Commentary

From Alzheimer's disease to skin tumors: The catenin connection

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Presenilins (PS) 1 and 2 play a key role in the pathogenesis of Alzheimer's disease (AD) by being essential for the intramembrane cleavage of amyloid precursor protein (APP), thus releasing the pathogenic Aβ peptides into the intercellular space (1). Recently, PS1 has been shown to exert an unusual aspartyl protease activity, possibly forming the active center of the γ secretase, one of the prime drug targets of an emerging AD therapy (2–4). In addition to the cleavage of APP, the physiological role of which is still not well understood, PS1 is indispensable for several crucial cellular signaling mechanisms mostly operative in tissue development and renewal. The best-studied example for its signaling functions is the proteolytic release of the signal transducing intracellular domain of notch receptors governing cell fate decisions, the inhibition of which causes the severe intrauterine lethal phenotype of PS1-deficient mice (5–7). PS1 also negatively regulates the cytoplasmic concentration of β-catenin, a signal integrator of the Wnt pathway.

In this issue of PNAS, Xia and colleagues (8) have used a highly elegant means to circumvent prenatal lethality in PS1-deficient mice to analyze the consequences of a PS1 loss of function in adult mice. By reintroducing wild-type PS1 under the control of a Thy1 promoter they have obtained normal levels of expression of PS1 in all tissues but skin keratinocytes. The mice survive up to a normal lifespan, a finding in line with two previous papers (9, 10) using the same approach. Loss of PS1 in keratinocytes is associated with the development of a stereotyped set of skin lesions during adulthood, encompassing multifocal hyperplasia and hyperkeratosis and the formation of epidermal skin tumors, ranging from the benign keratoacanthomas to malignant squamous cell carcinomas. Absence of PS1 expression in keratinocytes was correlated to an increased cytosolic concentration of β-catenin. As a consequence, cyclin D1, one of its target genes involved in cell proliferation control was activated even in normal-appearing skin samples. Again fitting into these data, the authors could demonstrate an increased fraction of cells re-entering cell division as a first step in the pathogenesis of the hyperproliferative skin lesions described above. A contribution of impaired notch signaling to the dysregulation of the β-catenin concentration but not to the formation of skin lesions was excluded by the introduction of either wild-type PS1 or a modified version of PS1 still active in notch cleavage but devoid of its β-catenin binding site (PS1 Δ cat) into PS1-deficient cells *in vitro*. Thereby, only wild-type PS1 was able to reduce the elevated cytosolic β-catenin to normal values, whereas a similar rescuing effect was not obtained by PS1 Δ cat, indicating that the notch signaling in these cells did not contribute to the observed phenotype.

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When discussing the PS1-mediated regulation of β-catenin and the effects of a malfunction of this system, it should be kept in mind that this only reflects one functional aspect of this protein.

β-Catenin exists in the cell in two different pools apparently fulfilling distinct functions. The majority of this protein is associated with the cell membrane, where it serves as a molecular bridge between cell adhesion molecules of the cadherin family and the actin cytoskeleton. The second, smaller fraction discussed here is present in the cytosol, where it acts as a signal transducer with the capacity to enter the nucleus, where it combines with DNA binding proteins of the T cell factor/lymphoid enhancer factor (TCF/LEF) family to form active transcription factors. This cytosolic concentration of β-catenin is normally kept at a low level by ubiquitination and targeting to the proteasome. The switch to initiate β-catenin degradation is its phosphorylation by glycogen synthase kinase (GSK 3-β). β-catenin phosphorylation in turn is regulated by binding of Wnt proteins to their membrane receptors, which reduces β-catenin phosphorylation and initiates or augments TCF/LEF activity (see refs. 10 and 11 for recent reviews of the literature).

The functional importance of β-catenin is directly illustrated by the effects of a β-catenin knockout, which terminates mouse development at the onset of gastrulation. Loss of this protein inhibits epithelial cell transdifferentiation and primitive streak formation (12). Conditional knockouts limiting β-catenin deficiency to regions of Wnt expression (13), resulting in severe brain and head malformations, surprisingly also encompassing a loss of cranial neural crest derivatives. Closer to the model investigated here, a pathologic stabilization of cytoplasmic β-catenin concentrations caused by mutations of either β-catenin itself or the catenin-binding proteins axin and adenomatous polyposis coli has been found in several malignancies like hepatoblastoma and adenomatous polyposis coli. Thus, independently or as a part of Wnt-dependent morphogenetic effects ranging from body axis formation to brain development, β-catenin signaling affects cell proliferation control. In skin morphogenesis, β-catenin has been shown to be essential for hair follicle formation, its overexpression causing the "furry" phenotype in mice, but also the development of skin malignancies (14, 15).

Analysis of the interaction between PS1 and β-catenin has originally created some confusion because of conflicting reports indicating effects in favor of either a stabilization (16, 17) or a degradation (18, 19) of this protein by PS1. This issue was recently re-evaluated by Soriano *et al.*

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(10), who provided strong evidence in favor of a PS1-induced β-catenin degradation. Somewhat surprisingly, their data indicate that PS1 might directly enhance ubiquitination of β-catenin instead of acting as a "workbench" binding both glycogen synthase kinase (GSK 3-β) and β-catenin to enable phosphorylation (19).

Thus, the data presented by Xia *et al.* (8) at first sight seem to perfectly link previous data on cytosolic β-catenin turnover and function, creating a new connection between a key factor in AD pathogenesis and tumorigenesis. Moreover, as already indicated in a previous report (10), this effect is independent of the proteolytic function of PS1, which still is an important target in the development of drugs to fight AD. Thus, the application of γ-secretase inhibitors to reduce Aβ formation is less likely to cause catenin-mediated side effects. Still, the puzzling question remains how PS mutations involved in AD pathogenesis interfere with the PS1/β catenin interaction, as they are apparently unable to restore normal catenin turnover in PS1-deficient cells (10). It should be noted that patients suffering from familial AD caused by PS1 mutations have so far not been found to have an increased risk to develop malignancies via this pathway, indicating that the remaining intact PS1 allele in this autosomal dominant condition suffices to maintain normal catenin levels (8).

However, a closer look into PS1- and β-catenin-dependent pathways operating in skin development still reveals several intriguing incongruencies between current concepts and the data presented here. First, selective deletion of β-catenin in keratinocytes in conditional knockouts or overexpression in keratinocytes controlled by the keratin 14 promoter bidirectionally modulate hair follicle morphogenesis (14, 20). Interestingly, overexpression of a stable β-catenin variant in keratinocytes, a condition directly related to the model described here, causes the excess eutopic formation of follicles, i.e., in haired skin regions (14), and the genesis of a different set of epidermal tumors exclusively associated with hair follicles in aged mice. It remains to be established, whether this phenotype difference could be explained by e.g., different concentrations or differences in intraepidermal expression of cytosolic β-catenin obtained under these different conditions.

Moreover, there is good evidence that PS1 function in skin development is not restricted to the regulation of β-catenin concentration. First, both notch receptors and ligands are expressed in the skin as well as in hair follicles (21). Notch receptor activation has repeatedly been discussed to trigger epidermal cell differentiation (22, 23). The control experiments described by Xia *et al.* (8) and Soriano *et al.* (10) expressing PS1 Δ cat with a preserved capacity for notch cleavage in PS1-negative cells have been done in cell culture so far, and it would obviously be highly interesting to find out how these two PS1-dependent signaling pathways interact *in vivo*.

Lastly, recent evidence indicates that the interaction between PS1 and β-catenin also may involve the membrane-associated fraction. It is unclear as yet under which conditions and to what extent changes in either pool are transmitted to the other, e.g., to modify the respective function. However, PS1 has been shown to be part of the cadherin-catenin complex (24, 25) where it stabilizes the cadherin-catenin interactions in adherens junctions, contributing to Ca^{2+}-mediated cell aggregation. Notably, the direct association of PS1 with the cadherin-catenin complex makes it a player of both epithelial and (synaptic) neuronal cell adhesion. Likewise, destabilization of cadherin-cytoskeleton interactions could explain the repeatedly observed disastrous hemorrhages in PS1-deficient mice.

The past years of research have made PS1 evolve from a mere AD-associated protein into a multifunctional maverick sitting at the heart of an expanding number of cellular signaling mechanisms. PSs are indispensable for regulated intramembrane proteolysis (RIP), a two-step processing scheme for transmembrane proteins creating cytosolic fragments acting as transcriptional activators in the nucleus. Very recently, amyloid precusor protein has joined the group of PS-RIPped proteins generating nuclear transcription signals (26). Now, research on the second major aspect of PS-mediated signaling, characterized by a nonenzymatic binding interaction as exemplified by β-catenin, seems to be on the rise.

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