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Commentary

Combining Immunofluorescence and Electron Microscopy with Quantum Dots

Charles Duyckaerts* and Jeanne Lainé†

From the Raymond Escourolle Neuropathology Laboratory, GHU Pitié-Salpêtrière, and CR ICM (UPMC, INSERM UMR S 975, CNRS UMR 7225), Paris; and the UMRS974 Institut de Myologie,† Paris, France*

The article by Uematsu et al[1] in this issue of *The American Journal of Pathology* reports the development of a new method of correlating information collected with immunofluorescence and electron microscopy. They apply the described method to Pick's body, an inclusion found in a rare but well-identified type of frontotemporal dementia.

Has the Time for Electron Microscopy Passed?

Electron microscopy (EM) had its heyday in the 1960s and 1970s. It was during that period that Kidd[2] described the paired helical filaments of Alzheimer's disease. Although EM opened a new chapter for morphology, it did not yield information on the protein content of what was seen—information that immunolabeling can disclose. Subsequently, interactions between proteins became accessible with double labeling, and confocal microscopy has been used to determine whether normal or pathological structures are colocalized or superimposed and to obtain three-dimensional imaging of the normal or pathological structures. With advances in immunohistochemistry, this approach has replaced EM for many routine clinical applications, including detection of specific cell markers, viruses, and protein accumulations in neurodegenerative diseases. The preparation of EM grids was considered too complex, the maintenance of the microscope too expensive, and the EM experts aged, retired, and were not replaced. As a consequence, a large number of electron microscopes have been removed from hospitals and research centers.

The need for ultrastructural details, however, remains as pressing as ever, and has even grown recently with the identification of new markers (in the field of neurodegenerative diseases, for instance) and the concurrent loss of EM expertise at many centers. For ultrastructural information to be useful, however, the data must now be associated with molecular information, such as that yielded by immunolabeling. Combining light and EM information (an approach known as correlative light and electron microscopy, or CLEM[3]) appears to be an important step in the unraveling of the relationship between structure and function or between an inclusion and the pathological mechanism with which it is associated.

Quantum Dots

Quantum dots (QD) are fluorescent nanocrystals with high quantum yield, minimal fading, and a narrow emission spectrum. They can be detected by EM, a process that originally included silver enhancement,[4] but that step does not appear to be required, as shown by Uematsu et al[1] and by others.[5] Up to three types of QDs with distinct sizes, shapes, and emission peaks can be used simultaneously to label different proteins and to identify them using both light and electron microscopes.[5]

Uematsu et al[1] used QD 655 and confirmed their presence at the ultrastructural level by energy-dispersive X-ray spot analysis showing peaks corresponding to cadmium and selenium. Localizing the structure to be examined can be quite challenging when moving from light microscopy to EM, but Uematsu et al[1] used the laser beam of a microdissector to punch holes around the selected cell.

The Possibilities of CLEM

Combining transmission EM and fluorescence confocal microscopy opens the way to several new research possibilities. The first, using multiple QD labeling, will be particularly useful in the investigation of the inclusions that typify most of the currently known neurodegenerative diseases. Uematsu et al[1] studied Pick's bodies, inclusions that are one of many abnormal structures that ac-

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Address reprint requests to Charles N. Duyckaerts, M.D., Ph.D., Laboratoire de Neuropathologie Escourolle, GHU Pitié-Salpêtrière, 47, Boulevard de l'Hôpital, 75651 Paris Cedex 13, France. E-mail: charles.duyckaerts@psl.aphp.fr.

cumulate tau protein in neurodegenerative diseases. Tau protein is indeed also found in the astrocytic tufts of progressive supranuclear palsy, in the astrocytic plaque of corticobasal degeneration, and in the postsynaptic compartment in argyrophilic grain diseases. In those disorders, the tau protein contains four repeats; in Pick's body, however, the tau protein contains three repeats. In Alzheimer's disease, the neurofibrillary tangles are made up of both three- and four-repeat tau. Why is tau accumulation so diverse in its composition and in the shape of the inclusions with which it is associated? CLEM could help elucidate this matter and explain why some tau isoforms associate with specific subcellular topography.

Neuronal inclusions are often thought of as being composed of one single protein; however, as shown by multiple labeling, they actually combine several proteins, sometimes sequestering essential functional components of the cells. Nuclear intraneuronal inclusions (NII) are good examples of such multiprotein aggregates.[6,7] Their molecular composition may be approached by immunohistochemistry, but knowledge of their subnuclear localization relies heavily on transmission EM studies, which are but poorly represented in the literature. This leads to the second new research possibility, fluorescence double labeling combined with EM. This method may determine whether two proteins colocalize or are sufficiently close to give rise to Förster resonance energy transfer (FRET). Using QD labeling, EM may then be applied to determine the subcellular topography of the protein or proteins involved. The third possibility, immunofluorescence combined with confocal microscopy, may also be used to reconstruct in three dimensions a labeled structure, as was done by Uematsu et al.[1] In this study, the volume rendering of Pick's body uncovered defects or lacunes in the inclusions. Study of the three-dimensional structure at the light microscopy level may secondarily be associated with transmission EM analysis to identify potentially involved organelles. It might even be possible, in some cases, to add to the confocal three-dimensional reconstruction a three-dimensional reconstruction at the submicron, ultrastructural scale, such as for synapses.

Summary

The questions that could be addressed by CLEM, particularly in neurodegenerative diseases, are numerous. They will be answered by scientists who master both transmission EM and immunofluorescence microscopy. The report[1] by Uchihara's team, with its combination of expertise, demonstrates the power of this new approach and the possibilities afforded to us in the future.

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