Journal of Human Genetics (2010) 55, 507–511

www.nature.com/jhg

ORIGINAL ARTICLE

Identification of alternatively spliced *GRIM-19* mRNA in kidney cancer tissues

Xuelian He[1] and Xinmin Cao

Gene associated with Retinoid-Interferon-induced Mortality (*GRIM*)-*19* was originally identified as a regulatory gene necessary for interferon-β and retinoic acid-induced cell death. Further studies revealed that GRIM-19 is a subunit of mitochondrial respiratory chain complex I. Previous studies show that the expression of GRIM-19 is lost or severely depressed in a number of primary renal cell carcinomas (RCCs) and in some urinogenital tumors. Four point mutations were found in the *GRIM-19* gene in mitochondria-rich (Hürthle) tumors and one point mutation was reported in RCC. In this study, we report an alternatively splicing form of *GRIM-19* mRNA with intron 3 by reverse transcriptase PCR. This splicing variant is found in kidney tumor tissues but not in matched normal tissues. Furthermore, we found that in addition to GRIM-19, the protein level of NDUFS3, which is another mitochondrial complex I subunit, was also diminished in kidney tumor tissues when compared with paired normal tissues. Our finding suggested that the alternative splicing form of GRIM-19 is tumor tissue specific.
Journal of Human Genetics (2010) **55,** 507–511; doi:10.1038/jhg.2010.57; published online 27 May 2010

Keywords: GRIM-19; kidney cancer; splicing form; mutation

INTRODUCTION

Kidney cancer represents the third leading cause of death among genitourinary malignancies and the 12th leading cause of cancer death overall in the United States.[1] It was estimated that 51 190 people would be diagnosed with and 12 890 deaths would be attributed to cancer of the kidney and renal pelvis in the United States in 2007.[2]

Rather than cigarette smoking, obesity, hypertension and some occupational factors,[3] genetic factors have an important role in the etiology of kidney cancers. For example, mutations in von Hippel–Lindau (*VHL*) gene, whose product functions as a tumor suppressor by inhibiting hypoxia-inducible genes involved in angiogenesis, cell growth and glucose uptake, are associated with renal cell carcinoma (RCC);[4,5] mutations in the MET proto-oncogene result in hereditary type 1 papillary RCC.[6] These rare genetic variants explain only small percentage of RCC, and most renal cancers are contributed by genetic variants with low or moderate risk.

The kidney is an organ rich in mitochondria. It has been known that cancer cells have many abnormalities in their mitochondria, and mutations in mitochondrial DNA and nuclear-encoded mitochondrial genes are related to cancers.[7–11] As an example, uterine leiomyomas and RCC have been linked to mutations in fumarate hydratase.[12]

Gene associated with Retinoid-Interferon-induced Mortality (*GRIM*)-*19* was originally defined as a cell death regulatory gene, as it is essential for the tumor cell death induced by interferon-β and retinoic acid.[13] Subsequently, GRIM-19 was demonstrated to be a new subunit of the mitochondrial NAPDH:ubiquinone oxidoredutase (respiratory chain complex I) and is essential for the complex I assembly, activity and maintenance of mitochondrial membrane potential.[14–17] Disruption of mitochondrial transmembrane potential by GRIM-19 mutants enhances the cells' sensitivity to apoptotic stimuli.[17] Furthermore, GRIM-19 has also been shown to repress the activity of the Signal Transducer and Activator of Transcription 3 (Stat3) and inhibit v-Src-induced oncogenic transformation and metastatic behavior of cells.[18,19] Thus, *GRIM-19* is a good candidate gene to study the etiopathogenesis of mitochondrion-rich tumors.

Recently, it has been reported that somatic and germline mutations (A26V, K88N, S83G and R198P) in GRIM-19 are linked to mitochondria-rich thyroid tumors and the expression of *ICAM1*, a downstream gene of Stat3, was significantly higher in tumors with mutations vs normal tissues.[20] Moreover, tumor-associated GRIM-19 mutations (K5N and R115P) have been demonstrated to impair the anti-v-Src effect of GRIM-19 and significantly reduce its ability to control v-Src-induced metastases *in vivo*.[21] With regard to renal cancer, a proteomic analysis revealed a loss of GRIM-19 expression in this cancer.[22] In view of the prominent role of *GRIM-19* in cancer, we initiated the study to examine whether there are mutations in this gene in kidney tumor tissues, as well as in paired normal tissues.

MATERIALS AND METHODS

Samples

After being approved by the National University of Singapore Institutional Review Board, we obtained RNA and corresponding protein samples from 37

Signal Transduction Laboratory, Institute of Molecular and Cell Biology, Agency for Science, Technology and Research, Singapore
Correspondence: Professor X Cao, Signal Transduction Laboratory, Institute of Molecular and Cell Biology, Agency for Science, Technology and Research, Proteos Building, 61 Biopolis Drive, Singapore.
E-mail: mcbcaoxm@imcb.a-star.edu.sg
[1]Current address: Clinical Research Center, Wuhan Children's Hospital, No. 100 Hongkong Road, Jiangan District, Wuhan 430016, China.
Received 15 January 2010; revised 4 March 2010; accepted 13 April 2010; published online 27 May 2010

kidney tumors (18 renal clear cell carcinomas, 3 papillary RCCs, 1 granular renal clear cell carcinoma, 6 unclassified RCCs, 5 nephroblastomas, 1 transitional cell carcinoma, 1 urothelial cell carcinoma, 1 Mullerian adenosarcoma and 1 giomyolipoma) and 20 paired normal tissues from the National University of Singapore Tissue Repository. These RNA and proteins were extracted using Trizol reagent (Invitrogen, Carlsbad, CA, USA) at the National University of Singapore Tissue Repository.

Detection of mutation in *GRIM-19* gene

First-strand cDNA was synthesized from 3–5 μg of total RNA by oligo$(dT)_{15}$-primed reverse transcription with Expand Reverse Transcriptase (Roche Applied Science, Mannheim, Germany). The synthesized cDNA was subjected to PCR by using a pair of primers: 5′-ATGCAAGAACCAAGGCGAGT-3′ and 5′-CAGAGCATTTCCGTCCCA-3′ to amplify the *GRIM-19*-encoding fragment. Aliquots of PCR products were separated on 2% agarose gel and visualized with ethidium bromide under UV light, and parts of the PCR products were sequenced directly using ABI PRISM BigDye Terminator (Applied Biosystems, Foster City, CA, USA).

Examining protein expression of GRIM-19

To further observe whether there are any differences in GRIM-19 protein between normal and tumor tissues, protein samples extracted from normal and kidney tumor tissues were subjected to western blot analysis with anti-GRIM-19 (Calbiochem, San Diego, CA, USA). For western blot assay, another complex I subunit, NDUFS3, was also detected using anti-NDUFS3 antibody (Invitrogen).

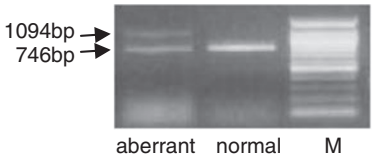

Figure 1 The agarose gel picture of normal and aberrant transcripts. A normal (746 bp) and a longer (1094 bp) transcripts were resolved on 1% agarose gel and visualized by ethidium bromide staining.

Overexpression of different *GRIM-19* transcripts in mammalian cells

HeLa cells were maintained in Dulbecco's modified Eagle's medium (Sigma, St Louis, IL, USA) supplemented with 10% fetal bovine serum (Hyclone, Logan, UT, USA) and cultured at 37 °C in a humidified 5% CO_2 atmosphere. GRIM-19 and its mutant transcript were cloned into mammalian expression vector pXJ40 by PCR using primers specific for *GRIM-19* sequence. Two restriction sites for *Bam*HI and *Kpn*I were added at the 5′ and 3′ ends, respectively. The forward primer is 5′-CGCGGATCCGCGATGGCGGTGGCAGTAAGT-3′ and the reverse primer is 5′-CGGGGTACCCCCTACGTGTACCACATGAA-3′. After PCR amplification, the products were digested with *Bam*HI and *Kpn*I and subcloned into pXJ40 vector. After transfection with resultant recombinants, cells were lysed and the cell lysate was subjected to western blot assay using anti-GRIM-19 antibody.

RESULTS

Novel GRIM-19 transcript generated by alternative splicing

The *GRIM-19* gene comprises five exons, spanning and encoding a protein with 144 amino acids. After amplifying the full-length GRIM-19 transcript with primers specific for *GRIM-19* cDNA using cDNA from kidney tissues as templates, only one PCR product with 746 bp was found in all normal tissues. In contrast, a longer product with 1094 bp was observed in more than 1/3 tumor tissues (Figure 1). After sequencing these two PCR products and comparing their sequences with the public human *GRIM-19* genomic sequence (GenBank accession number NC_000019) and with GRIM-19 cDNA sequence (GenBank accession number NM_015965), it was revealed that the short fragment is the normal transcript, whereas the long fragment contains sequence derived from intron 3, presumably through alternative splicing (Figure 2). However, no point mutation was

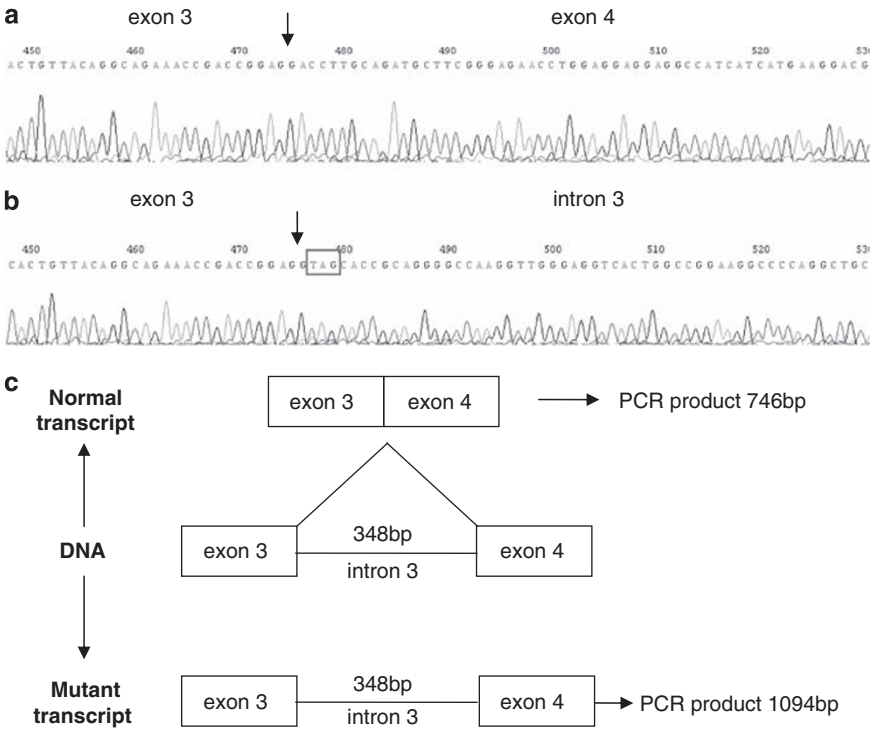

Figure 2 Sequence of normal and aberrant transcript. (**a**) Partial sequence of normal transcript and the adjunction site of exons 3 and 4 is marked by an arrow. (**b**) The alternative splicing form. The adjunction of exon and intron 3 is marked by an arrow. The sequence in red frame is the introduced premature stop codon (TAG). (**c**) Schematic representation of normal and mutant splicing forms and corresponding PCR products of *GRIM-19* gene. A full color version of this figure is available at the *Journal of Human Genetics* journal online.

detected in all samples. Among 37 RNA samples from kidney tumor tissues, 23 samples contain only normal transcript and 14 samples (38%) have been found to possess additional mutant transcript. Among the 14 samples, 10 are renal clear cell carcinomas and other 4 samples are from transitional cell carcinoma, nephroblastoma, angiomyolipoma and Mullerian adenosarcoma, respectively (Table 1). In contrast, the mutant transcript was absent in their matched normal samples. Thus, the abnormal splicing form is kidney tumor specific.

GRIM-19 and NDUFS3 protein expression was suppressed in tumor tissues

The alternatively transcribed mRNA with intron 3 introduces a TAG stop codon, leading to a premature mRNA, which is expected to encode a truncated GRIM-19 protein. To detect the presence of such protein, normal and paired tumor tissues were subjected to western blot analysis. However, we were unable to detect the expected truncated GRIM-19 protein (Figure 3). From western blot assay, we noticed that the protein level of GRIM-19 in tumors was decreased compared with the paired normal tissues, which is consistent with a previous report.[22] As, GRIM-19 is a subunit of complex I of the mitochondria, whether the decreased protein expression is specific for GRIM-19 or occurs in other subunits of complex is worthy to test. By examining the protein expression of another subunit of complex I, NUDFS3, we found that NUDFS3 expression was also reduced in the same trend, though in some samples, the decrease was not as obvious as GRIM-19 (Figure 3).

Alternative transcript of *GRIM-19* generated a truncated protein in mammalian cells

As we did not detect the expected truncated GRIM-19 protein, we were keen to know whether the alternative transcript can be translated into a shorter protein than the native GRIM-19 in cells. Thus, we constructed a plasmid expressing a normal or a mutant *GRIM-19* cDNA with intron 3 and expressed them in HeLa cells. After transfection, the expected truncated protein was detected (Figure 4). This suggested that the alternative transcript encoding a mutant GRIM-19 protein can be expressed in cells. As we did not observe

Table 1 Summary of subjects

Specimen ID	*Age (years)*	*Sex*	*Race*	*Diagnosis*	*Tumour or lesion (%)*	*Transcripts*
NT01/0087	43	F	Chinese	RCC, clear cell type	100	Variant
NT01/0089	63	F	Chinese	RCC, clear cell type	100	Variant
NT04/0067	50	M	Chinese	RCC, clear cell type	100	Variant
NT01/0084	56	M	Chinese	RCC, clear cell type	100	Normal
NT02/0001	48	F	Chinese	RCC, clear cell type	100	Normal
NT04/0042	39	M	Chinese	RCC, clear cell type	100	Normal
NT04/0081	65	F	Chinese	RCC, clear cell type	100	Normal
NT00/0151	44	M	Chinese	RCC, clear cell type	100	Normal
NT00/0231	60	M	Chinese	RCC, clear cell type	100	Normal
NT06/0120	42	F	Chinese	RCC, clear cell type	80	Variant
NT05/0111	75	F	Chinese	RCC, clear cell type	70	Variant
NT02/0085	76	M	Malay	RCC, clear cell type	100	Normal
NT05/0182	62	M	Malay	RCC, clear cell type		Normal
NT00/0238	53	M	Indian	RCC, clear cell type	100	Normal
NT00/0024	55	M	Indian	RCC, clear cell type	100	Variant
NT03/0107	63	M	Others	RCC, clear cell type	100	Normal
NT04/0048	51	M	Others	RCC, clear cell type	100	Variant
NT06/0187	52	M	Others	RCC, clear cell type	70	Normal
NT00/0106	76	M	Chinese	RCC, papillary subtype	100	Variant
NT01/0085	32	F	Chinese	RCC, papillary subtype	100	Normal
NT00/0116	71	F	Malay	RCC, papillary subtype	20	Normal
NT00/0085	50	M	Chinese	RCC, granular cell type	100	Normal
NT00/0138	62	M	Malay	RCC	100	Variant
NT01/0054	54	F	Chinese	RCC	100	Variant
NT01/0012	45	M	Chinese	RCC	100	Normal
NT00/0131	72	M	Chinese	RCC	100	Normal
NT00/0163	50	F	Chinese	RCC	100	Normal
NT01/0075	42	M	Chinese	RCC	100	Normal
NT00/0109	54	F	Chinese	Transitional cell carcinoma	100	Variant
NT04/0043	8	F	Chinese	Nephroblastoma	100	Variant
NT03/0066	4	F	Chinese	Nephroblastoma	100	Normal
NT03/0132	2	F	Malay	Nephroblastoma	100	Normal
NT06/0083	13	M	Malay	Nephroblastoma	30	Normal
NT05/0197	5	F	Chinese	Nephroblastoma	100	Normal
NT04/0001	73	F	Chinese	Urothelial carcinoma	100	Normal
NT03/0012	47	F	Chinese	Angiomyolipoma	100	Variant
NT04/0204	61	F	Chinese	Mullerian adenosarcoma	100	Variant

Abbreviations: F, female; M, male; RCC, renal cell carcinoma.
The underlined samples whose corresponding protein samples from normal and matched tumor tissues have been analyzed using western blot.

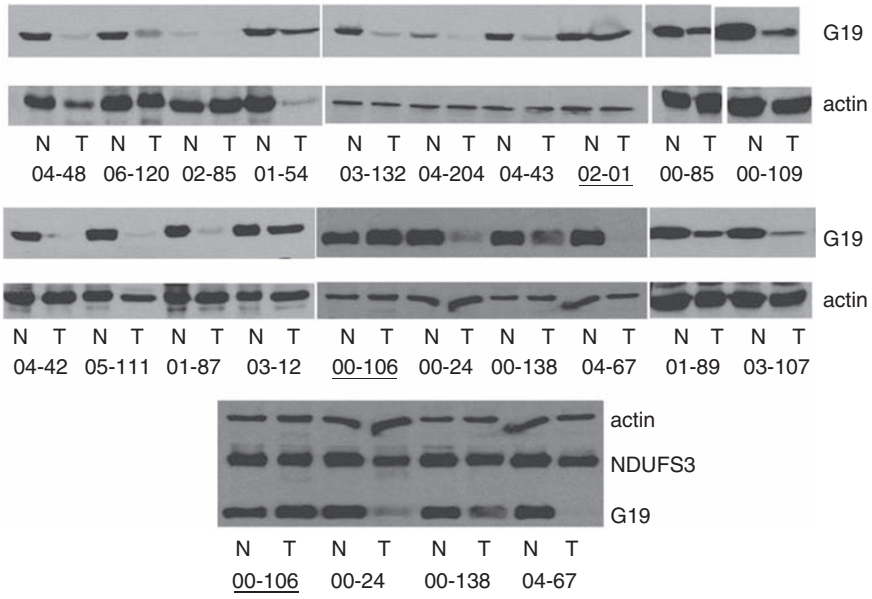

Figure 3 Protein expressions of GRIM-19 and NDUFS3 in normal and tumor tissues. Equal amount of protein was loaded into 12% polyacrylamide gel for western blot assay with actin as loading control. (Upper) Except for the two underlined samples, the G19 protein expression was decreased or lost in tumor tissues compared with matched normal tissues. (Lower) For these samples whose G19 protein levels were lowered or lost in tumor tissues, NDUFS3 protein expression was also suppressed detected by using NDUFS3 antibody to the same blot as G19. N, normal; T, tumor.

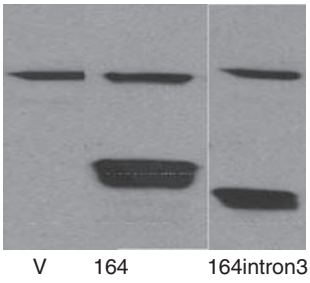

Figure 4 Overexpression of normal and alternative transcript, as well as empty vector in HeLa cells. The expected truncated protein was detected after transient transfection. V: empty vector; 164: plasmid expressing native GRIM-19; 164intron3: plasmid expressing alternative transcript with intron 3.

the truncated GRIM-19 protein from the clinical samples, it is unclear whether it is due to the instability of the truncated protein of the alternative transcript in tumor tissues. It is also feasible that protein encoded by the mutant GRIM-19 is modified after synthesis and lacks the critical epitope recognized by the anti-GRIM-19.

DISCUSSION

In this study, by screening *GRIM-19* mutation using clinical samples, we found that, in addition to a normal *GRIM-19* transcript, 14 from 37 patients harbored an additional transcript with intron 3 in the tumor tissues but not in the paired normal tissues. No point mutation was found among these tumor samples. However, the GRIM-19 protein level was downregulated and even lost in most patients (97%). This is in consistent with the previous report.[22] Furthermore, we have also shown that the downregulation at protein expression occurs not only in GRIM-19 but also in NUDFS3, another subunit of complex I. Although a protein with the expected smaller size presumably encoded by the splicing mutant was not detectable in these tissue samples, this variant transcript is able to be translated to a protein with expected lower molecular weight when it is transfected into HeLa cells.

Interestingly, 10 out of 14 RCC samples (~71%) have been detected to express the splicing variant, which seems to suggest a possible link between the expression of splicing mutant and the certain kidney tumor histotype. However, the splicing mutant transcript was also detected in the other four samples from transitional cell carcinoma, nephroblastoma, angiomyolipoma and Mullerian adenosarcoma, respectively. The link to these tumor types is unclear because of the small sample number. Altogether, the mutant transcript form is specific for kidney tumors, but further test of more samples is required to determine its relationship with different tumor histotypes.

Post-transcriptional regulations, such as splicing of immature messenger RNA (pre-mRNA), are fundamental for generating mature mRNA ready to be translated into proteins.[23] Aberrant splicing has been related to activation of oncogenes and inhibition of tumor-suppressor genes in a variety of human cancers.[24] Accumulating evidence suggests that cellular splicing machinery is changed during oncogenic transformation of cells, although the mechanism for these changes remains to be elucidated.[23,25] Being a cause of cancer, an alternative splicing variant must presumably be expressed at significant high level compared with the properly splicing products.[24] However, most aberrant mRNAs are degraded by nonsense-mediated mRNA decay pathway;[26] some new mRNA species can bring about a reduction of normal protein levels or produce different protein isoforms with potentially tumorigenic properties. In our case, there was no aberrant protein detected, although alternative splicing mRNA form was found. One possibility for this is that the alternative spliced mRNA is decayed before being translated. Alternatively, the transcript variant could be translated to an abnormal protein, which is unstable and degraded rapidly by proteinase(s). Currently, we are not clear whether the alternative splice form can make any significant contribution to the development of kidney cancers or it is generated during the development of kidney cancers. These issues remain to be further investigated.

It has been reported that the protein expression of GRIM-19 is lost or severely depressed in a number of primary RCC and in some urinogenital tumors.[22] Our results further confirmed the loss or

decreased expression of GRIM-19 in RCC, as well as in other kidney tumors, such as nephroblastoma, Mullerian adenosarcoma, transitional cell carcinoma and angiomyolipoma. Furthermore, the change is not unique to GRIM-19, but also occurred to NDUFS3, another subunit of complex I. The mechanism for the decreased level of GRIM-19 and NDUFS3 in RCC and other kidney tumors remains to be elucidated. A few possibilities exist. First, the genetic change may underlie the suppression of GRIM-19 protein. Considering the fact that no mutation was detected in the encoding region of this gene, whether there are some functional genetic alterations in the regulatory regions, such as promoter, 5′ and 3′ noncoding region, as well as other genes that control the expression of GRIM-19 and NDUFS3, is worthy for further investigation. Another possible explanation is that the rapid degradation of GRIM-19 protein, which may be mediated by some proteinases that are over active in the kidney tumors. The other possibility such as autophagy-mediated degradation of the mitochondria can also not be excluded.

The frequent loss or suppression of GRIM-19 protein expression in kidney cancers suggests a role of GRIM-19 in kidney tumorigenesis. How exactly GRIM-19 is involved in this process is mostly unknown. The primary function of GRIM-19 is a subunit of mitochondrial complex I. As GRIM-19 is essential for the complex I assembly and electron transfer activity, loss of its expression in the RCC and other kidney cancers may be related to an impaired function of mitochondrial energy production system. Similarly, reduced expression of NDUFS3 supports the hypothesis. Furthermore, we have shown that knockdown of GRIM-19 or NDUFS3 prevents apoptosis induced by interferon-β and retinoic acid.[16] It is also possible that the down-regulation of GRIM-19 and NUDFS3 not only disrupts complex I assembly and reduces mitochondrial respiratory chain activity but also decreases apoptotic capacity, the latter may benefit cancer cell growth. As GRIM-19 is also an inhibitor of Stat3, which is an oncogene, loss of GRIM-19 in kidney cancers may activate Stat3 and lead to the development of kidney cancer.

In summary, an alternative splice form of *GRIM-19* was found in kidney cancers and this aberrant splice form is kidney tumor specific. The specific role of GRIM-19, including its splicing variant, in kidney cancers and the mechanism involved are important issues for future investigation.

ACKNOWLEDGEMENTS

This work was supported by the Agency for Science, Technology and Research of Singapore.

1 Garcia, J. A. & Rini, B. I. Recent progress in the management of advanced renal cell carcinoma. *CA Cancer J. Clin.* **57,** 112–125 (2007).
2 Jemal, A., Siegel, R., Ward, E., Murray, T., Xu, J. & Thun, M. J. Cancer Statistics 2007. *CA Cancer J. Clin.* **57,** 43–66 (2007).
3 Scelo, G. & Brennan, P. The epidemiology of bladder and kidney cancer. *Nat. Clin. Pract. Urol.* **4,** 205–217 (2007).
4 Cohen, D. & Zhou, M. Molecular genetics of familial renal cell carcinoma syndromes. *Clin. Lab. Med.* **25,** 259–277 (2005).
5 Gallou, C., Chauveau, D., Richard, S., Joly, D., Giraud, S., Olschwang, S. *et al.* Genotype-phenotype correlation in von Hippel-Lindau families with renal lesions. *Hum. Mutat.* **24,** 215–224 (2004).
6 Walker, C. Molecular genetics of renal carcinogenesis. *Toxicol. Pathol.* **26,** 113–120 (1998).
7 Liu, V. W., Wang, Y., Yang, H. J., Tsang, P. C., Ng, T. Y., Wong, L. C. *et al.* Mitochondrial DNA variant 16189T>C is associated with susceptibility to endometrial cancer. *Hum. Mutat.* **22,** 173–174 (2003).
8 Herrmann, P. C., Gillespie, J. W., Charboneau, L., Bichsel, V. E., Paweletz, C. P., Calvert, V. S. *et al.* Mitochondrial proteome: altered cytochrome c oxidase subunit levels in prostate cancer. *Proteomics* **3,** 1801–1810 (2003).
9 Baysal, B. E., Ferrell, R. E., Willett-Brozick, J. E., Lawrence, E. C., Myssiorek, D., Bosch, A. *et al.* Mutations in SDHD, a mitochondrial complex II gene, in hereditary paraganglioma. *Science* **287,** 848–851 (2000).
10 Niemann, S. & Müller, U. Mutations in SDHC cause autosomal dominant paraganglioma, type 3. *Nat. Genet.* **26,** 268–270 (2000).
11 Vanharanta, S., Buchta, M., McWhinney, S. R., Virta, S. K., Peçzkowska, M., Morrison, C. D. *et al.* Early-onset renal cell carcinoma as a novel extraparaganglial component of SDHB-associated heritable paraganglioma. *Am. J. Hum. Genet.* **74,** 153–159 (2004).
12 Lehtonen, R., Kiuru, M., Vanharanta, S., Sjöberg, J., Aaltonen, L. M., Aittomäki, K. *et al.* Biallelic inactivation of fumarate hydratase (FH) occurs in nonsyndromic uterine leiomyomas but is rare in other tumors. *Am. J. Pathol.* **164,** 17–22 (2004).
13 Angell, J. E., Lindner, D. J., Shapiro, P. S., Hofmann, E. R. & Kalvakolanu, D. V. Identification of GRIM-19, a novel cell death-regulatory gene induced by the interferon-beta and retinoic acid combination, using a genetic approach. *J. Biol. Chem.* **275,** 33416–33426 (2000).
14 Fearnley, I. M., Carroll, J., Shannon, R. J., Runswick, M. J., Walker, J. E. & Hirst, J. GRIM-19, a cell death regulatory gene product, is a subunit of bovine mitochondrial NADH:ubiquinone oxidoreductase (complex I). *J. Biol. Chem.* **276,** 38345–38348 (2001).
15 Huang, G., Lu, H., Hao, A., Ng, D. C., Ponniah, S., Guo, K. *et al.* GRIM-19, a cell death regulatory protein, is essential for assembly and function of mitochondrial complex I. *Mol. Cell. Biol.* **24,** 8447–8456 (2004).
16 Huang, G., Chen, Y., Lu, H. & Cao, X. Coupling mitochondrial respiratory chain to cell death: an essential role of mitochondrial complex I in the interferon-beta and retinoic acid-induced cancer cell death. *Cell Death Differ.* **14,** 327–337 (2007).
17 Lu, H. & Cao, X. GRIM-19 is essential for maintenance of mitochondrial membrane potential. *Mol. Biol. Cell.* **19,** 1893–1902 (2008).
18 Lufei, C., Ma, J., Huang, G., Zhang, T., Novotny-Diermayr, V., Ong, C. T. *et al.* GRIM-19, a death-regulatory gene product, suppresses Stat3 activity via functional interaction. *EMBO J.* **22,** 1325–1335 (2003).
19 Kalakonda, S., Nallar, S. C., Gong, P., Lindner, D. J., Goldblum, S. E., Reddy, S. P. *et al.* Tumor suppressive protein gene associated with retinoid-interferon-induced mortality (GRIM)-19 inhibits src-induced oncogenic transformation at multiple levels. *Am. J. Pathol.* **171,** 1352–1368 (2007).
20 Máximo, V., Botelho, T., Capela, J., Soares, P., Lima, J., Taveira, A. *et al.* Somatic and germline mutation in GRIM-19, a dual function gene involved in mitochondrial metabolism and cell death, is linked to mitochondrion-rich (Hurthle cell) tumours of the thyroid. *Br. J. Cancer* **92,** 1892–1898 (2005).
21 Sun, P., Nallar, S. C., Kalakonda, S., Lindner, D. J., Martin, S. S. & Kalvakolanu, D. V. GRIM-19 inhibits v-Src-induced cell motility by interfering with cytoskeletal restructuring. *Oncogene* **28,** 1339–1347 (2009).
22 Alchanati, I., Nallar, S. C., Sun, P., Gao, L., Hu, J., Stein, A. *et al.* A proteomic analysis reveals the loss of expression of the cell death regulatory gene GRIM-19 in human renal cell carcinomas. *Oncogene* **25,** 7138–7147 (2006).
23 Pajares, M. J., Ezponda, T., Catena, R., Calvo, A., Pio, R. & Montuenga, L. M. Alternative splicing: an emerging topic in molecular and clinical oncology. *Lancet Oncol.* **8,** 349–357 (2007).
24 Venables, J. P. Unbalanced alternative splicing and its significance in cancer. *Bioessays* **28,** 378–386 (2006).
25 Pino, I., Pío, R., Toledo, G., Zabalegui, N., Vicent, S., Rey, N. *et al.* Altered patterns of expression of members of the heterogeneous nuclear ribonucleoprotein (hnRNP) family in lung cancer. *Lung Cancer* **41,** 131–143 (2003).
26 Cartegni, L., Chew, S. L. & Krainer, A. R. Listening to silence and understanding nonsense: exonic mutations that affect splicing. *Nat. Rev. Genet.* **3,** 285–298 (2002).