Signaling interactions among neurons impact cell fitness and death in Alzheimer’s disease

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From the Contents

Introduction 784
Search Strategy and Selection Criteria 785
Drosophila Melanogaster as a Model for Alzheimer’s Disease 785
Advantages of Modeling Alzheimer’s Disease in the Drosophila Eye 785
Aberrant c-Jun N-Terminal Kinase Signaling in Alzheimer’s Disease 785
Cell Competition for Intercellular Interactions 786
Context-Dependent Roles of Cell Competition and Intercellular Interactions 786
New Approaches to Understand Aberrant Cell-Cell Interactions in Alzheimer’s Disease 786
Conclusion 787

Abstract

The pathology of Alzheimer’s disease involves a long preclinical period, where the characteristic clinical symptoms of the changes in the brain are undetectable. During the preclinical period, homeostatic mechanisms may help prevent widespread cell death. Evidence has pointed towards selective cell death of diseased neurons playing a potentially protective role. As the disease progresses, dysregulation of signaling pathways that govern cell death contributes to neurodegeneration. Aberrant activation of the c-Jun N-terminal kinase pathway has been established in human and animal models of Alzheimer’s disease caused by amyloid-beta 42- or tau-mediated neurodegeneration. Closely related studies in Drosophila that examine amyloid-beta 42 in a subset of neurons suggest complex interplay between amyloid-beta 42-expressing and wild-type cells. This review examines the role of c-Jun N-terminal kinase signaling in the context of cell competition and short-range signaling interactions between amyloid-beta 42-expressing and wild-type neurons. Cell competition is a conserved phenomenon regulating tissue integrity by assessing the fitness of cells relative to their neighbors and eliminating suboptimal cells. Somatic clones of amyloid-beta 42 that juxtapose genetically distinct neuronal cell populations show promise for studying neurodegeneration. Generating genetic mosaics with labeled clones of amyloid-beta 42- or tau-expressing and wild-type neurons will allow us to understand how short-range signaling alterations trigger cell death in neurons and thereby contribute to the progression of Alzheimer’s disease. These approaches have the potential to uncover biomarkers for early Alzheimer’s disease detection and new therapeutic targets for intervention.

Key Words: Alzheimer’s disease; amyloid-beta 42 mediated neurodegeneration; cell competition; Drosophila; c-Jun N-terminal kinase signaling; suboptimal cell; super competition; super competitor cell; two clone-approach; wild type cell

Introduction

Alzheimer’s disease (AD) is a progressive neurodegenerative disease predominantly affecting people older than 65 (Knopman et al., 2021). During the onset of disease, impairments in memory and cognitive function occur, ultimately resulting in the death of the individual (McKhan et al., 1984; Knopman et al., 2021). Widespread cell death can be seen as a shrinkage in the size of the cortex, concomitant with cognitive function impairments seen in the disease (McKhan et al., 1984; Shankar et al., 2008; Knopman et al., 2021).

Yet, there is a long latency period before clinical symptoms appear, and the changes to the brain occurring prior to symptom manifestation have not been well understood (Hardy, 2009; Yeates et al., 2019). To date, there is no cure for AD, and only a few effective treatments have been developed. The vast majority of pharmaceutical treatments fail along the path from initial drug development and testing to animal models and finally, clinical trials (Goldman et al., 2018; Deshpande et al., 2019).

The mechanisms underlying disease etiology and progression have been a subject of considerable study. The hallmark of AD are extracellular accumulation of amyloid-beta 42 (Aβ42) plaques and intracellular accumulation of tau protein in neurofibrillary tangles. Aβ42, a 42 amino acid polypeptide, is generated after amyloid precursor protein is cleaved by β-secretase and γ-secretase (Glenner and Wong, 1984; Masters et al., 1985; Siegel et al., 2017). Familial AD is associated with mutations in amyloid precursor protein and the presenilin 1 and presenilin 2 genes (Magee, 1995; Lleó et al., 2002; Eggert et al., 2004; Kelleher and Shen, 2017). Aβ42 and prone to aggregation, forming extracellular Aβ plaques (Table 1; Tate et al., 2011; Sarkar et al., 2016; Cline et al., 2018; Yeates et al., 2019, 2020). Deposition of Aβ42 plaques follows a sequence, affecting the neocortex first, followed by the hippocampus and other brain regions (Thal et al., 2002; Sengoku, 2020). However, disease progression is complex as further cell signaling changes occur downstream of Aβ42 and neurofibrillary tangle accumulation (Cline et al., 2018).

A substantial body of research suggests that AD’s progression involves aberrant activation of signaling pathways that are normally tightly controlled, including those that regulate cell death. In this review, we will focus on alterations to signaling pathways that occur downstream of Aβ42 accumulation. Similar approaches are being pursued downstream of the expression of hyperphosphorylated forms of tau in Drosophila (Singh et al., unpublished data). The evolutionary conserved c-Jun N-terminal Kinase (JNK) pathway is one such pathway implicated in animal models of AD (Tare et al., 2011; Yeates et al., 2019; Irwin et al., 2020). JNK signaling plays an important role in development. Aberrant activation of JNK occurs during stress response, cell competition, and neurodegeneration, ultimately leading to cell death (Sun et al., 2022). An increase in JNK phosphorylation and activation has been reported in AD patients (Wang et al., 2014; Yarza et al., 2015), and robust evidence from animal models points to JNK activation as a key part of the neurodegeneration observed downstream of Aβ42 accumulation (Tare et al., 2011; Yeates et al., 2020; Goga et al., 2021). However, studies have largely focused on models in which Aβ42 is expressed uniformly throughout neuronal tissue (Cao et al., 2008; Tare et al., 2011; Moran et al., 2013; Sarkar et al., 2018; Yeates et al., 2019; Irwin et al., 2020). As a result, there is no information on how Aβ42-expressing versus wild-type neuronal cell populations respond to Aβ42 accumulation. Given the multitude of signaling pathways implicated in AD pathology and the progressive nature of the disease, it is necessary to understand how signaling dynamics between cell populations contribute to disease pathology (Yeates et al., 2019, 2020).

From the Contents

Introduction 784
Search Strategy and Selection Criteria 785
Drosophila Melanogaster as a Model for Alzheimer’s Disease 785
Advantages of Modeling Alzheimer’s Disease in the Drosophila Eye 785
Aberrant c-Jun N-Terminal Kinase Signaling in Alzheimer’s Disease 785
Cell Competition for Intercellular Interactions 786
Context-Dependent Roles of Cell Competition and Intercellular Interactions 786
New Approaches to Understand Aberrant Cell-Cell Interactions in Alzheimer’s Disease 786
Conclusion 787
Crosstalk among cells and their neighbors occurs through secreted signals, activation of intracellular signaling pathways as well as expression of cell surface markers. Cell competition is one such process implicated both in AD and in cell death mediated by JNK signaling. Cell competition is a process where the juxtaposition of genetically different cells (which are individually viable and contribute to tissue or organ development) causes the elimination of the less fit group of cells by active signaling from the more fit neighbors (Table 1) (Morata and Ripoll, 1975; Baker, 2020). Differential expression of cell surface markers and signaling pathway activity determines cellular fitness, and explains how less fit cells are eliminated. Determination of cell fitness depends on the context, with strong mutations resulting in super-competitor cells that proliferate while wild-type neighbors are eliminated (Table 1; Moreno and Basler, 2004). Introducing cell competition into our conceptualization of AD may help us understand how genetic and environmental factors combine to induce neurodegeneration. Neuronal degeneration in AD begins on a smaller scale, affecting fewer areas of the brain, and progressively affects more brain regions. By investigating local signaling changes and cell death in AD models, we gain a more comprehensive picture of disease progression and find new targets for intervention.

**Search Strategy and Selection Criteria**

We searched PubMed for studies published from 2012 to 2022 using the following keywords: Alzheimer’s disease, neurodegeneration, neuronal cell death, cell competition, super competition, FLP/FRT, Drosophila, MARCM, mosaic analysis, JNK signaling, and two clone-approach.

**Drosophila Melanogaster as a Model for Alzheimer’s Disease**

The fruit fly, *Drosophila melanogaster*, is an excellent model to study neurodegenerative diseases (Pandey and Nichols, 2011; Singh and Irvine, 2012; Fernandez-Funez et al., 2013; Bolus et al., 2020). Approximately 70% of the genes associated with human disease have homologs in *Drosophila* (Kaffman et al., 2005), and many components of synapse structure and neurotransmission are conserved between flies and humans (McGurk et al., 2015; Sarkar et al., 2016; Yeates et al., 2019). Furthermore, flies are well-suited to genetic and pharmacological screens, with numerous large-scale screens yielding insights into genetic mechanisms in neurodegeneration. Neurodegeneration in AD begins on a smaller scale, affecting fewer areas of the brain, and progressively affects more brain regions. By investigating local signaling changes and cell death in AD models, we gain a more comprehensive picture of disease progression and find new targets for intervention.

**Advantages of Modeling Alzheimer’s Disease in the Drosophila Eye**

*Drosophila* eye serves as an ideal organ system to assay the effects of neurodegeneration as (a) the genes involved in eye development exhibit consistent and functional similarities between insects and humans; (b) eyes are not essential for the viability or fertility of the fly (Fortini et al., 2000; Pandey and Nichols, 2011; Rincon-Limas et al., 2012; Singh and Irvine, 2012; Singh et al., 2012; Tare et al., 2013; Goga et al., 2020); and (c) *Drosophila* has a fully accessible nervous system with an architecture that separates specialized functions such as vision, olfaction, learning, and memory. This allows functional and behavioral studies (e.g., investigating neurotransmission or memory) in *Drosophila* neurodegeneration models. The adult compound eye of *Drosophila* consists of 800-unit eyes and develops from the eye imaginal disc of the larva (Figure 1; Garcia-Bellido and Merriam, 1969; Haynie and Bryant, 1986; Tare et al., 2013), which is a very well-studied system in terms of patterning, growth regulation, neural development and modeling human disease (Ready et al., 1976; Singh and Irvine, 2012; Singh et al., 2012; Tare et al., 2013; Goga et al., 2020). Retinal precursor cells are differentiated into photoreceptor clusters and restricted to the region posterior to the furrow (Wolff and Ready, 1993; Kumar, 2011). Thus, the *Drosophila* eye serves as an excellent model to study cell death in AD (Tare et al., 2011; Figures 1 and 2). The targeted misexpression genetic strategy of the Gal4-UAS (upstream activation sequence) system (Brand and Perrimon, 1993) is commonly used to model human diseases in *Drosophila*. This system allows the expression of a transgene (e.g., human Aβ42) in a specific tissue of interest (e.g., Drosophila retina). The transgenic expression of Aβ42 in flies is a model of disease pathology including amyloid plaque formation and aggregation, cell death, and defects in learning and memory (Iijima et al., 2004; Cao et al., 2008; Hirth, 2010; Tare et al., 2011; Moran et al., 2013; Steffensmeier et al., 2013; Sarkar et al., 2018). Irwin et al. (2020) have shown that the expression of Aβ42 in developing retinal neurons of the fly eye results in progressive neurodegeneration (Tare et al., 2011; Figure 2). Other AD models utilize different Gal4 drivers or express tau or other genetic lesions linked to this disease in *Drosophila*. However, among these, the Aβ42 model is a well-established model and a key focus of this review.

**Ablerrant c-Jun N-Terminal Kinase Signaling in Alzheimer’s Disease**

Aβ42 accumulation triggers neurodegeneration through aberrant activation of signaling pathways. Previous studies suggest upregulation of JNK signaling triggers neuronal cell death in AD (Tare et al., 2011; Yarza et al., 2015; Sarkar et al., 2016; Bonini et al., 2016; Yoon et al., 2017). JNKs are involved in the regulation of skin development in mammals (Geuken et al., 2005) and is activated in apoptosis, cell growth, inflammation, and immune response. JNK signaling is activated by binding of the ligand Egr (Egr) to its receptor(s) Wg and/or Grindelwald (Grind) (Kanda et al., 2002; Moreno et al., 2002). Activation of the receptors induces a cascade of kinases like hemipterous (hep) the Drosophila JNK and basket (bsk), the Drosophila JNK (Glise et al., 1995; Slus et al., 1996; Holland et al., 1997). Bsk phosphorylates and activates the transcription factor Jun that is then translocated to the nucleus and induces the expression of JNK target genes (Slus et al., 1996; Kockel et al., 2001). Puckered (Puc), a dual-specificity phosphatase, is the transcriptional target of JNK signaling and regulates the JNK pathway (Martin-Blanco et al., 1998; Stronach, 2005). JNK signaling induces cell death both through caspase-dependent mechanisms by activating pro-apoptotic factors like head involution defective (hid), reaper (rpr), grim, and through caspase-independent mechanisms (Martin-Blanco et al., 1998; Singh et al., 2006).

JNK phosphorylates tau and amyloid precursor protein in vitro (Yoon et al., 2012; Sun et al., 2022), thereby promoting the accumulation of hyperphosphorylated tau and Aβ42. In Drosophila, misexpression of Aβ42 in neurons of the brain leads to impaired locomotor function, age-dependent learning defects, progressive loss of neurons, and reduced lifespan (Iijima et al., 2004; Cao et al., 2008; Tare et al., 2011; Moran et al., 2013; Steffensmeier et al., 2013; Cutler et al., 2015; Irwin et al., 2020). Earlier, it has been reported that Aβ42 induces aberrant cellular morphology and increased cell death in the developing retina (Morata et al., 2021; Maruyama and Fujita, 2022). Although the protein is found in developing retinal neurons of the fly eye results in progressive neurodegeneration (Tare et al., 2011; Irwin et al., 2020). Activation of JNK signaling exacerbated Aβ42 toxicity, whereas pharmacological regulation of the JNK pathway (Martin-Blanco et al., 1998; Stronach, 2005). JNK signaling induces cell death both through caspase-dependent mechanisms by activating pro-apoptotic factors like head involution defective (hid), reaper (rpr), grim, and through caspase-independent mechanisms (Martin-Blanco et al., 1998; Singh et al., 2006).

**Do Cell Competition and/or Intercellular Interactions Drive Neurodegeneration?**

Cell competition is involved in the regulation of tissue integrity and homeostasis and has important implications in several diseases linked to impaired proliferation and/or survival, including neurodegeneration and cancer (Figure 3 and Table 1; Coelho and Moreno, 2019; Marques-Reis and Moreno, 2019; Morata et al., 2021; Morata and Ripoll, 1975), recent studies on the molecular mechanisms underlying cell competition revealed the role of membrane proteins. Differential expression of membrane proteins mediates information on cell fitness status, promoting apoptosis when cells are judged to be less fit than their neighbors. On the other hand, in tumorigenesis studies, cells with higher levels of Myc become super-competitive to the elimination of wild-type (WT) neighbors (Figure 3; Moreno and Basler, 2004; Parker et al., 2021). Interestingly, it has been suggested that cell competition may play a role in AD (Coelho et al., 2018; Coelho and Moreno, 2019; Yeates et al., 2020). The competition between different cell types, AD, and Alzheimers suggest that a complete understanding of interactions between neighboring cells may shed light on disease progression in AD.
Cell competition is first described in *Drosophila melanogaster*. The original study examined Minute mutants, which have impaired ribosomal activity due to mutations in ribosomal protein genes (*Morata and Ripoll, 1975; Marygold et al., 2007; Akiyama et al., 2018; Baumgartner et al., 2021*). Heterozygous Minute flies have a short developmental delay. In genetic mosaics of wild-type and heterozygous Minute cells, the Minute cells were eliminated (*Morata and Ripoll, 1975*). A similar type of cell competition has been identified in both *Drosophila* and mammals in homozygous Mahjong mutants (*Tamori et al., 2010*). In the *Drosophila* wing disc, Mahjong-knockout cells are eliminated when surrounded by wild-type cells. Mahjong knockdown cells in mammalian cell cultures are similarly eliminated. The mechanism of how these cells occurs through JNK-mediated apoptosis (*Tamori et al., 2010; Kajita and Fujita, 2015*).

The transmembrane protein flower (*Fwe*) is a key marker of cell fitness, determined through differential expression of three splice isoforms, Fwe$^{aa}$, Fwe$^{bb}$, and Fwe$^{cc}$ (*Coelho and Moreno, 2019*). Under normal circumstances, Fwe$^{aa}$ is ubiquitously expressed. Downregulation of Fwe$^{aa}$ associated with low fitness leads up to regulation of the Fwe$^{cc}$ isoforms. Cells are marked for death and eliminated when they express the Fwe$^{cc}$ isoform relative to neighboring cells (*Rihn et al., 2010*). In *Drosophila*, the *Drosophila* homolog of Secreted Protein, Acidic, Cysteine-Rich introduces an additional layer of regulation of cell competition. When transcriptionally upregulated in suboptimal “loser” cells, dsparc inhibited caspase activation, potentially allowing cells to recover from a transient reduction in their fitness (*Portela et al., 2010*). Downstream of Fwe and Secreted Protein, Acidic, Cysteine-Rich, transcription of ahuiuotl (azot) serves as a fitness checkpoint prior to initiation of pro-apoptotic signaling. Furthermore, azot knockouts showed reduced lifespan, providing further evidence that cell competition can promote tissue health by eliminating less fit cells (*Merino et al., 2015*).

Elimination of cells by cell competition and/or intercellular interactions has important implications for understanding the progression from the long asymptomatic phase of AD to the presentation of clinical symptoms. Deposition of Aβ$_{42}$ can be detected over many years prior to the onset of clinical symptoms in AD (*Mufson et al., 2016*). Moreover, higher levels of amyloid deposition in clinically normal subjects correlate with lower scores on episodic memory tests (*Sperling et al., 2013*). A wealth of evidence points to complex alterations to cells and cellular networks in AD; however, compensatory mechanisms may mask these alterations, delaying the onset of clinical symptoms (*De strooper and Karran, 2016*). The elimination of suboptimal neurons early in AD could delay the onset of widespread neurodegeneration and hence may play a protective role against AD. Enhancing this selective cell death before neurodegeneration becomes widespread, could be beneficial in treating AD (*Coelho et al., 2018; Coelho and Moreno, 2019*). In addition to the role cell competition and intercellular interactions may play in the asymptomatic phase of AD, insights from these studies may help explain the molecular underpinnings of AD neurodegeneration.

**Context-Dependent Roles of Cell Competition and Intercellular Interactions**

Cell competition is highly context-dependent and can lead to the death of either wild-type or mutant cells under different circumstances. Preferential death of wild-type cells was observed in cells expressing Aβ$_{42}$ as a reporter for cell fitness in a study on neurodegeneration in AD (*Yeates et al., 2020*). In cell competition studies, the death of wild-type cells is observed in cancer models. Further, cell competition can either contribute to or defend against mechanisms involved in cancer (*Kanda and Igaki, 2020*). Loss of the cell polarity gene *scrib* mutant tissue surrounded by wild-type cells fail to proliferate; the wild-type cells prevent proliferation by releasing JNK to activate Yorkie, with evidence supporting a bistable positive feedback loop between Yorkie and Myc (*Yeates et al., 2020; Zioz et al., 2010*). Thus, oncogenic cooperation showed a role in cell competition in tumor suppression. Alternatively, super-competition is a phenomenon associated with tumor growth where oncogenic cells proliferate while wild-type cells are eliminated. Cells expressing higher levels of the proto-oncogene *dMyc* become super-competitors, with neighboring wild-type cells judged as less fit in comparison (*Moreno and Basler, 2004*). Similar super-competition has also been described for Hippo pathway mutants, which results in proliferation of super competitor cell. This leads to the apoptosis of neighboring wild-type cells. Induction of apoptosis leads to cell fragmentation. In cell competition in tumor suppression, the elimination of suboptimal neurons early in AD could delay the onset of widespread neurodegeneration and hence may play a protective role against AD. Enhancing this selective cell death before neurodegeneration becomes widespread, could be beneficial in treating AD (*Coelho et al., 2018; Coelho and Moreno, 2019*). In addition to the role cell competition and intercellular interactions may play in the asymptomatic phase of AD, insights from these studies may help explain the molecular underpinnings of AD neurodegeneration.

**New Approaches to Understand Aberrant Cell-Cell Interactions in Alzheimer’s Disease**

Recently, we expanded upon existing *Drosophila* models of AD by creating a “two-clone” system to study the signaling interactions between Aβ$_{42}$-expressing and WT neurons (*Yeates et al., 2020*). This system uses multiple knockdown or overexpression of key genes, such as Fwe or FRT and the Gal4/UAS/Gal80 system to generate mosaics in developing retinal neurons (*Xu et al., 1995; Blair, 2003*). A cross produces *Drosophila* larvae with the following genetic components: a Fwe$^{aa}$ expressed under a...
heat shock promoter (hs-FLP), one copy of green fluorescent protein (GFP) under a ubiquitin promoter (ubi-GFP), one copy of GaIL80 under a tubulin promoter (TubGAL80), and Aβ42, expressed under the control of the GMR-Gal4 driver (GMR>Ub-Aβ42). Therefore, GMR>Ub-Aβ42 drives the expression of Aβ42 in neurons of the developing retina. Gal80 is a repressor protein that binds Gal4 and renders it inactive (Yeates et al., 2020). These components used in combination with the FLP/FRT system lead to the generation of two labeled cell populations. The Flippase directs recombination between mini-white clones that were located adjacent to ubi-GFP and TubGAL80. The cross generates progeny with the full genotype hsGFP; GMR>Ub-Aβ42/FRT82BubiGAL80/FRT82Bubi-GFP. A heat shock at 37°C triggers mitotic recombination at the FRT sites, which results in two clonal populations of neurons: one cell population that expresses the GFP reporter and Aβ42 in developing retinal neurons and the other cell population is WT. Both the Aβ42-expressing clone (GFP positive) and WT sister clone (GFP negative) are easily distinguished from the background, which weakly expresses GFP; facilitating quantification of the clone size (Figure 4).

**Figure 4** | A two-clone system to study interactions between Aβ42-expressing and WT cell populations.
(A) Generation of genetic mosaics in Drosophila eye imaginal discs. By using FLP/FRT system in conjunction with the Gal4/USP/Gal80 system and GFP expressed under an ubiquitin promoter, labeled clones can be generated (see Yeates et al., 2020). (B) Control clones. Mitotic recombination yields two sister clones: one with two copies of GFP and one with no GFP expression. Both clones are identifiable against the background, which weakly expresses GFP. GFP-positive and GFP-negative clones were found to be equivalent in size. (C) Aβ42-expressing experimental clones where GMR-Gal4 driver is used to drive expression of Aβ42 in the developing retina. Mitotic recombination generates two sister clones: one is GFP-positive and expresses Aβ42. The other is GFP-negative and WT, expressing no Aβ42 due to two copies of Gal4 repressor Gal80 (expressed under a tubulin promoter). Experimental data showed a surprising decrease in the size of WT clones adjacent to Aβ42-expressing clones. Evidence from DCP-1 (activated caspase) staining showed cell death in WT clones, suggesting that in this model, WT cells first, Aβ42-expressing cells appeared to die later. JNK activation was observed in Aβ42-expressing clones. Blocking JNK activation in Aβ42-expressing clones prevented the death of WT cells. AD: Alzheimer’s disease; Aβ42: amyloid beta 42; FLP/FRT: Flippase/Flippase recognition target; GFP: green fluorescent protein; GMR: glial multiple repeat; JNK: c-Jun N-terminal kinase; WT: wild type.

Because these two clones originate from a single progenitor cell, one would expect them to be equivalent in size. Indeed, in the absence of Aβ42, sister clones were not significantly different in size (Figure 4). However, in the presence of Aβ42-expressing clones, it was wild-type clones that were smaller, suggesting a more complex interplay between Aβ42-expressing and WT cells (Figure 4). Such differences in clone size may be related to different levels of cell death. A comparison of cell death revealed that many more cells were dying in the WT clones. Thus, Aβ42-expressing clones and their WT sister clones start under similar conditions, but WT cells die first, followed by Aβ42-expressing cells (Yeates et al., 2020; Figure 4).

The JNK signaling pathway is implicated in cell death observed in fly eye model and in mammalian AD models (Jen et al., 2020). The two-clone system demonstrated an activation of JNK in Aβ42-expressing clones in comparison to WT clones. Furthermore, misexpression of a constitutively active form of the JNK kinase homolog in flies, hemipterous (hep) in Aβ42-expressing clones (Glise et al., 1995; Tournier et al., 1997) resulted in significantly smaller WT clones in comparison to their sister clones expressing both Aβ42 and hep+. On the contrary, decreasing JNK activity in Aβ42-expressing clones by misexpression of a dominant-negative form of Drosophila JNK, basket (bsk+) in Aβ42-expressing clones restored the size of WT sister clones. These studies provided evidence for complex crosstalk between WT and Aβ42-expressing clones, ultimately leading to cell death mediated at least in part by the JNK pathway. Though cell death occurs in Aβ42-expressing clones, it appears to occur later. Interactions between these clones lead to sensitization of WT cells and eventually cell death, resulting in smaller WT clones (Figure 4).

The study of cell competition in AD has uncovered evidence for selective cell death of Aβ42-expressing neurons (Coelho et al., 2018; Coelho and Moreno, 2020; Costa-Rodrigues et al., 2021). Upregulation of markers of loser cell status, Fweα, and Aβ42 was restored following uniform expression of Aβ42 throughout the Drosophila retina. Then Aβ42 was expressed in small clones within the neuroepithelium of the fly eye disc. Flower and Aβ42 were upregulated within these clones, indicative of lower fitness, and the clones were eliminated over time by apoptosis (Coelho et al., 2018). Figure 3.

However, clones in this system were small, and Aβ42-expressing and WT clones were not labeled and terminated within the same disc. Fweα and cell death marker DCP-1 were observed outside of the Aβ42-expressing clones as well. On the whole, this selective death of Aβ42-expressing cells had a protective effect (Coelho et al., 2018). This was seen in related research on neuronal circuit pruning, in which selectively removing suboptimal cells increases the function of the circuit as a whole: hyperactive neurons may be eliminated through cell competition, restoring circuit function (Coelho and Moreno, 2020; Costa-Rodrigues et al., 2021). For cell competition to protect against neurodegeneration in AD, diseased neurons must be correctly identified as less fit and eliminated via an early compensatory mechanism before pathological changes accumulate.

**Conclusion**

AD is a multifactorial disease with no cure (Yeates et al., 2019; Jeon et al., 2020). Since neurons are post-mitotic cells and adult neurogenesis is limited, early detection of neurodegeneration is paramount to preserving as much cognitive function as possible. Understanding the molecular mechanisms underlying AD will give us better tools to delay or prevent widespread neurodegeneration.

Substantial evidence shows that dysregulation of physiological signaling pathways contributes to the etiology and progression of AD. JNK signaling plays important roles in development, including neuronal system development and neuronal pruning (Bornstein et al., 2015; Schellino et al., 2019; Zhu et al., 2019). Aberrant activation of JNK signaling is involved in the widespread neuronal degeneration seen in the two-clone system. JNK signaling is upregulated in Aβ42-expressing clones concomitant with the elimination of wild-type cells; blocking JNK in Aβ42-expressing clones prevented the death of WT clones (Yeates et al., 2020). These findings suggest the utility of components of the JNK signaling pathway as biomarkers.

The selective cell death that occurs during cell competition can improve tissue health overall. However, when JNK signaling and selective cell death are left unchecked, they contribute to the neurodegeneration seen in AD (Wang et al., 2014; Yarza et al., 2015). Research in cancer models demonstrates that cell competition can result in the death of wild-type cells, facilitating the proliferation of cancer cells. Cell competition and other processes regulating tissue homeostasis could protect against damage from Aβ plaques accumulation even on in AD only to effectively contribute to cell death and widespread neurodegeneration later in disease progression.

Clone models have promising utility to study the short-range signaling interactions contributing to neurodegeneration. Understanding the interplay between JNK signaling and cell competition may allow us to determine which molecules could best function as biomarkers in AD (Sharma et al., 2021). Cell fitness marker Fweα may have utility as a biomarker of early-stage AD and requires further investigation. Azot is a fitness sensor, which induces expression of pro-apoptotic factor head involution defective (hid) (Costa-Rodrigues et al., 2021). These components may also serve as therapeutic targets. Furthermore, emerging research has linked changes in microRNA expression with both AD and JNK-mediated neurodegeneration, generating interest in microRNAs as biomarkers (Angelucci et al., 2019; He et al., 2020). Selective cell death may help protect against neurodegeneration in AD, and for this reason, the molecular players underlying cell competition should be considered potential therapeutic targets. Understanding the conditions which prime cells for selective cell death or progressive neurodegeneration will improve our range of therapeutic targets and biomarkers for AD.

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