Effect of 42 amino acid long amyloid- β peptides on *Arabidopsis* plants

HanGyeol Lee · Ji Woo Kim · Sangyun Jeong · Jungeun An · Young-Cheon Kim · Hojin Ryu · Jeong Hwan Lee

Received: 21 October 2020 / Revised: 3 November 2020 / Accepted: 4 November 2020
© Korean Society for Plant Biotechnology

Abstract Although the evolution of *Arabidopsis thaliana* and humans diverged approximately 1.6 billion years ago, recent studies have demonstrated that protein function and cellular processes involved in disease response remain remarkably conserved. Particularly, γ -secretase, a multisubunit protein complex that participates in intramembrane proteolysis (RIP) regulation, is also known to mediate the cleavage of more than 80 substrates including the amyloid precursor protein (APP) and the Notch receptor. Although the genes (*PS1/2*, *APH-1*, *PEN-2*, and *NCT*) coding for the γ -secretase complex components are present in plant genomes, their function remains largely uncharacterized. Given that the deposition of 42 amino acid long amyloid- β peptides ($\text{hA}\beta_{42}$) is thought to be one of the main causes of Alzheimer's disease, we aimed to examine the physiological effects of $\text{hA}\beta_{42}$ peptides on plants. Interestingly, we found that *Arabidopsis* protoplast death increased after 24 h of exposure to 3 or 5 μM $\text{hA}\beta_{42}$ peptides. Furthermore, transgenic *Arabidopsis* plants overexpressing the *hA}\beta_{42}* gene exhibited changes in primary root length and silique phyllotaxy. Taken together, our results demonstrate that $\text{hA}\beta_{42}$ peptides, a metazoan protein, significantly affect *Arabidopsis* protoplast viability and plant morphology.

Keywords Alzheimer's disease, Amyloid- β peptide, *Arabidopsis* mesophyll protoplasts, γ -secretase, Transgenic plants

Introduction

Large functional genomics initiatives have revealed that many

human disorders are affected by multiple genes and their genetic and molecular interactions with each other and the environment. Thus, the use of various model systems such as human and mammalian cell lines, yeast (i.e., *Saccharomyces cerevisiae*), as well as other organisms such as *Caenorhabditis elegans* and *Drosophila melanogaster* contributes to important discoveries within human disease research and the design of new diagnostic tools and medical treatment strategies (Xu and Moller 2011).

Recent reports have shown that *Arabidopsis thaliana* known as a model organism for plant biology is a valuable model system to study molecular mechanisms underlying human disease states (Hays 2002). According to the comparison between *Arabidopsis* and human genome sequences, *Arabidopsis* genome encodes many orthologues of human proteins albeit *Arabidopsis* and humans diverged 1.6 billion years ago (Xu and Moller 2011). For example, TAIR WU-BLAS tool (<http://www.arabidopsis.org>) analysis showed that 71% of genes involved in neurological human diseases have *Arabidopsis* orthologues with high *E*-value cutoffs. Furthermore, *Arabidopsis* has some advantages for human disease research: (i) Simple cultivation and maintenance with low infrastructure and operating costs, (ii) Fast and simple transformation techniques for transgenic analyses, (iii) The availability of large mutants and genomic resources, and (iv) few ethical restrictions (Xu and Moller 2010).

Alzheimer's disease (AD) is the most common age-related neurodegenerative disorder of the central nervous system (Mattson 2004), which is characterized by the deposition of aggregation-prone amyloid- β peptides ($\text{A}\beta$ s) as the degraded products of the amyloid precursor protein (APP) and neurofibrillary tangles in the brain (Selkoe 1998). In humans, presenilin (PS) was identified from the genetic screens of patients suffering from AD and mutations in *PS1* or *PS2* led to improper cleavage of APP, which resulting in $\text{A}\beta$ s accumulation and progressive neurodegeneration (Sherrington et al. 1995). PS as a catalytic core protein is a component of γ -secretase, which is implicated in the process

H. G. Lee · J. W. Kim · S. Jeong · J. An · Y.-C. Kim · J. H. Lee (✉)
Division of Life Sciences, Jeonbuk National University, 567
Baekje-daero, Deokjin-gu, Jeonju, 54896, Republic of Korea
e-mail: jhwanlee90@jbnu.ac.kr

Hojin Ryu (✉)
Department of Biology, Chungbuk National University, Cheongju
28644, Republic of Korea
e-mail: hjryu96@chungbuk.ac.kr

of regulated intramembrane proteolysis (RIP) (Sannerud and Annaert 2009). Besides PS, nicastrin (NCT), presenilin enhancer 2 (PEN-2), and anterior pharynx-defective 1 (APH-1) as other constituents of γ -secretase are required for complex assembly, stabilization, trafficking, and substrate recognition (Parks and Curtis 2007).

APP as one of more than 80 substrates for γ -secretase is a metazoan protein, which is only present in genomes of multicellular animals (Haapasalo and Kovacs 2011). However, the components of γ -secretase have been identified in both metazoan and plants (Grigorenko et al. 2002; Kimberly and Wolfe 2003; Moliaka et al. 2004; Ponting et al. 2002; Wigge and Weigel 2001). The moss *Physcomitrella patens* also harbors homologues of all γ -secretase subunits with PS present in one copy. A lesion in moss PS (*PpPS*) showed several pleiotropic defects such as abnormal growth pattern, impaired chloroplast movement, and decreased uptake of an endocytosis tracer (Khandelwal et al. 2007). The mutation in PS of *Dictyostelium discoideum* as a slime mould affected cell fate determination and the regulation of phagocytosis (McMains et al. 2010). In *Arabidopsis* as a higher plant, all γ -secretase subunits interacted with each other and colocalized in endomembrane system compartments (Smolarkiewicz et al. 2014). These data suggest that γ -secretase is evolutionarily conserved and its role may be extended beyond RIP found from animal studies.

Here we presented the effect of 42 amino acids long-human amyloid- β peptide (hA β_{42}) on *Arabidopsis* protoplasts, tissues, and transgenic plants.

Materials and Methods

Plant materials and growth conditions

The wild-type [ecotype Columbia (Col-0)] and the transgenic *Arabidopsis* plants were grown in soil or a murashige and skoog (MS) medium at 23°C under long-day (LD) conditions (16 h light/8 h dark) at a light intensity of 120 $\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$.

For treatment of 42 amino acids long-human amyloid- β peptide (hA β_{42}) at cellular level, we followed the procedures for isolation of *Arabidopsis* mesophyll protoplasts, as previously described (Lee et al. 2017; Yoo et al. 2007). Isolated protoplasts (2×10^4) were treated with 3 and 5 mM hA β_{42} monomer or DMSO, and then the percentage of dead cells at indicated times was determined by staining protoplasts with Evans blue dye (Sigma-Aldrich), which was added to the samples to a final concentration of 0.04 % (Danon et

al. 2005; Wright et al. 2000). The number of stained cells was measured using a light microscope (Zeiss, Oberkochen, Germany). For treatment of hA β_{42} at tissue level, *Arabidopsis* plants were grown on soil or MS medium under LD conditions at 23°C. 10-d-old plants were treated with 3 mM hA β_{42} monomer or Dimethyl sulfoxide (DMSO) (Sigma-Aldrich, St. Louis, Missouri, USA), as previously described (Kim et al. 2017), and then hA β_{42} -treated plants were observed after 2 and 4 days under a light microscope (Nikon, Tokyo, Japan).

Preparation of hA β_{42} monomer

The procedures for were previously described (Dahlgren et al. 2002; Kook et al. 2012). hA β_{42} peptide was dissolved at a concentration of 1 $\text{mg} \cdot \text{ml}^{-1}$ in 3,3,3-Hexafluoro-2-propanol (HFIP) (Sigma-Aldrich). HFIP was evaporated using Speed Vacuum concentrator and lyophilized peptide was resolved in 10 ml DMSO. After calculation of the molar concentration, aliquots of 1 mM lyophilized hA β_{42} monomer were stored at -80°C until use.

Generation of transgenic Arabidopsis plants

For *p35S::hA β_{42}* construct, the region of human A β_{42} was polymerase chain reaction (PCR)-amplified using plasmid DNA template with the gene encoding human amyloid precursor protein (hAPP) [gifted from Dr. Jeong (Jeonbuk National University, South Korea) and the resulting products were cloned into the pCHF3 vector harboring *cauliflower mosaic virus* (CaMV) 35S promoter. The resulting recombinant plasmid was sequenced to verify the absence of the PCR errors during amplification. Oligonucleotide primers used for cloning were as follows: 5'-ACGAGCTC GGTACCCGGGATGCTGCCCCGTTTGGC -3' and 5'-CT CTAGAGGATCCCCGGGCGCTATGACAACACCGCCC -3'. This construct was transformed into *Agrobacterium tumefaciens* strain GV3101 and then infiltrated into the wild-type (Col-0) plants. Transgenic plants were generated using the floral dip method with minor modifications (Weigel and Glazebrook 2002). The transgenic seedlings were first selected using kanamycin for a pCHF3 vector and then verified using PCR-based DNA genotyping. At least 30 ~ 40 T₁ seedlings were analyzed for the construct.

To examine the expression of hA β_{42} in the transgenic *Arabidopsis* plants, we performed semi-quantitative reverse transcription (RT)-PCR, as described previously (Lee et al. 2007). Total RNA was extracted from 8-d-old seedlings using a RiboEx Total RNA kit (GeneAll, Seoul, Korea), and

first-strand complementary DNA (cDNA) was synthesized from 1 µg of total RNA, in accordance with the manufacturer's instructions (GeneAll). The following oligonucleotides were employed as gene-specific primers in the RT-PCR analyses: for *hAβ₄₂*, 5'-GAGTCATGCTGCCCCGTTTGG CACT -3' and 5'-TCTAGACGCTATGACAACACCGCCC ACC-3'; for *AtPP2AA3*, 5'-GCGGTTGTGGAGAACATGA TACG-3' and 5'-GAACCAAACACAATTCGTTGCTG-3'. *AtPP2AA3* was used as a reference gene.

Results and Discussion

The treatment of *hAβ₄₂* monomer in *Arabidopsis* mesophyll protoplasts affects the viability of the cells

Because the accumulation of aggregated form of *Aβ₄₂* in the brain leads to mitochondrial dysfunction and elevated reactive oxygen species (ROS), consequently resulting in cell death (Reddy and Beal 2008), we investigated whether *hAβ₄₂* monomer affects the viability of plant cells. Mesophyll protoplasts were isolated from 10-d-old *Arabidopsis* wild-type (Col-0) plants grown at 23°C under LD conditions and 2×10^4 protoplasts were treated with 3 or 5 mM *hAβ₄₂* monomer at 1, 2, 4, 8, and 24 h under continuous light conditions. The protoplasts treated with DMSO were used as a control. Because dead cells were specifically stained with Evans blue dye (Danon et al. 2005; Wright et al. 2000), we stained 3 or 5 mM *hAβ₄₂* monomer-treated protoplasts after 24 h with Evans blue dye. This indicates that dead cells were increased in the protoplasts treated with 3 or 5 mM *hAβ₄₂* monomer, compared with DMSO-incubated protoplasts (Fig. 1a). We also calculated the percentage of dead cells at 0, 1, 2, 4, 8, and 24 h under a light microscope. The percentage of dead cells in protoplasts treated with *hAβ₄₂* monomer gradually increased and reached approximately 60% after 24 h (Fig. 1b). The portion of dead cells was nearly similar irrespective of treatment of 3 or 5 mM *hAβ₄₂* monomer. In contrast, the percentage of dead cells in DMSO-treated mesophyll protoplasts remained almost constant until 8 h and showed approximately 40% after 24 h (Fig. 1b). These results indicated that *hAβ₄₂* monomer reduces the viability of *Arabidopsis* mesophyll protoplasts.

The treatment of *hAβ₄₂* monomer does not cause any morphological changes in *Arabidopsis* young seedlings

Because *hAβ₄₂* monomer affects the viability of *Arabidopsis* mesophyll protoplasts at cellular level (Fig. 1), we next exam-

Fig. 1 Effect of *hAβ₄₂* peptide treatment on *Arabidopsis* mesophyll protoplasts. (a) Morphology of protoplasts treated with 5 μM of *hAβ₄₂* monomer and DMSO. 10-d-old *Arabidopsis* (Col-0) plants were used for isolation of protoplasts, after which 2×10^4 protoplasts were incubated at 23°C under continuous light conditions and treated with 3 and 5 μM of *hAβ₄₂* monomer. After staining protoplasts with Evans blue dye, the dead cells were observed under the microscope (scale bars: 100 μm). (b) Percentage of dead cells at 0, 1, 2, 4, 8, and 24 hours. The asterisks indicate a significant difference in the percentage of dead cells in 3 and 5 μM *hAβ₄₂* monomer-treated protoplasts compared with DMSO-treated protoplasts (Student's t-test, **P* < 0.05)

ined the effect of *hAβ₄₂* monomer at plant tissue levels. Then, we sowed *Arabidopsis* wild-type (Col-0) seeds in solid MS medium, transferred 10-d-old seedlings into 6-well plates containing 1 mL of liquid MS medium with 3 mM *hAβ₄₂* monomer or DMSO, and observed the morphological changes of seedlings after 2 and 4 days. The 3 mM *hAβ₄₂* monomer-treated seedlings after 2 and 4 days did not show any defects of morphology such as leaves, hypocotyls, and roots (Fig. 2). This result indicated that *hAβ₄₂* monomer does not affect the growth of young seedlings at the tissue level. However, we cannot dismiss the possibility that young seedlings may be more resistant to physical and chemical treatment than protoplasts.

Fig. 2 Effect of A β 42 peptide treatment on *Arabidopsis* young seedlings. 10-d-old wild-type *Arabidopsis* (Col-0) plants grown at 23°C under long-day (LD) conditions were treated with 3 μ M hA β 42 monomer and morphological changes in *Arabidopsis* plants were observed after 2 and 4 days. DMSO-treated *Arabidopsis* seedlings were used as controls

The overexpression of hA β 42 in transgenic *Arabidopsis* plants leads to morphological changes in root length and silique phyllotaxy

In order to investigate the effect of the hA β 42 overexpression in *Arabidopsis* plants, we expressed the region of hA β 42 under the control of a 35S promoter (*p35S::hAβ42*) in *Arabidopsis* wild-type (Col-0) plants. We obtained 37 independent transgenic plants in the T₁ generation from DNA genotyping (Fig. 3a). To examine the morphological changes of the *p35S::hAβ42* plants in more detail, we generated the transgenic plants with a single *p35S::hAβ42* insertion in the T₃ generation and confirmed a higher expression level of introduced hA β 42 gene (Fig. 3b). Firstly, we compared root growth phenotypes of wild-type (Col-0) and *p35S::hAβ42* plants. The seedlings were grown on MS medium and primary root lengths were quantified 8 days after germination. We found that three independent *p35S::hAβ42* lines had long primary

roots than wild-type (Col-0) seedlings (Fig. 3c). The degree of primary root lengths was correlated with the expression levels of hA β 42 gene in independent *p35S::hAβ42* lines. For instance, a *p35S::hAβ42* line (#16) showing strong hA β 42 gene expression had more longer primary roots than *p35S::hAβ42* line (#8). Secondly, we measured the flowering time in six independent *p35S::hAβ42* lines under long-day (LD) conditions at 23°C. We found that the flowering time of four independent *p35S::hAβ42* lines (#14, #22, #26, and #36) was unchanged, whereas two independent lines (#8 and #16) has altered flowering time (Fig. 3d and e). However, the degree of flowering time was not correlated with the expression levels of hA β 42 gene in independent *p35S::hAβ42* lines. Lastly, we detected abnormal phyllotaxy in the inflorescence of two independent *p35S::hAβ42* lines (#16 and #22) (Fig. 3f), which showing that strong hA β 42 gene expression in *p35S::hAβ42* lines affected the degree of defects in phyllotaxy. These results indicated that the increased hA β 42 expression affects morphological changes in root length and silique phyllotaxy in the *p35S::hAβ42* plants.

Conclusions

Here we provide the first data on the effect of hA β 42 monomer as a metazoan protein in angiosperms. In a series of experiments, we showed that the viability of cells was reduced in hA β 42 monomer-treated *Arabidopsis* mesophyll protoplasts (Fig. 1), suggesting that hA β 42 monomer may lead to cell death. Because the protoplast system is known as a useful tool that allows an easy uptake of bioactive agents and a concise quantification of cell death response (Yoo et al. 2007), we will further investigate whether the features of the cellular destruction such as DNA cleavage, the activation of caspase-like molecules, and the release of mitochondrial components are observed in hA β 42 monomer-treated protoplasts (Reape and McCabe 2008). Although treatment of hA β 42 monomer did not affect the growth of young seedlings at the tissue level, hA β 42 gene expression in the transgenic *Arabidopsis* plants caused the morphological changes in primary root length and silique phyllotaxy (Fig. 3). A recent report have shown that *Arabidopsis ps1 ps2* double mutants have accelerated chlorosis in the dark (Smolarkiewicz et al. 2014). Because darkness and starvation induce senescence and autophagy in plants (Sobieszczuk-Nowicka et al. 2018), we will further examine the effect of prolonged darkness treatment on hA β 42 monomer-treated *Arabidopsis* seedlings and *p35S::hAβ42* plants.

Fig. 3 Effect of *hAβ₄₂* overexpression on transgenic *Arabidopsis* plants. (a) Confirmation of *hAβ₄₂* integration in *p35S::hAβ₄₂* plants at the T₃ generation. P was used as a control for DNA genotyping. (b) Overexpression of *hAβ₄₂* in *p35S::hAβ₄₂* plants at the T₃ generation. Wild-type *Arabidopsis* (Col-0) plant was used as a control for RT-PCR. (c) Root length measurements in *p35S::hAβ₄₂* plants. The number of measured plants is shown above each genotype in the bar graph. The error bars indicate the standard error of the mean of three biological replicates. Asterisks denote statistically significant differences compared with wild-type *Arabidopsis* (Col-0) plants (Student's *t*-test, ****P* < 0.001). (d and e) Flowering time phenotypes of *p35S::hAβ₄₂* plants at 23°C under LD conditions. Asterisks denote statistically significant differences compared with wild-type *Arabidopsis* (Col-0) plants (Student's *t*-test, **P* < 0.05, ****P* < 0.001, n.s., not significant). Photographs were taken when wild-type *Arabidopsis* (Col-0) plants flowered. Scale bars: 1 cm. (f) Silique phyllotaxy in *p35S::hAβ₄₂* plants. Scale bars: 1 cm

Acknowledgements

We thank Dr. Mook-Jung (Seoul National University) for kindly providing research methods. This work was supported by National University Promotion Program in 2019 (to J.H. Lee).

References

Dahlgren KN, Manelli AM, Stine WB, Jr., Baker LK, Krafft GA,

- LaDu MJ (2002) Oligomeric and fibrillar species of amyloid-beta peptides differentially affect neuronal viability. *J Biol Chem* 277:32046–32053
- Danon A, Miersch O, Felix G, Camp RG, Apel K (2005) Concurrent activation of cell death-regulating signaling pathways by singlet oxygen in *Arabidopsis thaliana*. *Plant J* 41:68–80
- Grigorenko AP, Moliaka YK, Korovaitseva GI, Rogaev EI (2002) Novel class of polytopic proteins with domains associated with putative protease activity. *Biochemistry (Mosc)* 67:826–835
- Haapasalo A, Kovacs DM (2011) The many substrates of presenilin/γ-secretase. *J Alzheimers Dis* 25:3–28
- Hays JB (2002) *Arabidopsis thaliana*, a versatile model system for

- study of eukaryotic genome-maintenance functions. *DNA Repair (Amst)* 1:579–600
- Khandelwal A, Chandu D, Roe CM, Kopan R, Quatrano RS (2007) Moonlighting activity of presenilin in plants is independent of gamma-secretase and evolutionarily conserved. *Proc Natl Acad Sci U S A* 104:13337–13342
- Kim HJ, Wu CY, Yu HM, Sheen J, Lee H (2017) Dual CLAVATA3 peptides in *Arabidopsis* shoot stem cell signaling. *J Plant Biol* 60:506–512
- Kimberly WT, Wolfe MS (2003) Identity and function of gamma-secretase. *J Neurosci Res* 74:353–360
- Kook SY, Hong HS, Moon M, Ha CM, Chang S, Mook-Jung I (2012) Abeta(1)-(4)(2)-RAGE interaction disrupts tight junctions of the blood-brain barrier via Ca(2)(+)-calcineurin signaling. *J Neurosci* 32:8845–8854
- Lee JH, Jin S, Kim SY, Kim W, Ahn JH (2017) A fast, efficient chromatin immunoprecipitation method for studying protein-DNA binding in *Arabidopsis* mesophyll protoplasts. *Plant Methods* 13:42
- Lee JH, Park SH, Lee JS, Ahn JH (2007) A conserved role of SHORT VEGETATIVE PHASE (SVP) in controlling flowering time of Brassica plants. *Biochim Biophys Acta* 1769:455–461
- Mattson MP (2004) Pathways towards and away from Alzheimer's disease. *Nature* 430:631–639
- McMains VC, Myre M, Kreppel L, Kimmel AR (2010) Dictyostelium possesses highly diverged presenilin/gamma-secretase that regulates growth and cell-fate specification and can accurately process human APP: a system for functional studies of the presenilin/gamma-secretase complex. *Dis Model Mech* 3:581–594
- Moliaka YK, Grigorenko A, Madera D, Rogaev EI (2004) Impas 1 possesses endoproteolytic activity against multipass membrane protein substrate cleaving the presenilin 1 holoprotein. *FEBS Lett* 557:185–192
- Parks AL, Curtis D (2007) Presenilin diversifies its portfolio. *Trends Genet* 23:140–150
- Ponting CP, Hutton M, Nyborg A, Baker M, Jansen K, Golde TE (2002) Identification of a novel family of presenilin homologues. *Hum Mol Genet* 11:1037–1044
- Reape TJ, McCabe PF (2008) Apoptotic-like programmed cell death in plants. *New Phytol* 180:13–26
- Reddy PH, Beal MF (2008) Amyloid beta, mitochondrial dysfunction and synaptic damage: implications for cognitive decline in aging and Alzheimer's disease. *Trends Mol Med* 14:45–53
- Sannerud R, Annaert W (2009) Trafficking, a key player in regulated intramembrane proteolysis. *Semin Cell Dev Biol* 20:183–190
- Selkoe DJ (1998) The cell biology of beta-amyloid precursor protein and presenilin in Alzheimer's disease. *Trends Cell Biol* 8:447–453
- Sherrington R, Rogaev EI, Liang Y, Rogaeva EA, Levesque G, Ikeda M, Chi H, Lin C, Li G, Holman K, Tsuda T, Mar L, Foncin JF, Bruni AC, Montesi MP, Sorbi S, Rainero I, Pinessi L, Nee L, Chumakov I, Pollen D, Brookes A, Sanseau P, Polinsky RJ, Wasco W, Da Silva HA, Haines JL, Pericak-Vance MA, Tanzi RE, Roses AD, Fraser PE, Rommens JM, St George-Hyslop PH (1995) Cloning of a gene bearing missense mutations in early-onset familial Alzheimer's disease. *Nature* 375:754–760
- Smolarkiewicz M, Skrzypczak T, Michalak M, Lesniewicz K, Walker JR, Ingram G, Wojtaszek P (2014) Gamma-secretase subunits associate in intracellular membrane compartments in *Arabidopsis thaliana*. *J Exp Bot* 65:3015–3027
- Sobieszczuk-Nowicka E, Wrzesinski T, Bagniewska-Zadworna A, Kubala S, Rucinska-Sobkowiak R, Polcyn W, Misztal L, Mattoo AK (2018) Physio-genetic dissection of dark-induced leaf senescence and Tyiming its reversal in barley. *Plant Physiol* 178:654–671
- Weigel D, Glazebrook J (2002) *Arabidopsis*: A laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
- Wigge PA, Weigel D (2001) *Arabidopsis* genome: life without notch. *Curr Biol* 11:R112–114
- Wright KM, Duncan GH, Pradel KS, Carr F, Wood S, Oparka KJ, Cruz SS (2000) Analysis of the N gene hypersensitive response induced by a fluorescently tagged tobacco mosaic virus. *Plant Physiol* 123:1375–1386
- Xu XM, Moller SG (2010) ROS removal by DJ-1: *Arabidopsis* as a new model to understand Parkinson's Disease. *Plant Signal Behav* 5:1034–1036
- Xu XM, Moller SG (2011) The value of *Arabidopsis* research in understanding human disease states. *Curr Opin Biotechnol* 22:300–307
- Yoo SD, Cho YH, Sheen J (2007) *Arabidopsis* mesophyll protoplasts: a versatile cell system for transient gene expression analysis. *Nat Protoc* 2:1565–1572