Abstract

UHMK1 (also known as KIS) is a serine/threonine kinase initially identified as a Stathmin interacting protein. UHMK1 is characterized by an N-terminal kinase domain and a C-terminal UHM motif. Through the UHM motif, the protein is capable of interacting with splicing factors, such as SF1 and SF3B1, involved in early steps of spliceosome assembly. UHMK1 is ubiquitously but preferentially expressed in the developing nervous system, where it plays a role in mRNA processing, translational enhancing, neurite outgrowth and postsynaptic plasticity. Protein interactions between UHMK1 and a range of proteins pointed to its function in different cellular processes, such as RNA metabolism, cell cycle progression, cell migration and membrane trafficking. More recently, a role of UHMK1 in cell differentiation has also been proposed.

Keywords
phosphorylation; splicing; cell-cycle control; nervous system

DNA/RNA

Location (base pair)
Starts at 162497174 and ends at 162529629 bp from pter (according to GRCh38.p7, 2016)

Description
The UHMK1 gene is located on the chromosome 1, band q23, orientated in the plus (+) strand. The genomic locus spans 32456 base pairs (NC_000001.11), contains 8 exons and two alternative first exons.

Transcription
Three alternatively spliced transcripts of 8535, 8194 and 8446 base pairs are formed (NM_175866, NM_001184763 and NM_144624, respectively). The transcript variant 1 (NM_175866) codes for the longest protein isoform, which has 419 amino acids in length (isoform 1; NP_787062). The transcript variant 2 (NM_001184763) differs in the 5' UTR and initiates translation at the alternative start codon. The resulting protein (isoform 2; NP_0011716921) of 345 amino acids has a distinct 15 amino acids N-terminal, encoded by the alternative exon 1, and the remaining 330 amino acids encoded by exons 2-8. The transcript variant 3 (NM_144624) lacks exon 7, which results in a frame shift and early stop codon within exon 8. The encoded protein (isoform 3; NP_653225) of 344 residues, shares the first 341 amino acids (exons 1-6) with isoform 1, differing only in the last 3 amino acids at the C-terminal
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(Figure 1). An additional processed transcript of 3345 bp (ENST00000282169.8) retaining intron 2 (between exons 2 and 3), which does not contain an open reading frame (ORF) has been annotated for this gene. Moreover, the first 540 nucleotides of UHMK1 locus (NC_000001.11) are shared with the LOC105371497 gene, which produces a 708 bp long non-coding RNA (XR_922225.1), transcribed in the opposite direction of UHMK1 (https://www.ncbi.nlm.nih.gov/gene/127933).

Figure 1. Genomic organization, alternative splicing and protein isoforms of UHMK1. Exons are represented by numbered blue boxes and introns by the black line. The positions of the exons within the genome (NC_000001.11) are numbered. Exon joining is represented for each transcript by light green, purple and orange continuous lines; dashed lines indicate the respective protein isoform; dotted lines indicate the alternatively first exon usage. Size of the transcript variants are shown in parentheses. The transcript variant 1 codes for the longer protein (UHMK1 isoform 1, light green). The transcript variant 2 comprises an alternative first exon (light purple box), which encodes the distinct 15 amino acids N-terminal of the protein (UHMK1 isoform 2, purple). The transcript variant 3 lacks exon 7, whose excision results in a frameshift and early stop codon in exon 8. The resulting UHMK1 isoform 3 (orange), exhibits a distinct C-terminal formed by 3 aminoacids encoded by the beginning of exon 8. Sizes were scaled up, where 0.5 cm symbolizes 150 bp of exonic region (blue boxes) and 624 bp of intronic regions (black line). Number and position of aminoacids are depicted for each isoform.

Protein

Figure 2. Diagram representing UHMK1 protein and the posttranslational modifications. UHMK1 is characterized by an N-terminal kinase core of 282 aminoacids, represented in green and a C-terminal UHM of 100 aminoacids, represented in violet. All residues described to be phosphorylated or ubiquitinated in large scale proteomic studies are depicted. Source: Phosphoproteomic databases PhosphoSitePlus (http://www.phosphosite.org) UHM: U2AF homology motif (modified from Archangelo, et al. 2013).

Description

UHMK1 is a serine/threonine kinase with calculated molecular weight of 46.5 kDa and a theoretical pI of 5.59 (PhosphoSite Plus). The primary sequence of the protein is characterized by an N-terminal kinase core (282 aminoacids) and the C-terminal UHM of 100 aminoacids (UHM), responsible for establishing protein interactions with UHM-ligand motifs (ULM), particularly present among splicing factors (Kielkopf et al., 2004; Manceau et al., 2006). UHMK1 phosphorylates preferentially proline directed serine residues on its target proteins (Maucuer et al., 2000). The lysine 54 within the N-terminal region is essential for its kinase activity and autophosphorylation activity has been observed (Boehm et al., 2002; Maucuer et al., 1997). A variety of large scale proteomic studies identified two types of posttranslational modifications within UHMK1.
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namely lysine-ubiquitination (K190-ub, K282-ub, K383-ub and K387-ub) and phosphorylation (Y197-p, S283-p and S290-p) as indicated at the phosphoproteomic database PhosphoSitePlus (http://www.phosphosite.org) (Figure 2).

Expression

UHMK1 is ubiquitously expressed throughout rat and human tissues, with enriched expression in the nervous system (Bieche et al., 2003; Caldwell et al., 1999; Maucuer et al., 1997). Uhmk1 mRNA is expressed during rat embryonic development and increases after birth and during the first month of brain development (Bieche et al., 2003). In the adult brain, in situ hybridization revealed remarkable expression in the substantia nigra and some sensorial and motor nuclei in the brain stem (Bieche et al., 2003). In the human brain, UHMK1 expression was detected in all regions examined, with highest levels in the deeper cortical layers. Strong expression was observed in dentate gyrus, CA1, CA3 and CA4 regions of the hippocampus, in Purkinje cells and granule cell layer of the cerebellum. No expression was detected in the white matter (Bristow et al., 2003).

In the hematopoietic compartment, high levels of UHMK1 transcripts were observed in differentiated lymphocytes (CD4+, CD8+ and CD19+) or leukemia cell lines. UHMK1 expression was upregulated in megakaryocytic-, monocytic- and granulocytic-induced differentiation of leukemia cell lines and in erythroid-induced differentiation of primary CD34+ cells (Barbutti et al., 2017).

Levels of UHMK1 protein are induced by mitogens. In serum starved cells, UHMK1 expression was reduced in contrast to serum stimulated cells (Boehm et al., 2002; Crook et al., 2008; Petrovic et al., 2008). UHMK1 expression increased after quiescent peripheral blood lymphocytes (PBLs) were induced to proliferate upon mitogen activation (Barbutti et al., 2017). Moreover, the amount of UHMK1 protein varies throughout the cell cycle. In synchronized cells, UHMK1 accumulates in G1 phase and decreases during S phase of the cell cycle (Archangelo et al., 2013).

Little is known about the transcriptional regulation of UHMK1, which was described as direct target of the transcription factors GABP (Crook et al., 2008) and FOXM1 (Petrovic et al., 2008). The core promoter region of UHMK1 was described within -141 to -41 base pairs upstream of the transcription start site and has no consensus sequences for TATA or CCAAT boxes. Instead, it has GC-box and 3 Ets-binding sites (EBS-1, EBS-2 and EBS-3), which are essential for the promoter activity, in vitro. The regions spanning EBS-1 and EBS-2 (-103/-73 bp), and EBS-3 (-52/-42 bp) bind GABP in response to serum, leading to UHMK1 expression, cell migration and cell cycle progression of VSCM cells (Crook et al., 2008). FoxM1 binds an internal regulatory region within UHMK1 and transactivates its expression in vitro. FoxM1 appears to be essential for serum-dependent activation of UHMK1 mRNA expression, as assessed in FoxM1/-/- MEF cells. It was suggested that FoxM1-induced UHMK1 expression is required for UHMK1-mediated phosphorylation and consequently degradation of CDKN1B (p27Kip1) (Petrovic et al., 2008).

Furthermore, UHMK1 was described as transcriptional target of the WD repeat domain 5 (WDR5), a core component of the KMT2A (MLL) / SETD1A complex, known for its methyltransferase activity on H3 lysine 4 (H3K4). The H3K4me3 epigenetic modification correlates with gene activation, thus it is suggested that WDR5-mediated H3K4me3 at UHMK1 locus promotes its expression (Chen et al., 2015).

Localisation

The UHMK1 protein localizes mainly to the nucleus and to a lesser extent to the cytoplasm (Boehm et al., 2002; Maucuer et al., 1997) (Figure 3). Shuttling between nucleus and cytoplasm has been described for the GFP-fused protein by fluorescence recovery after photobleaching (FRAP) (Francone et al., 2010).
The kinase domain is essential for the protein nuclear localization, since deletion mutants of this domain, particularly the residues 1-211, extinguished Uhmk1 signal in immunofluorescence analysis (Manceau et al., 2008). Overexpressed ha-tagged Uhmk1 localized to the RNA granules of axon and dendrites of cortical neurons (Cambray et al., 2009). Also, a nucleolar enriched localization was observed when ha-tagged Uhmk1 was co-expressed with its GFP-fused interacting partner PIMREG (Archangelo et al., 2013).

**Figure 4. Potential functions of UHMK1.** 1- UHMK1 interacts with and phosphorylates the splicing factors SF1 and SF3B1. 2- UHMK1 counteracts the inhibitory effect of p27[Kip1] on cell cycle. Upon mitogenic activation, UHMK1 is upregulated and phosphorylates p27[Kip1], which is exported from the nucleus and targeted for degradation by the proteasome. 3- UHMK1 impairs cell migration through negatively regulating the microtubule destabilizing protein Statmin (STMN). UHMK1-mediated phosphorylation of STMN on S38 targets the protein for degradation. 4- UHMK1 regulates the secretory pathway in neurons and endocrine cells through its interaction with the peptidylglycine α-amidating mono-oxigenase (PAM). 5- UHMK1 interacts with components of neuronal RNA granules, such as KIF3A, NonO and eEF1A. It also associates with RNP-transported mRNAs and stimulates translation driven by the β-actin 3' UTR. 6- UHMK1 interacts with and phosphorylates the proliferation marker PIMREG, suggesting a potential role in regulating proliferation. Black arrow: represents the mitogen-dependent activation of UHMK1. Grey arrows: indicate the UHMK1-mediated phosphorylation of target proteins. Grey dotted arrows: represent the fate of the UHMK1 phosphorylated proteins targeted for degradation. P: phosphorylation; Ub: ubiquitination. Illustration was drawn using Servier Medical Art.

**Function**

UHMK1 was described to interact with a range of proteins, shedding light on different functions of this protein in diverse cellular processes (Figure 4). UHMK1 is the only kinase that possesses the N-terminal kinase core juxtaposed to a C-terminal U2AF homology motif (UHM) (Maucuer et al., 1997). Through the UHM motif, UHMK1 interacts with the splicing factors SF1 and SF3B1 (Manceau et al., 2008). Upon interaction, UHMK1 phosphorylates SF1, which enhances SF1 specific binding to U2AF₆₅ and reduces the SF1-U2AF₆₅ binding to the 3' splice site RNA (Chatrikhi et al., 2016; Manceau et al., 2006). In addition, UHMK1 expression is necessary for normal phosphorylation of SF1 in vivo (Manceau et al., 2012). The fact that UHMK1 interacts with and regulates splicing factors suggests that UHMK1 might be involved in RNA metabolism.

Since UHMK1 is highly expressed in neurons, it is expected to exert important functions in the nervous system. It was demonstrated an abnormal phosphorylation of SF1 in brain extracts of neonate Uhmk1⁻/⁻ mice. Also, Uhmk1 deletion resulted in increased ratio of pre-mRNA relative to mRNA, and consequently down-regulation of brain specific genes, like cyst-loop ligand-gated ion channels and metabolic enzymes. Although adult Uhmk1⁻/⁻ mice did not present an obvious phenotype, animal
behavior was affected. The Uhmkl\textsuperscript{+−} mice displayed locomotor hyperactivity, reduced fear conditioning and learning capacities from aversive stimuli (Manceau et al., 2012). The murine Uhmkl was described to interact with known components of neuronal RNA granules, such as KIF3A, NONO and EEF1A1. The protein colocalizes with KIF3A kinesin in neurites and is required for neuritic outgrowth in cortical mouse neurons. Furthermore, Uhmkl associates with RNP-transported mRNAs and stimulate translation driven by the β-actin 3’ UTR, suggesting that Uhmkl contributes to modulate translation in RNA-transporting granules as a result of local signals (Cambray et al., 2009). Still, comparison of primary cultures derived from Uhmkl\textsuperscript{+−} mice did not reveal a significant difference in neuritic arborization of cortical neurons (Manceau et al., 2012). Furthermore, a study investigating Uhmkl action on hippocampal synaptic plasticity in mice, showed that Uhmkl knockdown impaired spine development, altered actin dynamics, and reduced postsynaptic responsiveness. Moreover, Uhmkl depletion resulted in decrease of the postsynaptic scaffolding protein PSD-95 and of AMPA receptor subunits. Thus Uhmkl enhances translation of AMPA receptors and stimulates dendritic spine remodeling (Pedraza et al., 2014).

Another described function of UHMK1 involves the regulation of secretory pathway in neurons and endocrine cells through its interaction with peptidylglycine α-amidating mono-oxygenase (PAM) (Alam et al., 1996). PAM cytosolic domain (CD) phosphorylation by UHMK1 (Ser-949) is required for the correct routing of this protein and consequently for its ability to affect trafficking in the regulated secretion pathway (Alam et al., 2001; Caldwell et al., 1999). Lately, it was described an intramembrane proteolysis pathway for PAM, generating a soluble fragment of the cytosolic domain (sf-CD), which accumulates in the nucleus in a phosphorylation-dependent manner, modulating the expression of genes involved in the secretory pathway. UHMK1 phosphorylates sf-CD, diminishing its localization in the nucleus and negatively regulating the expression of a subset of genes (Francone et al., 2010; Rajagopal et al., 2010).

An extensively documented function of UHMK1 is its ability to positively regulate cell cycle progression through phosphorylation and inhibition of the cyclin dependent kinase inhibitor (CDKI) p27\textsuperscript{Kip1}. Upon mitogenic activation, UHMK1 expression is upregulated and phosphorylates p27\textsuperscript{Kip1} on serine 10 (Ser10). As a consequence, p27\textsuperscript{Kip1} is exported from nucleus to cytoplasm, where it is targeted to the proteasome and degraded, and has no longer inhibitory effect on cell cycle. Thus, UHMK1 promotes cell cycle re-entry by inactivating p27\textsuperscript{Kip1} following growth factor stimulation (Boehm et al., 2002).

Another important target of UHMK1 is the microtubule-stabilizing protein, Stathmin (Maucuer et al., 1995). UHMK1 interacts with and phosphorylates Stathmin on serine 38 (Ser38), targeting this protein to proteasome. Through negative regulation of Stathmin, UHMK1 alter microtubule dynamics and consequently impairs cell migration (Langenickel et al., 2008).

UHMK1 expression is upregulated upon hematopoietic cell differentiation, thus a possible role of UHMK1 in cell differentiation was proposed (Barbutti et al., 2017). This idea was supported by the fact that UHMK1 mRNA is highly expressed in the mature brain and in terminally differentiated neural cells (Bieche et al., 2003) as well as during osteoclasts differentiation (Choi et al., 2016). The human UHMK1 shares high homology with a number of species as depicted in Table 1. PIMREG (previously known as FAM64A; CATS) is a proliferation marker shown to interact with UHMK1. The fact that UHMK1 interacts with and phosphorylates PIMREG suggests that UHMK1 regulates PIMREG function and/or localization. Nevertheless, the functional implication of this interaction remains elusive (Archangelo et al., 2013).

**Homology**

The human UHMK1 shares high homology with a number of species as depicted in Table 1. The human UHMK1 shares high homology with a number of species as depicted in Table 1.

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**Table 1. Homology between the human UHMK1 and other species**

<table>
<thead>
<tr>
<th>Homo sapiens UHMK1</th>
<th>Symbol</th>
<th>Protein (% Identity)</th>
<th>DNA (% Identity)</th>
</tr>
</thead>
<tbody>
<tr>
<td>vs. <em>P. troglodytes</em></td>
<td>UHMK1</td>
<td>99.8 (XP_001174268)</td>
<td>99.7 (XM_001174268)</td>
</tr>
<tr>
<td>vs. <em>M. mulatta</em></td>
<td>UHMK1</td>
<td>99.8 (NP_001253697)</td>
<td>99.0 (NM_001266768)</td>
</tr>
<tr>
<td>vs. <em>C. lupus</em></td>
<td>UHMK1</td>
<td>99.8 (XP_536143)</td>
<td>95.8 (XM_536143)</td>
</tr>
<tr>
<td>vs. <em>B. taurus</em></td>
<td>UHMK1</td>
<td>99.8 (NP_001192514)</td>
<td>95.9 (NM_001205585)</td>
</tr>
</tbody>
</table>

UHMK1 (U2AF homology motif kinase 1)

vs. *M. musculus* | Uhmk1 | 99.3 (NP_034763) | 93.0 (NM_010633) |
--- | --- | --- | --- |
vs. *R. norvegicus* | Uhmk1 | 99.3 (NP_058989) | 92.6 (NM_017293) |
vs. *G. gallus* | UHMK1 | 88.2 (XP_015145890) | 81.6 (XM_015290404) |
vs. *D. rerio* | uhmk1 | 73.6 (NP_001070127) | 69.4 (NM_001076659) |


### Mutations

#### Somatic

Recurrent mutations have not been identified for the UHMK1 gene. Nonetheless, more than 160 unique mutations were reported in this gene in the catalogue of somatic mutations in cancer database (COSMIC), mainly in lung, gastric, esophageal, colon, rectal and hepatocellular/liver cancer.

### Implicated in

#### Breast cancer

Erlotinib resistance in breast cancer treatment was attributed to p27\(^{kip1}\) cytoplasmic localization. UHMK1 depletion by siRNA enhanced erlotinib cytotoxicity in EGFR-expressing breast cancer cells, due to its accumulation in the nucleus and reduced p27\(^{kip1}\) cytoplasmic localization (Zhang et al., 2010). Besides, UHMK1 expression was reported to be inhibited in a dose-dependent manner by the anti-HER2 antibody trastuzumab, used for treatment of human metastatic breast cancer with HER2 overexpression (Le et al., 2005).

#### Neurological tumors

Higher levels of UHMK1 transcripts were observed in small cohort of neurological tumors associated with neurofibromatosis type 1 (NF1). Among the NF1-associated tumors analyzed, plexiform neurofibroma and malignant peripheral nerve sheath tumors (MPNSTs) presented higher UHMK1 mRNA levels compared to dermal neurofibroma (Bieche et al., 2003).

#### Bladder cancer

Silencing of WDR5, a protein shown to be upregulated in bladder cancer, reduced the H3K4me3 epigenetic marker on its target genes, such as UHMK1 and consequently downregulated UHMK1 expression in bladder cancer cells (Chen et al., 2015).

#### Hematological malignancies

No aberrant expression was observed in patient samples with myelodysplastic syndrome (MDS), acute myeloid (AML) or lymphoblastic (ALL) leukemia. Nonetheless, in MDS patients, increased levels of UHMK1 expression positively impacted event free and overall survival (Barbutti et al., 2017).

#### Schizophrenia

Puri and colleagues performed a fine mapping by genetic association and identified two SNPs within the UHMK1 gene (rs10494370, p = .004, and rs7513662, p = .043), which showed significant association with schizophrenia (Puri et al., 2007). The genetic association of these markers was confirmed in a second case-control (Puri et al., 2008). Nevertheless, the association of UHMK1 with schizophrenia is controversial since the data from different cohorts did not support the findings (Betcheva et al., 2009; Dumaine et al., 2011).

#### Osteoporosis

The SNP rs16863247 was identified within the UHMK1 locus in a genome-wide association study (GWAS) carried out to identify genetic variants that influence bone mineral density (BMD) in east Asians. Thus, UHMK1 was described as a bone mineral density susceptibility gene for this ethnical group. The authors also showed opposite expression levels of UHMK1 during osteoblast and osteoclast differentiation and proposed that UHMK1 may play a role in bone metabolism by controlling osteoclast and osteoblast differentiation (Choi et al., 2016).

#### Vascular remodeling and wound repair

Langenickel and coworkers demonstrated the importance of UHMK1 expression in controlling vascular remodeling and wound repair. These processes are characterized by vascular smooth muscle cell (VSMC) proliferation and cell migration, which can be achieved by inhibiting p27\(^{kip1}\) and Stathmin, two known substrates of UHMK1. In a mouse model, deletion of Uhmk1 led to accelerated neointima formation and vessel occlusion, caused by increased migratory activity of VSMCs, as a consequence of diminished degradation of Stathmin (Langenickel et al., 2008).

#### Corneal fibrosis

It was shown that FGF2-mediated proliferation of corneal endothelial cells (CECs) is partially dependent on UHMK1 upregulation and its inhibitory effects on CDK inhibitor p27\(^{kip1}\) (Lee and Kay, 2011; Lee et al., 2011).
**Cerebral visual impairment**

UHKM1 was recently reported among candidate genes for cerebral visual impairment (CVI), a major cause of low vision in childhood (Bosch et al., 2016).

**To be noted**

It is well accepted that UHKM1 promotes cell cycle re-entry by inactivating p27Kip1 following growth factor stimulation. Thus it is expected that abnormally elevated UHKM1 activity, which is supposed to relieve cells from p27Kip-dependent growth inhibition, could be involved in some aspects of tumor development. Nonetheless, no aberrant expression of UHKM1 has been reported amongst different cancer samples (Barbutti et al., 2017; Bieche et al., 2003), except in a few cases of neurological tumors associated with NF1 (Bieche et al., 2003). Hence, whether it plays a role in tumorigenesis or not remains largely elusive and must be further investigated.

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