



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

The authors suggest that dysregulation of *LARP4B* expression is an early event in tumorigenesis. It is likely that all tumors undergo a pre-invasive period before becoming metastatic and invasive and knowledge of the cumulative events that drive malignant change are instrumental for screening and treatment. Stepwise tumorigenesis has been best characterised in colorectal cancer where morphological and genetic alterations occur sequentially (to genes including *APC*, *DCC* and finally *TP53*) as the preinvasive colonic polyp transitions to an invasive cancer. The location of gliomas within the cranial cavity and their tendency to be diagnosed at symptomatic presentation makes biopsy and pre-invasive characterization almost impossible *in vivo*; any understanding of the molecular events that precede GB are mainly derived from post-mortem studies. The strength of the mutagenesis system described here is its ability to re-

capitulate the stepwise genetic changes that precede cancer 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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