

Alzheimer's disease linking neurodegeneration with neurodevelopment

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Summary

An association has recently been suggested between several of the genes and proteins that play a central role in early neuronal development, particularly in neuronal migration and axon elongation, and Alzheimer's disease (AD). This paper reviews the work of several investigators who have hypothesised the involvement of three pathways known to be active participants in neuronal maturation (those involving Notch, Reelin, and Wnt intracellular signalling) and also in the neurodegenerative events underlying AD. The choice of these intracellular pathways is based on the observation that there exist several points of convergence among these systems and amyloid precursor protein processing and neurofibrillary tangle formation. Pharmacological manipulation of the Notch/Wnt/Reelin intracellular signalling pathways may thus represent a novel approach to the regulation of neurodegenerative processes in AD.

KEY WORDS: APP, neurofibrillary tangles, Notch, Reelin, Wnt.

Introduction

Alzheimer's disease (AD) involves a progressive degeneration of selective neuronal subpopulations in cerebral and limbic cortices. Characteristic, although not exclusive, neuropathological hallmarks of AD are aggregated proteins in the form of intraparenchymal amyloid plaques or intracellular neurofibrillary tangles (NFTs) (1,2), as well as astrogliosis and microgliosis. The plaques are predominantly composed of a 40/42 amino acid peptide, defined amyloid β -peptide ($A\beta$), which is derived from the proteolysis of the amyloid pre-

cursor protein (APP) by enzymes called beta- and gamma-secretase (β - and γ -secretase), whereas NFTs are bundles of paired helical filaments composed of the microtubule-associated protein tau.

One of the most widely accredited working hypotheses is that excessive formation and deposition of $A\beta$ by its precursor APP is responsible for the neuronal loss/dysfunction that leads eventually to dementia. Notably, familial AD frequently results from mutations in the gene encoding APP or in genes encoding presenilins (PSs), which are involved in APP processing. Despite making considerable progress in understanding of the genetic and molecular basis of AD amyloidosis, researchers in the field have still to demonstrate that interference with plaque formation (by drugs or vaccine) constitutes a valid disease-modifying approach. An additional feature of the AD brain is the presence of dystrophic neurites, numerous contorted processes from cell bodies of NFT-bearing neurons and supernumerary basilar dendrites on hippocampal pyramidal cells. These observations suggest that AD may also be associated with an aberrant neural plasticity, understood, in more general terms, as an ability of selected neuronal cells to change morphology, including synaptic contacts.

In one particularly exciting line of research in the field, several of the genes and proteins associated with AD have been found to play a central role in early neuronal development, particularly neuronal migration and axon elongation. Many investigators have picked up on the potential significance of this observation, although a clear picture of the link between neurodevelopment and neurodegeneration has yet to emerge.

The pathfinder Reelin

Recent acquisitions in the study of physiological and pathological processes in the nervous system seem to paint a paradoxical picture of neuronal development and degenerative behaviour. The suggestion, based on an increasing number of observations, is that proteins formerly known as pro-differentiating molecules can be involved in neurodegenerative disorders, mainly AD. For example, it is known that the orderly migration of neuronal precursors from the ventricular germinal zone beneath the cortical plate to the outermost layer of the cortex is somehow guided by a protein called Reelin (3). This molecule is physiologically secreted by the Cajal-Retzius cells along the guiding track of the radial glia. In the absence of Reelin, migrating neurons fail in their attempt to reach the final cortical location and pile up in a disordered manner. The ability of Reelin to drive neuroblast migration and then differentiation is due to a membrane protein multi-complex capable of transducing Reelin binding in an intracellular signal, which is di-

rected down to the cytoskeleton and nucleus. In particular, along the signal cascade an adaptor protein called Disabled (Dab) becomes tyrosine phosphorylated, most probably by the non-receptor tyrosine kinase Abl. At present, very little is known about downstream targets of activated Dab. Interestingly, mutations in the cyclin-dependent kinase-5 (cdk5) gene produce defects in neuronal migration that resemble those caused by loss of the Reelin signal, thus proposing cdk5 as one of its potential effectors. Additionally, this kinase has been shown to be an important mediator of tau phosphorylation, hyperphosphorylated tau being typically abundant in NFTs. Although hypothesized, the idea that Reelin and APP signalling pathways may converge has not yet been fully explored.

It has recently been demonstrated that Reelin is a serine protease and that it induces detachment of cells from fibronectin- or laminin-coated substrates, thereby supporting the hypothesis that Reelin could modulate neuronal migration in the developing brain by regulating cell adhesion (4). In view of the recently discovered proteolytic activity of Reelin, one working hypothesis is that Reelin may be involved in the proteolytic processing of APP, or in the clearance of A β aggregates generated by APP processing. This hypothesis is supported by the following observations: a) both Reelin and APP are expressed during early embryogenesis as well as in the adult nervous system, and they trigger common intracellular signalling pathways involving adaptor proteins such as Dab and Ab1. Additionally, the Dab1-knockout mice phenotype includes neuronal migration disorders resembling the reeler phenotype (5); b) Reelin is a secreted serine protease, and the extracellular portion of two APP isoforms contains a serine protease inhibitor domain of the Kunitz type (KPI domain); c) *in vitro* studies suggest that both membrane-bound and soluble APP promote neuronal migration and neurite extension; d) double-transgenic mice that express both human mutant APP and human mutant PS1 show accumulation of Reelin in AD-typical plaques in the hippocampus and neocortex (6). Together, these observations suggest an interplay between APP and Reelin functions.

The Notch gene family

The Notch gene family has four members (Notch1, Notch2, Notch3, Notch4) encoding cell surface receptors involved in a wide variety of cellular interactions that determine cell fate during development. During neuronal differentiation, upon ligand-induced activation, this ubiquitous type I membrane family receptor undergoes a proteolytic cleavage of the intramembrane-spanning segment, releasing the intracellular domain. This fragment translocates into the nucleus where it is able to transactivate several genes involved in axon outgrowth and differentiation. Furthermore, Notch cooperates with Abl and other unknown tyrosine kinases to directly control growth cone motility and guidance, and this effect seems to be mediated by recruitment of Dab adaptor protein and Rho family GTPases, mainly Rac. Interestingly, the phenotype of PS1 knockout mice resembles that of Notch1 knockout mice (7) and the ex-

pression of Notch1 and its ligand Delta-like1 is diminished in the paraxial mesoderm of PS1 null embryos (8). These findings suggest that PS1 may regulate the Notch signalling pathway during development. Moreover, a possible involvement of the Notch family proteins in AD has been suggested. In fact, it has been shown that both Notch1 and APP can be cleaved by a PS1-associated γ -secretase activity (9,10), and Notch1 expression is markedly increased in AD hippocampal formation (11). Furthermore, Notch3 gene mutations can lead to CADASIL, an adult-onset autosomal dominant neurological disease characterized by progressive subcortical infarcts and dementia (12). Finally, Notch2 was found to be upregulated in an experimental model of excitotoxicity (13).

Recent *in vitro* studies of neurons from mouse cerebral cortex revealed that contact-mediated Notch signalling regulates the capacity of neurons to extend and elaborate neurites (14). Up-regulation of Notch1 and Notch2 activity was indeed concomitant with an increased number of interneuronal contacts and cessation of neurite growth. Given that members of the Notch signalling pathway are expressed in neurons in the adult cerebral cortex, it is plausible that Notch plays a role in maintaining the stability of neurites and connections. Thus, it is possible that an alteration of Notch activity (perhaps as a result of PS dysfunction) would contribute to aberrant neurite formation, such as that seen in the AD brain.

The Wnt pathway

Another molecule known to be involved in neuronal differentiation, adhesion and cell-fate is Wnt.

Upon interaction with specific membrane receptors such as Frizzleds (Fzd), Wnt activates the mammal homologue of *Drosophila* dishevelled protein, Dv1, thereby disabling glycogen-synthase kinase-3 β (GSK-3 β). As a result of GSK-3 β inactivation, intracellular levels of β -catenin, an Armadillo repeat-containing protein involved in cell adhesion, increases so that Wnt target genes are activated. In the absence of Wnt, GSK-3 β is activated, causing the phosphorylation of β -catenin and in turn its ubiquitin-proteasome mediated degradation with suppression of Wnt-related gene transcription. Interestingly, PS1 was found to interact both with β -catenin (stabilizing it) and GSK-3 β . This latter interaction seems to confer on GSK-3 β the ability to recognize tau proteins, suggesting that Wnt signalling may link both amyloid plaque biogenesis and the neurofibrillary changes observed in AD brains (15). Thus, dysregulation of Wnt signalling, caused by acute or chronic insults, may result in increased GSK-3 β activity with ensuing neuronal degeneration (16). Accordingly, lithium, which is endowed with neuroprotective properties, has the capacity to inhibit the activity of GSK-3 β through the activation of the AKT survival pathway (17-19). Moreover, the Wnt antagonist Dickkopf-1 (DKK-1), which potently inhibits the Wnt/ β -catenin signalling by linking the LPR co-receptor, is a transcriptional target of p53 and functions as a pro-apoptotic gene (20). Thus, a vast array of signals converging on GSK-3 β may influence neuronal fate.

Points of convergence

All these observations uncover an intriguing connection between development and cell death pathways that needs to be investigated further and suggest that it may be possible to identify a cluster of new cell-death molecular protagonists among the proteins involved in neuronal differentiation (21).

We suggest a possible involvement of three pathways known to be active participants in neuronal maturation (those involving Notch, Reelin, and Wnt intracellular signalling) in the neurodegenerative programme activated by APP proteolysis and/or in the pathogenesis of NFTs. The focus on the three above-mentioned intracellular pathways is justified by the observation that there exist several points of convergence (summarized schematically in figure 1) between these systems and the APP processing.

PSs, in particular PS1 appear to be key enzymes linking many of the players considered in this review. Gene knockout studies revealed that PS1 is required for the proteolytic processing of APP and Notch. The phenotype of PS1 knockout mice resembles that of Notch1 knockout mice and the expression of Notch1 and its ligand Delta-like1 is diminished in the paraxial mesoderm of PS1 null embryos. In addition, PS1 has been shown to interact with β -catenin and Wnt signalling. Wnt signalling is indeed tightly controlled by, among other things, cytosolic β -catenin levels. In the absence of Wnt ligands, cytosolic β -catenin is in fact rapidly phosphorylated by GSK-3 β . On the contrary, binding of Wnt ligands to cell surface receptors leads to the inactivation of GSK-3 β . It should be noted that GSK-3 β is one of the factors most likely to prove responsible for tau hyper-

phosphorylation. The Reelin signalling pathway involves membrane receptors belonging to the family of lipoprotein receptors, i.e., VLDL-R and ApoE-R2 (the latter also being the receptor for apolipoprotein E). Binding of Reelin to VLDL-R and ApoE-R2 induces activation of the cdk5/p35 pathway, which is another important mediator of tau phosphorylation. Mutation of Reelin or ApoE-R2 and VLDL-R also induces hyperphosphorylation of tau. Finally, double-transgenic mice that express both human mutant APP and human mutant PS1 show accumulation of Reelin in AD-associated amyloid plaques.

Another point of convergence is the Abl/Dab complex. In fact, APP, Reelin and Notch intracellular signalling require the cytosolic adaptor protein Dab and the tyrosine kinase Abl for the transduction of their corresponding intracellular signals. Thus, it is conceivable that alteration of one pathway may disrupt the efficiency of the others.

Concluding remarks

In summary, we believe that the Notch/Reelin/Wnt signalling pathways and APP processing and toxicity may influence each other at several and different levels, and that cross-talk should constitute the basis for detailed study of the relationship between the processes of neurodevelopment and neurodegeneration. Although it is unlikely that Reelin, Notch and Wnt are "Alzheimer genes" (given the lack of linkage between AD and the chromosomal regions where these genes are located), all of them could well play a role in the processing of APP or in modifying the phosphorylation state of tau proteins.

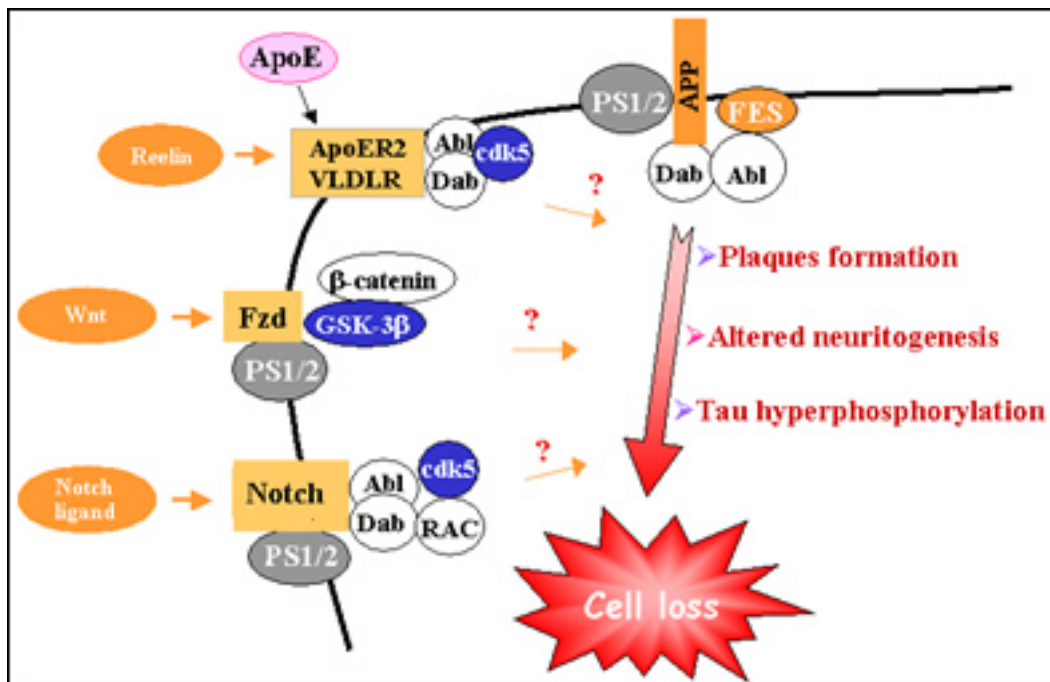


Figure 1 - Proteins and signalling pathways implicated in neuronal development and Alzheimer's disease.

Expectations generated by this hypothesis are twofold, conceptual and operational. Conceptually, identification of a link between two apparently distant physiopathological processes (i.e., neurodevelopment and neurodegeneration) may provide new perspectives from which to view AD pathophysiology, for instance in terms of abnormal reactivation of silent genetic programmes, and reveal new physiological roles for development-associated intracellular pathways in the mature brain. From an operational point of view, a connection between these pathways may provide a novel framework for the development of innovative therapeutic interventions in AD. Pharmacological modulations of Notch/Wnt/Reelin intracellular signalling pathways may indeed represent challenging new avenues in the regulation of APP processing and tau phosphorylation.

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References

1. Terry RD, Katzman R, Bick KL, Sisodia SS. Alzheimer Disease. Philadelphia PA; Lippincott Williams & Wilkins 1999
2. Selkoe DJ. Alzheimer's disease: genes, proteins, and therapy. *Physiol Rev* 2001;81:741-766
3. Rice DS, Curran T. Role of the reelin signaling pathway in central nervous system development. *Annu Rev Neurosci* 2001; 24:1005-1039
4. Quattrocchi CC, Wannenes F, Persico AM et al. Reelin is a serine protease of the extracellular matrix. *J Biol Chem* 2002; 277:303-309
5. Howell B, Hawkes R, Soriano P, Cooper JA. Neuronal position in the developing brain is regulated by mouse disabled-1. *Nature* 1997;389:733-737
6. Wirths O, Multhaup G, Czech C et al. Reelin in plaques of beta-amyloid precursor protein and presenilin-1 double-transgenic mice. *Neurosci Lett* 2001; 316:145-148
7. Swiatek PJ, Lindsell CE, Del Amo FF, Weinmaster G, Gridley T. Notch1 is essential for postimplantation development in mice. *Genes Dev* 1994; 8: 707-719
8. Wong PC, Zheng H, Chen H et al. Presenilin 1 is required for Notch1 and Dll1 expression in the paraxial mesoderm. *Nature* 1997;387:288-292
9. Song W, Nadeau P, Yuan M, Yang X, Shen J, Yankner BA. Proteolytic release and nuclear translocation of Notch-1 are induced by presenilin-1 and impaired by pathogenic presenilin-1 mutations. *Proc Natl Acad Sci USA* 1999;96:6959-6963
10. Selkoe DJ. Notch and presenilins in vertebrates and invertebrates: implications for neural development and degeneration. *Curr Opin Neurobiol* 2000;10:50-57
11. Berezovska O, Xia MQ, Hyman BT. Notch is expressed in adult brain, is coexpressed with Presenilin-1 and is altered in Alzheimer disease. *J Neuropathol Exp Neurol* 1998;57:738-795
12. Joutel A, Corpechot, C, Ducros et al. Notch3 mutations in cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL), a mendelian condition causing stroke and vascular dementia. *Ann NY Acad Sci* 1997;826:213-217
13. Ferrari Toninelli G, Bernardi C, Quarto M, Lozza G, Memo M, Grilli M. Long-lasting induction of Notch2 in the hippocampus of kainite-treated adult mice. *Neuroreport* 2003;14:917-921
14. Sestan N, Artavanis-Tsakonas S, Rakic P. Contact-dependent inhibition of cortical neurite growth mediated by Notch signaling. *Science* 1999; 286:741-746
15. De Ferrari GV, Inestrosa NC. Wnt signaling function in Alzheimer's disease. *Brain Res Brain Res Rev* 2000;33: 1-12
16. Caricasole A, Copani A, Caruso A et al. The Wnt pathway, cell cycle-activation and β -amyloid: novel therapeutic strategies in Alzheimer's disease. *Trends Pharmacol Sci* 2003; 24: 233-238
17. Alvarez G, Munoz-Montano JR, Satrustegui J et al. Lithium protects cultured neurons against beta-amyloid-induced neurodegeneration. *FEBS Lett* 1999; 453: 260-264
18. Chen RW, Chuang DM. Long term lithium treatment suppresses p53 and Bax expression but increases Bcl-2 expression. A prominent role in neuroprotection against excitotoxicity. *J Biol Chem* 1999;274: 6039-6042
19. Bhat RV, Shanley J, Correl MP et al. Regulation and localization of tyrosine 216 phosphorylation of glycogen synthase kinase-3 β in cellular and animal models of neurodegeneration. *Proc Natl Acad Sci USA* 2000;97:11074-11079
20. Shou J, Ali-Osman F, Multani AS et al. Human Dkk-1, a gene encoding a Wnt antagonist, responds to DNA damage and its overexpression sensitizes brain tumor cells to apoptosis following alkylation damage of DNA. *Oncogene* 2002; 21: 878-889
21. Bothwell M, Giniger E. Alzheimer's disease: neurodevelopment converges with neurodegeneration. *Cell* 2000; 102:271-273