Atlas of Genetics and Cytogenetics
in Oncology and Haematology

OPEN ACCESS JOURNAL

INIST-CNRS

Gene Section
Review

PIMREG (PICALM interacting mitotic regulator)

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Published in Atlas Database: January 2017
Online updated version : http://AtlasGeneticsOncology.org/Genes/PIMREGID54310ch17p13.html
Printable original version : http://documents.irevues.inist.fr/bitstream/handle/2042/68737/01-2017-PIMREGID54310ch17p13.pdf
DOI: 10.4267/2042/68737

Abstract

PIMREG (FAM64) was initially identified as CATS, the CALM (PICALM) interacting protein expressed in thymus and spleen. Mounting evidence suggests the involvement of FAM64A in tumorigenesis. Through interaction with PICALM, FAM64 was able to influence the subcellular localization of the leukemic fusion protein PICALM/MTT10 (CALM/AF10). FAM64A is highly expressed in leukemia, lymphoma and tumor cell lines and its levels are strongly correlated with cellular proliferation in both malignant and normal cells. FAM64A is a mitotic regulator that controls chromosome segregation during cell division, and its transcripts covariate with that of cell cycle regulation genes in tumorigenesis. Nevertheless, a precise role of FAM64A in tumorigenesis is yet to be defined.

Keywords:

PIMREG; FAM64; chromosome 17; proliferation; tumorigeneses; metaphase-anaphase transition; cell-cycle control; PICALM/MTT10

Identity

Other names: FAM64A, FLJ10156, FLJ10491, CATS, RCS1

HGNC (Hugo): PIMREG

Location: 17q13.2

Location (base pair): Starts at 6444415 and ends at 6451469 bp from pter (according to hg38 - Dec2013)

DNA/RNA

Description

PIMREG (FAM64) is located on chromosome 17 band p13.2, 6.29 megabase pairs from the telomere of the short arm (chromosome 17 genomic contig NT_010718). The genomic locus spans 7 Kb and contains 6 exons with a non-coding first exon.

Transcription

Two alternatively spliced transcripts of 1.5 Kb are formed (NM_019013 and NM_001195228) and code for two protein isoforms of 238 and 248 amino acids in length (isoform 1 and 2, respectively). Both isoforms share the first 228 residues, encoded by exons 2-4.

The two proteins differ in their C-termini. The 10 last amino acids of isoform 1 are encoded by exon 5, which is absent in the transcript coding for isoform 2.

The last 20 amino acids of isoform 2 are encoded by exon 6 (Figure 1) (Archangelo, et al. 2006). An additional transcript variant with retained intron 5 (between exons 5 and 6) was described (Archangelo, et al. 2006; Coulombe-Huntington, et al. 2009). There are 4 processed pseudogenes of FAM64A at other chromosomal locations (2q33, 4p15, 4q24 and 6q15).

Protein

Description

The FAM64A calculated molecular weight is about 27 kDa.

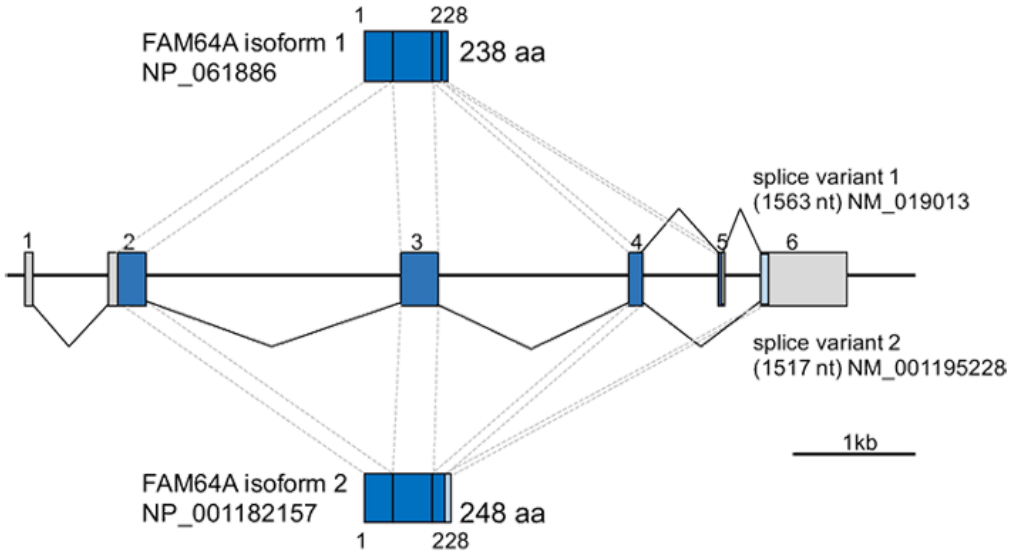

Figure 1: Genomic organization, alternative splicing, and protein isoforms of PIMREG (FAM64). Boxes represent exons, with blue parts symbolizing coding region and gray parts the 5' and 3' UTRs. There are 2 splice variants (sizes are indicated in parentheses). In splice variant 2, exon 5 is spliced out resulting in a longer protein (FAM64A isoform 2) encoded by exons 2, 3, 4 and 6. The FAM64A isoform 1 (shorter form) is encoded by exons 2, 3, 4 and 5 from splice variants 1 and the transcript with retained intron 5 (see in the text). Both FAM64A isoforms are identical from amino acids 1-228, they differ in their last 10 and 20 C-terminal residues (isoform 1 and 2, respectively). (Figure modified from Archangelo, et al. 2006)

The protein contains two putative destruction box motifs (RxxL) at amino acids 14-17 and 53-56 (DB1 and DB2, respectively), the second being a recognition motif for the ubiquitin ligase anaphase-promoting complex/cyclosome (APC/C) (Zhao, et al. 2008).

There are three types of posttranslational modifications identified within FAM64A isoforms, namely lysine- acetylation (K-ac), lysine-ubiquitination (K-ub) and phosphorylation (S-p and T-p). A total of 22 residues were described as phosphorylated in FAM64A in a variety of large scale proteomic studies as revealed by the phosphoproteomic databases PhosphoSitePlus™ (http://www.phosphosite.org) and Phosida (http://www.phosida.com/) (Figure 2).

Expression

In normal adult tissue FAM64A is predominantly expressed in thymus, spleen and colon and to a lesser extent in small intestines, ovary and brain (Archangelo, et al. 2006; Archangelo, et al. 2008). Moreover, FAM64A is highly expressed in leukemia, lymphoma and tumor cell lines. The protein levels vary throughout the cell cycle. In synchronized cells FAM64A accumulates in S and G_2 phases of the cell cycle, peaks in the mitosis and drops drastically as cells exit from mitosis into the G_1 phase (Archangelo, et al. 2008; Zhao, et al. 2008). FAM64A transcripts covariate with that of cell cycle regulation genes in tumorigenesis (Zhao, et al. 2008).

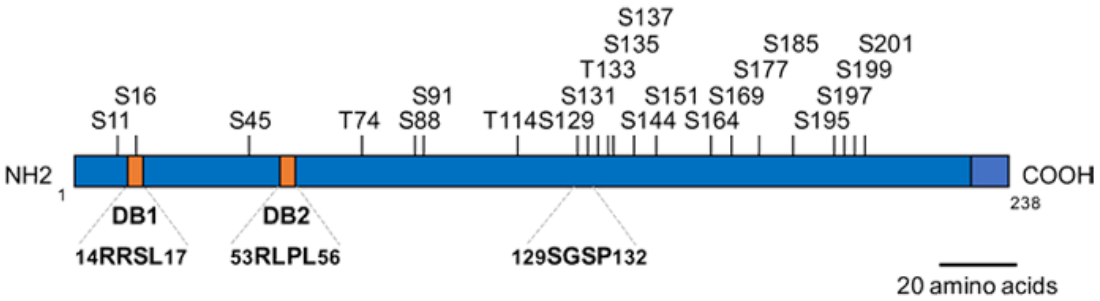

Figure 2: Diagram representing the protein structure of PIMREG (FAM64). FAM64A is a phosphoprotein. All residues described to be phosphorylated in large scale proteomic studies are depicted. Source: Phosphoproteomic databases PhosphoSitePlus™ (http://www.phosphosite.org) and Phosida (http://www.phosida.com/). Destruction box motifs are depicted as orange boxes (DB1 and DB2). Aminoacids comprising the DB motifs and the unique phospho-residue characterized (S131) are shown. (Figure modified from Archangelo, et al. 2013)

Resting human peripheral blood lymphocytes (PBLs), which are not selected for growth in culture, do not express FAM64A protein.
However, when these cells are induced to proliferate upon mitogen activation they do express FAM64A. Likewise, glioblastoma cells induced to proliferate, show a progressive upregulation of FAM64A. Conversely, FAM64A expression drastically decreases when highly proliferative leukemia cells cease to proliferate upon exposure to differentiation agents (Barbutti, et al. 2016).Thus, FAM64A expression positively correlates with the proliferative state of the cells (Archangelo, et al. 2008).
Expression analysis of the murine Fam64a homolog revealed a strong expression throughout mouse embryogenesis, in particular at the developing central nervous system (CNS). The expression decreases gradually and proportionally as embryos develop to later stages (Archangelo, et al. 2008). In the hematopoietic compartment, Fam64A is widely expressed in different cell subpopulations (Archangelo, et al. 2008). Moreover, RNA-Seq analysis of individual hematopoietic stem cells (HSCs) to resolve heterogeneity within HSC population, revealed that Fam64a is highly expressed in the HSC population primed for proliferation (Wilson, et al. 2015).

Localisation

FAM64A is a nuclear protein enriched in the nucleoli. Expression levels in the nucleoplasm and nucleolar accumulation are different from cell to cell and dependent on the cell cycle phase (Archangelo, et al. 2008) (Figure 3). In mitotic cells FAM64A-GFP is diffusely distributed throughout cells without specific association with chromosomes, kinetochores, spindle, or centrosome (Zhao, et al. 2008).

Function

Based on the fact that levels of FAM64A protein strongly correlate with cellular proliferation in both normal and malignant cells, this protein was described as a marker for proliferation with a possible role in the control of cell proliferation and tumorigenesis (Archangelo, et al. 2008). In fact, silencing of FAM64A protein in the U937 leukemia cell line resulted in reduced proliferation and altered cell cycle progression, as attested by diminished expression of the cell cycle regulators CCNA1, CCNE1, CCNB1 (cyclin-A, -E and -B1) in FAM64A depleted cells (Barbutti, et al. 2016).
Furthermore, Zhao and coworkers described FAM64A as a regulator of chromosome segregation. FAM64A is a substrate of the APC/C complex that controls the metaphase to anaphase transition and its knock-down resulted in accelerated anaphase onset. Thus FAM64A plays an important role in determining the kinetics of mitotic progression (Zhao, et al. 2008). Although the literature indicates a putative function of FAM64A in controlling cell cycle progression, division and tumorigenesis, the impairment of cell proliferation upon FAM64A depletion was modest and not sufficient to inhibit tumor growth of xenotransplanted U937 cells in an in vivo model (Barbutti, et al. 2016). Similarly, the faster metaphase-to-anaphase transition observed in FAM64A depleted cells was not sufficient to produce any of the mitotic defects often observed in cancer cells. Rather, mitotic index, spindle assembly, chromosome congregation/segregation and cytokinesis proceeded normally in FAM64A depleted cells (Zhao, et al. 2008).
FAM64A has also been described to function as a transcriptional repressor in a GAL4-based reporter gene assay (Archangelo, et al. 2006). Additionally, FAM64A was able to repress the transactivation capacity of the leukemic fusion protein PICALM/MTT10 (CALM/AF10) (Archangelo, et al. 2013). Evidence further supporting the transcriptional inhibitory properties of FAM64A were provided by Zhao and colleagues, who described the interaction between FAM64A and components of the nucleosome remodeling and deacetylase (NuRD) complex, such as MTA2, HDAC1/HDAC2 and RBBP7 / RBBP4 (RBAp46/48) (Zhao, et al. 2008).

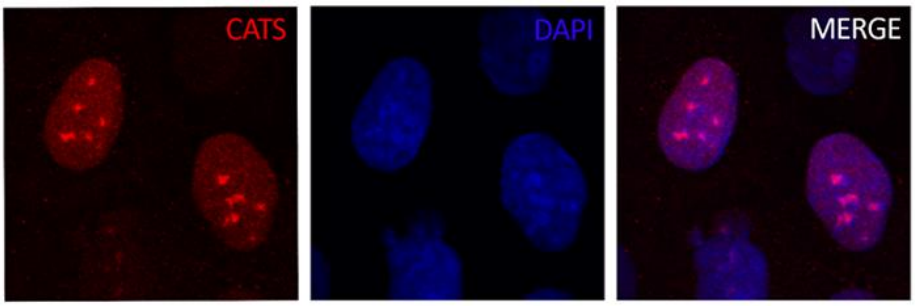

Figure 3: Subcellular localization of endogenous PIMREG (FAM64). Endogenous protein was visualized by immunofluorescence and confocal microscopy in non-synchronized U2OS cells. FAM64A is a nuclear protein enriched in the nucleolus of some cells. Note the different levels of FAM64A expression from cell to cell. FAM64A protein levels and nucleolar localization are cell cycle dependent. The figure shows cells labeled with Cy3 (FAM64A) and DAPI with channels merged.

The NuRD complex is implicated in transcriptional regulation and plays a role in modifying chromatin structures to initiate and maintain gene repression.
Since its discovery a number of FAM64 interacting proteins have been described (e.g. PICALM (Archangelo, et al. 2006), UHMK1 (KIS) (Archangelo, et al. 2013), PRNP (PrPC) (Satoh, et al. 2009), PTBP3 (ROD1) (Brazao, et al. 2012) and NuRD complex (Zhao, et al. 2008), shedding light on the putative function of this protein.
The interaction of FAM64A with the Phosphatidylinositol Binding Clathrin Assembly Protein (PICALM) was identified in a yeast two hybrid screen (Y2H) and confirmed by GST pull-down and co-immunoprecipitation experiments for both FAM64A isoforms. The FAM64A interaction region was mapped to amino acids 221 to 294 of PICALM. Through this interaction FAM64A was able to alter the subcellular localization of PICALM. The fluorescently-tagged protein YFP-PICALM localized mainly in the cytoplasm and plasma membrane of transfected NIH3T3 cells. However, when CFP-FAM64A was co-expressed, PICALM was observed both at the cytoplasm/membrane and in the nucleus of some cells at almost equal levels (Archangelo, et al. 2006). The interaction between FAM64A and the Kinase Interacting Stathmin (KIS and UHMK1) was identified in Y2H screen and confirmed by GST pull-down, co-immunoprecipitation and co-localization experiments. KIS interacts with amino-acids 136-187 of FAM64A. Kinase assay showed that FAM64A is a substrate of KIS at serine 131 (S131). In a GAL4-based reporter gene assay KIS enhanced the transcriptional repressor activity of FAM64A, independently of phosphorylation on S131 but dependent on KIS kinase activity (Archangelo, et al. 2013). RNA immunoprecipitation sequencing (RIP-seq) described the interaction between FAM64A and ROD1 (PTBP3), an RNA-binding protein involved in nonsense-mediated decay (NMD) (Brazao, et al. 2012). Protein microarray analysis identified FAM64A as an interacting partner of the cellular prion protein (PrPC). The interaction was confirmed by co-immunoprecipitation of the overexpressed tagged-proteins (Satoh, et al. 2009).

Homology

The human FAM64A shares homology with the species described in Table 1.

Mutations

Somatic

Mutations in the FAM64A gene were reported in the catalogue of somatic mutations in cancer (COSMIC) database in 53 out of the 28710 samples tested, of which 6 are nonsense substitutions, 33 missense substitutions and 10 synonymous substitutions (http://cancer.sanger.ac.uk/cancergenome/projects/cosmic)

***Homo sapiens* FAM64A**	**Symbol**	**Identity (%) Protein**	**DNA**
vs. P.troglodytes	FAM64A	96.6	98.6
vs. vs. M. mulatta	LOC712701	91.6	95.9
vs. C. lupus	FAM64A	73.3	81.2
vs. B. taurus	FAM64A	83.2	85.9
vs. M. musculus	Fam64a	74.8	77.5
vs. R. norvegicus	Fam64a	76.7	79.1

Table 1. Homology between the human FAM64A and other species (Source: http://www.ncbi.nlm.nih.gov/homologene/)

Implicated in

Leukemia

FAM64A is highly expressed in leukemia and lymphoma cell lines (Archangelo, et al. 2008). FAM64A interacts with the central domain of PICALM, a region contained in the PICALM moiety of the PICALM/MLLT10 (CALM/AF10) leukemic fusion protein (Archangelo, et al. 2006). FAM64A expression markedly increased the nuclear localization of PICALM/MLLT10 (CALM/AF10) and counteracted the ability of this fusion protein to activate transcription in vitro (Archangelo, et al. 2006; Archangelo, et al. 2013). Additionally, the murine Fam64a transcripts were up-regulated in hematopoietic cells (B220+ lymphoid cells) transformed by PICALM/MLLT10 (CALM/AF10) in comparison to the same subpopulation from non-leukemic mice (Archangelo, et al. 2008).
In a recent report, FAM64A silencing in the PICALM/MLLT10 (CALM/AF10)-positive U937 leukemia cell line resulted in somewhat reduced proliferation, altered cell cycle progression, lower migratory ability in vitro, and reduced clonogenicity of FAM64A-depleted U937 cells. Nonetheless, FAM64A silencing was not capable of interfering with the expression of the PICALM/MLLT10 (CALM/AF10)-leukemia deregulated genes (HOXA gene cluster, MEIS1 and BMI1) nor sufficient to hinder tumor growth of U937 xenografts (Barbutti, et al. 2016).

Breast cancer

FAM64A was identified together with BIRC5 and CENPA as the three genes specifically upregulated in triple-negative breast cancer (TNBC), an aggressive type of cancer with poor outcome and short survival. In a broader clinical set analysis,

FAM64A upregulation correlated with poor survival, despite the molecular type of breast cancer (Zhang, et al. 2014).

Lung cancer

The fusion gene XAF1 (17p13.1) / FAM64A (17p13.2) was identified in a single lung adenocarcinoma patient from a large scale RNA sequencing study. The fusion gene was validated by Sanger sequencing and showed no co-occurrence with other canonical driver point mutation in that sample. The breakpoints were described at position chr17:6663920, within exon 4 of the donor transcript (XAF1), and at position chr17:6348396, within the 5' untranslated region (5'UTR) of the acceptor gene FAM64A (Seo, et al. 2012).
In the same study FAM64A was also detected among a number of cancer outlier genes (CoGs), being highly expressed in 8 cancer tissue samples (Seo, et al. 2012).

Head and Neck squamous cell carcinoma

Single gene clustering of RNA sequencing in a set of 177 lung and 279 head and neck squamous cell carcinomas, reveled a differential alternative isoform usage pattern of the FAM64A locus, with lower expression of a 152 bp cluster within the final exon of the gene. The implication of the differential isoform usage remains to be determined (Kimes, et al. 2014).

To be noted

FAM64A is a marker for proliferation (Archangelo, et al. 2008) with potential use as a prognostic marker (Zhang, et al. 2014). FAM64A plays a role in the regulation of chromosome segregation during cell division (Zhao, et al. 2008) and in the control of cell cycle progression and clonogenicity of the U937 leukemia cell line (Barbutti, et al. 2016). However, FAM64A depletion is not sufficient to impair either mitosis or hinder cell growth (Barbutti, et al. 2016; Zhao, et al. 2008). Hence, it still to be addressed whether the strong correlation of FAM64A expression with proliferation is determinant for tumorigenesis or if it is a consequence.

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This article should be referenced as such:

Archangelo LF. PIMREG (PICALM interacting mitotic regulator). Atlas Genet Cytogenet Oncol Haematol. 2017; 21(10):358-362.