

P015 Generation and characterization of mutant cell lines defective in γ -secretase processing of Notch and APP

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Several type I integral membrane protein, like Notch receptor or Amyloid Precursor Protein (APP), are cleaved in their intramembrane domain by γ -secretase activity, which is carried within a multiproteic complex. These cleavages generate molecules (NICD, A β peptide, AICD) involved in intracellular or extracellular signalings. At least four transmembrane proteins belong to the γ -secretase complex : Presenilin (PS1 or PS2), Nicastrin (Nct), Aph-1 and Pen-2. It is still unclear whether these proteins are the only components of the complex and whether a unique complex is involved in the different γ -secretase processes. Here we have set up a genetic screen based on the permanent acquisition or loss of resistances to various antibiotics, depending on the ability of a chimeric substrate derived from Notch to be γ -secretase-cleaved. Using this screen on randomly mutagenized mammalian cells allowed us to select clones deficient in γ -secretase activity. We further analyzed two of these clones and identified different mutations in the Nct gene. The first one abolishes the Nct production and the second one, a point mutation in the ectodomain, abolishes Nct maturation process. In both cases, γ -secretase activity on Notch and APP is impaired.