BUB3 (BUB3 mitotic checkpoint protein)

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Abstract

BUB3 is a WD40 protein that belongs to spindle mitotic checkpoint complex, which monitors the chromosome attachment to mitotic (or meiotic) fuse and prevents premature chromosome segregation. Alterations in BUB3 have been associated with chromosomal instability and aneuploidy, but their contribution for cancer development and progression are poorly understood, and appear to differ depending on the type of cancer.

The present review contains data on BUB3 DNA, RNA, protein encoded and function.

Keywords
BUB3; Mitotic checkpoint protein BUB3; WD40 protein; Spindle checkpoint; Cell cycle; Anaphase-promoting complex.

DNA/RNA

Description
The entire BUB3 gene is approximately 16.2 Kb (start: 123154277 and end: 123170467 bp; orientation: Forward strand).

Transcription
The BUB3 gene encodes for 4 transcript variants: there are two transcript variants deposited in the NCBI database (https://www.ncbi.nlm.nih.gov/gene) and two additional transcript variants reported in Ensembl (http://www.ensembl.org/). The transcript variant 1 is the longest transcript variant (exons: 8, coding exons: 7, transcript length: 7828 bp) and encodes the isoform a (328 amino acids [aa]). The transcript variant 2 present an alternative splice site in 3’ coding region (exons: 8, coding exons: 7, transcript length: 1361 bp), which leads to a frameshift and a shorter and distinct C-terminus compared to isoform a (isoform b, 326 aa).

Identity

Other names: BUB3L, hBUB3
HGNC (Hugo): BUB3
Location: 10q26.13

Figure 1. BUB3 protein structure. BUB3 protein presents seven WD40 repeat domain, a nearly 40 amino acids (aa) motif rich in tryptophan-aspartic acids (W-D). The position of aa are indicated.
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The transcript variant 3 present 6 coding exons, a transcript length of 895 bp and a translation length of 278 aa.
The transcript variant 4 is shorter transcript presenting 5 exons (being 4 coding exons), a transcript length 667 bp and resulting protein of 145 aa.

Protein

Description
BUB3 is a WD40 protein that create a symmetric circular wall around a central pore or funnel region with its seven-blade β-propeller structure, acting as a scaffolding protein for its binding partners BUB1B (BUBR1) and BUB1 (Prinz et al., 2016; Seeley et al., 1999).
The primary structure of BUB3 is illustrated in Figure 1.

Expression
Ubiquitous.

Localisation
BUB3 is localized preferentially in unattached kinetochores during mitosis (especially prometaphase), participating of mitotic checkpoint complex (Sudakin et al., 2001). In interphase cells, BUB3 is localized in cytosol (Yoon et al., 2004).

Function
BUB3 protein is a component of the mitotic checkpoint complex (MCC), which present a crucial activity monitoring the state of chromosome attachment to the mitotic (or meiotic) fuse and prevents loss of sister chromatid cohesion and premature chromosome segregation in the presence of unattached or incorrectly attached chromosomes to the spindle by inhibition of the anaphase-promoting complex/cyclosome (APC/C) (Lopes et al., 2005; Musacchio, 2015). The canonical BUB3-related cellular signaling is illustrated in Figure 2.
Using a Drosophila melanogaster model, Morais da Silva and colleagues (Morais da Silva et al., 2013) reported that BUB3 inhibition resulted in increased proliferative potential, promoted tumorigenesis and widespread chromosomal aneuploidy. Interesting, loss of cytoplasmatic, but not attached-kinetochore, BUB3 pool was found to be driven tumorigenesis, indicating a novel non-kinetochore-dependent tumor suppressing function for BUB3 (Morais da Silva et al., 2013). Additional non-canonical functions of BUB3 had been described during interphase. BUB3 and CDC20 form a complex with histone

Figure 2: BUB3, a component of mitotic checkpoint complex (MCC). (Upper panel) The presence of unattached kinetochores induces the formation of MCC composed by BUB3, BUBR1, MAD2L1, which sequester CDC20, leads the anaphase-promoting complex/cyclosome (APC/C) inhibition and cell cycle arrest. (Lower panel) In the presence of attached kinetochores, the MCC complex is disassembled and the CDC20 is released, which leads to APC/C activation (which in turn triggers degradation of securin and cyclin B), chromosomal segregation and completion of mitosis.
deacetylases, which seems to confer transcriptional repressor activity (Yoon et al., 2004). Wan and colleagues (Wan et al., 2015) reported that BUB3 regulates RNA splicing, R-loop formation, DNA damage, and TP53 activation.

Homology

The BUB3 gene and protein is highly homologous among different species, as shown in Table 1.

<table>
<thead>
<tr>
<th>% Identity for: Homo sapiens BUB3</th>
<th>Symbol</th>
<th>Protein</th>
<th>DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>vs. P. troglodytes</td>
<td>BUB3</td>
<td>100.0</td>
<td>99.8</td>
</tr>
<tr>
<td>vs. M. mulatta</td>
<td>BUB3</td>
<td>100.0</td>
<td>98.8</td>
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<tr>
<td>vs. B. taurus</td>
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<td>100.0</td>
<td>93.5</td>
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<td>vs. M. musculus</td>
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<td>100.0</td>
<td>90.4</td>
</tr>
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<td>vs. R. norvegicus</td>
<td>Bub3</td>
<td>100.0</td>
<td>91.1</td>
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<td>vs. G. gallus</td>
<td>BUB3</td>
<td>96.9</td>
<td>84.0</td>
</tr>
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<td>vs. X. tropicalis</td>
<td>bub3</td>
<td>93.2</td>
<td>79.5</td>
</tr>
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<td>vs. D. rerio</td>
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<td>59.2</td>
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<td>vs. S. pombe</td>
<td>bub3</td>
<td>38.0</td>
<td>45.0</td>
</tr>
<tr>
<td>vs. A. thaliana</td>
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<td>BUB3.2</td>
<td>56.3</td>
<td>55.8</td>
</tr>
<tr>
<td>vs. O. sativa</td>
<td>Ox03g0448600</td>
<td>56.3</td>
<td>57.4</td>
</tr>
</tbody>
</table>

Table 1. Comparative identity of human BUB3 with other species. (Source: http://www.ncbi.nlm.nih.gov/homologene)

Mutations

Somatic

Recurrent mutations in the BUB3 gene are rare. A total of 81 unique samples presented BUB3 mutations, which are distributed on 75 different mutations (60 missense substitutions, 10 synonymous substitutions, 1 nonsense substitutions, 2 inframe deletions and 2 frameshift deletions), were found among the 47120 unique samples reported in COSMIC (Catalogue of Somatic Mutations in Cancer; http://cancer.sanger.ac.uk/cancergenome/projects/cosmic). In agreement, 0.2% of 74247 tested samples presented BUB3 somatic mutation as reported in cBioPortal (http://www.cbioportal.org), which correspond to 154 mutations: 132 missense substitutions, 17 truncating and 5 inframe mutations. Of note, there are 55 duplicate mutations in patients with multiple samples. A total of 472 (0.8%) samples presented any type of genetic alteration in BUB3, when mutations, amplifications, deep deletions and multiple alterations were considered in 55817 cancer samples. These findings corroborate initial studies in different types of cancer (Hernando et al., 2001).

Implicated in

Adrenocortical carcinoma

In a cohort of 79 adrenocortical carcinoma patients, increased levels of BUB3 mRNA levels was associated with high grade tumors and poor clinical outcomes (Subramanian and Cohen, 2019). In addition, Oncomine Giordano ACC data analysis revealed increased levels of BUB3 in adrenocortical carcinoma compared to normal adrenal samples (Subramanian and Cohen, 2019).

Breast cancer

BUB3 promoter polymorphisms (rs3763740, rs3763741, rs17014712, rs3808960 and rs3808961) did not impact familial breast cancer risk in a German cohort of 441 breast cancer patients and 552 controls matched by age, ethnicity and geographical region (Vaclavicek et al., 2007). Similarly, the polymorphisms rs11248416, rs11248419, and rs6599657 in BUB3 gene were not associated with risk of breast cancer development in a Chinese cohort of 462 breast cancer patients and 529 controls (Wang et al., 2014).

BUB3 was highly expressed in primary and cell lines from breast cancer compared to immortalized breast cancer epithelium or normal primary mammary cells (Yuan et al., 2006). BUB3 gene region (10q26.3) was found to be amplified and overexpressed in breast cancer samples (Turner et al., 2010). BUB3 mRNA levels were upregulated in doxorubicin and cyclophosphamide sensitive breast cancer tumors (Cleator et al., 2006). In MDA-MB-231 breast cancer cells, BUB3 expression was induced by BMP signaling (Yan et al., 2012).

Cervical cancer

Using proteomics approach, BUB3 was found to be downregulated by paclitaxel and 5-fluorouracil in HeLa cells (Lee et al., 2005; Yim et al., 2004). In addition, BUB3 inhibition by siRNA reduced paclitaxel-induced cell cycle arrest (Lee et al., 2005).

Chronic myeloid leukemia

BCR/ABL1 expression repressed spindle checkpoint components, including BUB3, to escape from metaphase arrest (Wolanin et al., 2010).

Colorectal cancer

Three missense variants in BUB3 [predicted to be pathogenic: c.790T>C (p.F264L), c.63G>C (p.K21N) and c.446G>A (p.R149Q)] were observed in familial or early onset colorectal cancer patients (n=185), which were not found in large cohort of control (n=1154) (de Voer et al., 2013). In agreement, Mur and colleagues (Mur et al., 2018) reported that BUB3 was found to be mutated [BUB3 c.77C>T (p.T26I)] in familial colorectal cancer.
**Gastric cancer**

High BUB3 gene expression was observed in 34 out of 43 gastric cancer tumors compared with their matched normal mucosa counterpart, which was associated with Ki-67 expression, but not with aneuploidy (Grabisch et al., 2003). The BUB3 polymorphism, rs7897156, did not impact gastric cancer susceptibility in a study including 164 gastric cancer patients and 381 ethnicity matched controls (Mesic et al., 2017).

**Glioblastoma**

he genetic variation C>T in the position -6 of the BUB3 gene, but not in coding sequence, was detected in 4 out of 22 glioblastoma samples (Reis et al., 2001). Bie and colleagues (Bie et al., 2011) reported that BUB3 was highly expressed in grade III and IV gliomas compared to normal brain samples. On the other hand, Morales and colleagues (Morales et al., 2013) reported a downregulation of BUB3 in primary samples and cell lines derived from glioblastoma compared to non-neoplastic white matter from epileptic patients. In BUB3-depleted U87MG glioblastoma cells, the expression of BUB3<sup>Y207F</sup>, which mimics an unphosphorylated form, strongly reduced tumor growth and improved survival compared to the expression BUB3<sup>WT</sup> in intracranial tumor mice model, indicating that BUB3 phosphorylation at Y207 site is required for tumorigenesis (Jiang et al., 2014).

**Lung cancer**

Initial observations did not identify genetic defects in BUB3 gene in lung cancer patients (Haruki et al., 2001). In cohort of 766 non-small cell lung cancer patients, the presence of allele T of polymorphism in BUB3 (rs7897156C>T) was associated with poor overall survival (Kang et al., 2017). Using H1299 and A549 non-small cell lung cell lines, functional studies based in luciferase assays indicated that T allele induced enhancement of BUB3 expression. In addition, increased BUB3 expression was observed in lung tumor tissues compared to non-malignant lung tissues (Kang et al., 2017).

**Osteosarcoma**

Loss of heterozygosity at 10q26, but not BUB3 mutations, was found in osteosarcoma samples (Mendoza et al., 2005). In Saos-2 osteosarcoma cells, TAp73x (an isoform of TP73) binds to BUB3 and BUB1, which leads to potential alteration of mitotic checkpoint function of these proteins and aneuploidy (Vernole et al., 2009). In U2OS osteosarcoma cells, but not normal fibroblast, BUB3 silencing reduces cell proliferation and clonogenicity and induces DNA fragmentation (Prinz et al., 2016).

**Ovarian cystadenoma**

In ML10 cells, a human ovarian cystadenoma model, BUB3 nuclear expression and phosphorylation were increased in low compared to high replicative age cells, which may be involved in prolonged mitotic arrest and cytokinesis failure observed in this cellular model (Austria et al., 2018).

**Pancreatic cancer**

A mutation in BUB3 (c.576+1G>A) was found among deleterious germline mutations (n=33) in sporadic pancreatic adenocarcinoma patients (n=854) (Shindo et al., 2017). In pancreatic cells, DMAP1/BUB3 complex repressed antiapoptotic genes transcription mediating mitotic stress-induced apoptosis and improved in vivo paclitaxel-induced tumor growth inhibition, which are impaired by SRC activity (Li et al., 2018).

**Renal cell carcinoma**

BUB3 gene expression profile was similar between samples from papillary renal cell carcinoma, chromophobe renal cell carcinoma and clear cell renal cell carcinoma patients and normal kidney tissues (Pinto et al., 2007; Pinto et al., 2008).

**To be noted**

BUB3 knockout mice presented accumulation of mitotic errors during embryogenesis and were unviable after day 6.5-7.5 postcoitus (Kalitsis et al., 2000). BUB3 haploinsufficiency led to chromosome instability and predisposition to carcinogen-induced, but not to spontaneous, tumors development in mice (Babu et al., 2003; Kalitsis et al., 2005; Rao et al., 2009). Induced-BUB3 mutations presented no effect on the genome rearrangements rate in Saccharomyces cerevisiae (Myung et al., 2001). In healthy humans, BUB3 genetic variations were not associated with non-specific chromosomal aberrations (Forsti et al., 2016).

**References**


(Adriamycin) and cyclophosphamide (cytoxan) (AC) response and resistance. Breast Cancer Res Treat. 2006 Feb;95(3):229-33


Morales AG, Pezuk JA, Brassesco MS, de Oliveira JC, de Paula Queiroz RG, Machado HR, Carlotti CG Jr, Neder L, de Oliveira HF, Scriddel CA, Tone LG. BUB1 and BUBR1 inhibition decreases proliferation and colony formation, and enhances radiation sensitivity in pediatric glioblastoma cells. Childs Nerv Syst. 2013 Dec;29(12):2241-8


Myung K, Datta A, Kolodner RD. Suppression of spontaneous chromosomal rearrangements by S phase checkpoint functions in Saccharomyces cerevisiae Cell 2001 Feb 9;104(3):397-408


Rao CV, Yamada HY, Yoo Y, Dai W. Enhanced genomic instabilities caused by deregulated microtubule dynamics and chromosome segregation: a perspective from genetic studies in mice Carcinogenesis 2009 Sep;30(9):1685-74


Seeley TW, Wang L, Zhen JY. Phosphorylation of human MAD1 by the BUB1 kinase in vitro Biochem Biophys Res Commun 1999 Apr 13;257(2):589-95


Sudakin V, Chan GK, Yen TJ. Checkpoint inhibition of the APC/C in HeLa cells is mediated by a complex of BUB1, BUB3, CDC20, and MAD2 J Cell Biol 2001 Sep 1;154(5):925-36

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Yim EK, Lee KH, Bae JS, Namkoong SE, Um SJ, Park JS. Proteomic analysis of antiproliferative effects by treatment of 5-fluorouracil in cervical cancer cells DNA Cell Biol 2004 Nov;23(11):769-76


