Laser capture microdissection (LCM) is a new technology that is becoming increasingly important for studies of neurodegenerative disorders. A characteristic feature of all neurodegenerative diseases is “selective vulnerability.” In each of the disorders, there is selective degeneration of particular types of neurons, with relative preservation of much of the rest of the brain. Familiar examples are the selective degeneration of dopaminergic neurons in Parkinson disease, the striking depletion of basal forebrain cholinergic neurons in Alzheimer disease, the selective atrophy of the caudate nucleus in Huntington disease, and the loss of spinal motor neurons in amyotrophic lateral sclerosis. Closer examination reveals an even more fine-grained pattern of neuronal injury. For example, in Parkinson disease there is striking depletion of dopaminergic neurons in the ventral tier of the substantia nigra pars compacta, with very little injury to dopaminergic neurons located only a few millimeters away in the dorsal tier of the nucleus. In Huntington disease, there is an exquisite degree of selectivity among the different types of neurons found within the caudate and putamen, with striking depletion of the medium spiny projection neurons, especially those projecting to the globus pallidus, in contrast to preservation of the intermingled interneurons. These characteristic patterns of injury are evident even in genetically determined forms of neurodegenerative disease where the fundamental defect can be shown to be present in all neurons. Understanding the basis for these patterns of neuronal loss may provide important insight into the mechanisms of neurodegeneration.

Selective vulnerability creates difficult technical challenges for investigators attempting to apply modern molecular methods to the study of disease. Many of the current methods for analyzing DNA, messenger RNA (mRNA) and proteins rely on homogenization and extraction of the chemical elements of interest. Until recently, most studies of this nature have relied on studies of anatomical regions at least several millimeters in size, and often much larger. These areas are large enough that they unavoidably encompass both affected and unaffected neuronal populations. They also include a large component of glial structures, which may have their own distinct role in and response to neurodegeneration. The data obtained by homogenization of such heterogeneous samples are often difficult to reconcile with alterations in the biology of specific populations of component cells. Researchers can use LCM as an accessible method for separating the different cell types present in a brain region, and are able to study the biology of homogenous cell populations with specific characteristics.

METHODS

Laser capture microdissection was developed at the National Cancer Institute for studies of the cellular components of systemic neoplasms. At present there are several types of LCM instrumentation commercially available for routine laboratory
use. All of the instruments are capable of isolating populations of cells from histological tissue sections. The most widely used instrument is marketed by Arcturus Inc (Mountain View, Calif) and is a variant of the original method developed at the National Cancer Institute. This instrument uses an ethylene vinyl acetate (EVA) film and an infrared laser to capture cells. Histological sections mounted on glass microscope slides are placed on the stage of an inverted microscope. A plastic cap topped with EVA film is placed so the film is in contact with the tissue. The cells of interest are identified through the microscope and the laser is used to melt the film overlying individual cells, resulting in adhesion of the cells to the film. A variety of other technical approaches to LCM have been developed. Some of these approaches require mounting the sections on plastic film and using a laser to cut away unwanted sample. Other techniques use the pressure generated by intense light to catapult cells out of a tissue section and into a receptacle.

An essential part of the design of an LCM experiment is determining how the collected cells will be identified. It is important to consider both specificity of the staining method as well as the effect the staining reagents may have on the properties of the cells. All of the LCM instruments provide optics for visualization of both fluorescent as well as opaque histological stains. For many types of examinations of the brain, simple histological stains such as methylene blue dye may be sufficient; however, cellular isolation depends on the ability of the operator to recognize anatomical landmarks and features. This approach is sufficient, for example, to isolate the melanized dopamine neurons of the substantia nigra (Figure), or the motor neurons of the ventral horn of the spinal cord. For other kinds of studies, immunohistochemical staining for specific neurochemical markers can be helpful. For example, a stain for choline acetyltransferase provides definite identification of cholinergic neurons in the basal forebrain, and a stain for nitric oxide synthase identifies interneurons in the caudate and putamen. Although specific immunohistochemical stains may provide important information about a specimen’s specificity, they may introduce other problems. This is particularly evident in studies of RNA, which is very labile. The staining protocols used in pathological studies may call for incubating the tissue for hours in antibody solutions, which may result in complete degradation of the RNA present. It is possible to develop protocols for rapid immunohistochemical staining to circumvent this problem.

**ANALYSIS OF LCM SPECIMENS**

In practice, the analysis of LCM specimens is considerably more challenging than their collection. At present, 3 distinct classes of biomolecules can be analyzed in LCM specimens: DNA, RNA, and proteins. Each of these requires distinct approaches to sample collection and markedly different sample sizes.

The most sensitive detection methods are for the study of DNA. This is largely the result of 2 factors: the relative stability of DNA under a variety of tissue storage and processing conditions, and the sensitivity of polymerase chain reaction–based methods for DNA amplification. DNA analysis has been performed on both somatic genes encoded within the nucleus of neurons as well as on genes present in the mitochondrial genome. For example, it is possible to study the size of CAG repeat expansions in single neurons in disorders such as Huntington disease and dentatorubropallidoluysian atrophy. The extreme sensitivity of this method is demonstrated by the fact that a single neuron contains only one molecule of DNA with the expanded allele. Such studies prove that although the primary genetic defect is inherited, the genetic abnormality may be further modified in some neurons during development and adult life. Studies of mitochondrial DNA can detect the heteroplasmy that arises because each cell has several mitochondria, each of which may have different DNA defects.

The most frequent application of LCM is for studies of mRNA levels in individual cells. These studies are of great interest because they provide information about the synthetic activities of the cells. Two general kinds of analytic methods are used. To study the expression of individual genes, mRNA can be isolated, converted into DNA using a reverse transcriptase enzyme, and studied with quantitative polymerase chain reaction methods. This approach can be used to study small numbers of neurons, even single cells in some cases; however, sample sizes generally range from 10 to 100 neurons. A broader view of cellular transcription activity can be obtained by gene array profiling using microarrays. This approach allows the simultaneous study of a very large number of genes, up to 65000 using current methods. Array methods require amplification of the mRNA. Several approaches for
this are available, but each may introduce artifacts that need careful controls. Sample sizes for gene array studies are typically 500 to 2000 neurons.

Direct analysis of proteins in LCM samples would be of great interest to researchers. It is clear that many regulatory steps in cell biology depend on post-translational modifications, such as phosphorylation. In addition, mRNA levels in many cases are not directly correlated with the abundance of the encoded protein. A variety of proteomic techniques have been employed both in direct studies of the human brain, including both automated gel electrophoresis as well as mass spectrometry. A major barrier to the application of these powerful techniques to LCM is the lack of a reliable method for protein amplification. In some cases, this problem has been circumvented by the use of very large sample sizes, typically 10000 to 50000 cells. This approach is impractical in the neurodegenerative disorders, where the number of cells constituting a homogeneous population is limited.

USE OF LCM IN THE STUDY OF NEUROLOGICAL DISORDERS

Although LCM is still a new technology, several studies have made use of this approach to draw interesting and novel conclusions. Laser capture microdissection has been employed both in direct studies of the human brain, as well as in animal models of neurological disease. In amyotrophic lateral sclerosis, LCM has been used to isolate motor neurons and demonstrate alterations in oxidative metabolism and proteosomal function. Several studies have made use of LCM to study the expression of viruses among different classes of neurons and glia, most notably human immunodeficiency virus. In rodent models, LCM has been used to isolate dopaminergic neurons from the ventral tegmental area, and study alterations in gene expression induced by cocaine administration. Laser capture microdissection has also been applied to gene array profiling of single neurons from the rat hippocampus, and for revealing distinct patterns of gene expression in neurons and interneurons. In our laboratory, we have applied LCM to studies of gene expression profiles in human dopamine neurons as well as rodent models of Huntington disease.

Laser capture microdissection methods have been in development for more than a decade, but it is only recently that the technology used for LCM has reached a sufficient level of accuracy for widespread use in neurological disease. In the future, this powerful new tool will provide important insights into the etiology of neurodegenerative disorders and further explain the basis of selective vulnerability.

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