Секция «Клеточная биология и гистология»

Localization of amyloid beta peptides inside the cells by fluorescent microscopy

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Alzheimer's disease is one of many age-associated diseases and the most common dementia in elderly (60-80% of all known dementias). Currently, AD and related dementias affect about 47 million people worldwide (Alzheimer's Disease International, 2016). The highest risk factor for AD is age. It is expected that the number will double and triple by 2030 and 2050, respectively, if no significant progress has been made in research for early diagnosis and new efficient therapy. Aβ peptides are continuously produced throughout life in the healthy brain and an increase in either total levels of Aβ or of relative concentrations of Aβ_{40} and Aβ_{42} have been implicated in the late-onset of AD pathogenesis. Different mechanisms of the contribution of Aβ peptides in AD have been proposed. There are still discussions on whether Aβ fibrils, which form extracellular plaques, or amyloid beta monomers and oligomers are the major contributor in the pathogenesis of AD. According to the "amyloid hypothesis", aggregates of amyloid fibrils that are deposited outside neurons in dense formations (senile or neuritic plaques) are the causative agent of AD, possibly in combination with neurofibrilary tangles (NFTs). Neuron loss, vascular damage, and dementia follow Aβ peptide deposition (Hardy & Higgins, 1992). However, cases of AD without plaques (Price & Morris, 1999) or their presence in non-demented people were documented (Crystal et al., 1988; Price & Morris, 1999).

In order to study cellular and organelle trafficking of Aß, to identify its target(s) in our current studies the Aß$_{42}$ peptide was disaggregated to form monomers/small oligomers that are externally applied to mammalian cells (human neuroblastoma cell line and rat oligodendroglia cell line). In contrast to plaques composed of aggregated Aβ fibrils, the monomeric/oligomeric peptide is able to enter cells, this is proven by confocal microscopy. Cells were treated with fluorescently labeled Aβ_{42} peptide. We are able to track in time and space the pathway of the Aß peptides from the outside of the cell across the plasma membrane to the internal targets - specific organelles. This way for both human neuroblastoma and rat oligodendroglia cell lines we have shown that fluorescently labeled Aβ colocolized with plasma membrane and started to enter the cells only after some hours of incubation. In both cell lines Aβ colocalization with acidic organelles was found. Concerning the kinetics of Aβ penetration into the cells, we observed differences between cell lines and also between Aβ peptides with different fluorescent labels.

Иллюстрации

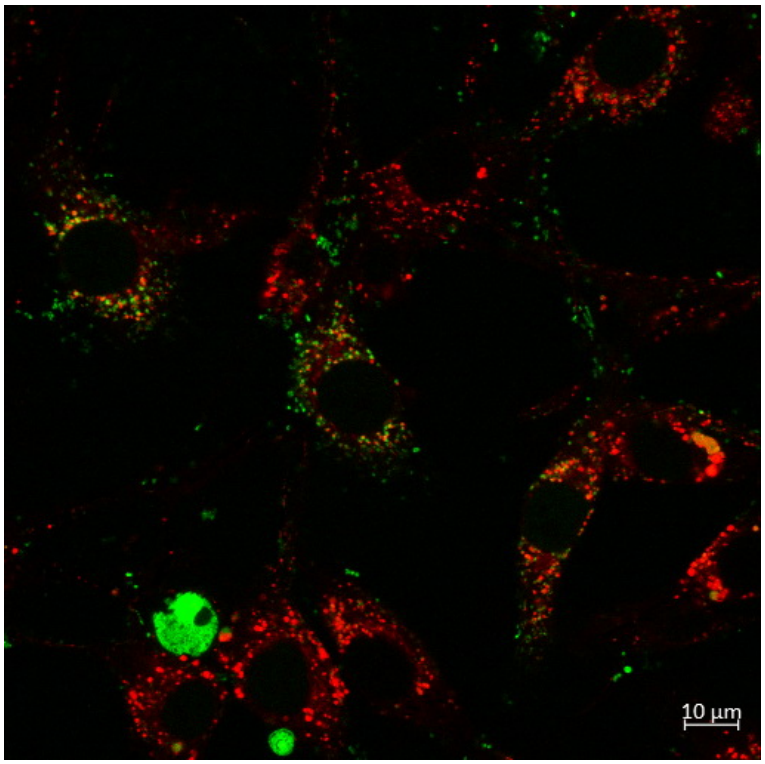

Рис. 1. Green: FITC-Aβ, after 16h of incubation; Red: LysoTracker Red;

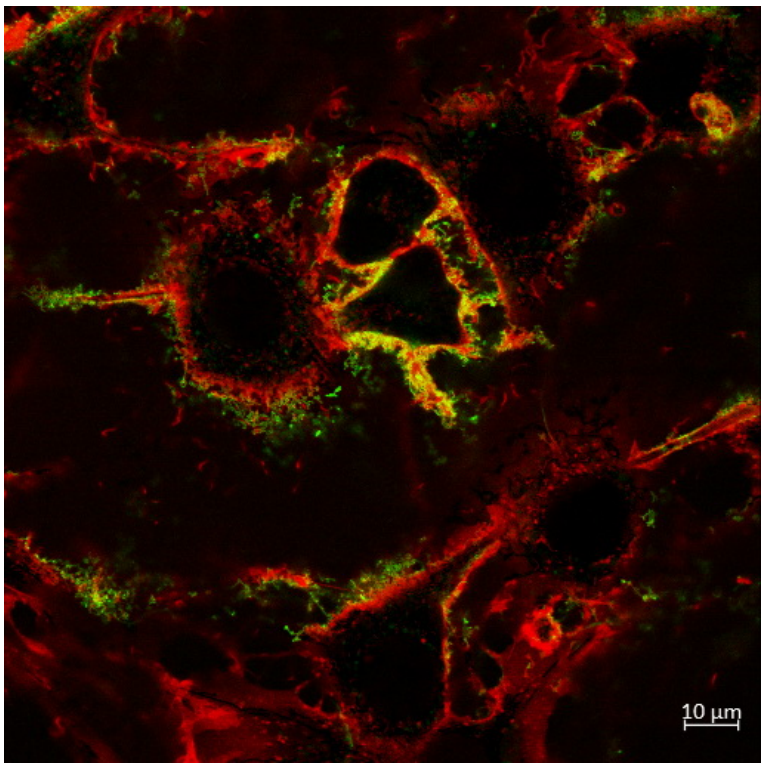

Рис. 2. Green: FITC-Aβ, after 6h of incubation; Red: CellMask plasma membrane stain;