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Loss of p53 in quaking viable mice leads to Purkinje cell defects and reduced survival

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The *qk*^{v} mutation is a one megabase deletion resulting in abnormal expression of the *qkI* gene. *qk*^{v} mice exhibit hypomyelination of the central nervous system and display rapid tremors and seizures as adults. The *qkI* locus on 6q26-27 has also been implicated as a candidate tumor suppressor gene as the *qkI* locus maps to a region of genetic instability in Glioblastoma Multiforme (GBM), an aggressive brain tumor of astrocytic lineage. As GBM frequently harbors mutations affecting *p53*, we crossbred *qk*^{v} and *p53* mutant mice to examine whether *qk*^{v} mice on a *p53*$^{-/-}$ background have an increased incidence of GBM. *qk*$^{v/v}$*; p53*$^{-/-}$ mice had a reduced survival rate compared to *p53*$^{-/-}$ littermates, and the cause of death of the majority of the mice remains unknown. In addition, immunohistochemistry revealed Purkinje cell degeneration in the cerebellum. These results suggest that *p53* and *qkI* are genetically linked for neuronal maintenance and survival.

The study of neurological diseases has been greatly advanced with the use of mouse models. In particular, spontaneously occurring mutant mice have played an important role in identifying key components required for proper myelination. One such mouse, the autosomal recessive *qk*^{v} mutant, was first described in 1964 by Sidman and colleagues. The *qk*$^{v/v}$ mouse exhibited severe hypomyelination of the central and peripheral nervous system and severe hindlimb shaking[1]. The mutation responsible for the *qk*$^{v/v}$ phenotype was later identified as a one megabase deletion that results in loss of *parkin* (*park2*) and *parkin co-regulated* (*pacrg*) function, whereas *qkI* expression becomes abnormally expressed[2–4]. In *qk*$^{v/v}$ mutants, *qkI-6/7* expression is preferentially lost in myelin producing cells of the central and peripheral nervous systems, while for the most part expression remains unaffected in all other cell types[3]. The *qkI* gene encodes for the alternatively spliced KH domain RNA binding proteins QKI-5, -6, and -7[5]. QKI has been shown to regulate important mRNA targets involved in myelination and oligodendrocyte differentiation[6–11]. QKI is implicated in myelinogenesis by stabilizing myelin basic protein (MBP) mRNA[6,12], regulating the alternative splicing pattern of myelin associated glycoprotein (MAG)[10,13], and oligodendrocyte differentiation and Schwann cell differentiation[7,14]. The over-expression of QKI-6 in *qk*^{v} mutants has been shown to rescue the myelination defect[15].

In addition to myelination defects, *qk*^{v} mice also exhibit ciliopathies including male sterility and mild hydrocephalus[16–17]. Cilia lining the ventricles of the brain are responsible for proper circulation of cerebral spinal fluid (CSF). Ciliogenesis is normal in *qk*^{v} mice, however the cilia are functionally impaired leading to decreased CSF flow and hydrocephalus[16]. Hydrocephalus in *qk*^{v} mice becomes fatal on a *patched1* (*ptch1*) heterozygous background[18].

The long arm of chromosome 6 is known to harbor a common fragile site that is frequently lost or mutated in a variety of cancers[19–20]. The breakpoint includes several genes, one of which has been identified as the *qkI* gene[21–22]. Chromosomal aberrations at 6q26-27 are common in Glioblastoma Multiforme (GBM)[21]. In addition to mapping to a region of genomic instability, expression of *qkI* mRNA transcripts have been shown to be altered specifically in human gliomas compared to other brain cancers[23]. Thus, it has been proposed that QKI may function as a tumor suppressor and play a role in GBM progression. Evidence for a suppressor role was shown for the *C. elegans* GLD-1, a QKI homolog[24]. The GLD-1 *C. elegans* homolog of QKI is known to associate with the p53 cep-1 mRNA, influencing its activity[25]. In addition, QKI was discovered to be down-regulated in colorectal cancers[26]. QKI over-expression in the colon epithelium resulted in increased levels of p27^{kip1} as well as an increase in membrane bound β-catenin, an indicator of gastric cell differentiation[26]. These observations along with our data

that QKI-6/-7 cause cell cycle arrest[7], a property often observed for tumor suppressors, suggest that the absence of QKI proteins may promote tumorigenesis.

Loss of heterozygosity and mutations of the *p53* gene is commonly associated with a variety of cancers in multiple organ sites[27]. Individuals with the Li Fraumeni syndrome, in part caused by mutations in the *p53* gene, suffer from early onset of many different types of cancer[28]. Mutations abrogating p53 function and allelic loss of chromosome 17p were among the first genetic lesions identified in GBM[29]. *p53* lesions are present in all grades of astrocytoma at an average of 30%[30], suggesting that the inactivation of *p53* is an early event in gliomagenesis. $p53^{+/-}$ and $p53^{-/-}$ mice have been found to develop a broad spectrum of tumors, including lymphomas, osteosarcomas, and fibroscarcomas[31]. $p53^{+/-}$ and $p53^{-/-}$ mice have been used as a background strain to breed with other mutant strains to produce entirely novel tumor spectrums that were not found in mice with a mutation in *p53* alone[32]. Although $p53^{+/-}$ mice showed no incidence of any brain malignancy[31,33], $nf1^{+/-}$; $p53^{+/-}$ mice developed astrocytomas with a penetrance of 92% by 6 months of age[34].

Here we report that $qk^{v/v}$ mice on a $p53^{-/-}$ background had a reduced survival rate compared to $qk^{v/+}$; $p53^{-/-}$ and $p53^{-/-}$ controls, and the cause of death of the majority of the mice remains unknown. $qk^{v/v}$; $p53^{-/-}$ mice also displayed neurological defects including Purkinje neuron degeneration.

Results

The $qk^{v/v}$ mice contain a recessive autosomal mutation of ~1.1 Mb on chromosome 17 affecting the expression of *quaking* (*qkI*), *parkin-coregulated gene* (*pacrg*) and *parkin* (*park2*)[4]. The mutation results in aberrant expression of alternatively spliced *qkI* transcripts, leading to the dysmyelination defects observed in the $qk^{v/v}$ mice[4]. Deletion of the *pacrg* gene in qk^{v} mice causes ciliopathies including hydrocephalus[16] and male sterility[35]. To examine whether the *p53* pathway genetically interacts with the *pacrg-parkin-qkI* locus in the regulation of the phenotypes of the $qk^{v/v}$ mice, we crossbred the $qk^{v/v}$ mice with *p53* null mice. We initially assessed the myelin sheath of resulting $qk^{v/v}$; $p53^{-/-}$ progeny. Post-natal day 30 (P30) coronal sections of the corpus callosum were stained with an antibody specific to myelin basic protein (MBP). As expected, $qk^{v/v}$ mice showed reduced thickness of the corpus callosum (98.8 ± 16.4 μm) compared to *wild-type* (*wt*) (172.7± 7.03 μm) and $qk^{v/+}$; $p53^{-/-}$ (191.8 ± 5.99 μm) littermates (Figure 1). However, $qk^{v/v}$; $p53^{-/-}$ mice did not show an increased severity of hypomyelination compared to $qk^{v/v}$ mice (86.9 ± 8.89 μm, Figure 1), and corpus callosum thickness was not found to be statistically different between the mice ($p = 0.287$). Although corpus callosum thickness was reduced in mice with the qk^{v} mutation, sparse high intensity MBP staining was observed along the myelin tracts. These MBP-positive "blebs" have previously been observed in spinal cord white matter of $qk^{v/v}$ mice, corresponding to redundant loops of uncompacted myelin[36]. These results suggest that *p53* does not cooperate with *qkI* in the regulation of myelin formation *in vivo*. The loss of both *wt p53* alleles did not affect the onset of hindlimb shaking in mice pups, since both $qk^{v/v}$; $p53^{-/-}$ mice, $qk^{v/v}$; $p53^{+/-}$ mice and $qk^{v/v}$ mice displayed tremors by P14 (data not shown). Common husbandry procedures and animal handling are sources of acute stress and elicit tonic clonic seizures in qk^{v} mice[37–39]. The onset of stress induced tonic-clonic seizures in $qk^{v/v}$; $p53^{-/-}$ mice was significantly earlier than that of $qk^{v/v}$ mice. $qk^{v/v}$ mice began demonstrating stress-induced seizures at 12 weeks of age, whereas $qk^{v/v}$; $p53^{+/-}$ mice and $qk^{v/v}$; $p53^{-/-}$ mice were observed to have seizures and ataxic movements as early as 4 weeks of age (data not shown). As expected, mice heterozygous for the qk^{v} mutation did not demonstrate tonic-clonic seizures or hindlimb shaking.

We assessed the cellular morphology of Purkinje cells by immunostaining P30 coronal brain sections with an antibody against Calbindin, a Purkinje neuron-specific marker. Immunostaining of the brains of $qk^{v/v}$; $p53^{-/-}$ mice revealed Purkinje cell body loss as well as loss of Calbindin-positive Purkinje cell dendritic arbors, whereas control mice demonstrated normal Purkinje cell appearance

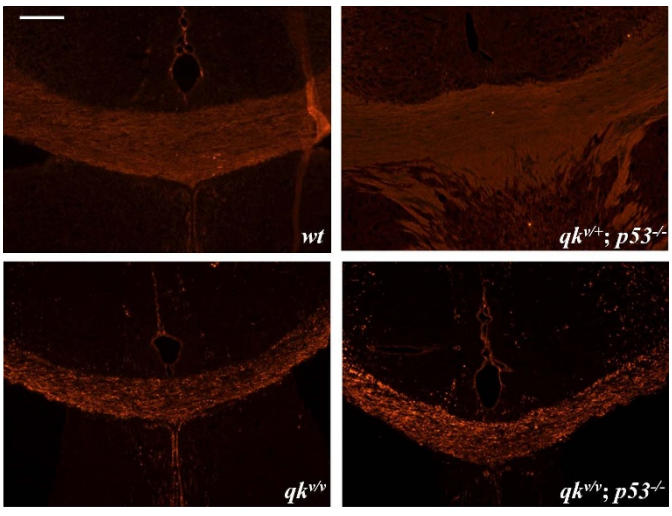

Figure 1 | qk^{v}-associated hypomyelination is not exacerbated on a $p53^{-/-}$ background. Brains were frozen over acetone-dry ice and cryostat sectioned at a thickness of 10 μm. Coronal sections of age and sex-matched mouse cortexes were stained with anti-MBP antibody. Scale bar represents 100 μm.

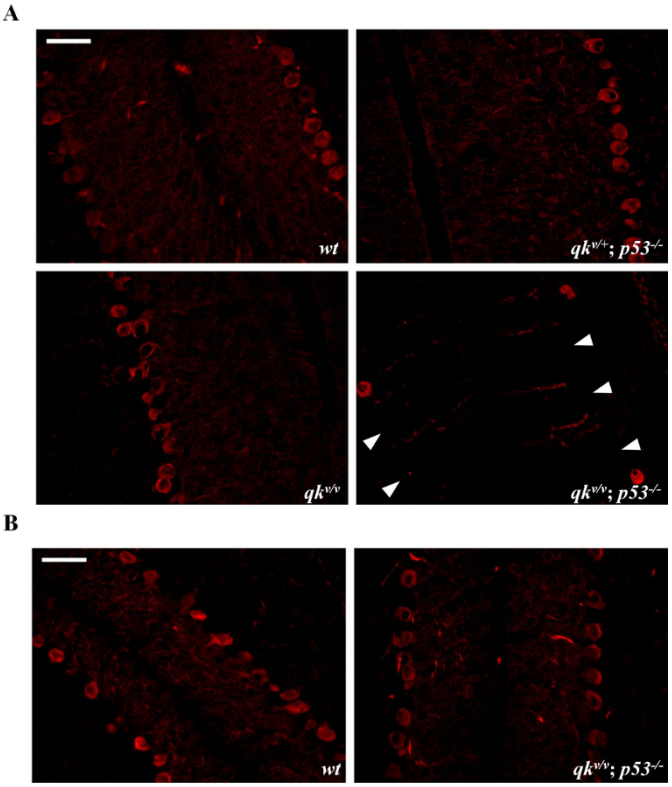

Figure 2 | $qk^{v/v}$; $p53^{-/-}$ mice display Purkinje cell defects. (A) $qk^{v/v}$; $p53^{-/-}$ mice display Purkinje cell body and dendritic arbor loss at P30 (arrows), whereas control littermates exhibit normal cerebellar architecture. Sections were stained with anti-Calbindin antibody. Scale bar represents 50 μm. (B) $qk^{v/v}$; $p53^{-/-}$ show normal Purkinje cell morphology of the cerebellum at P14. Coronal sections of the cerebellum were stained with anti-Calbindin antibody. Scale bar represents 50 μm.

(Figure 2A). In order to quantitatively assess differences in Purkinje cell numbers, we analyzed the Purkinje cell linear density of P30 mice. $qk^{v/v}$; $p53^{-/-}$ mice were found to have a significantly lower Purkinje cell density (16.36 ± 1.42 cells/mm) compared with *wt* (38.77 ± 2.52 cells/mm; $p < 0.001$), $qk^{v/+}$; $p53^{-/-}$ (42.45 ± 6.5 cells/mm; $p = 0.006$), and $qk^{v/v}$ controls (40.16 ± 7.9 cells/mm; $p = 0.01$). Misplaced Purkinje neurons have occasionally been observed in $qk^{v/v}$ mice[40]. To determine if the Purkinje defects observed were due to impaired migration or other developmental defects, immunostaining was performed on brains of P14 mice. Purkinje cell morphology was normal in $qk^{v/v}$; $p53^{-/-}$ P14 mice, suggesting that Purkinje cell loss was not due to failure in neuronal migration at earlier stages of development (Figure 2B). These results suggest that the Purkinje cell defects observed in $qk^{v/v}$; $p53^{-/-}$ mice were not due to impaired development, but cell body and dendrite degeneration and defects in neuronal maintenance and survival. Indeed, *qkI* has been shown play a role in apoptosis in 3T3 mouse fibroblasts, HeLa cells, and primary rat oligodendrocytes[41–42].

$p53^{+/-}$ mice develop primarily sarcomas, with an incidence of 28% over 17 months. $p53^{-/-}$ mice have a much accelerated rate of tumorigenesis, with the majority of mice succumbing to lymphomas by six months of age[31]. *qkI* has been implicated as a candidate tumor suppressor gene as the *qkI* locus maps to a region of genetic instability in GBM[22], therefore we examined whether $qk^{v/v}$; $p53^{-/-}$ mice would display a higher incidence of brain tumors and thus may die earlier than mice deficient for *p53* alone. Kaplan-Meier curve analysis was performed in order to compare mouse survival rates (Figure 3A). In agreement with previous observations[1], none of the $qk^{v/v}$ mice cohort succumbed to tumor formation at the end of the 261 day observation period. Overall, $qk^{v/v}$; $p53^{-/-}$ mice were found to have a reduced survival time compared to the other groups, with a median survival time (MST) of 119 days. $qk^{v/v}$; $p53^{-/-}$ mice showed a significantly reduced survival time compared to $qk^{v/+}$; $p53^{-/-}$ mice (MST = 142 days; $p = 0.0135$) as well as $p53^{-/-}$ mice (MST = 172 days; $p = 0.0443$) according to the log rank/Mantel Cox test. There was no significant difference between the survival rates of $qk^{v/+}$; $p53^{-/-}$and $p53^{-/-}$ mice according to the Mantel Cox test ($p = 0.9175$).

Previous studies have shown that the majority of $p53^{-/-}$ mice develop tumors of the lymphatic system and soft tissue sarcomas, with only one instance of a brain tumor reported in a $p53^{+/-}$ mouse[31,33]. Similarly, the $p53^{-/-}$ and $qk^{v/+}$; $p53^{-/-}$ mice we generated developed primarily sarcomas and lymphomas, with no occurrence

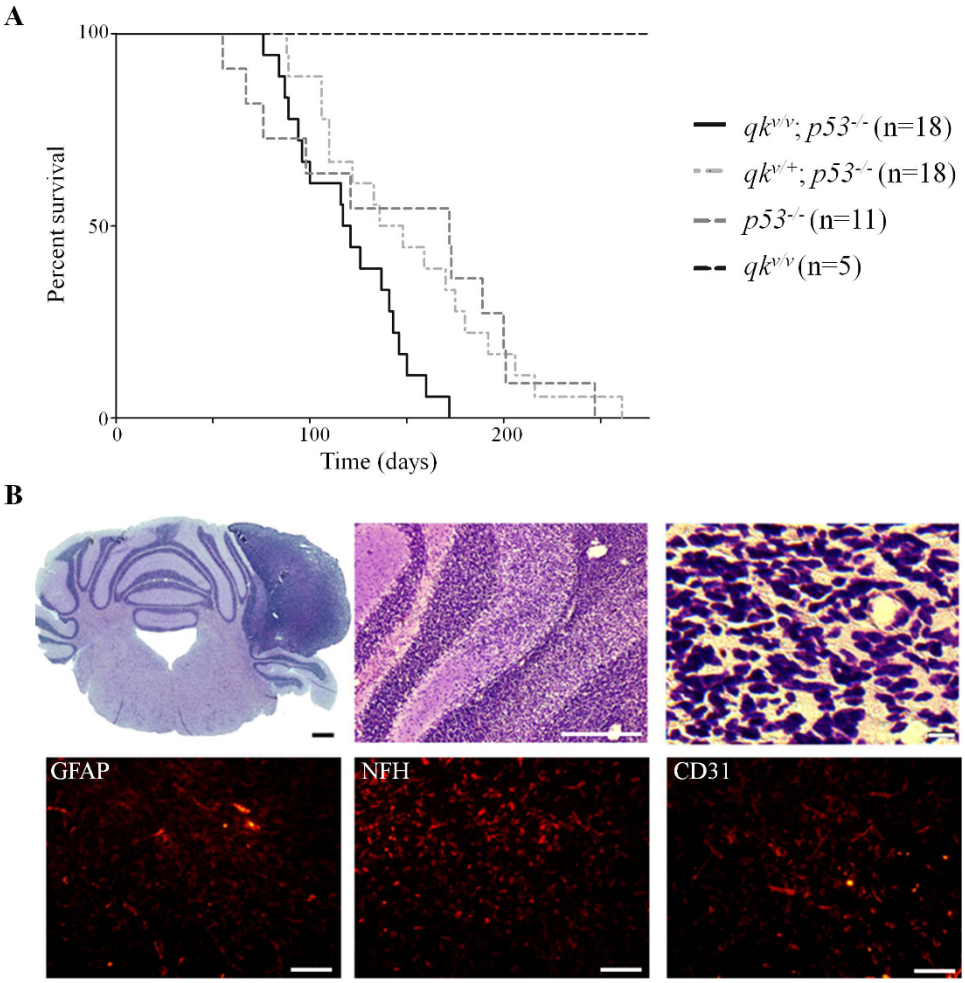

Figure 3 | $qk^{v/v}$ homozygous mice show a reduced survival rate in the absence of *p53*. (A) $qk^{v/v}$; $p53^{-/-}$ mice ($n = 18$) were observed to have a median survival time of 119 days, significantly lower compared to $qk^{v/+}$; $p53^{-/-}$ mice (142 days, $n = 18$) and $p53^{-/-}$ mice (172 days, $n = 11$) according to the log rank/Mantel Cox test ($p = 0.0135$ and $p = 0.0443$, respectively). The loss of survival was measured by mice that were found dead or that had to be sacrificed due to illness according to the guidelines of the Canadian Animal Care Committee. (B) Low-power magnification showing macroscopic appearance of medulloblastoma occurring in the cerebellum, with an arrow denoting the tumor area. Higher magnification of medulloblastoma, demonstrating the invasion of the molecular layer by the over proliferation of granular like tumor cells. Section was stained with H&E. Immunohistochemistry performed on the medulloblastoma reveals tumor reactivity to GFAP, NFH, and CD31, specific markers for different cell lineages.

of any brain malignancies (Table 1). In contrast, none of the $qk^{v/v}$; $p53^{-/-}$ mice demonstrated any occurrence of sarcomas, lymphomas, or tumors previously described in $p53^{-/-}$ mice (Table 1). Several $qk^{v/v}$; $p53^{-/-}$ and $qk^{v/v}$; $p53^{+/-}$ mice displayed neurological symptoms characteristic of hydrocephalus. Brains examined from these mice showed enlarged lateral ventricles with an accumulation of CSF, similar to the hydrocephalic phenotype observed in $qk^{v/v}$; $ptch1^{+/-}$ mice[18]. The brain of one $qk^{v/v}$; $p53^{-/-}$ mouse showed increased vascularization of the cerebellum and abnormal gross cerebellar architecture. Further histological analysis of cerebellar sections revealed granule cell layer invasion of the surrounding parenchyma, consistent with medulloblastoma (Figure 3B). Cytological analysis confirmed the presence of medulloblastoma, as tumor cell nuclei were densely packed and polygonal in shape. Tumor cells were also found to express markers of astrocytic (GFAP), neuronal (NFH) and vascular (CD31) lineage (Fig. 3B). Cells expressing markers of multiple lineages indicate cells of a stem cell precursor nature, consistent with primitive neuroectodermal tumors such as medulloblastoma. Medulloblastoma has not previously been observed to occur spontaneously in either $p53^{-/-}$ or $qk^{v/v}$ mice[1,31]. One study identified only one instance of a brain malignancy out of a cohort of $p53^{+/-}$ mice ($n = 40$), whereas another study did not observe any tumors of the nervous system in a cohort of $p53^{-/-}$ mice ($n = 35$) or $p53^{+/-}$ ($n = 96$) mice[31,33]. The one $p53^{+/-}$ brain tumor identified was an ependymoma, a tumor originating from the walls of the ventricular system[43]. *p53* mutations contribute to approximately 10% of human medulloblastoma cases[44]; in addition, many mouse models of medulloblastoma have been developed by the combination of a specific tumor suppressor mutation and the loss of *p53*[32]. Although *qkI* has been mapped to the common fragile site 6q25-26 in GBM, loss of 6q25-26 in medulloblastoma is rare[45], suggesting that somatic *qkI* mutations in clinical medulloblastoma do not confer any specific tumorigenetic advantage. QKI has been shown to be expressed in post-natal neural progenitor cells of the SVZ[46], suggesting that QKI may also be expressed in other populations of multi-potent precursor cells including the rostral rhombic lip progenitor cells that give rise to granule neuronal precursor cells. It has not been determined if the qk^{v} mutation affects QKI expression in neural progenitor cells including granule neuronal precursor cells, therefore it remains a possibility that the loss of *qkI-6/7* in the absence of *p53* could provide sufficient oncogenic stress to induce transformation.

Discussion

Our study indicates that $qk^{v/v}$; $p53^{-/-}$ mice do not have decreased corpus callosum thickness or MBP-staining compared to $qk^{v/v}$ littermate controls, suggesting that the qk^{v}-associated myelination defect is not further impaired by the loss of *p53*. These findings indicate that *qkI* does not cooperate with *p53 in vivo* to regulate the oligodendrocyte differentiation pathway. $qk^{v/v}$; $p53^{-/-}$ mice displayed Purkinje-cell defects in the cerebellum characterized by dendritic arborization defects and cell body loss, suggesting that *qkI* is required for neuronal cell maintenance in the absence of *p53*. However, the exact mechanism by which this phenotype occurs has yet to be elucidated. Cerebellar defects have previously been documented in mice homozygous for the qk^{v} mutation. Three month old $qk^{v/v}$ mice displayed axonal swellings in both the Purkinje and granular cell layer, indicative of axonal injury[40]. Axonal swelling is characteristic of inflammatory lesions in Multiple Sclerosis and experimental autoimmune encephalitis, suggesting that axonal pathology is secondary to myelination defects[48–49]. While our work showed that the qk^{v} dysmyelinating phenotype was not exacerbated on a $p53^{-/-}$ background, Purkinje cell degeneration was only observed in $qk^{v/v}$; $p53^{-/-}$ mice and not $qk^{v/v}$ controls, suggesting that the neuronal pathology was not due to myelination defects. Interestingly, $qk^{v/v}$; $p53^{-/-}$ mice displayed stress-induced tonic-clonic seizures and ataxic movements at about one month of age that was not observed in their $qk^{v/v}$ counterparts. Disruption in normal cerebellar architecture is one cause of ataxia[50], thus Purkinje cell degeneration may be related to the ataxic-like phenotype observed in $qk^{v/v}$; $p53^{-/-}$ mice. Indeed, 29 QKI-interacting partners were identified in a protein interaction network generated for human inherited cerebellar ataxias[51].

$qk^{v/v}$; $p53^{-/-}$ mice failed to develop tumors characteristic of $p53^{-/-}$ mice, but demonstrated a reduced survival rate compared to both $qk^{v/+}$; $p53^{-/-}$ and $p53^{-/-}$ mice. One case of medulloblastoma was documented in a cohort of $qk^{v/v}$; $p53^{-/-}$ mice (n = 11), while 2 other mice demonstrated signs of hydrocephalus. Recently, we have shown that $qk^{v/v}$ mice on a $ptch1^{+/-}$background have a reduced survival rate due to the development of fatal hydrocephalus[18]. A mild hydrocephalic phenotype occurs in qk^{v} mice due the loss of PACRG expression in the ciliated ependymal cells lining the ventricular walls, leading to cilia dysfunction[16]. Similarly, $qk^{v/v}$; $ptch1^{+/-}$ mice showed abnormal cilia function, leading to accumulation of CSF in the ventricles and hydrocephalus[18].

The majority of $p53^{-/-}$ and $qk^{v/+}$; $p53^{-/-}$ mice in our study succumbed to thymic lymphoma, demonstrating clear symptoms including difficulty breathing due to tumor-induced compression of the lungs, enlarged ribcages, and lethargy due to tumor burden. Necropsy of these mice revealed highly vascularized, enlarged thymuses that completely encompassed the heart and the majority of the lungs, and in some instances, extended outside the ribcage into the subcutaneous layers. These symptoms were not observed in any of the $qk^{v/v}$; $p53^{-/-}$ mice before death, and necropsy revealed normal thymic morphology. In addition, necropsy of $qk^{v/v}$; $p53^{-/-}$ mice did not reveal the presence of any overt cancers. Thus, the cause of death of the majority of the mice remains undetermined. Several possible explanations exist for the reduced survival rate observed in $qk^{v/v}$; $p53^{-/-}$ mice. The $qk^{v/v}$ mutation results in brain-specific loss of *qkI* isoforms *-6/7*, resulting in preferential sensitization of the nervous system to tumorigenesis in the absence of *p53*. As mentioned earlier, our post-mortem pathological observation methods were limited, thus it is possible that $qk^{v/v}$ mice developed additional brain tumors that remained undetected, including medulloblastomas. Although we suspect that *qkI* and *p53* participate in different signaling pathways since double mutant $qk^{v/v}$; $p53^{-/-}$ mice displayed a novel phenotype not observed in either parent strain, a bioinformatics analysis has identified *p53* as a putative target of QKI in mice. The *p53* mRNA harbors a putative QRE (QUAKING Response Element) within its 3'UTR (UACUAACnnnnGAAG)[47]. These findings indicate that QKI may regulate *p53* mRNA levels *in vivo*. Consistent with these findings, the *C. elegans* homolog of

Table 1 | Tumors in $qk^{v/v}$; $p53^{-/-}$ mice [a]

Genotype	Total no. of mice	Brain tumors	Hydrocephalus	Subcutaneous tumors	Thymic lymphoma	Intraperitoneal tumors	Other
$qk^{v/v}$; $p53^{-/-}$	11	1(11%)	2(18%)	0	0	0	0
$qk^{v/v}$; $p53^{+/-}$	33	0	5(15%)	0	0	0	0
$qk^{v/+}$; $p53^{-/-}$	12	0	0	2(15%)	8(61%)	3(23%)	0
$p53^{-/-}$	9	0	0	0	5(55%)	3(33%)	1(11%)

a. tumors were classified according to tumor location.

QKI, GLD-1, is known to associate with the *p53 cep-1* mRNA and regulate its activity[25].

In conclusion, QKI proteins are highly conserved among species and have been implicated in multiple diverse biological pathways, highlighting the pivotal role of RNA binding proteins in cellular function. Our data suggests that *qkI* interacts with *p53 in vivo* to regulate neuronal maintenance and survival.

Methods

Antibodies. Monoclonal MBP and NFH antibodies were obtained from Sternberger Monoclonals (Baltimore, MD). Monoclonal Calbindin-D-28K and GFAP antibodies were purchased from Sigma (St. Louis, MO). Anti-CD31 was purchased from Millipore (Billerica, MA). Antibodies against QKI-5,-6, and -7 were generated as described previously[7,42].

Animals. Mice were monitored daily and were handled and sacrificed in accordance with a protocol approved by the Animal Care Committee at McGill University. The mice were housed in ventilated cages with a 12/12 hour light/dark cycle. $p53^{-/-}$ mice (lab stock # 002101) and qk^{v} mice (lab stock # 000506) were obtained from The Jackson Laboratory (Bar Harbor, ME). Mice colonies were maintained on a C57BL/6 background. Mice homozygous for the *p53* mutation were crossbred to heterozygous qk^{v} mice and subsequent trans-heterozygous crosses were used to generate $qk^{v/+}$; $p53^{-/-}$ males. These males were subsequently crossbred to trans-heterozygous females to generate double mutant mice. Mice were screened for the *p53 wild-type* allele via genomic PCR using oligonucleotides (5′-ATA GGT CGG CGG TTC AT-3′) and (5′-CCC GAG TAT CTG GAA GAC AG-3′). The mutant *p53* allele was identified using the following oligonucleotides: (5′-CTT GGG TGG AGA GGC TAT TCG-3′) and (5′-GTC GGG CAT GCG CGC CTT GAG-3′). The qk^{v} mutation was amplified using primers directed against the breakpoint (5′-TCT AAA GAG CAT TTT CGA AGT-3′) and (5′-TTG CTA ACT GAA TAT TAC T-3′).

Immunohistochemistry. Mice were anaesthetized with isoflurane and perfused with ice-cold phosphate buffered saline followed by 4% paraformaldehyde. Brains were cryoprotected in 30% sucrose overnight and embedded in OCT compound (Tissue-Tek, Markham, ON) over dry ice in acetone. Tissues were cryostat sectioned at a thickness of 10 μm and collected on +/+ slides (Fisher, Ottawa, ON). Tissue sections were blocked in 10% goat serum in Tris-buffered saline + 0.5 % Triton X-100 for 1 hr followed by incubation with primary antibodies overnight at room temperature. Slides were incubated with Alexa-flour 488 or 546 Immunoglobulin G (Invitrogen, Carlsbad, CA) at a dilution of 1:400 for 4 hr. Corpus callosum thickness was visualized by MBP staining and quantified for 4 different areas using Axiovision software (Zeiss). P14 and P30 mice cerebellar coronal sections were evaluated at bregma ~−5.8 mm, and representative images were taken of the simple lobule adjacent to the primary fissure. Purkinje cell linear density was quantified for 3 different areas using Axiovision software. Purkinje cell linear density was quantified as Calbindin-positive cell bodies/mm Purkinje cell layer. Statistical significance was calculated according to a two-sample two-tailed paired student *t*-test.

1. Sidman, R. L., Dickie, M. M. & Appel, S. H. Mutant mice (quaking and jimpy) with deficient myelination in the central nervous system. *Science* **144**, 309-311 (1964).
2. Lockhart, P. J., O'Farrell, C. A. & Farrer, M. J. It's a double knock-out! The quaking mouse is a spontaneous deletion of parkin and parkin co-regulated gene (PACRG). *Mov. Disord.* **19**, 101-104 (2004).
3. Hardy, R. J. *et al.* Neural cell type-specific expression of QKI proteins is altered in the *quaking* viable mutant mice. *J. Neuroscience* **16**, 7941-7949 (1996).
4. Ebersole, T. A., Chen, Q., Justice, M. J. & Artzt, K. The quaking gene product necessary in embryogenesis and myelination combines features of RNA binding and signal transduction proteins. *Nat Genet* **12**, 260-265 (1996).
5. Kondo, T. *et al.* Genomic organization and expression analysis of the mouse qkI locus.*Mammalian Genome* **10**, 662-669 (1999).
6. Larocque, D. *et al.* Nuclear retention of MBP mRNAs in the Quaking viable mice. *Neuron* **36**, 815-829 (2002).
7. Larocque, D. *et al.* Protection of the p27KIP1 mRNA by quaking RNA binding proteins promotes oligodendrocyte differentiation. *Nat. Neurosci* **8**, 27-33 (2005).
8. Wu, J. I., Reed, R. B., Grabowski, P. J. & Artzt, K. Function of quaking in myelination: regulation of alternative splicing. *Proc. Natl. Acad. Sci. USA* **99**, 4233-4238 (2002).
9. Zhang, Y. *et al.* Tyrosine phosphorylation of QKI mediates developmental signals to regulate mRNA metabolism. *EMBO J.* **22**, 1801-1810 (2003).
10. Zhao, L., Mandler, M. D., Yi, H. & Feng, Y. Quaking I controls a unique cytoplasmic pathway that regulates alternative splicing of myelin-associated glycoprotein. *Proc Natl Acad Sci U S A* **107**, 19061-19066, doi:1007487107 [pii]10.1073/pnas.1007487107 (2010).
11. Doukhanine, E., Gavino, C., Haines, J. D., Almazan, G. & Richard, S. The QKI-6 RNA binding protein regulates actin-interacting protein-1 mRNA stability during oligodendrocyte differentiation. *Mol Biol Cell* **21**, 3029-3040, doi:E10-04-0305 [pii] 10.1091/mbc.E10-04-0305 (2010).
12. Li, Z., Zhang, Y., Li, D. & Feng, Y. Destabilization and mislocalization of the myelin basic protein mRNAs in quaking dysmyelination lacking the Qk1 RNA-binding proteins. *J. Neurosci.* **20**, 4944-4953 (2000).
13. Zearfoss, N. R., Clingman, C. C., Farley, B. M., McCoig, L. M. & Ryder, S. P. Quaking regulates Hnrnpa1 expression through its 3' UTR in oligodendrocyte precursor cells. *PLoS Genet* **7**, e1001269, doi:10.1371/journal.pgen.1001269 (2011).
14. Larocque, D. *et al.* The QKI-6 and QKI-7 RNA binding proteins block proliferation and promote Schwann cell myelination. *PLoS ONE* **4**, e5867 (2009).
15. Zhao, L., Tian, D., Xia, M., Macklin, W. B. & Feng, Y. Rescuing qkV dysmyelination by a single isoform of the selective RNA-binding protein QKI. *J Neurosci* **26**, 11278-11286 (2006).
16. Wilson, G. R. *et al.* Deletion of the Parkin co-regulated gene causes defects in ependymal ciliary motility and hydrocephalus in the quakingviable mutant mouse. *Hum Mol Genet* **19**, 1593-1602, doi:ddq031 [pii]10.1093/hmg/ddq031 (2010).
17. Lorenzetti, D., Bishop, C. E. & Justice, M. J. Deletion of the Parkin coregulated gene causes male sterility in the quaking(viable) mouse mutant. *Proc Natl Acad Sci U S A* **101**, 8402-8407, doi:10.1073/pnas.0401832101 [pii] (2004).
18. Gavino, C. & Richard, S. Patched1 haploinsufficiency impairs ependymal cilia function of the quaking viable mice, leading to fatal hydrocephalus. *Mol Cell Neurosci*, doi:S1044-7431(11)00067-4 [pii]10.1016/j.mcn.2011.03.004 (2011).
19. Cesari, R. *et al.* Parkin, a gene implicated in autosomal recessive juvenile parkinsonism, is a candidate tumor suppressor gene on chromosome 6q25-q27. *Proc Natl Acad Sci U S A* **100**, 5956-5961, doi:10.1073/pnas.0931262100931262100 [pii] (2003).
20. Smith, D. I., Zhu, Y., McAvoy, S. & Kuhn, R. Common fragile sites, extremely large genes, neural development and cancer. *Cancer Lett* **232**, 48-57, doi:S0304-3835(05)00823-2 [pii]10.1016/j.canlet.2005.06.049 (2006).
21. Mulholland, P. J. *et al.* Genomic profiling identifies discrete deletions associated with translocations in glioblastoma multiforme. *Cell Cycle* **5**, 783-791 (2006).
22. Ichimura, K. *et al.* Small regions of overlapping deletions on 6q26 in human astrocytic tumours identified using chromosome 6 tile path array-CGH. *Oncogene* **25**, 1261-1271 (2006).
23. Li, Z. Z. *et al.* Expression of Hqk encoding a KH RNA binding protein is altered in human glioma. *Jpn J Cancer Res* **93**, 167-177. (2002).
24. Jones, A. R. & Schedl, T. Mutations in GLD-1, a female germ cell-specific tumor suppressor gene in *C.elegans*, affect a conserved domain also found in Sam68. *Genes & Dev.* **9**, 1491-1504 (1995).
25. Schumacher, B. *et al.* Translational Repression of C. elegans p53 by GLD-1 Regulates DNA Damage-Induced Apoptosis. *Cell* **120**, 357-368 (2005).
26. Yang, G. *et al.* RNA-binding protein quaking, a critical regulator of colon epithelial differentiation and a suppressor of colon cancer. *Gastroenterology* **138**, 231-240 e231-235, doi:S0016-5085(09)01392-4 [pii]10.1053/j.gastro.2009.08.001 (2010).
27. Hollstein, M., Sidransky, D., Vogelstein, B. & Harris, C. C. p53 mutations in human cancers. *Science* **253**, 49-53 (1991).
28. Bougeard, G. *et al.* Screening for TP53 rearrangements in families with the Li-Fraumeni syndrome reveals a complete deletion of the TP53 gene. *Oncogene* **22**, 840-846, doi:10.1038/sj.onc.12061551206155 [pii] (2003).
29. Nigro, J. M. *et al.* Mutations in the p53 gene occur in diverse human tumour types. *Nature* **342**, 705-708, doi:10.1038/342705a0 (1989).
30. Nozaki, M. *et al.* Roles of the functional loss of p53 and other genes in astrocytoma tumorigenesis and progression. *Neuro Oncol* **1**, 124-137 (1999).
31. Jacks, T. *et al.* Tumor spectrum analysis in p53-mutant mice. *Curr Biol* **4**, 1-7 (1994).
32. Huse, J. T. & Holland, E. C. Genetically engineered mouse models of brain cancer and the promise of preclinical testing. *Brain Pathol* **19**, 132-143, doi:BPA234 [pii] 10.1111/j.1750-3639.2008.00234.x (2009).
33. Donehower, L. A. *et al.* Mice deficient for p53 are developmentally normal but susceptible to spontaneous tumours. *Nature* **356**, 215-221 (1992).
34. Reilly, K. M., Loisel, D. A., Bronson, R. T., McLaughlin, M. E. & Jacks, T. Nf1;Trp53 mutant mice develop glioblastoma with evidence of strain-specific effects. *Nat Genet* **26**, 109-113, doi:10.1038/79075 (2000).
35. Lorenzetti, D. *et al.* The neurological mutant quaking(viable) is Parkin deficient. *Mamm Genome* **15**, 210-217 (2004).
36. Bo, L. *et al.* Endocytic depletion of L-MAG from CNS myelin in quaking mice. *J Cell Biol* **131**, 1811-1820 (1995).
37. Taylor, S. M., Bennett, G. D., Abbott, L. C. & Finnell, R. H. Seizure control following administration of anticonvulsant drugs in the quaking mouse. *Eur J Pharmacol* **118**, 163-170, doi:0014-2999(85)90675-2 [pii] (1985).
38. Gioanni, Y., Gioanni, H. & Mitrovic, N. Seizures can be triggered by stimulating non-cortical structures in the quaking mutant mouse. *Epilepsy Res* **9**, 19-31, doi:0920-1211(91)90043-F [pii] (1991).
39. Van Loo, P. L. *et al.* Long-term effects of husbandry procedures on stress-related parameters in male mice of two strains. *Lab Anim* **38**, 169-177, doi:10.1258/002367704322968858 (2004).
40. Suzuki, K. & Zagoren, J. C. Focal axonal swelling in cerebellum of quaking mouse: light and electron microscopic studies. *Brain Res* **85**, 38-43, doi:0006-8993(75)91001-X [pii] (1975).

41. Pilotte, J., Larocque, D. & Richard, S. Nuclear translocation controlled by alternatively spliced isoforms inactivates the QUAKING apoptotic inducer. *Genes & Dev.* **15**, 845-858 (2001).
42. Chen, T. & Richard, S. Structure-function analysis of Qk1: a lethal point mutation in mouse quaking prevents homodimerization. *Mol Cell Biol* **18**, 4863-4871. (1998).
43. Poppleton, H. & Gilbertson, R. J. Stem cells of ependymoma. *Br J Cancer* **96**, 6-10, doi:6603519 [pii]10.1038/sj.bjc.6603519 (2007).
44. Kleihues, P., Schauble, B., zur Hausen, A., Esteve, J. & Ohgaki, H. Tumors associated with p53 germline mutations: a synopsis of 91 families. *Am J Pathol* **150**, 1-13 (1997).
45. Thompson, M. C. *et al.* Genomics identifies medulloblastoma subgroups that are enriched for specific genetic alterations. *J Clin Oncol* **24**, 1924-1931, doi:JCO.2005.04.4974 [pii]10.1200/JCO.2005.04.4974 (2006).
46. Hardy, R. J. QKI expression is regulated during neuron-glial cell fate decisions. *J Neurosci Res* **54**, 46-57. (1998).
47. Galarneau, A. & Richard, S. Target RNA motif and target mRNAs of the Quaking STAR protein. *Nat Struct Mol Biol* **12**, 691-698 (2005).
48. Trapp, B. D. *et al.* Axonal transection in the lesions of multiple sclerosis. *N Engl J Med* **338**, 278-285, doi:10.1056/NEJM199801293380502 (1998).
49. Raine, C. S. & Cross, A. H. Axonal dystrophy as a consequence of long-term demyelination. *Lab Invest* **60**, 714-725 (1989).
50. Vaillant, C. & Monard, D. SHH pathway and cerebellar development. *Cerebellum* **8**, 291-301, doi:10.1007/s12311-009-0094-8 (2009).
51. Lim, J. *et al.* A protein-protein interaction network for human inherited ataxias and disorders of purkinje cell degeneration. *Cell* **125**, 801-814 (2006).

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Author contributions

The project and all experiments were designed by C.G. and S.R. All experiments and statistical analysis were performed by C.G. The manuscript was written by C.G. and S.R.

Additional information

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