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A-kinase anchoring protein 12 regulates the completion of cytokinesis

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ABSTRACT

A-kinase anchoring protein 12 (AKAP12) gene is frequently inactivated in human gastric cancer and in several other cancers due to promoter hypermethylation. However, the biological function of AKAP12 in tumorigenesis remains to be identified. Aneuploidy, a hallmark of cancer cells, is often caused by abnormal cell division. In the present study, AKAP12 was found to localize to the cell periphery during interphase and to the actomyosin contractile ring during cytokinesis. Furthermore, AKAP12 depletion using small interfering RNA increased the number of multinucleated cells, and disrupted the completion of cytokinesis. Interestingly, the inhibition of myosin light chain kinase (MLCK), a key regulator of actomyosin contractility, removed AKAP12 from the cell periphery during interphase and from the contractile ring during cytokinesis, suggesting that AKAP12 might be a downstream effector of MLCK. Our findings implicate AKAP12 in the regulation of cytokinesis progression, and suggest a novel role for AKAP12 tumor suppressor.

A-kinase anchoring proteins (AKAPs) define an expanding group of scaffold proteins that possess a signature binding site for protein kinase A (PKA). In addition to compartmentalizing cAMP signaling, AKAPs assemble multi-protein complexes with other signaling enzymes and also participate in cytoskeletal signaling events [1].

AKAP12, one of the AKAPs [2], is the official gene name of a group of orthologous proteins that includes human gravin and the rodent Src-Suppressed C Kinase Substrate (SSeCKS). Evidence indicates that AKAP12 plays a tumor suppressor role in cancers, for example, human AKAP12/gravin is down-regulated in human prostate and gastric cancers and in myeloid malignancies [3–6], and suppresses cancer cell growth when overexpressed in human gastric cancer and fibrosarcoma cells [4,7]. In addition, aberrant DNA methylation of AKAP12 is believed to be an important aspect of loss of tumor suppressor function in gastric and colon cancers and in myeloid malignancies [4,8,9].

The rodent homolog of AKAP12 (SSeCKS) was originally reported to be down-regulated in response to oncogene activations, by Src or Ras [10], and the re-expression of SSeCKS reinstated Src-induced tumor growth [11,12]. Likewise, the overexpression of SSeCKS, one of the targets down-regulated by v-Jun in mouse fibroblasts, suppressed Jun-induced transformation [13]. Moreover, SSeCKS is thought to inhibit cell cycle progression at the G1-S transition in NIH3T3 cells [14], and has been implicated in prostate cancer metastasis [3,15]. These reports suggest that AKAP12 acts as a tumor suppressor. However, how AKAP12 functions as a tumor suppressor remains to be elucidated.

Cytokinesis, the process where by newly formed daughter cells separate, is the last action in the cell cycle, and it is critical that this process be accurately regulated to ensure the equal division of genetic and biochemical materials [16]. Cytoskeletal structures play many important roles in this process. Microtubules of the mitotic spindle take part in chromosome segregation, and the contractile ring is composed of parallel actin and myosin filaments, which provide the force required for cellular contraction about the midzone [17]. Myosin II is one of the main components of the contractile ring, and thus, the absence of myosin II causes serious cell division defect, e.g., the formation of multinucleated cells. In higher eukaryotes, non-muscle myosin II is activated by the phosphorylation of its regulatory light chain at Ser19/Thr18. Furthermore, myosin light chain (MLC) phosphorylation controls both the assembly of the actomyosin contractile apparatus and its contractility via a process that is regulated by several kinases, including myosin light chain kinase (MLCK), Rho-Kinase (ROCK), and citron kinase [18]. These kinases have been reported to localize to cleavage furrows and to modulate MLC phosphorylation status via different pathways [19,20]. Several other molecules that affect cytokinesis include anillin, LATS1 tumor suppressor, and ARF6 [16,21–23]. These proteins also localize to the contractile rings and/or midbodies of cells during mitosis, and their knock-down using small

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interfering RNA (siRNA) results in multinucleate phenotypes. However, although more research is being conducted on mitosis, relatively little is known about the events involving the regulation of cytokinesis, especially in animal cells.

In this study, we examined AKAP12 localization during cell division in human cells, and found that AKAP12 is involved in the final stages of cytokinesis. In addition, MLCK was identified to be an upstream regulator of AKAP12.

Materials and methods

Materials. ML-9 was purchased from Tocris Bioscience and Calbiochem; Y-27632 and ML-7 were from Calbiochem; thymidine, nocodazole, anti-actin, and anti-α-tubulin antibodies from Sigma; and anti-myosin light chain 2 and anti-phospho-myosin light chain 2 (Thr18/Ser19) from Cell Signaling Technology. SiRNAs were purchased from PROLIGO. Polyclonal anti-AKAP12 antibody was used, as previously described [2,4].

Cell culture. HEK293 (human embryonic kidney), HeLa (human cervical carcinoma) and COS7 (monkey embryonic kidney) cells were purchased from the American Type Culture Collection (Manassas, VA), and maintained in DMEM supplemented with 10% fetal bovine serum (WELGENE Inc., Korea) and gentamycin (10 μg/ml). Human hepatocellular cancer cells (SNU-449) and colon cancer cells (HCT116) were purchased from the Korean Cell Line Bank (Seoul), and grown in RPMI1640 supplemented with 10% FBS and gentamycin. All cell types used were incubated under standard culture condition (20% O_2 and 5% CO_2 at 37 °C).

RNA interference. Cells were seeded and transfected separately with siRNA oligonucleotides specific for AKAP12A or AKAP12B, or with siRNA oligonucleotides common to both, or with an unspecific control using Lipofectamine 2000 (Invitrogen). The following sequences were used; unspecific control sense (5′-UUCUCCGAACG UGUCACGUtt-3′) and antisense (5′-ACGUGACACGUUCGGAGAAtt-3′). AKAP12A sense (5′-CACCAUCAAUGGCGUAGCUtt-3′) and antisense (5′-AGCUACGCCAUUGAUGGUGtt-3′). AKAP12B sense (5′-G UACAGUAGUGCUACUUAAtt-3’) and antisense (5′-UUAAGUAGCA CUACUGUACtt-3′). AKAP12A and B common sense (5′-UCUGCAG AAUCUCCGACUAtt-3′) and antisense (5′-UAGUCGGAGAUUCUGCA GAtt-3′).

Cell synchronization. Cell synchronization was performed as previously described [23] with some modification. Briefly, cells transfected with siRNA were incubated with thymidine (2 mM for 16 h) to achieve S phase block. Thymidine-containing medium was then exchanged for normal growth medium, and cells were further incubated for 5 h. Nocodazole (100 ng/ml) was then added, and cells were cultured for an additional 9 h. After collecting mitotic cells by centrifugation, they were resuspended in normal growth medium and seeded on poly-L-lysine coated coverslips.

Fluorescence microscopy. Cells grown on coverslips in six-well plates were washed twice with PBS, and fixed by incubating them with ice-cold methanol for 10 min at −20 °C. For the synchronization experiment, cells were fixed and permeabilized using formaldehyde solution (2% for 10 min) followed by 0.1% Triton X-100 in PBS for 5 min at room temperature. Cells were then blocked with 1% bovine serum albumin (BSA) in PBS and subsequently incubated with primary and secondary antibodies in PBS containing 1% BSA. The secondary antibodies used were FITC-conjugated anti-mouse IgG and TRITC-conjugated anti-rabbit IgG (Cortex Biochem, San Leandro, CA). Cells were imaged under a confocal fluorescence microscope (model LSM5; Carl Zeiss).

Immunoblotting. Cells were washed with PBS and then lysed in extraction buffer (50 mM Tris–Cl, pH 7.4, 150 mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, 1 mM sodium orthovanadate, 1 mM PMSF, 0.1 mM pepstatin A, 0.1 mM antipain, 0.2 mM leupeptin, and 10 μg/ml aprotinin) on ice for 30 min. Lysates were cleared by centrifugation (13,000 rpm for 20 min). Equal amounts of cell extracts were then resolved by SDS–PAGE, transferred to nitrocellulose membranes, and probed with antibodies. Blots were detected using an ECL system (Amersham).

Results and discussion

AKAP12 localizes to the actomyosin contractile ring during cytokinesis

To investigate the function of AKAP12 in cell division, we first examined its subcellular localization by confocal microscopy in SNU-449 human hepatocellular cancer cells. As shown in Fig. 1A, endogenous AKAP12 seemed to localize at the cell periphery during interphase. In contrast, during metaphase and anaphase, most AKAP12 staining was diffusely localized to cytoplasm. Interestingly, as cells proceeded to telophase/cytokinesis, a substantial proportion of AKAP12 accumulated specifically at the contractile ring. Similar results were also obtained from the experiments in which HEK293 cells were used (Supplementary Fig. 1). Moreover, AKAP12 colocalized with actin during cytokinesis (Fig. 1B), thus supporting the targeting of AKAP12 to the actomyosin contractile ring.

Knock-down of AKAP12 increased multinucleated cell numbers

Failure in cytokinesis can lead to a genome amplification in cancers [24]. The depletion of several cytokinesis-related proteins

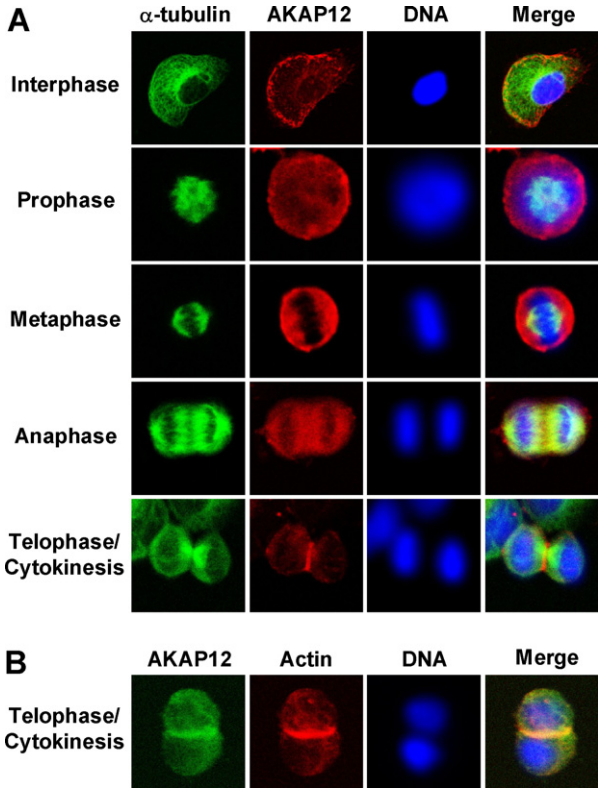

Fig. 1. AKAP12 localizes to the actomyosin contractile ring during cytokinesis. (A) SNU-449 cells fixed with ice-cold methanol were stained sequentially with anti-AKAP12 antibody, a TRITC-conjugated secondary antibody (red), anti-α-tubulin antibody, and an FITC-conjugated secondary antibody (green). DNA (blue) was visualized by DAPI staining. (B) Co-localization of AKAP12 (green) and actin (red) was observed at actomyosin contractile rings during cytokinesis in SNU-449 cells. (For interpretation of color mentioned in this figure the reader is referred to the web version of the article.)

including LATS1 tumor suppressor disrupts cytokinesis and increases multinucleate cell proportions [16,21–23]. Although many proteins involved in cytokinesis have been identified by isolating midbodies from synchronized CHO cells and by RNA interference (RNAi) screening in *Drosophila* cells [25–27], relatively little is known of the process at the molecular level in human cells, and in particular, the involvement of AKAP12 in cytokinesis remains unknown. Thus, we examined the effect of depletion of AKAP12 tumor suppressor on cytokinesis. The depletion of AKAP12 in SNU-449 cells was achieved by siRNA transfection. siRNAs for each isoform (si-AKAP12A or si-AKAP12B) and for both (si-common) were used to selectively disrupt AKAP12 isoforms. Treatment of SNU-449 cells with siRNAs against human AKAP12 isoforms resulted in the depletion of the corresponding proteins (Fig. 2A), whereas treatment with a non-specific control siRNA had no effect. Interestingly, AKAP12 knock-down resulted in a 4- to 5-fold increase in multinucleate cells versus control siRNA-transfected cells (Fig. 2B); although few multinucleate cells were observed. This low frequency may be due to limitations of the transient siRNA system used, and is often observed in vertebrate cells, which have complicated supportive mechanisms that ensure the completion of cytokinesis [16,23]. Nevertheless, the primary defect in AKAP12-depleted cells appeared to be a failure to complete cytokinesis. Moreover, we were unable to find any significant difference between single isoform-depleted or both isoform-depleted SNU-449 cells, which suggests that both isoforms might play a function during cytokinesis.

Depletion of AKAP12 disrupted cytokinesis completion

Next, we investigated whether AKAP12 functions during the late-stage of cytokinesis. In fact, several proteins, such as, ARF6 and dynamin, are known to be required for the completion of cytokinesis [28]. HeLa cells were chosen for cell synchronization because they have been used for similar experiments [23], and basally express AKAP12 isoforms (Fig. 2C). HeLa cells transfected with control or AKAP12 siRNA were synchronized using a thymidine block in the S phase and then subjected to nocodazole arrest at G2/M. Subsequently, mitotic cells were fixed at different times following release from nocodazole arrest. During the first 60–90 min of mitosis, AKAP12-depleted cells appeared to progress through anaphase and telophase in the same manner as controls. However, 2 h after mitotic entry, AKAP12 siRNA-transfected cells were not spread as well and showed a more rounded morphology than control siRNA-transfected cells (Fig. 2D and Table 1), suggesting that AKAP12 is required for the later stages of cytokinesis. Furthermore, 5 h after mitotic entry, an increased number of binucleate cells was observed among AKAP12-depleted cells.

MLCK regulated AKAP12 localization during interphase and cytokinesis

The phosphorylation of myosin light chain (MLC) at Ser19/Thr18 is essential for the activity of myosin II, which mechanically drives cytokinesis. Furthermore, it is well known that MLC is phosphorylated by several kinases, including Rho-Kinase (ROCK) and

Table 1
Depletion of AKAP12 by siRNA inhibited late-stage cytokinesis

Transfection	Cells at cytokinesis	Appositioned and rounded cells (percent of cells at cytokinesis)
2 h after mitotic entry		
Control siRNA	151	49 (32.5%)
AKAP12 siRNA	121	80 (66.1%)
	Total cells	Binucleated cells (percent of total cells)
5 h after mitotic entry		
Control siRNA	494	39 (7.9%)
AKAP12 siRNA	430	82 (19%)

HeLa cells transfected with control or AKAP12 siRNA were synchronized and fixed 2 or 5 h after entry into mitosis. Immunofluorescence microscopy was performed as described in Fig. 2D.

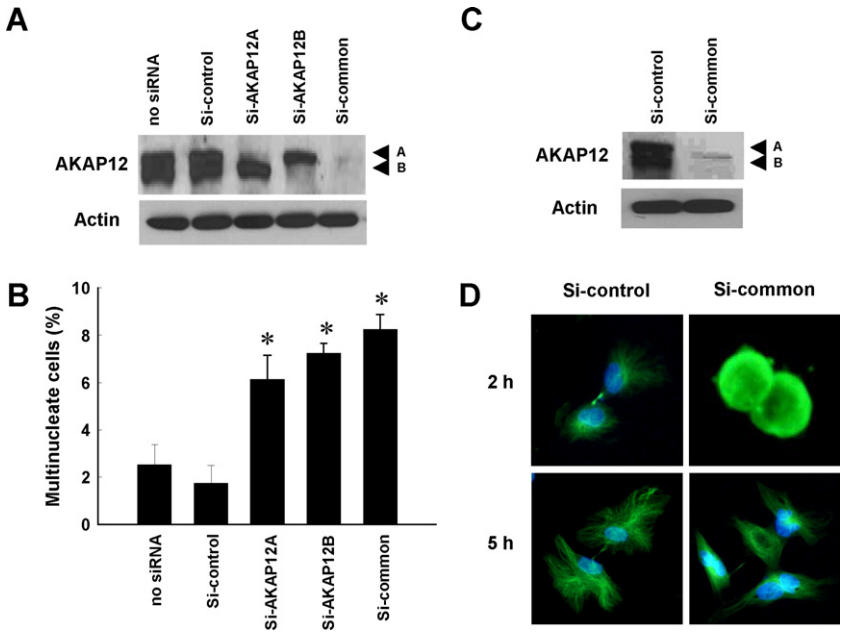

Fig. 2. Depletion of AKAP12 by siRNA increased multinucleate cell proportions by disrupting cytokinesis completion. (A,B) Depletion of AKAP12 isoforms by siRNA in SNU-449 cells. SNU-449 cells were transfected without or with control siRNA or siRNAs against AKAP12 isoforms for 3 days. (A) Western blots of whole cell lysates using anti-AKAP12 and anti-actin antibodies. (B) Percentages of multinucleate cells. Columns, means; bars, SD ($n = 5$). $^{*}P < 0.001$ versus control siRNA-transfected cells. (C,D) Knockdown of AKAP12 prevented cytokinesis completion in HeLa cells. (C) Western blot of the lysates of HeLa cells lysates treated with control siRNA (si-control) or an AKAP12 siRNA (si-common) using anti-AKAP12 and anti-actin antibodies. (D) HeLa cells transfected with control or AKAP12 siRNA were synchronized (described in Materials and methods), and mitotic cells were fixed for 2 or 5 h following release from nocodazole arrest, and examined under an immunofluorescence microscope for α-tubulin and DNA staining.

myosin light chain kinase (MLCK). Thus, we examined whether these kinases could regulate the expression and subcellular localization of AKAP12. SNU-449 cells and HEK293 cells were treated with ML-9 (an MLCK inhibitor) or Y-27632 (a ROCK inhibitor). Western blot analysis showed that AKAP12 expression was not altered in Y-27632 or ML-9-treated HEK293 cells, but that it decreased slightly in ML-9 treated SNU-449 cells (Fig. 3A). Furthermore, in SNU-449 cells, AKAP12 expression and MLC phosphorylation were found to be dose-dependently decreased by ML-9 (Supplementary Fig. 2), and confocal microscopy showed that Y-27632 did not affect AKAP12 localization in either cell type, although it did cause a morphological change in HEK293 cells (Fig. 3B). Notably, ML-9 caused AKAP12 protein to dramatically translocate from the plasma membrane to the cytoplasm during interphase in both cells. Moreover, ML-9 was found to have similar effects in HCT116 and COS7 cells (Supplementary Fig. 3). Furthermore, the localization of AKAP12 protein at the contractile ring during cytokinesis was prevented by ML-9 (Fig. 3C), and a similar result was obtained using ML-7 (another MLCK inhibitor, data not shown), which confirmed that MLCK, but not ROCK, is important for AKAP12 localization during interphase and cytokinesis. It is known that MLCK phosphorylates MLC at the cell periphery, whereas ROCK is responsible for MLC phosphorylation in the center of cells [29]. In view of the peripheral localization of AKAP12 under basal conditions, it may be that MLCK (but not ROCK) regulates the localization of AKAP12 during interphase and cytokinesis. Furthermore, it has been reported that in interphase cells, rodent SSeCKS colocalizes with F-actin in an FAK-dependent manner [30], and that several other AKAPs, including WAVE1, AKAP-Lbc, ezrin and MAP2, also participate in cytoskeletal signaling events [1,31–33]. Additional studies are required to determine how AKAP12 affects the cytoskeletal organization of the contractile ring required for cytokinesis. Thus, we suggest that it worthwhile identifying binding partners of AKAP12, though MLCK and myosin II are obvious candidates.

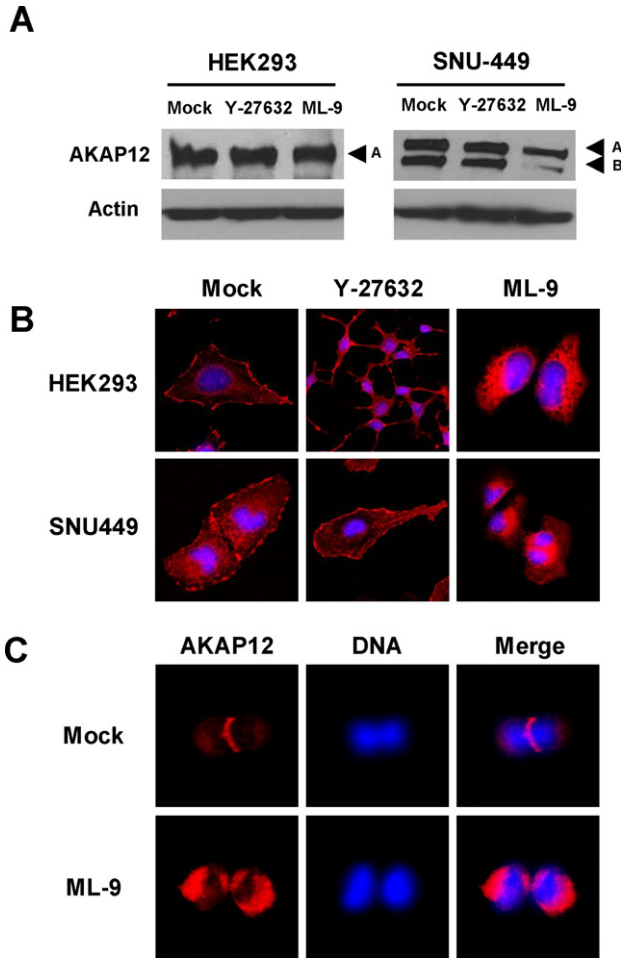

Fig. 3. MLCK regulated AKAP12 localization during interphase and cytokinesis. HEK293 and SNU-449 cells were treated with Y-27632 (15 μM; a ROCK inhibitor) or with ML-9 (30 μM; a MLCK inhibitor) for 24 h, and AKAP12 expressions and its subcellular localizations were determined by western blotting (A) and confocal microscopy (B), respectively. (C) Location changes of AKAP12 during cytokinesis in ML-9-treated SNU-449 cells. Cells treated or not with ML-9 were stained for AKAP12 (red) and DNA (blue). (For interpretation of color mentioned in this figure the reader is referred to the web version of the article.)

Understanding of the molecular mechanism of cytokinesis is important, because cytokinesis failure almost certainly contributes to cancer progression. The major finding of this study is that AKAP12 probably contributes cytokinesis, because AKAP12 depletion was found to cause abnormal cytokinesis and to increase the proportions of multinucleate cells. Our results also support the notion that AKAP12 acts as a potential tumor suppressor.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2008.05.184.

References

[1] W. Wong, J.D. Scott, AKAP signalling complexes: focal points in space and time, Nat. Rev. Mol. Cell Biol. 5 (2004) 959–970.
[2] J.B. Nauert, T.M. Klauck, L.K. Langeberg, J.D. Scott, Gravin an autoantigen recognized by serum from myasthenia gravis patients is a kinase scaffold protein, Curr. Biol. 7 (1997) 52–62.
[3] W. Xia, P. Unger, L. Miller, J. Nelson, I.H. Gelman, The Src-suppressed C kinase substrate, SSeCKS, is a potential metastasis inhibitor in prostate cancer, Cancer Res. 61 (2001) 5644–5651.
[4] M.C. Choi, H.S. Jong, T.Y. Kim, S.H. Song, D.S. Lee, J.W. Lee, N.K. Kim, Y.J. Bang, AKAP12/Gravin is inactivated by epigenetic mechanism in human gastric carcinoma and shows growth suppressor activity, Oncogene 23 (2004) 7095–7103.
[5] J. Boultwood, A. Pellagatti, F. Watkins, L.J. Campbell, N. Esoof, N.C. Cross, H. Eagleton, T.J. Littlewood, C. Fidler, J.S. Wainscoat, Low expression of the putative tumour suppressor gene gravin in chronic myeloid leukaemia, myelodysplastic syndromes and acute myeloid leukaemia, Br. J. Haematol. 126 (2004) 508–511.
[6] M. Yildirim, S. Paydas, K. Tanriverdi, G. Seydaoglu, U. Disel, S. Yavuz, Gravin gene expression in acute leukaemias: clinical importance and review of the literature, Leuk. Lymphoma 48 (2007) 1167–1172.
[7] D.K. Yoon, C.H. Jeong, H.O. Jun, K.H. Chun, J.H. Cha, J.H. Seo, H.Y. Lee, Y.K. Choi, B.J. Ahn, S.K. Lee, K.W. Kim, AKAP12 induces apoptotic cell death in human fibrosarcoma cells by regulating CDKI-cyclin D1 and caspase-3 activity, Cancer Lett. 254 (2007) 111–118.
[8] Y. Mori, K. Cai, Y. Cheng, S. Wang, B. Paun, J.P. Hamilton, Z. Jin, F. Sato, A.T. Berki, T. Kan, T. Ito, C. Mantzur, J.M. Abraham, S.J. Meltzer, A genome-wide search identifies epigenetic silencing of somatostatin, tachykinin-1, and 5 other genes in colon cancer, Gastroenterology 131 (2006) 797–808.
[9] C. Flotho, A. Paulun, C. Batz, C.M. Niemeyer, AKAP12, a gene with tumour suppressor properties, is a target of promoter DNA methylation in childhood myeloid malignancies, Br. J. Haematol. 138 (2007) 644–650.
[10] X. Lin, P.J. Nelson, B. Frankfort, E. Tombler, R. Johnson, I.H. Gelman, Isolation and characterization of a novel mitogenic regulatory gene, 322, which is transcriptionally suppressed in cells transformed by src and ras, Mol. Cell. Biol. 15 (1995) 2754–2762.
[11] X. Lin, I.H. Gelman, Reexpression of the major protein kinase C substrate, SSeCKS, suppresses v-src-induced morphological transformation and tumorigenesis, Cancer Res. 57 (1997) 2304–2312.

[12] Y. Liu, L. Gao, I.H. Gelman, SSeCKS/Gravin/AKAP12 attenuates expression of proliferative and angiogenic genes during suppression of v-Src-induced oncogenesis, BMC Cancer 6 (2006) 105.
[13] S.B. Cohen, A. Waha, I.H. Gelman, P.K. Vogt, Expression of a down-regulated target, SSeCKS, reverses v-Jun-induced transformation of 10T1/2 murine fibroblasts, Oncogene 20 (2001) 141–146.
[14] X. Lin, P. Nelson, I.H. Gelman, SSeCKS, a major protein kinase C substrate with tumor suppressor activity, regulates G(1) → S progression by controlling the expression and cellular compartmentalization of cyclin D, Mol. Cell. Biol. 20 (2000) 7259–7272.
[15] B. Su, Q. Zheng, M.M. Vaughan, Y. Bu, I.H. Gelman, SSeCKS metastasis-suppressing activity in MatLyLu prostate cancer cells correlates with vascular endothelial growth factor inhibition, Cancer Res. 66 (2006) 5599–5607.
[16] A.F. Straight, C.M. Field, T.J. Mitchison, Anillin binds nonmuscle myosin II and regulates the contractile ring, Mol. Biol. Cell 16 (2005) 193–201.
[17] S.O. Dean, S.L. Rogers, N. Stuurman, R.D. Vale, J.A. Spudich, Distinct pathways control recruitment and maintenance of myosin II at the cleavage furrow during cytokinesis, Proc. Natl. Acad. Sci. USA 102 (2005) 13473–13478.
[18] F. Matsumura, G. Totsukawa, Y. Yamakita, S. Yamashiro, Role of myosin light chain phosphorylation in the regulation of cytokinesis, Cell Struct. Funct. 26 (2001) 639–644.
[19] A. Poperechnaya, O. Varlamova, P.J. Lin, J.T. Stull, A.R. Bresnick, Localization and activity of myosin light chain kinase isoforms during the cell cycle, J. Cell Biol. 151 (2000) 697–708.
[20] F. Matsumura, Regulation of myosin II during cytokinesis in higher eukaryotes, Trends Cell Biol. 15 (2005) 371–377.
[21] X. Yang, K. Yu, Y. Hao, D.M. Li, R. Stewart, K.L. Insogna, T. Xu, LATS1 tumour suppressor affects cytokinesis by inhibiting LIMK1, Nat. Cell Biol. 6 (2004) 609–617.
[22] J.K. Schweitzer, C. D'Souza-Schorey, Localization and activation of the ARF6 GTPase during cleavage furrow ingression and cytokinesis, J. Biol. Chem. 277 (2002) 27210–27216.
[23] J.K. Schweitzer, C. D'Souza-Schorey, A requirement for ARF6 during the completion of cytokinesis, Exp. Cell Res. 311 (2005) 74–83.
[24] M. Carmena, W.C. Earnshaw, The cellular geography of aurora kinases, Nat. Rev. Mol. Cell Biol. 4 (2003) 842–854.
[25] A.R. Skop, H. Liu, J. Yates 3rd, B.J. Meyer, R. Heald, Dissection of the mammalian midbody proteome reveals conserved cytokinesis mechanisms, Science 305 (2004) 61–66.
[26] U.S. Eggert, A.A. Kiger, C. Richter, Z.E. Perlman, N. Perrimon, T.J. Mitchison, C.M. Field, Parallel chemical genetic and genome-wide RNAi screens identify cytokinesis inhibitors and targets, PLoS Biol. 2 (2004) e379.
[27] U.S. Eggert, T.J. Mitchison, C.M. Field, Animal cytokinesis: from parts list to mechanisms, Annu. Rev. Biochem. 75 (2006) 543–566.
[28] J.K. Schweitzer, C. D'Souza-Schorey, Finishing the job: cytoskeletal and membrane events bring cytokinesis to an end, Exp. Cell Res. 295 (2004) 1–8.
[29] G. Totsukawa, Y. Yamakita, S. Yamashiro, D.J. Hartshorne, Y. Sasaki, F. Matsumura, Distinct roles of ROCK (Rho-kinase) and MLCK in spatial regulation of MLC phosphorylation for assembly of stress fibers and focal adhesions in 3T3 fibroblasts, J. Cell Biol. 150 (2000) 797–806.
[30] W. Xia, I.H. Gelman, Mitogen-induced FAK-dependent tyrosine phosphorylation of the SSeCKS scaffolding protein, Exp. Cell Res. 277 (2002) 139–151.
[31] D. Diviani, J.D. Scott, AKAP signaling complexes at the cytoskeleton, J. Cell Sci. 114 (2001) 1431–1437.
[32] A. Feliciello, M.E. Gottesman, E.V. Avvedimento, The biological functions of A-kinase anchor proteins, J. Mol. Biol. 308 (2001) 99–114.
[33] D.L. Beene, J.D. Scott, A-kinase anchoring proteins take shape, Curr. Opin. Cell Biol. 19 (2007) 192–198.