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APP phosphorylation at S655 correlates with F-actin cytoskeleton dynamics – relevance in neuronal differentiation

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The Alzheimer's Amyloid Precursor Protein (APP) is a type 1 transmembranar protein that has been implicated in roles such as cell adherence, survival, migration and differentiation. Although a role in neuritogenesis has been attributed to APP, some contradictory results have been reported regarding the benefits of knocking-down or overexpressing APP [1]. Our preliminary work indicated that pAPP (APP phosphorylated at the S655 residue) may potentially modulate APP-mediated neuronal differentiation, through mechanisms that may involve increased APP trafficking and cleavage, and altered APP-mediated cytoskeleton rearrangements [2, 3]. In order to address the ability of APP and pAPP to mediate neuronal differentiation, we used APP cDNA constructs fused to the Green Fluorescent Protein (GFP): wild-type APP and APP S655 mutants (S655A dephosphomimicking and S655E phosphomimicking mutants). SH-SY5Y neuroblastoma cells, a well documented neuronal-like cell model, were used, and the conditions for their retinoic-acid mediated differentiation and concomitant APP-GFP transfection were first optimized [4].

In the work here described, neuritogenic and cytoskeleton alterations were assayed for APP-GFP expressing cells at key regulatory differentiation periods, with cells being transfected at day 2, 4 and 6 of differentiation, and collected after 48h of transient transfection. The evaluation of cell differentiation included analysis of the neuritogenic output by bright field and epifluorescence microscopy, and microtubules and actin cytoskeletons were monitored by confocal microscopy, using an acetylated alpha-Tubulin antibody and red fluorescent-labeled phalloidin, respectively.

APP transfection was observed to induce specific effects in each of the differentiation periods tested (data not shown), and in the earlier differentiation period (4 days of differentiation), APP overexpression decreased the number of processes longer that 20 μm per cell, although being able to slightly increase processes length (Figure 1, N1 vector vs APP-GFP). This was particularly true for the phosphomimicking S655E mutant, which was more capable of inducing neuritic elongation. APP may alter neuritogenesis through the induction of cytoskeleton rearrangements, and indeed we could observe that Wt and S655A APP-GFP transfection led to the depolymerization of F-actin stress fibers, resulting in a more cortical phenotype, while S655E did not have a significant effect in F-actin dynamic (Figure 1, 'F-actin phalloidin'). In contrast, no major differences were observed in acetylated tubulin in APP-GFP transfected versus non-transfected cells (Figure 2, exemplified for the S655 phosphomutants). Neuritogenesis is a process involving highly regulated cytoskeleton rearrangements and it appears that at least part of the APP effects in cellular differentiation may occur via alterations on the actin dynamics.

References

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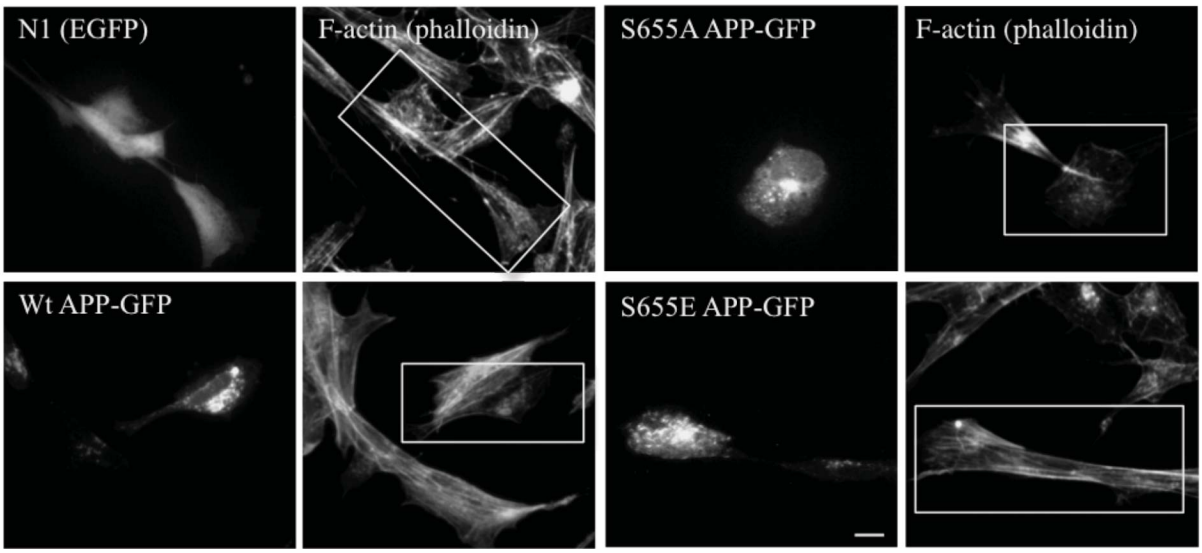

Figure 1. F-actin was evaluated using fluorescent-labelled phalloidin, in SH-SY5Y cells differentiated for 4 days and transfected with N1 EGFP and Wt, S655A, S655E at the 2nd day of differentiation. A more cortical phenotype can be observed for Wt and S655A expressing cells, where actin stress fibers are drastically diminished. Scale bar, 10 µm.

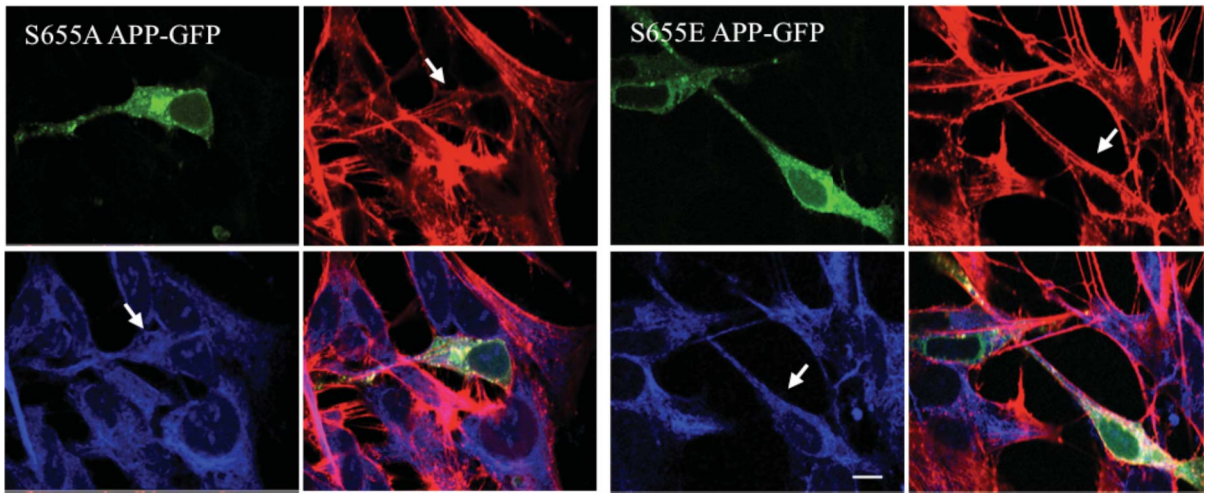

Figure 2. SH-SY5Y cells differentiated for 4 days and transfected with S655A and S655E APP-GFP (green fluorescence) at the 2nd day of differentiation. F-actin was evaluated using red fluorescent-labelled phalloidin, and the levels of acetylated tubulin were monitored by immunocytochemistry (blue fluorescence). Arrows indicate APP-GFP transfected cells. Scale bar, 10 µm.

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