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Decrease in catalytic capacity of γ-secretase can facilitate pathogenesis in sporadic and Familial Alzheimer's disease

Željko M. Svedružić [a,b,*], Katarina Popović [c], Vesna Šendula-Jengić [a,c]

[a] Medical Biochemistry, PB Rab, Faculty of Medicine, University of Rijeka, Rab, Croatia
[b] Department of Biotechnology, University of Rijeka, Rijeka, Croatia
[c] Neurology and Geriatrics, PB Rab, Faculty of Medicine, University of Rijeka, Rab, Croatia

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ABSTRACT

Background: Alzheimer's disease can be a result of an age-induced disparity between increase in cellular metabolism of Aβ peptides and decrease in maximal activity of a membrane-embedded protease γ-secretase.
Results: We compared activity of WT γ-secretase with the activity of 6 FAD mutants in its presenilin-1 component and 5 FAD mutants in Aβ-part of its APP substrate (Familial Alzheimer's disease). All 11 FAD mutations show linear correlation between the decrease in maximal activity and the clinically observed age-of-onset and age-of-death. Biphasic-inhibitors showed that a higher ratio between physiological Aβ-production and the maximal activity of γ-secretase can be observed in cells that can facilitate pathogenic changes in Aβ-products. For example, Aβ production in cells with WT γ-secretase is at 11% of its maximal activity, with delta-exon-9 mutant at 26%, while with M139V mutant is at 28% of the maximal activity. In the same conditions, G384A mutant is fully saturated and at its maximal activity. Similarly, Aβ production in cells with γ-secretase complex carrying Aph1A$_L$ component is 12% of its maximal activity, while in cells with Aph1B complex is 26% of its maximal activity. Similar to the cell-based studies, clinical studies of biphasic dose–response in plasma samples of 54 healthy individuals showed variable ratios between physiological Aβ production and the maximal activity of γ-secretase.
Conclusions: The increase in the ratio between physiological Aβ production and maximal activity of γ-secretase can be an early sign of pathogenic processes in enzyme-based, cell-based, and clinical studies of sporadic and Familiar Alzheimer's disease.

1. Introduction

Alzheimer's disease is a slowly progressing fatal neurodegenerative disorder that represents the biggest financial burden for the health care providers in developed countries (Holtzman et al., 2011). Impressive efforts in basic and pharmaceutical research have lead to more than a hundred of different therapeutic approaches. Many of them reached clinical trials, including the phase III (Doody et al., 2013; Sambamurti et al., 2011). Sadly, all of those trials led to disappointments and in some cases surprisingly daunting results (Doody et al., 2013; Tong et al., 2012). Most notably we do not understand to what extent the future efforts can be concentrated on the "amyloid hypothesis" or on some of the alternative therapeutic approaches (Hunter and Brayne, 2014). We also lack reliable early diagnostic methods that can facilitate therapeutic approaches before the onset of irreversible neurodegenerative processes (Hunter and Brayne, 2014; Holtzman et al., 2011).

The studies based on "amyloid hypothesis" have explored different evidences that the pathogenesis can be driven by changes in metabolism of Amyloid precursor protein (APP), in particular its C terminal fragment (β-CTF-APP), and the resulting Aβ peptides (Shen and Kelleher, 2007; Hunter and Brayne, 2014; Sambamurti et al., 2011). Contrary to frequent beliefs, the "amyloid hypothesis" is just a fraction of the total research effort. At the time of writing of this manuscript a Pubmed search for "Alzheimer's" disease gives more than 102,234 entries! Only about 61% of all publications on Alzheimer's disease (60,600 entries), can be retrieved using a search that is focused on the "Alzheimer's AND Aβ OR amyloid". Interestingly, only about 6% of all of the Alzheimer's disease publications, or about 5876 entries, could be retrieved with a search focused on "Alzheimer AND gamma-secretase OR beta-secretase". These numbers indicate that a wide range of possible pathogenic processes have been explored, and the main problem could be lack of insights at the key drug-target enzymes (Svedruzic et al., 2012, 2013; Sambamurti et al., 2011; Shen and Kelleher, 2007). Without adequate insights in the catalytic mechanism of γ-secretase, development of the new drug candidates and the early diagnostic methods will remain an expensive guess-work with a high risk of failure (Doody et al., 2013; Tong et al., 2012; Svedruzic et al., 2013).

* Corresponding author at: Medical Biochemistry, PB Rab, Faculty of Medicine, University of Rijeka, Rab, Croatia.
E-mail address: zsvedruz@biol.pmf.hr (Ž.M. Svedružić).

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FAD mutations (Familial Alzheimer's disease), offer unique opportunities for analysis of pathogenic changes in γ-secretase activity and Aβ production (Chavez-Gutierrez et al., 2012; Pera et al., 2013; Potter et al., 2013; Shen and Kelleher, 2007; Svedruzic et al., 2012; Jonsson et al., 2012; Seidner et al., 2006; Kumar-Singh et al., 2006; Citron et al., 1992). More than 400 mutations have been identified in the last 24 years (www.molgen.ua.ac.be/ADMutations). The mutations affect all steps in APP metabolism however the majority affects the final steps, i.e., presenilin components of γ-secretase, or Aβ part of APP protein, or apo-lipoprotein ApoE (Hunter et al., 2013; Sambamurti et al., 2011; Shen and Kelleher, 2007). There is also a fascinating protective mutation A673T-APP (Jonsson et al., 2012). Different FAD mutations can trigger disease at different age, which indicates that comparative analysis of the underlining mechanism can provide quantitative insights in the pathogenic processes (Kumar-Singh et al., 2006; Seidner et al., 2006). Unfortunately comparative studies of different FAD mutations and WT γ-secretase are still inconclusive. We do not understand why some FAD mutations can increase and some can decrease Aβ production relative to the healthy WT controls (Pera et al., 2013; Potter et al., 2013; Kumar-Singh et al., 2006; Shen and Kelleher, 2007; Citron et al., 1992; Jonsson et al., 2012), or why some of the FAD mutations can both increase and decrease Aβ production depending on the experimental approach (i.e., "gain-of-function" and "loss-of-function" debate (Potter et al., 2013; Kumar-Singh et al., 2006; Shen and Kelleher, 2007)). Finally we do not understand to what extent changes in γ-secretase activity produced by different FAD mutations can be related to the aging processes that lead to sporadic Alzheimer's disease (Hunter et al., 2013; Sambamurti et al., 2011; Kumar-Singh et al., 2006; Shen and Kelleher, 2007; Fukumoto et al., 2004; Kern and Behl, 2009; Kern et al., 2006).

In this study we provide some answers to the presented questions. Different FAD mutants and WT γ-secretase are compared using activity assays that can measure all three parameters that define the enzyme activity in cells (Ferscht, 1998; Svedruzic et al., 2013), namely: the ongoing physiological activity of γ-secretase, the maximal possible activity, and the extent of γ-secretase saturation with its substrate. Similar approaches have been used successfully to describe activation and inhibition of γ-secretase by different drug-candidates (Burton et al., 2008; Svedruzic et al., 2013), or to describe the changes in enzymatic mechanism of γ-secretase that support pathogenic shift in Aβ products and Aβ42/Aβ40 ratio (Kakuda et al., 2006; Svedruzic et al., 2012; Yin et al., 2007).

2. Results

2.1. Correlation between decrease in maximal activity of γ-secretase and "age-of-onset" and "age-of-death" for different FAD mutations

We find that decrease in γ-secretase activity caused by different FAD mutations shows linear correlation with clinically observed "age-of-onset" or "age-of-death" for each mutation (Fig. 1 and Table 1). The presented data come from our previous enzyme-based studies (Svedruzic et al., 2012), and from subsequent enzyme-based studies by a large research group (Chavez-Gutierrez et al., 2012). We combined data from two different studies to maximize statistical significance of the presented analysis, and to show that the presented correlations are not affected by different experimental approaches. The data from different studies can be normalized to the same scale by always setting the WT measurements as 100% activity, so that the corresponding FAD mutants can be presented as a percentage of the WT activity (Table 1).

Two different types of FAD mutations are included in the analysis, the mutations in presenilin 1 core of γ-secretase, and the mutations in Aβ sequence of its APP substrate (Table 1). The FAD mutations in presenilin 1 are shown as the maximal turnover rates (data taken from Fig. 8A in ref. (Svedruzic et al., 2012) and Table 2 in ref. (Chavez-Gutierrez et al., 2012)). Eleven different experiments, with WT gamma secretase and six different FAD mutations in presenilin 1 show linear correlation between maximal activity and clinically observed age-of-onset or age-of-death (Fig. 1, $R^2 = 0.88$ and 0.86 respectively). The linear correlation spans from the most aggressive FAD mutations to the least aggressive mutations and the WT enzyme. Similar linear correlations can be also observed between 5 FAD mutations in Aβ sequence of APP substrate and the WT substrate (Fig. 1, $R^2 = 0.90$ and 0.94, respectively). However for analysis of FAD mutations in the substrate, the different turnover rates represent the readings at the lowest substrate concentrations when substrate dimerization/oligomerization is at the lowest level (Svedruzic et al., 2012). This was necessary since those mutations can affect substrate dimerization (Gorman et al., 2008), and thus γ-secretase's activity in response to increasing substrate concentrations (Svedruzic et al., 2012). The readings at the lowest substrate concentrations are directly proportional to the maximal activity (Ferscht, 1998), and therefore can be used in evaluating the ratios between different maximal activities.

At the end we also show that the correlations can be observed even when all FAD mutations in presenilin 1 and Aβ part of APP substrate are combined together (Fig. 1, $R^2 = 0.9$ and 0.91 respectively). The combined approach strengthens the credibility of the presented analysis and indicates that the presented analysis could be a universal approach for studies of all FAD mutations (www.molgen.ua.ac.be/ADMutations). Following observed correlations when both mutations are combined together the predicted age-of-onset for WT is 51.1, and age-of-death is 58.5 (Fig. 1), so that the duration of the disease is about 8 years. The calculated "age-of-onset" and "age-of-death" are about 20 years earlier than the clinically observed age (Holtzman et al., 2011). Such underestimate can be expected, since the pathogenic processes driven by FAD mutations in young individuals are more aggressive than the pathogenic processes driven by age-induced slow changes in γ-secretase activity and APP metabolism (Kern et al., 2006; Theuns et al., 2003; Fukumoto et al., 2004).

The observed correlations between catalytic activity and "age-of-onset" or "age-of-death" for different FAD mutations cannot be an accidental coincidence. The presented correlations are result of a number of different measurements from different laboratories that used different experimental setup.

2.2. Biphasic inhibitors can be reliable indicators of pathogenic changes in γ-secretase activity in cells

The insights from enzyme-based studies presented in Fig. 1 can be used to analyze pathogenic processes in cells. Measurements of maximal activity of γ-secretase in cell-based assays are more complex than in the enzyme-based assays (Ferscht, 1998; Svedruzic et al., 2013). In enzyme-based assay saturation of γ-secretase with its substrate is an experimentally controlled variable (Svedruzic et al., 2012), while in cell-based assays the saturation of γ-secretase is controlled by the cell physiology (Ferscht, 1998; Svedruzic et al., 2013). Thus, to understand γ-secretase activity in cells, we have to measure the ongoing physiological activity, the maximal possible activity, and the extent of γ-secretase saturation with its β-CTF-APP substrate (Ferscht, 1998; Svedruzic et al., 2012). In earlier studies we showed that all three parameters can be quantitatively measured using biphasic inhibitors of γ-secretase (Svedruzic et al., 2013).

We measured biphasic dose response curves for DAPT using presenilin 1 and 2 double knockout MEF cells that have been transfected with human WT presenilin 1 or ΔE9, M139V and G384A FAD mutations in presenilin 1 (Bentahir et al., 2006). These cells have no modifications in their APP genes, therefore Aβ 1–40 production in the absence of DAPT represents the physiological γ-secretase activity for these cells (Svedruzic et al., 2013). The biphasic profiles show that for WT γ-secretase the physiological activity is 36.3 ± 3 pM Aβ (1–40) secreted while the maximal possible activity is 324 ± 90 pM of Aβ (1–40)