained using pan-RSK inhibitors reveal a complex balance between the sometimes divergent functions of RSK

isoforms that renders the therapeutic efficacy of these compounds hard to predict. Therefore, more selective inhibitors will be required if we intend to translate our understanding of RSK biology into the clinic.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: R. Lara, M.J. Seckl
Writing, review, and/or revision of the manuscript: R. Lara, M.J. Seckl, O.E Pardo

Received December 5, 2012; revised June 4, 2013; accepted June 27, 2013; published OnlineFirst August 22, 2013.

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