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Proteomic Analysis of Mammalian Primary Cilia

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Summary

The primary cilium is a microtubule-based organelle that senses extracellular signals as a cellular antenna [1]. Primary cilia are found on many types of cells in our body and play important roles in development and physiology. Defects of primary cilia cause a broad class of human genetic diseases called ciliopathies. To gain new insights into ciliary functions and better understand the molecular mechanisms underlying ciliopathies, it is of high importance to generate a catalog of primary cilia proteins. In this study, we isolated primary cilia from mouse kidney cells by using a calcium shock method and identified 195 candidate primary cilia proteins by MudPIT (multidimensional protein identification technology), protein correlation profiling, and subtractive proteomic analysis. Based on comparisons with other proteomic studies of cilia, around 75% of our candidate primary cilia proteins are shared components with motile or specialized sensory cilia. The remaining 25% of the candidate proteins are possible primary cilia specific proteins. These possible primary cilia specific proteins include Evc2, Inpp5e and Inversin, several of which have been linked to known ciliopathies. We have performed the first reported proteomic analysis of primary cilia from mammalian cells. These results provide new insights into primary cilia structure and function.

Results

Isolation of Mammalian Primary Cilia

To identify the proteins that localize to mammalian primary cilia, we isolated primary cilia from the mouse IMCD3 cell line, which are derived from kidney collecting ducts, by using a modified calcium shock method (see Supplemental Experimental Procedures). The calcium shock method is conventionally employed for olfactory cilia isolation [2] and has been used for proteomic analysis of human bronchial and rat olfactory cilia [3, 4]. Immunofluorescence imaging confirmed that our modified calcium shock method efficiently removed primary cilia from cultured IMCD3 cells and isolated fraction includes primary cilia (Figure 1).

Not surprisingly for such a method, we found that the isolated primary cilia fraction included non-ciliary proteins when analyzed by Western blot (data not shown). To separate primary cilia from non-ciliary proteins, we fractionated the isolated primary cilia fraction by

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velocity sedimentation through a 30–45% continuous sucrose gradient. Western blot analysis of these fractions showed that the peak of acetylated-tubulin (fraction 13–17), a marker for primary cilia, was displaced from the peak of actin (fraction 6–9), which was used as a non-ciliary marker (Figure 2A, B). We confirmed that actin is not enriched in the primary cilium by immunofluorescence microscopy (data not shown). A recent study also showed that F-actin is excluded from cilia and the area around the base of cilia [5].

MudPIT analysis

Since the actin and acetylated-tubulin rich peaks overlapped extensively, it was not possible to simply pick one fraction and consider it a pure ciliary fraction. Instead, we analyzed each of the fractions 5–22 from the continuous sucrose gradient, which included both the actin and acetylated-tubulin peaks, by MudPIT, a mass-spectrometry-based method in which complex mixtures of proteins can be analyzed without prior electrophoretic separation [6]. MudPIT analysis of the fractions 5–22 detected 2,937 proteins in total (based on a cutoff of 10 spectrum counts), which included known ciliary proteins as well as known non-ciliary proteins (Table S4).

Protein Correlation Profiling

To distinguish ciliary proteins from non-ciliary proteins we employed protein correlation profiling [7]. We calculated the relative abundance of each protein in each of the sucrose gradient fractions using the spectrum count of MudPIT data (see Supplemental Experimental Procedures). Once the abundance profiles were calculated for every protein, we next identified possible candidate primary cilia proteins by correlating their profiles to profiles of known ciliary proteins. The profiles of several known intraflagellar transport (IFT) proteins, plotted in Figure 2C, showed that they all had very similar profile patterns. These IFT protein abundance profiles all peaked between fractions 13–17, which coincided with the cilia peak as judged by Western blotting analysis for acetylated-tubulin (Figure 2A, B). In contrast, non-ciliary proteins had a fractionation profile that was very different from that of IFT proteins (Figure 2D). We performed protein correlation profiling as described in Supplemental Experimental Procedures, comparing the profile of every detected protein with the IFT-derived consensus profile. This analysis identified 379 candidate proteins with similar profile patterns to the IFT proteins (Table S4). These proteins were selected as our initial candidate set for the primary cilia proteome.

Subtractive Proteomic Analysis

Protein correlation profiling is a purely statistical tool. While it can indicate which proteins are more likely than others to be ciliary proteins it will always generate false positives. We therefore augmented our analysis with a subtractive proteomic strategy for discriminating ciliary from non-ciliary proteins independent of profile correlations. We generated IMCD3 cells lacking cilia by treating cells with nocodazole and incubating at a cold temperature (Figure 3). We then performed cilia isolations using the calcium shock method from both normal ciliated IMCD3 cells and non-ciliated nocodazole/cold treated IMCD3 cells. The isolated fractions from ciliated and non-ciliated cells were individually analyzed by MudPIT and compared. Any proteins detected twice or more in the fraction from non-ciliated cells were classified as likely non-ciliary proteins and subtracted from the original list of 379 candidates.

As a final step, we corrected for multiple isoforms and redundant protein identification. (Table S4). The final result of our combined correlation profiling and subtractive proteomics analysis was a list of 195 candidate primary cilia proteins (Table S1).

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Validation of Candidate Primary Cilia Proteins

To validate our candidate list, we compared our candidate primary cilia protein list with other systematic studies, including proteomic, comparative genomic, and promoter-analysis based studies of cilia (Table S2) [3, 4, 8–19]. We found that more than 80% of proteins in our candidate list were also identified in one or more prior systematic studies of cilia and around 75% of candidate proteins were also identified in other proteomic studies of cilia. Thus we infer that our proteomic analysis of primary cilia is likely to contain many bona fide ciliary proteins. We note that 138 of our candidate proteins were also identified in proteomic studies of specialized sensory cilia [3, 8, 9]. Significantly, out of our list of 195 candidates, 46 were never identified in other proteomic studies of cilia.

To more directly verify our candidate list, we randomly cloned a subset of candidate proteins, and stably expressed C-terminal green fluorescent protein (GFP)-tagged and N-terminal FLAG-tagged candidate proteins in IMCD3 cells (Table S3). 8 of 18 candidate proteins were localized to primary cilia in these stably expressing lines as judged by GFP fluorescence (Figure 4) or FLAG-tag staining (data not shown). Thus only about half of the proteins tested showed localization in cilia, and while some of these could well be false negatives due to the peptide tags, it is clear that not all of the proteins in our candidate list are bona fide ciliary proteins. As with any complex organelle proteome, our results are best viewed as giving an enrichment of ciliary proteins, rather than an absolute purification.

During the course of our studies, some of these candidate proteins have already been confirmed their ciliary localization in other tissues or cell lines by other recent studies [20–22]. Interestingly, among these candidates we found that Gtl3 (a homologue of *Chlamydomonas* BUG22) [23] is localized to both primary cilia and nuclei (Figure 4B), and it has a domain consistent with it being a possible transcription factor. Wdr11 is mainly localized to centrosomes but is also weakly localized to cilia (Figure 4D). One particularly interesting candidate that we localized to primary cilia, Tsga14 (CEP41, Figure 4C), had been identified in the proteomic analysis of the human centrosome [7] but was not previously localized to cilia.

Discussion

We identified 195 proteins as potential primary cilia proteins by using the calcium shock isolation method, MudPIT correlation profiling, and subtractive proteomic analysis. This study is the first proteomic analysis of generic mammalian primary cilia. Previous proteomic analyses of non-motile mammalian cilia focused on specialized sensory cilia, namely the connecting cilium of photoreceptors and olfactory cilia [3, 8, 9], which differ in appearance from “generic” primary cilia but share the same fundamental structure of a microtubule axoneme surrounded by a membrane.

Our primary cilia candidate list includes almost all known IFT proteins except Ift43 (Table S5). We also identified kinesin and dynein IFT motor proteins. Notably absent were any BBS proteins, which are the causative gene products of Bardet-Biedl syndrome and are localized to primary cilia [24]. We do not understand why most of the IFT proteins, but none of the BBS proteins, were detected in our analysis. Nevertheless, the fact that we recovered almost every known IFT proteins, including IFT proteins not used to construct the reference protein profile, confirms that our calcium shock method and protein correlation profiling were effective for identifying bona fide ciliary proteins. Our candidate list also includes proteins that potentially regulate protein import into the cilium. We identified the septins (Sept2, Sept7 and Sept9) in our ciliary proteome. Septins have been reported to form a diffusion barrier that controls protein entry into cilia and regulates ciliogenesis [25, 26]. We also detected the small GTPase Ran as well as importin, which regulate both nuclear and

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ciliary entry of proteins [27]. However, we failed to detect many known ciliary membrane proteins, such as polycystin-1 and polycystin-2. This may reflect the reduced solubility of such proteins under extraction conditions used for protein separation in MudPIT.

Around 75% of our candidate primary cilia proteins are shared components with motile or specialized sensory cilia (Table S2). Especially, most of IFT proteins and Nme7 were also identified in comparative genomics and X-box promoter analysis (Table S2). We speculate that Nme7 is a part of IFT complex or related with IFT. However, Nme7 may regulate protein transport or signal transduction rather than assembly of cilia [28]. This indicates these proteins are necessary for all types of cilia and flagella. On the other hand, prior to our analysis we had expected to recover two major classes of axonemal proteins in our candidate list: tektins and pf-ribbon proteins [29, 30]. As these proteins are known to be stable and abundant components of the axoneme, we assumed that they would be among the most abundant hits in our list, just as they have been reported to be common in proteomic analysis of motile cilia/flagella and basal bodies [4, 10, 23]. Structural studies have suggested that tektins and pf-ribbon proteins form filamentous structures tightly associated with the outer doublets of the axoneme, which are conserved among all animal cilia, and may therefore be critical determinants of outer doublet architecture in all cilia [31]. Surprisingly, neither tektin nor pf-ribbon proteins were found in the candidate list from our primary cilia proteome. Tektin and pf-ribbon proteins have also not been identified in the proteomes of specialized sensory cilia [3, 8, 9]. Moreover, knock out or knock down of tektin induces defects in ciliary motility but not in ciliogenesis per se [32, 33]. Therefore, it is possible that tektin and pf-ribbon proteins are only components of basal bodies and motile cilia, but not primary cilia.

The remaining 25% of our candidate proteins are possible primary cilia specific proteins because these proteins have not been identified in proteomic studies of motile or specialized sensory cilia. However, some primary cilia specific proteins might be also localized to sensory or motile cilia because Inpp5e was also identified in inner segment of photoreceptor [34]. Some of the primary cilia specific proteins are apparently involved in signal transduction, such as, Evc2, Inpp5e, Inversin, Broad-Minded (Bromi) and Nphp3. Evc2 and Bromi function in hedgehog signal transduction [20, 35], while Inpp5e is involved with phosphatidylinositol and PDGF signaling [34, 36].

Because ciliary defects are linked to many genetic disorders, we were particularly interested to see if any ciliary disease loci would turn up in our candidate list. In fact, multiple candidate proteins were found to correspond to known causative gene products of genetic disorders (Table S6). We identified several known ciliopathy gene products, including Inpp5e, Evc2, Nphp3, Nek8 and Lca5 (see referenes in Supplemental Information). A recent study reported that TSGA14 is a plausible causative gene of autism spectrum disorders [37]. Moreover, WDR11 has been also recently reported as a causative gene of idiopathic hypogonadotropic hypogonadism and Kallmann syndrome [38]. In light of our results that Tsga14 and Wdr11 are present within primary cilia (Figure 4C, D), we hypothesize that the autism and abnormal genital development in such patients might illustrate new symptoms of ciliopathy.

In this study, we isolated primary cilia from cultured mammalian cells and utilized this material to generate the first reported proteome of mammalian primary cilia. Our analysis revealed that the majority of primary cilia components are shared with motile and specialized sensory cilia. However, around 25% of the candidate proteins were only identified in this analysis, including Evc2, Inpp5e, Inversin, Nphp3 and Broad-Minded. These novel candidates, a number of which have already been implicated in ciliopathies and signal transduction, are potentially specific to primary cilia. Tsga14 and Wdr11 are new

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candidates of causative genes of ciliopathy. The results of this study should serve as a starting point toward a greater understanding of primary cilia functions and mechanistic insights into ciliary diseases.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations List

BBS	Bardet-Biedl Syndrome
GFP	green fluorescent protein
IFT	intraflagellar transport
IMCD3	inner medullary collecting duct 3
MudPIT	multidimensional protein identification technology

References

1. Veland IR, Awan A, Pedersen LB, Yoder BK, Christensen ST. Primary cilia and signaling pathways in mammalian development, health and disease. Nephron Physiol. 2009; 111:39–53.
2. Rhein LD, Cagan RH. Biochemical studies of olfaction: isolation, characterization, and odorant binding activity of cilia from rainbow trout olfactory rosettes. Proc. Natl. Acad. Sci. U.S.A. 1980; 77:4412–4416. [PubMed: 6449006]
3. Mayer U, Ungerer N, Klimmeck D, Warnken U, Schnölzer M, Frings S, Möhrlen F. Proteomic analysis of a membrane preparation from rat olfactory sensory cilia. Chem. Senses. 2008; 33:145–162. [PubMed: 18032372]
4. Ostrowski LE, Blackburn K, Radde KM, Moyer MB, Schlatzer DM, Moseley A, Boucher RC. A proteomic analysis of human cilia: identification of novel components. Mol. Cell Proteomics. 2002; 1:451–465. [PubMed: 12169685]
5. Francis SS, Sfakianos J, Lo B, Mellman I. A hierarchy of signals regulates entry of membrane proteins into the ciliary membrane domain in epithelial cells. J. Cell Biol. 2011; 193:219–233. [PubMed: 21444686]
6. Washburn MP, Wolters D, Yates JR. Large-scale analysis of the yeast proteome by multidimensional protein identification technology. Nat. Biotechnol. 2001; 19:242–247. [PubMed: 11231557]
7. Andersen JS, Wilkinson CJ, Mayor T, Mortensen P, Nigg EA, Mann M. Proteomic characterization of the human centrosome by protein correlation profiling. Nature. 2003; 426:570–574. [PubMed: 14654843]
8. Liu Q, Tan G, Levenkova N, Li T, Pugh EN Jr, Rux JJ, Speicher DW, Pierce EA. The proteome of the mouse photoreceptor sensory cilium complex. Mol. Cell Proteomics. 2007; 6:1299–1317. [PubMed: 17494944]
9. Mayer U, Küller A, Daiber PC, Neudorf I, Warnken U, Schnölzer M, Frings S, Möhrlen F. The proteome of rat olfactory sensory cilia. Proteomics. 2009; 9:322–334. [PubMed: 19086097]
10. Pazour GJ, Agrin N, Leszyk J, Witman GB. Proteomic analysis of a eukaryotic cilium. J. Cell Biol. 2005; 170:103–113. [PubMed: 15998802]

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11. Avidor-Reiss T, Maer AM, Koundakjian E, Polyanovsky A, Keil T, Subramaniam S, Zuker CS. Decoding cilia function: defining specialized genes required for compartmentalized cilia biogenesis. Cell. 2004; 117:527–539. [PubMed: 15137945]
12. Li JB, Gerdes JM, Haycraft CJ, Fan Y, Teslovich TM, May-Simera H, Li H, Blacque OE, Li L, Leitch CC, et al. Comparative genomics identifies a flagellar and basal body proteome that includes the BBS5 human disease gene. Cell. 2004; 117:541–552. [PubMed: 15137946]
13. Blacque OE, Perens EA, Boroevich KA, Inglis PN, Li C, Warner A, Khattra J, Holt RA, Ou G, Mah AK, et al. Functional genomics of the cilium, a sensory organelle. Curr. Biol. 2005; 15:935–941. [PubMed: 15916950]
14. Efimenko E, Bubb K, Mak HY, Holzman T, Leroux MR, Ruvkun G, Thomas JH, Swoboda P. Analysis of xbx genes in C. elegans. Development. 2005; 132:1923–1934. [PubMed: 15790967]
15. Smith JC, Northey JGB, Garg J, Pearlman RE, Siu KWM. Robust method for proteome analysis by MS/MS using an entire translated genome: demonstration on the ciliome of Tetrahymena thermophila. J. Proteome Res. 2005; 4:909–919. [PubMed: 15952738]
16. Broadhead R, Dawe HR, Farr H, Griffiths S, Hart SR, Portman N, Shaw MK, Ginger ML, Gaskell SJ, McKean PG, et al. Flagellar motility is required for the viability of the bloodstream trypanosome. Nature. 2006; 440:224–227. [PubMed: 16525475]
17. Laurençon A, Dubruille R, Efimenko E, Grenier G, Bissett R, Cortier E, Rolland V, Swoboda P, Durand B. Identification of novel regulatory factor X (RFX) target genes by comparative genomics in Drosophila species. Genome Biol. 2007; 8:R195. [PubMed: 17875208]
18. Merchant SS, Prochnik SE, Vallon O, Harris EH, Karpowicz SJ, Witman GB, Terry A, Salamov A, Fritz-Laylin LK, Maréchal-Drouard L, et al. The Chlamydomonas genome reveals the evolution of key animal and plant functions. Science. 2007; 318:245–250. [PubMed: 17932292]
19. Arnaiz O, Malinowska A, Klotz C, Sperling L, Dadlez M, Koll F, Cohen J. Cildb: a knowledgebase for centrosomes and cilia. Database (Oxford). 20092009:bap022. [PubMed: 20428338]
20. Blair HJ, Tompson S, Liu Y-N, Campbell J, MacArthur K, Ponting CP, Ruiz-Perez VL, Goodship JA. Evc2 is a positive modulator of Hedgehog signalling that interacts with Evc at the cilia membrane and is also found in the nucleus. BMC Biol. 2011; 9:14. [PubMed: 21356043]
21. Massinen S, Hokkanen M-E, Matsson H, Tammimies K, Tapia-Páez I, Dahlström-Heuser V, Kuja-Panula J, Burghoorn J, Jeppsson KE, Swoboda P, et al. Increased expression of the dyslexia candidate gene DCDC2 affects length and signaling of primary cilia in neurons. PLoS ONE. 2011; 6:e20580. [PubMed: 21698230]
22. Mill P, Lockhart PJ, Fitzpatrick E, Mountford HS, Hall EA, Reijns MAM, Keighren M, Bahlo M, Bromhead CJ, Budd P, et al. Human and mouse mutations in WDR35 cause short-rib polydactyly syndromes due to abnormal ciliogenesis. Am. J. Hum. Genet. 2011; 88:508–515. [PubMed: 21473986]
23. Keller LC, Romijn EP, Zamora I, Yates JR, Marshall WF. Proteomic analysis of isolated chlamydomonas centrioles reveals orthologs of ciliary-disease genes. Curr. Biol. 2005; 15:1090–1098. [PubMed: 15964273]
24. Nachury MV, Loktev AV, Zhang Q, Westlake CJ, Peränen J, Merdes A, Slusarski DC, Scheller RH, Bazan JF, Sheffield VC, et al. A core complex of BBS proteins cooperates with the GTPase Rab8 to promote ciliary membrane biogenesis. Cell. 2007; 129:1201–1213. [PubMed: 17574030]
25. Hu Q, Milenkovic L, Jin H, Scott MP, Nachury MV, Spiliotis ET, Nelson WJ. A septin diffusion barrier at the base of the primary cilium maintains ciliary membrane protein distribution. Science. 2010; 329:436–439. [PubMed: 20558667]
26. Kim SK, Shindo A, Park TJ, Oh EC, Ghosh S, Gray RS, Lewis RA, Johnson CA, Attie-Bittach T, Katsanis N, et al. Planar cell polarity acts through septins to control collective cell movement and ciliogenesis. Science. 2010; 329:1337–1340. [PubMed: 20671153]
27. Dishinger JF, Kee HL, Jenkins PM, Fan S, Hurd TW, Hammond JW, Truong YN-T, Margolis B, Martens JR, Verhey KJ. Ciliary entry of the kinesin-2 motor KIF17 is regulated by importin-beta2 and RanGTP. Nat. Cell Biol. 2010; 12:703–710. [PubMed: 20526328]

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28. Lai CK, Gupta N, Wen X, Rangell L, Chih B, Peterson AS, Bazan JF, Li L, Scales SJ. Functional characterization of putative cilia genes by high-content analysis. Mol. Biol. Cell. 2011; 22:1104–1119. [PubMed: 21289087]
29. Linck RW, Norrander JM. Protofilament ribbon compartments of ciliary and flagellar microtubules. Protist. 2003; 154:299–311. [PubMed: 14658491]
30. Amos LA. The tektin family of microtubule-stabilizing proteins. Genome Biol. 2008; 9:229. [PubMed: 18671835]
31. Nojima D, Linck RW, Egelman EH. At least one of the protofilaments in flagellar microtubules is not composed of tubulin. Curr. Biol. 1995; 5:158–167. [PubMed: 7743179]
32. Tanaka H, Iguchi N, Toyama Y, Kitamura K, Takahashi T, Kaseda K, Maekawa M, Nishimune Y. Mice deficient in the axonemal protein Tektin-t exhibit male infertility and immotile-cilium syndrome due to impaired inner arm dynein function. Mol. Cell. Biol. 2004; 24:7958–7964. [PubMed: 15340058]
33. Mitchell B, Jacobs R, Li J, Chien S, Kintner C. A positive feedback mechanism governs the polarity and motion of motile cilia. Nature. 2007; 447:97–101. [PubMed: 17450123]
34. Bielas SL, Silhavy JL, Brancati F, Kisseleva MV, Al-Gazali L, Sztriha L, Bayoumi RA, Zaki MS, Abdel-Aleem A, Rosti RO, et al. Mutations in INPP5E, encoding inositol polyphosphate-5-phosphatase E, link phosphatidyl inositol signaling to the ciliopathies. Nat. Genet. 2009; 41:1032–1036. [PubMed: 19668216]
35. Ko HW, Norman RX, Tran J, Fuller KP, Fukuda M, Eggenschwiler JT. Broad-minded links cell cycle-related kinase to cilia assembly and hedgehog signal transduction. Dev. Cell. 2010; 18:237–247. [PubMed: 20159594]
36. Jacoby M, Cox JJ, Gayral S, Hampshire DJ, Ayub M, Blockmans M, Pernot E, Kisseleva MV, Compère P, Schiffmann SN, et al. INPP5E mutations cause primary cilium signaling defects, ciliary instability and ciliopathies in human and mouse. Nat. Genet. 2009; 41:1027–1031. [PubMed: 19668215]
37. Korvatska O, Estes A, Munson J, Dawson G, Bekris LM, Kohen R, Yu C-E, Schellenberg GD, Raskind WH. Mutations in the TSGA14 gene in families with autism spectrum disorders. Am. J. Med. Genet. B Neuropsychiatr. Genet. 2011; 156B:303–311. [PubMed: 21438139]
38. Kim H-G, Ahn J-W, Kurth I, Ullmann R, Kim H-T, Kulharya A, Ha K-S, Itokawa Y, Meliciani I, Wenzel W, et al. WDR11, a WD protein that interacts with transcription factor EMX1, is mutated in idiopathic hypogonadotropic hypogonadism and Kallmann syndrome. Am. J. Hum. Genet. 2010; 87:465–479. [PubMed: 20887964]

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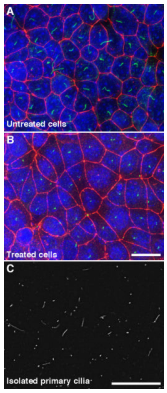

Figure 1.

The calcium shock method efficiently isolates primary cilia from IMCD3 cells. (A, B) Immunofluorescence images of IMCD3 cells stained with acetylated-tubulin (green; primary cilia), ZO-1 (red; cell-cell junctions) and DAPI (blue; nuclei). Untreated cells (A) and cells treated with deciliation solution (B). (C) Isolated fraction includes primary cilia, which stained with acetylated-tubulin. Scale bars, 10 μm.

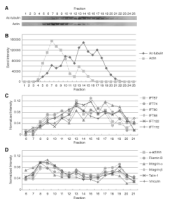

Figure 2.

Fractionation of isolated ciliary proteins and protein correlation profiling. (A) Western blotting images of isolated primary cilia fractions, fractionated on a 30–45% continuous sucrose gradient. Acetylated-tubulin was used as a marker for ciliary proteins, while actin represents a typical non-ciliary contaminant. (B) Quantification of band intensities from Western blotting in A showing displaced but overlapping peaks of acetylated-tubulin and actin. (C, D) Graphs display the normalized abundance profiles of a set of known ciliary proteins (IFT proteins: C) and known non-ciliary proteins (focal adhesion proteins: D) based on MudPIT mass spectrometry analysis of the fractions.

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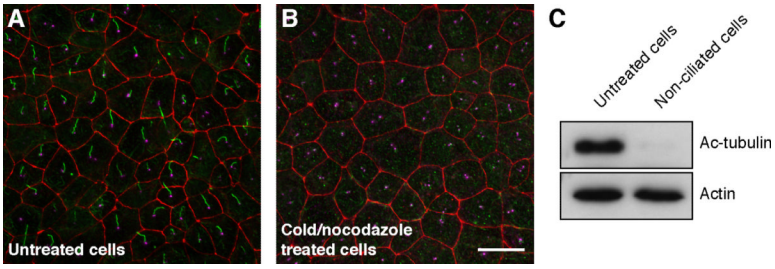

Figure 3.

Nocodazole and cold treatment generate non-ciliated cells for subtractive proteomic analysis.

(A, B) Immunofluorescence images of IMCD3 cells stained with acetylated-tubulin (green; primary cilia), ZO-1 (red; cell-cell junctions) and pericentrin (magenta; centrosomes). Untreated cells (A) and cells treated with cold and nocodazole to retract cilia (B). Scale bars, 10 μm. (C) Western blots of isolated fractions from normal IMCD3 cells and non-ciliated (cold and nocodazole treated) cells, probed with antibodies to acetylated-tubulin (Ac-tubulin) and actin. These non-ciliated cells were used to prepare a reference fraction for subtractive proteomics.

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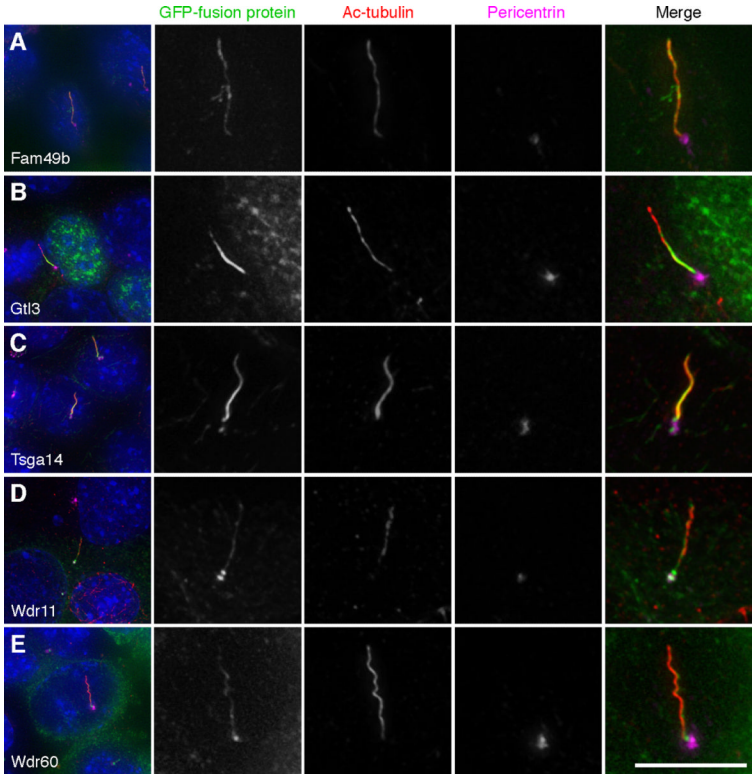

Figure 4.

Localization of candidate primary cilia proteins.

(A–E) GFP-tagged candidate primary cilia protein constructs (GFP-fusion protein, green) stably expressed in transfected IMCD3 cells. Acetylated-tubulin (Ac-tubulin, red) antibody stains primary cilia. Pericentrin antibody stains centrosomes (magenta). Far left column shows merged image and DAPI (blue; nuclei). Right four columns are enlarged images. Scale bars, 5 μm.