The substrate repertoire of γ-secretase/presenilin

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Abstract

The intramembrane protease γ-secretase is a hetero-tetrameric protein complex with presenilin as the catalytic subunit and cleaves its membrane protein substrates within their single transmembrane domains. γ-Secretase is well known for its role in Notch signalling and in Alzheimer's disease, where it catalyzes the formation of the pathogenic amyloid β (Aβ) peptide. However, in the 21 years since its discovery many more substrates and substrate candidates of γ-secretase were identified. Although the physiological relevance of the cleavage of many substrates remains to be studied in more detail, the substrates demonstrate a broad role for γ-secretase in embryonic development, adult tissue homeostasis, signal transduction and protein degradation. Consequently, chronic γ-secretase inhibition may cause significant side effects due to inhibition of cleavage of multiple substrates. This review provides a list of 149 γ-secretase substrates identified to date and highlights whether substrate cleavage was validated and characterized in vitro or also in vivo. Additionally, the review lists the cleavage sites where they are known and discusses the functional implications of γ-secretase cleavage with a focus on substrates identified in the recent past, such as CHL1, TREM2 and TNFR1. A comparative analysis demonstrates that γ-secretase substrates mostly have a long extracellular domain and require ectodomain shedding before γ-secretase cleavage, but that γ-secretase is also able to cleave naturally short substrates, such as the B cell maturation antigen. Taken together, the list of substrates provides a resource that may help in the future development of drugs inhibiting or modulating γ-secretase activity in a substrate-specific manner.

Keywords (max 6): γ-secretase, Alzheimer's disease, TREM2, intramembrane proteolysis, CACHD1

1. Introduction

Intramembrane proteases are a fascinating group of proteases that are buried within the lipid bilayer of the membrane and cleave their substrates within or very close to their transmembrane domains [1-3]. The best studied intramembrane protease in mammals is γ-secretase, because of its role in Alzheimer's disease (AD), Notch signalling and Notch-dependent tumors [4]. Besides γ-secretase, the intramembrane proteases comprise signal peptide peptidase (SPP) and its homologs, the signal peptide peptidase-like (SPPL) proteases SPPL2a, SPPL2b, SPPL2c and SPPL3 as well as the rhomboid proteases, the site-2-protease and the glutamyl protease Rce1 [3, 5].

With the molecular identification of γ-secretase 21 years ago [6], both the AD-linked amyloid precursor protein (APP) and Notch were shown to be substrates [7, 8]. γ-Secretase inhibitors were then developed as drugs targeting AD and Notch-dependent tumors [9]. Since then, more than one hundred substrates and substrate candidates have been identified and, continuously, more are being revealed. The last comprehensive reviews on γ-secretase substrates date back several years [10, 11], but numerous additional γ-secretase substrates have been identified since then. The purpose of this review is to provide an updated, comprehensive list of the 149 γ-secretase substrates identified to date with a special focus on the recently identified ones. This review will highlight the degree of validation of the substrates and indicate whether their cleavage by γ-secretase was seen under overexpression or endogenous expression conditions of the substrate and whether substrate cleavage was detected in vivo and shown to be relevant for a physiological process. At the beginning, we start out with a brief description of the subunits and the cleavage mechanism of γ-secretase.

2. Subunits of γ-secretase

γ-Secretase is a hetero-tetrameric protein complex consisting of one proteolytically active subunit, presenilin (PS), and the three non-proteolytic subunits nicastrin, APH-1 (anterior pharynx defective-1) and presenilin enhancer 2 (PEN-2) (Fig. 1). Two homologous genes exist for PS, named PS1 and PS2, while APH1 is encoded by two homologous genes in humans (APH-1a and APH-1b), with APH-1a being present as long (APH-1aL) and a short (APH-1aS) splice form, and in rodents, there is even an additional APH-1 gene, APH-1c [12-14]. Given the different possible subunit combinations, γ-secretase is present in human cells and tissues as up to six homologous complexes, containing either PS1 or PS2 and either one of the three APH-1 homologs (APH-1aL, APH-1aS, and APH-1b) [12, 14]. The γ-secretase complexes differ in their subcellular localization, with PS1-containing complexes found in different secretory pathway compartments including at the plasma membrane, whereas PS2-containing complexes localize more to endosomes and lysosomes [15, 16]. As a consequence, some substrates, such as PMEL and TRP-1, which localize to lysosome-related melanosomes, are mostly cleaved by PS2-, but barely by PS1-containing γ-secretase complexes. Thus, it is possible that the six different γ-secretase complexes generally have a different, but probably overlapping substrate spectrum, but this still remains to be analysed in more detail [15]. The relative abundance of the six γ-secretase complexes may also differ among cell types and tissues [17]. For example, it has been reported that microglia cells predominantly utilize PS2, but not PS1 in the γ-secretase complex [18].

Upon assembly of the heterotetramer in early biosynthetic compartments of the secretory pathway, the PS subunit undergoes autoproteolytic cleavage within the large loop between

transmembrane domains 6 and 7, resulting in the active protease complex [6, 19, 20] (Fig. 1). γ-Secretase is an aspartyl protease, where the two catalytically active aspartic acids are located at amino acids 257 and 385 in transmembrane domains 6 and 7 of PS1, respectively [6]. PS1 and PS2 also have a non-proteolytic function in calcium homeostasis, autophagy and lysosomal acidification, which is reviewed elsewhere [21, 22] and not covered in our review. The heterotetrameric nature of γ-secretase was initially demonstrated functionally in yeast [20], later by biochemical experiments [23-25], and finally in cryo-electron microscopy studies with a high resolution of less than 4 Å [26]. These structures, which are now also available together with a substrate, derived from either Notch or APP [27, 28], demonstrate, that the four γ-secretase subunits form a horseshoe-like structure in the membrane (Fig. 2). γ-Secretase is known to exclusively cleave substrate transmembrane domains with a type I orientation (Table 1) and this is also seen in the cryo-electron microscopy structure, where the N-terminus of the substrate helix is oriented towards the luminal/extracellular membrane surface and the C-terminus directed towards the cytoplasmic membrane boundary.

3. Cleavage mechanism of γ-secretase

While PS, APH-1 and PEN-2 have multiple transmembrane domains, Nicastrin only has a single transmembrane domain, which, however, is connected to a large extracellular domain, which folds in such a way that it forms a lid-like structure on top of the membrane-buried horseshoe structure of γ-secretase (Fig. 2). This lid-like structure is essential for substrate selection by γ-secretase, because it helps to anchor the substrate in the complex [29]. Additionally, it sterically prevents membrane proteins with a long extracellular domain to fit under the nicastrin lid, thereby excluding their direct cleavage by γ-secretase [30, 31]. Yet, nearly all known γ-secretase substrates have long ectodomains (Fig. 2). As a consequence, these membrane proteins undergo a first proteolytic cleavage by another protease, which cleaves off the large ectodomain and generates a C-terminal fragment (CTF) with a remaining ectodomain stub of typically not more than 50 amino acids. This cleavage in the membrane-proximal juxtamembrane domain is referred to as ectodomain shedding and is mediated by a diverse group of typically membrane-bound proteases, such as ADAM and BACE proteases [32] that have their active site within their ectodomain and are able to cleave the substrates within their ectodomain close to the membrane surface (Fig. 2) [32] . Thus, when we refer to a membrane protein as being a substrate for γ-secretase, it is typically not the full-length form but the CTF of that protein that is the real substrate. The combination of ectodomain shedding and subsequent intramembrane proteolysis, e.g. by γ-secretase, is seen for numerous membrane proteins, such as APP and Notch, and is generally referred to as regulated intramembrane proteolysis (RIP) [1, 32]. Taken together, γ-secretase only cleaves substrates with a short ectodomain and a transmembrane domain of type I orientation.

Notably, one substrate, the B cell maturation antigen (BCMA), is directly cleaved by γ-secretase without a prior requirement for ectodomain shedding. This is possible because the ectodomain is naturally short with only 54 amino acids in the human sequence. At present, it is not known whether more naturally short substrates exist for γ-secretase. Interestingly, the APP-homolog APLP1, which has a long ectodomain was recently suggested to be directly cleaved by γ-secretase, at least to a small extent, whereas such a direct cleavage was not observed with APP and the other homolog APLP2, which both require prior ectodomain shedding before being cleaved by γ-secretase [33-35]. How the long ectodomain of APLP1 bypasses the strict length requirements imposed by nicastrin remains unclear, but this

demonstrates that the cleavage mechanism of γ-secretase appears more complex than what is known and requires more research to be better understood.

In fact, while 149 membrane proteins (discussed in section 4) have been shown to be cleavable by γ-secretase, the exact cleavage mechanism has only been studied for few of them, most notably APP and Notch. From the available cryo-electron microscopy of the substrate-bound γ-secretase [27, 28] it is assumed that the helical substrate transmembrane domain unwinds at its C-terminus, where it gets cleaved by γ-secretase (referred to as the ε-cleavage site), leading to release of the intracellular domain (ICD) into the cytosol (Fig. 2). ICDs may act as signalling molecules, at least in the case of some substrates, such as Notch [8], or may simply be further degraded. The remaining membrane-bound fragment then further unwinds its helical transmembrane domain in a stepwise fashion and is further truncated by about 3 amino acids (one turn of a transmembrane domain helix, termed ζ-cleavage) in each step. This carboxypeptidase-like activity of γ-secretase proceeds until the enzyme-substrate complex is not anymore stable enough, because the remaining part of the transmembrane domain is shortened and can slip out of the membrane [36]. This last cleavage (termed γ-cleavage), thereby gives rise to a short, secreted peptide (Fig. 3). In case of APP this is the Aβ peptide (Fig. 2). In agreement with the stepwise cleavage mechanism there are Aβ peptides of different length, ranging mostly from 37 to 43 amino acid (Aβ37-43), with the longer ones (Aβ42/43) being the pathogenic species in AD. In most familial forms of AD with a dominant inheritance, mutations in PS1 or the substrate APP interfere with efficient cleavage from the ε- to the γ-site [37]. As a result longer Aβ peptides are generated, resulting in an earlier onset of AD (for a review see [38]). Mechanistically, the AD-linked mutations decrease the stability of the enzyme-substrate complex, so that substrate dissociation may occur prematurely, effectively leading to longer, and thus, more toxic Aβ peptides [39, 40]. Additionally, it is known that experimental mutations within the APP-transmembrane domain affect either the ε-cleavage or the subsequent trimming towards the γ-cleavage site and thus affect the ratio of short versus long Aβ (e.g. [41-46]). This demonstrates that the transmembrane sequence of a substrate has a significant influence about whether and how it is cleaved by γ-secretase. A more detailed review of the cleavage mechanism of γ-secretase has been published elsewhere [47].

Taken together, a wealth of biochemical and now also structural data provided exciting insights into the cleavage mechanism of γ-secretase. However, because most studies focused on only few substrates – in particular APP and Notch –, crucial points in the mechanism are still not well understood, such as how substrates are recognized by γ-secretase and then transferred to the active site and whether different substrates use the same or different mechanisms. A better understanding of these topics requires a more comprehensive knowledge of the spectrum of γ-secretase substrates and their cleavage patterns by γ-secretase.

4. Substrates of γ-secretase

During the past 21 years since the discovery of the molecular identity of γ-secretase, 149 membrane proteins have been shown to be cleavable by γ-secretase and, thus, can be considered as the currently known γ-secretase substrates (Table 1). This total number comprises the membrane proteins listed in a previous review in 2011 [10] plus the results of a Pubmed search for the years 2010 until 2019 for the keywords “gamma secretase AND (substrate OR shedding OR cleavage)”. While a few of these substrates were identified in

proteomics and candidate screening studies (e.g.[48]), most of the membrane proteins were identified by individual candidate approaches.

The criteria to define a membrane protein as a substrate of γ-secretase varied throughout these studies. Some studies used a single criterium, others used multiple criteria. Most studies blocked γ-secretase activity in cell lines pharmacologically or genetically and monitored by immunoblot analysis the accumulation of the substrate CTF, which results from the inhibited CTF cleavage. This is a popular approach (see Table 1), because antibodies are often available to the cytoplasmic tail of a given membrane protein. However, detection of the cleavage products – the ICD and the short, secreted peptide (Aβ in case of APP) – is mostly more difficult. The ICD is typically short-lived and, thus, present at very low concentrations. An inhibition of the proteasome or of insulin-degrading enzyme was used in some studies to stabilize and visualize the ICD (e.g.[49]) . The short, secreted peptide is often not detected, simply because no suitable antibody is available for this short peptide. One way around this limitation is the expression of the CTF – and not the full-length protein – with an N-terminal epitope tag, which then allows detection of the short, secreted peptide (e.g. [50, 51]).

The different substrate identification strategies have their advantages and limitations. Thus, for an initial screen of several substrate candidates it may be sufficient to only consider one of the above criteria, but if a substrate is studied in more detail, its cleavage by γ-secretase should be investigated by different of the above approaches. Ideally, substrate cleavage should also be confirmed in an in vitro assay, where different approaches are feasible. For example, it is possible to use a) purified γ-secretase and/or substrate or b) detergent-solubilized membrane fractions containing endogenous γ-secretase isolated from cultured cells [23, 25, 52, 53], or c) membrane fractions from (transfected) cells which can be incubated in vitro [54-56].

Another method to inform about whether a protein is cleaved by γ-secretase or not, is the determination of the cleavage site, which should be within the substrate's transmembrane domain. Cleavage sites have so far been determined for a subset of substrates (Table 2), mostly by Edman degradation, where single amino acids from the N-terminus of a peptide are sequentially removed and identified, or by matrix-assisted laser desorption-ionization (MALDI) mass spectrometry, which allows to measure exact peptide masses. Such analysis can be done on both cleavage products, either the ICD or the short, secreted peptide, and is, thus, able to give information about either the initial ε-cleavage or the final γ-cleavage. Mass spectrometric methods even allowed to detect the products of the intermediate ζ–cleavages [57].

Finally, it is highly desirable to show that a membrane protein is a γ-secretase substrate not only when the substrate is overexpressed, but also under endogenous expression conditions of the substrate. While γ-secretase is ubiquitously expressed, this approach requires a suitable cell line or primary cell that expresses the substrate endogenously. Ideally, the cleavage is also shown in vivo in tissue or it is demonstrated that the cleavage has a physiological consequence.

Taken together, various experimental approaches are available to demonstrate that a membrane protein can be considered as a substrate for γ-secretase. Table 1 indicates which of the above methods were used for the 149 known γ-secretase substrates.

A comparison of the substrates reveals differences and commonalities among the substrates. Clear differences are seen in the wide range of physiological functions of the γ-

secretase substrates and their cleavage fragments (discussed in the next section) as well as in their amino acid sequences, including within the transmembrane domain. But there are also commonalities as discussed above, such as a short extracellular domain and a type I orientation in the membrane (Table 1).

Notably, some substrates in the table are annotated as having a membrane orientation other than type I. While they may appear to break the general rule for γ-secretase substrates to have a transmembrane domain with type I orientation, these proteins in fact are not against the rule because of the following reasons. A type III membrane protein has the same orientation as a type I membrane protein with the only difference that it lacks an N-terminal signal peptide. One such γ-secretase substrate is BCMA [58]. Type IV membrane proteins are multipass membrane proteins. One example is neuregulin-1 type III (NRG1) which has a hairpin loop structure with two transmembrane domains. After shedding within the loop that connects both TMDs, the protein consists of two single span membrane protein subunits. One has a type I orientation and is further cleaved by γ-secretase, whereas the other one has a type II orientation and is further cleaved by the distant presenilin homologs SPPL2a and SPPL2b, which only cleave type II membrane proteins [59]. One type II membrane protein (GnT-V or MGAT5) was previously suggested to be a γ-secretase substrate [60], but this finding was corrected when it was discovered that GnT-V is instead cleaved by the distant presenilin homolog SPPL3, which is known to cleave type II membrane proteins [61, 62].

In contrast to some proteases with high sequence specificity, such as trypsin or caspases, γ-secretase does not appear to have a unique sequence motif that is preferentially cleaved and that would allow prediction of further γ-secretase substrates (Table 2) [63]. Identification of such a motif has been difficult because very few non-substrates of γ-secretase have been unequivocally established, such as integrin β1 [48], which would allow systematic sequence comparisons between substrates and non-substrates. Despite the low cleavage specificity of γ-secretase, there is also evidence that mutations close to the ε- or γ-site can affect the efficiency of γ-secretase cleavage at either site, as shown for example for APP, but also Notch [64], and as described above in the section on the cleavage mechanism, in particular for APP.

5. Functions of γ-secretase cleavage

The physiological functions of the full-length forms of γ-secretase substrates vary strongly. Because most substrates require ectodomain shedding before being further cleaved by γ-secretase, the function of the full-length proteins may not necessarily be affected by γ-secretase cleavage. Generally, it is assumed that γ-secretase cleavage serves one of three purposes or a combination thereof; a) signal transduction mediated by the released ICD or b) secretion of a short peptide which may have a (patho-)physiological function or c) degradation of the membrane-tethered CTF. The former is best exemplified by cleavage and signalling of the Notch receptor [8, 65, 66]. Notch can be seen as a membrane-bound transcriptional activator which requires γ-secretase cleavage to become transcriptionally active, e.g. during development [67]. Because hyperactivation of this signalling contributes to tumorigenesis [68], γ-secretase inhibitors are now used/tested for the treatment of Notch-signalling-dependent tumors, such as leukemias [69, 70]. Other, more recent examples, where γ-secretase cleavage has functional consequences for a substrate, are described further below and listed in Table 1.

In contrast to Notch, many other γ-secretase substrates have short cytoplasmic domains such that their ICD may not have a signalling function or, at least, not a known function.

Similar to the ICD, it is largely unknown whether the second γ-secretase cleavage product, the short, secreted peptide (Aβ in case of APP), has a physiological function. In case of APP, a potential physiological function of both Aβ and ICD remain controversially discussed [71]. However, Aβ has an established pathophysiological function early in AD pathogenesis and mutations in APP or PS1 or PS2 cause dominantly inherited forms of AD. Consequently, γ-secretase inhibitors have been developed and tested for AD treatment, but were discontinued due to the occurrence of, most likely, Notch signalling mediated side effects [72, 73]. Proposed beneficial functions for Aβ include an antimicrobial role [74], and memory regulation [75].

Given the large range of γ-secretase substrates, it has been proposed that γ-secretase cleavage may also serve the function of protein degradation similar to the function of the proteasome for cytoplasmic and nuclear proteins [76]. A CTF that arises through ectodomain shedding cannot simply be extracted from the membrane and be degraded, e.g. by the proteasome. Instead, it may be transported to the lysosomes for degradation. Alternatively, γ-secretase cleavage appears as an attractive mechanism to cleave within the CTF's transmembrane domain, which allows the ICD and the short, secreted peptide to be released from the membrane and then be further degraded by soluble proteases. Yet, the general function of γ-secretase on CTF degradation has been questioned by a proteomic study [48], which found that only few CTFs accumulated in γ-secretase inhibitor-treated HeLa cells. However, since then many more proteins were shown to be endogenous γ-secretase substrates (Table 1), so that a function of γ-secretase acting as a CTF-degrading enzyme still remains entirely possible.

Physiological and pathophysiological consequences of γ-secretase cleavage, in particular of APP and Notch, but also of other γ-secretase substrates, have been described in previous review articles [4, 11]. Thus, in the following we list selected γ-secretase substrates identified since 2011 and highlight how γ-secretase cleavage controls their function. The comprehensive list of γ-secretase substrates and available information on the functional outcome of γ-secretase cleavage is found in Table 1, which lists the first paper describing γ-secretase cleavage of a given membrane protein and its functional implication.

5.1. Receptors with immune functions

The B cell maturation antigen (BCMA) is a surface receptor expressed on B cells, where it activates NF-κB and allows survival of plasma cells after activation with its ligands BAFF or APRIL. BCMA was found to be a naturally short γ-secretase substrate [58]. Due to its short ectodomain length of only 54 amino acids, it apparently directly fits under the nicastrin domain, so that a shedding step prior to γ-secretase cleavage is not required. The physiological consequence of BCMA cleavage by γ-secretase is two-fold. First, the released, short BCMA ectodomain acts as a decoy receptor that competes with the membrane-bound full-length BCMA for ligand binding. Second, γ-secretase cleavage reduces the levels of full-length BCMA. As a result of both processes, γ-secretase cleavage reduces and thereby controls the physiological BCMA-mediated NF-κB activation in vitro and survival of plasma cells in vivo. Interestingly, pharmacological inhibition of γ-secretase cleavage of BCMA is now exploited in a clinical trial to improve the efficacy of BCMA-specific chimeric antigen receptor (CAR) T cells in multiple myeloma [77]. The rationale behind this approach, which has already been successfully tested in mice, is the following. γ-Secretase inhibition blocks

release of the soluble, shed BCMA, which normally would inhibit CAR-T cell function. Additionally, γ-secretase inhibition increases full-length BCMA protein levels at the surface [58], which in turn allows improved tumor recognition by the CAR-T cells.

Triggering receptor expressed on myeloid cells 2 (TREM2) is a major genetic risk factor of AD [78]. Through association with the coreceptor DAP12, TREM2 acts as a cell surface receptor controlling phagocytic activity of myeloid cells, such as microglia. The long ectodomain of TREM2 is shed by ADAM proteases [79-81]. The resulting TREM2 CTF can be further processed by γ-secretase [82, 83], which controls in microglial BV2 cells the extent of the inflammatory response [84] and TREM2-dependent calcium signalling and phagocytosis [85].

Tumor necrosis factor (TNF) receptor (TNFR) signalling is a key pathway in inflammation, and an established clinical target for inflammatory disorders. TNFR1 is the major receptor for soluble TNF, which is released mostly from immune cells after proteolytic processing by ADAM17 [86, 87]. Ligand-bound TNFR1 can signal at two cellular localizations. At the plasma membrane TNFR1 signalling activates the classical NF-κB pathway and triggers a prosurvival response, whereas in endosomes, TNFR1 signalling can lead to apoptosis [87, 88]. Similar to the ligand TNF, TNFR1 is also subjected to proteolytic processing by ADAM17 [86]. The resulting membrane bound TNFR1 CTF can be further processed by γ-secretase, and this processing was shown to be important in TNF-mediated JNK/MAPK activation and TNF-induced apoptosis [89].

5.2. Receptors and cell adhesion proteins in the nervous system

The close homolog of L1 (CHL1) is a transmembrane cell adhesion protein with essential functions during development, most notably in axon guidance and growth cone collapse, where it is recruited to the neuropilin 1 (NRP1)/plexin A receptor upon NRP1 stimulation with semaphorin 3A (Sema3A). This stimulation induces shedding of CHL1 by BACE1 [90], generating the CHL1 CTF, which in turn transmits the Sema3A signal to the neuronal cytoskeleton, resulting in growth cone collapse in thalamic neurons in vitro. Inhibition of γ-secretase was found to enhance the CTF-dependent growth cone collapse [90], demonstrating that γ-secretase cleavage is a mechanism to control the signalling function of the CHL1 CTF.

A Notch-like signalling mechanism was reported for the immunoglobulin and cell adhesion receptors DSCAM and DSCAML1 [91]. Both are substrates for γ-secretase and their ICDs move to the nucleus where they are able to stimulate transcription of target genes involved in neuronal differentiation, apoptosis and synapse formation. In primary murine neurons expression of the ICDs impaired neurite growth and synapse numbers [91].

Neurexins (NRX) and neuroligins (NGLN) constitute two protein families and are central interaction partners in synapse development [92]. They are trans-synaptic cell adhesion proteins that may undergo shedding, for example by BACE1 and ADAM10 [93-95], or by MMP9 [96]. All three neurexin family members as well as two out of five neuroligin homologues are further subjected to γ-secretase cleavage. Activity dependent processing by sheddases and γ-secretase was proposed to control synaptic autoregulation [97].

NGL-3, a recently identified γ-secretase substrate, is also subjected to proteolytic processing at synapses, where shedding and γ-secretase cleavage are stimulated with induction of long-term depression. These cleavages were suggested to regulate synaptic adhesion [98].

5.3. Additional substrates and functions of γ-secretase

Candidate approaches and systematic screenings identified a number of receptor tyrosine kinases (RTKs) as substrates for γ-secretase and demonstrated that their cleavage may provide a new signalling mechanism for these receptor tyrosine kinases, controlling cell proliferation [99], but also chemoresistance in non-small-cell lung cancer cells, through cleavage of Axl [100].

The oncogenic role is not limited to RTKs. CD147, also known as Basigin, is cleaved by γ-secretase, and the generated ICD induces Notch1 transcription in hepatocellular carcinoma (HCC). The role of γ-secretase cleavage was also tested in HCC xenographs, where γ-secretase inhibitor treatment together with Basigin antibody improved outcome compared to antibody-only treatment [101]. Trop2 is a cell surface protein, overexpressed in many epithelial cells. Upon γ-secretase cleavage, Trop2-ICD acts through β-catenin signalling and promotes proliferation [102].

Additional γ-secretase substrates were identified, such as seizure protein 6 (SEZ6), which is genetically linked to schizophrenia, autism and AD [103, 104], as well as the TNFR superfamily member DR6, which acts as a death receptor but also in peripheral myelination [105, 106], and CACHD1 [107], that modulates voltage-gated calcium channel activity [108, 109]. While functions were identified for the full-length forms or the shed ectodomains of the above proteins, it is not yet clear whether the generated ICDs have a signalling function or are simply further degraded.

Taken together, for at least 61 of the 149 identified γ-secretase substrates there is some or good evidence for a functional consequence of their cleavage by γ-secretase (Table 1). It is well possible that functions will also be discovered in the future for some of the remaining substrates.

6. Conclusion and Outlook

The function of a protease is determined by its substrates. For γ-secretase the number of its substrates is continuously increasing, reaching currently 149, and it is likely that this number will increase further. A fundamental question that arises is whether – in principle – γ-secretase is able to cleave most or even all type I membrane proteins, provided that their ectodomain length is short enough or is shed and that the CTF is found in the same place where γ-secretase is active in cells. At present little is known about non-substrates, i.e. type I membrane proteins that are proven to not be cleaved by γ-secretase. One such protein is integrin β1 [48]. Although it does not appear to be a substrate for γ-secretase, integrin β1 still binds γ-secretase as shown by immunoprecipitation. Another example is ICAM-5, also known as telencephalin. PS1 interacts with ICAM-5 [110], but does not cleave it. Instead, PS1 mediates the turnover of the ICAM-5 through the autophagy pathway, independent of the catalytic activity of PS1 [111].

What exactly prevents the cleavage of the non-substrates is not known, but mutations within a substrate's transmembrane domain can affect the cleavage efficiency as discussed above. Thus, we consider the possibility that γ-secretase may accept most type I membrane proteins as potential substrates, but that the cleavage rates may differ significantly, for example based on the specific transmembrane domain sequence or even sequences outside of the transmembrane domain. As a result, some proteins may be cleaved with such slow rates that their cleavage by γ-secretase is not meaningful in quantitative terms for physiological processes in the cell. This would qualify such a protein effectively as a non-

substrate. In this context, it is important to note that γ-secretase itself has slow cleavage kinetics, as was shown for the two well-characterized substrates APP and Notch [30, 112]. Alternatively, more real non-substrates may be identified in the future, which would not be cleaved even upon prolonged cleavage reaction times. Such non-substrates would be ideal to compare their sequences and features to the ones of the real substrates and thereby better understand which features are essential to make a type I membrane protein a substrate for γ-secretase. A detailed understanding would also help to develop safer, substrate-preferring γ-secretase inhibitors, for example for targeting specifically the cleavage of APP in AD, but not of other γ-secretase substrates, such as Notch [17].

Another open question for future studies relates to the functional consequence of γ-secretase cleavage. It will be interesting to see whether more ICDs and short, secreted peptides have a physiological function or not. It will also be important to understand which of the many γ-secretase substrates identified in vitro are also functionally relevant in vivo.

An additional topic for further investigation is the substrate specificity of the up to six individual γ-secretase complexes. It will be important to see whether γ-secretase complexes with different PS or APH-1 isoforms have a generally different or overlapping substrate spectrum. If the spectrum is different, the distinct complexes may be targeted individually for the development of substrate-preferring γ-secretase inhibitors as it is now tested for T cell acute lymphoblastic leukemia [69].

Taken together, γ-secretase is an unusual, but fascinating protease that is able to cleave at least 149 substrates within their transmembrane domains. 21 years after the discovery of the PS subunit of γ-secretase an enormous amount of insight into the function and cleavage mechanism of γ-secretase has been gathered, and the enzyme is a major drug target for AD and Notch-dependent leukemias. However, as this review demonstrates, much more still needs to be understood about the substrates, their recruitment and cleavage by γ-secretase. This will allow a better mechanistic understanding of the function of this multi-purpose enzyme in embryonic development and adult tissue homeostasis, and may pave the way towards a targeted development of substrate-selective and, thus, safer inhibitors of γ-secretase.

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References

[1] M.S. Brown, J. Ye, R.B. Rawson, J.L. Goldstein, Regulated Intramembrane Proteolysis, Cell 100(4) (2000) 391-398.
[2] S.F. Lichtenthaler, C. Haass, H. Steiner, Regulated intramembrane proteolysis--lessons from amyloid precursor protein processing, J Neurochem 117(5) (2011) 779-96.
[3] N. Kühnle, V. Dederer, M.K. Lemberg, Intramembrane proteolysis at a glance: from signalling to protein degradation, Journal of Cell Science 132(16) (2019) jcs217745.
[4] A. Medoro, S. Bartollino, D. Mignogna, D. Passarella, C. Porcile, A. Pagano, T. Florio, M. Nizzari, G. Guerra, R. Di Marco, M. Intrieri, G. Raimo, C. Russo, Complexity and Selectivity of γ-Secretase

Cleavage on Multiple Substrates: Consequences in Alzheimer's Disease and Cancer, J Alzheimers Dis 61(1) (2018) 1-15.
[5] D. Langosch, C. Scharnagl, H. Steiner, M.K. Lemberg, Understanding intramembrane proteolysis: from protein dynamics to reaction kinetics, Trends Biochem Sci 40(6) (2015) 318-27.
[6] M.S. Wolfe, W. Xia, B.L. Ostaszewski, T.S. Diehl, W.T. Kimberly, D.J. Selkoe, Two transmembrane aspartates in presenilin-1 required for presenilin endoproteolysis and γ-secretase activity, Nature 398(6727) (1999) 513-517.
[7] B. De Strooper, P. Saftig, K. Craessaerts, H. Vanderstichele, G. Guhde, W. Annaert, K. Von Figura, F. Van Leuven, Deficiency of presenilin-1 inhibits the normal cleavage of amyloid precursor protein, Nature 391(6665) (1998) 387-390.
[8] B. De Strooper, W. Annaert, P. Cupers, P. Saftig, K. Craessaerts, J.S. Mumm, E.H. Schroeter, V. Schrijvers, M.S. Wolfe, W.J. Ray, A. Goate, R. Kopan, A presenilin-1-dependent γ-secretase-like protease mediates release of Notch intracellular domain, Nature 398(6727) (1999) 518-522.
[9] T.E. Golde, E.H. Koo, K.M. Felsenstein, B.A. Osborne, L. Miele, γ-Secretase inhibitors and modulators, Biochimica et biophysica acta 1828(12) (2013) 2898-2907.
[10] A. Haapasalo, D.M. Kovacs, The many substrates of presenilin/γ-secretase, J Alzheimers Dis 25(1) (2011) 3-28.
[11] N. Jurisch-Yaksi, R. Sannerud, W. Annaert, A fast growing spectrum of biological functions of γ-secretase in development and disease, Biochim Biophys Acta 1828(12) (2013) 2815-27.
[12] S.S. Hébert, L. Serneels, T. Dejaegere, K. Horré, M. Dabrowski, V. Baert, W. Annaert, D. Hartmann, B. De Strooper, Coordinated and widespread expression of γ-secretase in vivo: evidence for size and molecular heterogeneity, Neurobiology of Disease 17(2) (2004) 260-272.
[13] L. Serneels, T. Dejaegere, K. Craessaerts, K. Horré, E. Jorissen, T. Tousseyn, S. Hébert, M. Coolen, G. Martens, A. Zwijsen, W. Annaert, D. Hartmann, B. De Strooper, Differential contribution of the three Aph1 genes to γ-secretase activity *in vivo*, Proceedings of the National Academy of Sciences of the United States of America 102(5) (2005) 1719-1724.
[14] K. Shirotani, D. Edbauer, S. Prokop, C. Haass, H. Steiner, Identification of Distinct γ-Secretase Complexes with Different APH-1 Variants, Journal of Biological Chemistry 279(40) (2004) 41340-41345.
[15] R. Sannerud, C. Esselens, P. Ejsmont, R. Mattera, L. Rochin, A.K. Tharkeshwar, G. De Baets, V. De Wever, R. Habets, V. Baert, W. Vermeire, C. Michiels, A.J. Groot, R. Wouters, K. Dillen, K. Vints, P. Baatsen, S. Munck, R. Derua, E. Waelkens, G.S. Basi, M. Mercken, M. Vooijs, M. Bollen, J. Schymkowitz, F. Rousseau, J.S. Bonifacino, G. Van Niel, B. De Strooper, W. Annaert, Restricted Location of PSEN2/γ-Secretase Determines Substrate Specificity and Generates an Intracellular Abeta Pool, Cell 166(1) (2016) 193-208.
[16] X. Meckler, F. Checler, Presenilin 1 and Presenilin 2 Target γ-Secretase Complexes to Distinct Cellular Compartments, J Biol Chem 291(24) (2016) 12821-37.
[17] L. Serneels, J. Van Biervliet, K. Craessaerts, T. Dejaegere, K. Horré, T. Van Houtvin, H. Esselmann, S. Paul, M.K. Schäfer, O. Berezovska, B.T. Hyman, B. Sprangers, R. Sciot, L. Moons, M. Jucker, Z. Yang, P.C. May, E. Karran, J. Wiltfang, R. D'Hooge, B. De Strooper, γ-Secretase Heterogeneity in the Aph1 Subunit: Relevance for Alzheimer's Disease, Science 324(5927) (2009) 639-642.
[18] S. Jayadev, A. Case, A.J. Eastman, H. Nguyen, J. Pollak, J.C. Wiley, T. Moller, R.S. Morrison, G.A. Garden, Presenilin 2 is the predominant γ-secretase in microglia and modulates cytokine release, PLoS One 5(12) (2010) e15743.
[19] G. Thinakaran, D.R. Borchelt, M.K. Lee, H.H. Slunt, L. Spitzer, G. Kim, T. Ratovitsky, F. Davenport, C. Nordstedt, M. Seeger, J. Hardy, A.I. Levey, S.E. Gandy, N.A. Jenkins, N.G. Copeland, D.L. Price, S.S. Sisodia, Endoproteolysis of presenilin 1 and accumulation of processed derivatives in vivo, Neuron 17(1) (1996) 181-90.
[20] D. Edbauer, E. Winkler, J.T. Regula, B. Pesold, H. Steiner, C. Haass, Reconstitution of γ-secretase activity, Nat Cell Biol 5(5) (2003) 486-8.

[21] A. Peric, W. Annaert, Early etiology of Alzheimer's disease: tipping the balance toward autophagy or endosomal dysfunction?, Acta Neuropathol 129(3) (2015) 363-81.
[22] S.P. Duggan, J.V. McCarthy, Beyond γ-secretase activity: The multifunctional nature of presenilins in cell signalling pathways, Cell Signal 28(1) (2016) 1-11.
[23] P.C. Fraering, W. Ye, J.M. Strub, G. Dolios, M.J. LaVoie, B.L. Ostaszewski, A. van Dorsselaer, R. Wang, D.J. Selkoe, M.S. Wolfe, Purification and characterization of the human γ-secretase complex, Biochemistry 43(30) (2004) 9774-89.
[24] T. Sato, T.S. Diehl, S. Narayanan, S. Funamoto, Y. Ihara, B. De Strooper, H. Steiner, C. Haass, M.S. Wolfe, Active γ-secretase complexes contain only one of each component, J Biol Chem 282(47) (2007) 33985-93.
[25] E. Winkler, S. Hobson, A. Fukumori, B. Dumpelfeld, T. Luebbers, K. Baumann, C. Haass, C. Hopf, H. Steiner, Purification, pharmacological modulation, and biochemical characterization of interactors of endogenous human γ-secretase, Biochemistry 48(6) (2009) 1183-97.
[26] X.C. Bai, C. Yan, G. Yang, P. Lu, D. Ma, L. Sun, R. Zhou, S.H.W. Scheres, Y. Shi, An atomic structure of human γ-secretase, Nature 525(7568) (2015) 212-217.
[27] G. Yang, R. Zhou, Q. Zhou, X. Guo, C. Yan, M. Ke, J. Lei, Y. Shi, Structural basis of Notch recognition by human γ-secretase, Nature 565(7738) (2019) 192-197.
[28] R. Zhou, G. Yang, X. Guo, Q. Zhou, J. Lei, Y. Shi, Recognition of the amyloid precursor protein by human γ-secretase, Science 363(6428) (2019).
[29] D. Petit, M. Hitzenberger, S. Lismont, K.M. Zoltowska, N.S. Ryan, M. Mercken, F. Bischoff, M. Zacharias, L. Chavez-Gutierrez, Extracellular interface between APP and Nicastrin regulates Abeta length and response to γ-secretase modulators, EMBO J (2019).
[30] D.M. Bolduc, D.R. Montagna, Y. Gu, D.J. Selkoe, M.S. Wolfe, Nicastrin functions to sterically hinder γ-secretase-substrate interactions driven by substrate transmembrane domain, Proc Natl Acad Sci U S A 113(5) (2016) E509-18.
[31] G. Struhl, A. Adachi, Requirements for Presenilin-Dependent Cleavage of Notch and Other Transmembrane Proteins, Molecular Cell 6(3) (2000) 625-636.
[32] S.F. Lichtenthaler, M.K. Lemberg, R. Fluhrer, Proteolytic ectodomain shedding of membrane proteins in mammals-hardware, concepts, and recent developments, EMBO J 37(15) (2018).
[33] L. Schauenburg, F. Liebsch, M. Eravci, M.C. Mayer, C. Weise, G. Multhaup, APLP1 is endoproteolytically cleaved by γ-secretase without previous ectodomain shedding, Sci Rep 8(1) (2018) 1916.
[34] M.H. Scheinfeld, E. Ghersi, K. Laky, B.J. Fowlkes, L. D'Adamio, Processing of beta-amyloid precursor-like protein-1 and -2 by γ-secretase regulates transcription, J Biol Chem 277(46) (2002) 44195-201.
[35] S. Hogl, P.-H. Kuhn, A. Colombo, S.F. Lichtenthaler, Determination of the proteolytic cleavage sites of the amyloid precursor-like protein 2 by the proteases ADAM10, BACE1 and γ-secretase, PloS one 6(6) (2011) e21337-e21337.
[36] M. Takami, Y. Nagashima, Y. Sano, S. Ishihara, M. Morishima-Kawashima, S. Funamoto, Y. Ihara, γ-Secretase: successive tripeptide and tetrapeptide release from the transmembrane domain of beta-carboxyl terminal fragment, J Neurosci 29(41) (2009) 13042-52.
[37] M. Szaruga, S. Veugelen, M. Benurwar, S. Lismont, D. Sepulveda-Falla, A. Lleo, N.S. Ryan, T. Lashley, N.C. Fox, S. Murayama, H. Gijsen, B. De Strooper, L. Chavez-Gutierrez, Qualitative changes in human γ-secretase underlie familial Alzheimer's disease, J Exp Med 212(12) (2015) 2003-13.
[38] H. Steiner, A. Fukumori, S. Tagami, M. Okochi, Making the final cut: pathogenic amyloid-beta peptide generation by γ-secretase, Cell Stress 2(11) (2018) 292-310.
[39] M. Szaruga, B. Munteanu, S. Lismont, S. Veugelen, K. Horre, M. Mercken, T.C. Saido, N.S. Ryan, T. De Vos, S.N. Savvides, R. Gallardo, J. Schymkowitz, F. Rousseau, N.C. Fox, C. Hopf, B. De Strooper, L. Chavez-Gutierrez, Alzheimer's-Causing Mutations Shift Abeta Length by Destabilizing γ-Secretase-Aβn Interactions, Cell 170(3) (2017) 443-456 e14.

[40] M. Okochi, S. Tagami, K. Yanagida, M. Takami, T.S. Kodama, K. Mori, T. Nakayama, Y. Ihara, M. Takeda, γ-Secretase modulators and presenilin 1 mutants act differently on presenilin/γ-secretase function to cleave Aβ42 and Aβ43, Cell Rep 3(1) (2013) 42-51.
[41] S.F. Lichtenthaler, R. Wang, H. Grimm, S.N. Uljon, C.L. Masters, K. Beyreuther, Mechanism of the cleavage specificity of Alzheimer's disease γ-secretase identified by phenylalanine-scanning mutagenesis of the transmembrane domain of the amyloid precursor protein, Proc Natl Acad Sci U S A 96(6) (1999) 3053-8.
[42] S.F. Lichtenthaler, D. Beher, H.S. Grimm, R. Wang, M.S. Shearman, C.L. Masters, K. Beyreuther, The intramembrane cleavage site of the amyloid precursor protein depends on the length of its transmembrane domain, Proc Natl Acad Sci U S A 99(3) (2002) 1365-70.
[43] S.F. Lichtenthaler, N. Ida, G. Multhaup, C.L. Masters, K. Beyreuther, Mutations in the transmembrane domain of APP altering γ-secretase specificity, Biochemistry 36(49) (1997) 15396-403.
[44] E. Tischer, B. Cordell, Beta-amyloid precursor protein. Location of transmembrane domain and specificity of γ-secretase cleavage, J Biol Chem 271(36) (1996) 21914-9.
[45] D.M. Bolduc, D.R. Montagna, M.C. Seghers, M.S. Wolfe, D.J. Selkoe, The amyloid-beta forming tripeptide cleavage mechanism of γ-secretase, Elife 5 (2016).
[46] M.P. Murphy, L.J. Hickman, C.B. Eckman, S.N. Uljon, R. Wang, T.E. Golde, γ-Secretase, evidence for multiple proteolytic activities and influence of membrane positioning of substrate on generation of amyloid beta peptides of varying length, J Biol Chem 274(17) (1999) 11914-23.
[47] T. Tomita, Molecular mechanism of intramembrane proteolysis by γ-secretase, J Biochem 156(4) (2014) 195-201.
[48] M.L. Hemming, J.E. Elias, S.P. Gygi, D.J. Selkoe, Proteomic Profiling of γ-Secretase Substrates and Mapping of Substrate Requirements, PLOS Biology 6(10) (2008) e257.
[49] D. Edbauer, M. Willem, S. Lammich, H. Steiner, C. Haass, Insulin-degrading enzyme rapidly removes the beta-amyloid precursor protein intracellular domain (AICD), J Biol Chem 277(16) (2002) 13389-93.
[50] Y. Araki, N. Miyagi, N. Kato, T. Yoshida, S. Wada, M. Nishimura, H. Komano, T. Yamamoto, B. De Strooper, K. Yamamoto, T. Suzuki, Coordinated metabolism of Alcadein and amyloid beta-protein precursor regulates FE65-dependent gene transactivation, J Biol Chem 279(23) (2004) 24343-54.
[51] C. Bohm, N.M. Seibel, B. Henkel, H. Steiner, C. Haass, W. Hampe, SorLA signaling by regulated intramembrane proteolysis, J Biol Chem 281(21) (2006) 14547-53.
[52] Y.M. Li, M.T. Lai, M. Xu, Q. Huang, J. DiMuzio-Mower, M.K. Sardana, X.P. Shi, K.C. Yin, J.A. Shafer, S.J. Gardell, Presenilin 1 is linked with γ-secretase activity in the detergent solubilized state, Proc Natl Acad Sci U S A 97(11) (2000) 6138-43.
[53] I. Khan, S. Krishnaswamy, M. Sabale, D. Groth, L. Wijaya, M. Morici, I. Berger, C. Schaffitzel, P.E. Fraser, R.N. Martins, G. Verdile, Efficient production of a mature and functional γ-secretase protease, Sci Rep 8(1) (2018) 12834.
[54] T. Moehlmann, E. Winkler, X. Xia, D. Edbauer, J. Murrell, A. Capell, C. Kaether, H. Zheng, B. Ghetti, C. Haass, H. Steiner, Presenilin-1 mutations of leucine 166 equally affect the generation of the Notch and APP intracellular domains independent of their effect on Abeta 42 production, Proc Natl Acad Sci U S A 99(12) (2002) 8025-30.
[55] M. Sastre, H. Steiner, K. Fuchs, A. Capell, G. Multhaup, M.M. Condron, D.B. Teplow, C. Haass, Presenilin-dependent γ-secretase processing of beta-amyloid precursor protein at a site corresponding to the S3 cleavage of Notch, EMBO Rep 2(9) (2001) 835-41.
[56] I. Pinnix, U. Musunuru, H. Tun, A. Sridharan, T. Golde, C. Eckman, C. Ziani-Cherif, L. Onstead, K. Sambamurti, A novel γ-secretase assay based on detection of the putative C-terminal fragment-γ of amyloid beta protein precursor, J Biol Chem 276(1) (2001) 481-7.
[57] S. Tagami, K. Yanagida, T.S. Kodama, M. Takami, N. Mizuta, H. Oyama, K. Nishitomi, Y.W. Chiu, T. Okamoto, T. Ikeuchi, G. Sakaguchi, T. Kudo, Y. Matsuura, A. Fukumori, M. Takeda, Y. Ihara, M. Okochi, Semagacestat Is a Pseudo-Inhibitor of γ-Secretase, Cell Rep 21(1) (2017) 259-273.

[58] S.A. Laurent, F.S. Hoffmann, P.H. Kuhn, Q. Cheng, Y. Chu, M. Schmidt-Supprian, S.M. Hauck, E. Schuh, M. Krumbholz, H. Rubsamen, J. Wanngren, M. Khademi, T. Olsson, T. Alexander, F. Hiepe, H.W. Pfister, F. Weber, D. Jenne, H. Wekerle, R. Hohlfeld, S.F. Lichtenthaler, E. Meinl, γ-Secretase directly sheds the survival receptor BCMA from plasma cells, Nat Commun 6 (2015) 7333.
[59] D. Fleck, M. Voss, B. Brankatschk, C. Giudici, H. Hampel, B. Schwenk, D. Edbauer, A. Fukumori, H. Steiner, E. Kremmer, M. Haug-Kroper, M.J. Rossner, R. Fluhrer, M. Willem, C. Haass, Proteolytic Processing of Neuregulin 1 Type III by Three Intramembrane-cleaving Proteases, J Biol Chem 291(1) (2016) 318-33.
[60] S. Nakahara, T. Saito, N. Kondo, K. Moriwaki, K. Noda, S. Ihara, M. Takahashi, Y. Ide, J. Gu, H. Inohara, T. Katayama, M. Tohyama, T. Kubo, N. Taniguchi, E. Miyoshi, A secreted type of beta1,6 N-acetylglucosaminyltransferase V (GnT-V), a novel angiogenesis inducer, is regulated by γ-secretase, FASEB J 20(14) (2006) 2451-9.
[61] M. Voss, U. Kunzel, F. Higel, P.H. Kuhn, A. Colombo, A. Fukumori, M. Haug-Kroper, B. Klier, G. Grammer, A. Seidl, B. Schroder, R. Obst, H. Steiner, S.F. Lichtenthaler, C. Haass, R. Fluhrer, Shedding of glycan-modifying enzymes by signal peptide peptidase-like 3 (SPPL3) regulates cellular N-glycosylation, EMBO J 33(24) (2014) 2890-905.
[62] P.H. Kuhn, M. Voss, M. Haug-Kroper, B. Schroder, U. Schepers, S. Brase, C. Haass, S.F. Lichtenthaler, R. Fluhrer, Secretome analysis identifies novel signal Peptide peptidase-like 3 (Sppl3) substrates and reveals a role of Sppl3 in multiple Golgi glycosylation pathways, Mol Cell Proteomics 14(6) (2015) 1584-98.
[63] A.J. Beel, C.R. Sanders, Substrate specificity of γ-secretase and other intramembrane proteases, Cell Mol Life Sci 65(9) (2008) 1311-34.
[64] E.H. Schroeter, J.A. Kisslinger, R. Kopan, Notch-1 signalling requires ligand-induced proteolytic release of intracellular domain, Nature 393(6683) (1998) 382-6.
[65] C. Brou, F. Logeat, N. Gupta, C. Bessia, O. LeBail, J.R. Doedens, A. Cumano, P. Roux, R.A. Black, A. Israel, A novel proteolytic cleavage involved in Notch signaling: the role of the disintegrin-metalloprotease TACE, Mol Cell 5(2) (2000) 207-16.
[66] D. Hartmann, B. de Strooper, L. Serneels, K. Craessaerts, A. Herreman, W. Annaert, L. Umans, T. Lubke, A. Lena Illert, K. von Figura, P. Saftig, The disintegrin/metalloprotease ADAM 10 is essential for Notch signalling but not for alpha-secretase activity in fibroblasts, Hum Mol Genet 11(21) (2002) 2615-24.
[67] S. Artavanis-Tsakonas, M.D. Rand, R.J. Lake, Notch signaling: cell fate control and signal integration in development, Science 284(5415) (1999) 770-6.
[68] M. Shih Ie, T.L. Wang, Notch signaling, γ-secretase inhibitors, and cancer therapy, Cancer Res 67(5) (2007) 1879-82.
[69] R.A. Habets, C.E. de Bock, L. Serneels, I. Lodewijckx, D. Verbeke, D. Nittner, R. Narlawar, S. Demeyer, J. Dooley, A. Liston, T. Taghon, J. Cools, B. de Strooper, Safe targeting of T cell acute lymphoblastic leukemia by pathology-specific NOTCH inhibition, Science Translational Medicine 11(494) (2019) eaau6246.
[70] E.R. Andersson, U. Lendahl, Therapeutic modulation of Notch signalling--are we there yet?, Nat Rev Drug Discov 13(5) (2014) 357-78.
[71] V.W. Chow, M.P. Mattson, P.C. Wong, M. Gleichmann, An overview of APP processing enzymes and products, Neuromolecular Med 12(1) (2010) 1-12.
[72] B. De Strooper, Lessons from a failed γ-secretase Alzheimer trial, Cell 159(4) (2014) 721-6.
[73] D.B. Henley, K.L. Sundell, G. Sethuraman, S.A. Dowsett, P.C. May, Safety profile of semagacestat, a γ-secretase inhibitor: IDENTITY trial findings, Current Medical Research and Opinion 30(10) (2014) 2021-2032.
[74] M.L. Gosztyla, H.M. Brothers, S.R. Robinson, Alzheimer's Amyloid-β is an Antimicrobial Peptide: A Review of the Evidence, J Alzheimers Dis 62(4) (2018) 1495-1506.
[75] J.E. Morley, S.A. Farr, The role of amyloid-beta in the regulation of memory, Biochemical pharmacology 88(4) (2014) 479-85.

[76] R. Kopan, M.X.G. Ilagan, γ-Secretase: proteasome of the membrane?, Nature Reviews Molecular Cell Biology 5(6) (2004) 499-504.
[77] M.J. Pont, T. Hill, G.O. Cole, J.J. Abbott, J. Kelliher, A.I. Salter, M. Hudecek, M.L. Comstock, A. Rajan, B.K.R. Patel, J.M. Voutsinas, Q. Wu, L. Liu, A.J. Cowan, B.L. Wood, D.J. Green, S.R. Riddell, γ-Secretase inhibition increases efficacy of BCMA-specific chimeric antigen receptor T cells in multiple myeloma, Blood 134(19) (2019) 1585-1597.
[78] J.D. Ulrich, T.K. Ulland, M. Colonna, D.M. Holtzman, Elucidating the Role of TREM2 in Alzheimer's Disease, Neuron 94(2) (2017) 237-248.
[79] D. Feuerbach, P. Schindler, C. Barske, S. Joller, E. Beng-Louka, K.A. Worringer, S. Kommineni, A. Kaykas, D.J. Ho, C. Ye, K. Welzenbach, G. Elain, L. Klein, I. Brzak, A.K. Mir, C.J. Farady, R. Aichholz, S. Popp, N. George, U. Neumann, ADAM17 is the main sheddase for the generation of human triggering receptor expressed in myeloid cells (hTREM2) ectodomain and cleaves TREM2 after Histidine 157, Neuroscience Letters 660 (2017) 109-114.
[80] K. Schlepckow, G. Kleinberger, A. Fukumori, R. Feederle, S.F. Lichtenthaler, H. Steiner, C. Haass, An Alzheimer-associated TREM2 variant occurs at the ADAM cleavage site and affects shedding and phagocytic function, EMBO Molecular Medicine 9(10) (2017) 1356-1365.
[81] P. Thornton, J. Sevalle, M.J. Deery, G. Fraser, Y. Zhou, S. Ståhl, E.H. Franssen, R.B. Dodd, S. Qamar, B. Gomez Perez-Nievas, L.S. Nicol, S. Eketjäll, J. Revell, C. Jones, A. Billinton, P.H. St George-Hyslop, I. Chessell, D.C. Crowther, TREM2 shedding by cleavage at the H157-S158 bond is accelerated for the Alzheimer's disease-associated H157Y variant, EMBO Molecular Medicine 9(10) (2017) 1366-1378.
[82] G. Kleinberger, Y. Yamanishi, M. Suárez-Calvet, E. Czirr, E. Lohmann, E. Cuyvers, H. Struyfs, N. Pettkus, A. Wenninger-Weinzierl, F. Mazaheri, S. Tahirovic, A. Lleó, D. Alcolea, J. Fortea, M. Willem, S. Lammich, J.L. Molinuevo, R. Sánchez-Valle, A. Antonell, A. Ramirez, M.T. Heneka, K. Sleegers, J. van der Zee, J.-J. Martin, S. Engelborghs, A. Demirtas-Tatlidede, H. Zetterberg, C. Van Broeckhoven, H. Gurvit, T. Wyss-Coray, J. Hardy, M. Colonna, C. Haass, TREM2 mutations implicated in neurodegeneration impair cell surface transport and phagocytosis, Science Translational Medicine 6(243) (2014) 243ra86-243ra86.
[83] P. Wunderlich, K. Glebov, N. Kemmerling, N.T. Tien, H. Neumann, J. Walter, Sequential proteolytic processing of the triggering receptor expressed on myeloid cells-2 (TREM2) protein by ectodomain shedding and γ-secretase-dependent intramembranous cleavage, J Biol Chem 288(46) (2013) 33027-36.
[84] L. Zhong, X.F. Chen, Z.L. Zhang, Z. Wang, X.Z. Shi, K. Xu, Y.W. Zhang, H. Xu, G. Bu, DAP12 Stabilizes the C-terminal Fragment of the Triggering Receptor Expressed on Myeloid Cells-2 (TREM2) and Protects against LPS-induced Pro-inflammatory Response, J Biol Chem 290(25) (2015) 15866-77.
[85] K. Glebov, P. Wunderlich, I. Karaca, J. Walter, Functional involvement of γ-secretase in signaling of the triggering receptor expressed on myeloid cells-2 (TREM2), J Neuroinflammation 13 (2016) 17.
[86] F. Zunke, S. Rose-John, The shedding protease ADAM17: Physiology and pathophysiology, Biochimica et Biophysica Acta (BBA) - Molecular Cell Research 1864(11, Part B) (2017) 2059-2070.
[87] J. Fritsch, P. Zingler, V. Sarchen, A.L. Heck, S. Schutze, Role of ubiquitination and proteolysis in the regulation of pro- and anti-apoptotic TNF-R1 signaling, Biochim Biophys Acta Mol Cell Res 1864(11 Pt B) (2017) 2138-2146.
[88] A.T. Ting, M.J.M. Bertrand, More to Life than NF-kappaB in TNFR1 Signaling, Trends Immunol 37(8) (2016) 535-545.
[89] J. Chhibber-Goel, C. Coleman-Vaughan, V. Agrawal, N. Sawhney, E. Hickey, J.C. Powell, J.V. McCarthy, γ-Secretase Activity Is Required for Regulated Intramembrane Proteolysis of Tumor Necrosis Factor (TNF) Receptor 1 and TNF-mediated Pro-apoptotic Signaling, J Biol Chem 291(11) (2016) 5971-85.
[90] S. Barao, A. Gartner, E. Leyva-Diaz, G. Demyanenko, S. Munck, T. Vanhoutvin, L. Zhou, M. Schachner, G. Lopez-Bendito, P.F. Maness, B. De Strooper, Antagonistic Effects of BACE1 and APH1B-

γ-Secretase Control Axonal Guidance by Regulating Growth Cone Collapse, Cell Rep 12(9) (2015) 1367-76.
[91] S.M. Sachse, S. Lievens, L.F. Ribeiro, D. Dascenco, D. Masschaele, K. Horre, A. Misbaer, N. Vanderroost, A.S. De Smet, E. Salta, M.L. Erfurth, Y. Kise, S. Nebel, W. Van Delm, S. Plaisance, J. Tavernier, B. De Strooper, J. De Wit, D. Schmucker, Nuclear import of the DSCAM-cytoplasmic domain drives signaling capable of inhibiting synapse formation, EMBO J 38(6) (2019).
[92] A.M. Craig, Y. Kang, Neurexin-neuroligin signaling in synapse development, Curr Opin Neurobiol 17(1) (2007) 43-52.
[93] P.H. Kuhn, A.V. Colombo, B. Schusser, D. Dreymueller, S. Wetzel, U. Schepers, J. Herber, A. Ludwig, E. Kremmer, D. Montag, U. Muller, M. Schweizer, P. Saftig, S. Brase, S.F. Lichtenthaler, Systematic substrate identification indicates a central role for the metalloprotease ADAM10 in axon targeting and synapse function, Elife 5 (2016).
[94] P.H. Kuhn, K. Koroniak, S. Hogl, A. Colombo, U. Zeitschel, M. Willem, C. Volbracht, U. Schepers, A. Imhof, A. Hoffmeister, C. Haass, S. Rossner, S. Brase, S.F. Lichtenthaler, Secretome protein enrichment identifies physiological BACE1 protease substrates in neurons, EMBO J 31(14) (2012) 3157-68.
[95] K. Suzuki, Y. Hayashi, S. Nakahara, H. Kumazaki, J. Prox, K. Horiuchi, M. Zeng, S. Tanimura, Y. Nishiyama, S. Osawa, A. Sehara-Fujisawa, P. Saftig, S. Yokoshima, T. Fukuyama, N. Matsuki, R. Koyama, T. Tomita, T. Iwatsubo, Activity-dependent proteolytic cleavage of neuroligin-1, Neuron 76(2) (2012) 410-22.
[96] R.T. Peixoto, P.A. Kunz, H. Kwon, A.M. Mabb, B.L. Sabatini, B.D. Philpot, M.D. Ehlers, Transsynaptic signaling by activity-dependent cleavage of neuroligin-1, Neuron 76(2) (2012) 396-409.
[97] S. Restituito, L. Khatri, I. Ninan, P.M. Mathews, X. Liu, R.J. Weinberg, E.B. Ziff, Synaptic autoregulation by metalloproteases and γ-secretase, J Neurosci 31(34) (2011) 12083-93.
[98] H. Lee, E.J. Lee, Y.S. Song, E. Kim, Long-term depression-inducing stimuli promote cleavage of the synaptic adhesion molecule NGL-3 through NMDA receptors, matrix metalloproteinases and presenilin/γ-secretase, Philos Trans R Soc Lond B Biol Sci 369(1633) (2014) 20130158.
[99] J.A.M. Merilahti, V.K. Ojala, A.M. Knittle, A.T. Pulliainen, K. Elenius, Genome-wide screen of γ-secretase-mediated intramembrane cleavage of receptor tyrosine kinases, Mol Biol Cell 28(22) (2017) 3123-3131.
[100] Y. Lu, J. Wan, Z. Yang, X. Lei, Q. Niu, L. Jiang, W.M. Passtoors, A. Zang, P.C. Fraering, F. Wu, Regulated intramembrane proteolysis of the AXL receptor kinase generates an intracellular domain that localizes in the nucleus of cancer cells, FASEB J 31(4) (2017) 1382-1397.
[101] Y.L. Yong, R.Y. Zhang, Z.K. Liu, D. Wei, Y.K. Shang, J. Wu, Z.Y. Zhang, C. Li, Z.N. Chen, H. Bian, γ-Secretase complex-dependent intramembrane proteolysis of CD147 regulates the Notch1 signaling pathway in hepatocellular carcinoma, J Pathol (2019).
[102] T. Stoyanova, A.S. Goldstein, H. Cai, J.M. Drake, J. Huang, O.N. Witte, Regulated proteolysis of Trop2 drives epithelial hyperplasia and stem cell self-renewal via beta-catenin signaling, Genes Dev 26(20) (2012) 2271-85.
[103] M. Pigoni, J. Wanngren, P.H. Kuhn, K.M. Munro, J.M. Gunnersen, H. Takeshima, R. Feederle, I. Voytyuk, B. De Strooper, M.D. Levasseur, B.J. Hrupka, S.A. Muller, S.F. Lichtenthaler, Seizure protein 6 and its homolog seizure 6-like protein are physiological substrates of BACE1 in neurons, Mol Neurodegener 11(1) (2016) 67.
[104] A. Ambalavanan, S.L. Girard, K. Ahn, S. Zhou, A. Dionne-Laporte, D. Spiegelman, C.V. Bourassa, J. Gauthier, F.F. Hamdan, L. Xiong, P.A. Dion, R. Joober, J. Rapoport, G.A. Rouleau, De novo variants in sporadic cases of childhood onset schizophrenia, Eur J Hum Genet 24(6) (2016) 944-948.
[105] S. Mi, X. Lee, Y. Hu, B. Ji, Z. Shao, W. Yang, G. Huang, L. Walus, K. Rhodes, B.J. Gong, R.H. Miller, R.B. Pepinsky, Death receptor 6 negatively regulates oligodendrocyte survival, maturation and myelination, Nat Med 17(7) (2011) 816-21.

[106] A. Colombo, H.E. Hsia, M. Wang, P.H. Kuhn, M.S. Brill, P. Canevazzi, R. Feederle, C. Taveggia, T. Misgeld, S.F. Lichtenthaler, Non-cell-autonomous function of DR6 in Schwann cell proliferation, EMBO J 37(7) (2018).
[107] J. Rudan Njavro, J. Klotz, B. Dislich, J. Wanngren, M.D. Shmueli, J. Herber, P.H. Kuhn, R. Kumar, T. Koeglsperger, M. Conrad, W. Wurst, R. Feederle, A. Vlachos, S. Michalakis, P. Jedlicka, S.A. Muller, S.F. Lichtenthaler, Mouse brain proteomics establishes MDGA1 and CACHD1 as in vivo substrates of the Alzheimer protease BACE1, FASEB J 34(2) (2020) 2465-2482.
[108] S. Dahimene, K.M. Page, I. Kadurin, L. Ferron, D.Y. Ho, G.T. Powell, W.S. Pratt, S.W. Wilson, A.C. Dolphin, The α2δ-like Protein Cachd1 Increases N-type Calcium Currents and Cell Surface Expression and Competes with α2δ-1, Cell Reports 25(6) (2018) 1610-1621.e5.
[109] G.S. Cottrell, C.H. Soubrane, J.A. Hounshell, H. Lin, V. Owenson, M. Rigby, P.J. Cox, B.S. Barker, M. Ottolini, S. Ince, C.C. Bauer, E. Perez-Reyes, M.K. Patel, E.B. Stevens, G.J. Stephens, CACHD1 is an α2δ-Like Protein That Modulates Ca_V3 Voltage-Gated Calcium Channel Activity, The Journal of Neuroscience 38(43) (2018) 9186-9201.
[110] W.G. Annaert, C. Esselens, V. Baert, C. Boeve, G. Snellings, P. Cupers, K. Craessaerts, B. De Strooper, Interaction with telencephalin and the amyloid precursor protein predicts a ring structure for presenilins, Neuron 32(4) (2001) 579-89.
[111] C. Esselens, V. Oorschot, V. Baert, T. Raemaekers, K. Spittaels, L. Serneels, H. Zheng, P. Saftig, B. De Strooper, J. Klumperman, W. Annaert, Presenilin 1 mediates the turnover of telencephalin in hippocampal neurons via an autophagic degradative pathway, J Cell Biol 166(7) (2004) 1041-54.
[112] F. Kamp, E. Winkler, J. Trambauer, A. Ebke, R. Fluhrer, H. Steiner, Intramembrane proteolysis of beta-amyloid precursor protein by γ-secretase is an unusually slow process, Biophys J 108(5) (2015) 1229-37.
[113] B. De Strooper, Nicastrin: gatekeeper of the γ-secretase complex, Cell 122(3) (2005) 318-20.
[114] I. Stutzer, N. Selevsek, D. Esterhazy, A. Schmidt, R. Aebersold, M. Stoffel, Systematic proteomic analysis identifies beta-site amyloid precursor protein cleaving enzyme 2 and 1 (BACE2 and BACE1) substrates in pancreatic beta-cells, J Biol Chem 288(15) (2013) 10536-47.
[115] P. May, H.H. Bock, J. Nimpf, J. Herz, Differential glycosylation regulates processing of lipoprotein receptors by γ-secretase, J Biol Chem 278(39) (2003) 37386-92.
[116] A. Stoeck, L. Shang, P.J. Dempsey, Sequential and γ-secretase-dependent processing of the betacellulin precursor generates a palmitoylated intracellular-domain fragment that inhibits cell growth, J Cell Sci 123(Pt 13) (2010) 2319-31.
[117] C.R. Blair, J.B. Stone, R.G. Wells, The type III TGF-beta receptor betaglycan transmembrane-cytoplasmic domain fragment is stable after ectodomain cleavage and is a substrate of the intramembrane protease γ-secretase, Biochim Biophys Acta 1813(2) (2011) 332-9.
[118] A.T. Schiffmacher, R. Padmanabhan, S. Jhingory, L.A. Taneyhill, Cadherin-6B is proteolytically processed during epithelial-to-mesenchymal transitions of the cranial neural crest, Mol Biol Cell 25(1) (2014) 41-54.
[119] Y. Nagara, M. Hagiyama, N. Hatano, E. Futai, S. Suo, Y. Takaoka, Y. Murakami, A. Ito, S. Ishiura, Tumor suppressor cell adhesion molecule 1 (CADM1) is cleaved by a disintegrin and metalloprotease 10 (ADAM10) and subsequently cleaved by γ-secretase complex, Biochem Biophys Res Commun 417(1) (2012) 462-7.
[120] N. Houri, K.C. Huang, J. Nalbantoglu, The Coxsackievirus and Adenovirus Receptor (CAR) undergoes ectodomain shedding and regulated intramembrane proteolysis (RIP), PLoS One 8(8) (2013) e73296.
[121] Z. Chen, A. Kapus, I. Khatri, O. Kos, F. Zhu, R.M. Gorczynski, Cell membrane-bound CD200 signals both via an extracellular domain and following nuclear translocation of a cytoplasmic fragment, Leuk Res 69 (2018) 72-80.
[122] Christian X. Andersson, J. Fernandez-Rodriguez, S. Laos, D. Baeckström, C. Haass, Gunnar C. Hansson, Shedding and γ-secretase-mediated intramembrane proteolysis of the mucin-type molecule CD43, Biochemical Journal 387(2) (2005) 377-384.

[123] D. Murakami, I. Okamoto, O. Nagano, Y. Kawano, T. Tomita, T. Iwatsubo, B. De Strooper, E. Yumoto, H. Saya, Presenilin-dependent γ-secretase activity mediates the intramembranous cleavage of CD44, Oncogene 22(10) (2003) 1511-1516.
[124] T. Bedau, F. Peters, J. Prox, P. Arnold, F. Schmidt, M. Finkernagel, S. Kollmann, R. Wichert, A. Otte, A. Ohler, M. Stirnberg, R. Lucius, T. Koudelka, A. Tholey, V. Biasin, C.U. Pietrzik, G. Kwapiszewska, C. Becker-Pauly, Ectodomain shedding of CD99 within highly conserved regions is mediated by the metalloprotease meprin beta and promotes transendothelial cell migration, FASEB J 31(3) (2017) 1226-1237.
[125] K. Wilhelmsen, P. van der Geer, Phorbol 12-myristate 13-acetate-induced release of the colony-stimulating factor 1 receptor cytoplasmic domain into the cytosol involves two separate cleavage events, Mol Cell Biol 24(1) (2004) 454-64.
[126] A. Schulte, B. Schulz, M.G. Andrzejewski, C. Hundhausen, S. Mletzko, J. Achilles, K. Reiss, K. Paliga, C. Weber, S.R. John, A. Ludwig, Sequential processing of the transmembrane chemokines CX3CL1 and CXCL16 by α- and γ-secretases, Biochem Biophys Res Commun 358(1) (2007) 233-40.
[127] Y. Taniguchi, S.H. Kim, S.S. Sisodia, Presenilin-dependent "γ-secretase" processing of deleted in colorectal cancer (DCC), J Biol Chem 278(33) (2003) 30425-8.
[128] T. Ikeuchi, S.S. Sisodia, The Notch ligands, Delta1 and Jagged2, are substrates for presenilin-dependent "γ-secretase" cleavage, J Biol Chem 278(10) (2003) 7751-4.
[129] M.J. LaVoie, D.J. Selkoe, The Notch ligands, Jagged and Delta, are sequentially processed by α-secretase and presenilin/γ-secretase and release signaling fragments, J Biol Chem 278(36) (2003) 34427-37.
[130] E. Six, D. Ndiaye, Y. Laâbi, C. Brou, N. Gupta-Rossi, A. Israël, F. Logeat, The Notch ligand Delta1 is sequentially cleaved by an ADAM protease and γ-secretase, Proceedings of the National Academy of Sciences 100(13) (2003) 7638-7643.
[131] P. Marambaud, J. Shioi, G. Serban, A. Georgakopoulos, S. Sarner, V. Nagy, L. Baki, P. Wen, S. Efthimiopoulos, Z. Shao, T. Wisniewski, N.K. Robakis, A presenilin-1/γ-secretase cleavage releases the E-cadherin intracellular domain and regulates disassembly of adherens junctions, The EMBO Journal 21(8) (2002) 1948-1956.
[132] D. Maetzel, S. Denzel, B. Mack, M. Canis, P. Went, M. Benk, C. Kieu, P. Papior, P.A. Baeuerle, M. Munz, O. Gires, Nuclear signalling by tumour-associated antigen EpCAM, Nat Cell Biol 11(2) (2009) 162-71.
[133] E. Inoue, M. Deguchi-Tawarada, A. Togawa, C. Matsui, K. Arita, S. Katahira-Tayama, T. Sato, E. Yamauchi, Y. Oda, Y. Takai, Synaptic activity prompts γ-secretase-mediated cleavage of EphA4 and dendritic spine formation, J Cell Biol 185(3) (2009) 551-64.
[134] C. Litterst, A. Georgakopoulos, J. Shioi, E. Ghersi, T. Wisniewski, R. Wang, A. Ludwig, N.K. Robakis, Ligand binding and calcium influx induce distinct ectodomain/γ-secretase-processing pathways of EphB2 receptor, J Biol Chem 282(22) (2007) 16155-63.
[135] T. Tomita, S. Tanaka, Y. Morohashi, T. Iwatsubo, Presenilin-dependent intramembrane cleavage of ephrin-B1, Mol Neurodegener 1 (2006) 2.
[136] A. Georgakopoulos, C. Litterst, E. Ghersi, L. Baki, C. Xu, G. Serban, N.K. Robakis, Metalloproteinase/Presenilin1 processing of ephrinB regulates EphB-induced Src phosphorylation and signaling, EMBO J 25(6) (2006) 1242-52.
[137] C.-Y. Ni, M.P. Murphy, T.E. Golde, G. Carpenter, γ-Secretase Cleavage and Nuclear Localization of ErbB-4 Receptor Tyrosine Kinase, Science 294(5549) (2001) 2179-2181.
[138] C.R. Degnin, M.B. Laederich, W.A. Horton, Ligand activation leads to regulated intramembrane proteolysis of fibroblast growth factor receptor 3, Mol Biol Cell 22(20) (2011) 3861-73.
[139] J.W. Cowan, X. Wang, R. Guan, K. He, J. Jiang, G. Baumann, R.A. Black, M.S. Wolfe, S.J. Frank, Growth hormone receptor is a target for presenilin-dependent γ-secretase cleavage, J Biol Chem 280(19) (2005) 19331-42.

[140] E.L. Meyer, N. Strutz, L.C. Gahring, S.W. Rogers, Glutamate receptor subunit 3 is modified by site-specific limited proteolysis including cleavage by γ-secretase, J Biol Chem 278(26) (2003) 23786-96.
[141] B.W. Carey, D.Y. Kim, D.M. Kovacs, Presenilin/γ-secretase and α-secretase-like peptidases cleave human MHC Class I proteins, Biochem J 401(1) (2007) 121-7.
[142] A.Z.M. Saleh, A.T. Fang, A.E. Arch, D. Neupane, A.E. Fiky, J.J. Krolewski, Regulated proteolysis of the IFNaR2 subunit of the interferon-alpha receptor, Oncogene 23(42) (2004) 7076-7086.
[143] B. McElroy, J.C. Powell, J.V. McCarthy, The insulin-like growth factor 1 (IGF-1) receptor is a substrate for γ-secretase-mediated intramembrane proteolysis, Biochem Biophys Res Commun 358(4) (2007) 1136-41.
[144] J. Lokau, C.M. Flynn, C. Garbers, Cleavage of the Interleukin-11 receptor induces processing of its C-terminal fragments by the γ-secretase and the proteasome, Biochem Biophys Res Commun 491(2) (2017) 296-302.
[145] B.M. Elzinga, C. Twomey, J.C. Powell, F. Harte, J.V. McCarthy, Interleukin-1 receptor type 1 is a substrate for γ-secretase-dependent regulated intramembrane proteolysis, J Biol Chem 284(3) (2009) 1394-409.
[146] P.H. Kuhn, E. Marjaux, A. Imhof, B. De Strooper, C. Haass, S.F. Lichtenthaler, Regulated intramembrane proteolysis of the interleukin-1 receptor II by α-, β-, and γ-secretase, J Biol Chem 282(16) (2007) 11982-95.
[147] A. Chalaris, J. Gewiese, K. Paliga, L. Fleig, A. Schneede, K. Krieger, S. Rose-John, J. Scheller, ADAM17-mediated shedding of the IL6R induces cleavage of the membrane stub by γ-secretase, Biochim Biophys Acta 1803(2) (2010) 234-45.
[148] K. Kasuga, H. Kaneko, M. Nishizawa, O. Onodera, T. Ikeuchi, Generation of intracellular domain of insulin receptor tyrosine kinase by γ-secretase, Biochem Biophys Res Commun 360(1) (2007) 90-6.
[149] M. Niwa, C. Sidrauski, R.J. Kaufman, P. Walter, A Role for Presenilin-1 in Nuclear Accumulation of Ire1 Fragments and Induction of the Mammalian Unfolded Protein Response, Cell 99(7) (1999) 691-702.
[150] C.C. Sachse, Y.H. Kim, M. Agsten, T. Huth, C. Alzheimer, D.M. Kovacs, D.Y. Kim, BACE1 and presenilin/γ-secretase regulate proteolytic processing of KCNE1 and 2, auxiliary subunits of voltage-gated potassium channels, FASEB J 27(6) (2013) 2458-67.
[151] L. Bloch, O. Sineshchekova, D. Reichenbach, K. Reiss, P. Saftig, M. Kuro-o, C. Kaether, Klotho is a substrate for α-, β- and γ-secretase, FEBS Letters 583(19) (2009) 3221-3224.
[152] T. Maretzky, M. Schulte, A. Ludwig, S. Rose-John, C. Blobel, D. Hartmann, P. Altevogt, P. Saftig, K. Reiss, L1 is sequentially processed by two differently activated metalloproteases and presenilin/γ-secretase and regulates neural cell adhesion, cell migration, and neurite outgrowth, Mol Cell Biol 25(20) (2005) 9040-53.
[153] A. Haapasalo, D.Y. Kim, B.W. Carey, M.K. Turunen, W.H. Pettingell, D.M. Kovacs, Presenilin/γ-secretase-mediated cleavage regulates association of leukocyte-common antigen-related (LAR) receptor tyrosine phosphatase with beta-catenin, J Biol Chem 282(12) (2007) 9063-72.
[154] P. May, Y.K. Reddy, J. Herz, Proteolytic processing of low density lipoprotein receptor-related protein mediates regulated release of its intracellular domain, J Biol Chem 277(21) (2002) 18736-43.
[155] C.X. Liu, S. Ranganathan, S. Robinson, D.K. Strickland, γ-Secretase-mediated release of the low density lipoprotein receptor-related protein 1B intracellular domain suppresses anchorage-independent growth of neuroglioma cells, J Biol Chem 282(10) (2007) 7504-11.
[156] K. Mi, G.V. Johnson, Regulated proteolytic processing of LRP6 results in release of its intracellular domain, J Neurochem 101(2) (2007) 517-29.
[157] Z. Zou, B. Chung, T. Nguyen, S. Mentone, B. Thomson, D. Biemesderfer, Linking receptor-mediated endocytosis and cell signaling: evidence for regulated intramembrane proteolysis of megalin in proximal tubule, J Biol Chem 279(33) (2004) 34302-10.

[158] B. Foveau, F. Ancot, C. Leroy, A. Petrelli, K. Reiss, V. Vingtdeux, S. Giordano, V. Fafeur, D. Tulasne, Down-regulation of the met receptor tyrosine kinase by presenilin-dependent regulated intramembrane proteolysis, Mol Biol Cell 20(9) (2009) 2495-507.
[159] J. Julian, N. Dharmaraj, D.D. Carson, MUC1 is a substrate for γ-secretase, J Cell Biochem 108(4) (2009) 802-15.
[160] P. Marambaud, P.H. Wen, A. Dutt, J. Shioi, A. Takashima, R. Siman, N.K. Robakis, A CBP binding transcriptional repressor produced by the PS1/ε-cleavage of N-cadherin is inhibited by PS1 FAD mutations, Cell 114(5) (2003) 635-45.
[161] D.Y. Kim, L.A. Ingano, D.M. Kovacs, Nectin-1alpha, an immunoglobulin-like receptor involved in the formation of synapses, is a substrate for presenilin/γ-secretase-like cleavage, J Biol Chem 277(51) (2002) 49976-81.
[162] J. Kim, A. Chang, A. Dudak, H.J. Federoff, S.T. Lim, Characterization of nectin processing mediated by presenilin-dependent γ-secretase, J Neurochem 119(5) (2011) 945-56.
[163] D. Goldschneider, N. Rama, C. Guix, P. Mehlen, The neogenin intracellular domain regulates gene transcription via nuclear translocation, Mol Cell Biol 28(12) (2008) 4068-79.
[164] J. Bao, D. Wolpowitz, L.W. Role, D.A. Talmage, Back signaling by the Nrg-1 intracellular domain, J Cell Biol 161(6) (2003) 1133-41.
[165] M. Czarnek, J. Bereta, Proteolytic Processing of Neuregulin 2, Mol Neurobiol (2019).
[166] C.A. Saura, E. Servian-Morilla, F.G. Scholl, Presenilin/γ-secretase regulates neurexin processing at synapses, PLoS One 6(4) (2011) e19430.
[167] N. Bot, C. Schweizer, S. Ben Halima, P.C. Fraering, Processing of the synaptic cell adhesion molecule neurexin-3beta by Alzheimer disease α- and γ-secretases, J Biol Chem 286(4) (2011) 2762-73.
[168] D. Sakry, A. Neitz, J. Singh, R. Frischknecht, D. Marongiu, F. Biname, S.S. Perera, K. Endres, B. Lutz, K. Radyushkin, J. Trotter, T. Mittmann, Oligodendrocyte precursor cells modulate the neuronal network by activity-dependent ectodomain cleavage of glial NG2, PLoS Biol 12(11) (2014) e1001993.
[169] J. Akter, A. Takatori, M.S. Islam, A. Nakazawa, T. Ozaki, H. Nagase, A. Nakagawara, Intracellular fragment of NLRR3 (NLRR3-ICD) stimulates ATRA-dependent neuroblastoma differentiation, Biochem Biophys Res Commun 453(1) (2014) 86-93.
[170] M.T. Saxena, E.H. Schroeter, J.S. Mumm, R. Kopan, Murine notch homologs (N1-4) undergo presenilin-dependent proteolysis, J Biol Chem 276(43) (2001) 40268-73.
[171] K. Gowrishankar, M.G. Zeidler, C. Vincenz, Release of a membrane-bound death domain by γ-secretase processing of the p75NTR homolog NRADD, J Cell Sci 117(Pt 18) (2004) 4099-111.
[172] K.C. Kanning, M. Hudson, P.S. Amieux, J.C. Wiley, M. Bothwell, L.C. Schecterson, Proteolytic Processing of the p75 Neurotrophin Receptor and Two Homologs Generates C-Terminal Fragments with Signaling Capability, The Journal of Neuroscience 23(13) (2003) 5425-5436.
[173] C. Rajagopal, K.L. Stone, R.E. Mains, B.A. Eipper, Secretion stimulates intramembrane proteolysis of a secretory granule membrane enzyme, J Biol Chem 285(45) (2010) 34632-42.
[174] S. Biswas, M. Adrian, J. Weber, K. Evdokimov, M. Winkler, C. Geraud, Posttranslational proteolytic processing of Leda-1/Pianp involves cleavage by MMPs, ADAM10/17 and γ-secretase, Biochem Biophys Res Commun 477(4) (2016) 661-666.
[175] M.P. Kummer, H. Maruyama, C. Huelsmann, S. Baches, S. Weggen, E.H. Koo, Formation of Pmel17 amyloid is regulated by juxtamembrane metalloproteinase cleavage, and the resulting C-terminal fragment is a substrate for γ-secretase, J Biol Chem 284(4) (2009) 2296-306.
[176] M.M. Yurrita, B. Fernandez-Munoz, G. Del Castillo, E. Martin-Villar, J. Renart, M. Quintanilla, Podoplanin is a substrate of presenilin-1/γ-secretase, Int J Biochem Cell Biol 46 (2014) 68-75.
[177] D. Merrick, H. Chapin, J.E. Baggs, Z. Yu, S. Somlo, Z. Sun, J.B. Hogenesch, M.J. Caplan, The γ-secretase cleavage product of polycystin-1 regulates TCF and CHOP-mediated transcriptional activation through a p300-dependent mechanism, Dev Cell 22(1) (2012) 197-210.

[178] J.Y. Kaimori, Y. Nagasawa, L.F. Menezes, M.A. Garcia-Gonzalez, J. Deng, E. Imai, L.F. Onuchic, L.M. Guay-Woodford, G.G. Germino, Polyductin undergoes notch-like processing and regulated release from primary cilia, Hum Mol Genet 16(8) (2007) 942-56.
[179] M.S. Garcia-Ayllon, M.L. Campanari, M.F. Montenegro, I. Cuchillo-Ibanez, O. Belbin, A. Lleo, K. Tsim, C.J. Vidal, J. Saez-Valero, Presenilin-1 influences processing of the acetylcholinesterase membrane anchor PRiMA, Neurobiol Aging 35(7) (2014) 1526-36.
[180] S. Bouillot, E. Tillet, G. Carmona, M.H. Prandini, A.S. Gauchez, P. Hoffmann, N. Alfaidy, F. Cand, P. Huber, Protocadherin-12 cleavage is a regulated process mediated by ADAM10 protein: evidence of shedding up-regulation in pre-eclampsia, J Biol Chem 286(17) (2011) 15195-204.
[181] S. Bonn, P.H. Seeburg, M.K. Schwarz, Combinatorial expression of α- and γ-protocadherins alters their presenilin-dependent processing, Mol Cell Biol 27(11) (2007) 4121-32.
[182] I.G. Haas, M. Frank, N. Veron, R. Kemler, Presenilin-dependent processing and nuclear function of γ-protocadherins, J Biol Chem 280(10) (2005) 9313-9.
[183] Y. Watanabe, H. Nakamura, Nuclear translocation of intracellular domain of Protogenin by proteolytic cleavage, Dev Growth Differ 54(2) (2012) 167-76.
[184] H.W. Na, W.S. Shin, A. Ludwig, S.T. Lee, The cytosolic domain of protein-tyrosine kinase 7 (PTK7), generated from sequential cleavage by a disintegrin and metalloprotease 17 (ADAM17) and γ-secretase, enhances cell proliferation and migration in colon cancer cells, J Biol Chem 287(30) (2012) 25001-9.
[185] J.P. Chow, A. Fujikawa, H. Shimizu, R. Suzuki, M. Noda, Metalloproteinase- and γ-secretase-mediated cleavage of protein-tyrosine phosphatase receptor type Z, J Biol Chem 283(45) (2008) 30879-89.
[186] A. Galichet, M. Weibel, C.W. Heizmann, Calcium-regulated intramembrane proteolysis of the RAGE receptor, Biochem Biophys Res Commun 370(1) (2008) 1-5.
[187] M. Seki, A. Watanabe, S. Enomoto, T. Kawamura, H. Ito, T. Kodama, T. Hamakubo, H. Aburatani, Human ROBO1 is cleaved by metalloproteinases and γ-secretase and migrates to the nucleus in cancer cells, FEBS Lett 584(13) (2010) 2909-15.
[188] L. Anders, P. Mertins, S. Lammich, M. Murgia, D. Hartmann, P. Saftig, C. Haass, A. Ullrich, Furin-, ADAM 10-, and γ-secretase-mediated cleavage of a receptor tyrosine phosphatase and regulation of beta-catenin's transcriptional activity, Mol Cell Biol 26(10) (2006) 3917-34.
[189] J.D. Londino, D. Gulick, J.S. Isenberg, R.K. Mallampalli, Cleavage of Signal Regulatory Protein alpha (SIRPalpha) Enhances Inflammatory Signaling, J Biol Chem 290(52) (2015) 31113-25.
[190] A.C. Nyborg, T.B. Ladd, C.W. Zwizinski, J.J. Lah, T.E. Golde, Sortilin, SorCS1b, and SorLA Vps10p sorting receptors, are novel γ-secretase substrates, Mol Neurodegener 1 (2006) 3.
[191] G. Hermey, S.S. Sjogaard, C.M. Petersen, A. Nykjaer, J. Gliemann, Tumour necrosis factor alpha-converting enzyme mediates ectodomain shedding of Vps10p-domain receptor family members, Biochem J 395(2) (2006) 285-93.
[192] J.G. Schulz, W. Annaert, J. Vandekerckhove, P. Zimmermann, B. De Strooper, G. David, Syndecan 3 intramembrane proteolysis is presenilin/γ-secretase-dependent and modulates cytosolic signaling, J Biol Chem 278(49) (2003) 48651-7.
[193] M.B. Marron, H. Singh, T.A. Tahir, J. Kavumkal, H.Z. Kim, G.Y. Koh, N.P. Brindle, Regulated proteolytic processing of Tie1 modulates ligand responsiveness of the receptor-tyrosine kinase Tie2, J Biol Chem 282(42) (2007) 30509-17.
[194] G.S. Tejeda, S. Ayuso-Dolado, R. Arbeteta, G.M. Esteban-Ortega, O.G. Vidaurre, M. Diaz-Guerra, Brain ischaemia induces shedding of a BDNF-scavenger ectodomain from TrkB receptors by excitotoxicity activation of metalloproteinases and γ-secretases, J Pathol 238(5) (2016) 627-40.
[195] N. Ali, V. Knauper, Phorbol ester-induced shedding of the prostate cancer marker transmembrane protein with epidermal growth factor and two follistatin motifs 2 is mediated by the disintegrin and metalloproteinase-17, J Biol Chem 282(52) (2007) 37378-88.
[196] R. Wang, P. Tang, P. Wang, R.E. Boissy, H. Zheng, Regulation of tyrosinase trafficking and processing by presenilins: partial loss of function by familial Alzheimer's disease mutation,

Proceedings of the National Academy of Sciences of the United States of America 103(2) (2006) 353-358.
[197] B. Schulz, J. Pruessmeyer, T. Maretzky, A. Ludwig, C.P. Blobel, P. Saftig, K. Reiss, ADAM10 regulates endothelial permeability and T-Cell transmigration by proteolysis of vascular endothelial cadherin, Circ Res 102(10) (2008) 1192-1201.
[198] J. Cai, W.G. Jiang, M.B. Grant, M. Boulton, Pigment epithelium-derived factor inhibits angiogenesis via regulated intracellular proteolysis of vascular endothelial growth factor receptor 1, J Biol Chem 281(6) (2006) 3604-13.
[199] H.K. Wong, T. Sakurai, F. Oyama, K. Kaneko, K. Wada, H. Miyazaki, M. Kurosawa, B. De Strooper, P. Saftig, N. Nukina, β Subunits of voltage-gated sodium channels are novel substrates of beta-site amyloid precursor protein-cleaving enzyme (BACE1) and γ-secretase, J Biol Chem 280(24) (2005) 23009-17.
[200] D.Y. Kim, L.A. Ingano, B.W. Carey, W.H. Pettingell, D.M. Kovacs, Presenilin/γ-secretase-mediated cleavage of the voltage-gated sodium channel beta2-subunit regulates cell adhesion and migration, J Biol Chem 280(24) (2005) 23251-61.
[201] H.S. Hoe, G.W. Rebeck, Regulation of ApoE receptor proteolysis by ligand binding, Brain Res Mol Brain Res 137(1-2) (2005) 31-9.
[202] Y. Piao, A. Kimura, S. Urano, Y. Saito, H. Taru, T. Yamamoto, S. Hata, T. Suzuki, Mechanism of intramembrane cleavage of alcadeins by γ-secretase, PLoS One 8(4) (2013) e62431.
[203] K. Yanagida, M. Okochi, S. Tagami, T. Nakayama, T.S. Kodama, K. Nishitomi, J. Jiang, K. Mori, S. Tatsumi, T. Arai, T. Ikeuchi, K. Kasuga, T. Tokuda, M. Kondo, M. Ikeda, K. Deguchi, H. Kazui, T. Tanaka, T. Morihara, R. Hashimoto, T. Kudo, H. Steiner, C. Haass, K. Tsuchiya, H. Akiyama, R. Kuwano, M. Takeda, The 28-amino acid form of an APLP1-derived Aβ-like peptide is a surrogate marker for Aβ42 production in the central nervous system, EMBO Mol Med 1(4) (2009) 223-35.
[204] Y. Gu, H. Misonou, T. Sato, N. Dohmae, K. Takio, Y. Ihara, Distinct intramembrane cleavage of the beta-amyloid precursor protein family resembling γ-secretase-like cleavage of Notch, The Journal of biological chemistry 276(38) (2001) 35235-35238.
[205] C.L. Masters, G. Simms, N.A. Weinman, G. Multhaup, B.L. McDonald, K. Beyreuther, Amyloid plaque core protein in Alzheimer disease and Down syndrome, Proceedings of the National Academy of Sciences 82(12) (1985) 4245-4249.
[206] A. Weidemann, S. Eggert, F.B.M. Reinhard, M. Vogel, K. Paliga, G. Baier, C.L. Masters, K. Beyreuther, G. Evin, A Novel ε-Cleavage within the Transmembrane Domain of the Alzheimer Amyloid Precursor Protein Demonstrates Homology with Notch Processing, Biochemistry 41(8) (2002) 2825-2835.
[207] S. Lammich, M. Okochi, M. Takeda, C. Kaether, A. Capell, A.K. Zimmer, D. Edbauer, J. Walter, H. Steiner, C. Haass, Presenilin-dependent intramembrane proteolysis of CD44 leads to the liberation of its intracellular domain and the secretion of an Abeta-like peptide, J Biol Chem 277(47) (2002) 44754-9.
[208] I. Okamoto , Y. Kawano , D. Murakami , T. Sasayama , N. Araki , T. Miki , A.J. Wong , H. Saya Proteolytic release of CD44 intracellular domain and its role in the CD44 signaling pathway, Journal of Cell Biology 155(5) (2001) 755-762.
[209] A. Vahidi, G. Glenn, P. van der Geer, Identification and mutagenesis of the TACE and γ-secretase cleavage sites in the colony-stimulating factor 1 receptor, Biochem Biophys Res Commun 450(1) (2014) 782-7.
[210] T. Tsaktanis, H. Kremling, M. Pavsic, R. von Stackelberg, B. Mack, A. Fukumori, H. Steiner, F. Vielmuth, V. Spindler, Z. Huang, J. Jakubowski, N.H. Stoecklein, E. Luxenburger, K. Lauber, B. Lenarcic, O. Gires, Cleavage and cell adhesion properties of human epithelial cell adhesion molecule (HEPCAM), J Biol Chem 290(40) (2015) 24574-91.
[211] X. Wang, J.W. Cowan, M. Gerhart, B.R. Zelickson, J. Jiang, K. He, M.S. Wolfe, R.A. Black, S.J. Frank, γ-Secretase-mediated growth hormone receptor proteolysis: mapping of the intramembranous cleavage site, Biochem Biophys Res Commun 408(3) (2011) 432-6.

[212] M. Okochi, H. Steiner, A. Fukumori, H. Tanii, T. Tomita, T. Tanaka, T. Iwatsubo, T. Kudo, M. Takeda, C. Haass, Presenilins mediate a dual intramembranous γ-secretase cleavage of Notch-1, Embo j 21(20) (2002) 5408-16.
[213] Y. Ran, F. Hossain, A. Pannuti, C.B. Lessard, G.Z. Ladd, J.I. Jung, L.M. Minter, B.A. Osborne, L. Miele, T.E. Golde, γ-Secretase inhibitors in cancer clinical trials are pharmacologically and functionally distinct, EMBO Mol Med 9(7) (2017) 950-966.
[214] K.M. Jung, S. Tan, N. Landman, K. Petrova, S. Murray, R. Lewis, P.K. Kim, D.S. Kim, S.H. Ryu, M.V. Chao, T.W. Kim, Regulated intramembrane proteolysis of the p75 neurotrophin receptor modulates its association with the TrkA receptor, J Biol Chem 278(43) (2003) 42161-9.

Figures and legends

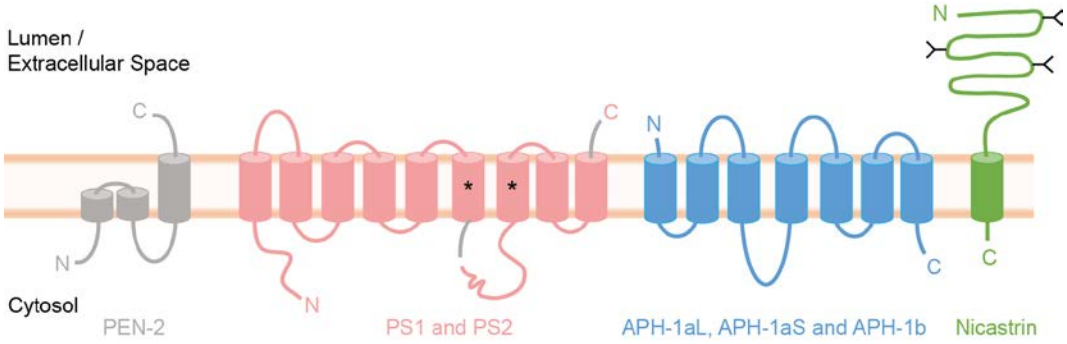

Figure 1: The four subunits of γ-secretase. Presenilins (PS1, PS2) undergo endoproteolytic cleavage within the loop between transmembrane domains 6 and 7, which results in proteolytic activity of γ-secretase towards its substrates [6, 19, 20]. Human PS1 and PS2 have 65% identity on the amino acid level, with most variability within the loop regions. APH-1aL differs from Aph-1aS in the C-terminal tail, and human APH-1b is 57% identical with human APH-1aL. Nicastrin has a large, glycosylated extracellular domain [30, 113]. Asterisks indicate the catalytic aspartic acid residues.

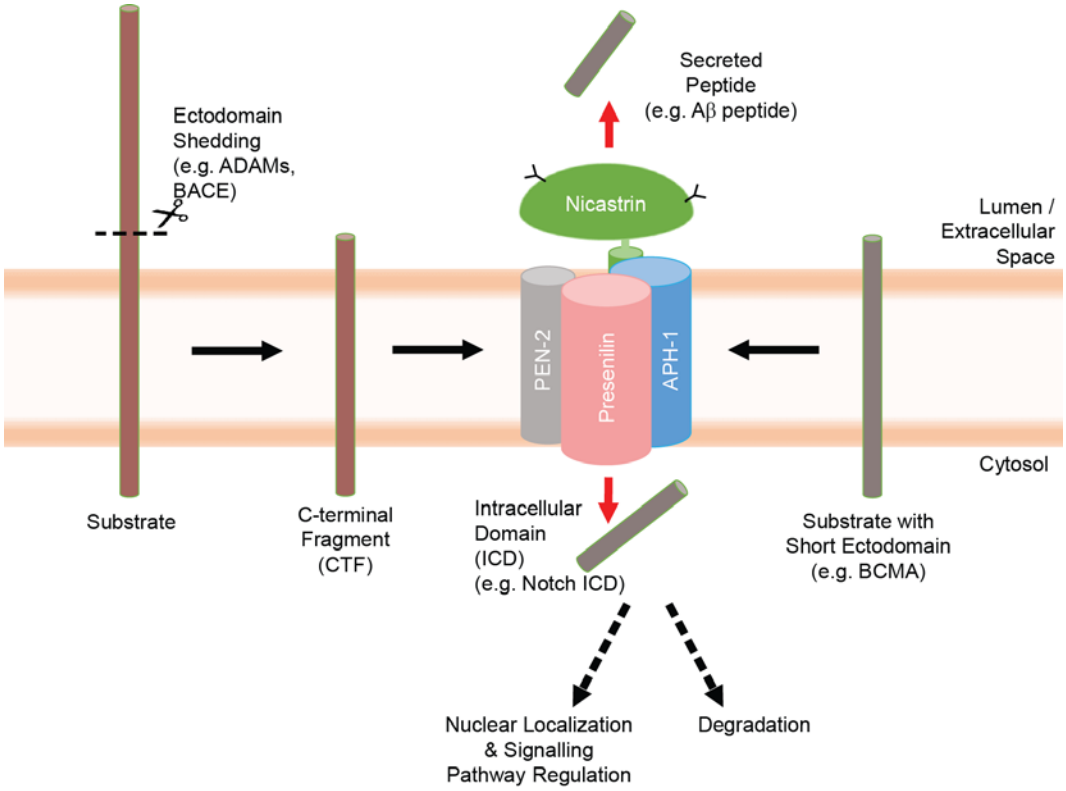

Figure 2: γ-Secretase substrate recognition and cleavage. γ-Secretase forms a hetero-tetrameric, horseshoe-shaped complex with one copy of each subunit. The subunits are mostly buried within the membrane. An exception is Nicastrin. It has a large extracellular domain that acts as a gatekeeper which only allows proteins with short ectodomains to be transferred to the active site [30] and provides a mechanism to anchor the substrates in the active site [29]. While the majority of substrates undergo ectodomain shedding to fit under the nicastrin lid-like structure, a substrate with a naturally short ectodomain may be directly cleaved by γ-secretase without prior shedding [58]. The γ-secretase cleavage releases the intracellular domain (ICD) to the cytosol and a short secreted peptide to the extracellular space.

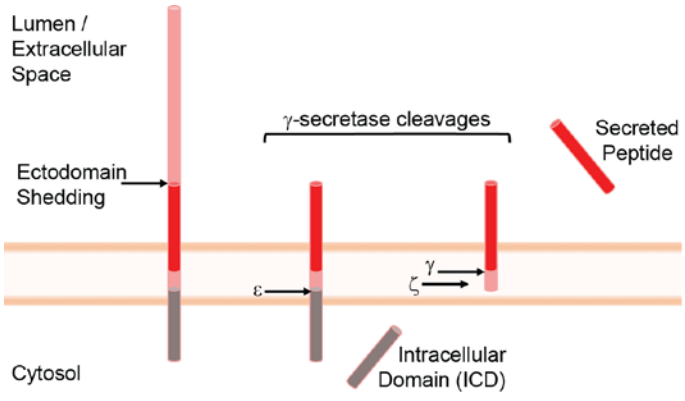

Figure 3: Processivity of γ-secretase. After the ectodomain cleavage, the CTF fragment is recruited to the catalytic site of γ-secretase. The initial cleavage at the ε-site, causes the release of ICD. The remaining membrane bound part is then subjected to cleavages at the ζ-sites and continues to be processed, until the final cleavage at the γ-site takes place, which leads to release of an N-terminal peptide to the extracellular space.

Table 1: List of γ-secretase substrates. The table comprises membrane proteins listed in a previous review in 2011 [10] plus the results of a Pubmed search for the years 2010 until 2019 for the keywords "gamma secretase AND (substrate OR shedding OR cleavage)". The information is collected from the papers that made the initial identification of the corresponding substrate. Proteins are sorted alphabetically by their name. Be aware that some proteins may have multiple names and, thus, may be at a different position in the table than expected. For several substrates the cleavage by γ-secretase was subsequently further characterized, for example with regard to the cleavage mechanism, the functional consequences or its in vivo relevance. These additional publications and their content are not listed in order to not overload the table. In the detected product column, if no experimental method (e.g. MEM or LUC) or condition (e.g. dnPS1 or PS1 KO) is indicated next to the detected product, then the detection was made in the presence of γ-secretase inhibitors (GSIs). Use of GSI is only indicated when it has been applied together with some other method or condition. In this column, if two terms are connected with 'and', the information in the following parenthesis is applied for both [e.g. CTF and ICD (PS dKO, GSI) shows both CTF and ICD detected in PS dKO cells and in presence of GSI]. In the proposed function column, if it is stated as 'ICD by itself …', this shows that the ICD fragment was expressed separately and the observation was done independent of γ-secretase cleavage. In the "Tissue/cell line" column, information about whether the endogenous protein being studied or it has been exogenously overexpressed (including the method) has been indicated within the parentheses. The following abbreviations are used in the table. #: Cleavage has shown in vivo. **: Type I transmembrane protein. ***: Type III transmembrane protein. ****: Type IV membrane protein. CTF: C-terminal fragment. dKO: double knock-out. dnPS1: Dominant negative presenilin 1. E.: Endogenous protein. FL: Full-length. GSI: γ-Secretase inhibitor. ICD: Intracellular domain. IF: Immunofluorescence. IP: Immunoprecipitation. LUC: Luciferase reporter. MEM: Isolated membrane incubation assay. OE: Overexpression. Sec.Pep.: Short, secreted peptide. sECD: Soluble ectodomain. sT.: Stable transfection. TD: Transduction. tT. : Transient transfection

Protein name	Organism	Detected Product	Tissue/cell line (Overexpres-sion / Endogenous)	Proposed function of cleavage	Ref.
ACP2	Human (P11117)	CTF	MIN6 (tT)	-	[114]
Alcadein α (Calsyntenin-1)	Human (O94985)	CTF, Sec.Pep., ICD (MEM)	HEK293 (tT)	ICD by itself locate to nucleus and regulates gene activation	[50]
		Sec.Pep.	MEF (tT)		
	Mouse (Q9EPL2)	CTF (PS1/2 KO)	MEF (E)		
		CTF, ICD	Brain (E)		
Alcadein β (Calsyntenin-3)	Human (Q9BQT9)	ICD (MEM)	HEK293 (tT)	-	[50]
Alcadein γ (Calsyntenin-2)	Human (Q9H4D0)	CTF, ICD (MEM)	HEK293 (tT)	-	[50]
APLP1	Mouse (Q03157)	CTF	N2a (E)	-	[34]
		CTF (PS1	Brain (E)		

		KO)			
	Human (P51693)	ICD (LUC), ICD	HEK293 (tT)		
		ICD (LUC)	N2a (tT)		
	Human (P51693)	sECD	HEK293, SH-SY5Y (tT)	-	[33]
		sECD (IP)	SH-SY5Y (E)		
APLP2	Human (Q06481)	CTF	HEK293 (E)	-	[34]
		ICD (LUC), ICD	HEK293 (tT)		
		ICD (LUC)	N2a (tT)		
	Mouse (Q06335)	CTF	N2a (E)		
		CTF (PS1 KO)	Brain (E)		
apoER2	Mouse (Q924X6)	CTF	Primary neurons (E)	-	[115]
		CTF, ICD (LUC)	CHO (tT)		
APP	Mouse (P12023)	CTF, Aβ	Primary neurons (E)	-	[7]
	Human (P05067)	CTF, Aβ	Primary neurons (TD)		
AXL	Human (P30530)	CTF, ICD	H1299, A549, AsPC-1, Panc-1, Panc-28, LN-18, LN-229 (E), HEK293T (tT, TD)	ICD by itself locates to nucleus, growth promotion (OE in 3T3 cells), ICD translocation to nucleus (A431 cells)	[100]
		CTF	HeLa, SGC7901, AGS, MDA-MB-231, HCT116 (E)		
		CTF (PS dKO)	MEF (TD)		
		Activity assay	Cell-free (Purified protein)		
		CTF	MCF-7 (tT)		[99]
	Mouse (Q00993)	CTF (PS dKO, GSI)	MEF (E)		[100]
BCMA[#,*]	Human (Q02223)	FL on surface (PS 1/2 KO, GSI), sBCMA	B cells, PBMC (E), MEF (TD), HeLa (tT)	Released sBCMA acts as a decoy receptor	[58]
	Mouse (O88472)	FL on surface	Imm. splenocytes, CD138+ plasma cells, spleen and bone		

			marrow plasma cells (In vivo)		
Betacellulin	Human (P35070)	CTF, ICD	IMPE (TD)	ICD locates to nucleus, inhibits cell growth	[116]
		CTF	A431 (E)		
		ICD (PS dKO)	MEF (TD)		
Betaglycan	Rat (P26342)	CTF	COS7 (tT)	TGF-β2 signalling regulation	[117]
	Human (Q03167)	CTF	HepG2 (E)		
	Hamster (A0A3L7HB54)	CTF	CHO (E)		
	Mouse (O88393)	CTF	NIH-3T3 (E)		
CACHD1	Mouse (Q6PDJ1)	CTF	Primary neurons (E)	-	[107]
Cadherin-6B (Cad6B)	Chicken (Q90762)	CTF	CHO (tT)	-	[118]
CADM1	Mouse (Q8R5M8)	CTF, ICD	COS7 (sT)	-	[119]
		CTF (PS1/2 KO, NCT KO)	MEF (E)		
CAR	Human (P78310)	CTF, ICD	U87 (TD)	ICD by itself locates to nucleus	[120]
		ICD (PS1/2 KO)	MEF (TD)		
CD147 (Basigin)	Human (P35613)	ICD	SMMC-7721 (tT)	Nuclear translocation of ICD, regulates Notch1 expression. CD147 Ab + GSI application treated human HCC - Mouse xenografts.	[101]
CD200	Human (P41217)	ICD	Patient CLL (E)	ICD by itself locates to nucleus and regulate TF expression	[121]
CD43 (Leukosialin)	Human (P16150)	CTF	COLO 205, K562, Jurkat (E), MCF-7, SW80 (sT)	-	[122]
		CTF (PS1 KO, GSI)	HEK293 (tT)		
CD44	Human (P16070)	CTF, ICD	U251MG (E)	-	[123]
		CTF, ICD (PS1 KO)	MEF (tT)		
CD99	Human (P14209)	CTF	HeLa (tT)	-	[124]
CSF-1R	Mouse (P09581)	CTF (dnPS1)	HEK293 (tT)	-	[125]

		CTF	P388D1 (E)		
CX3CL1 (Fractalkine)	Human (P78423)	CTF	HEK293 (tT)	-	[126]
CXCL16	Human (Q9H2A7)	CTF, ICD	HEK293 (tT)	-	[126]
		CTF	MEF (tT)		
DCC	Rat (Q63155)	ICD (LUC), ICD	HEK293 (tT)	-	[127]
		CTF (dnPS1), ICD	N2a (tT)		
	Mouse (P70211)	CTF (PS1 KO)	Spinal cord (E)		
Delta1	Mouse (Q61483)	CTF (dnPS1, GSI), ICD (dnPS1, GSI, IP)	N2a (sT)	-	[128]
		ICD (LUC)	HEK293 (tT)		
	Rat (P97677)	CTF (dnPS1)	CHO (tT)		[129]
	Murine	CTF, ICD	MEF (TD)		[130]
Desmoglein-2	Human (Q14126)	CTF, ICD	HEK293 (sT)	-	[48]
DNER	Human (Q8NFT8)	CTF (dnPS1)	MEF (sT)	-	[48]
		CTF, ICD	HEK293 (sT)		
DR6	Human (O75509)	CTF	HEK293 (tT)	-	[106]
DSCAM	Mouse (Q9ERC8)	CTF, ICD (LUC, WB)	HEK293 (sT)	ICD locates to nucleus, regulates transcription in neurons	[91]
		ICD (IF)	SH-SY5Y (tT)		
		ICD (IF)	Primary hippocampal neurons (TF)		
DSCAML1	Mouse (Q4VA61)	ICD (LUC)	HEK293 (sT)	-	[91]
Dystroglycan	Human (Q14118)	CTF (dnPS1)	MEF (sT)	-	[48]
		CTF, ICD	HEK293 (sT)		
		CTF	HeLa (sT)		
E-Cadherin	Mouse (P09803)	CTF (PS dKO)	Embryo (E)	ICD binds to β-catenin. Promoting disassembly of adherens junctions	[131]
	Human (P12830)	CTF (PS1 KO, GSI)	MEF (sT, tT)		
		CTF, ICD	A431, HEK239 (E)		

EpCAM[#]	Human (P16422)	ICD (IP)	HCT-8 (E)	ICD locates to nucleus, promotes proliferation. EpCAM-ICD expressing HEK293 graft to SCID mice cause tumor. Cleavage is PS2 dependent.	[132]
		ICD (IP, IF)	FaDu (E)		
		CTF, ICD (IP)	HEK293 (sT)		
		ICD (IF)	Colon carcinoma (Human tissue)		
EphA2	Human (P29317)	CTF	MCF-7 (tT)	-	[99]
EphA4	Rat (D3ZZK3)	CTF (PS dKO, GSI), ICD (MEM)	HEK293 (tT)	ICD promotes dendritic spine formation	[133]
		CTF	Primary hippocampal neurons (E)		
EphA5	Human (P54756)	CTF	MCF-7 (tT)	-	[99]
EphA7	Human (Q15375)	CTF	MCF-7 (tT)	-	[99]
EphB2	Mouse (P54763)	CTF (PS1 KO)	Brain (E)	-	[134]
		CTF (PS1 KO), ICD (MEM)	MEF (tT)		
		CTF, ICD	HEK293T (TD)		
		CTF and ICD (MEM, GSI)	N2a (TD)		
EphB2	Rat (B2B9B0)	CTF	Primary cortical neurons (E)		
EphB3	Human (P54753)	CTF	MCF-7 (tT)	-	[99]
EphB4	Human (P54760)	CTF	MCF-7 (tT)	-	[99]
EphB6	Human (O15197)	CTF	MCF-7 (tT)	Growth promotion (OE in 3T3 cells)	[99]
Ephrin-B1	Mouse (P52795)	CTF (PS dKO)	MEF (E)	ICD by itself locates to nucleus	[135]
		CTF	Lung (E)		
	Monkey	CTF	COS7 (E)		
	Human (P98172)	ICD (in vivo, MEM)	COS7 (tT)		
Ephrin-B2	Mouse (P52800)	CTF (PS1 KO)	Brain (E)	ICD activates Src signalling	[136]
		CTF (PS1 KO, GSI)	MEF (E)		
		CTF, ICD (dnPS1, GSI)	HEK293 (tT)		

ErbB4	Human (Q15303)	CTF, ICD	NIH-3T3 (sT)	ICD locates to nucleus	[137]
		ICD (dnPS1)	HEK293 (sT)		
F11R	Human (Q9Y624)	CTF	MIN6 (tT)	-	[114]
FGFR3	Mouse (Q61851)	ICD	HEK293 (tT)	ICD locates to nucleus	[138]
FGFR4	Human (P22455)	CTF	MCF-7 (tT)	-	[99]
GHR	Mouse (P16882)	CTF, ICD	3T3-F442A (E)	ICD locates to nucleus	[139]
	Rabbit (P19941)	CTF and ICD (PS dKO, GSI)	MEF (sT, TD)		
GLG1	Human (Q92896)	CTF	MIN6 (tT)	-	[114]
GluR3**	Mouse (Q9Z2W9)	ICD-like	HEK293 (tT)	-	[140]
HLA-A2	Human (P01892)	CTF (dnPS1, GSI), ICD (in vivo, MEM)	CHO (sT)	-	[141]
		CTF	Jurkat (E), B104 (sT)		
IFNαR2	Human (P48551)	ICD (LUC)	CHO (tT)	ICD by itself locates to nucleus and repress transcription	[142]
IGF-1R	Human (P08069)	CTF, ICD	MEF (sT)	-	[143]
IL11R	Human (Q14626)	CTF	HEK293 (tT)	-	[144]
IL-1R1	Human (P14778)	CTF and ICD (dnPS1, GSI)	HEK293T (tT)	IL-1R1 signalling regulation	[145]
		CTF and ICD (PS dKO)	MEF (tT)		
IL-1R2	Human (P27930)	CTF (dnPS1, GSI), ICD (MEM)	HEK293 (tT)	-	[146]
		CTF (PS dKO)	MEF (TD)		
IL6R	Human (P08887)	CTF	HepG2 (tT)	-	[147]
		CTF (PS dKO)	MEF (TD)		
IR	Human (P06213)	CTF (dnPS1, GSI), ICD	HEK293 (tT)	ICD by itself locates to nucleus	[148]

	Mouse (P15208)	CTF	Primary neurons (E)		
Ire1α	Human (O75460)	CTF	HeLa (tT)	Nuclear translocation	[149]
Ire1β	Human (Q76MJ5)	CTF	HeLa (tT)	Nuclear translocation	[149]
Jagged1	Rat (Q63722)	CTF (GSI), ICD (MEM)	Embryo (E)	-	[129]
		CTF (dnPS1, GSI)	CHO (tT)		
		ICD	COS7 (tT)		
Jagged2	Human (Q9Y219)	CTF and ICD (dnPS1, GSI)	NIH-3T3 (sT)	-	[128]
KCNE1	Human (P15382)	CTF, ICD	B104 (sT)	-	[150]
		CTF	HEK293 (tT)		
	Mouse (P23299)	CTF	Cardiomyocyte (E)		
KCNE2	Human (Q9Y6J6)	CTF, ICD	B104 (sT)	-	[150]
	Mouse (Q9D808)	CTF	Primary cortical neurons (E)		
Klotho	Human (Q9UEF7)	CTF (dnPS1, GSI)	HEK293 (sT)	-	[151]
		CTF (dnPS1)	MEF (tT)		
L1	Mouse (P11627)	CTF and ICD (PS dKO, GSI)	MEF (E)	-	[152]
LAR	Human (P10586)	CTF (GSI), ICD (MEM)	CHO (sT)	ICD locates to nucleus, regulates β-catenin mediated transcription	[153]
		CTF (PS dKO dnPS1)	CHO (tT)		
	Rat (Q64604)	CTF	Cortical neurons (E)		
LDLR	Human (P01130)	CTF	MIN6 (tT)	-	[114]
LRP1	Human (Q07954)	CTF (GSI), ICD (LUC, MEM)	HEK293 (tT)	-	[154]
		CTF (PS1 KO), ICD (LUC)	N2a (tT)		
LRP1b	Human (Q9NZR2)	CTF, ICD (LUC)	HEK293 (tT)	ICD by itself locates to nucleus, leads tumor suppression	[155]
		CTF, ICD	H4 (tT)		

LRP6	Human (O75581)	CTF, ICD	CHO (sT)	-	[156]
Megalin	Opossum (Q6E0K3)	CTF	Kidney (E)	-	[157]
MER	Human (Q12866)	CTF	MCF-7 (tT)	-	[99]
MET	Human (P08581)	CTF	HeLa (E)	Downregulation of receptor activity	[158]
		CTF, ICD	MDCK (E)		
	Mouse (P16056)	CTF	MEF (E)		
MUC1	Human (P15941)	CTF (Nct siRNA, GSI)	Uterine epithelial cells (E)	-	[159]
		CTF	ZR75, T47D (E)		
	Mouse (Q02496)	CTF	Uterine epithelial cells (E)		
MUSK	Human (O15146)	CTF	HEK293 (tT)	Growth promotion (OE in 3T3 cells)	[99]
N-cadherin	Human (P19022)	ICD (dnPS1 MEM)	HEK293 (E)		[160]
	Mouse (P15116)	CTF (PS dKO)	Brain (E)	ICD represses CREB-mediated transcription	
		ICD (MEM)	Neuronal cultures, N2a (E)		
		CTF (PS dKO, GSI), ICD (MEM)	MEF (E)		
Nectin-1α	Mouse (Q9JKF6)	CTF (dnPS1, GSI), ICD (MEM)	CHO (sT)	Cell junction remodelling	[161]
		CTF	Primary cortical neurons (E)		
Nectin-3	Mouse (Q9JLB9)	CTF, ICD (PS1 KO, GSI)	MEF (E)	-	[162]
		CTF, ICD	Rat hippocampal neurons (TD)		
Neogenin	Human (Q92859)	ICD	MCF-7, 67NK (E), HEK293T (tT)	ICD locates to nucleus, regulates transcription in neurons	[163]
	Mouse (P97798)	ICD	Brain (E)		
Neuregulin-1	Mouse (Q6DR98)	ICD (LUC)	HEK293 (tT)	Retrograde signalling	[164]
Neuregulin-2	Mouse (P56974)	CTF	B16F10, MC38CEA (sT)	-	[165]

Neurexin-1-β#	Rat (Q63373)	CTF	HEK293, COS (tT)	Regulation of synaptic function and processing of neurexins at glutamatergic synapses	[166]
		CTF (dnPS1, GSI)	Cultured hippocampal neurons (E)		
	Mouse (P0DI97)	CTF (PS1 KO)	Brain (In vivo)		
Neurexin-2-β#	Rat (Q63376)	CTF	HEK293, COS (tT)	Regulation of synaptic function and processing of neurexins at glutamatergic synapses	[166]
		CTF (dnPS1, GSI)	Cultured hippocampal neurons (E)		
	Mouse	CTF (PS KOs)	Brain (In vivo)		
Neurexin-3-β	Human (Q9HDB5)	CTF	HEK293T (tT)	-	[167]
		CTF (GSI), ICD (MEM)	CHO (sT)		
		CTF (PS1/2 KO)	MEF (tT)		
		ICD (LUC)	HeLa (tT)		
		ICD	Cell-free (Purified protein)		
	Mouse (Q8C985)	CTF	Primary cortical neurons (E)		
Neuroligin-1	Rat (Q62765)	ICD (MEM)	Adult cortex (E)	Regulates cell-surface levels	[95, 96]
	Mouse (Q99K10)	ICD (MEM)	Brain synapto-neurosome (E)		
		CTF	Primary neurons (E), COS7 (tT)		
		CTF (PS1/2 KO)	MEF (tT)		
Neuroligin-2	Rat (Q62888)	ICD (MEM)	Adult cortex (E)	-	[95]
	Mouse (Q69ZK9)	CTF	Primary neurons (E)		
		CTF (PS1/2 KO)	MEF (tT)		
NG2	Mouse (Q8VHY0)	CTF	OPC, HEK293 (tT)	-	[168]
		ICD	OPC (E)		
NGL-3	Rat (P0CC10)	CTF	Hippocampal neurons (E)	Synaptic adhesion regulation during LTD	[98]
NLRR3	Human (Q9H3W5)	ICD	SH-SY5Y (E), SK-N-BE (tT)	ICD by itself supresses growth and promotes neurite extension	[169]

Notch-1	Mouse (Q01705)	ICD (PS1 KO, GSI)	Primary neurons (TD)	ICD locates to nucleus	[8]
		ICD (PS1 KO)	MEF (tT)		
Notch-2	Mouse (O35516)	ICD (dnPS1, GSI)	CHO (tT)	-	[170]
		ICD (LUC)	MEF (tT)		
Notch-3	Mouse (Q61982)	ICD (dnPS1, GSI)	CHO (tT)	-	[170]
		ICD (LUC)	MEF (tT)		
Notch-4	Mouse (P31695)	ICD (dnPS1, GSI)	CHO (tT)	-	[170]
		ICD (LUC)	MEF (tT)		
NPR-C	Mouse (P70180)	CTF (dnPS1)	MEF (sT)	-	[48]
		CTF	HEK293 (sT)		
NRADD*	Mouse (Q8CJ26)	ICD (LUC) (PS dKO, GSI)	N2a (tT)	ICD locates to nucleus	[171]
		ICD (MEM)	N2a (E)		
		ICD (LUC)	BD (tT)		
		ICD	NIH-3T3 (E)		
p75 NTR	Human (P08138)	CTF, ICD	RN22F (E), HEK293 (sT)	ICD locates to nucleus	[172]
PAM	Rat (P14925)	CTF, ICD	AtT-20 (sT)	-	[173]
Pianp	Mouse (Q6P1B4)	CTF, ICD	B16F10 (TD)	-	[174]
		CTF and ICD (PS1 KO, GSI)	MEF (TD)		
PLXDC2	Human (Q6UX71)	CTF	HEK293 (sT)	-	[48]
Pmel17	Human (P40967)	CTF (GSI), ICD (MEM)	MNT1 (E)	-	[175]
		ICD (PS1/2 KO)	MEF (tT)		
	Mouse (Q60696)	CTF	B16F10 (E)		
Podoplanin (PDPN)	Human (Q86YL7)	CTF	PS1 KO MEFs, HEK293-PS1 (tT)	Cleavage is PS1 specific	[176]
Polycystin-1**	Human (P98161)	ICD (LUC)	HEK293 (tT)	ICD locates to nucleus, transcriptional regulation of pathways involved in proliferation and apoptosis	[177]
		ICD	LLC (sT)		

Polyductin	Human (P08F94)	ICD	HEK293 (sT)	ICD locates to nucleus	[178]
PRiMA	Mouse (Q810F0)	CTF	CHO (tT)	PRiMA CTF nuclear localization	[179]
Protocadherin-12	Human (Q9NPG4)	ICD, CTF	CHO (sT)	-	[180]
Protocadherin-α4	Rat (Q767I8)	CTF (PS dKO, GSI), ICD	SH-SY5Y (tT)	ICD by itself locates to nucleus	[181]
		CTF, ICD	Primary neurons (TD)		
Protocadherin-γ-A1	Rat (I6LBW6)	CTF	SH-SY5Y (tT)	-	[181]
Protocadherin-γ-A3	Mouse (Q91XY5)	CTF	HEK293 (sT)	-	[182]
Protocadherin-γ-C3	Mouse (Q91XX1)	CTF, ICD	HEK293 (sT)	ICD by itself locates to nucleus	[182]
		CTF (PS dKO, GSI)	MEF (E)		
Protogenin	Chicken (Q589G5)	ICD	HEK293 (tT)	ICD by itself locates to nucleus	[183]
PTK7	Human (Q13308)	CTF, ICD	SW480 (tT, sT)	ICD by itself increases oncogenic potential	[184]
Ptprζ	Rat (Q62656)	ICD (IF, WB)	HEK293 (tT)	-	[185]
PVRL2	Mouse (P32507)	CTF	MIN6 (tT)	-	[114]
RAGE	Human (Q15109)	CTF	HEK293 (sT)	-	[186]
Robo1	Human (Q9Y6N7)	CTF, ICD	PLC/PRF/5 (E), HEK293 (tT)	ICD locates to nucleus	[187]
RPTPκ	Mouse (P35822)	CTF (dnPS1), ICD	HEK293 (tT)	ICD locates to nucleus, activates β-catenin mediated transcription	[188]
		CTF and ICD (PS dKO, GSI)	MEF (E)		
	Human (Q15262)	CTF	768-O (E)		
RPTPμ	Mouse (P28828)	CTF, ICD (PS dKO)	MEF (E)	-	[188]
SEZ6	Mouse (Q7TSK2)	CTF	HEK293T (sT)	-	[103]
SEZ6L	Human (Q9BYH1)	CTF	MIN6 (tT)	-	[114]
SEZ6L2	Human (Q6UXD5)	CTF	MIN6 (tT)	-	[114]
SIRPα	Human (P78324)	CTF, ICD	THP-1, RAW264.7, BEAS-2B (E)	NF-κB signalling regulation	[189]
		ICD	HeLa (tT)		
SorCS1	Human (Q8WY21)	CTF (dnPS1)	MEF (tT)	-	[190]

		CTF (MEM)	HEK293 (sT)		
		CTF	CHO (sT)		[191]
SorLA	Human (Q92673)	CTF	NT2 (E), CHO (sT)	ICD by itself locates to nucleus	[51, 190, 191]
		CTF (dnPS1)	MEF (tT)		
		CTF (dnPS1, GSI), ICD (MEM), Sec.Pep.	HEK293 (sT)		
Sortilin	Human (Q99523)	CTF	HEK293 (tT)	-	[190]
		CTF (dnPS1)	MEF (tT)		
Syndecan-1	Human (P18827)	Mass Spectrometry (CTF with GSI)	HeLa (E)	-	[48]
Syndecan-2	Human (P34741)	Mass Spectrometry (CTF with GSI)	HeLa (E)	-	[48]
Syndecan-3	Human (O75056)	ICD (MEM)	MEF (sT)		[192]
		Sec.Pep.	MEF (TD)		
		ICD (LUC)	HEK293 (tT)		
	Mouse (Q64519)	CTF (PS1 KO, GSI)	MEF (E)	Regulates CASK transcription factor localization	
		ICD (MEM)	Brain membrane (E)		
Tie1	Human (P35590)	CTF, ICD	HUVEC (E)	Regulates signalling via degradation	[193]
TkrB	Mouse (P15209)	CTF	Primary cortical neurons (E)	-	[194]
		CTF, ICD	Primary cortical neurons (TD)		
TMEFF2	Human (Q9UIK5)	ICD	HEK293, CHO (sT), PC3 (tT)	-	[195]
TNFR1	Human (P19438)	CTF and ICD (dnPS1, GSI)	HEK293T (tT)	TNF-stimulated apoptosis	[89]
		CTF, ICD	MCF-7 (E)		
		CTF and ICD (PS dKO)	MEF (E)		
TREM2	Human (Q9NZC2)	CTF, ICD	HEK293 (tT)	Maintenance of proper TREM2 signalling	[83]
		CTF (IF)	COS7 (tT)		
TRKA	Human (P04629)	CTF	MCF-7 (tT)	Growth promotion (OE in 3T3 cells)	[99]

TROP2	Mouse (Q8BGV3)	CTF (PS1/2 siRNA, GSI)	Prostate epithelial basal (TD)	ICD locates to nucleus. ICD signalling through β-catenin interaction. Required for protein's self-renewal and proliferative effect.	[102]
		Functional assay (GSI and mutants)	LSCThi (TD)		
		ICD (IP)	TRAMP-C2 (TD)		
	Human (P09758)	ICD (IF)	Patient Prostate Cancer (E)		
TYRO3	Human (Q06418)	CTF	MCF-7 (tT)	Growth promotion (OE in 3T3 cells)	[99]
Tyrosinase	Mouse (P11344)	CTF	Primary melanocytes (E)	-	[196]
		CTF (PS1 KO)	MEF (tT)		
TYRP1 (TRP1)	Mouse (P07147)	CTF	Primary melanocytes (E)	-	[196]
TYRP2	Mouse (P29812)	CTF	Primary melanocytes (E)	-	[196]
Vasorin	Human (Q6EMK4)	CTF (dnPS1)	MEF (sT)	-	[48]
		CTF, ICD	HEK293 (sT)		
VE-cadherin	Human (P33151)	CTF	HUVEC (E)	-	[197]
VEGFR-1 (FLT1)	Bovine (F1MDD9)	ICD	BRMEC (E)	Angiogenesis inhibition	[198]
VEGFR3	Human (P35916)	CTF	MCF-7 (tT)	Growth promotion (OE in 3T3 cells)	[99]
VGSC β1	Mouse (P97952)	CTF	HEK293 (tT)	-	[199]
		ICD	N2a (tT)		
VGSC β2	Human (O60939)	CTF (dnPS1, GSI), ICD	CHO (tT)		[200]
		CTF (PS dKO)	ES cells (tT)		
		CTF	B104 (sT)		
	Mouse (Q56A07)	CTF	Primary cortical neurons (E), HEK293 (tT)	Regulation of cell adhesion and migration	[199]
		ICD	N2a (tT)		
VGSC β3	Mouse (Q8BHK2)	CTF	HEK293 (tT)	-	[199]
VGSC β4	Mouse (Q7M729)	CTF	Primary cortical neurons (E), HEK293 (tT)	-	[199]
		ICD	N2a (tT)		
VLDLR	Mouse (P98156)	CTF	COS7 (tT)	-	[201]

Table 2: Identified major γ-secretase cleavage sites. The table shows the substrates which γ-secretase cleavage sites have been experimentally determined. The sequences comprise transmembrane domain (underlined), including the flanking 5 amino acid (in one letter code). Asterisk ('*') signs indicate where cleavage sites were determined. Note that only the major cleavage site(s) identified are indicated. Detailed cleavage sites including alternative cleavage sites and ζ-cleavage events that are available for few selected substrates (e.g. APP) are omitted. Cleavage sites that were only predicted based on sequence similarity or mutational screening are not included in this table. Data from cellular based cleavage assays are included if multiple sources exists (e.g.CD44).

Protein Name	Sequence	Ref.
Alcadein α (Calsyntenin-1)	VVPSTAT*VVIVVCVSFLVFMIIL*GVFRIRAA	[202]
Alcadein β (Calsyntenin-3)	MIPSAATLII*VVCVGFLVLMVVLG*LVRIHSLH	[202]
Alcadein γ (Calsyntenin-2)	SVVPSIAT*VVIIISVCMLVFVVAM*GVYRVRIA	[202]
APLP1	GVSREAVSGLLIMGAGG*GSLIVL*SMLLLRRKKP	[203, 204]
APLP2	FSLSSSA*LIGL*LVIAVAIATVIVISL*VMLRKRQY	[35]
APP	VGSNKGAIIGLMVGGVV*IATVIVITL*VMLKKKQY	[205, 206]
CD44	PQIPEWLIILASLLA*LALILAVCI*AVNSRRR	[207, 208]
CSF-1R	SLFTPVVVACMSVMSLLVLLLL*LLLYKYKQK	[209]
E-Cadherin	LQIPAILGILGGILALLILILLLLLFL*RRRRA	[131]
EpCAM	MQGLKAGVIAVIV*VVVIAVVAGIV*VLVISRKKR	[210]
EphB2	EKLPLIVGSSAAGLVFLIAVVVI*AIVCNRRG	[134]
GHR	EEDFRFPWFLIIIFGIFGLTVMLFV*FIFSKQQRI	[211]
IL-1R2	TTVKEASSTFSWGIV*LAPLS*LAFLVLGGIWMHRRCK	[146]
Neuregulin-1	LYQKRVLTITGICIAL*LVVGIMC*VVAYCKTKKQ	[59]
Notch-1	PAQLHFMYVAA*AAFVLLFFVGCG*VLLSRKRR	[64, 212]
Notch-2	PERTQLLYLLAVAVV*IILFIILLG*VIMAKRKRK	[213]
Notch-3	PSVPLLPLLVA*GAVLLLVILVLG*VMVARRKRE	[213]
Notch-4	ANQLPWPVLCSPVAG*VILLALGALL*VLQLIRR	[213]
p75 NTR	GTTDNLIPVYCSILAAVV*VGLVAYIAFKRWNS	[214]
Podoplanin (PDPN)	STVTLVGIIVGVLLAIGFIGAIIV*VVMRKMS	[176]
PTK7	KMIQTIGLSVGAAVAYIIAVLG*LMFYCKKRC	[184]
Syndecan-3	ERKEVLVAVIVGGVVGALFAAL*VTLLIYRMKK	[192]
VEGFR-1 (FLT1)	KSNLELITLTCT*CVAATLFWLLL*TLFIRKMKR	[213]