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Discussion

Live discussion: How the other half lives – or the what, how, and where, of the AβPP intracellular domain[1]

Live discussion held 20 September 2002 on the Alzheimer Research Forum, featuring Frank LaFerla. Moderated by Paul Coleman.
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Background text by Frank LaFerla

The Notch receptor (Notch) and the amyloid β protein precursor (AβPP) are examples of type-I integral membrane proteins that are substrates for γ-secretase. Notch is a vital signaling molecule that regulates cell-fate determination during development. Signaling through the Notch pathway is triggered by the binding of ligands such as Delta and Jagged, which induces cleavage of Notch. A subsequent β-secretase mediated cleavage releases the Notch intracellular domain (NICD), which binds to transcription factors (e.g., Supressor of Hairless) and translocates to the nucleus, where it regulates transcription of selective genes.

Similarities between the processing of Notch and AβPP have prompted speculation that AβPP may play an analogous signaling role. Once regarded as nothing more than a throw-away fragment that happens to border the Aβ sequence, the carboxy-terminal sequence of AβPP has emerged as a potential nuclear signaling molecule [1]. This long ignored fragment, which is referred to as the AβPP intracellular domain (AICD), was initially described by Passer et al., who showed that AICD-like peptides occur in both normal and Alzheimer's disease (AD) brain [2].

AICD consists of the last 50 carboxy-terminal residues of the AβPP protein. Surprisingly, this does not correspond to the entire sequence downstream of the γ-secretase site, which would have led to an AICD species containing either 57 or 59 residues, depending on whether cleavage occurred at the Aβ42 or Aβ40 site, respectively. Several groups have recently demonstrated that an additional proteolytic event must occur (either before or after γ-secretase processing) that cleaves at a conserved valine downstream of the canonical γ-secretase cleavage sites (either by γ-secretase or another protease) [3–5].

The Alzheimer's AICD fragment, like NICD, can also complex with transcription factors. Kimberly and colleagues [6] have shown that the cytoplasmic domain of AβPP is highly labile, but that it is stabilized by forming a complex with Fe65, which is then capable of entering the nucleus. Fe65, in turn, interacts with the transcription factor CP2/LSF/LBP1 and Tip60, a histone acetyltransferase. Sudhof's group re-

[1] Note: The transcript has been edited for clarity and accuracy.

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cently showed that AICD complexed with Fe65 and Tip60 can potently regulate the expression of artificial expression constructs in transfected cells [7]. Pimplikar's group [8] also showed that AICD (they called it C) exerts effects in the nucleus, and found that the 59-residue-long fragment, but not the 57 amino acid fragment, potently represses retinoic acid-responsive gene expression.

What role in cell signaling does AICD mediate? My lab has recently demonstrated a functional role for AICD in regulating phosphoinositide-mediated calcium signaling [9]. Genetic ablation of the presenilins or pharmacological inhibition of β-secretase activity (and thereby AICD production) greatly attenuated calcium signaling in a dose-dependent and reversible manner through a mechanism involving the modulation of endoplasmic reticulum calcium stores. Cells lacking AβPP (and hence AICD) exhibited similar calcium signaling deficits, and – notably – these disturbances could be reversed by transfection with AβPP constructs containing an intact AICD, but not by constructs lacking this domain. We noted that there was a three-hour time lag after inhibition of γ-secretase and inhibition of calcium signaling, which would be adequate time for transcriptional-mediated events. One aspect of this work that is presently unresolved is why there was no compensation in the AβPP null cells by the homologues APLP1 or APLP2. It could be that there is a disparity in expression levels or stability of these molecules in fibroblasts, or that they don't bind efficiently to Fe65 (all of which need to be addressed).

Is modulating calcium signaling the only role for AICD? Things are hardly ever this simple, as recent findings by Luciano D'Adamio and Brad Hyman's groups point to additional roles. In the first case, D'Adamio's lab has shown that AICD also binds to cytosolic Notch inhibitors Numb and Numb-like, which can represses Notch activity [10]. Kinoshita has shown that γ-secretase generated C-terminal domain of AβPP may also be involved in apoptosis [11].

Paul Coleman: Frank, would you like to start with a brief overview of C-terminal in AD and its recent attention?

Frank LaFerla: I think the first description of this fragment was provided by Luciano D'Adamio's group several years ago. They also named the fragment AID for AβPP intracellular domain.

Paul Coleman: So, what gave it the current impetus?

Frank LaFerla: The field has now seemed to have adopted the name AICD because of similarities between it and Notch; the same fragment in Notch is referred to as NICD. Its impetus: Probably the most exciting aspect is that it is involved in nuclear signaling and, therefore, has the potential to amplify cellular responses.

Malcolm Leissring: Has Rachael Neve not been heralding the idea that the CTF of AβPP is the neurotoxic portion of AβPP?

Frank LaFerla: Yes, Rachael has been suggesting that it is neurotoxic, and several other groups as well, including Luciano D'Adamio's and Brad Hyman's.

Jason Shepherd: Perhaps it is neurotoxic through abnormal calcium signaling, as Frank LaFerla has shown?

Frank LaFerla: Good point, Jason.

Paul Coleman: It appears that Rachael is not here now, but it is correct that she championed the C100 molecule for some time. However, as Frank LaFerla points out in his background, the situation may be more complex than that. In fact, a recent Cell paper [12] suggests additional complexes.

Malcolm Leissring: So there is very little evidence for a ligand for AβPP at this moment – and this is not for lack of trying by many groups. Does it make sense, then, that the AICD should have some critical nuclear signaling role?

Frank LaFerla: Yes, it does.

Jason Shepherd: I would be surprised if it did not, but the BACE1 knockouts seem to have no observable phenotype, although, of course, there may be compensation by other BACE-like enzymes.

Malcolm Leissring: What is the message that is being signaled?

Della: One paper [13] showed Aβ to bind to AβPP. Does this seem reasonable?

Frank LaFerla: That remains to be worked out here.

Malcolm Leissring: I suppose it could be saying, "I am attached to the extracellular matrix" or some other, chronic signal.

Frank LaFerla: I suppose it could seem reasonable, but Aβ is a notoriously sticky molecule, so the significance of its interaction would need to be worked out.

Malcolm Leissring: It would be really nifty if Aβ itself had some signaling role.

Frank LaFerla: I agree, particularly if there was a disparity between Aβ40 and 42.

Malcolm Leissring: Or perhaps tragic for the therapeutic efforts aimed at removing Aβ . . .

Jason Shepherd: If Aβ does have a function, it does not seem to be essential for normal cognitive function . . . at least in mouse models.

Malcolm Leissring: But AβPP knockout mice have definite electrophysiological deficits.

Frank LaFerla: This is true, and BACE knockouts may also have similar deficits once they get measured.

Jason Shepherd: True, but AβPP itself has a host of other putative functions.

Glenda Bishop and Andrea Wilson: So far, the physiological function of Aβ is completely unknown; no tests have really been performed to work that out.

Malcolm Leissring: And APPL knockout flies show interesting phenotypes that, interestingly enough, can be rescued by AICD. Or, I should say, overexpression in flies of constructs containing AICD produce interesting phenotypes.

Frank LaFerla: As do mice with AβPP, APLP1/2 knockouts.

June Kinoshita: Regarding the effects of AβPP knockout, I suppose one would have to work out the contributions of all of the different fragments to the electrophysiological deficit.

Glenda Bishop and Andrea Wilson: Does the AICD contain any part of the Aβ sequence?

Frank LaFerla: Nope. It corresponds to the last 50 amino acids of AβPP.

Glenda Bishop and Andrea Wilson: Then, in some ways, the function of Aβ is irrelevant when determining the effects of AICD.

Jason Shepherd: What do people think about Aβ forming a calcium permeable pore?

June Kinoshita: Is that Lansbury's proposal?

Malcolm Leissring: That idea has been talked about and published on for years.

Frank LaFerla: Lansbury has been proposing this recently, but the original idea as far as I recall is from Arispe. I think there is more and more data supporting a role for a calcium or other ion-permeable pore.

Malcolm Leissring: There are lots of papers.

Della: If AβPP is involved in transport, and as Aβ was proved to be present in the vesicles, could Aβ be released as a signal to other neurons or to astrocytes?

Malcolm Leissring: That is an interesting idea, but the release would seem to occur at sites very distal from the nucleus, according to the evidence on the transport idea.

June Kinoshita: We are drifting a bit off-topic. Let us get back to AICD! (Aβ gets plenty of attention already!)

Jason Shepherd: What are those results in flies, Malcolm?

Malcolm Leissring: Flies overexpressing constructs that contain an intact AICD show increased synaptic boutons at the neuromuscular junction. And flies overexpressing AβPP constructs in the wing show a "blistered wing" phenotype.

Frank LaFerla: AICD may be pleiotrophic.

Malcolm Leissring: These fly papers are very illuminating and I recommend them to everyone.

Jason Shepherd: References, Malcolm?

Malcolm Leissring: Kalpana White has done the most work in this area. (Also see [14])

Della: Does anyone know what sort of DNA-binding site would be recognized by the C-terminus?

Frank LaFerla: Della, some of this has already been worked out, but I suppose it would depend on what transcription factors it ultimately binds to and whether this is a cell-dependent effect. Yama Akbari in my lab will be giving a talk at the Society for Neuroscience meeting [15] detailing at least one gene that is affected.

Jason Shepherd: I think recently it was found that AICD can bind to Src homology domains, which are very common motifs on other proteins.

Glenda Bishop and Andrea Wilson: Frank, what do you mean by AICD being pleiotrophic. What effects are you talking about?

Frank LaFerla: Glenda and Andrea, I mean that it may have multiple roles. Malcolm Leissring, when he was in my lab, showed that AICD could play a physiologic signaling role by modulating phosphoinositide calcium signaling. Other groups have shown that overexpression of AICD is neurotoxic and can induce apoptosis, although it is not yet clear if these are overexpression artifacts.

Glenda Bishop and Andrea Wilson: Frank, sounds a lot like Aβ. Some think it is good, some think it is bad ...

Frank LaFerla: Glenda and Andrea, it could be that the signaling role, especially with regard to its function in modulating calcium signaling, underlies the effects on apoptosis.

June Kinoshita: Malcolm, can you go a bit more into why you think these fly papers are enlightening?

Malcolm Leissring: Well, the fly papers show the effects of overexpression of AβPP constructs in an intact, living organism. The take-home message seems to be that the AICD is a necessary domain for the effects. The effects seem to be of two types, depending on the locus of expression: (1) synaptic bouton differentiation and morphology, and (2) interaction with the extracellular matrix.

Glenda Bishop and Andrea Wilson: Has anyone tried overexpressing the AICD in mice yet?

Malcolm Leissring: I am working on it. I have 12 founders!

Frank LaFerla: You have 12 founders that overexpress AICD?

Malcolm Leissring: And Fe65, using a bicistronic construct.

Frank LaFerla: Cool – are you using an inducible approach?

Malcolm Leissring: In a way ... I am using the CaMKII promoter, which does not come on until P14 or so.

Jason Shepherd: There seems to be evidence that AICD can be phosphorylated, which seems to regulate its binding to Fe65 ... This would be interesting in terms of regulation.

Glenda Bishop and Andrea Wilson: Malcolm, do you have cell lines that overexpress AICD, too?

Jason Shepherd: Is AICD not "naturally" overexpressed in cells with PS1 mutations?

Malcolm Leissring: Jason, no, the ratio of Aβ42 to total changes, not the overall amount of γ-secretase. In fact, some data suggest that the amount of overall γ-secretase activity actually goes down for PS mutants, at least for Notch proteolysis.

Della: I would like to know, as Mark Bothwell seems to think, if the C-terminus really has a PEST sequence, as you could mutate this to stabilize the C-terminus in the transgenic mice or cells.

Jason Shepherd: I can dig out the reference for you, Della.

Malcolm Leissring: Della, what is a PEST sequence?

Della: Malcolm, this is a signal to the proteasome system for degradation.

Frank LaFerla: It is a degradation sequence.

Malcolm Leissring: AICD gets degraded by insulin-degrading enzyme – not by the proteasome.

Frank LaFerla: Christian Haas's group recently showed that AICD is degraded by IDE.

Della: AICD has been shown last year to be degraded *in vitro* by the proteasome. (See [16])

Frank LaFerla: By the way, getting back to the AICD story, Luciano's group has a paper that is out in JBC [17] showing that APLP1 and 2 also release an AICD-like domain.

Malcolm Leissring: No big surprise.

Glenda Bishop and Andrea Wilson: Is the AICD-like domain from APLP molecules very similar to AICD from AβPP?

Malcolm Leissring: Glenda and Andrea, yes. Almost totally identical.

Frank LaFerla: APLPs lack the Aβ sequence, but the AICD are very similar.

Malcolm Leissring: All contain a GYENPTY sequence.

Glenda Bishop and Andrea Wilson: Malcolm, is that sequence the "active" part?

Malcolm Leissring: Glenda and Andrea, GYENPTY is a domain that is involved in internalization and interaction with other proteins like Fe65. Whether these two functions are related is not known.

Jason Shepherd: Does overexpression of AβPP itself lead to increased AICD, though?

Malcolm Leissring: Jason, it should, which makes the reports that AICD is toxic quite curious.

Frank LaFerla: Jason, it may be that AICD levels are very tightly regulated and that overexpressing AβPP will not lead to increased AICD. Christian Haas's/Steiner's group has shown that presenilin mutations which increase Aβ42 levels do not necessarily lead to a concomitant increase in AICD.

Malcolm Leissring: Frank, but if Aβ goes up (as it does), then by definition AICD also goes up. I suppose that the amount of "signaling-competent" AICD might be regulated, though.

Frank LaFerla: Malcolm, the processing may go up, but the steady levels may not change.

Jason Shepherd: Perhaps the AICD-like fragments from APLPs compensate for the function of AICD in the BACE1 KO and other models where AICD production is inhibited; thus, the actual function of AICD may be masked.

Rong Wang: Frank, I got here late. Can you update for me on the relationship between the generation of Aβ and AICD? Are they the same processing event or independent from each other? Thanks.

Frank LaFerla: Rong, it appears as though AICD is liberated by a γ-secretase type activity that occurs at a downstream site that is referred to as the epsilon cleavage site. Cleavage at this epsilon site is presenilin-dependent.

Rong Wang: Frank, so the generation of Aβ and generation of AICD are independent proteolytic events, right?

Frank LaFerla: Rong, there are three papers (Sisodia [18], Beyreuther [19], and Haas [20]) showing that AICD is cleaved downstream of the Aβ sequence.

June Kinoshita: Frank, would the AICD be different depending on whether you have Aβ40 or 42?

Frank LaFerla: June, good question. Since cleavage occurs downstream at position 49 or 50 (relative to Aβ), it would be expected that AICD is not that different. Otherwise, we would have predicted that an AICD 57 or 59 would have emerged, depending on whether Aβ42 or 40 was generated. June, that does not mean that AICD 57 or 59 does not exist. Luciano's group did some mass spect analysis and showed that a small amount can be found *in vivo*.

June Kinoshita: Interesting, Frank. And there have been reports of elevated C99 in AD brain. So do those brains have more or less AICD? Also, is it possible that there might be functional differences between the AICD 57 and 59 fragments?

Frank LaFerla: June, as far as I know, no one has quantified AICD 57 or 59 levels in different stages of AD; the closest report was by Luciano–his group in their original paper published in JAD [21].

June Kinoshita: Rong, is that something you are looking at (quantifying AICD 57 and 59) in AD brain?

Rong Wang: June, not yet.

Glenda Bishop and Andrea Wilson: Can the AICD fragment be cleaved from AβPP while Aβ is still attached to the N-terminal region, or can it only be cleaved after Aβ is removed?

Frank LaFerla: Andrea, according to those three reports, it looks like AICD is liberated first, followed by cleavage at the γ-secretase site.

Glenda Bishop and Andrea Wilson: Will AICD cleavage always, then, lead to γ-cleavage?

Frank LaFerla: Curiously, it seems like the β-CTF (C99) is much more a substrate for generating AICD than the α-CTF (C83).

Glenda Bishop and Andrea Wilson: So can AICD be cleaved from the full AβPP as well as from CTF?

Frank LaFerla: I think it needs to be cleaved from C99. Not sure if it can be generated from the holoprotein. It might not be a good substrate.

Stavros Therianos: Would different levels of AICD 57 and 59 have a differential effect on Fe65 nuclear localization?

Frank LaFerla: Stavros, I do not think that has been addressed, yet. They are differentially unstable, at least in cell culture.

June Kinoshita: Frank, what are the most pressing open questions with respect to AICD that you would like to see answered?

Frank LaFerla: 1) What genes are specifically upregulated by AICD?; 2) Do the APLP1 and 2 AICD-like domains compensate for AβPP AICD?; 3) I would like to see AICD be overexpressed in a mouse model; I think it would have to be done in an inducible fashion; 4) What effect does AICD have on the pathogenesis of AD? Is it a normal physiologic function, or does it truly lead to a pathological consequence, as well (perhaps apoptosis, as some groups have suggested)? I think those are some of the important questions that need to be addressed.

June Kinoshita: Frank, thanks so much for leading today's discussion.

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