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REVIEW

Kank proteins: structure, functions and diseases

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Abstract The Kank family of proteins, Kank1–Kank4, are characterized by their unique structure, coiled-coil motifs in the N-terminal region, and ankyrin-repeats in the C-terminal region, with an additional motif, the KN motif, at the N-terminus. *Kank1* was obtained by positional cloning of a tumor suppressor gene in renal cell carcinoma, while the other members were found by homology search. The family is involved in the regulation of actin polymerization and cell motility through signaling pathways containing PI3K/Akt and/or unidentified modulators/effectors. Their relationship to diseases such as cancer, and to neuronal and developmental disorders, will be an important subject of future study.

Keywords Ankyrin-repeat · Coiled-coil motif · Tumor suppressor gene · Actin stress fiber · Nucleo-cytoplasmic shuttling · Renal cell carcinoma · Signal transduction

Introduction

The human *Kank1* gene (*KANK1*) was found as a candidate tumor suppressor gene for renal cell carcinoma mapped to chromosome 9 at p24, and encodes an ankyrin-repeat domain-containing protein [1]. Kank1 protein was found to form a new family of proteins with three other members, Kank2, Kank3, and Kank4, based on domain and phylogenetic analyses [2]. The names of the family members and their official symbols (all upper-case letters for human genes and upper-case followed by lower-case letters for other or unspecified species) approved by the Human Genome Organisation (HUGO; http://www.hugo-international.org) are summarized in Table 1. *Kank* genes are characterized by conserved ankyrin-repeat and coiled-coil domains, and a motif (KN motif) at the N-terminus containing potential motifs for nuclear localization and export signals (Fig. 1a) [2]. Here, we summarize reports published to date.

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A new gene family

The Kank family are predominantly distributed in the cytoplasm in several human kidney cell lines [2]. In the cytoplasm, Kank1 has a role in controlling the formation of the cytoskeleton by regulating the polymerization of actin [1, 3–6]. In addition, an orthologue of the Kank family in *C. elegans*, VAB-19, was reported to act in epidermal morphogenesis and to play a significant role in the regulation of the actin cytoskeleton [7]. However, Kank1 is not only located in the cytoplasm but also acts as a nucleo-cytoplasmic shuttling protein responsible for the relocalization of β-catenin to the nucleus to activate β-catenin-dependent transcription [8]. The other Kank proteins, Kank2, Kank3, and Kank4, also function in the formation of actin stress fibers. The formation of actin stress fibers is significantly decreased in cells expressing each of the Kank proteins (Fig. 1b) [2]. These results suggest that members of the family have a similar function at least in the formation of actin stress fibers, although the mechanism of this function, especially its signaling cascade, is not fully understood. The Rho family of GTPases, such as Rho, Rac,

Table 1 Human *Kank*-family genes

Gene Previous names
KANK1 (KN motif and ankyrin-repeat domains 1, GeneID: 23189)
KANK; *ANKRD15*; *KIAA0172*; MGC43128; DKFZp451G231
KANK2 (KN motif and ankyrin-repeat domains 2, GeneID: 25959)
SIP; *MXRA3*; *ANKRD25*; FLJ20004; *KIAA1518*; MGC119707; DKFZp434N161
KANK3 (KN motif and ankyrin-repeat domains 3, GeneID: 256949)
ANKRD47; FLJ46061
KANK4 (KN motif and ankyrin-repeat domains 4, GeneID: 163782)
ANKRD38; FLJ10884; dJ1078M7.1; RP5-1155K23.5

Official symbols and names in the Entrez Gene database approved by the HUGO Gene Nomenclature Committee (updated in December 2008) are listed along with the names of the genes (in italics) and cDNA (in roman type) reported previously

and Cdc42, serves as molecular switches in the regulation of a wide variety of signal transduction pathways; in particular, membrane ruffling and stress fiber formation [9–11]. Kank1 can negatively regulate actin polymerization by inhibiting the binding of Rac1 and insulin receptor substrate p53 (IRSp53) [5]. Kank1 is a substrate of Akt and inhibits RhoA's activation mediated by 14-3-3 protein [6] (discussed below). Other Kank proteins were examined by conducting a pull-down assay using a GST-fused RhoA-binding domain of rhotekin (GST-RBD) [2]. The results showed that the amount of active RhoA (GTP-RhoA) was decreased when each family member was overexpressed. Thus, Kank proteins may share a common function in regulating the polymerization of actin through the inhibition of RhoA activity.

Transcriptional variants

There are only 20,000–25,000 protein-encoding genes in the human genome, although there were thought to be many more at the beginning of the Human Genome Project [12]. Since proteins outnumber genes in the human genome, there must be some mechanism to achieve proteomic diversity with a limited number of genes. It has been reported that 40–60% of human genes have alternatively spliced forms, suggesting alternative splicing to be one of the most significant mechanisms for creating the functional complexity of the human genome [13]. This is strengthened by the fact that more than half of human genes have alternative promoters, and alternative promoters are positively correlated with alternative splicing at the genomic level [14].

The *KANK1* gene has several alternative promoters, by which different types of transcripts are generated from the *KANK1* locus (Fig. 2a) [15]. While mRNA from VMRC-RCW cells encodes the KANK1 protein reported previously [1], mRNA from normal kidney tissue encodes a novel form (herein referred to as KANK1-L), which contains an

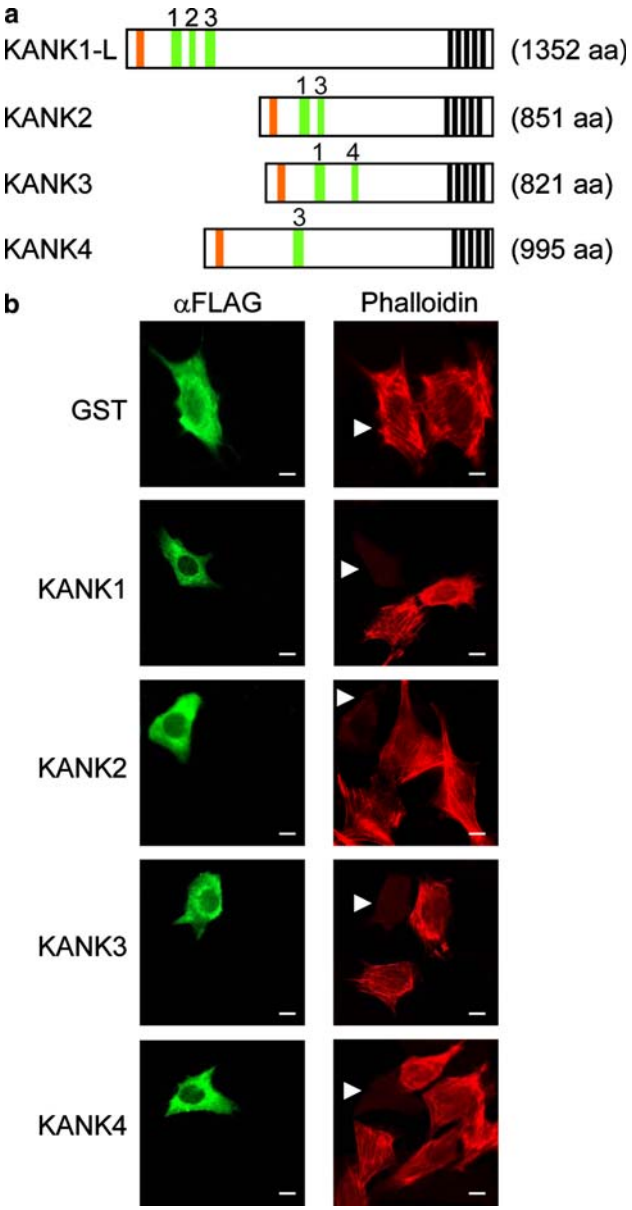

Fig. 1 Structure and functions of human Kank proteins. **a** Schematic structure of human Kank proteins. The human Kank family (KANK1, KANK2, KANK3, and KANK4) share a common structural feature; the Kank N-terminal (KN) motif (*orange boxes*), and coiled-coil and ankyrin-repeat domains from the N- to C-terminal. The coiled-coil domain contains coiled-coil motifs (*green boxes*) and the ankyrin-repeat domain contains ankyrin-repeats (*black boxes*). The variants of the coiled-coil motifs are numbered. **b** Inhibition of the formation of actin stress fibers by overexpression of Kank proteins in NIH3T3 cells. Each of the Kank proteins was detected by immunostaining (*green*). Actin stress fibers were detected with rhodamine-conjugated phalloidin (*red*). Cells expressing FLAG-GST were used as a control. The cells expressing Kank proteins or FLAG-GST are indicated by *arrowheads*. *Scale bar*s 20 μm

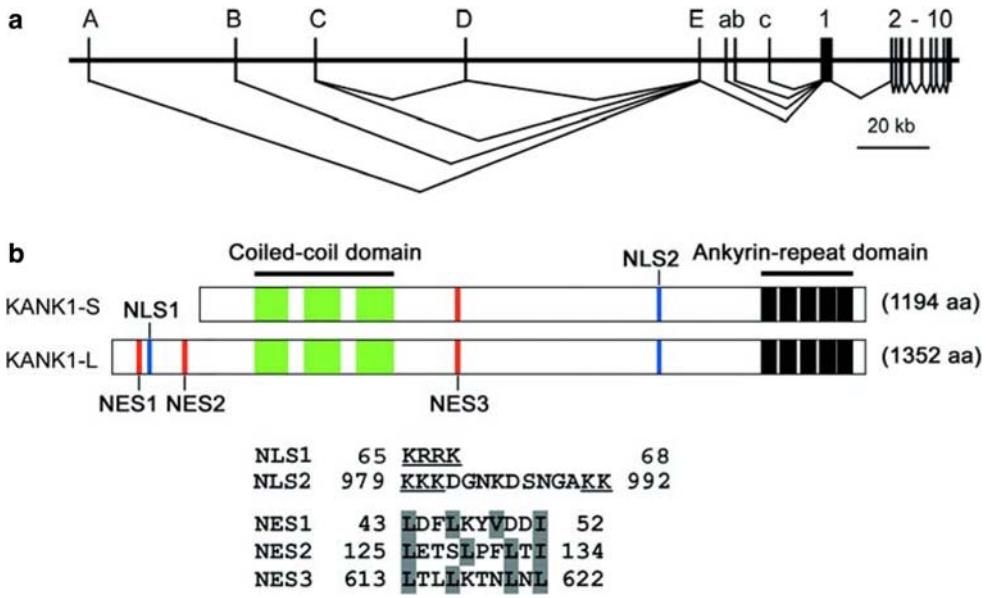

Fig. 2 Structure of the *Kank1* gene and Kank1 protein. **a** The genomic organization of the human *Kank1* gene (*KANK1*). The alternative exons detected in the normal human kidney are labeled in *upper case letters*, whereas the alternative exons detected in VMRC-RCW cells are labeled in *lower case*. **b** Schematic structure of the human KANK1-L and KANK1-S proteins (*KANK1-L* and *KANK1-S*), showing identified domains and motifs. The sequences of NLS (*NLS1* and *NLS2*) and NES (*NES1* to *NES3*) motifs identified in KANK1-L are shown, with conserved sites *underlined* (NLSs) or *shaded* (NESs)

additional N-terminal sequence 158 amino acids long, as well as the entire length of the previously reported KANK1 protein (referred to as KANK1-S) (Fig. 1a). In molecular mass, KANK1-L and KANK1-S generated from cDNA clones correspond to the two bands of endogenous KANK1 present in OS-RC-2 and VMRC-RCW cells, respectively [15]. The tissue distribution patterns of these two types of transcripts differ: the expression of human KANK1-L is somewhat tissue-specific, whereas the expression of human KANK1-S is ubiquitous. The difference in distribution between KANK1-L and total *KANK1* mRNAs suggests that the expression of the isoforms is regulated by distinct mechanisms. Recent research has suggested that multiple first exons are generated by a mechanism of cell- and tissue-specific gene regulation [16]. Since human KANK1-L and KANK1-S have different first exons and their expression is driven by different promoters, it is likely that KANK1-S has a basic level of expression in almost all tissues while KANK1-L is expressed differentially among tissues (predominantly in heart and kidney) [15].

A comprehensive summary of alternative splicing of the *KANK1* gene can be found in the AceView database [17], in which there are at least 26 spliced variants based on 361 GenBank entries from 340 cDNA clones (http://www.ncbi.nlm.nih.gov/IEB/Research/Acembly/).

Domains and motifs

There are five ankyrin-repeats at the C-terminus and three coiled-coil motifs close to the N-terminus of Kank1 (Fig. 1a; see also the Uniprot database: http://www.uniprot.org/ [18]). Nearly 6% of eukaryotic protein sequences contain ankyrin-repeat domains, which consist of several repeats and often function in binding [19]. Each repeat consists of 30–34 amino acid residues with a helix-turn-helix conformation, and strings of such tandem repeats are packed in a nearly linear array to form helix-turn-helix bundles with relatively flexible loops [20]. Kank1 interacts with KIF21A, which is involved in intracellular transportation, through its ankyrin-repeat domain (Kakinuma et al., unpublished data). However, we have not yet identified any specific amino acid position in this domain which is responsible for the interaction. This may be reasonable because recent studies demonstrated that proteins containing ankyrin-repeats do not recognize specific sequences, and interacting residues are discontinuously dispersed throughout both the ankyrin-repeat-containing protein and its partner [20].

Long α-helical coiled-coil proteins are involved in a variety of organizational and regulatory processes in eukaryotic cells, and a number of human diseases seem to be caused by mutations in long proteins with coiled-coil motifs [21]. Kank1 binds specifically to IRSp53 through its coiled-coil domain (Fig. 1a) [5]. In addition, the coiled-coil domain is required to form a homodimer of Kank1 (Kakinuma et al., unpublished data). Moreover, there is a 14-3-3 binding motif located between the coiled-coil motifs 1 and 2, and phosphorylation of the motif by Akt is required for the interaction between Kank1 and 14-3-3 [6].

A number of proteins shuttle continuously back and forth between the nucleus and the cytoplasm, and thus act as key factors in conveying information on nuclear and cytoplasmic activities within the cell [22]. A nuclear export signal (NES) and/or a nuclear localization signal (NLS) are often required for this transportation process. With the help

of bioinformatic tools, three NESs and two NLSs were experimentally identified in KANK1-L (Fig. 2b) [8]. The localization of Kank1 in cells before and after treatment with leptomycin B suggests that the transportation of Kank1 from the nucleus to the cytoplasm is mediated by a CRM1-dependent mechanism. Further analysis revealed that the nuclear import of Kank1 was involved in the activation of β-catenin-dependent transcription. It was also confirmed that Kank1 can bind to β-catenin. Probably, Kank1 has a role in the regulation of the subcellular distribution of β-catenin. It seems that Kank1 has multiple functions in cells and plays different roles in the cytoplasm and the nucleus.

Regulation of actin polymerization

One of the functions to which Kank1 contributes is the regulation of actin polymerization and the inhibition of cell migration (Fig. 3). This function is mediated through two signaling pathways: the regulation of RhoA activity through phosphoinositide 3-kinase (PI3K)/Akt signaling [6], and the regulation of Rac1 signaling by inhibition of IRSp53 [5].

PI3K/Akt signaling is activated by growth factors such as insulin, insulin-like growth factor (IGF), and epidermal growth factor (EGF). These growth factors activate receptor-type tyrosine kinases (RTKs), which leads to the activation of PI3K. Activated PI3K phosphorylates phosphatidylinositol 4,5-bisphosphate (PIP2) to form phosphatidylinositol-3,4,5-trisphosphate (PIP3), while Akt is activated by binding to PIP3 and by phosphorylation with its specific kinase, 3-phosphoinositide-dependent kinase-1 (PDK1). Akt is a serine/threonine kinase and is known to phosphorylate various substrates such as $p27^{Kip1}$ and BAD [23, 24]. The serine residue at 167 on Kank1 (KANK1-S) is phosphorylated through PI3K/Akt signaling after the stimulation of cells with growth factors, and this phosphorylation results in the binding of Kank1 to 14-3-3θ [6]. 14-3-3 is a group of adaptor proteins with seven isoforms in mammals and controls several signal transduction pathways such as those involved in the cell cycle, and in the growth, differentiation, survival, migration, and spreading of cells [25]. On the other hand, Akt activates RhoA through 14-3-3θ [6]. While Kank1 inhibits RhoA activity through the 14-3-3-binding motif, phosphorylation of the serine residue at 167 on Kank1 may competitively inhibit the function of 14-3-3θ. Through this mechanism, Kank1 reduces the formation of actin fibers and thus regulates cell migration.

IRSp53 is an adaptor protein linked to WAVE2 and Rac1/Cdc42, which are activated through RTKs [26, 27]. IRSp53 has a Rac1-binding domain (RBD) and a half-CRIB (Cdc42/Rac1 interactive binding) domain. The association of the RBD with active Rac1 leads to the formation of lamellipodia, while the association of the half-CRIB domain with active Cdc42 leads to the formation of filopodia. Kank1 inhibits the formation of lamellipodia by competing with active Rac1 to bind to the RBD of IRSp53 [5]. However, Kank1 cannot inhibit the active Cdc42-dependent formation of filopodia. Moreover, Kank1 inhibits fibronectin-stimulated cell spreading. This function is partially mediated by IRSp53. However, the interaction between active Rac1 and IRSp53 is not related to this function. The shape of spreading cells can be categorized into three phenotypes; filopodial, smooth-edged, and ruffling [28]. The filopodial phenotype is characterized by filopodia, with dynamic finger-like protrusions. The smooth-edged phenotype is characterized by outer perimeters characteristic of protrusions, such as in lamellipodia, showing sheet-like actin layers revealed by phalloidin staining. The ruffling phenotype is defined by phase-dense wave-like structures. Kank1 inhibits the filopodial and the IRSp53-induced smooth-edged phenotypes in spreading

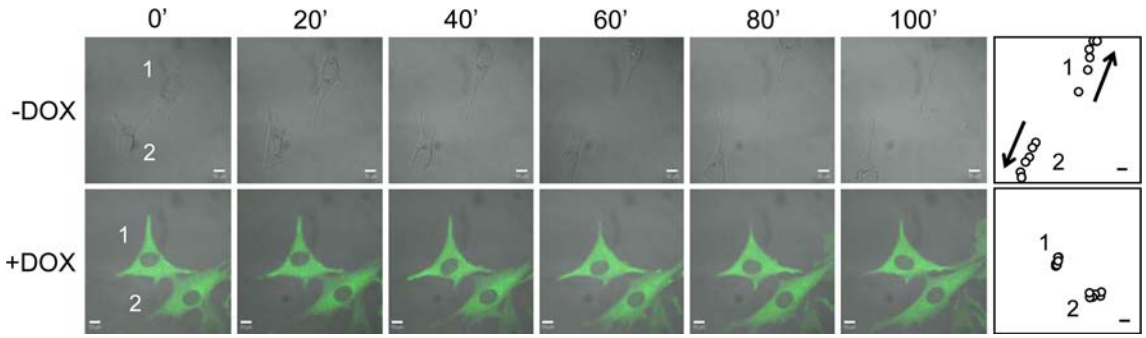

Fig. 3 Inhibition of cell migration by Kank1. Time-lapse confocal laser microscopic images of the tetracycline-inducible NIH3T3 cells are shown in the *six left panels*. The expression of GFP-Kank1 was induced with doxycycline (*+DOX*; *lower panels*), while the control cells were treated with ethanol (*−DOX*; *upper panels*). The images were taken every 20 min. The images are fluorescence micrographs merged with phase-contrast micrographs, with the cells containing GFP-Kank1 shown in *green*. The migration of cells was traced (*rightmost panels*), where the centers of the nuclei are marked. *Solid arrows* indicate the direction of cell movement. *Scale bars* 10 μm

NIH3T3 cells, and delays cell spreading and increases the number of cells with the ruffling phenotype [5]. In addition, Kank1 inhibits both the IRSp53-dependent and independent formation of neurites in mouse neuroblastoma N1E115 cells.

The N-terminal region of IRSp53 includes IMD (IRSp53/MIM homology domain), which is homologous to missing-in-metastasis (MIM) protein and almost entirely occupied by the classic RBD [29]. This domain functions in association with active Rac1, lipids, and actin filaments. A dimerized IMD, when associated with the membrane, can deform the membrane outward, resulting in tubulation of the membrane, which is called the inverse-BAR (Bin/amphiphysin/Rvs) domain-like effect, and IRSp53 binds to actin in the IMD and the C-terminal WH2 domain. Hence, IRSp53 may form membrane tubules and link the membrane to actin, and thereby induce the formation of Rac1-independent filopodia [30]. Overexpression of Kank1 inhibits actin from binding to IRSp53, while Kank1 may inhibit the membrane's linkage to actin by IRSp53, resulting in the inhibition of the filipodial phenotype in spreading NIH3T3 cells and the IRSp53-dependent formation of neurites in N1E115 cells [5].

In conclusion, Kank1 inactivates RhoA through PI3K/Akt signaling, and inhibited the formation of Rac1-dependent-lamellipodia, and possibly Rac1-indepdendent-filopodia, too, through IRSp53, resulting in negative regulation of cell migration. Although this mechanism is not fully understood, Kank1 regulates cell migration via inhibition of actin polymerization. A hypothetical mechanism for this regulation is summarized in Fig. 4. When cells are migrating, RTKs are activated and signals are transmitted through active Rac1 and active RhoA (Fig. 4, upper), resulting in membrane ruffling and the formation of

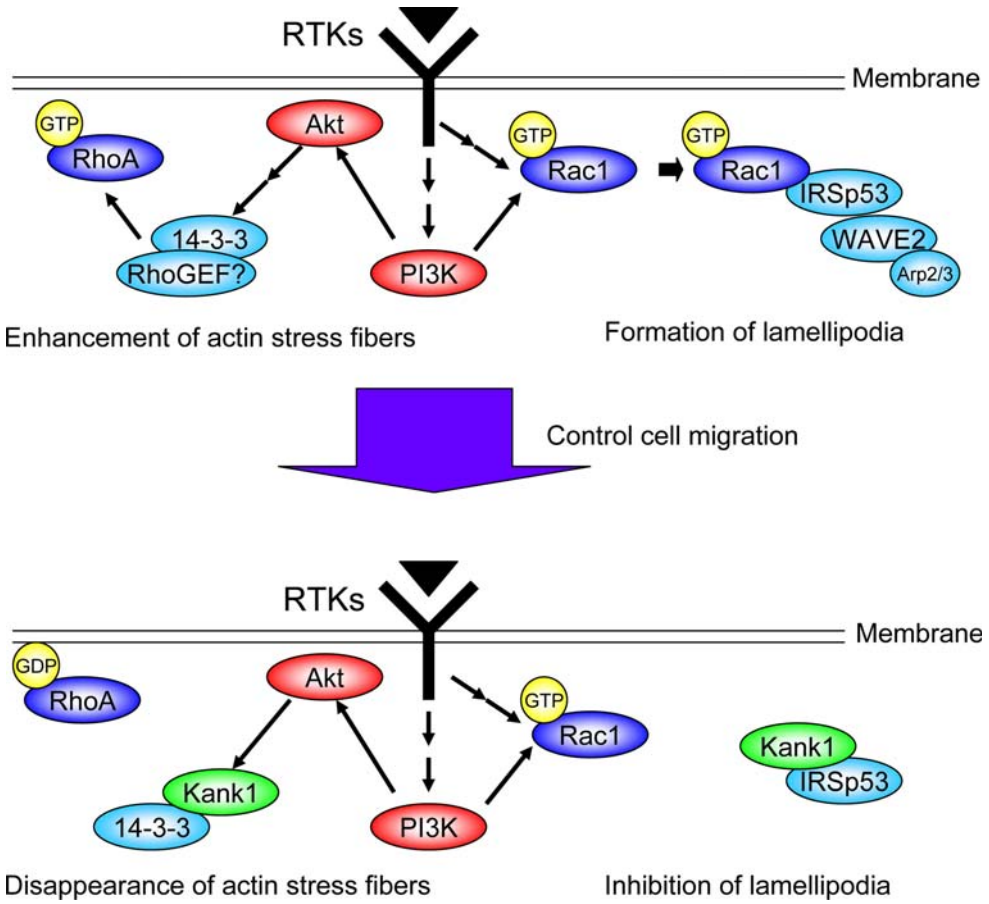

Fig. 4 A model of the role of Kank1. A hypothetical model of the function of Kank1 at the leading edge of cells is shown. When cells are moving, stimulated RTKs activate Rac1, and active Rac1 (GTP-loaded form of Rac1) associates with the IRSp53/WAVE2/Arp2/3 complex, resulting in the formation of lamellipodia (*upper*, *right*). Stimulated RTKs also activate RhoA through a complex containing 14-3-3, and active RhoA (GTP-loaded form of RhoA) enhances the development of actin stress fibers in the leading edge (*upper, left*). When the cells need to control the migration or stop the movement, Kank1 is activated at the leading edge or translocated there, where it associates with IRSp53 and competes with active Rac1 to bind to IRSp53 (*lower*, *right*). Kank1 is also phosphorylated by Akt during this process, secludes 14-3-3 from an activation complex for RhoA, resulting in the inhibition of RhoA, and thereby decreases the formation of actin stress fibers at the leading edge (*lower*, *left*). Both these signal pathways could be inhibited by Kank1

Table 2 *KANK1*-locus related diseases

Disease	Affected locus	Reference
Cancers		
Renal cell carcinoma	9p24	Hatano et al. [32], Roy et al. [4]
Cervical carcinoma	9p	Zimonjic et al. [33]
Bladder cancer	9p	Simon et al. [34]
Hepatocellular carcinoma	9p24	Huang et al. [35]
	9p	Shao et al. [36]
Pancreatic carcinoma	9p24	Heidenblad et al. [37]
Lung cancer	9p24	Sato et al. [38]
	9p	Lo et al. [39]
Acute lymphocytic leukemia	9p24	Heyman et al. [40]
Breast cancer	9p24	An et al. [41]
Other diseases		
Obsessive-compulsive disorder	9p24	Willour et al. [45]
Myeloproliferative disorders	*KANK1*[a]	Kralovics et al. [42]
Cerebral palsy	9p24.3	Lerer et al. [43]
Monosomy 9p syndrome	9p24	Vinci et al. [44]
Renal metanephric adenoma	t(9;15) (p24;q24)	Rakheja et al. [50]

[a] The expression of the *KANK1* gene is down-regulated, although there is no change in the genomic DNA

a leading edge. When the control of cell migration is needed, it is regulated by Kank1 as follows (Fig. 4, lower). First, Kank1 is activated at or translocated to the leading edge of cells. Second, the association between active Rac1 and IRSp53 is inhibited by Kank1, which inhibits the formation of lamellipodia, and activated Kank inactivates RhoA and inhibits the formation of actin stress fibers at the leading edge. Third, the leading edge disappears. Fourth, cells stop moving or change their direction. According to this hypothesis, cell migration could be enhanced in renal cell carcinoma by the disappearance of Kank1 or by a decrease in its amount. Since the enhancement of cell migration is related to metastasis, Kank1 might be related to the malignancy of renal cell carcinoma.

Mutations

The inactivation of genes by mutations and loss of heterozygosity (LOH) often occur in carcinomas. LOH at 9p24, where *KANK1* is located, was detected not only in renal cell carcinomas (RCCs) but also in other diseases described below (Table 2). Mutational inactivation, as revealed by deletions and non-sense mutations, which leads to the generation of truncated or premature proteins, often plays an important role in malignancy. Despite extensive mutational studies of *KANK1* using RCC samples, no deletions or non-sense mutations have been found to date. However, several missense and insertional mutations have been found (Table 3). Single-nucleotide missense mutations, such as G to C at nucleotide 1429 of the Kank1 cDNA sequence (Accession No.: NM_015158), resulting in a change in amino acid from Glu to Gln (position 432), T to G at nucleotide 1525, resulting in the replacement of Ser with Ala (position 464), and G to A at nucleotide 2135, resulting in a change from Arg to His (position 667), were found in 9.3, 6.7, and 10.6% of RCC samples, respectively. Several other sporadic single-nucleotide missense mutations were also observed. A six-nucleotide insertion, adding Ala-Val, was found in a sample. In addition, several silent mutations were detected (Roy et al. unpublished results).

Table 3 Mutations found in the *KANK1* gene

Nucleotide position	Codon change	Amino acid position	Amino acid change	Frequency (%)
765	CAC → CAG[a]	210	His → Gln	1/75 (1.3)
1112	GCG → GTG	326	Ala → Val	1/75 (1.3)
1415	GTA → GGA	427	Val → Gly	1/75 (1.3)
1416-1417	+GCTGTA	427–428	(Ala-Val) insertion	1/75 (1.3)
1429	GAG → CAG[a]	432	Glu → Gln	7/75 (9.3)
1525	TCC → GCC[a]	464	Ser → Ala	5/75 (6.7)
2126	GCA → GTA[a]	664	Ala → Val	2/75 (2.6)
2135	CGT → CAT[a]	667	Arg → His	8/75 (10.6)
Total				19/75 (25.3)

[a] These nucleotide changes can also be found in dbSNP (http://www.ncbi.nlm.nih.gov/SNP/)

To be physiologically functional, proteins need proper conformational changes, and a single missense mutation causing a change in the amino acid sequence can have a significant effect on conformation and often impairs functions. However, we still do not know the significance of these mutations and further study is needed to understand their biological importance.

Pathology

The presence of KANK1 in many tissues was detected at transcriptional and protein levels (see the HUGE database in Kazusa DNA Research Institute for the entry name, KIAA0172: http://www.kazusa.or.jp/huge/) [4, 31]. The cytosolic localization of KANK1 protein is observed widely in renal tubular cells and the glandular cells of the colon, stomach, and other digestive organs. The glandular cells in endocrine glands such as the thyroid, pancreas, adrenal, and prostate also show cytosolic staining of KANK1 protein. Fast growing cells in the epithelium showed prominent

staining of KANK1 protein [4], suggesting a role for KANK1 in tissue development. In a recent study, Kank1 was found at the frontal edge of moving epithelial cells and co-localized with IRSp53 [5]. Kank1 inhibits IRSp53 from binding with active Rac1 and thus inhibits integrin-induced cell spreading and the formation of insulin-induced lamellipodia in NIH3T3 cells. Kank1 negatively regulates the formation of actin stress fibers and cell migration through the inhibition of RhoA activity, which is controlled by the binding of Kank1 to 14-3-3 [6]. A growth inhibitory effect of Kank1 was reported in a previous study [1]. These findings demonstrated that Kank1 can regulate the growth of tissues and thus suggest that Kank1 can also regulate the abnormal growth of cancer cells. In an immunohistochemical analysis of specimens derived from both KANK1-positive and negative RCCs, however, there was no apparent correlation between the expression of KANK1 and clinical stage or histological grade [4], suggesting an as yet unidentified role for Kank1 in tumorigenesis. Kank1 may exert its growth inhibitory effect under more specific conditions; for example, dependent on cell type (e.g., glandular cells), cell shape (e.g., lamellipodium), and the type of physiological stimulation (e.g., EGF). Such information is essential for its pathological characterization.

Diseases

The deletion of chromosome 9p has been reported in a variety of carcinomas and other diseases (Table 2). Deletions of the *KANK1* locus have been reported in RCC [1, 3, 4, 32], cervical carcinoma [33], bladder cancer [34], hepatocellular carcinoma [35, 36], pancreatic carcinoma [37], lung cancer [38, 39] acute lymphocytic leukemia [40], and breast cancer [41]. Therefore, the loss of *KANK1* may be related to carcinogenesis. The reduced expression of *KANK1* was also reported in myeloproliferative disorders (MPDs), where oncogenic Jak2/Stat signaling was activated [42]. MPDs are a heterogeneous group of diseases characterized by increased numbers of nonlymphoid cells or platelets in peripheral blood. A group of researchers in Israel reported the deletion of *KANK1* at 9p24.3 in cerebral palsy, the deletion causing an imprinting-like inheritance [43]. Cerebral palsy is a non-progressive chronic disorder and impairs the control of movement and posture caused by damage to the developing central nervous system. Terminal deletion of 9p is associated with monosomy 9p syndrome (also known as Afi syndrome), which is characterized by mental retardation, gonadal dysgenesis, autistic spectrum disorder, etc. [44]. An obsessive-compulsive disorder is associated with a locus mapped to 9p24 [45].

There are several genes at chromosome 9p24 potentially responsible for behavioral phenotypes, including *FOXD4, DOCK8, and KANK1* [44]. Forkhead transcription factor genes act as key regulators in embryogenesis and tumorigenesis [46], and mutations in these genes cause specific diseases, including speech and language disorders [47]. The *DOCK8* gene was disrupted in two unrelated mental retardation patients [48]. The DOCK-family proteins regulate the cytoskeletal reorganization of the actin filament system [49]. *Kank1* also plays a potential role in cytoskeletal reorganization and neurite outgrowth [5, 6]. So, it is reasonable to speculate that *Kank1* might be involved in cytogenetic behavioral and nervous system-related diseases.

Renal metanephric adenoma, a rare neoplasm, shows the translocation t(9;15)(p24;q24), which leads to inactivation of the second *KANK1* allele, while the first allele is likely to be inactivated by promoter methylation [50].

Therapeutic impact

Cell adhesion is a hallmark of carcinoma cells, and potentiates and promotes cancer-defining biological processes such as growth, survival, migration, and metastasis. Abnormal expression of adhesive markers is a feature shared by most malignancies, and these markers could be a therapeutic target in various diseases including cancer. Kank1 inhibits cell spreading and migration induced by small G-proteins, and knockdown of Kank1 results in enhanced cell migration [5, 6]. Importantly, the *KANK1* locus is deleted not only in various carcinomas but also in a variety of other diseases (discussed above). Thus, the functional domain of KANK1 (the coiled-coil domain, for example) could be a potential therapeutic target based on its inhibitory function in cell spreading and cell migration.

Kank2, Kank3, and Kank4

Several groups reported proteins identical to Kank2. A study of co-expression of human cDNA libraries revealed Kank2, or matrix-remodeling-associated 3 (MXRA3), in association with cell adhesion and matrix remodeling [51]. Using a high-content screening technology, Kank2, or Ankrd25, was identified as a growth regulatory factor [52]. Overexpression of this gene increases growth kinetics in primary fibroblast cells. Using a yeast two-hybrid system, a protein, named steroid receptor coactivators (SRC)-interacting protein (SIP), was found [53]. This protein affects the localization of SRC and regulates its transcriptional activity. The information about the genes and cDNA clones for Kank2 can be found in the following databases: FLJ20004 (a cDNA clone obtained by the NEDO human cDNA sequencing project) (see UniprotKB: http://www.uniprot.org/); KIAA1518 (a gene reported by the Kazusa

DNA Research Institute: http://www.kazusa.or.jp/); MGC119707 (a clone obtained by the I.M.A.G.E. consortium: http://image.hudsonalpha.org/), DKFZp434N161 (a clone obtained by the German Cancer Research Center, Heidelberg, Germany) (see the Entrez database of the National Center for Biotechnology Information: http://www.ncbi.nlm.nih.gov/).

Although there were no reports for the function of Kank3 and Kank4, the information about their cDNA clones can be found at: FLJ46061 and FLJ10884 (see UniprotKB above); dJ1078M7.1 and RP5-1155K23.5 (see the Entrez database shown above).

Conclusions and perspective

Kank-family genes were obtained by positional cloning of the original gene *KANK1* at 9p24, followed by phylogenetic and homology-based searches for other genes having coiled-coil and ankyrin-repeat domains. Among proteins with ankyrin-repeats, four (Kank1–Kank4) were found to have similarly positioned N-terminal coiled-coil motifs and C-terminal ankyrin-repeats, together with an additional motif (KN motif), and form a distinguished family of proteins in mammals. They are characterized by inhibition of the formation of actin stress fibers upon overexpression, and thus play a role in cell motility. Among the family members, Kank1 was investigated by examining interacting proteins, among which 14-3-3 and IRSp53 were found to be functionally related. The signaling from receptors stimulated by insulin, EGF, and others is transduced through PI3K/Akt to actin polymerization, leading to cell motility as a phenotype. This line of investigation will provide information on the role of the family in diseases. Other than renal cell carcinoma, the first *KANK1*-related malignancy found, the *KANK1* gene or locus has been associated with cancer, and neuronal and developmental diseases. The mutations found in *KANK1* will give insight into signaling pathways based on the functional analysis of domains/motifs, interacting proteins, receptors/growth factors, nucleo-cytoplasmic shuttling, and cellular phenotypes.

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