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Parafibromin inhibits cancer cell growth and causes G1 phase arrest

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Abstract

The HRPT2 (hereditary hyperparathyroidism type 2) tumor suppressor gene encodes a ubiquitously expressed 531 amino acid protein termed parafibromin. Inactivation of parafibromin predisposes one to the development of HPT-JT syndrome. To date, the role of parafibromin in tumorigenesis is largely unknown. Here, we report that parafibromin is a nuclear protein that possesses anti-proliferative properties. We show that overexpression of parafibromin inhibits colony formation and cellular proliferation and induces cell cycle arrest in the G1 phase. Moreover, HPT-JT syndrome-derived mutations in HRPT2 behave in a dominant-negative manner by abolishing the ability of wild-type parafibromin to suppress cell proliferation. These findings suggest that parafibromin has a critical role in cell growth, and mutations in HRPT2 can directly inhibit this role.

Keywords: HRPT2; Parafibromin; G1 phase arrest; Cell cycle

Hyperparathyroidism-jaw tumor syndrome (HPT-JT) is an autosomal dominant multi-tumor syndrome characterized by hyperparathyroidism as a result of parathyroid adenoma or carcinoma, ossifying tumors of the skull bones, and a variety of unusual kidney manifestations [1,18,19]. The underlying genetic disorder is caused by mutations in the HRPT2 gene, which was mapped to 1q21–q23 [18] and further refined to a critical interval of 12 cM by genotyping 26 HPT-JT kindreds [10,14]. Using a positional candidate approach, one of the 67 candidate genes in this region was confirmed to be the HRPT2 gene [2,9]. Subsequently, somatic inactivating HRPT2 gene mutations have also been identified in sporadic parathyroid carcinomas [3,17]. These loss-of-function mutations identified in both familial and sporadic forms of cancer, together with studies showing loss of heterozygosity (LOH) involving wild-type alleles of HRPT2, strongly suggest that HRPT2 functions as a tumor suppressor gene.

The HRPT2 gene, containing 17 exons and spanning 18.5 kb in the genome, encodes a 2.7-kb transcript that is expressed at varying levels in the kidney, heart, liver, pancreas, skeletal muscles, brain, and lung, suggesting a potentially broad function [2]. Conceptual translation of the human HRPT2 gene predicts a protein of 531 amino acids, termed parafibromin. Parafibromin is highly conserved during evolution with clear recognizable orthologues present in *Mus musculus* (95% identical), *Xenopus* (89%), *Danio rerio* (85%), *Drosophila* (54%), and *Caenorhabditis elegans* (26%). Parafibromin does not contain significant sequence similarity to known protein domains but its C-terminal

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domain contains 100 amino acids that exhibit a significant similarity to the *Saccharomyces cerevisiae* Cdc73 protein (32%). In yeast, Cdc73 is a component of yeast Paf1 protein complex, which interacts with RNA polymerase II and functions in the regulation of transcriptional elongation, histone methylation, and RNA processing [11,15]. Recently, parafibromin has been confirmed to interact with the human homologs of the yeast Paf1 complex and associate with human RNA polymerase II [19,21]. However, the cellular functions and biochemical mechanisms underlying the tumor suppression activities of parafibromin remain unknown. This study provides evidence that parafibromin functions as a tumor suppressor through the inhibition of cell growth and regulation of the cell cycle.

Materials and methods

Antibodies and immunological procedures. Hygromycin B, Geneticin, and anti-Myc monoclonal antibody were purchased from Invitrogen Corporation (Carlsbad, CA). Antibodies against green fluorescent protein (GFP), c-jun, and Ras were purchased from Santa Cruz Biotechnology (Santa Cruz, CA), and the antibody against β-actin was purchased from Oncogene Research Products (Boston, MA). The monoclonal antibody against human parafibromin was produced as described [12].

Plasmids. The plasmid pGFP/ERTM and pGFP/ERTM/HRPT2 were obtained by cutting the estrogen receptor LBD domain from pCre-ERT2 (kindly provided by Dr. Xiang Gao in Model Animal Research Center of Nanjing University) and in-frame ligating into pEGFP/C3 vector at *Xho*I and *Eco*RI sites, then inserting the HRPT2 cDNA after the GFP-ER domain. Similarly, we constructed pcDNA3/ERTM and pcDNA3/HRPT2/ERTM.

Cell fractionation, Western blotting, and immunoassay. To prepare whole cell extracts, 3×10^6 cells were washed in phosphate-buffered saline (PBS), resuspended in 100 μl of cell lysis buffer (50 mM Hepes–KOH, pH 7.5, 150 mM NaCl, 1% Triton X-100, 1.5 mM $MgCl_2$, 1 mM EGTA, 0.1 mM phenylmethanesulfonyl fluoride, 10 μg/ml each leupeptin and aprotinin, 200 μM sodium vanadate, 100 mM NaF, and 10% glycerol), and incubated on ice for 10 min. The cell lysates were clarified by centrifugation at 9000*g* for 10 min, and the supernatant was collected. To prepare nuclear extracts, 3×10^6 cells were washed in PBS, pelleted, and then resuspended in an equal volume of lysis buffer (10 mM Tris–HCl, pH 8.0, 60 mM KCl, 1 mM EDTA, 1 mM dithiothreitol, 0.1 mM PMSF, and 0.5% NP-40). After incubating the cells on ice for 5 min, the lysates were centrifuged at 500*g* for 5 min. The pelleted nuclei were rinsed with lysis buffer without NP-40 and resuspended in an equal volume of extraction buffer (20 mM Tris–HCl, pH 8.0, 420 mM NaCl, 1.5 mM $MgCl_2$, 0.2 mM EDTA, and 25% glycerol); 5 M NaCl was added to a final concentration of 400 mM. After further incubation on ice for 10 min, the nuclei were briefly vortexed and centrifuged at 300*g* for 5 min and the supernatant was collected. Protein concentrations were determined by the Lowry method [4]. For Western analysis, cell extracts (20 μg of protein) were separated on an SDS–polyacrylamide gel and transferred onto a nitrocellulose membrane. After blocking with TBST (20 mM Tris, pH 7.5, 0.1% Tween-20, 37 mM NaCl in PBS) containing 5% nonfat dry milk, the membrane was incubated with the indicated primary antibody in the same buffer. After washing three times with TBST, the membrane was further incubated with HRP-conjugated anti-IgG antibody. Results were visualized by chemiluminescence using an ECL kit (Amersham).

For indirect immunostaining, Cos-7 cells (3×10^4) were cultured in a chamber slide (Nalge Nunc International, Rochester, NY) and transfected with the appropriate expression vectors. Forty-eight hours after transfection, the cells were washed with PBS and fixed in cold acetone/methanol (1:1) for 10 min and then rehydrated through 70% ethanol, 50% ethanol, and PBS at room temperature. After fixation, the cells were washed twice with PBS, permeabilized in cold PBS containing 0.2% Triton X-100 for 5 min, and blocked in PBS/0.5% BSA for 30 min. Primary antibody diluted in blocking buffer was added and incubated at RT for 1 h, then with an appropriate fluorochrome-conjugated secondary antibody for another hour. Between different antibody incubations, the cells were washed three times with PBS. Stained cells were covered by anti-fluorescent mounting medium (DAKO) and examined under ultraviolet microscope. 4, 6-Diamidino-2-phenylindole dihydrochloride (DAPI) was used to stain nuclei [20].

Cell culture, transient expression, and inducible HRPT2-expressing cell lines. Cos-7, HeLa Tet-On, 293 Tet-On, and NIH3T3 cell lines were cultured in DMEM containing 10% FBS supplemented with 100 U/ml penicillin and 100 mg/ml streptomycin. For transient expression, plasmid DNA was vortexed with an appropriate amount of Lipofectamine2000 reagent (Invitrogen Corporation) and added to the cells at 70–80% confluence. Under these conditions, the transfection efficiency was 15–30% when assayed using β-galactosidase-expressing plasmid.

To establish inducible HRPT2-expressing cell lines, HeLa Tet-On cells were transfected with 9 μg of pTRE2/HRPT2 plasmid DNA and 1 μg of pTK-Hyg plasmid using Lipofectamine2000, and the cells were plated at a density of 3×10^5 cells per 100-mm-diameter culture dish in DMEM (Invitrogen Corporation) containing 10% FBS. Two days after transfection, hygromycin was added to the medium to a final concentration of 500 μg/ml. Two weeks later, hygromycin-resistant colonies were selected using glass cloning cylinders (Bellco Glass, Vineland, NJ) and expanded into stable cell lines. After adding doxycycline (Sigma–Aldrich, St. Louis, MO) in a series of concentrations, the stable cell lines were checked for HRPT2 expression by immunoblotting using anti-c-Myc antibody. Control experiments were performed by replacing the pTRE2/HRPT2 vector with the pTRE2 vector.

Fluorescence-activated cell sorting (FACS) analysis. HeLa Tet-On HRPT2 cells and their control cells (3×10^5) were treated with 2 μg/ml doxycycline and cultured at 37 °C for 0, 24, or 48 h. The attached and floating cells were combined, washed twice with PBS, and permeabilized using 0.1% Triton X-100. After staining with propidium iodide (50 μg/ml), the cells were subjected to fluorescence-activated cell sorting analysis of DNA content. The percentage of cells with G1 DNA content was taken as a measure of the cell population. Experiments were repeated using the 293 Tet-On cell lines.

Cell proliferation, colony forming, and soft-agar assays. Cells (3×10^5) were seeded onto 35 mm plates triplicate. Cell proliferation was analyzed by counting the cells every day for up to 6 days. Both the attached and floating cells were centrifuged at 500*g* for 5 min, and then the cell pellets were resuspended in PBS and stained with 0.2% trypan blue. Visible cells were counted. The number of viable cells was also assessed using the Cell Titer 96AQueous Assay system (MTS assay, Promega Corp., Madison, WI). After the addition of 100 μl PMS solution to 2.0 ml of MTS solution, 20 μl of combined MTS/PMS solution was pipetted into 96-well plate containing 100 μl cells in each well The reaction was performed by incubating the plate for 1–4 h at 37 °C in a humidified, 5% CO_2 atmosphere. Absorbance at 490 nm was recorded in ELISA plate reader.

For the focus formation assay. cells transfected with equal amounts of pcDNA3/HRPT2, pDCR/H-rasV12, and control pcDNA3 were plated (1000 cells/well) in triplicate in a six-well tissue culture plate, and G418 was added 2 days after transfection (400 μg/ml). Cells were continuously cultured in the presence of G418 for another 15 days, and G418-resistant colonies were counted after crystal violet staining.

To perform the soft agar assay, bottom agarose (0.7%, Invitrogen) in DMEM was cast on plastic six-well plates. Cells (2×10^4) were mixed in 0.3% agarose in DMEM containing 10% FBS at 37 °C and plated over the bottom agarose. The inoculated plates were incubated for 10–15 days. The number of colonies was determined by direct counting under a microscope. Quantitative data were expressed as mean + SD. Statistical analyses were performed using Student's *t*-test. Differences were considered significant when $p < 0.05$.

Results

Overexpression of parafibromin results in cell growth suppression in vitro

To examine the cellular function of parafibromin, we transfected NIH3T3 cells with parafibromin (c-Myc-HRPT2) and H-Ras (pDCR/H-ras) expression vectors and performed focus forming assays. The results show that overexpression of activated H-RasV12 oncoprotein doubled the number of colonies formed compared to that of in empty-vector (pcDNA) transfected control cells (Fig. 1). Interestingly, overexpression of parafibromin resulted in a significant reduction of H-RasV12-induced colony formation (Fig. 1A and B). The expression of parafibromin and H-Ras was confirmed by direct immunoblotting (Supplemental Fig. I). These results demonstrated that overexpression of parafibromin can inhibit cell proliferation and suppress Ras-mediated cell transformation.

Parafibromin inhibits cancer cell growth and causes G1 phase arrest

To evaluate the growth suppression activity of parafibromin, we established a parafibromin-expressing HeLa cell line in a doxycycline (Dox)-inducible (Tet-On) system and examined the effects of parafibromin on cell proliferation and survival. Dose-dependent treatment of the cells with Dox demonstrated that parafibromin expression increased with increasing concentration of Dox (Fig. 2A). Induction of parafibromin with 2.0 μg/ml Dox significantly inhibited cell proliferation (Fig. 2B). This observation was further confirmed by a Dox-time course experiment (Fig. 2C) and soft agar assay (Supplemental Fig. IIA). Similar results were also obtained using Dox-inducible-parafibromin-expressing 293 cells (data not shown).

Next, we examined the effect of parafibromin overexpression on cell cycle progression using fluorescence-activated cell sorting (FACS) analysis. The inducible HRPT2-expressing and the control HeLa cells were exposed to Dox (2 μg/ml) for 0, 24, or 48 h, stained with propidium iodide, and then analyzed by FACS. The results show that Dox treatment resulted in a significant increase (10%) in the G1-phase populations of the parafibromin-expressing cells compared to the control cells (Fig. 2D and Supplemental Fig. IIB). Accordingly, the S-phase and the G2/M phase populations were decreased in parafibromin-expressing cells compared to parental HeLa cells. In contrast, in the absence of Dox treatment, no change in the cell cycle was observed. Similar results were also obtained when Dox-inducible 293 cells were examined (data not shown). Taken together, these results suggest that overexpression of parafibromin causes cell cycle arrest.

Parafibromin is predominantly localized to the nucleus

The subcellular localization of parafibromin has been controversial with reports of both nuclear and nucleocytoplasmic localization [6,15,19]. To clarify this issue, we performed subcellular localization and immunostaining

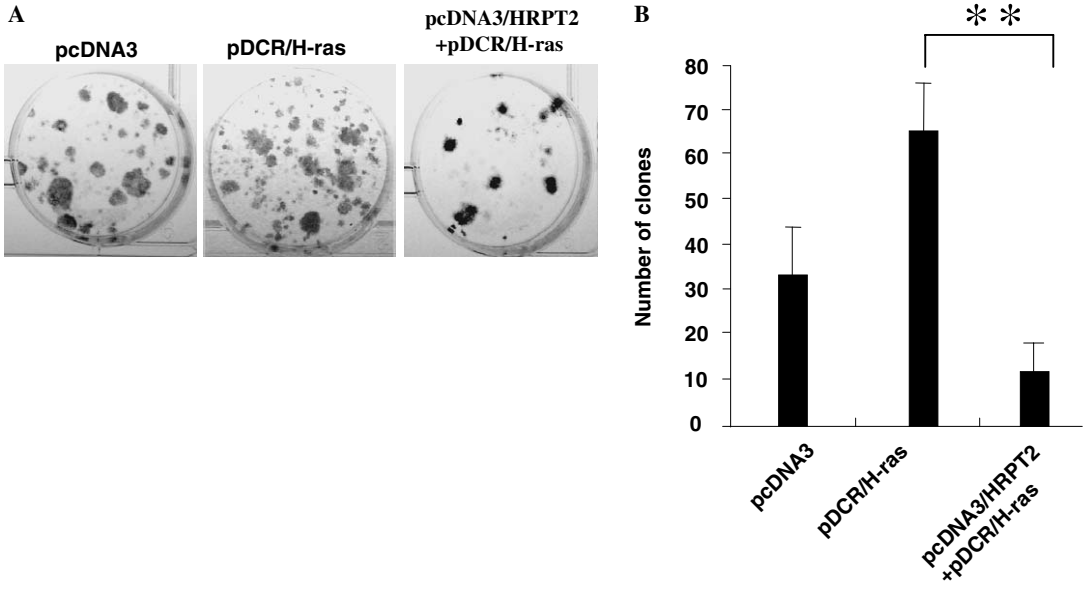
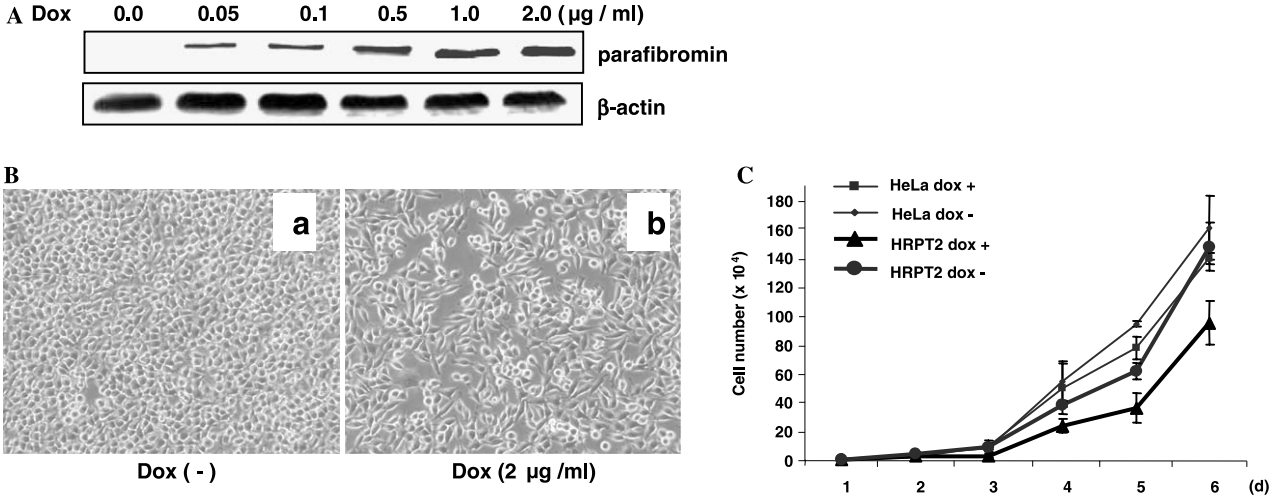


Fig. 1. Overexpression of HRPT2 inhibited colony formation. (A) NIH3T3 cells were transfected with a pcDNA3 empty vector, with a Ras-expressing plasmid, pCDR/H-ras; or with the combination of the pCDR/H-ras and pcDNA3/HRPT2 plasmids. (B) After equal molar pcDNA3/HRPT2 or pcDNA3 plasmid was cotransfected with oncogenic H-ras expression plasmid into NIH3T3 cells, a focus formation assay was performed. The number of colonies was calculated from three individual experiments with 1×10^3 pcDNA3/HRPT2/ras cells or pcDNA3/ras cells seeded into six-well dishes. The double asterisks denote a statistically significant decrease in the number of colonies in the dishes transfected with pcDNA3/HRPT2 and pDCR/H-ras, in comparison with the cells transfected with both pcDNA3 and pDCR/H-ras ($p < 0.01$). Columns indicate the mean number of colonies (clones) from three dishes; error bars are the standard deviation.



D

		Sub G1	G1	S	G2/M
HeLa Control Cells	Dox -	1.82± 0.05	56.89 ± 0.69	20.36 ± 1.63	21.25 ± 2.45
	Dox+ 24h	3.04 ± 0.36	54.16 ± 0.68	18.71 ± 0.30	26.04 ± 1.49
	Dox+ 48h	1.81 ± 0.56	58.75 ± 2.18	18.79 ± 0.93	20.96 ± 1.85
HeLa HRPT2 Cells	Dox -	1.24 ± 0.11	56.84 ± 0.72	17.99 ± 0.72	24.33 ± 0.58
	Dox+ 24h	3.54 ± 3.69	61.07 ± 1.29	17.11 ± 2.10	18.43 ± 0.07
	Dox+ 48h	4.56± 0.05	* 69.36 ± 3.21	14.11 ± 0.46	12.31 ± 3.75

Fig. 2. Overexpression of HRPT2 inhibited cell proliferation and caused G1 cell cycle arrest. (A) HeLa Tet-On HRPT2 cells were treated with 0, 0.05, 0.1, 0.5, 1, or 2 μg/ml of doxycycline for 24 h. The whole cell extracts (20 μg of protein) were separated by SDS–PAGE and assayed by Western blot analysis using antibody specific to HRPT2. (B) The HeLa Tet-On HRPT2 cells were treated with (b) or without (a) doxycycline (2 μg/ml) for 48 h before being photographed. (C) HeLa Tet-On HRPT2 cells and the control HeLa Tet-On cells were seeded in 60-mm-diameter dishes and cultured in the presence of the indicated amount of doxycycline (2 μg/ml) for 1–6 days. The number of surviving cells at different time points was counted after trypan blue staining. Statistically significant inhibition is indicated ($p < 0.005$). (D) Asynchronously growing HeLa Tet-On HRPT2 cells and the control HeLa Tet-On cells were incubated for 0, 24, and 48 h with or without doxycycline (2 μg/ml). The cells were harvested and subjected to flow cytometry analysis of DNA content. The values represent the means ± SD from three independent experiments. The asterisk indicates $p < 0.05$, as found in the comparison of the G1 cell number changes between the control cells and HRPT2-expressing cells after 48 h of incubation with doxycycline.

experiments. We constructed a pEGFP-HRPT2 plasmid expressing an EGFP–parafibromin fusion protein and transfected the construct into Cos-7 cells. The EGFP alone was evenly distributed throughout both the nucleus and cytoplasm. Fusion to parafibromin resulted in the nuclear accumulation of GFP (Fig. 3A). The expression of EGFP–parafibromin protein in Cos-7 cells was also confirmed by direct immunoblotting using a rabbit anti-GFP antibody (Supplemental Fig. IIIA). The localization of endogenous parafibromin was detected using an anti-parafibromin monoclonal antibody. The endogenous protein was localized predominantly to the nucleus, with a small fraction localized to the cytoplasm (Supplemental Fig.III). These results were then confirmed by nuclear and cytoplasmic subcellular fractions followed by immunoblotting (Fig. 3B). These data suggest that parafibromin is predominantly localized to the nucleus. The results are consistent with the recent identification of a bipartite nuclear localization signal at amino acid residues 125–139 [7].

Nuclear localization of parafibromin is essential to its anti-proliferative effect

To address the relationship between subcellular localization and negative effect on cell cycle proceeding (while maintaining the intact original protein), we utilized an ER (estrogen receptor)-inducible system [5,8,22]. After fusing a modified form of the estrogen receptor ligand binding domain (LBD, Gly400, Arg521) to the N-terminus of the HRPT2 cDNA, we demonstrated that HRPT2-ERTM is an efficient carrier to translocalize exogenous parafibromin from cytoplasm to nucleus upon the addition of 4-hydroxytamoxifen (4-OHT). An EGFP tag was fused to the construct as a visualized marker so that expression could be detected by both immunofluorescence (Fig. 3C) and cell fractionation (Fig. 3D). After the addition of 4-OHT, the original cytoplasmic-expressed fusion proteins greatly relocalized into nucleus. Then we performed a colony formation assay to study the anti-proliferative function due to the overexpression of parafibromin. Besides the pair of

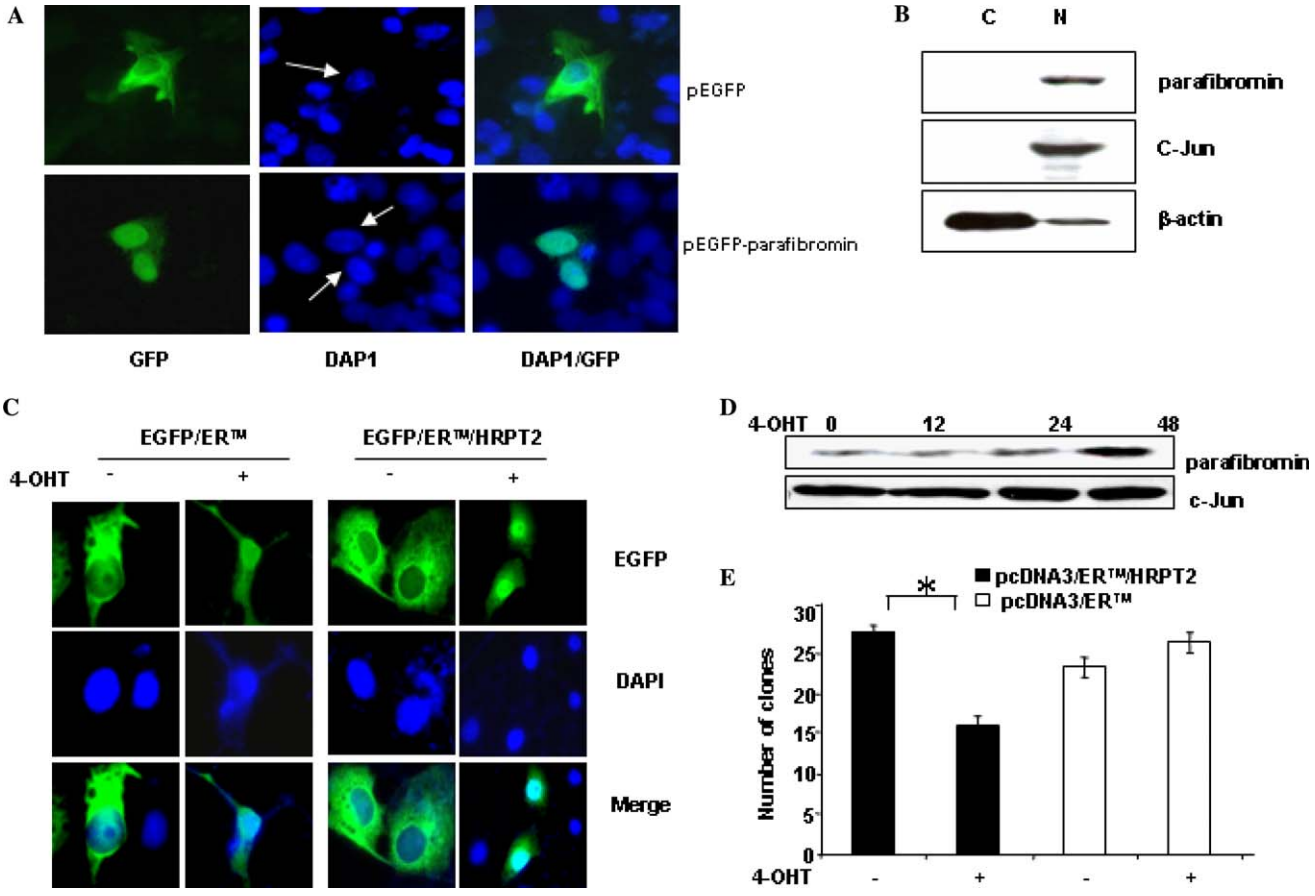


Fig. 3. Nuclear localization of parafibromin is essential for maintaining its anti-proliferative effect. Parafibromin protein fused at the C-terminus to GFP (pEGFP-HRPT2) was transiently expressed in Cos-7 cells. (A) At 24 h post-transfection, GFP was detected in the cells transfected with an empty vector, pEGFP, or with pEGFP-HRPT2. Nuclei were also stained by DAPI. (B) Nuclear (N) and cytoplasmic (C) extracts were prepared from Cos-7 cells. After the SDS–PAGE separation, the endogenous expression of parafibromin (top panel), c-Jun (middle panel, as a nuclear protein marker), and β-actin (bottom panel, as a predominantly cytoplasmic protein marker) were examined by immunoblotting. (C) Cos-7 cells were transiently transfected with pEGFP/ERTM or pEGFP/ERTM/HRPT2 plasmids. After 24 h culture with serum, 100 nM 4-OHT was added into the medium. In control cells, equal amount of ethanol was added. Forty-four hours later, the cells were fixed, and stained by DAPI. (D) In pcDNA3/ERTM/HRPT2-transfected Cos-7 cells, after 24 h of transfection, 4-OHT was added. At a serial of time points, Western blotting was performed on nuclear extracts using anti-parafibromin antibody. (E) The pcDNA3/ERTM/HRPT2-transfected NIH3T3 cells were cultured with or without 4-OHT and screened by colony formation assay.

pEGFP/ERTM and pEGFP/ERTM/HRPT2 (data not shown), we also transfected pcDNA3/ERTM/HRPT2 in pair with pcDNA3/ERTM into NIH3T3 cells (Fig. 3E). The result indicates that overexpression of nuclear parafibromin significantly reduced colony formation by about 35% compared with non-treated cells, while the overexpression of cytoplasmic protein has no such a capability. These data suggest that the nuclear localization of parafibromin is essential to its anti-growth effect caused by its overexpression.

Dominant-negative HRPT2 mutations suppress the effects of parafibromin on cell cycle arrest

Various mutations in the HRPT2 gene have been identified in both familial HPT-JT syndrome and sporadic parathyroid tumors in humans. To investigate whether tumor-derived HRPT2 mutations function in cell growth inhibition, we generated four disease-associated HRPT2 mutant constructs (HRPT2 (Q415Δ, (R227Δ, L64P), and (Y97H)) by site-directed mutagenesis. The expression of these constructs was confirmed by immunoblotting (Fig. 4A). The mutant HRPT2 genes were assayed for their ability to affect colony formation. All four mutants substantially reduced the ability of parafibromin to suppress colony formation (Fig. 4B). These mutants appear to function as dominant negatives, inhibiting the ability of the endogenous wild-type parafibromin to suppress cell growth. To probe the molecular basis for the loss of function in parafibromin, we also explored the effects of these mutations on subcellular localization. Each of the four disease-associated mutations was introduced into a pEGFP expression plasmid and assayed for subcellular localization by immunoflorescence microscopy. Each of the mutants retained the ability to localize to the nucleus (data not shown).

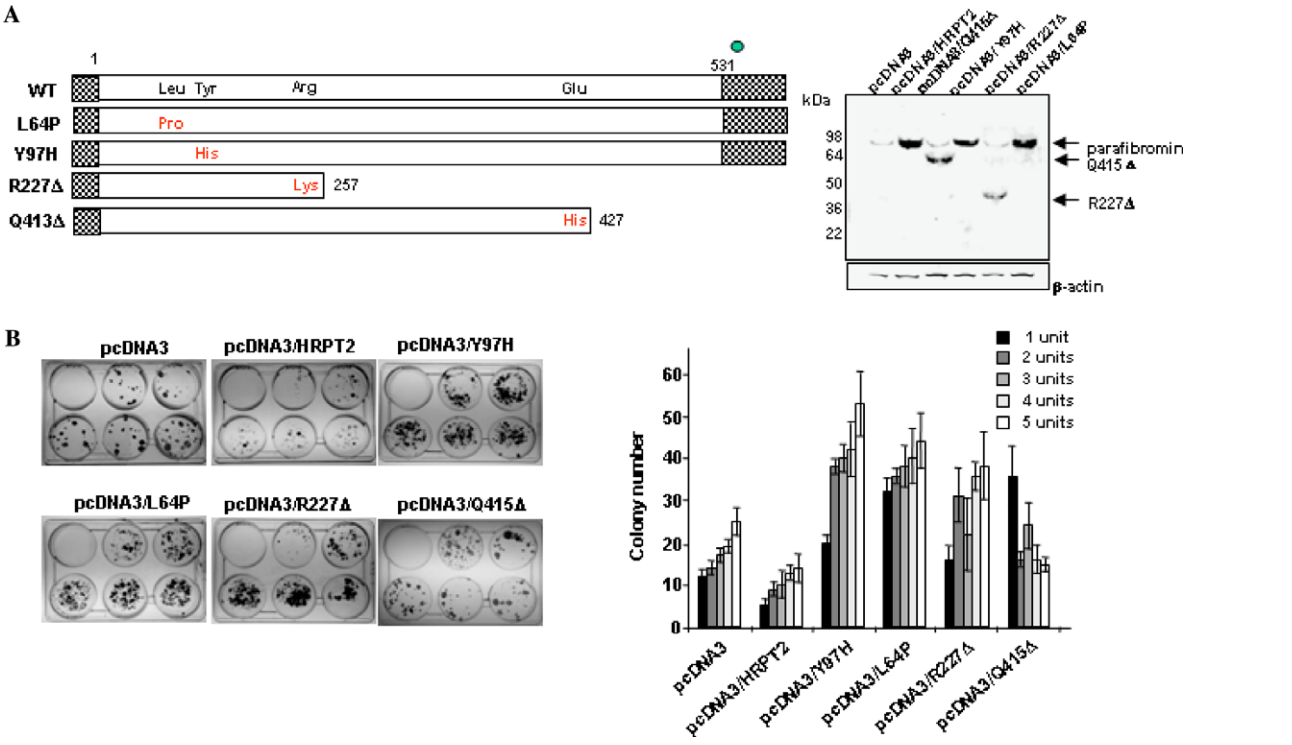


Fig. 4. The effect of parafibromin mutants in cell proliferation. (A) The top panel is a schematic representation of mutant human parafibromin constructs based on known patient mutations. Expression of the mutant proteins were confirmed by Western blotting with an anti-HRPT2 antibody. (B) Plasmids with mutated HRPT2 were transfected into NIH3T3 cells and assessed by foci formation. The mean number of colonies from three dishes is indicated in the right chart. One unit stands for a specific molar amount of plasmid. Results represent the means + SD from three independent experiments.

Discussion

HPT-JT syndrome is a classical hereditary cancer syndrome inherited in an autosomal dominant pattern. The inactivating nature of the mutations in these patients coupled with the LOH of the wild-type alleles in some tumors point to a tumor suppressive role of the gene [16]. Although recent studies have identified a role of parafibromin in the Paf1 transcriptional complex [15], the mechanism through which parafibromin contributes to tumorigenesis is unknown. In this study, we have shown that overexpression of parafibromin can inhibit colony formation and anchorage-dependent cell growth, supporting the role of HRPT2 as a tumor suppressor gene. We have also demonstrated that this decrease in cell proliferation is likely a result of cell cycle arrest at the G1 phase. These findings suggest a direct role of parafibromin in cell cycle regulation; however the critical molecular mechanism and biochemical properties responsible for the cell cycle arrest and decreased cell proliferation require further study.

We additionally confirmed that parafibromin is predominantly localized to the nucleus, consistent with it documented function in the Paf1 transcriptional complex [15,21]. By using the estrogen-receptor system to control subcellular transportation, we observed that nuclear localization of parafibromin is essential for cell cycle arrest. Interestingly, overexpression of cytoplasmic parafibromin does not cause cell cycle arrest. These data show that overexpression of nuclear parafibromin can inhibit cell proliferation. Since the function of parafibromin in the nucleus may be directly linked to the Paf1 complex, the involvement of the Paf1 complex in cell cycle regulation is worth further study.

Previous studies have identified three somatic inactivating mutations and 13 germline mutations of HRPT2 in HPT-JT patients [9,19]. These mutations are spread throughout the HRPT2 gene. No particular region, including the Cdc73-homologous domain, appears to be a hot spot for oncogenic mutations. In addition to nonsense and frameshift mutations, a number of missense mutations have been identified. The functional consequences of these missense mutations in the development of HPT-JT syndrome and parathyroid carcinoma had been unclear. Our data showing that induced overexpression of parafibromin causes growth inhibition and cell cycle arrest provided the first functional assay for evaluating the physiological consequences of these tumor-derived mutations. We have found that all four mutations in HRPT2 that we tested abolished the function of parafibromin in suppressing colony formation (Fig. 4). Protein phosphorylation, a modulator of protein function and stability, can occur at Tyr residues and is mediated by specific protein kinases [13]. In HRPT2/Y97H, the change from Tyr to His on exon 3 of HRPT2, and in HRPT2/L67P, the change from Leu to Pro on exon 2 of HRPT2, lead to the loss of a potential site of phosphorylation. In contrast to the point mutants, the

HRPT2 (R227Δ) and HRPT2 (Q415Δ) mutations are each frameshifts at exon 7 and exon 14, respectively, and thus generate two truncated proteins that have lost the cell cycle—inhibitory functions of parafibromin. The expression of these parafibromin mutations leads to suppression of the growth-arrested phenotype and enhanced survival (Fig. 4). The C-terminus of parafibromin shares a high sequence similarity with the yeast protein Cdc73 [9], a component of the Paf1 complex that is associated with RNA polymerase II [11]. Interestingly, parafibromin truncation mutants R227Δ and Q415Δ lie within this conserved region. Therefore, this shared region may be important for an interaction with RNA polymerase II. We show that these mutants act in a dominant negative manner in the presence of the wild-type allele. This is compatible with the clinico-pathological observation that in a significant percentage of HPT-JT-related tumors, no LOH was found, suggesting a possible dominant negative mechanism. These findings also provide an assay for evaluating the functional consequence of other disease-associated mutations in HRPT2 and may help to establish HRPT2 as a promising target for anticancer therapies.

In summary, we addressed the cellular and biochemical mechanisms underlying tumor suppression by parafibromin. First, overexpression of parafibromin suppressed colony formation and anchorage-independent cell growth, likely a direct result of G1 cell cycle arrest (Figs. 1 and 2). Second, parafibromin was predominantly localized in the nucleus, which was essential to its anti-proliferative effect in tumor cell lines (Fig. 3). Lastly, disease-associated mutations in parafibromin abolished its ability to suppress colony formation (Fig. 4).

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2006.08.169.

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