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Review

Proteolytic processing of Neuregulin-1

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ABSTRACT

Neuregulin-1 (NRG1), known also as heregulin, acetylcholine receptor inducing activity (ARIA), glial growth factor (GGF), or sensory and motor neuron derived factor (SMDF), is a key factor for many developmental processes and in adult brain. All known splice variants contain an epidermal growth factor (EGF)-like domain, which is mediating signaling *via* receptors of the ErbB family. In particular, NRG1 acts as an essential signaling molecule expressed on the axonal surface, where it signals to Schwann cells throughout development and regulates the thickness of the myelin sheath. NRG1 is required also by other cell types in the nervous system, for instance as an axonal signal released by proprioceptive afferents to induce development of the muscle spindle, and it controls aspects of cortical interneuron development as well as the formation of thalamo-cortical projections. The precursor protein of NRG1 can be activated and released from the membrane through limited proteolysis by the β-Secretase (β-site amyloid precursor protein cleaving enzyme 1, BACE1) which was first identified through its function as the rate limiting enzyme of amyloid-β-peptide (Aβ) production. Aβ is the major component of amyloid plaques in Alzheimer's disease (AD). Due to the hairpin nature of NRG1 type III two membrane-bound stubs with a type 1 and a type 2 orientation are generated by an initial proteolytic cleavage and successive release of the EGF-like domain either by dual cleavage by BACE1 or by ADAM17 (a disintegrin and metalloprotease) which is also called TACE (Tumor Necrosis Factor-α-converting enzyme). The cleavages activate NRG1 to allow juxtacrine or paracrine signaling. The type 1 oriented stub is further cleaved by γ-secretase in the transmembrane domain with a putative role in intracellular domain (ICD) signaling, while the type II oriented stub is cleaved by signal peptidase like proteases (SPPLs). Neuregulin-1 was identified as a major physiological substrate of BACE1 during early postnatal development when similarities in BACE1 KO mice and NRG1 heterozygous mice were discovered. Both display severe hypomyelination of peripheral nerves. Later it was shown with genetic and pharmacological evidence that the developmental effect of type I NRG1 on the formation and the maintenance of muscle spindles is BACE1 dependent. Thus, NRG1 functions in PNS and CNS are likely to set limits to an Alzheimer disease therapy with relatively strong BACE1 inhibition.

Contents

Abbreviations: AD, Alzheimer's disease; ADAM, a disintegrin and metalloprotease; Aβ, Amyloid β-peptide; APP, β-amyloid precursor protein; BACE1, beta-site APP cleaving enzyme 1; CTF, C-terminal fragment; EGF, epidermal growth factor; ICD, intracellular domain; I-CLiP, intramembrane cleaving protease; MMP, matrix-metalloprotease; NRG1, Neuregulin-1; PS, presenilin; RIP, regulated intramembrane proteolysis; SPP, signal peptide peptidase; SNP, single nucleotide polymorphism; TACE, tumor necrosis factor-α converting enzyme; TMD, transmembrane domain.

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1. NRG1 shedding is mediated by α- and β-secretases

Shedding proteases cleaving the ectodomain of membrane-bound pro-NRG1 play a central role in Alzheimer's disease (AD), the most prevalent neurodegenerative disease worldwide which is without any cure so far. The major pathological hallmarks of AD are intracellular neurofibrillary tangles, which mainly consist of the hyperphosphorylated tau protein and extracellular amyloid plaques (Hardy and Selkoe, 2002). Amyloid plaques are predominantly composed of the amyloid-β-peptide (Aβ), a hydrophobic peptide of 37–43 amino acids (Glenner and Wong, 1984; Haass and Selkoe, 2007). Cleavage of amyloid precursor protein (APP) by β-secretase liberates the soluble N-terminus of APP with the cleavage at the β-site while the C-terminal fragment (APP-CTF) remains bound to the membrane. The APP-CTF is proteolysed within the membrane by γ-secretase giving rise to Aβ and the intracellular domain of APP (AICD) which are liberated to the luminal and the cytoplasmic sides, respectively (Haass, 2004; Steiner et al., 2008a). β-Secretase was discovered independently in 1999 by several groups (Hussain et al., 1999; Lin et al., 2000; Sinha et al., 1999; Vassar et al., 1999; Yan et al., 1999) and has been called BACE1 (for β-site APP cleaving enzyme 1; (Vassar et al., 1999)). BACE1 is a member of the pepsin-like family of aspartyl proteases. It is a type I membrane protein, which contains the characteristic dual active site motif (D-T/S-G-T/S) of aspartic proteases in its ectodomain (Hong et al., 2000; Vassar et al., 1999). High BACE1 enzymatic activity was found in human brain extracts which is consistent with the finding that neurons produce the highest levels of Aβ (Citron et al., 1992). Due to its role in the generation of Aβ, the β-secretase BACE1 is an attractive target for the therapeutic treatment of AD, while attempts to treat the disease with γ-secretase inhibitors failed due to the inevitable inhibition of substrate functions *e.g.* of Notch-1 (De Strooper, 2014; Doody et al., 2013). In an alternative proteolytic pathway, APP can be cleaved in the middle of the Aβ domain by α-secretase, thereby precluding the formation of Aβ or at the more N-terminal secretase cleavage site. Thereby MT5-MMP is cleaving first and the following cleavages by BACE1 or by α-secretase result in liberation of Aη-β or Aη-α respectively (Willem et al., 2015). Three members of the ADAM family of metalloproteases, ADAMs 9, 10 and 17 are described to have α-secretase activity (Buxbaum et al., 1998; Koike et al., 1999; Lammich et al., 1999). The ADAMs family consists of many members with a variety of identified substrates and functions (Reiss and Saftig, 2009). Spatial and temporal expression patterns determine which proteases are physiologically relevant for specific substrates in different cell types. Moreover, the activity of ADAMs and of MMPs is antagonized by certain tissue inhibitors of metalloproteases (TIMPs, (Rosenberg, 2009)). Mechanisms underlying pathological conditions like in cancer or in neurodegenerative diseases could cause a disturbance of the normal regulation and function of these enzymes (Murphy, 2008; Rosenberg, 2009).

2. Liberation of the EGF-like domain of type III NRG1-β1

In humans there appears to be six main classes of protein (type I–VI) with distinct N-termini produced by alternative promoter usage, all containing the biologically active epidermal growth factor (EGF)-like domain (Esper et al., 2006; Falls, 2003; Mei and Xiong, 2008). Most of the NRG1 isoforms are membrane associated proteins, however, some are secreted. A detailed discussion of NRG1 structure is provided in recent reviews (Falls, 2003; Mei and Xiong, 2008). Both membrane-bound type I and type III oriented isoforms are highly *O*-glycosylated in close proximity to the EGF-like domain. Type I NRG1 isoforms contains an N-terminal heparin-binding immunoglobulin-like domain (HBD-Ig) followed by a spacer (containing *O*-gylcosylation sites) and the EGF-like domain in the extracellular part, with a single membrane-spanning segment. The transmembrane domain is followed by a C-terminal cytoplasmic domain of which several forms exist (a/b/c) through alternative splicing (Esper et al., 2006; Falls, 2003; Mei and Xiong, 2008). The N-terminal domain of membrane-bound type III NRG1differs from that of Type I and includes a hydrophobic cysteine-rich domain (CRD). The CRD domain leads to retention of the N-terminus within the cytosol, and itself inserts into the membrane, thus leading to the formation of an extracellular hairpin loop containing the EGF-like domain followed by the transmembrane and cytoplasmic domains that are shared with those of the type I isoform. Alternative splicing causes changes in a small stretch of amino acids at the C-terminal side of the EGF-like domain giving rise to an α and β variant of this domain, with a predominance of the β variant, which has higher affinity for the receptors ErbB3 and ErbB4, being found in the brain (Burgess et al., 1995; Wen et al., 1994). Further variety in different NRG1 isoforms results from alternative splicing of the sequences encoding the juxtamembrane region, which are denoted by numerical suffices following the EGF variant (*e.g.* type I-β1 or type III-β1, membrane-bound isoforms, or type III-β3, a soluble type III isoform, see also the review of Douglas Falls for a more complete nomenclature of the NRG1 variants (Falls, 2003)). It is exactly within these variable sites close to the membrane after the EGF-like domain that both membrane-bound type I and type III NRG1 isoforms are cleaved (Horiuchi et al., 2005; Montero et al., 2000, 2002). While the same cleavage was originally thought to be sufficient to activate even NRG1 type III-β1, subsequent studies from our laboratory revealed in addition a dual cleavage by BACE1 and of ADAMs (Fig. 1) for the liberation of the sole EGF-like domain and demonstrated the signaling capacity of such released peptides for example in the process of peripheral myelination irrespective to the exact C-terminal ending (Fleck et al., 2012, 2013; van Bebber et al., 2013). The cleavage site of BACE1 in the juxtamembrane region of NRG1-β1 has been identified and is conserved in NRG3 (Fleck et al., 2013; Hu et al., 2008) encoded by one of three other homologous genes (*NRG2-4*), with functions in the CNS (Loos et al., 2014). In contrast to type I NRG1 isoforms, a single cleavage C-terminal of the EGF-like domain of NRG1 type III-β1 results in retention of the membrane bound NTF (N-terminal fragment), which could activate ErbB receptors on target cells in a juxtamembrane fashion as discussed in Taveggia et al. (Taveggia et al., 2005). Since ErbB receptor internalization is likely to be necessary for NRG1 signaling (Liu et al., 2007), it seems plausible that a membrane-bound form of NRG1 is impaired in signaling. A dual cleavage at either side of the EGF-like domain of type III NRG1 liberates the growth factor to enable paracrine signaling as shown for type I NRG1 (Fleck et al., 2013). In the case of type I NRG1 the liberated EGF-like domain with or without the heparin-binding domain can provide paracrine signals after shedding (Eto et al., 2006; Falls, 2003; Loeb and Fischbach, 1995; Montero et al., 2000). In contrast to the CRD-containing isoform, the attached heparin-binding domain of type I NRG1 might allow for signal enhancement by binding and storage within the extracellular matrix where further processing and liberation of the EGF-like domain could occur (Loeb and Fischbach, 1995). However, the shed extracellular domain of type I NRG1 (HBD plus EGF-like domain) can also be detected in cerebrospinal fluid and is more potent in signaling than the EGF-like domain alone (Pankonin et al., 2009), suggesting that a single cleavage suffices for activation of this isoform.

3. *In vivo* activation of NRG1 signaling by limited proteolysis

Already in 2006 studies with BACE1 knockout mice revealed that NRG1 is a substrate for BACE1 (Hu et al., 2006; Willem