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Pericentrin in health and disease

Exploring the patchwork of Pericentrin splice variants

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Abbreviations: Pcnt, Pericentrin (protein); PCNT, Pericentrin (gene); PCM, pericentriolar material; MOPD II, Majewski/microcephalic osteodysplastic primordial dwarfism type II

Researchers around the world perform large-scale screens to identify disease-related gene defects in humans. One of the genes of interest is Pericentrin (PCNT), a gene which codes for a large coiled-coil protein with multiple functions in the cell. Recently, we showed that different Pericentrin (Pcnt) splice variants are differentially distributed among sensory tissues of the mouse, emphasizing the importance of a protein's spliceome for the function of a cell.

Pericentrin (Pcnt)

Pcnt, a component of the pericentriolar material (PCM), is a highly conserved protein throughout the animal kingdom up to human. At the centrosomes, Pcnt serves as a multifunctional scaffold for various proteins and protein complexes and has important functions in microtubule organization, cell division, cell cycle progression, assembly of cilia and probably in various other fundamental cellular processes.[1-4] Pericentrin (PCNT) mutations are associated with various diseases, most prominent the Majewski/microcephalic osteodysplastic primordial dwarfism type II (MOPD II), a rare human autosomal recessive genetic disorder.[4-6] The link between Pcnt and other diseases like human cancer, mental disorders and ciliopathies is not as strong as for MOPD II, but nevertheless cellular and molecular evidence supports a role for Pcnt in these disorders.[4] The localization of Pcnt at the base of primary cilia was shown a few years ago,[7] and since then Pcnt proved to play important roles in ciliary function.[7-10] Only very recently, we showed the specific expression of a Pcnt splice variant at the basal body complex of the connecting cilium in photoreceptor cells of mice,[11] raising questions concerning the function of the Pcnt spliceome in general.

Pcnt is a large coiled-coil protein with so far three known splice variants from orthologous genes in mice and humans. The largest form, Pcnt B, has a molecular weight of ~360 kDa in mice and ~380 kDa in humans. Moreover, two smaller forms with a size of ~220–250 kDa, Pcnt A and Pcnt S, are known from mice (**Fig. 1A**).[11-15]

Pcnt in the Mouse

In our study of the cellular expression and distribution of Pcnt in neuronal tissues of the mouse with a focus on the retina and its sensory neurons, the photoreceptors, we showed that Pcnt splice variants are differentially distributed in the examined tissues and even within the cells of one tissue.[11] The photoreceptors of mice contain predominantly one of the smaller Pcnt splice variants, most likely a modified variant of Pcnt S. In contrast, the larger Pcnt B splice variant is present in much lesser amounts in photoreceptors, but it is strongly expressed in other retinal cells or other neuronal tissues.[11] This specific distribution of the various Pcnt splice variants provides an explanation for the findings of Miyoshi and colleagues, who reported ciliary defects in the olfactory system of mice with a hypomorphic mutation in the PCNT gene in the region of exon 1, and the absence of a ciliary phenotype in other ciliated tissues like the retina with its photoreceptors.[10] The Pcnt splice variants present in the olfactory epithelium—Pcnt B and Pcnt A[11]—both start with exon 1 and thus are affected by the hypomorphic mutation (**Fig. 1A**). As mouse photoreceptors predominantly express Pcnt S, which is most likely still functional in the hypomorphic animals, the lack of a retinal phenotype is not astonishing.[11]

We conclude that in the mouse different Pcnt splice variants are expressed in different tissues and even in different cells of one tissue. The consequence of such a patchwork of splice variants is that most mutations in the PCNT gene will not have global but tissue and even cell specific effects.

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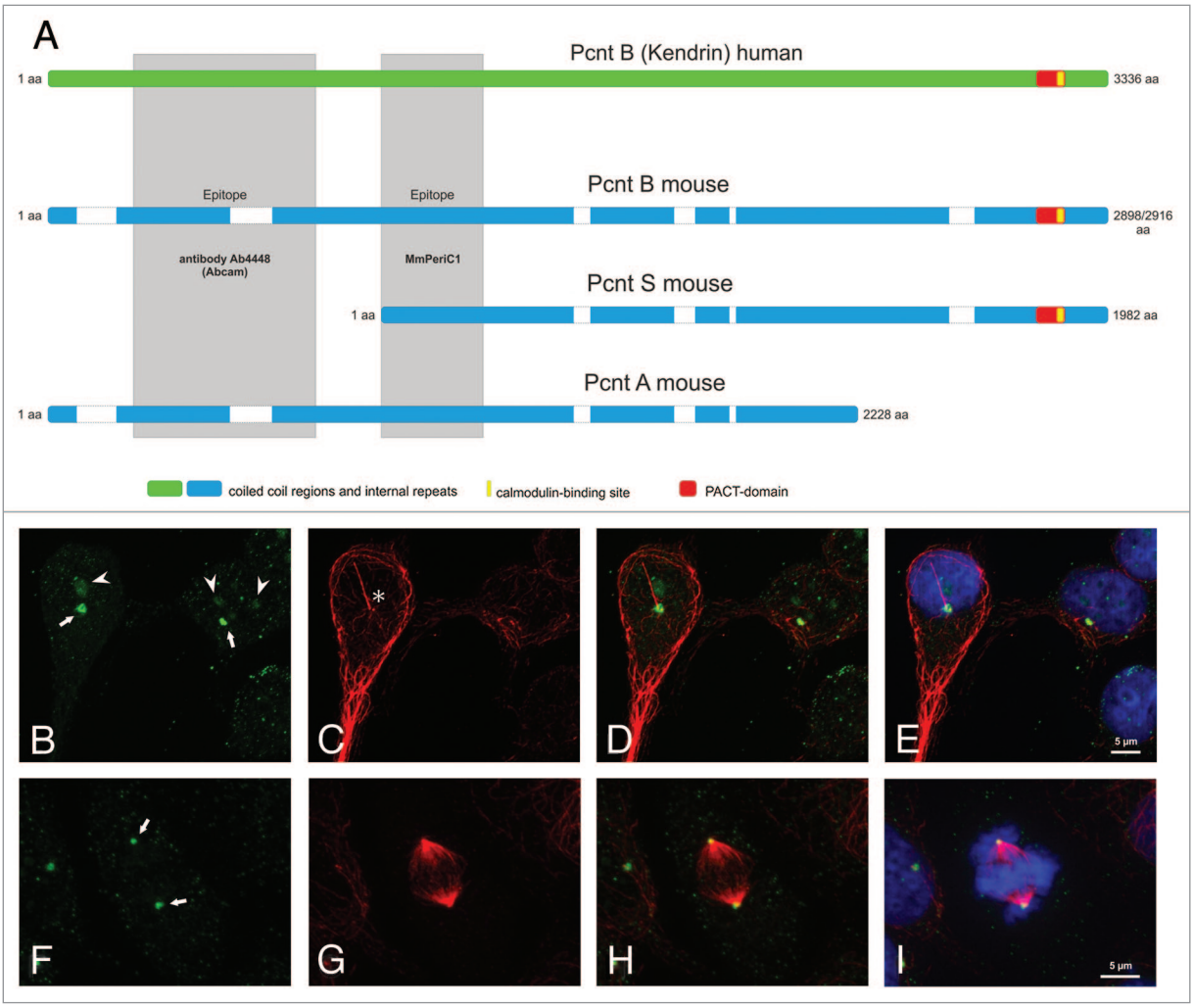

Figure 1. Mouse and human Pericentrin variants and Pericentrin distribution in human embryonic kidney cells. (A) Scheme of the known and published Pericentrin (Pcnt) splice variants: Pcnt B human (Kendrin, accession number (AN: NP_006022), Pcnt B mouse (Pcnt 360, AN: NP_032813 or BAF36559), Pcnt A mouse (AN: partial, AAO24322.1) and Pcnt S mouse (Pcnt 250, BAF36560). Human Pcnt is larger than mouse Pcnt—white bars in the mouse Pcnt variants indicate missing sequence parts. The homology between human and mouse Pcnt is about 60%. The epitopes of the affinity purified MmPeriC1 antibody and the Ab4448 antibody are indicated in gray. (B-I) Triple labeling of Pcnt (green, B and F), ac. tubulin (marker particularly for primary cilia, red, C and G), and DAPI (blue, E and I) in human embryonic kidney cells (HEK-293T cells). Pcnt is localized at the centrosomes of resting and dividing cells and at the basal body complex of primary cilia (arrows; primary cilium marked with an asterisk). Moreover it accumulates in the nucleoli of interphase cells (arrowheads) and can be found distributed throughout the cytoplasm at granular appearing structures. (D, H) Merge of the Pcnt and ac. tubulin staining. (E, I) Merge of the Pcnt, ac. tubulin and DAPI staining. Scale bar: 5 µm (E, I).

Pcnt in Human

In the last years, a lot of effort has been invested into deciphering the role of Pcnt in human. Diseases associated with mutations in the PCNT gene display heterogeneous clinical manifestations, making it difficult to pinpoint the functional role of Pcnt (for a review see ref. 4). The issue is further complicated by the fact that little is known about the expression of Pcnt splice variants in the various human tissues. Immunocytochemical stainings of human embryonic kidney cells (HEK-293T cells) with our affinity purified Pcnt antibody MmPeriC1, which should detect all splice variants that are known to date (**Fig. 1A**), showed a localization of Pcnt at the centrosomes (see also refs. 1–3, 11), at the basis of primary cilia (see also refs. 7, 8, 11), throughout the cytoplasm [probably at granular structures (see also refs. 16 and 17)], and in the nucleoli of interphase cells (see also ref. 18) (**Fig. 1B–I**). This wide distribution pattern most likely reflects the functional diversity of Pcnt and its splice variants in human cells. Based on what we know about the functional Pcnt patchwork in the mouse, we started to search for possible Pcnt splice variants in human tissues. We performed western blot experiments with our antibody MmPeriC1 using various human cell lines, i.e., HEK-293T cells, cervical cancer cells (HeLa cells), and two breast cancer cell lines, MCF-7 and MDA-MB 231 (**Fig. 2B**). Mouse tissues and 3T3 mouse fibroblasts served as controls (**Fig. 2A**). We found in the human cells, like in the mouse, different Pcnt positive bands with varying intensities on the protein level (**Fig. 2**). These findings corroborate earlier

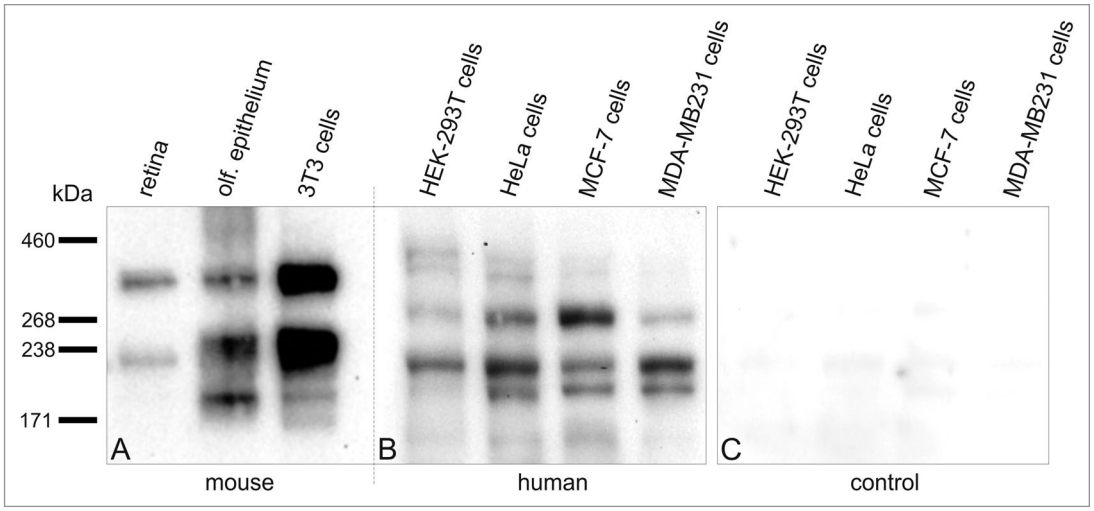

Figure 2. Expression of Pericentrin splice variants in different mouse and human tissues and cells. Every lane is loaded with approximately the same amount of protein. (A) Western blot analysis of mouse protein extracts of retina, olfactory epithelium and NIH 3T3 mouse fibroblasts using the MmPeriC1 antibody. A ~360 kDa protein band—mouse Pericentrin (Pcnt) B—is detected in all three samples. A second protein band with varying molecular weight—~250 kDa (most likely mouse Pcnt A and/or S) in olfactory epithelium and NIH 3T3 mouse fibroblasts, ~225 kDa (most likely a variant of mouse Pcnt S) in retina—suggests the existence of different Pcnt variants in different tissues, which are expressed at different protein levels. The third band in the olfactory epithelium at ~190 kDa might be a cleaved part of Pcnt, since it does not appear constantly in every experiment. (B) Western blot analysis of human protein extracts of HEK-293T cells (embryonic kidney), HeLa cells (cervical cancer), MCF-7 cells and MDA-MB 231 cells (both breast cancer) using the MmPeriC1 antibody. The ~380 kDa human Pcnt B is detected as a double band with different expression levels in the different cell lines. The constantly appearing double band might show a posttranslational modification of Pcnt B resulting in a weight shift. All four human cell extracts show a second and a third band with a molecular weights of ~270 kDa and 220 kDa (potentially human Pcnt A and S). In the three cancer cell lines an additional Pcnt positive band at ~200 kDa appears. All human cell lines show different expression levels of distinct bands, suggesting a unique expression pattern of different Pcnt splice variants in every human tissue. (C) Control western blot analysis of the human cell extracts used in B. Preadsorption of the MmPeriC1 antibody with the respective antigen in saturating concentrations blocks the detection of the protein bands in all human cell samples. For detailed experimental information see ref. 11.

results from northern blot experiments, showing the existence of more than one Pcnt variant in humans.[12,19]

In MOPD II studies the loss of Pcnt positive bands in western blots of lymphoblastoid cells of patients was reported.[5,14,20] For the detection of Pcnt an antibody recognizing the N-terminal part of Pcnt (Ab4448, Abcam) was used. However, the use of such an antibody causes an experimental problem because it might not detect all Pcnt variants, e.g., Pcnt S or any other variants lacking the N-terminal region of Pcnt (**Fig. 1A**). Indeed, using our antibody MmPeriC1, we find a different protein pattern in western blots of human tissues compared with the control cells in the MOPD II studies (**Fig. 2B**). MOPD II patients show a severe and complex phenotype,[21,22] but they are viable. As it is assumed for humans that a complete loss of Pcnt will lead to prenatal death,[23] and knockout mice with a complete loss of Pcnt are nonviable,[15] the question arises why the MOPD II phenotype is not lethal. There are only few possible explanations: The mutations in the PCNT gene are hypomorphic, or they are compensated for by the expression of other genes,[23] or, much simpler, not all splice variants of Pcnt are affected by the mutations.

Like in the mouse, our findings suggest also for humans a patchwork of different Pcnt splice variants in different tissues, which may explain why mutations in the human PCNT gene generate a multitude of different phenotypes.

For a final answer the Pcnt spliceome in human tissues has to be deciphered—a task that exceeds the possibilities of our group focusing on animal models. Nonetheless, we believe that the hypothesis of a functional Pcnt patchwork in humans is worth following up, as it will lead to a better understanding of disorders linked to PCNT mutations. In fact, this would be a good starting point for an interdisciplinary research project with basic and clinical research working hand in hand.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Supplemental Material

Supplemental materials may be found at: www.landesbioscience.com/journals/cib/article/20363

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