Epidermal growth factor receptor is a preferred target for treating Amyloid-β–induced memory loss

Lei Wang[a,1], Hsueh-Cheng Chiang[b,c,1,2], Wenjuan Wu[a,d,1], Bin Liang[a,e,1], Zuolei Xie[f], Xinsheng Yao[d,g], Weiwei Ma[f], Shuwen Du[f], and Yi Zhong[a,b,3]

[a]Key Laboratory for Protein Sciences of Ministry of Education, School of Life Sciences, Tsinghua University, Beijing 100084, China; [b]Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724; [c]Department of Neuroscience, State University of New York, Stony Brook, NY 11794; [d]Division of Life Science and Health, Graduate School at Shenzhen, Tsinghua University, Shenzhen 518055, China; [e]Key Laboratory for Tropical Animal and Plant Ecology of Ministry of Education, College of Life Sciences, Hainan Normal University, Haikou 571158, China; [f]JoeKai Biotech, LLC, Beijing 100084, China; and [g]Institute of Traditional Chinese Medicine and Natural Products, College of Pharmacy, Jinan University, Guangzhou 510632, China

Edited by Leslie Lars Iversen, University of Oxford, Oxford, United Kingdom, and approved September 4, 2012 (received for review May 11, 2012)

Current understanding of amyloid-β (Aβ) metabolism and toxicity provides an extensive list of potential targets for developing drugs for treating Alzheimer's disease. We took two independent approaches, including synaptic-plasticity–based analysis and behavioral screening of synthetic compounds, for identifying single compounds that are capable of rescuing the Aβ-induced memory loss in both transgenic fruit fly and transgenic mouse models. Two clinically available drugs and three synthetic compounds not only showed positive effects in behavioral tests but also antagonized the Aβ oligomers-induced activation of the epidermal growth factor receptor (EGFR). Such surprising converging outcomes from two parallel approaches lead us to conclude that EGFR is a preferred target for treating Aβ-induced memory loss.

Amyloid-β (Aβ) oligomers-induced memory loss is thought to be a hallmark of Alzheimer's disease (AD) progression (1–3). Aβ peptides are cleaved from a membrane protein APP via β- and γ-secretases' activities (4). They can be removed through activities of neprilysin, insulin-degradation enzyme, and possibly other mechanisms (5–7). Aβ is also able to bind with a large array of target proteins, such as EphB2, TNF-R1, RAGE1, and NMDA receptor and prion (8–12) to exert a wide range of effects, including synaptic transmission, protein transportation, mitochondrial functions, and others (13–15). Thus, there are a large number of potential targets for developing AD treatment based on the Aβ hypothesis, for example, the mechanisms either reducing the production of Aβ peptides or enhancing the degradation process. It remains, however, an open question as to whether some of these targets are, at the organism level, better suited for drug development than others. One important reason is that manipulating activities of such production and degradation enzymes may affect many other physiological proteins. Thus, reported failures in a number of Aβ-based drug efforts (16) stress the necessity of identifying such preferred targets.

To evaluate the possibility of finding preferred targets, we looked for overlapping and converging effects of identified targets with multiple independent approaches. First, following the direction of a synaptic-plasticity–based, mechanism-guided study, we continued to work on the signal transduction pathway of PI3-kinase that has been shown to mediate an Aβ-induced change in long-term synaptic depression as well as the Aβ-induced memory loss in Aβ42-expressing *Drosophila*, which recapitulates several AD-like symptoms (17). These efforts led us to find Aβ42 oligomers-induced activation of the epidermal growth factor receptor (EGFR) and the rescue of Aβ-induced memory loss in transgenic *Drosophila* and APP/PS1 double transgenic mouse models through treatments with clinically available EGFR inhibitors. Second, we worked to identify single compounds capable of rescuing Aβ-induced memory loss through large-scale behavioral screening with Aβ42-expressing transgenic fruit flies, followed by a confirmative behavioral assay in APP/PS1 double transgenic mice with overloaded Aβ deposits. The behavioral screening included 2,000 synthetic compounds that are potential inhibitors of protein kinases (randomly selected on the basis of the backbone structures of the chemicals). We were surprised to learn that four compounds rescued memory loss in both fly and mouse models, and three of them were capable of suppressing oligomeric Aβ42-induced EGFR activation. The current work suggests that EGFR is an important factor that mediates Aβ42 toxicity and inhibition of oligomeric Aβ42-induced EGFR activation is an effective way to treat Aβ42-induced memory loss.

Results

Ameliorating Aβ42-Induced Memory Loss by Inhibition of EGFRs in Transgenic Fruit Flies. Aβ-induced memory loss is observed across a wide range of organisms including *Drosophila*, mice, and humans, suggesting a conserved underlying molecular mechanism (18, 19). In *Drosophila*, expression of a secretory form of human Aβ42 in the brain recapitulates AD-like features, such as age-dependent accumulation of Aβ deposits, memory loss, and late-onset severe neurodegeneration (20, 21). Our earlier work reveals a role of PI3-kinase in Aβ42-induced alteration in long-term depression and memory loss (17). To find receptors associated with this PI3-kinase effect, we first evaluated behavioral effects of overloaded insulin receptor (InR) or EGFR in Aβ42-expressing *Drosophila* brains.

The severity of the memory loss induced by pan-neuronal expression of Aβ42 (*elav-Gal4 > UAS-Aβ42*) depended on the amount of expressed Aβ42. Female adult transgenic flies (*elav-Gal4/+;UAS-Aβ42/+*) were selected for the following genetic interaction study because of their lower expression level of Aβ42 resulting from gene-dosage effects (20).

Memory was measured through a well-established aversive Pavlovian olfactory conditioning assay (22), in which fruit flies learn to avoid an odor paired with electric shock in training trials. In 5-d-old transgenic female flies, expression of Aβ42 showed no detectable memory loss, and overexpressing InR or EGFR (*elav-Gal4/+;UAS-InR^{wt}/+* or *elav-Gal4/+;;UAS-EGFR^{wt}/+*) had very little effect on memory performance (Fig. 1*A*). In contrast, coexpression of EGFR with Aβ42 (*elav-Gal4/+;UAS-Aβ42/+;UAS-EGFR^{wt}/+*) produced a synergistic effect in reducing the immediate memory, whereas coexpression of InR with Aβ42 (*elav-Gal4/+;UAS-Aβ42/UAS-InR^{wt}*) yielded a memory score similar to that found with

Author contributions: L.W., H.-C.C., W.W., B.L., X.Y., W.M., S.D., and Y.Z. designed research; L.W., H.-C.C., W.W., B.L., and Z.X. performed research; L.W., H.-C.C. and X.Y. contributed new reagents/analytic tools; L.W., H.-C.C., W.W., B.L., Z.X., X.Y., W.M., S.D., and Y.Z. analyzed data; and L.W. and Y.Z. wrote the paper.

Conflict of interest statement: Behaviorally effective compounds reported in this study are under patent filings entitled Methods and compositions for treating Alzheimer's disease.

This article is a PNAS Direct Submission.

[1]L.W., H.-C.C., W.W., and B.L. contributed equally to this work.

[2]Present address: National Institute of Neurological Disorders and Stroke, Bethesda, MD 20892.

[3]To whom correspondence should be addressed. E-mail: zhongyi@cshl.edu.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1208011109/-/DCSupplemental.

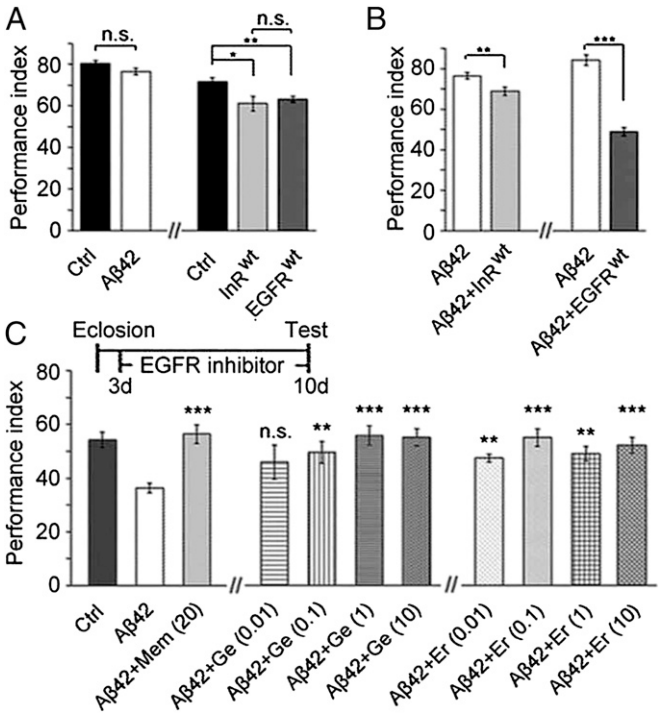

Fig. 1. Identification of the involvement of EGFR in Aβ42-induced immediate memory loss in *Drosophila*. (*A*) In 5-d-old adult female flies, no memory effects were observed in Aβ42-expressing flies, but slight defects were shown owing to pan-neuronal expression of either InR or EGFR (*elav/+;UAS-InR^{wt}/+* or *elav/+;;UAS-EGFR^{wt}/+*). n = 10–13 PIs. All data in this and following figures, unless otherwise indicated, are means ± SEM, and for t test: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$; n.s., not significant, $P > 0.05$. (*B*) Synergistic effect of EGFR, but not InR, on Aβ42-induced memory loss (*elav/+;UAS-Aβ42/+;UAS-EGFR^{wt}/+* and *elav/+;UAS-Aβ42/UAS-InR^{wt}*). n = 14–29 PIs. (*C*) Rescue of Aβ42-induced memory loss. Drug-feeding paradigm for fruit flies is illustrated for this and following figures (*Upper*). The histograms represent immediate memory for effects of drug feeding (*Lower*). Drug concentrations (in μg/mL) are indicated in brackets and the control is treated with sucrose. Mem, memantine; Ge, gefitinib; Er, erlotinib. n = 8 PIs for each group.

overexpression of InR alone (Fig. 1*B*). Such genetic interaction data imply that increased EGFR activity, instead of InR, might be relevant in causing the Aβ42-induced memory loss.

To verify this genetic observation, we tested effects of two EGFR inhibitors, gefitinib (Ge) and erlotinib (Er), which are used in clinical cancer therapy (23). Both drugs inhibit the tyrosine kinase activity of EGFR by binding with the ATP-binding sites that are conserved between fruit flies and humans (24). For the purpose of seeing a stronger memory-loss phenotype, we selected 3-d-old male adults (*elav-Gal4/Y;UAS-Aβ42/+*) for drug treatment. Memory scores were measured at day 10 of posteclosion following 4 h of drug feeding each day for 7 consecutive days (17) (Fig. 1*C* and Fig. S1*A*). It was intriguing to note that memantine (Mem), the clinically available drug for AD treatment, was capable of preventing memory loss in Aβ42-expressing flies (Fig. 1*C*). Feeding of either EGFR inhibitor also prevented memory loss in 10-d-old Aβ42-expressing males over a range of concentrations (0.01, 0.1, 1, and 10 μg/mL; Fig. 1*C*). Memory was not affected in control flies fed with higher concentrations of either Ge (100 μg/mL) or Er (40 μg/mL) (Fig. S1*B*). Behavior data suggest that inhibition of EGFRs prevents the Aβ42-induced memory loss in *Drosophila*.

Rescuing Memory Loss by Oral Administration of an EGFR Inhibitor in APP/PS1 Double Transgenic Mice. To determine the general significance of such observations, we tested the effects of Ge, which penetrates the blood–brain barrier and has some effectiveness in treating brain tumors (25, 26), in Tg(APPswe.PSEN1dE9) double transgenic mice with the expression of two mutated AD-linked transgenes: a chimeric human amyloid beta (A4) precursor protein (APPswe) and a "DeltaE9" mutant of human presenilin 1 (27). Extensive plaques are reported to be visible in early ages and the memory-loss phenotype is evident around 6–9 mo of age in double transgenic mice (27–31). The Morris water maze was used for the behavioral assay, in which mice learned to find a hidden platform (32). For drug treatment, we chose a very short period of feeding paradigm, including only a 7-d pretesting drug treatment followed with a 2-d adaption to the water maze environment and then 9 d of training and testing (Fig. 2*A*). We reasoned that such a short period of treatment is sufficient to reverse behavioral defects if the mechanism we identified is indeed a direct cause of the Aβ-induced memory loss. For this reason, we used 8-mo-old mice in which the memory-loss phenotype was evident.

Indeed, the treatment was sufficient in rescuing memory loss. Mice were trained four times a day over a period of 9 d. After training, the escape latency of wild-type (control) mice decreased (Fig. 2 *B–E*, open circles), indicating that the mice learned the location of the hidden platform. However, the double transgenic mice did not improve their performance (Fig. 2 *B–E*, filled circles). The memory loss of APP/PS1 mice was rescued after feeding with Ge in an optimal concentration (0.01 mg·kg^{-1}·d^{-1}; Fig. 2*D*) and in a wide range of concentrations (Fig. 2 *C, D, F*), which were hundreds, if not thousands, of times lower than that used for treating tumors in mice (26, 33). In contrast, with such a short period of treatment Mem was unable to improve the memory-loss phenotype (Fig. 2*E*), although it was reported to be effective with much longer treatment times (32, 34). Effects of Ge were also consistent in the plot of representative paths (Fig. 2*G*) and in quadrant occupancy times (Fig. 2 *H* and *I*). Thus, inhibition of EGFR activity is capable of rescuing the Aβ-induced memory loss in mice.

Oligomeric Aβ42-Induced Activation of EGFRs. To determine molecular mechanisms underlying observed genetic and pharmacological effects of EGFR in Aβ-induced memory loss, we assayed the level of EGFR activation in the hippocampus region through Western blotting of p-EGFR^{Tyr1068}, the site for binding with Grb2 that leads to activation of MAPK (35). The p-EGFR level was significantly increased in the hippocampus of the double transgenic mice (Fig. 3). Importantly, the increased p-EGFR level was brought back to a level similar to the control after 18 d (the duration used for memory rescue) of Ge treatment (10 mg·kg^{-1}·d^{-1}, Fig. 3), showing that elevated EGFR activity correlated well with the Aβ-induced memory loss.

To make sure that the EGFR activation was caused by expressed Aβ, we measured p-EGFR levels in cultured COS-7 cells, a cell line derived from monkey kidney with low background affinity with Aβ42 (12), transfected with human wild-type EGFR (EGFR^{wt}). Monomeric and oligomeric Aβ42 preparations were produced separately (36). We had no estimation of precise concentrations of each aggregation form (Fig. S2*A*). Western blotting showed that although expression levels of EGFRs were not affected after application of 25 μg/mL Aβ42 from oligomeric preparation, the level of p-EGFR or activated EGFR was significantly elevated (Fig. 4*A* and Fig. S2*B*). In contrast, treatment with monomeric Aβ42 preparation had the opposite effect on EGFR activation (Fig. S2*C*). EGFR inhibitors, Er and Ge, suppressed both EGF- and Aβ42 oligomers-activated EGFR (Fig. 4*B*).

To gain further insights into such Aβ-induced activation of EGFR, we performed immunoprecipitation to determine whether endogenously produced Aβ42 could directly bind to EGFRs. COS-7 cells were cotransfected with genes encoding a secretory form of Aβ42 and a human EGFR^{wt}. Cell lysates were assayed for coimmunoprecipitation with rabbit anti-EGFR 48 h later. We found that both Aβ42 monomers and oligomers, probably dimers

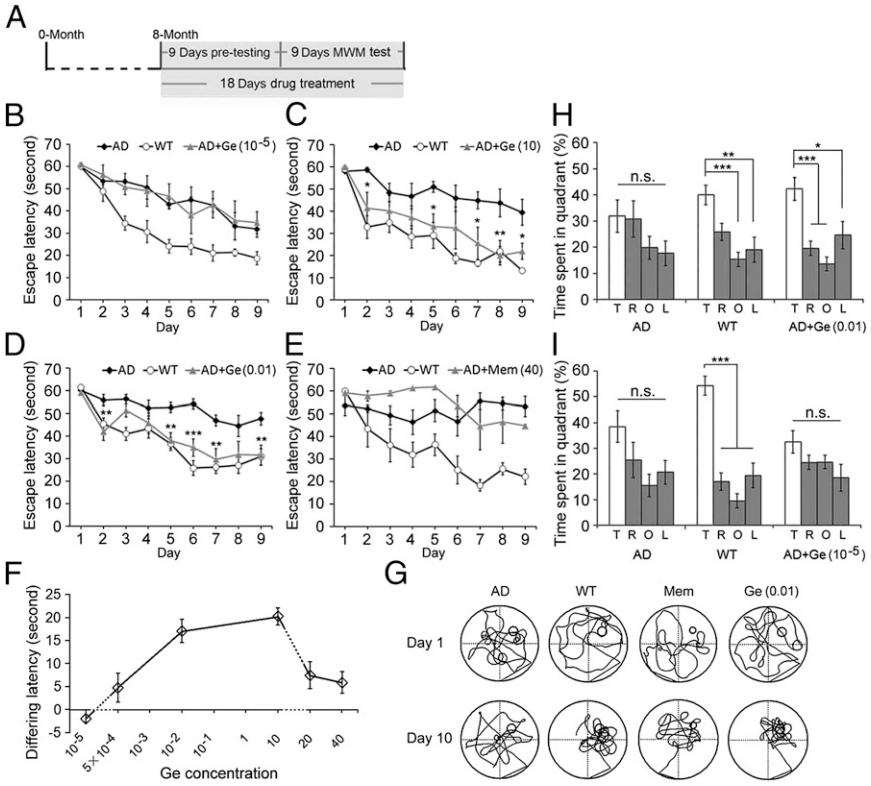

Fig. 2. Rescue of Aβ-induced memory loss via pharmacological inhibition of EGFR in mice in Morris water maze (MWM) test. (*A*) Drug-feeding scheme to 8-mo-old double transgenic mice (AD mice). Drug dosage is specified in each figure in milligrams per kilogram per day. (*B–D*) Memory rescuing effects through treatment with Ge at different concentrations. (*E*) No improvement in memory after Mem treatment. $n = 6–8$ in B; $n = 6–7$ in C; $n = 12–16$ in D; $n = 6–8$ in E. (*F*) Dosage effect of Ge on memory performance. Escape latency results from days 7–9 were included for calculation. (*G*) Representative swimming traces for probe trails on days 1 and 10. (*H* and *I*) Memory rescue is also evident in the test with removed submerged platform. Results are indicated as time spent within different quadrants. Control (WT) and drug-treated mice spent more time in the target quadrant where the platform was located (ANOVA, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$). T, target; R, right; O, opposite; L, left.

according to the molecular weight, were pulled down with $EGFR^{wt}$ (Fig. 4*C*).

Taken together, these results obtained from mechanism-guided study support the hypothesis that EGFR functions as a cell membrane receptor of Aβ peptides, and the Aβ oligomers-induced activation of EGFR plays a critical role in leading to memory loss.

Behaviorally Identified Effective Synthetic Compounds Antagonize Oligomeric Aβ42-Induced EGFR Activation. In parallel with the mechanism-guided study presented above, we performed behavioral screening of 2,000 synthetic compounds (purchased from TimTec LLC) with structures that are presumably targeted to protein kinase activities. The drug feeding scheme for fruit flies is indicated in Fig. 1*C*. Only male flies were selected for 7-d drug treatment and then subjected to behavioral assay at day 10 after eclosion. Going through an initial $n = 2$ screening followed with $n > 6$ confirmation, 45 synthetic compounds were found to be effective in rescuing memory loss in Aβ42 transgenic flies. Among them, nine were tested in double transgenic mice, and four compounds were indicated to have positive results after 2 mo of treatment (6–8 mo of age). The summary of behavioral screening and testing is depicted in Fig. 5*A*. Behavioral data are provided for these four compounds related to rescuing memory loss in flies (Fig. 5*B*) and mice (Fig. 5 *C–G*). We were amazed to find that three compounds, designated as JKF-006, JKF-011, and JKF-027 (Table S1 shows their structures), not only showed effective results in rescuing memory loss, but also significantly antagonized a 10 μg/mL oligomeric Aβ42-induced phosphorylation of human EGFR expressed in COS-7 cells (Fig. 5*H*).

Discussion

Data presented above demonstrate that overactivation of EGFRs plays a critical role in causing Aβ-induced memory loss. We first showed that expression of additional EGFRs exacerbated memory loss, whereas inhibition of EGFR activity through oral administration of two different drugs rescued memory loss in Aβ42-expressing fruit flies. One would expect that reduced EGFR activity through either genetic manipulation or pharmacological treatment should prevent or slow Aβ42-induced memory loss. Genetic manipulation, such as the use of a loss-of-function mutation or RNAi knockdown, however, was not adopted in the study because of potential complications during development. Second, we showed that the level of p-EGFR (activated EGFR) was elevated in the hippocampal tissues of APP/PS1 double transgenic mice and this increase was suppressed by feeding Ge. Treatment with this EGFR inhibitor completely rescued impaired memory in mice. It is worth noting that a dose as low as 0.01 $mg \cdot kg^{-1} \cdot d^{-1}$ is behaviorally effective in mice, whereas the rescuing effect is moderate with higher doses, such as 40 $mg \cdot kg^{-1} \cdot d^{-1}$ (Fig. S3). The effective low dose suggests that drug treatment is highly specific to EGFRs. Therefore, suppression of EGFR activity, but not other proteins' activities, is more likely responsible for the rescued memory. The ineffectiveness of Ge at higher doses suggests that an appropriate level of EGFR activity is critical in maintaining normal physiological function. Alternatively, such ineffectiveness might result from the unidentified side effects of EGFR inhibitors on other protein targets at higher doses. Thus, enhanced EGFR activity appears to be a conserved mechanism in mediating Aβ-induced memory loss in both *Drosophila* and the mouse.

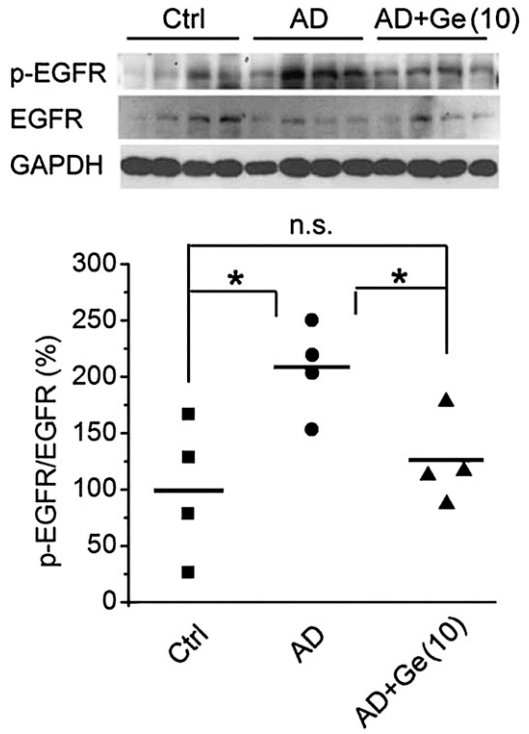

Fig. 3. Treatment with Ge suppresses the elevation of p-EGFR level in hippocampuses of double transgenic mice. In the hippocampus of 12-mo-old AD mice, the relative p-EGFR level was significantly increased compared with controls. Feeding mice with 10 mg·kg^{-1}·d^{-1} of Ge for 18 d suppressed the elevated EGFR activation. (*Upper*) Representative Western blotting. (*Lower*) Individual data points and means (horizontal lines) are shown. n = 4. Same presentation of Western blotting for following figures.

The EGFR pathway functions to enhance the differentiation, maturation, and survival of a variety of cell types (37). Accumulating evidence also indicates that the EGFR pathway can regulate neuronal plasticity by changing the intracellular Ca^{2+} concentration or glutamate release in postmitotic neurons (38, 39). In human patients, it is well studied that an increased level of *egfr* expression is closely related to tumorigenesis (40); however, the relationship between the EGFR pathway and AD is not clear. Previous reports showed that presenilin and Aβ could regulate the expression and metabolism of EGFR, which suggested the involvement of EGFR in the AD process (15, 41, 42). In our study, enhanced EGFR activity likely resulted from Aβ oligomers-dependent activation, possibly through direct binding. We have shown that oligomeric Aβ42 peptides were capable of stimulating EGFR activities, whereas monomeric Aβ42 expressed opposite effects. In combination with observations of elevated EGFR activities in the hippocampus and the rescuing effects of EGFR inhibitors on memory loss, we propose that Aβ oligomers-induced EGFR activation results in memory loss. However, which forms of oligomers lead to such activation remains to be determined. Immunoprecipitation assays suggested a potential binding between Aβ oligomers and EGFRs. However, we should point out that there is no direct evidence showing whether such binding leads to activation of EGFRs and, in particular, how oligomers bind with the receptors. It is interesting to note that Aβ oligomers also bind with EphB2, another receptor tyrosine kinase, and such binding is reported to play a critical role in mediating memory loss in another mouse model of AD (8).

The memory loss dependent on an oligomeric Aβ-induced activation of EGFR may reflect an acute toxic effect of Aβ, which might be independent of synaptic and neuronal degeneration. To gain insights into the nature of observed EGFR effects, we purposely shifted the drug feeding paradigm of Ge to a short-period treatment. Only 18 d (the shortest we tested) of drug treatment was sufficient to rescue memory loss of 8-mo-old APP/PS1 mice, which had severe memory loss but few morphological changes in the brain. In the current study, behavior results of Ge treatments in the mouse model contrasted sharply with those of Mem, which had no memory-rescuing effects with the short-period treatment. However, in other reports, Mem was effective after oral administration for several months (32, 34). We also noted that treating APP/PS1 mice with Ge for 18 d did not significantly influence the Aβ aggregation (oligomers and amyloid plaques) level in the brain (Fig. S4). These observations lead us to hypothesize that EGFR hyperactivity-dependent memory loss is due to acute effects of Aβ oligomers. It is known that Aβ oligomers are capable of disrupting long-term synaptic plasticity (1) and the downstream signaling pathways of EGFR, such as PI3-kinase and Ras, which are important in regulating long-term synaptic plasticity (17, 37, 43).

This acute role of Aβ oligomers could be a major mechanism causing Aβ oligomers-dependent memory loss. We were surprised to find that three out of four behavior-screening identified compounds were capable of suppressing the oligomeric Aβ-induced activation of EGFRs. One possible explanation for such a high ratio is that EGFR activation might be a major component leading to Aβ42-induced memory loss in 10-d-old fruit flies. Such mechanisms, however, might be more complex in mice because of their genomic complexity and longer progression toward developing memory deficits. As a result, mouse-based drug screening would yield a much lower ratio in converging to one component. Simpler

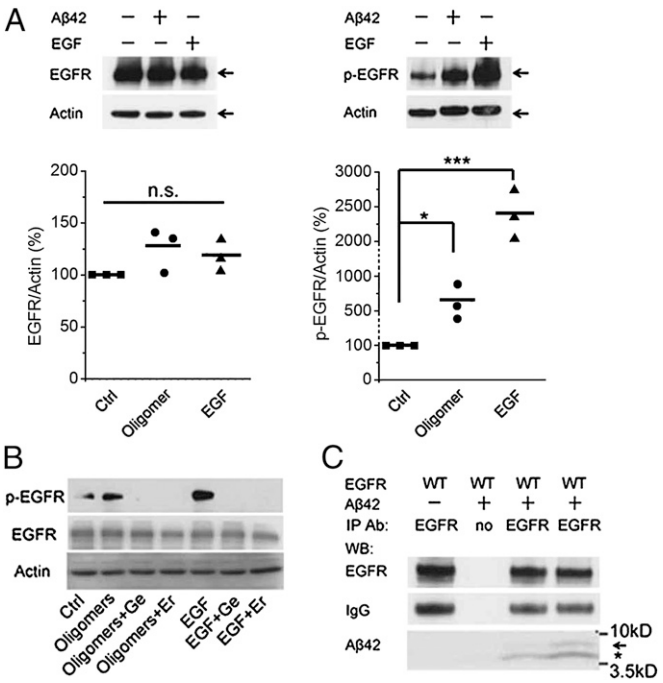

Fig. 4. Oligomeric Aβ42 activates and coprecipitates with EGFR in cultured cells. (*A*) Elevated p-EGFR (*Right*), but not total EGFR (*Left*), in response to oligomeric Aβ42 and EGF applications. COS-7 cells were transfected with human EGFR^{wt} plasmid for 48 h then incubated with 25 μg/mL of oligomeric Aβ42 preparation for 15 min. Human EGF (0.5 μg/mL) for positive control. n = 3. (*B*) Inhibition of Aβ42- and EGF-induced activations of EGFR by EGFR inhibitors Ge and Er, respectively. (*C*) Coprecipitation of Aβ42 with EGFRs. COS-7 cells were transfected with Aβ42 and human EGFR^{wt} plasmids for 48 h then cell lysates were incubated with protein G-agarose beads conjugated with rabbit anti-EGFR for immunoprecipitation. Monomer (star) and dimer (arrow) of Aβ42 are visible.

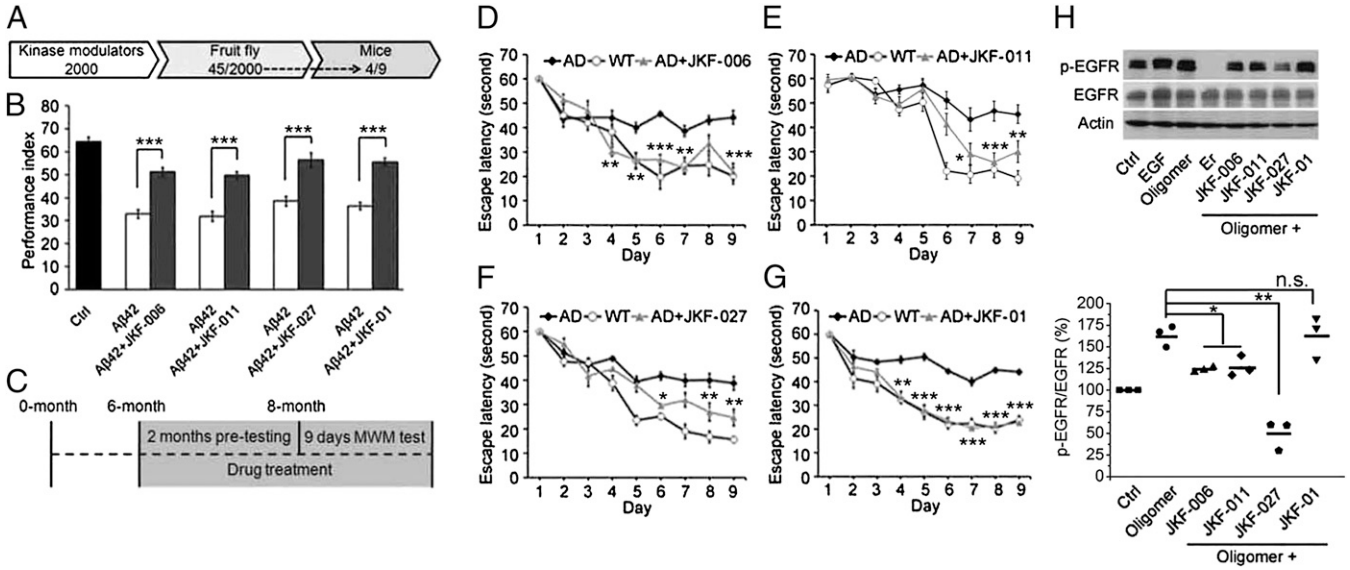

Fig. 5. Behavioral screening of synthetic compounds. (*A*) Schematic illustration of screening processes and summary of results. (*B*) Prevention of Aβ42-induced memory loss in *Drosophila*. Effects of four representative compounds are shown. Concentrations: 50 μg/mL, n = 6–8 PIs. (*C*) Drug feeding scheme for double transgenic mice. Six-month-old mice were subject to a 2-mo drug treatment and then to the MWM test. (*D*–*G*) Rescue of Aβ-induced memory loss in mice. Results of the four screened compounds as in *B* are shown. Concentrations (in milligrams per kilogram per day) are 58 for JKF-006, 14.4 for JKF-011, 55 for JKF-027, and 40 for JKF-01. n = 6–9. (*H*) Effects of four positive compounds (all in 100 μg/mL) on 10 μg/mL oligomeric Aβ42-induced EGFR activation in COS-7 cells transfected with $EGFR^{wt}$ plasmid. Except JKF-01, the other three compounds were capable of antagonizing the induced p-EGFR elevation. n = 3.

systems at every level in *Drosophila* might be favored in helping to identify drug-preferred and conserved targets.

Taken together, convergent outcomes from mechanism-guided study and behavioral screening of synthetic chemicals lead us to conclude that EGFR is a preferred target for treating Aβ-induced memory loss. Because inhibition of EGFR activity represents an efficient treatment for Aβ42-induced deficits in transgenic animals, it would be of interest to see further effects of EGFR inhibitors as well as behaviorally screened chemicals in treatments of AD patients.

Materials and Methods

***Drosophila* Stock.** Human wild-type Aβ42 transgenic flies (*UAS-Aβ42*) used in this study have been previously described (21). *UAS-InR*^{wt} and *UAS-EGFR*^{wt} were obtained from the Bloomington Drosophila Stock Center. *elav-Gal4* is a laboratory stock. Flies were raised and maintained at room temperature (22–24 °C). All stock used for Pavlovian olfactory conditioning were equilibrated by five generations of out-cross to the isogenic line, w^{1118} (*isoCJ1*).

Pavlovian Olfactory Associative Immediate Memory. The training and testing procedures were the same as previously described (22). During one training session, a group of 100 flies was sequentially exposed for 60 s to two odors, 3-octanol (OCT, Fluka) or 4-methylcyclohexanol (MCH, Fluka), with 45 s of fresh air between presentations. Flies were subjected to foot-shock (1.5-s duration with 3.5-s interstimulus interval, 60 V) during exposure to the first odor (CS+) but not to the second (CS−). To measure "immediate memory" (also referred to as "learning"), flies were transferred immediately after training to the choice point of a T-maze and forced to choose between the two odors for 2 min. Then flies were trapped in their respective T-maze arms, anesthetized, and counted. A performance index (PI) was calculated from the distribution of this group of flies in the T-maze. A reciprocal group of flies was trained and tested by using OCT as the CS+ or MCH as the CS+, respectively. The so-called half-PIs, PI (OCT) and PI (MCH), were finally averaged and multiplied by 100, yielding an n = 1. A PI of 0 indicated a distribution of 50:50 (no learning), whereas a PI of 100 indicated "perfect learning": 100% of the flies avoided the CS+ previously paired with foot shock. Control groups were age-matched to the experimental groups in each test.

Mouse Strains and Genotyping. Double transgenic mice that express a mutant chimeric mouse/human APPswe and a mutant human presenilin 1 (Delta E9), both driven by the prion protein promoter, were purchased from The Jackson Laboratory [strain B6C3-Tg(APPswe.PSEN1dE9) 85Dbo/J]. Transgenic mice were derived from B6C3/Tg+ × B6C3 crosses. Genotyping is done by PCR following Jackson Laboratory protocols [primers for Tg(APP): 5′ AAT AGA GAA CGG CAG GAG CA 3′ and 5′ GCC ATG AGG GCA CTA ATC AT 3′; primers for Tg(PSEN1): 5′ AGG ACT GAC CAC TCG ACC AG 3′ and 5′ CGG GGG TCT AGT TCT GCA T 3′]. Tg+ and their Tg− littermates were randomly assigned to various groups for drug treatment or vehicle control. All animal experiments were approved by the Institutional Animal Care and Use Committee of Tsinghua University.

Morris Water Maze. The Morris water maze was performed as in previous reports (30, 32). A water tank (120 cm in diameter) was filled with room-temperature water (19–20 °C) that was made opaque with milk. A transparent platform (15 cm in diameter) was placed in the center of one of the four virtually divided quadrants and 2 cm below the water surface. Distal cues were provided in all experiments as spatial references. Mice swam until they found the platform and remained on it for at least 5 s. During the first day of the experiment (day 1), if a mouse did not find the platform within 60 s, it was gently guided to the platform and given the 5-s stay. From days 2–9, if animals did not find the platform within 60 s, they were given a nominal latency of 60 s. Four trials were performed each day. A video tracking system (Jiliang Software Technology) was used to record data.

Latency to find the platform (maximum of 60 s) was recorded for each trial and the four daily trials were averaged for statistical analysis. Averaged escape latencies of double transgenic mice (AD mice) and drug-treated AD mice on days 7–9 were calculated. Their differences were used to indicate the dosage effect of drug at different concentrations (Fig. 2*F*).

Probe Trial. The platform was removed on day 10, 1 d after the memory ability test. Mice were allowed to swim for 60 s. The time spent in each quadrant and path length were recorded.

Drug Treatment of Animals. Gefitinib and erlotinib were purchased from LC Laboratories. Memantine was bought from Sigma-Aldrich.

For drug feeding treatments for flies, all chemicals (gefitinib, erlotinib, memantine, and synthetic compounds) were dissolved in dimethyl sulfoxide (DMSO; Sigma-Aldrich) and stored at −20 °C. Flies were maintained at 29 °C after eclosion. Flies were starved for 3 h in empty vials and then fed with drugs, diluted in 4% sucrose (chemical concentrations are vol/vol for liquid and wt/vol for solid), for another 4 h. Flies were transferred to normal food after treatment. Drug feeding was carried out once each day during the treatment period.

For treating mice, all chemicals were dissolved in physiological saline containing 0.5% Tween-80. Drugs were prepared every week. Tg+ and Tg− mice received one daily dose of drugs or vehicle by intragastric administration. Except gefitinib and memantine, which were subjected to an 18-d procedure

with 8-mo-old mice, other drugs were used for a 2-mo treatment on 6-mo-old mice until the end of experiments. Drug treatments were carried out after the last trial every day during the testing period.

Statistical Analysis. Data were analyzed by *t* test or post hoc test following ANOVA (Origin version 8; OriginLab Corporation). Statistical results are presented as means ± SEM. Western blotting results are shown as individual data points and means (horizontal lines). Asterisks indicates critical values (*$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$).

ACKNOWLEDGMENTS. We thank Dr. Sarah Parsons (University of Virginia), the James Tissue Culture Facility of Cold Spring Harbor Laboratory, Bloomington *Drosophila* Stock Center, Jackson Laboratory, and Center of Biomedical Analysis of Tsinghua University for providing experimental materials and equipment. This work was supported by grants (all to Y.Z.) from the National Basic Research Project (973 program) of the Ministry of Science and Technology of China (2009CB941301 and 2013CB835102), the Tsinghua University Initiative Scientific Research Program (20111080956), the Tsinghua-Yue-Yuen Medical Sciences Fund, the Cold Spring Harbor Laboratory, and the Dart Neuroscience (San Diego).

1. Haass C, Selkoe DJ (2007) Soluble protein oligomers in neurodegeneration: Lessons from the Alzheimer's amyloid beta-peptide. *Nat Rev Mol Cell Biol* 8:101–112.
2. Lesné S, et al. (2006) A specific amyloid-beta protein assembly in the brain impairs memory. *Nature* 440:352–357.
3. Shankar GM, et al. (2008) Amyloid-beta protein dimers isolated directly from Alzheimer's brains impair synaptic plasticity and memory. *Nat Med* 14:837–842.
4. Nunan J, Small DH (2000) Regulation of APP cleavage by alpha-, beta- and gamma-secretases. *FEBS Lett* 483:6–10.
5. Leissring MA, et al. (2003) Enhanced proteolysis of beta-amyloid in APP transgenic mice prevents plaque formation, secondary pathology, and premature death. *Neuron* 40:1087–1093.
6. Du Y, et al. (1997) alpha2-Macroglobulin as a beta-amyloid peptide-binding plasma protein. *J Neurochem* 69:299–305.
7. Carson JA, Turner AJ (2002) Beta-amyloid catabolism: Roles for neprilysin (NEP) and other metallopeptidases? *J Neurochem* 81:1–8.
8. Cissé M, et al. (2011) Reversing EphB2 depletion rescues cognitive functions in Alzheimer model. *Nature* 469:47–52.
9. Li R, et al. (2004) Tumor necrosis factor death receptor signaling cascade is required for amyloid-beta protein-induced neuron death. *J Neurosci* 24:1760–1771.
10. Cai W, He JC, Zhu L, Lu C, Vlassara H (2006) Advanced glycation end product (AGE) receptor 1 suppresses cell oxidant stress and activation signaling via EGF receptor. *Proc Natl Acad Sci USA* 103:13801–13806.
11. Li S, et al. (2009) Soluble oligomers of amyloid Beta protein facilitate hippocampal long-term depression by disrupting neuronal glutamate uptake. *Neuron* 62:788–801.
12. Laurén J, Gimbel DA, Nygaard HB, Gilbert JW, Strittmatter SM (2009) Cellular prion protein mediates impairment of synaptic plasticity by amyloid-beta oligomers. *Nature* 457:1128–1132.
13. Mancuso M, Coppedè F, Murri L, Siciliano G (2007) Mitochondrial cascade hypothesis of Alzheimer's disease: Myth or reality? *Antioxid Redox Signal* 9:1631–1646.
14. Selkoe DJ (2002) Alzheimer's disease is a synaptic failure. *Science* 298:789–791.
15. Almeida CG, Takahashi RH, Gouras GK (2006) Beta-amyloid accumulation impairs multivesicular body sorting by inhibiting the ubiquitin-proteasome system. *J Neurosci* 26:4277–4288.
16. Mangialasche F, Solomon A, Winblad B, Mecocci P, Kivipelto M (2010) Alzheimer's disease: Clinical trials and drug development. *Lancet Neurol* 9:702–716.
17. Chiang HC, Wang L, Xie Z, Yau A, Zhong Y (2010) PI3 kinase signaling is involved in Abeta-induced memory loss in Drosophila. *Proc Natl Acad Sci USA* 107:7060–7065.
18. Götz J, et al. (2004) Transgenic animal models of Alzheimer's disease and related disorders: histopathology, behavior and therapy. *Mol Psychiatry* 9:664–683.
19. Walsh DM, Selkoe DJ (2004) Deciphering the molecular basis of memory failure in Alzheimer's disease. *Neuron* 44:181–193.
20. Iijima K, et al. (2004) Dissecting the pathological effects of human Abeta40 and Abeta42 in Drosophila: A potential model for Alzheimer's disease. *Proc Natl Acad Sci USA* 101:6623–6628.
21. Iijima K, et al. (2008) Abeta42 mutants with different aggregation profiles induce distinct pathologies in Drosophila. *PLoS One* 3:e1703.
22. Tully T, Quinn WG (1985) Classical conditioning and retention in normal and mutant Drosophila melanogaster. *J Comp Physiol A Neuroethol Sens Neural Behav Physiol* 157:263–277.
23. Gschwind A, Fischer OM, Ullrich A (2004) The discovery of receptor tyrosine kinases: Targets for cancer therapy. *Nat Rev Cancer* 4:361–370.
24. Birnbaum A, Ready N (2005) Gefitinib therapy for non-small cell lung cancer. *Curr Treat Options Oncol* 6:75–81.
25. Ceresoli GL, et al. (2004) Gefitinib in patients with brain metastases from non-small-cell lung cancer: A prospective trial. *Ann Oncol* 15:1042–1047.
26. Heimberger AB, et al. (2002) Brain tumors in mice are susceptible to blockade of epidermal growth factor receptor (EGFR) with the oral, specific, EGFR-tyrosine kinase inhibitor ZD1839 (iressa). *Clin Cancer Res* 8:3496–3502.
27. Jankowsky JL, et al. (2001) Co-expression of multiple transgenes in mouse CNS: A comparison of strategies. *Biomol Eng* 17:157–165.
28. Reiserer RS, Harrison FE, Syverud DC, McDonald MP (2007) Impaired spatial learning in the APPSwe + PSEN1DeltaE9 bigenic mouse model of Alzheimer's disease. *Genes Brain Behav* 6:54–65.
29. Savonenko A, et al. (2005) Episodic-like memory deficits in the APPswe/PS1dE9 mouse model of Alzheimer's disease: Relationships to beta-amyloid deposition and neurotransmitter abnormalities. *Neurobiol Dis* 18:602–617.
30. Cohen E, et al. (2009) Reduced IGF-1 signaling delays age-associated proteotoxicity in mice. *Cell* 139:1157–1169.
31. Jankowsky JL, et al. (2005) Environmental enrichment mitigates cognitive deficits in a mouse model of Alzheimer's disease. *J Neurosci* 25:5217–5224.
32. Martinez-Coria H, et al. (2010) Memantine improves cognition and reduces Alzheimer's-like neuropathology in transgenic mice. *Am J Pathol* 176:870–880.
33. Wakeling AE, et al. (2002) ZD1839 (Iressa): An orally active inhibitor of epidermal growth factor signaling with potential for cancer therapy. *Cancer Res* 62:5749–5754.
34. Scholtzova H, et al. (2008) Memantine leads to behavioral improvement and amyloid reduction in Alzheimer's-disease-model transgenic mice shown as by micromagnetic resonance imaging. *J Neurosci Res* 86:2784–2791.
35. Rojas M, Yao S, Lin YZ (1996) Controlling epidermal growth factor (EGF)-stimulated Ras activation in intact cells by a cell-permeable peptide mimicking phosphorylated EGF receptor. *J Biol Chem* 271:27456–27461.
36. Dahlgren KN, et al. (2002) Oligomeric and fibrillar species of amyloid-beta peptides differentially affect neuronal viability. *J Biol Chem* 277:32046–32053.
37. Wong RW, Guillaud L (2004) The role of epidermal growth factor and its receptors in mammalian CNS. *Cytokine Growth Factor Rev* 15:147–156.
38. Abe K, Saito H (1992) Epidermal growth factor selectively enhances NMDA receptor-mediated increase of intracellular Ca2+ concentration in rat hippocampal neurons. *Brain Res* 587:102–108.
39. Barrie A, Chieregatti E, Miloso M, Benfenati F, Valtorta F (1996) Epidermal growth factor-mediated inhibition of neurotransmitter glutamate release from rat forebrain synaptosomes. *Mol Pharmacol* 49:399–403.
40. Sharma SV, Bell DW, Settleman J, Haber DA (2007) Epidermal growth factor receptor mutations in lung cancer. *Nat Rev Cancer* 7:169–181.
41. Repetto E, Yoon IS, Zheng H, Kang DE (2007) Presenilin 1 regulates epidermal growth factor receptor turnover and signaling in the endosomal-lysosomal pathway. *J Biol Chem* 282:31504–31516.
42. Zhang YW, et al. (2007) Presenilin/gamma-secretase-dependent processing of beta-amyloid precursor protein regulates EGF receptor expression. *Proc Natl Acad Sci USA* 104:10613–10618.
43. Mazzucchelli C, Brambilla R (2000) Ras-related and MAPK signalling in neuronal plasticity and memory formation. *Cell Mol Life Sci* 57:604–611.