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Review

A fast growing spectrum of biological functions of γ-secretase in development and disease☆

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ABSTRACT

γ-secretase, which assembles as a tetrameric complex, is an aspartyl protease that proteolytically cleaves substrate proteins within their membrane-spanning domain; a process also known as regulated intramembrane proteolysis (RIP). RIP regulates signaling pathways by abrogating or releasing signaling molecules. Since the discovery, already >15 years ago, of its catalytic component, presenilin, and even much earlier with the identification of amyloid precursor protein as its first substrate, γ-secretase has been commonly associated with Alzheimer's disease. However, starting with Notch and thereafter a continuously increasing number of novel substrates, γ-secretase is becoming linked to an equally broader range of biological processes. This review presents an updated overview of the current knowledge on the diverse molecular mechanisms and signaling pathways controlled by γ-secretase, with a focus on organ development, homeostasis and dysfunction. This article is part of a Special Issue entitled: Intramembrane Proteases.

Contents

1. Introduction

γ-Secretase is a promiscuous di-aspartyl protease responsible for the cleavage of a series of integral membrane proteins, almost all being type I transmembrane proteins. To date, more than 90 proteins have been identified as substrates among which the most notorious

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are Notch and Amyloid precursor protein (APP). Interestingly, γ-secretase emerged first as an enigmatic enzymatic activity with the discovery of amyloid β and APP as scientists realized at that time that proteolysis within the transmembrane domain was required to generate this peptide [1]. Now we know that γ-secretase is a multiprotein complex consisting of presenilin (abbreviated as PSEN independently of its origin), nicastrin (NCT), anterior-pharynx defective-1 (APH1) and PSEN enhancer-2 (PEN2) [2–5]. Originally discovered by geneticists as the protein products of genes mutated in families with autosomal dominant Alzheimer's disease (AD), PSEN harbors the catalytic activity of the complex ([6,7], reviewed in [8]). However, PSEN absolutely requires the co-factors NCT, APH1 and PEN2 to form a functional γ-secretase complex. Additionally, the γ-secretase complex is heterogenous in nature due to the existence of two PSEN homologues (PSEN1 & 2) and several APH1 isoforms. Different complexes are not only present in different tissues [9–11]; they have as well heterogeneous biochemical and physiological properties [12,13]. However, little is known with respect to the cell biology of these complexes and how (or whether) they might contribute to substrate and/or cleavage specificity [14]. In addition, PSEN also functions outside the γ-secretase complex, for instance, in vesicular trafficking, Ca2 + homeostasis, β-catenin stabilization and cell adhesion [5,15].

γ-Secretase created thus far a tremendous attraction through its intimate involvement in the pathophysiology of AD by catalyzing the final cleavage in the production of amyloid β peptides, thereby overshadowing its increasing involvement in major physiological processes during development as well as adulthood. In this review we summarize our current knowledge not only on the function of PSEN and γ-secretase in the broader physiological context, but also provide an updated catalogue of mutations in γ-secretase components discovered so far in human diseases.

2. Complex structure and formation

At least 19 transmembrane domains (TMD) contribute to the hydrophobicity of γ-secretase, of which nine are from PSEN and seven from APH1. PEN2 has a hairpin-like topology containing two TMDs, whereas NCT is the only type I transmembrane glycoprotein of the complex. The crystal structure of an ancestral PSEN/signal peptide peptidase (SPP) homologue has been recently revealed [16] (discussed in more detail in the article of Mike Wolfe in this review series) and remarkably confirms earlier biochemical studies on its genuine 9 transmembrane [17–19] and ring-structure topology [20]. Genetic ablation of only one component results in mislocalization (notably retention in the endoplasmic reticulum (ER)), incomplete maturation and destabilization of the remaining components, clearly indicating that inter- and intramolecular interactions are crucial in the course of assembly, transport and activation of the γ-secretase complex [14].

All γ-secretase components colocalize initially in the ER where proper posttranslational modifications and quality control systems ensure their correct folding and assembly. Their assembly is not a random process but occurs sequentially and stoichiometrically and is superimposed on transport regulation that ensures cell- and tissue-specific levels of γ-secretase activity. Most studies agree on the initial formation of an NCT–APH1 subcomplex as the first step [21,22] that is stable even in the absence of PSEN and PEN2 [23]. The formation of this intermediate NCT–APH1 scaffold is regulated by the Golgi-to-ER cargo receptor Rer1p (retrieval to ER 1 protein) [24]. Rer1p binds to the same polar residues on the NCT TMD that are essential for interaction with APH1, and thus blocks APH1 interaction with NCT during the early stages of γ-secretase complex assembly. Hence, Rer1p controls formation of γ-secretase subcomplexes and, concomitantly, total cellular γ-secretase levels and activity [14,24].

Thereafter, the sequence of events leading to the formation of a mature PSEN complex may involve direct binding of the APH1–NCT scaffold to PSEN followed by the incorporation of PEN2 [25,26]. This is thought to trigger the endoproteolysis of PSEN to form N- and C-terminal fragments (NTF and CTF) that stably associate into heterodimers. Although both fragments are part of the catalytic γ-secretase, endoproteolysis is not a requirement for activity. Alternatively, the APH1–NCT pre-complex combination may bind directly to a preformed PSEN1–PEN2 structure to generate the mature, active γ-secretase complex [23,27]. This alternative assembly process is based on the detergent-based identification of two additional intermediate complexes, NCT–APH1–PSEN1 CTF and PEN2–PSEN1 NTF; also the fact that PEN2 can bind full-length PSEN independently of NCT and APH1 supports this view [28,29].

3. Regulated Intramembrane Proteolysis (RIP)

γ-Secretase belongs to the family of intramembrane cleaving proteases (i-CLiPs) which contains in addition to the PSENs, the zinc metalloprotease site-2 protease (S2P), the signal peptide peptidases (SSP) and the rhomboid proteases. All i-CLiPs enzymatically cleave their substrate proteins within the plane of the lipid bilayer in a process termed regulated intramembrane proteolysis (RIP)([30,31]). With the exception of rhomboid proteases, RIP requires the initial ectodomain shedding of the substrate. Hence, the magnitude of RIP depends on the rate-limiting regulation of these shedding enzymes. Ectodomain shedding can be constitutive but it may also be induced by several stimuli such as ligand binding, protein kinase C (PKC) activation by phorbol esters or Ca^{2+} influx. The initial ectodomain shedding in γ-secretase-associated RIP is essentially carried out by either of two protease families, i.e. members of the 'α-disintegrin and metalloprotease' (ADAM) family commonly referred to as α-secretases (for review see [32]) and the aspartyl proteases BACE1 and BACE2 also called β-secretase [33]. The shedding results in the release of a soluble ectodomain into the extracellular environment and generation of a truncated membrane-associated carboxyl terminal fragment (CTF). The CTF is in turn cleaved by the i-CLiP that releases the intracellular domains (ICD) and a small peptide such as P3 or Aβ in case of APP.

γ-Secretase-mediated RIP may activate, turn off or switch the signaling properties of the transmembrane protein involved (Fig. 1). For instance, RIP activates signaling pathways like Notch, by allowing intracellular domains (ICD) to translocate to the nucleus where it incorporates into a transcriptional complex and regulates gene transcription (Fig. 1A) [34]. Alternatively, RIP may turn off signaling events in which the transmembrane anchored protein is responsible for signaling, and the cleavage event terminates the signal. The cleavage of Deleted in Colorectal Cancer (DCC), for instance, attenuates downstream signaling (Fig. 1B) [35]. A third possibility is that the cleavage serves as a switch between signaling modes with the uncleaved transmembrane form activating one pathway at the membrane and the soluble ICD carrying out a different function in another cellular compartment (Fig. 1C). This has been shown for various substrates including ErbB4 [36] and APP [37].

4. The most prominent γ-secretase substrates

To date more than 90 substrates have been identified for γ-secretase (reviewed in [38]). Although they are diverse in their structure, localization and physiological functions, the majority of these proteins share some common features. For instance, almost all substrates are type-I transmembrane proteins. The only exceptions identified so far are the polytopic membrane proteins glutamate receptor GluR3 and polycystin-1 as well as the type-II transmembrane protein glucosaminyltransferase (GnT-V). Typically, they contain a large ectodomain often including cell adhesion molecule-like domains, a single-pass transmembrane domain and a cytosoplasmic tail frequently capable of initiating or mediating intracellular signaling. Interestingly, the γ-secretase-mediated RIP does not depend critically on recognizing particular sequences in the transmembrane domains of its substrates but rather on the size of the extracellular domain remaining after

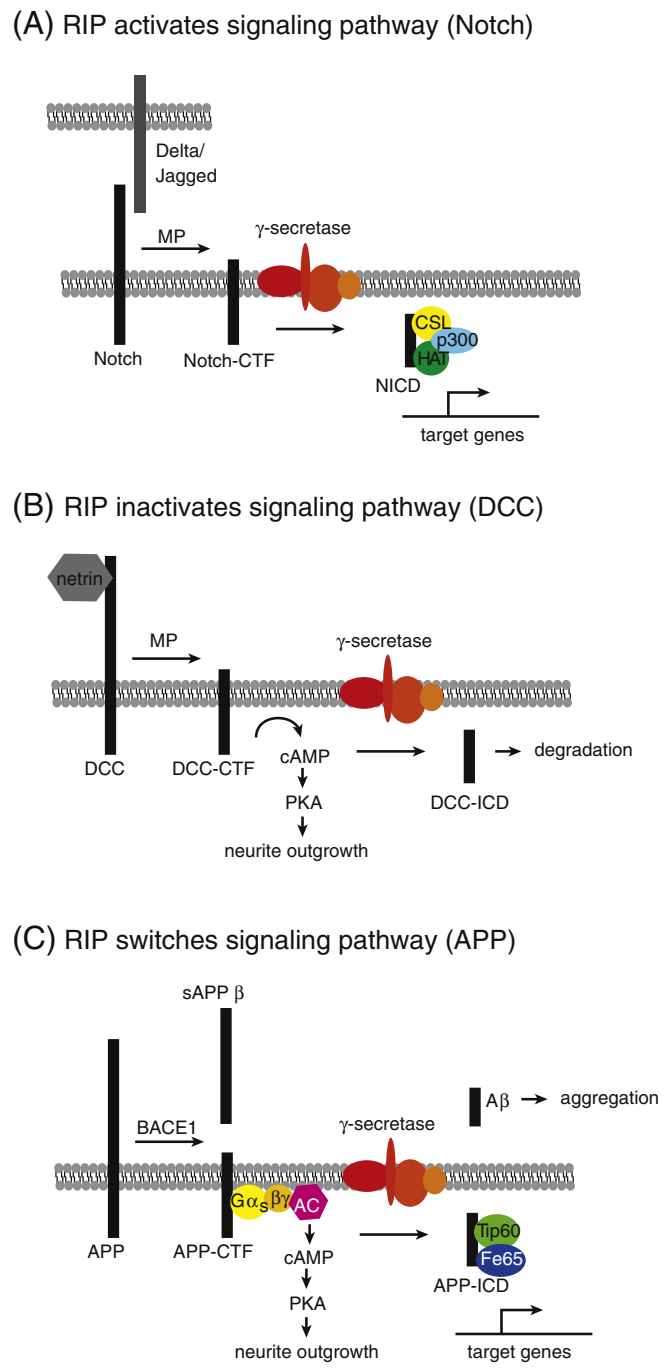

Fig. 1. γ-Secretase-mediated cleavage regulates signaling pathways. γ-Secretase-mediated RIP which takes place after ectodomain shedding by a sheddase (metalloproteinase (MP) or BACE) may activate, turn off or switch the signaling properties of the transmembrane protein involved. (A) RIP activates signaling pathways such as Notch, by allowing intracellular domains (ICD) to translocate to the nucleus where it incorporates into a transcriptional complex with CSL transcription factors, p300 co-activator and histone acetyltransferase (HAT) and regulates gene transcription [34]. (B) RIP turn off signaling events in which the transmembrane anchored protein is responsible for signaling, and the cleavage event terminates the signal. The cleavage of Deleted in colorectal cancer (DCC) attenuates downstream cAMP/PKA signaling involved in neurite outgrowth [35]. (C) RIP serves as a switch between signaling modes with the uncleaved transmembrane form activating one pathway at the membrane and the soluble ICD carrying out a different function in another cellular compartment. APP-CTF upon direct binding to Gαs activates cAMP/PKA signaling pathway and regulates neurite outgrowth [37]. Upon γ-secretase cleavage, the released APP-ICD binds to the histone acetyl-transferase Tip60 and Fe65 and regulates gene transcription [202].

shedding, with shorter lengths being associated with a higher efficiency of cleavage [39]. Also, γ-secretase substrates appear to function as signaling proteins and regulate a wide variety of cellular events such as cell fate determination, adhesion, migration, neurite outgrowth, axon guidance or formation and maintenance of synapses. Interestingly, many of these events are disrupted during neurodegeneration e.g. in AD [40]. The most studied γ-secretase substrates are APP for its role in AD, and Notch, a signaling molecule critically required in development and cell fate determination in multicellular organisms. Therefore, a brief description of both substrates is given in the following paragraphs.

4.1. Notch signaling pathway

By virtue of its determining role in development, the Notch signaling pathway is probably one of the most extensively studied γ-secretase-related functions in living organisms. Notch signaling is evolutionarily conserved and plays a critical role in short-range communication during the development of multicellular organisms, as it controls cell fate by regulating cell proliferation, survival, positioning and differentiation (reviewed in [41,34]). The importance of Notch signaling even extends into adulthood where it regulates stem cell maintenance, binary cell fate decisions, such as in the T- or B-lymphocyte lineage, and differentiation in self-renewing organs. Notch proteins are a family of transmembrane receptors encoded by four distinct genes (Notch1–4) in mammals. Binding of Notch receptor to one of its five Jagged/Delta ligands (Jag1 and Jag2 and delta-like 1 (Dll1), Dll3 and Dll4) presented by neighboring cells initiates a cascade of sequential proteolytic cleavage events mediated by α-secretase and γ-secretase, resulting in the release of the intracellular domain of the Notch receptor (NICD) [34]. The pivotal role of γ-secretase in activating the Notch cascade was shown in knock-in mouse models, where a single point mutation V1744G near the transmembrane cleavage site in Notch1 sufficient to inhibit intramembrane proteolysis, phenocopied *Notch1*-deficient mice [42]. After cleavage, NICD translocates to the nucleus where it modulates gene expression by binding to CBF1/RBPJk, suppressor of hairless Su(H), Lag-1 (CSL) transcription factors. These interactions convert CSL from a transcriptional repressor to an activator by displacing co-repressor and histone deacetylases and recruiting histone acetyltransferases and co-activators such as p300 [41]. Notch was originally discovered in *Drosophila* where it induced "notched wings". Its first human homologue, *NOTCH1*, was identified in T-cell acute lymphoblastic leukemia (T-ALL) patients with the chromosomal translocation t(7;9) (q34; q34.3) found in less than 1% of T-ALLs [43]. The translocation breakpoints in this original case of T-ALL were mapped within *NOTCH1* on chromosome 9 and the T-cell receptor beta locus on chromosome 7, resulting in the expression of a truncated, constitutively active Notch 1 receptor [43].

Aberrant Notch signaling interferes with embryonic development and has been detected in various human diseases including several types of cancer. In some cancers including lymphocytic leukemia and B-cell malignancies, constitutive activation of the Notch pathway and increased levels of NICD are induced by molecular genetic alterations including chromosomal translocation, point mutations and chromosomal amplification at the Notch receptor loci [44]. While for a long time Notch has been thought to be solely a tumor promoter, few studies provide now evidence that Notch acts also as tumor suppressor. This is the case in squamous cell carcinomas of the head and neck, lung and skin where loss-of-function mutations of Notch have been identified [45–48].

Interestingly, not only the Notch receptor undergoes γ-secretase mediated RIP, but also both its ligands, Delta and Jagged. Their shedding is mediated by ADAM17 followed by γ-secretase mediated RIP, which releases the respective intracellular C-terminal domains termed DICD and JICD, in analogy to NICD [49,50]. Their ectodomain processing can be stimulated by expressing Notch, as well as regulated by phorbol ester/PKC activation. The fact that the soluble Delta ectodomain is not an efficient Notch activator [51,52] suggests that the ectodomain

cleavage of Delta serves to limit the amount of ligand available at the cell surface. Alternatively, the membrane tethered CTF of Jagged/Delta may compete with Notch for γ-secretase processing. This scenario would be particularly relevant during early lateral inhibition when both Notch and its ligands are expressed in the same cell; competition would then decrease the ability of a stimulated cell to release NICD and therefore allow that particular cell to adopt its cell fate. Similar to NICD, both JICD and DICD translocate to the nucleus where they affect gene expression, including AP-1 for JICD [50]. While DICD was shown to have a transcriptional activity [49], JICD does not and most probably affects transcription by associating with a transcriptional activating complex [50].

4.2. Amyloid precursor protein and Alzheimer's disease

Accumulation of β-amyloid (Aβ) plaques and hyperphosphorylated tau bearing tangles in the brain of patients together with synaptic dysfunction and cognitive decline are hallmark features of AD. Biochemical and genetic studies have identified Aβ as a trigger for AD pathogenesis. Aβ is generated by the successive proteolytic cleavage of APP by BACE1 and γ-secretase [29].

Understanding the physiological functions of APP and its proteolytic fragments as well as the regulation of its processing is of immediate relevance for AD pathogenesis. One particularity of this substrate is that beside the amyloidogenic pathway mentioned above, a non-amyloidogenic pathway can take place. Herein, APP is first cleaved by α-secretase (mediated mainly through ADAM10 as well as ADAM17) to generate a secreted N-terminal sAPP-α fragment. The remaining APP-CTF 83 is further cleaved by γ-secretase to release a P3 peptide and APP intracellular domain (AICD). *In vitro* and *in vivo* studies have shown important functions for APP in various neuronal and synaptic processes. They can be executed either as a full-length protein or as one of the processing products, often the soluble fragments, or the AICD but also the membrane-tethered CTF [37], meaning that processing of APP is required and must be highly regulated (review in [53]).

To add to the complexity, γ-secretase cleaves APP-CTF fragment at multiple sites and likely successively (ε-, ζ- and γ-cleavage) [54]. Following the tripeptide processing model, γ-secretase cleaves within the transmembrane domain of APP-CTF sequentially, N-terminally directed, releasing three residues at each step [55,56]. The initial ε-cleavage occurring at two major sites (Aβ48 or Aβ49) influences the subsequent cleavages, meaning that Aβ48 is converted to Aβ42/38 and Aβ49 to Aβ43/40. At each step, the substrate is either further processed or released from γ-secretase. This results in Aβ peptides of different lengths, from 38 to 42 amino acids, the principal peptide being Aβ40 and the most toxic being Aβ42 [57–59]. The region between the γ- and ε-cleavage sites is shown to contribute to the interaction scaffold with PSEN1 [20] and, interestingly, translocation prone peptides mimicking this region selectively inhibit APP processing and Aβ production but not Notch cleavage [60].

In general, overproduction of Aβ leads to neurotoxicity, synaptic damage and eventually neuron loss [61]. In familial AD (FAD), mutations in PSENs (1 & 2, http://www.molgen.vib-ua.be/ADMutations) have shown to directly affect the processing of APP mostly by either increasing the production of Aβ42 or decreasing the production of Aβ40 thereby affecting the ratio Aβ42/40 [54]. The molecular mechanisms linking the different FAD mutations with the observed pathology are not yet fully understood; what is becoming clear however is that all FAD mutations cannot be explained with one common molecular mechanism. Recently, *in vitro* measurement of the kinetics of the activity of different FAD–PSENs demonstrated that not all mutations resulted in a loss of function at the ε cleavage site. However, they all cause qualitative changes in Aβ profiles by various mechanisms [59,62]. Moreover, besides APP, FAD mutations affect also the cleavage of other substrates, such as Notch [59,63], N-Cadherin [59,64], ErbB4 [59] and EphrinB [65,66] and this even in distinct manners [59]. In addition to these mutations, in late-onset AD changes in membrane trafficking have been suggested to affect APP processing and therefore membrane trafficking is emerging as a potent regulator of RIP [29,67,68].

Finally, efforts in the scientific community have led to the identification of a number of potential therapeutic targets for AD based on the facts that they can modulate Aβ production without affecting Notch cleavage. Such proteins can enhance Aβ production by controlling the assembly of γ-secretase, such as Rer1p ([24]; c.f. supra and infra) or redistribution of the complex to membrane micro-domains as suggested for the orphan G-protein coupled receptor GPR3 and β-arrestin [69,70]. Alternatively they may help the interaction between APP and γ-secretase as observed in case of γ-secretase activating protein (GSAP) [71]. *In vivo* diminished processing of APP upon genetic ablation of these aforementioned proteins prevents plaque formation in AD mouse models. Still, the role of this interaction has not been studied in a context distinct from AD and may be of relevance in other diseases and developmental processes regulated by γ-secretase.

5. Role of γ-secretase in embryonic development

In mammals, there are 2 highly homologous PSENs, PSEN1 and PSEN2. PSEN1 is ubiquitously expressed in all tissues, including embryonic and adult brain. Its expression is developmentally regulated and changes in expression are associated with neuronal differentiation and synaptogenesis [72,73]. Initial clues of its function within the Notch signaling pathway have come from the *C. elegans* homolog sel-12, which shares about 50% amino acid identity with PSEN1 and is a suppressor/enhancer of LIN-12, a member of the LIN-12/Notch family of receptors [74]. Throughout metazoan evolution, PSENs play essential roles in development. Mice lacking either *PSEN1* or both *PSENs* die perinatally or during early embryonic development, respectively. More particularly, *PSEN1* knock-out mice exhibit severely abnormal development of various tissues, consisting of a neuronal migration disorder, midline defects of the body wall and defective somitogenesis [6,75–77]. Additionally, vascular lesions within the central nervous system cause several hemorrhages into the parenchyma and lateral ventricle. PSEN1 plays also a critical role in neurogenesis, where it prevents neural progenitor cells from differentiating into post mitotic neurons [75,78–80]. PSEN1 is also required for normal neuronal migration and cortical lamination as well as survival of Cajal–Retzius neurons, with PSEN1-deficient mice presenting a pathology very similar to human type 2 lissencephaly [76]. It has been suggested that many of these lesions and in particular the impaired somitogenesis and neurogenesis are a consequence of disturbed Notch signaling in mice. However, it should be noticed that *PSEN1*-deficient mice differ as well in several aspects from the Notch1 deficient phenotype. While *PSEN1*$^{-/-}$ embryos are viable until birth, *Notch1* deficient animals do not survive beyond embryonic day E11 [81,82].

PSEN2 shares overall 63% amino acid identity with PSEN1 and is predicted to have a very similar structure. It has been initially identified as a functional PSEN1 homologue in *C. elegans* where both wild-type PSEN1 and PSEN2 could complement sel-12 function [83]. Unlike *PSEN1*-deficient animals, *PSEN2*-knockout mice are viable and fertile and develop only mild pulmonary fibrosis and hemorrhage with age [84]. Definitive evidences that PSEN2 and PSEN1 are functional homologues and both involved in the Notch signaling pathway were obtained in double *PSEN1*/*PSEN2*-deficient embryos, which closely resembled *Notch-1* deficient animals. Indeed, at E9.5, embryos were developmentally retarded with delayed yolk sac vasculogenesis, absence of blood circulation and posterior truncation [84,85].

APH1 is encoded by a single gene in C. elegans [86,87] and Drosophila melanogaster [88], whose loss of function causes overt Notch signaling deficiencies. By contrast, mammalians harbor two APH1 (APH1a and APH1b in humans) or three *APH1* (*APH1a*, *APH1b* and its duplicated gene *APH1c* in rodents [9]) genes. Because alternative splicing results in two isoforms of APH1a, APH-1a long form (APH-1aL) and APH-1a short

form (APH-1aS), a total of three to four distinct APH-1 isoforms can be found in humans and mice, respectively. This combined with two PSENs results in up to 6 or 8 distinctly composed γ-secretase complexes that may co-exist in tissues and cells [9]. The APH1a complexes are crucial for Notch signaling during embryogenesis. Similar to Notch1 null embryos, *APH1a*$^{-/-}$ embryos display defects in vascular morphogenesis of the yolk sac, distention of the pericardial sac, underdeveloped first branchial arch and heart chambers, as well as kinks in the caudal part of the neural tube, and mild defects in somite patterning, particularly in the rostral somites [89,90]. APH1b and APH1c are highly similar (96.3% identity at the nucleotide level) but interestingly are differentially expressed. While *APH1b* is uniformly expressed in all tissues, *APH1c* distribution is limited to the kidney and the testis [9]. In contrast to *APH1a*$^{-/-}$, APH1b$^{-/-}$, APH1c$^{-/-}$ and APH1bc$^{-/-}$ homozygous mice are viable and fertile [90] and present only a mild disturbance in prepulse inhibition (APH1bc$^{-/-}$ mice) [91]. Furthermore, APH1bc deficiency does not affect expression of Notch and its target genes. This agrees well with the observation that Notch is predominantly expressed in non-neuronal and neuronal precursor cells where APH1a but not APH1b is expressed.

Finally, similar to *PSEN1*$^{-/-}$/*PSEN2*$^{-/-}$ double KO and *APH1a*$^{-/-}$ single KO mice, *NCT*$^{-/-}$ and *PEN2*$^{-/-}$ embryos die by embryonic day 10.5 and E9.5, respectively, and exhibit several patterning defects, reminiscent of Notch signaling defects. These include defects in somite segmentation and in angiogenic vascular morphogenesis in the yolk sac, as well as kinks in the neural tube, and distention of the pericardial sac [92,93].

The role of γ-secretase in embryonic development has been mostly investigated in loss-of-function experiments. We recently reported that a gain of γ-secretase activity through loss of its negative regulator Rer1p also affects normal embryonic development in zebrafish. Because of the selective high expression of Rer1p in ciliated cells and organs, (morpholino-mediated) downregulation during development gives rise to shortened cilia and impairment of their motile and sensory functions [94]. Cilia, which are antennal organelles emanating from the surface of most vertebrate cells, generate fluid flow (motile cilia) or orchestrate signaling pathways including Hedgehog, PDGFRα and non-canonical Wnt (primary cilia). Genetic defects in genes encoding ciliary proteins gives rise to a class of human syndromes term ciliopathies. In line with this, ciliary dysfunction caused by loss of Rer1p and gain-of-function of γ-secretase activity in zebrafish results in developmental defects that resemble some clinical features of human ciliopathies. Altogether, loss- and gain-of-function experiments highlight the critical importance of balanced γ-secretase activity during development [94].

6. Role of γ-secretase in adulthood and disease

The investigation of γ-secretase function in adulthood has been hampered by the facts that the genetic inactivation of most γ-secretase components causes prenatal lethal Notch phenotypes. To overcome early lethality and study the function of γ-secretase in adulthood, groups have turned towards mouse lines heterozygous/homozygous for one or more components of γ-secretase that are compatible with survival or towards conditional knock-out mice models.

In addition, the identification of mutations in genes encoding γ-secretase components in human diseases has enabled scientists to explore and appreciate the broad range of physiological implications of altering γ-secretase activities in health and disease development. Such mutations have been so far described in five diseases including frontotemporal dementia (FTD) and AD [95], acne inversa [96–98], dilated cardiomyopathy [99–102] and breast cancer [103](see Table 1). Interestingly, in rare cases, mutations such as e.g. PSEN2 R62H have been found in distinct diseases including AD, breast cancer and dilated cardiomyopathy, suggesting a common underlying molecular mechanism [101]. In contrary, so far, no overlapping mutations have been identified in acne inversa and AD that would indicate different implications of γ-secretase in those diseases.

6.1. γ-Secretase in adult brain

γ-Secretase plays a decisive role in the adult brain, first recognized upon discoveries of FAD mutations in the *PSEN* genes and since then corroborated in various animal models and human diseases.

Conditional loss of both *PSEN1* and *PSEN2* or *NCT* in the cortex results in progressive synaptic and memory impairment prior to age-dependent neurodegeneration accompanied by increased levels of hyperphosphorylated tau, another hallmark of AD [104–108]. In contrast, conditional *PSEN1* knock-out mice exhibit only mild cognitive deficits in long-term spatial memory [108]. Interestingly, these deficits appear to be independent of the Notch signaling pathway [108,109]. PSENs are also essential for regulating neurotransmitter release during synaptic transmission as shown in mice with specific deletion of both *PSENs* in pre-synaptic (CA3) and post-synaptic (CA1) neurons of the hippocampus [106]. Finally during adult neurogenesis, PSEN1 sustains the proliferation of neuronal progenitor cells and restricts their differentiation through EGF receptor and β-catenin pathways [110]. While most of the neurological defects observed during embryonic development have been ascribed to disturbed Notch signaling, it appears a different case during postnatal life where other γ-secretase substrates come into play. For instance, Neuregulin-1 (Nrg1) may contribute to the pharmacological and behavioral abnormalities in *APH1bc*-deficient mice that can be reversed by antipsychotic drugs [91]. Interestingly, Nrg1, which is a prominent candidate gene associated with increased risk for schizophrenia, and its receptor ErbB4 are both γ-secretase substrates [91].

6.1.1. γ-Secretase in axon guidance

To ensure proper synaptic connections and plasticity (and therefore brain function), the development of axonal and dendritic processes must be tightly regulated in time and space. The neuronal wiring relies on the coordination of at least two events: the secretion/presentation of extracellular cues acting as guidance signals and the expression on the moving neurons of the respective receptors, which eventually process the signals [111]. γ-Secretase plays a potentially prominent role in regulating these processes as many of its substrates are axon guidance molecules or receptors [38,111]. The functional consequence of γ-secretase cleavage are best defined for the Netrin receptor, DCC, as its processing is required for the sensitivity of axons to midline guidance cues (Fig. 1B). Netrin-1 was identified as the first guidance cue implicated in neuronal guidance [112,113]. It is expressed in the floor plate of the neural tube, to first guide/attract commissural axons during nervous system development [112,114]. When crossing the midline, the commissural axons lose their sensitivity to Netrin-1 and acquire responsiveness to repellents such as Slit, Ephrin and Semaphorin family members [115]. Importantly, the expression and localization of these guidance cues and receptors is exquisitely tailored to allow growth cones to rapidly switch their responsiveness at specific times and places throughout development. DCC-CTF stimulates neurite outgrowth by orchestrating signaling pathways including cAMP/PKA and protein kinases [35] which become later on abrogated through γ-secretase cleavage that releases the DCC intracellular domain. As such, in cultured neuroblastoma, preventing γ-secretase activity results in the accumulation of DCC-CTF and enhanced neurite outgrowth (Fig.1B) [35,116]. Similarly, overexpression of a myristoylated form of DCC causes axon growth defects by activating downstream kinases [117]. Finally, overexpression of a DCC-CTF variant which cannot be cleaved by γ-secretase causes motor neurons to remain responsive to Netrin-1. They are unable to switch and respond to repellent neurons and therefore cannot exit the neural tube [117]. These findings are further supported by the observation of aberrant axonal growth in a PSEN1 mouse mutant generated from a mouse ENU mutagenesis screen [118]. In these so-called Columbus

Table 1
Non-exhaustive list of diseases related to a defect in γ-secretase mediated signaling pathway.

Organ	Disease and development	Mutations of γ-secretase components in diseases	Potential substrates or molecular mechanism
Brain	• Neurodegeneration (AD, FTD) [95] • Cognitive defects [104–108] • Adult neurogenesis [110] • Axon guidance [118] • Synaptogenesis [122,127–129]	• PSEN1 (AD, FTD) [95] • PSEN2 (AD, FTD) [95]	• APP [119] • Neuregulin1 [91] • DCC [35,116,118] • EphrinB2 [122] • Neuroligin [127,128] • EphrinA4 [129]
Kidney	• Nephrogenesis [131,132]		• Notch [133] • Polycystin-1 [142] • Fibrocystin/polyductin [149]
Skin	• Hair follicle development [150] • Acne inversa (AI) [96,155]	• PSEN1 (AI) [96,155] • NCT (AI) [96,155,200] • PEN2 (AI) [96,155]	• Notch [150]
Immune system	• Splenomegaly [151,152,161] • Autoimmune disease [152,161] • T cell development [162,163] • B cell development [164]		• Notch [165] • HLA-A2 [201] • IL-1R2 [171] • IL-1R1 [172] • LRP1 [173] • CX3CL1 [174] • CXCL16 [174] • CD46 [175]
Heart vascular	• Cardiac morphogenesis [85,178] • Calcium homeostasis [100] • Dilated cardiomyopathy (DCM) [100] • Diabetic retinopathy [188,189] • Age-related macular degeneration [191]	• PSEN1 (DCM) [99,100] • PSEN2 (DCM) [99]	• Notch [101] • Ca2 + [100] • IGF-1R [177] • ErbB4 [177] • VEGFR [177] • APP [177]
Cancer	• Hyperplasia and skin cancer [151–153,156] • Breast cancer[103] • Glioma [193] • T-LL [166] • Leukemia [169]	• PSEN2 (breast) [103] • NCT (leukemia) [169]	• E-cadherin [192] • Notch [44] • Nrg1/Erb4 [193] • EpCAM [195] • β-catenin [153]

AD, Alzheimer's disease; APP, Alzheimer precursor protein; FTD, frontotemporal dementia; AI, acne inversa; DCM, dilated cardiomyopathy.

mutant mice, lack of γ-secretase processing promotes aberrant growth response to netrin-1, due to accumulation of DCC-CTF [118]. As a consequence these mice present aberrant growth of some motor axons into the floor plate at the midline instead of exiting laterally at their normal ventral root sites. APP was shown recently to be also part of the DCC receptor complex which mediates netrin-1-dependent axon guidance [119]. APP interacts with DCC in the presence of netrin-1 and enhances netrin-1-mediated DCC intracellular signaling, such as MAPK activation [119]. Finally, netrin-1 binding to APP regulates its processing resulting in lower Aβ secretion [120].

Bidirectional signaling through another γ-secretase substrate, namely the ephrinB ligand and its EphrinB receptor (EphB), is also important for several neuronal functions such as axon guidance, neuronal plasticity, spine maturation and synaptogenesis [65,66,121,122]. For instance, binding of the receptor EphB to its ligand ephrinB stimulates ephrinB processing by γ-secretase. Liberation of ephrinB2-ICD activates Src signaling pathway which in turn phosphorylates ephrinB2 ligand [65]. Phosphorylated ephrinB enables the binding to Grb4 which will further modulate spine morphogenesis and synapse formation [65,123]. In addition, phosphorylation of the ligand prevents its processing and thereby creates an inhibitory feedback mechanism. Importantly, this pathway was shown to be inhibited by PSEN1 FAD mutations [65,122].

Overall, it becomes clear that disturbance of γ-secretase activity results in abnormal axonal guidance, contributes to defect in the maintenance and repair of neuronal circuits and thereby may be potentially involved in the development of AD [111]. Future studies on the regulation of guidance signaling pathways by γ-secretase could provide new insight into the molecular relationships between neural development and degeneration.

6.1.2. γ-Secretase at the synapse

The nervous system also depends on proper neuronal communication that takes place at the synapse. Formation and maintenance of the synaptic structure is a dynamic process that requires bidirectional interaction between pre- and postsynaptic elements [124]. Understanding how (altered) γ-secretase activity may modulate this is of potential value to AD. Also, it is generally appreciated that synaptic dysfunction and in particular defects in neurotransmitter release precedes neurodegeneration [125]. Several cell adhesion molecules, some of which are γ-secretase substrates, are present at the synapses and are crucial for their function and organization. One well characterized synaptic cell-adhesion molecule is the receptor neuroligin (NLG). NLG is expressed at the post-synaptic membrane and binds to its presynaptic ligand neurexin (NRX) to initiate signaling across the synapse. Mutations in these proteins have been linked to distinct cognitive diseases, such as autism spectrum disorders, Tourette's syndrome, schizophrenia and learning disability [126]. The levels of NLG within the synaptic membrane are presumed to directly modulate synaptic function. Interestingly, recent findings suggest that processing of NLG by ADAM10 [127] or metalloproteinase 9 [128] followed by γ-secretase regulates its presence at the synapse [127]. NLG processing was shown to be stimulated by NMDA receptor and the soluble NRX fragment. Cleavage of NLG results in lower synaptic strength and reduces presynaptic release without affecting the postsynaptic structure and function [128]. In addition, by analyzing the amount of NLG ectodomain shedded fragment in the aging mouse brain, Peixoto and co-authors showed that NLG processing is upregulated during early stages of development [128], but it remains to be defined if NLG processing is involved in synaptic maturation during development.

Inoue and co-authors [129] found that γ-secretase-mediated EphrinA4 receptor (EphA4) processing regulates the morphogenesis of dendritic spines. This EphA4-cleavage is disrupted by FAD mutations in *PSEN1*, raising the possibility that abnormal processing of EphA4 may contribute to AD pathogenesis or affect the maintenance and repair of neuronal circuits [129]. The authors also showed that the processing is enhanced by synaptic activity [129]. High levels of EphA4 ICD were shown to increase the number of dendritic spines via activation of the Rac signaling pathway, consistent with the fact

that γ-secretase inhibitor treatment reduces spine density *in vivo* [130].

6.2. γ-Secretase and kidney: Notch and beyond

γ-Secretase deficiency in the kidney leads to severe defects. *PSEN1*, *PSEN2*-double knock-out embryos present no comma- or S-shaped bodies, as well as no mature glomeruli [131]. Additionally, treatment of cultured isolated mouse embryonic metanephroi with γ-secretase inhibitor caused a severe deficiency in proximal tubules and glomeruli [132]. Although both studies suggested that the Notch pathway may be the underlying mediator for PSEN activity in kidney development, up to three γ-secretase substrates including Notch, polycystin-1 and fibrocystin/polyductin are recognized for their renal functions.

Notch signaling molecules are expressed throughout kidney development and are involved in pronephros development in *Xenopus*, mice and zebrafish (reviewed in [133]). In *Xenopus*, blocking Notch signaling upon injection with a dominant negative CSL converts tubular cell fate to ductal cell fate [134]. Mice with a hypomorphic allele for *Notch2* or mice with a targeted deletion in the kidney showed hypoplastic kidneys with glomerular defects [135]. Additionally in the zebrafish, Notch signaling controls the formation of single versus multi-ciliated cells (MCC) in the pronephros, likely required for chemosensing/signal transduction and fluid propulsion, respectively [136]. Loss- and gain-of-function of Notch signaling pathway components has been associated with many renal disorders. Mutations in the genes encoding for *Jagged1* and *Notch2* are responsible for Alagille syndrome (AGS), an inherited autosomal dominant disease characterized by multi-organ dysfunction and a wide spectrum of renal abnormalities [137]. Renal cysts are also observed in Hajdu Cheney and serpentine fibula polycystic kidney syndromes that display mutations in *Notch2* [138,139]. The expression of Notch receptors and ligands is strongly decreased in the mature kidney, but abnormal Notch activation is observed in different models of acute and chronic kidney disease including diabetic nephropathy, focal segmental glomerulosclerosis and disease associated with fibrosis [133]. For instance, conditional overexpression of NICD in podocytes or in tubular epithelial cells results in glomerulosclerosis or tubulointerstitial fibrosis, respectively [140,141]. Furthermore, genetic deletion of a Notch transcriptional partner (*Rbpj*) specifically in podocytes or proximal tubules or pharmacological inhibition of Notch signaling using γ-secretase inhibitors showed protective effects in rats with proteinuric kidney diseases or against fibrosis development, respectively [140,141]. Hence, Notch is a key regulator of kidney development, repair and injury and both loss- and gain-of-function result in kidney diseases.

Polycystin-1 (PC1), which has been recently discovered as a novel γ-secretase substrate [142], plays also an essential role in kidney development and disease. Importantly, the gene encoding PC1 (*Pkd1*) is mutated in 85% of the cases of autosomal-dominant polycystic kidney disease (ADPKD), a common genetic disease producing fluid-filled renal cysts that disrupt the normal tubular architecture and that can ultimately lead to kidney failure [143]. The other 15% of the cases result from mutations in the gene encoding polycystin-2 (*Pkd2*), a non-selective calcium permeable cation channel that interacts and forms a complex with PC1. PC1 is an atypical γ-secretase substrate by the fact that it is composed of 11 TMDs, while almost all γ-secretase substrates are type-I transmembrane proteins. To be fully functional PC1 must undergo N-terminal autocatalytic cleavage in the secretory pathway [144] which could be seen as the functional equivalent of ectodomain shedding in classical substrates. Knock-in mice expressing the non-cleavable PC1 protein develop severe polycystic kidney disease after birth [145] and so do missense mutations in humans. Later, the PC1 C-terminal tail (PC1-CTT) is released by γ-secretase mediated RIP and translocates to the nucleus. Reduced fluid flow in the kidney increases PC1 cleavage and nuclear translocation, suggesting that it may initiate cellular responses to mechanical stress [146]. In agreement with this, once in the nucleus PC1-CTT regulates several signaling pathways including Wnt and activator protein-1 (AP-1) [146]. Additionally it inhibits TCF and CHOP by disrupting their interaction with p300. Importantly, PC1-CTT is sufficient to restore normal tubular morphology and dorsal axis curvature in cells or zebrafish null for *pkd1* or treated with γ-secretase inhibitor, respectively, thereby inferring that γ-secretase-mediated cleavage of PC1 plays an obligate role in at least a subset of its physiological functions [142].

The third γ-secretase substrate involved in renal function, fibrocystin/polyductin is the gene product of the human autosomal recessive polycystic kidney disease gene (ARPKD), PKHD1. Patients with defects in this gene develop severe cystic kidney disease along with defects in the lung, pancreas, and liver [147,148]. Fibrocystin is a large type-I transmembrane protein that is predicted to function either as a receptor or ligand but there is no evidence so far. Moreover, it is suggested to maintain the planar cell polarity (PCP) in the kidney. The mechanism by which mutations in *PKHD1* produce ARPKD is currently not known. Fibrocystin which localizes to cilia and centrosomes in mammalian cells undergoes shedding by ADAM metalloproteinases followed by the release of an ICD fragment by γ-secretase [149]. However, so far no nuclear function to the fibrocystin ICD has been attributed and further studies are required to evaluate the impact of γ-secretase cleavage on the development of ARPKD.

6.3. γ-Secretase function in skin development and disease

Both human and animal data functionally support a role for γ-secretase in skin development and skin cancer. First, PSENs are required during skin development to maintain the differentiated cellular identity within the hair follicle and their absence leads to the conversion of hair follicles into epidermal cysts [150]. Yet, they are not involved in skin patterning or cell fate acquisition of the hair follicle as shown in conditional knock-out mice (*msx2*-cre driven ablation of *PSEN1*/*PSEN2*). These mice also show a severely hyperplastic epidermis and the absence of sebaceous glands. Additionally, partial loss of γ-secretase activity (of at least 50%) in skin leads to the development of epidermal hyperplasia and skin tumors including squamous cell carcinomas (SCC) in adult mice. This was observed in many different mouse models including $Nct^{+/-}$ and $Nct^{+/-}$/$PSEN1^{+/-}$ knock-out mice [151], $PSEN1^{+/-}PSEN2^{-/-}$ mice older than 6 months [152] and *PSEN1* knockout mice that are rescued through neuronal expression of a human *PSEN1* transgene [153].

In human, heterozygous mutations of the γ-secretase genes *PEN2*, *PSEN1* and *NCT* were recently reported in acne inversa (AI, also called hidradenitis suppurative), a chronic inflammatory skin condition that presents with comedones, painful nodules, abscesses and sinus tracts in apocrine gland-bearing areas [154]. Longstanding disease can result in fibrosis, dermal contractures, scaring, formation of fistulae and rarely malignant transformation to SCC. To date, 17 *NCT*, 3 *PEN2* and 1 *PSEN1* mutations have been reported in eleven kindreds and four sporadic cases, of which three are nonsense mutations, seven result in frameshifts, seven in altered splicing and four are missense mutations (reviewed in [155]). Haploinsufficiency of the γ-secretase component genes suggests that critical levels of γ-secretase activity are required to maintain skin homeostasis. This is supported by the findings that a number of individuals involved in the clinical trial of the γ-secretase inhibitor Semagacestat for AD reported cutaneous side effects and an increased risk of skin cancer [156].

Most of the skin developmental defects observed in γ-secretase-deficient mice have been related to Notch signaling pathway defects. Indeed, compound loss of the different Notch receptors phenocopies loss of γ-secretase in hair follicles therefore inferring that Notch proteolysis is a major signaling function of γ-secretase in skin [150]. Interestingly a recent study showed that Notch and PSEN2 are present in ciliary structures of suprabasal epithelial cells and that the cilium may function directly in fine-tuning the Notch-regulated balance between proliferation and differentiation in developing skin [157].

Alternatively, an activated β-catenin pathway in absence of PSEN1 contributes to the skin tumor phenotype. This was previously thought to relate to a γ-secretase independent function of PSEN1 where direct interaction of PSEN1 with β-catenin or N- and E-cadherins at the adherent junction regulates β-catenin stability and downstream signaling [153,158]. However, rescue of this phenotype is possible with a synthetic mutant of PSEN1 that disrupts the cadherin/catenin interaction with PSEN1 but maintains γ-secretase-mediated Notch signaling [159]. Also, because the interaction between PSEN1 and β-catenin concerns only the membrane-associated pool, the absence of PSEN1 may have little effect on the soluble cytoplasmic pool of β-catenin, which is more directly regulated by GSK/Axin/APC signaling. Therefore, these skin tumors are now thought to be another manifestation of the many different defects caused by deficient Notch signaling in *PSEN1* knockout mice. This is in agreement with the findings that Notch1 acts as a tumor suppressor in skin via regulation of β-catenin signaling [160].

6.4. γ-Secretase in immune system homeostasis and disease

The phenotypic characterization of several *in vivo* γ-secretase defective mouse models has revealed strong links between γ-secretase and the immune system. For instance, reducing levels of γ-secretase by at least 30% ($Nct^{+/-}$ and $Nct^{+/-}PSEN1^{+/-}$ mice) increases risk for development of splenomegaly characterized by hyperproliferation of granulocytes and reduction of T-cell populations [151]. Interestingly this phenotype is age-dependent as enlarged spleens were only observed in mice older than 15 months. Adult $PSEN1^{-/+}PSEN2^{-/-}$ mice also develop splenomegaly with granulocyte infiltration in addition to a serious autoimmune phenotype characterized by B-cell dominated infiltrates, hypergammaglobulinemia with immune deposits in tissues including skin and kidney, high titer nuclear autoantibodies and increased CD4+/CD8+ ratio [152,161].

Mice with conditionally deleted *PSEN* genes in developing T cells (*cd4*-Cre-$PSEN1\Delta/\Delta$,$PSEN2^{-/-}$) have a defective CD4+ T cell development associated with impaired TCR signaling as a consequence of poor positive selection of thymocytes. Re-expression of NICD rescues this phenotype thereby inferring a role of PSEN-dependent Notch signaling in thymocyte development [162]. Moreover these conditional *PSEN* KO mice displayed a significant decrease in the number of CD8+ cell on the periphery in addition to having CD4+CD8+ double-positive cells resistant to anti-CD3-induced apoptosis [163]. PSENs also play a role in B-cell function as PSEN-deficient B cells (*CD19*-cre, $PSEN1\Delta/\Delta$, $PSEN2^{-/-}$) displayed a defective marginal zone and T2 B cell development that is reminiscent of observations in *Notch2*-deficient and *RBPJ*-deficient B cells [164]. Additionally, they display deficits in signal transduction events including lipopolysaccharide (LPS)-induced proliferation and anti-IgM-mediated calcium flux. These defects are likely to be independent of defects in Notch activation because both were normal in B cells lacking *RBPJk*.

Most of the hematopoietic defects observed in γ-secretase defective animals have been ascribed to the Notch signaling pathway, being consistent with the fact that Notch is required for proper hematopoiesis. More particularly, Notch1 signaling is crucial for T-cell specification in early hematopoietic progenitors, namely the T- versus B-cell lineage choice in early hematopoietic progenitors, by promoting their proliferation, differentiation and survival in the thymus (for review, see [165]). Notch also plays a clear pathological role in T-cell lymphoblastic leukemia (T-LL) in which the majority of human and murine tumors have acquired mutations that lead to aberrant increases in Notch signaling [166]. For instance, *Notch1* gain-of-function mutations have been found in roughly 60% of primary human T-LL [167]. Most *Notch1* mutations in human T-LL fall either into the heterodimerization (HD) domain or into the C-terminal PEST (polypeptide enriched in proline, glutamate, serine and threonine) domain. HD domain mutations are found in 40 to 50% of T-LLs patients and usually consist of point mutations or small in-frame insertions or deletions. They destabilize Notch sufficiently to permit ligand-independent metalloproteinase shedding, albeit still requiring γ-secretase activity [168]. The PEST domain targets the protein for degradation through the proteasome pathway and thereby modulates the half-life of NICD in the nucleus [34]. Hence, T-LL associated mutations or deletions of the PEST domain accentuate Notch activity by increasing NICD half-life, a process independent of γ-secretase activity (see for review [165,166]). Interestingly, few loss-of-function mutations in the Notch signaling pathway including a null mutation in NCT (NCTA433T) have been identified in leukemia patients, suggesting again that Notch acts not only as a tumor promoter but also as a tumor suppressor gene in leukemia [169].

Additional γ-secretase substrates play as well roles in the development and function of hematopoietic cells; albeit the direct function of some of their cleavage products has remained elusive. These include (1) HLA-A2 (human leukocyte antigen-A2), a major histocompatibility complex class I protein with primary functions in T-cell development and initiation of immune cell response [170]; (2) IL-1R2 (interleukin-1 receptor type 2) [171] and (3) IL-1R1 that play important roles in innate immunity and inflammatory responses and Il-1R1 cleavage potentiates downstream MAPK activation [172]; (4) LRP1 (lipoprotein receptor-related protein 1) whose cleavage product restricts LPS-stimulated production of proinflammatory cytokines by macrophages [173]; (5) the transmembrane chemokines CX3CL1 and CXCL16 [174]; as well as (6) CD46 whose cleavage is important for T cell activation, proliferation and cytokine production [175].

6.5. γ-Secretase functions in the cardiovascular system

Important developmental processes like vasculogenesis, angiogenesis [90,176] (for review [177]) and cardiac morphogenesis [85,178] also do not escape from a functional involvement of γ-secretase activities. *PSEN1*, *APH1a* and *PEN2* knockout mice show abnormal blood vessel development and a disorganized vascular system [90,92,176]. Up-regulation of PSEN2 was also reported in a retinal model of retinopathy of prematurity [179]. This elevation was due to an increased binding of HIF-1-DNA to the *PSEN2* gene promoter.

Finally, mutations of *PSEN1* and *PSEN2* genes have been described in dilated cardiomyopathy (DCM) patients [99,100,102]. DCM is characterized by ventricular enlargement and contractile dysfunction with normal left ventricular wall thickness. Since the mutations described in this pathology were already listed as FAD mutations, Gianni and coworkers have hypothesized that protein aggregation could be the leading cause of the disease as in AD. The presence of fibrillar and oligomeric assemblies in the hearts of DCM patients suggest abnormal protein aggregation and could explain the alteration on $Ca2+$ homeostasis [100]. Alternatively, using *Drosophila* as a model to study the role of PSEN in heart function, Li and co-authors have shown that cardiac dysfunction resulted from aberrant calcium channel receptor activities and disturbed Wnt signaling transduction due to affected Notch processing [101].

While most of the effects of γ-secretase activity on the cardiovascular system have been associated to its substrate Notch, which is an important modulator of endothelial behavior, other substrates like VEGFR, ErbB4, cadherin and IGF1R also contribute significantly.

For instance, VEGF signaling which plays a critical role in angiogenesis is modulated by γ-secretase. Upon binding to its ligand VEGF, VEGFR-1 gets processed by γ-secretase generating a VEGFR-1-ICD fragment that acts as a negative regulator of VEGFR-2-mediated neovascularization [180]. Also, upon pigment epithelium-derived factor (PEDF) treatment, γ-secretase is actively transported to the plasma membrane which induces its activity and thereby prevents angiogenesis in microvascular endothelial cells.

Upon binding to epidermal growth factor (EGF), its receptor ErbB4 undergoes γ-secretase-mediated processing resulting in the release and nuclear translocation of the ErbB4-ICD fragment where it regulates transcription promoting migration and proliferation, although

the nuclear binding complex is yet to be identified [181,182]. It has also been shown that neuregulin-2 binding to ErbB4 prevents angiogenesis [183].

In the case of E- and N-cadherin, their processing will result in dissociation of the adhesion complex required for proper cell–cell junctions [184–186]. In the case of the blood vessel, the vascular endothelial (VE)-cadherin regulates the permeability and prevent leakage [187]. However, γ-secretase-mediating processing of VE-cadherin remains to be reported.

IGF-1 (insulin-like growth factor-1) is a pro-angiogenic factor that significantly contributes in aberrant neovascularization associated with diabetic retinopathy [188,189]. Its receptor, IGF-R has been recently reported to be a substrate of γ-secretase although the downstream signaling pathway initiated by its cleavage product remains to be elucidated [190].

Finally, as Aβ has been reported to be present in extracellular deposits called Drusen which are a hallmark of age-related macular degeneration (AMD), APP appears to also participate to diabetic retinopathy and AMD in the eye [191].

6.6. Role of γ-secretase in cancers

In addition to the critical roles of γ-secretase in skin cancer and leukemia described in previous paragraphs, two FAD mutations in PSEN2, namely R62H and R71W, have been identified in breast cancer patients [103]. To and coworkers showed that both mutations alter the stability of PSEN2 and therefore may induce a loss-of-function effect. Although the role of these mutations in FAD is still unclear, both mutations do not affect Aβ(42/40) ratio but rather alter Notch signaling as shown in *C. elegans* and promote cell growth inhibition in mouse embryonic fibroblasts [103]. Besides FAD mutations, upregulation of γ-secretase components has also been reported in cancer. NCT levels were shown to be elevated in invasive breast cancer [192], *PSEN2* in brain tumors [193] and *PSEN1* in human malignant melanoma [194]. Liu and coworkers demonstrated that in malignant glioma tissue, upregulation of *PSEN2* was due to its promoter demethylation. Consequently, downregulating the expression of either NCT or PSEN2 prevented the development of cancer.

Besides hyperactivation of the Notch signaling described in cancer cells, E-cadherin cleavage by γ-secretase releases cell–cell contact and promotes invasion and metastasis. Therefore downregulating γ-secretase prevents E-Cadherin cleavage, dissociation of the cell–cell contact and metastatic cell transformation [192].

EpCam (epithelial cell adhesion molecule, CD326), another γ-secretase substrate, mediates epithelial-specific intracellular cell-adhesion. Its overexpression in most carcinomas has been proposed as a potent protein marker in oncology [195]. Recently, the molecular mechanism of EpCam in oncogenesis was demonstrated and involves the γ-secretase mediated processing of EpCAM and subsequent release of an EpCam-ICD fragment that forms a nuclear complex with FHL2, β-catenin and Lef-1 to activate gene expression [196].

7. Concluding remarks

Since its discovery more than fifteen years, the complex biological implications of γ-secretase activity are becoming increasingly evident. Because of its many substrates, γ-secretase is involved in vital physiological processes in health and unfortunately also in diseases. Therefore, understanding the proper regulatory mechanisms of its activity for each substrate in its specialized cell type is highly relevant to understand the molecular mechanism of diseases and develop novel therapeutical approaches. Especially how mutations in γ-secretase can either lead to a neurodegenerative disease or a cancer needs to be addressed. Furthermore, as previously mentioned up to six different γ-secretase complexes are present in humans and eight in rodents and it is becoming increasingly evident that different complexes have heterogeneous biochemical and physiological properties. Therefore, understanding the nature of each γ-secretase complex is becoming highly significant as their diversity become apparent but far from understood. On the other hand, while the list of γ-secretase substrates is constantly growing, the function(s) of most of them are at most partially known or currently under investigation. While at first one needs to understand the critical function of each substrate in health and disease by using various ablation methods in animal models, we also need to fully comprehend the physiological consequences of its processing by γ-secretase. Approaches similar to the Val1744 mutation knock-in in Notch that phenocopies Notch-deficiency are therefore needed for other substrates.

The tremendous progress made in the past fifteen years has as well boosted major interests from academic and pharmaceutical industry to find treatments and to maximally exploit γ-secretase as a relevant drug target [197]. We are likely coming into an era in which, after multiple failed clinical trials for AD, γ-secretase inhibitors are now entering clinical trials for treatment of distinct diseases. More particularly, the inhibitor R04929097 is currently used in phase I/II clinical trials to see how well it works in treating young patients with relapsed or refractory solid tumors, CNS tumors, lymphoma, or T-cell leukemia (http://clinicaltrials.gov/show/NCT01088763). Despite intense drug development programs, no therapeutic drugs have yet reached the clinic for AD mainly caused by the great danger of creating unexpected side effects which are not fully understood. Certainly, a better understanding on the heterogeneity of γ-secretase complexes function will help in developing substrate specific inhibitors or modulators. As well, another hope of therapy aims at further developing γ-secretase modulators which instead of blocking its activity shift it in order to produce shorter Aβ peptides without affecting the Notch signaling pathway [71,198,199].

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