

Loss of *LARP4B*, an early event in the tumorigenesis of brain cancer?

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In April 2016, Koso and colleagues from professor Sumiko Watanabe's group at University of Tokyo published a paper in *Cancer Research* describing *LARP4B* as a tumour suppressor gene (TSG) in glioma (1). Glioblastoma (GB) is the most common, aggressive and locally invasive form of glioma with a median survival from diagnosis of 12–15 months despite maximal treatment (2). Survival has improved little over the last 30 years and effective therapies are still a major unmet clinical need. Prof. Watanabe's group developed a two-step method based around *Sleeping Beauty* transposon mutagenesis to identify genes required for cancer initiation and progression. The group has previously used this technique to characterise bowel cancer development (3), but here they focused on gliomas. They first mutagenised neural stem cells (NSCs) *in vitro* into glioma-initiating cells that are believed to be the progenitors of gliomas. These cells were subcutaneously injected into the flanks of immunocompromised (SCID) mice to allow tumour development following a second round of mutagenesis. Using this technique, *LARP4B* was identified as one of over 80 likely TSGs capable of transforming NSCs (4). By interrogating The Cancer Genome Atlas (TCGA) database, the authors observed heterozygous *LARP4B* deletion in 80% primary GB samples in association with methylation-induced transcriptional silencing of the second *LARP4B* allele and observed a correlation between low *LARP4B* copy number and adverse patient survival outcome.

This is the first time the RNA binding protein (RBP) *LARP4B* has been associated with glioma. *LARP4B* was initially described in 2002 as a component of an mRNA translation-regulating complex in rat neuronal synapses

(5,6). *LARP4B* belongs to a seven-member family of La-related proteins (LARPs) and its closest paralogue is *LARP4* (also known as *LARP4a*) (7). The individual functions of members of this evolutionarily conserved family of proteins are gradually being elucidated. *LARP1*, 1B, 4, 4B and 6 are cytosolic factors involved in mRNA metabolism whilst *LARP3* and 7 primarily function in transcription regulation. *LARP4* and 4B have been shown to bind and stabilise target mRNAs via an N-terminal “La module”, an RNA-binding motif comprised of a La domain and an adjacent RRM conserved across every member of the LARP family. Both *LARP4* and 4B have also been separately identified as existing in complexes with poly(A)-binding protein (PABP) and the ribosome-associated factor RACK1. Whilst *LARP1*, *LARP3* (also known as “genuine La”) and *LARP6* appear to be proto-oncogenic, *LARP4* and 4B have tumour suppressive activity. In prostate and more recently breast cancer cell lines, depletion of *LARP4* has been shown to induce cell migration, invasion and increase cell motility (8,9). Here, Koso *et al.* demonstrate that over-expression of *LARP4B* suppresses tumour proliferation and induces mitotic arrest and apoptosis in glioma cell lines. After *LARP4B* over-expression, mRNA levels of the mitotic regulatory gene *CDKN1A* and the Bcl2 family member *BAX* are upregulated. Interestingly, these factors have also been described as *LARP4B* targets in HEK293 cells (10). This indicates that *LARP4B* controls their stability and/or translation.

The *LARP4B* phenotype is reminiscent of that of its cousin, *LARP1*. *LARP1* is a proto-oncogene that protects cancer cells from apoptotic cell death by directly binding multiple mRNAs including *BIK* and *BCL2* (11,12).

65 Whilst LARP1 destabilises the pro-apoptotic regulator
 66 *BIK*, it stabilises the anti-apoptotic *BCL2* with the net
 67 result of avoiding apoptosis in cancer cells in which
 68 LARP1 is strongly upregulated. As a tumour suppressor,
 69 LARP4B exerts the opposite effect by facilitating cell
 70 death through apoptosis. It is interesting to observe that
 71 a number of pro- and anti-apoptotic *BCL2*-related factors
 72 are present in the LARP4B interactome as defined by
 73 Photoactivatable Ribonucleoside-Enhanced Crosslinking
 74 and Immunoprecipitation (PAR-CLIP) (10). This suggests
 75 that, like LARP1, LARP4B might exert a dual effect on
 76 its target mRNAs, by stabilising the proapoptotic but
 77 destabilising the anti-apoptotic transcripts. The *BCL2*
 78 family of proteins that includes Bcl-xL (also known as
 79 *BCL2L1*), Bad, Bid, *BIK* and *BAX* are components of
 80 the intrinsic or mitochondrial pathway of cell death. On
 81 death signalling they translocate from the cytoplasm to the
 82 mitochondria to release cytochrome C and induce caspase-
 83 mediated apoptosis. It is interesting to note the presence
 84 of numerous mitochondrially-associated genes in both the
 85 LARP1 and LARP4B interactomes (10,11), particularly in
 86 light of the recent finding that *Drosophila* LARP1 acts
 87 to track mRNAs to the mitochondrial outer membrane
 88 whereupon they are released for localised protein
 89 synthesis (13). It is possible this mRNA-to-
 90 mitochondrial tracking function is conserved across all
 91 other translation-associated LARPs (including LARP1B,
 92 LARP4, LARP4B and LARP6), although this cannot be
 93 assumed with any certainty until their full interactomes
 94 are known.

95 The authors suggest that dysregulation of *LARP4B*
 96 expression is an early event in tumorigenesis. It is likely that
 97 all tumors undergo a pre-invasive period before becoming
 98 metastatic and invasive and knowledge of the cumulative
 99 events that drive malignant change are instrumental for
 100 screening and treatment. Stepwise tumorigenesis has been
 101 best characterised in colorectal cancer where morphological
 102 and genetic alterations occur sequentially (to genes
 103 including *APC*, *DCC* and finally *TP53*) as the preinvasive
 104 colonic polyp transitions to an invasive cancer. The location
 105 of gliomas within the cranial cavity and their tendency to be
 106 diagnosed at symptomatic presentation makes biopsy and
 107 pre-invasive characterization almost impossible *in vivo*; any
 108 understanding of the molecular events that precede GB are
 109 mainly derived from post-mortem studies. The strength of
 110 the mutagenesis system described here is its ability to re-
 111 capitulate the stepwise genetic changes that precede cancer
 112 development. But it raises the question of whether it gives

an accurate representation of events *in vivo*. It is known that
 extrinsic pressures such as the stromal and immunological
 milieu significantly contribute to tumorigenesis, in addition
 to competitive pressures within a heterogeneous population
 of cells. Also there is still debate as to the cell of origin
 for glioblastoma, it is probable that this differs between
 subtypes and that early genetic events are unlikely to be
 shared between them.

The gene encoding *LARP4B* is carried on the short
 arm of chromosome 10 at position 10p15.3. Loss of
 heterozygosity (LOH) of chromosome 10 is commonly
 observed in primary and secondary gliomas at frequencies of
 60–80%. This is caused by complete loss of the chromosome
 or deletion of three “hot spots”, one of which is 10p14–15,
 indicating that TSGs are carried in these loci. So far, those
 identified in 10p have included *KLF6* (found on 10p15.2)
 and *PFKFB3* (found on 10p15.1). Although deletion of
 chromosome 10 is more commonly observed in primary
 than secondary GBs, there is debate as to whether it is an
 early or late event. It is interesting to note that another gene
 identified from Koso’s mutagenesis screen was *MLLT10*
 also encoded on 10p (at 10p12.3) and previously associated
 (by SNP genotyping) with risk of meningioma (14). The
 data from other glioma studies support the authors’ findings
 that a TSG located at 10p (like *LARP4B*) is likely to be
 influential on tumour pathophysiology.

As information from TCGA and other sources has
 become available, gliomas have been subclassified into
 classical, proneural, neural and mesenchymal types. By
 correlating their genomic and morphological features,
 the authors aligned their mutagenized NSC cells with
 the “mesenchymal” subtype of GB. This subtype is
 characterised by frequent mutations to *NF1*, *PTEN* and
TP53 as well as upregulation of genes associated with
 glycolysis and gluconeogenesis (e.g., *ALDH1A*) (15).
 Glycolysis and gluconeogenesis are both metabolic
 processes and, like the apoptotic regulators, genes encoding
ALDH1A and other glucose-metabolism proteins are
 strongly enriched within the LARP4B interactome. Again
 this would position LARP4B within this cancer subtype
 and perhaps indicate that loss of *LARP4B* could activate
 tumorigenesis by de-repressing the expression of these
 genes.

While the authors find *LARP4B* deletion in 80% of GB
 cases, it is surprising this aberration has not previously
 been described in large genome-wide mapping studies
 in glioma (16). For many years, genes encoding RBPs
 were thought to have insignificant roles in pathogenesis

and were largely over-looked. Work by Vogel (17) indicated a disparity between levels of gene and protein expression in GB cell lines suggesting a significant post-transcriptional contribution to gene expression. This contradiction to the central dogma is becoming accepted in cancer where dysregulation of RBPs, splicing factors, long non-coding (lnc) and micro (mi) RNAs are increasingly recognised as altering if not dominating gene expression (18). It is therefore possible that RBPs identified in previous tumor mapping studies were disregarded. Alternatively, the expression of many RBPs is post-transcriptionally regulated (often they are self-regulated) and they are infrequently mutated and therefore genetic analyses and/or mutational screens would fail to identify them. In that respect *LARP4B* is unusual as deletion/mutation of LARPs has not yet been convincingly shown in other diseases.

That a methylation “hit” to the second allele of *LARP4B* is required to inactivate the gene indicates a potential therapeutic avenue to reactivate *LARP4B* using methylation inhibitors. Epigenetic changes are frequently observed in tumor initiating cells and these cells can be reprogrammed by removal of the cancer specific methylation marks (19). In the context of an established tumor, it is unclear whether restoring one allele of *LARP4B* is likely to have any clinical impact. Without knowing the chronology of genetic mutations it is difficult to know whether extrinsic *LARP4B* is likely to reverse changes or whether its functional relevance has diminished as genetic alterations accumulate in the *in vivo* setting.

The LARP family are emerging as having fundamental roles in tumorigenesis and as potential drug targets. RBPs like the LARPs are powerful targets as they regulate the expression of entire networks of proteins and can significantly alter the behaviour of a cancer cell. It is possible that, with advances in whole proteome sequencing, where proteomics and genomics can be conducted in parallel, a more comprehensive picture of cancer biology will emerge. With this, the role of genes such as *LARP4B* will become more evident. Thus the development of drugs that target post-transcriptional regulators like *LARP4B* could broaden the therapeutic options for many cancers that have failed to benefit from the genome-centric approach that has hitherto dominated drug discovery. Here, Koso and team present intriguing evidence that loss of *LARP4B* tumor suppressor function is an important early event that occurs during glioma tumorigenesis.

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Footnote

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