Atlas of Genetics and Cytogenetics
in Oncology and Haematology

OPEN ACCESS JOURNAL

INIST-CNRS

Gene Section
Review

KNL1 (cancer susceptibility candidate 5)

Masato Takimoto, Jean-Loup Huret

Institute for Genetic Medicine, Hokkaido University, Sapporo, Hokkaido, Japan. takimoto@igm.hokudai.ac.jp (MT); Genetics, Dept Medical Information, University of Poitiers, CHU Poitiers Hospital, F-86021 Poitiers, France. jean-loup.huret@chu-poitiers.fr (JLH)

Published in Atlas Database: October 2016

Online updated version : http://AtlasGeneticsOncology.org/Genes/AF15q14ID318.html

Printable original version : http://documents.irevues.inist.fr/bitstream/handle/2042/68251/10-2016-AF15q14ID318.pdf

DOI: 10.4267/2042/68251

This article is an update of :
Takimoto M. CASC5 (cancer susceptibility candidate 5). Atlas Genet Cytogenet Oncol Haematol 2013;17(1)
Takimoto M. CASC5 (Cancer Sensitibity Candidate 5). Atlas Genet Cytogenet Oncol Haematol 2007;11(1)
Huret JL, Charrin C. AF15q14 (ALL1 fused gene from 15q14). Atlas Genet Cytogenet Oncol Haematol 2000;4(2)

Abstract

Review on KNL1, with data on DNA, on the protein encoded, and where the gene is implicated.

Keywords
KNL1

Identity

Other names: CT29, CASC5, AF15Q14, D40, PPP1R5, hKNL-1, hSpc105, AF15q14, KIAA1570

HGNC (Hugo): KNL1

Location: 15q15.1

DNA/RNA

Note
Whole genomic size is about 70 kbp, but consists of 27 exons.

Transcription

KNL1 mRNA expression is dominant in normal human testis and slight expression are observed in other organs, such as placenta. Analysis on cancer cell lines, such as HeLa, gave single band with 8.5 kb. There is another alternative splicing site at the 5' side of this gene that generates a short exon with 78 bp in cDNA. There are potential other alternative splicing at cancer cell lines.
Analysis on testis mRNA shows two bands with size of approximately 6 and 8,5 kb which are probably derived from the two isoforms.

Protein

Description

Encodes 1833 amino acids and 2342 amino acids.
The KLN1 protein contains: conserved motifs, which are the following: a (S/G)ILK motif (aa 25-28), a RRVSF motif (aa 57-61), and, for BUB3 recognition, MELT repeats (aa 140-161, 308-329, 474-494, 562-582, 750-769, 859-882, 902-924, 940-962, 1025-1044, 1073-1096, 1114-1136, 1152-1174). The Bubs recognition KI motifs KI(D/N)XXXF(L/I)XXLK, are KIDTTSFLANLK (aa 202-213) for BUB1, and KIDFNDFIKRLK (aa 238-249) for BUB1B: (BUBR1); a nuclear localization signal (aa 1789-1803); a coiled coil region (aa 1942-2133) and the ZWINT (Zwint-1) binding region (aa 1834 or 19811 for a smaller region -2108); and RWD repeats (aa 2109- 2353) With the NSL1 (hMis14)-binding region (aa 2109-2316), according to Kiyomitsu et al., 2011, VEGA checking, and SwissProt.

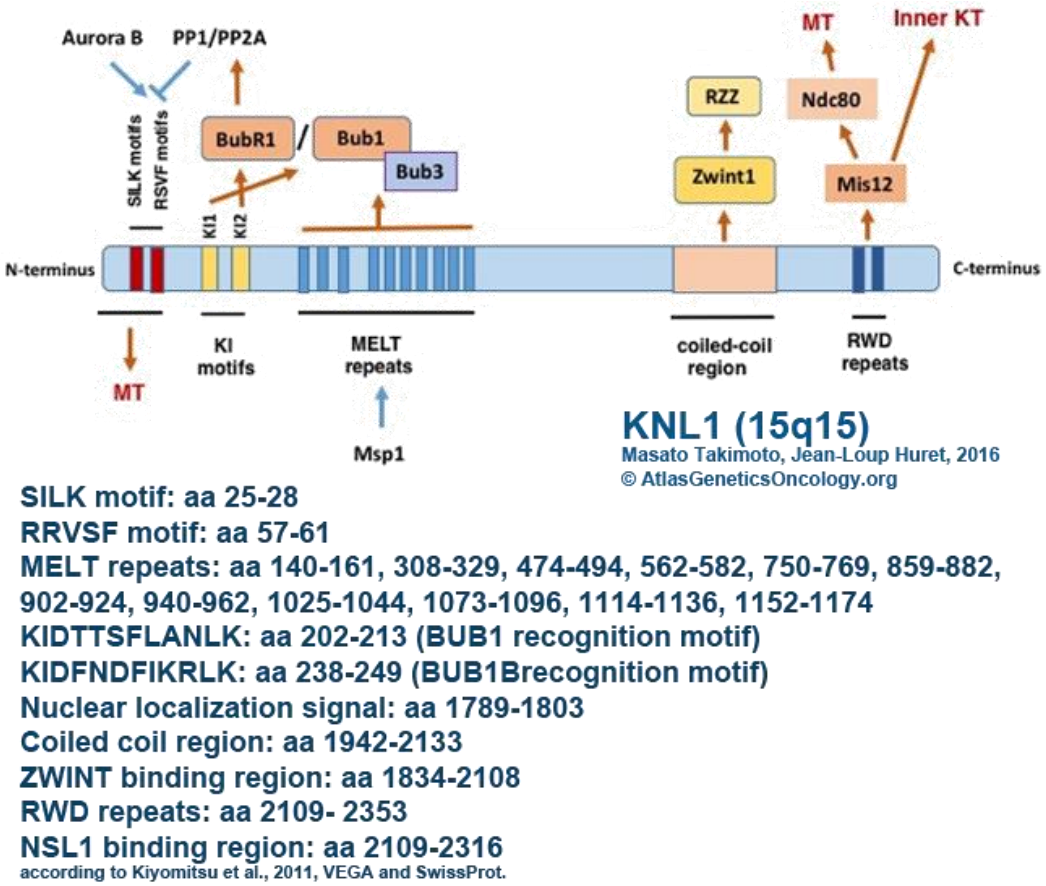

Figure 1. Domain structure of KNL1 protein. Brown arrows indicate protein interactions, and blue arrows and line with T-shape indicate phosphorylation and dephosphorylation, respectively. MT :microtubules. KT : kinetochore.

Expression

KNL1 protein expressions with molecular weight of approximately 250 kDa and 300 kDa are observed in human testicular germ cells and cancer cell lines.

Localisation

In germ cell of testis, significant high expressions of KNL1 protein are observed in nucleus of spermatocytes and slightly in spermatogonia. It is noteworthy that round spermatids express significantly high KNL1 protein in their pre-acrosome. As KNL1 protein has no hydrophobic signal peptide in its amino terminus, it probably localizes outer surface of pre-acrosome membrane of spermatids inside of the cells..
In mitosis KNL1 protein is localized in kinetochore in a human cancer cell line.

Function

KNL1 is a large of kinetochore protein, constituting KMN (KNL1/ MIS12 complex/ NDC80 complex) network. KMN network is the central hub of outer kinetochore, not only connecting mitotic chromosomes and spindles but also coordinating microtubule-binding, chromosome congression and spindle assembly checkpoint (SAC) signaling. There are two microtubule binding activity in KMN network, one in NDC80 and the other in KNL1. KNL1 protein binds directly microtubule through its far N-terminal region, consisting of 68 amino acids, in vitro, and indirectly through the interactions with MIS12 and NDC80 complexes at its C-terminus. KNL1 depletion affects metaphase chromosome congression. In C. elegans,
The depletion preclude metaphase chromosomal congression, while in yeast, Drosophila and human cells, the depletions show partial alignment phenotypes, in which some chromosomes congress to the equator but many chromosomes remain stranded near the spindle poles. In the N-terminus of KNL1 protein, AURKB (Aurora kinase B) phosphorylates SILK and RVSF motifs, and then disrupts the interaction between KNL1 and protein phosphatase I (PP1), which also binds the motifs. KNL1 augments Aurora B kinase activity that phosphorylates outer kinetochore protein, such as NDC80, resulting in reduction its microtubule-binding activity. Protein phosphatase 2A (PP2A) is recruited by BUB1B (BubR1), one of SAC protein. Both PP1 and PPA2 are suggested to counteract Aurora B kinase activity. Especially, PP1 was shown to stabilize microtubule attachments to kinetochores probably through KNL1, while Aurora B kinase destabilizes microtubule-binding by NDC80

phosphorylation as described. The destabilized binding of KNL1 to microtubules is presumably important for correcting and eliminating erroneous kinetochore-microtubule attachment during SAC.
It is suggested that the bindings with microtubule and with PP1 also play roles in SAC silencing. Although their binding sites are in close proximity, one of their bindings do not affect the other, and they contribute independently to the silencing of SAC. Two KI motifs, KI1 and KI2, localized in the N-terminus, bind SAC protein, BUB1 and BUB1B, respectively, through tetratricopeptide repeats (TPRs) of the proteins, resulting into folding the motifs into short alpha-helices. Although BUB1 fragment with mutation in KI-binding domain was not able to bind to KNL1 in vitro, BUB1 and BUB1B mutants with KI-binding sites were able to attach to kinetochore. Mutations in the BUB3-binding domain (BUB3-BD) in BUB1 and BUB1B prevent kinetochore localization of the SAC protein. BUB1 fragment consisting only of N-terminus with TPRs does not localize to kinetochore and longer fragments that accommodate BUB3-BD did. Those results suggest that BUB3-BD of BUB1 and BUB1B, rather than TPRs, is critical for their recruitment to kinetochore and that the interaction between TPRs of Bub proteins and KI-motif of KNL1 might play a subsidiary role in the localization of BUB1 and BUB1B to kinetochore. In response to SAC signal, the first step of this response is phosphorylation of MELT motifs of KNL1, located in the N-terminal and central region, by Mps1 kinase, and then the phosphorylated MELT motifs bind BUB3/BUB1complex, mediating SAC signaling. KNL1- BUB3-BUB1(KBB) complex binds MXD1 (MAD1)/ MAD2L1 (MAD2) complex, and then, together with MAD1 phosphorylation by Msp1, the binding leads to CDC20/MAD2 formation, an essential part of Mitotic Checkpoint Complex (MCC) that inhibits Anaphase Promoting Complex/Cyclosome (APC/C).
Recently, it suggested that there are two pathways for recruiting MAD1-MAD2 that results in SAC activation. One is the pathway through KBB, as described above, the other is KNTC1 (ROD)-RW10-ZWILCH, (RZZ) complex, which interacts with KNL1 through ZWINT (Zwint-1 protein). The former is required for SAC activation when kinetochores are misaligned but is not essential when kinetochores are unattached from microtubules. The latter binds SPDL1 (Spindly protein) and MAD1-MAD2, and causes the anaphase-onset delay in response to unattached kinetochore independently of the former.
It was suggested that the binding of KNL1 with microtubules and with PP1 contribute to silencing of SAC, in which motor protein dynein, moving along on microtubules, is suggested to work to strip MAD1-MAD2. The C-terminal region of KNL1 interacts with MIS12 and Zwint-1 protein, through RWD motif and coiled-coiled region, respectively. The binding with the former plays role in connecting inner kinetochore with KNL1 and the latter mediates the interaction of KNL1 with RZZ complex which works in SAC regulation as described above.

Implicated in

Leukemia

A small subset of leukemia with a t(11;15)(q23;q14) has been described for long and has often be referred as: t(11;15)(q23;q15) MLL/AF15q14. KMT2A, (previous symbol: mixed leukemia gene (MLL)) is translocated with KNL1 (previous symbol CASC5, originally described as AF15q14), which makes research of published cases often arduous.

t(11;15)(q23;q15) and/or KMT2A/KNL1 t(11;15)(q23;q14-15)

Data on 16 cases with a t(11;15)(q23;q14-15), according to (Yang et al., 2014) are the following: there was 2 of myelodysplastic syndrome (MDS) cases, 10 acute myeloid leukemia (AML) cases (2 M1, 4 M2, 3 M4, and 1 NOS), and 4 acute lymphoblastic leukemia (ALL) cases. Mean age of the patients was 20.6 years (range 1-54); there were 11 males and 5 females. Abnormalities of chromosome 3 were seen in 10 out of 16 cases. Out of 8 patients for whom clinical data were available, only 3 are in complete remission, whereas 5 patients died with a mean survival period of 10.4 months.

t(11;15)(q23;q15) and KMT2A/KNL1

Of 7 cases with a t(11;15)(q23;q15) and KMT2A/KNL1 hybrid gene and fusion protein ((Chinwalla et al., 2003; Kuefer et al., 2003; Meyer et al., 2006; Yang et al., 2014). Diagnosis was: therapy related MDS (t-MDS) in 2 cases, AML in 4 cases (1 M2, 2 AML-M4, 1 AML-NOS), and 1 de novo T-ALL. Sex ratio was 5M:1F; There was 3 children and 4 adult patients. Of three cases with data on survival, patients died at: 8 mths, 8 mths, and 22 mths. Of four cases with documented karyotypes: the karyotype was a complex karyotype with markers in two cases, abnormalities of chromosome 3 were seen in three cases, +21 in two cases. KMT2A (MLL) exon 8, 9, or 10 were fused to exon 10, 11 or 12 of KNL1, the fusion protein contains the 1362 or 1418 first aa from MLL with aa 1796, 1818 or 1819 from KNL1 (according to authors and/or VEGA).

A t(3;15;p14;q15) KNL1/ ADAMTS9-AS2 is mentioned, without further data in the ChiTARS database (Gorohovski et al., 2016) as a chimeric EST (dbEST Id:12413828; accession BQ375909).

Lung cancer

In one study on primary lung cancer, KNL1 mRNA expression was observed in more than 40% of the cases, which is the highest among all the different types of cancers examined. The study also revealed

that clinicopathological findings correlates with KNL1 expression. KNL1 mRNA expression is more frequent in the tumors with low differentiation than the ones with moderate and high differentiation. Further, the tumors derived from smoker express higher incidence of KNL1 mRNA than the ones from non-smoker.

Spermatogenesis

KNL1 mRNA was highly expressed in normal testis. As KNL1 protein expressions were observed in spermatogonia and spermatocytes in seminiferous tube of human testes, this protein may also play a role in cell division as a kinetochore protein in meiotic cells. It is noteworthy that KNL1 protein is also significantly expressed in pre-acrosome of spermatids, especially from its early stage, suggesting that KNL1 might be playing important role in the formation of acrosome, an essential organelle for fertilization. KNL1 expressions in testes of the patients with infertility were significantly lower than normal ones.

Microcephaly

Disease

Autosomal recessive primary microcephaly (MCPH) is a very rare neuro-developmental disorder with brain size reduction, no structural malformation of the brain at birth, mild-to moderate mental retardation and absence of other neurological or somatic disease.

There are 12 genetic loci responsible for MCPH, and it was suggested that one of the responsible genetic locus, MCPH4, resides on chromosome 15. Subsequently, the study on the patients with MCPH4 of consanguineous families in Morocco revealed that a homozygous missense mutation was observed in exon 18 of KNL1 gene.

This point mutation caused skipping this exon in splicing reaction in the mRNA maturation of KNL1, suggesting that the affected nucleotide is a part of Exonic splicing enhancers.

The mutation resulted in frame-shift and truncation of KNL1 protein.

As RWD repeats at near its carboxy terminus were deleted by this mutation, the truncated protein is no longer able to bind to Mis12, leading to the defected recruitment of KNL1 protein to kinetochore. One of the patients in the Morroco families has cryptorchidism in addition to microcephaly. The results of studies on MCH4 is a direct demonstration that KNL1 is essential to cell division in vivo.

References

Blencowe BJ. Exonic splicing enhancers: mechanism of action, diversity and role in human genetic diseases. Trends Biochem Sci. 2000 Mar;25(3):106-10

Bollen M. Kinetochore signalling: the KIss that MELTs Knl1. Curr Biol. 2014 Jan 20;24(2):R68-70

Caldas GV, DeLuca JG. KNL1: bringing order to the kinetochore. Chromosoma. 2014 Jun;123(3):169-81

Cheeseman IM, Chappie JS, Wilson-Kubalek EM, Desai A. The conserved KMN network constitutes the core microtubule-binding site of the kinetochore. Cell. 2006 Dec 1;127(5):983-97

Chinwalla V, Chien A, Odero M, Neilly MB, Zeleznik-Le NJ, Rowley JD. A t(11;15) fuses MLL to two different genes, AF15q14 and a novel gene MPFYVE on chromosome 15. Oncogene. 2003 Mar 6;22(9):1400-10

Espeut J, Cheerambathur DK, Krenning L, Oegema K, Desai A. Microtubule binding by KNL-1 contributes to spindle checkpoint silencing at the kinetochore. J Cell Biol. 2012 Feb 20;196(4):469-82

Faesen AC, Musacchio A. The (phospho) needle in the (MELT) Haystack. Mol Cell. 2015 Mar 5;57(5):765-6

Genin A, Desir J, Lambert N, Biervliet M, Van Der Aa N, Pierquin G, Killian A, Tosi M, Urbina M, Lefort A, Libert F, Pirson I, Abramowicz M. Kinetochore KMN network gene CASC5 mutated in primary microcephaly. Hum Mol Genet. 2012 Dec 15;21(24):5306-17

Gorohovski A, Tagore S, Palande V, Malka A, Raviv-Shay D, Frenkel-Morgenstern M. ChiTaRS-3.1-the enhanced chimeric transcripts and RNA-seq database matched with protein-protein interactions. Nucleic Acids Res. 2017 Jan 4;45(D1):D790-D795

Hayette S, Tigaud I, Vanier A, Martel S, Corbo L, Charrin C, Beillard E, Deleage G, Magaud JP, Rimokh R. AF15q14, a novel partner gene fused to the MLL gene in an acute myeloid leukaemia with a t(11;15)(q23;q14). Oncogene. 2000 Sep 7;19(38):4446-50

Jamieson CR, Govaerts C, Abramowicz MJ. Primary autosomal recessive microcephaly: homozygosity mapping of MCPH4 to chromosome 15. Am J Hum Genet. 1999 Nov;65(5):1465-9

Kerres A, Vietmeier-Decker C, Ortiz J, Karig I, Beuter C, Hegemann J, Lechner J, Fleig U. The fission yeast kinetochore component Spc7 associates with the EB1 family member Mal3 and is required for kinetochore-spindle association. Mol Biol Cell. 2004 Dec;15(12):5255-67

Kiyomitsu T, Murakami H, Yanagida M. Protein interaction domain mapping of human kinetochore protein Blinkin reveals a consensus motif for binding of spindle assembly checkpoint proteins Bub1 and BubR1. Mol Cell Biol. 2011 Mar;31(5):998-1011

Kops GJ, Saurin AT, Meraldi P. Finding the middle ground: how kinetochores power chromosome congression. Cell Mol Life Sci. 2010 Jul;67(13):2145-61

Krenn V, Wehenkel A, Li X, Santaguida S, Musacchio A. Structural analysis reveals features of the spindle checkpoint kinase Bub1-kinetochore subunit Knl1 interaction. J Cell Biol. 2012 Feb 20;196(4):451-67

Kuefer MU, Chinwalla V, Zeleznik-Le NJ, Behm FG, Naeve CW, Rakestraw KM, Mukatira ST, Raimondi SC, Morris

SW. Characterization of the MLL partner gene AF15q14 involved in t(11;15)(q23;q14). Oncogene. 2003 Mar 6;22(9):1418-24

Liu D, Vleugel M, Backer CB, Hori T, Fukagawa T, Cheeseman IM, Lampson MA. Regulated targeting of protein phosphatase 1 to the outer kinetochore by KNL1 opposes Aurora B kinase. J Cell Biol. 2010 Mar 22;188(6):809-20

Meyer C, Schneider B, Jakob S, Strehl S, Attarbaschi A, Schnittger S, Schoch C, Jansen MW, van Dongen JJ, den Boer ML, Pieters R, Ennas MG, Angelucci E, Koehl U, Greil J, Griesinger F, Zur Stadt U, Eckert C, Szczepański T, Niggli FK, Schäfer BW, Kempski H, Brady HJ, Zuna J, Trka J, Nigro LL, Biondi A, Delabesse E, Macintyre E, Stanulla M, Schrappe M, Haas OA, Burmeister T, Dingermann T, Klingebiel T, Marschalek R. The MLL recombinome of acute leukemias. Leukemia. 2006 May;20(5):777-84

Obuse C, Iwasaki O, Kiyomitsu T, Goshima G, Toyoda Y, Yanagida M. A conserved Mis12 centromere complex is linked to heterochromatic HP1 and outer kinetochore protein Zwint-1. Nat Cell Biol. 2004 Nov;6(11):1135-41

Przewloka MR, Zhang W, Costa P, Archambault V, D'Avino PP, Lilley KS, Laue ED, McAinsh AD, Glover DM. Molecular analysis of core kinetochore composition and assembly in Drosophila melanogaster. PLoS One. 2007 May 30;2(5):e478

Sasao T, Itoh N, Takano H, Watanabe S, Wei G, Tsukamoto T, Kuzumaki N, Takimoto M. The protein encoded by cancer/testis gene D40/AF15q14 is localized in spermatocytes, acrosomes of spermatids and ejaculated spermatozoa. Reproduction. 2004 Dec;128(6):709-16

Sasao T, Takimoto M, Itoh N, Maeda T, Tanaka T, Masumori N, Tsukamoto T. Testis cancer gene D40 expression and its relationship with clinicopathological features in infertile men. Int J Urol. 2011 Feb;18(2):175-9

Silió V, McAinsh AD, Millar JB. KNL1-Bubs and RZZ

Provide Two Separable Pathways for Checkpoint Activation at Human Kinetochores. Dev Cell. 2015 Dec 7;35(5):600-13

Simpson AJ, Caballero OL, Jungbluth A, Chen YT, Old LJ. Cancer/testis antigens, gametogenesis and cancer. Nat Rev Cancer. 2005 Aug;5(8):615-25

Takimoto M, Wei G, Dosaka-Akita H, Mao P, Kondo S, Sakuragi N, Chiba I, Miura T, Itoh N, Sasao T, Koya RC, Tsukamoto T, Fujimoto S, Katoh H, Kuzumaki N. Frequent expression of new cancer/testis gene D40/AF15q14 in lung cancers of smokers. Br J Cancer. 2002 Jun 5;86(11):1757-62

Taylor SS, Hussein D, Wang Y, Elderkin S, Morrow CJ. Kinetochore localisation and phosphorylation of the mitotic checkpoint components Bub1 and BubR1 are differentially regulated by spindle events in human cells. J Cell Sci. 2001 Dec;114(Pt 24):4385-95

Wei G, Takimoto M, Yoshida I, Mao PZ, Koya RC, Miura T, Kuzumaki N. Chromosomal assignment of a novel human gene D40. Nucleic Acids Symp Ser. 1999;(42):71-2

Welburn JP, Vleugel M, Liu D, Yates JR 3rd, Lampson MA, Fukagawa T, Cheeseman IM. Aurora B phosphorylates spatially distinct targets to differentially regulate the kinetochore-microtubule interface. Mol Cell. 2010 May 14;38(3):383-92

Yang JJ, Park TS, Lee ST, Seo JY, Oh SH, Cho EH, Strehl S, Mühlegger N, Dworzak MN, Zuna J, Pospisilova D, Meyer C, Marschalek R, Kim HJ, Kim SH. Molecular characterization and clinical impact of t(11;15)(q23;q14-15) MLL-CASC5 rearrangement. Haematologica. 2014 Jan;99(1):e11-3

This article should be referenced as such:

Takimoto M, Huret JL. KNL1 (cancer susceptibility candidate 5). Atlas Genet Cytogenet Oncol Haematol. 2017; 21(6):200-204.