Specific Targeting of Microglia with Quantum Dots

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Introduction: Microglia are resident immune cells in the brain, responsible for the innate immune responses. Accumulating evidence suggests that microglia play important roles in neurological disorders. They become readily activated in response to brain injuries or to immunological stimuli, leading to release of a variety of soluble factors that contribute to neurodegeneration. In Alzheimer disease (AD), neurodegeneration may be exacerbated by chronic inflammatory reactions of microglial cells [1]. We report a simple method using semiconductor quantum dots (QDs) to visualize and modulate microglia in culture and in mouse brains with high specificity. By covalently linking with Qds, toxin saporin led to selective elimination of microglia, and increased neuronal survival against amyloid toxicity in primary cortical cultures.

Methods: The ability of QDs to target microglia suggests that they can be modified to deliver bioactive compounds to microglia specifically. To investigate this hypothesis, we modified QDs to deliver a cytoxin saporin, which belongs to a family of single-chain ribosome inactivating proteins (RIPs). Saporin was biotinylated to couple with QD-streptoavidin with a relative stoichiometry of 1:100 to 1:10. The solution was incubated at room temperature with continuous shaking for 12hrs before it was used for further analysis. X-ray photoelectron spectroscopy was used to confirm covalent linkage of saporin on QDs surfaces. We applied QDs conjugated with saporin to primary cultures for 2 days and quantified microglia after the treatment. Microglia were labeled with antibodies against cd11b or Iba-1.

Results/Discussion: Presently, specific targeting of QDs in biological systems has been achieved with targeting-ligands or surface-immobilized antibodies. Due to the phagocytotic ability of microglia, we reasoned that microglia may have the natural ability to uptake QDs without any targeting signal. To test this hypothesis, we investigated the passive uptake of QDs in primary cultures derived from neonatal mouse cortices, which contain three main cell types in the brain: MAP2-positive neurons, GFAP-positive astrocytes, and CD11b-positive microglia. The QDs (QD-streptavidin, Invitrogen) were applied at different concentrations to the mixed culture system and visualized 2–24 h later. Notably, QDs (4 nM) were selectively internalized by microglia labeled with CD11b antibody. No significant internalization of dots was observed in MAP-2 positive neurons or GFAP-positive astroglia (Figure 1).

Conclusion: Treatment with non-conjugated Qds or saporin did not significantly affect the number of microglia compared with non-treated control. Application of QD-SAP resulted in a marked reduction of microglia labeled with Iba1 or cd11b antibodies, without affecting the number and morphology of neurons or astroglia.

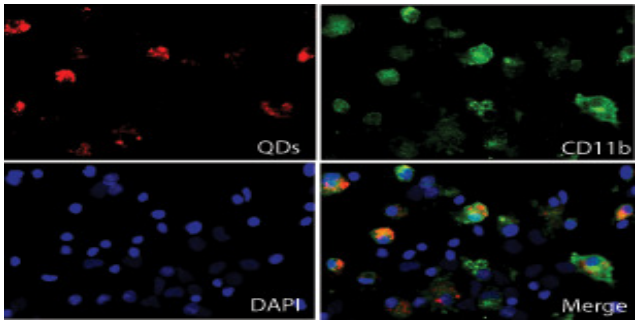

Fig. 1 QDs are specifically targeted to microglia in mixed primary cultures from rat cortices. QDs (red) are localized primarily in microglia labeled with an anti-cd11b antibody (green). DAPI (blue) stains the nuclei.

References:

1. Chen, J., et al., *SIRT1 Protects against Microglia-dependent Amyloid-{beta} Toxicity through Inhibiting NF-{kappa}B Signaling*. J Biol Chem, 2005. **280**(48): p. 40364-74.