The Role of BUB3 in Human Cancers

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The BUB3 protein plays a key role in the activation of the spindle assembly checkpoint (SAC), a ubiquitous surveillance mechanism that ensures the fidelity of chromosome segregation in mitosis and, consequently, prevents chromosome mis-segregation and aneuploidy. Besides its role in SAC signaling, BUB3 regulates chromosome attachment to the spindle microtubules. It is also involved in telomere replication and maintenance. Deficiency of the BUB3 gene has been closely linked to premature aging. Upregulation of the BUB3 gene has been found in a variety of human cancers and is associated with poor prognoses.

Keywords: BUB3 ; spindle assembly checkpoint ; mitosis ; cancer ; senescence ; anticancer target

1. BUB3 in Aging

BUB3 shares extensive sequence homology with each of the four WD repeat motifs, and over the entire length of the RAE1 protein, indicative of functional similarity [1][2]. While BUB3 functions in the SAC pathway, RAE1 (also called Gle2 or mrnp41) is involved in mRNA export in interphase [2][3][4][5][6]. Binding to RAE1 is mediated by a GLEBS motif present in the nucleoporin Nup98 [5]. Strikingly, RAE1 also binds to the GLEBS motif of BUB1 [7]. The discovery that BUB3 also binds to the GLEBS motifs of the SAC proteins BUB1 and BUBR1 has led to the hypothesis that RAE1 might have a role as an SAC protein [7]. Homologous recombination-mediated mouse *Rae1* gene disruption showed that the loss of a single *Rae1* allele causes a SAC defect and chromosome mis-segregation. Besides the 34% identity and 52% similarity of the human RAE1 and BUB3, *Bub3* haploinsufficient cells exhibit a strikingly similar mitotic phenotype, suggesting that RAE1 and BUB3 are functionally analogous, namely, by playing a specific or perhaps a redundant role in BUB1 targeting to unattached kinetochores and subsequent SAC activation [8][9]. Interestingly, double *Rae1*/*Bub3* haploinsufficiency causes a much more severe chromosomal instability phenotype than single haploinsufficiencies, suggesting a cooperative role of RAE1 and BUB3 in regulating the SAC activities to prevent chromosomal mis-segregation [9]. Long-term phenotype analysis showed a reduced lifespan of mice harboring the combined *Bub3* and *Rae1* haploinsufficiency, with phenotypes associated with aging appearing early in double haploinsufficient mice, while mice with single *Bub3* or *Rae1* haploinsufficiency were viable and had a normal appearance [9][10][11]. Aneuploidy in single haploinsufficient *Bub3* or *Rae1* mice increased dramatically with age, and increased further in double *Bub3*/*Rae1* haploinsufficient mice [9][11][12]. Curiously, mice with single or combined disruption of *Bub3* and *Rae1* were not predisposed to spontaneous tumorigenesis. Instead, *Bub3*/*Rae1* haploinsufficiency caused early onset of cellular senescence, which was due to SAC weakening, rather than to aneuploidy itself. Since the age-associated phenotypes exhibited by haploinsufficient *Bub3*/*Rae1* mice also occur in very old wild-type mice, then *Rae1* and *Bub3* were proposed to accelerate the aging process. Molecularly, haploinsufficient *Bub3*/*Rae1* mice embryonic fibroblasts (MEFs) accumulate high levels of cellular senescence inductors, including p16, p19, p21, and p53, but, surprisingly, no major signs of apoptosis, suggesting that haploinsufficiency of *Bub3* and *Rae1* accelerates aging through induction of cellular senescence [9][10][11][12]. Significantly, and similarly to haploinsufficient *Bub3*/*Rae1* mice, hypomorphic *BubR1* mice develop several aging-associated phenotypes at a very young age, including cataracts, lordokyphosis, loss of subcutaneous fat, and impaired wound healing [13]. However, hypomorphic *BubR1* mice had a much earlier onset of aging phenotypes, with many more senescent cells, than haploinsufficient *Bub3*/*Rae1* mice, indicating that the rate of premature aging is correlated with the level of induction of senescence. Therefore, in addition to oncogenic transformation, accelerated aging seems to be another major biological manifestation of a weakened SAC [11][13]. What determines if it is oncogenic transformation or accelerated aging that will take place in a deficient SAC background is unknown. It might depend on the extent of SAC deficiency and/or SAC component depletion.

2. BUB3 in Cancer

Defects in SAC activity lead to chromosome mis-segregation, which is thought to be responsible, at least in part, for aneuploidy generation in human malignancies [14][15][16][17][18]. SAC deficiency is often associated with deregulated SAC

genes [14][16][18]. The researchers examined the expression of *BUB3* in various human cancer types. To this end, *BUB3* gene expression and clinical data for 35 cancer types retrieved from the UALCAN data portal (http://ualcan.path.uab.edu/index.html, accessed on 24 December 2021) were analyzed [19]. *BUB3* transcript levels were compared between cancers and normal tissue in 18 cancer types; 17 cancer types were excluded from the analysis due to lack of normal samples.

BUB3 protein levels are also elevated in a wide variety of human cancers compared to normal tissue (**Figure 1**). The researchers analyzed BUB3 protein levels in TP53-mutant cancers, as TP53-dependent SAC has been described [20][21][22]. TP53 is a transcription factor that acts as a tumor suppressor by inducing cell cycle arrest, cellular senescence, or apoptosis in response to cellular stresses, such as hypoxia, DNA and spindle damage [23]. *TP53* gene mutations are universal across cancer types, and this contributes to human cancers in different ways [24]. The TP53 pathway regulates the expression of a network of genes that are targeted to respond to a variety of intrinsic and extrinsic stress signals to ensure, among other things, accurate DNA replication, chromosome segregation, and cell division [25]. Interestingly, in most of the cancer types analyzed, BUB3 levels are significantly higher in *TP53*-mutant cancers than in *TP53*-wild-type cancers, suggesting that wild-type TP53 represses *BUB3* gene expression in physiological conditions, and that the TP53–BUB3 pathway may play an important role in carcinogenesis (**Figure 1**).

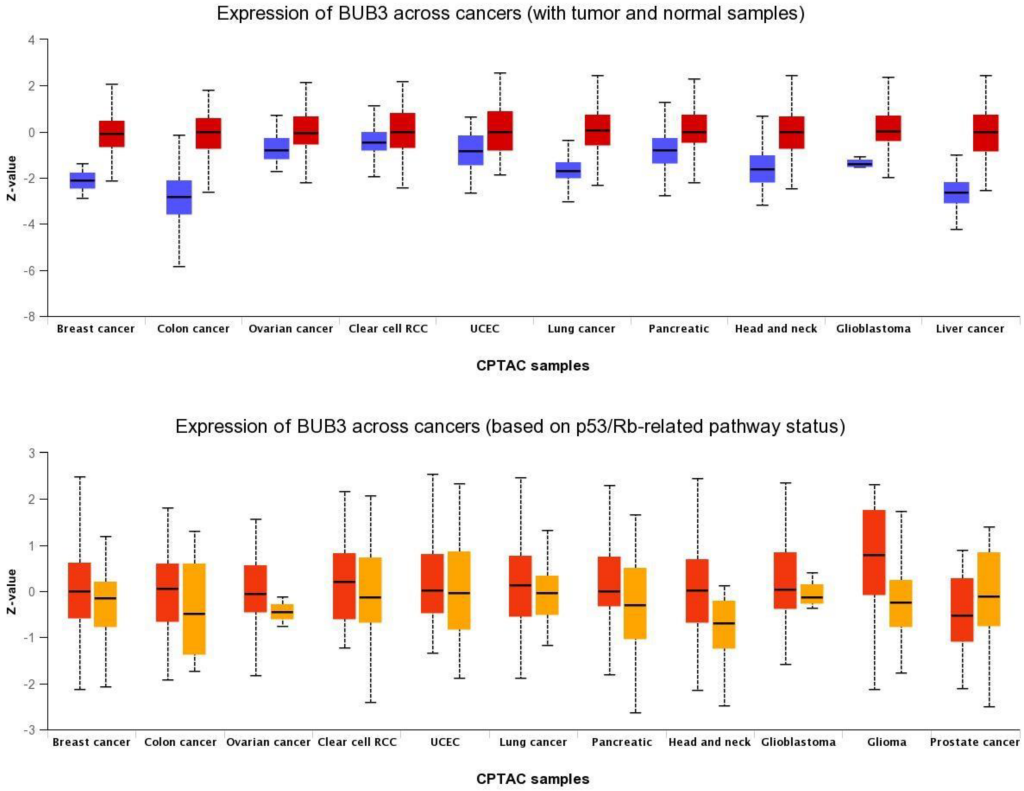

Figure 1. Pan-cancer view of expression of BUB3 protein across cancers. (**Upper panel**) Comparison between normal (blue) and primary tumors (red); (**lower panel**) Comparison between TP53-mutant (red) and TP53-non-mutant (orange) tumor samples. RCC: renal cell carcinoma; UCEC: Uterine corpus endometrial carcinoma; CPTAC: Clinical Proteomic Tumor Analysis Consortium. Data were retrieved from UALCAN portal (http://ualcan.path.uab.edu/index.html) on 24 December 2021.

Previous studies have reported *BUB3* overexpression, at both RNA and protein levels, in a variety of human cancers compared with normal tissue. In most cancers, this upregulation was associated with poor prognoses. The researchers reported that *BUB3* is upregulated and is associated with poor prognosis in oral squamous cell carcinoma [26]. The positive expression of cytoplasmic BUB3, together with that of cyclin B1 and the pituitary tumor-transforming gene 1, was significantly correlated with recurrence in prostate cancer [27]. *BUB3* was upregulated in 79% of gastric cancers, being a proliferation-dependent phenomenon in gastric cancer [28]. BUB3 levels were reported to be higher in sarcoma samples, and higher expression levels of BUB3 were associated with lower overall and disease-free survival in patients with sarcomas [29]. High expression of *BUB3* was associated with increased mortality in hepatocellular carcinoma [30]. In other studies, however, high protein expression of BUB3 in low-grade breast cancers was associated with longer overall survival, whereas lower expression resulted in poorer outcomes [31]. Upregulated *BUB3* was also reported in breast cancer samples [32]. Polymorphism in the *BUB3* gene was associated with the worst survival outcomes in early-stage non-small-cell lung cancer [33]. As with other SAC genes, epigenetic deregulation remains the most common alteration in the *BUB3* gene, while mutations at the sequence levels are rather rare and confer no increased cancer risk [14]. For instance,

genetic variation in the *BUB3* gene did not affect familial breast cancer risk, and mutations in the *BUB3* gene were shown to be rare in bladder tumors and glioblastomas [34][35][36]. Overall, these studies confirm that the overexpression of the BUB3 gene and protein is a common feature of human cancers, being associated with poor prognosis.

Why is *BUB3* overexpressed in cancer cells? This question still remains unanswered. *BUB3* and other SAC genes are frequently overexpressed in cancer, and such overexpression is correlated with chromosomal instability [37]. It was reported that loss of major tumor suppressor pathways, such as RB and TP53 pathways, can lead to transcriptional upregulation of SAC genes through E2F promoters and, subsequently, to chromosome mis-segregation [38][39][40]. As suggested by the researchers' analysis (**Figure 1**), TP53 loss could also lead to *BUB3* upregulation, which should fuel chromosomal instability in cancer cells.

The role of *BUB3* in carcinogenesis is still unclear. Contradictory results have been reported from animal models. For instance, haploinsufficiency of *Bub3* causes an increase in chromosome instability in mice, but is not clearly associated with the frequency or the rate at which tumors appear in the animal [41]. Analysis of mice with reduced levels of *Bub3* has shown that mice have significant increases in the number of aneuploid fibroblasts, and are predisposed to chemical-induced lung tumorigenesis rather than spontaneous tumor development [9]. A tumor suppressor role has been suggested for Bub3 in a *Drosophila melanogaster* tumorigenesis model derived from knocking down SAC genes [42]. Indeed, when transplanted into adult flies, Bub3-deficient tumors displayed neoplastic growth, widespread chromosomal aneuploidy, and high proliferative potential. Overall, these studies reveal that aneuploidy induced by *BUB3* downregulation might not be sufficient to initiate tumorigenesis but might still facilitate it.

3. BUB3 as an Anticancer Therapeutic Target

For many years, the role of BUB3 has been reduced to the recruitment of its partners BUB1 and BUBR1 to unattached kinetochores. Probably for this reason, BUB3 has not been regarded as a potential anticancer target. Nevertheless, and as referred to above, BUB3 itself has a specific role in regulating kinetochore–microtubule attachments, and is involved in telomere replication maintenance and premature aging [11][43][44]. Importantly, the *BUB3* gene is upregulated in most cancers studied, which is generally associated with poor outcomes. Thus, BUB3 is not just a simple partner, and its targeting deserves attention. Today, there are no small molecules against BUB3, and the unique attempt to target BUB3 makes use of RNAi [26]. The researchers here have shown that RNAi-mediated inhibition of BUB3 was cytotoxic to OSCC cells and enhanced their chemosensitivity to cisplatin [26]. This antiproliferative activity of BUB3 inhibition against OSCC cells was recently confirmed by another group [45]. Very recently, the researchers showed that inhibition of BUB3 compromises glioblastoma cell proliferation, mainly through senescence induction rather than by apoptosis, suggesting that premature senescence can be a viable approach to restrain cancer propagation [46]. Thus, oligonucleotide-based targeting of BUB3 could be a viable therapeutic approach. However, small-molecule inhibitors should be a better option due to RNAi security and stability issues. As BUB3 is a non-enzyme protein, and, thus, an “undruggable target”, the development of an anti-BUB3 drug may be a challenging task. To circumvent this, one should design small molecules that target protein–protein interactions to interfere with biological processes by modulating the formation of protein–protein complexes. In this sense, targeting the interaction of BUB3 with BUB1 and BUBR1 is an attractive option. This would prevent MCC formation, leading to SAC inactivation, which is expected to kill cancer cells as a consequence of massive chromosome mis-segregation. Strategies to mimic *Bub3*/*Rae1* haploinsufficiency in order to induce premature senescence of cancer cells should be explored. Indeed, cellular senescence has also been considered a suppressive mechanism of tumorigenesis, making therapy-induced senescence a plausible approach for cancer treatment, by irreversibly arresting the cell cycle [47].

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