Understanding Disease Cell by Cell

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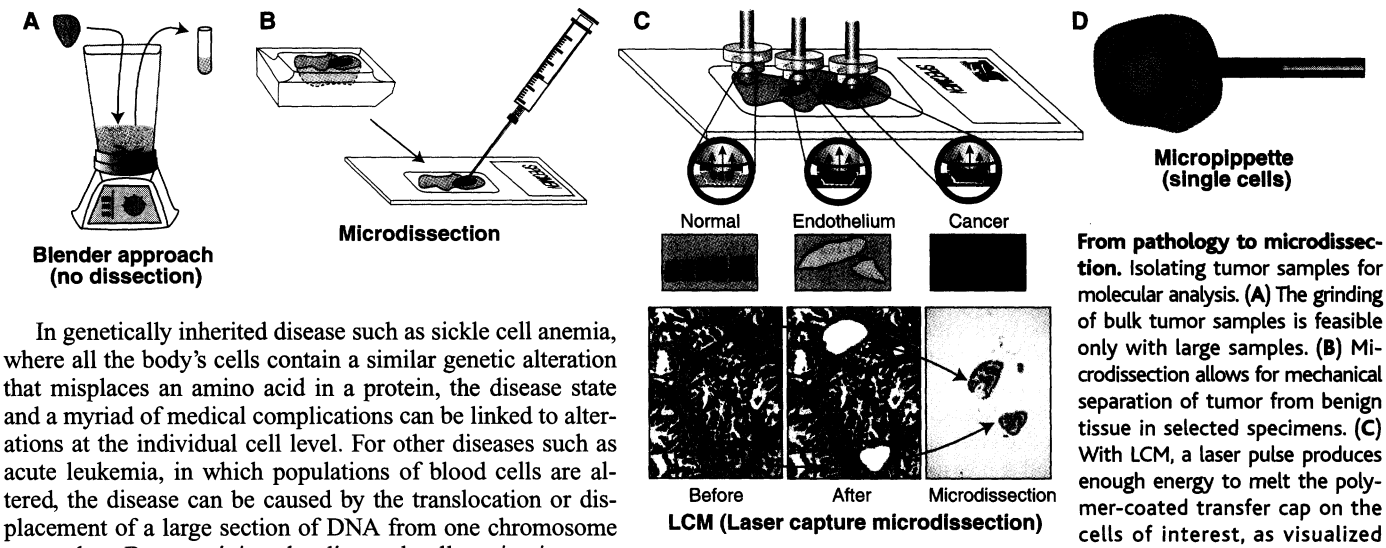
In 1858, Rudolf Virchow published a series of lectures as a book entitled *Cellular Pathology* (*1*), which altered medical research by turning the focus from the diseased cell's form to its function (*2*). Virchow postulated that the cell constitutes the basic unit of disease, a new paradigm that helped create fields of science to explore the physiology of disease as opposed to the pathology of disease, which, as practiced in the mid-1800s, mainly described disease from visual inspection of organs at autopsy.

In genetically inherited disease such as sickle cell anemia, where all the body's cells contain a similar genetic alteration that misplaces an amino acid in a protein, the disease state and a myriad of medical complications can be linked to alterations at the individual cell level. For other diseases such as acute leukemia, in which populations of blood cells are altered, the disease can be caused by the translocation or displacement of a large section of DNA from one chromosome to another. By examining the diseased cells, scientists can understand these alterations and develop treatment to correct them. Still, the vast majority of human disease—particularly solid tumors in humans—cannot be explained so simply.

One limitation to understanding disease at the cellular level has been our inability to isolate pure populations of diseased cells. With acute leukemia, a neoplasm affecting blood cells, viable tumor cells can be easily collected from an affected individual's blood. However, solid tumors (also referred to as carcinomas) like those found in the prostate and breast, cannot be easily isolated. Carcinomas expand by infiltrating among preexisting nonmalignant structures. Tumors develop their own blood supply (i.e., tumor endothelium), traverse supporting tissues, and enter into blood and lymphatic channels, allowing tumor spread to distant sites. Populations of benign lymphoid cells may also be "recruited," making the isolation of pure populations of tumor cells difficult. To investigate and better understand carcinogenesis, researchers must dissect malignant prostate cells away from the neighboring benign structures. Microdissection acheives this goal via precise mechanical separation.

Early work in tumor biology often relied on the grinding of tumor samples [see (A) in the figure below], an approach that was feasible before the advent of prescreening because detected tumors were typically large. A fresh sample would yield relatively pure populations of tumor cells that could be isolated by gross examination, ground up, and evaluated. This approach led to an important understanding of colon cancer development by Bert Vogelstein's group (*3*, *4*). However, two factors necessitated the development of microdissection: (i) the increasing difficulty in obtaining adequate-size tumor samples and (ii) new molecular techniques that require pure populations of cells.

Clinical screening tests help doctors identify tumors earlier than before, thus dramatically reducing the size of an obtainable sample at the time of detection. For example, the stool blood test can help identify early colonic and rectal cancers. And blood tests for carcinoembryonic antigen (CEA), CA-125, and prostate specific antigen (PSA) can help detect early-stage colon, ovarian, and prostate tumors, respectively. In the early 1990s, surgically resected breast and even prostate cancers could be identified by gross visual inspection. Over the last several years, however, these and other tumor types have been extremely small because of early detection. Computer tomography or mammography can provide images of some of the earliest tumors before they become clinically significant. Even the most seasoned surgical pathologist cannot easily identify tumors that are only readily visible under the microscope.



From pathology to microdissection. Isolating tumor samples for molecular analysis. **(A)** The grinding of bulk tumor samples is feasible only with large samples. **(B)** Microdissection allows for mechanical separation of tumor from benign tissue in selected specimens. **(C)** With LCM, a laser pulse produces enough energy to melt the polymer-coated transfer cap on the cells of interest, as visualized through a microscope. The before and after images show removal of prostate cancer cells. **(D)** Micromanipulation with a micropipette allows the contents of the entire nucleus to be removed.

Emerging molecular technology also drove development of microdissection methods. In the early 1990s, for example, tumor biologists screened for genetic mutations by looking for molecular alterations along the genome with a technique called loss of heterozygosity (LOH) analysis. This approach used small, known sequences of DNA to inspect chromosomes segment by segment in search of mismatches or microsatellite lesions. LOH requires the comparison of relatively pure populations of tumor and normal cells from the same individual. For large solid tumors, lymphomas, or melanomas, this could be readily performed with the use of existing approaches. However, prostate cancer, because of its infiltrative nature, required a new form of tumor isolation.

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The first type of microdissection used a standard syringe to dissect a tumor viewed under a microscope [see (B) of the figure]. Pulling and tugging tissues with a syringe dislodges the tumor, which could be transferred with a pipette for analysis. This technique allows one to work with relatively small samples. It is also easy to perform, because it requires only a syringe, alcohol, and a microscope. This low-tech, low-cost method allowed many tumor biologists to look for LOH and then determine any molecular mutations by sequencing the DNA. Early work on alterations in chromosome 8 in prostate cancer, using clinical samples that required microdissection, was made possible by this approach (*5*). But the early methodology had some limitations because the tumors still needed to be well delimited at a microscopic level. Thus, in a series of cases studied, only a small number could be used with this method, potentially biasing results to reflect the biology of larger and more advanced tumors. Pulling and tugging indeed dislodged the cells and glands of interest, but other cells could come along for the ride, making the dissected sample less than pure. In short, early forms of microdissection excluded many cases from molecular examination and thus was limited to well-circumscribed groups of cells.

Laser Capture Microdissection

Microdissection techniques progressed in a logical way, and the end result was Laser Capture Microdissection (LCM) [see (C) of the figure]. It offers laser precision and can achieve transfer and isolation of single cells. LCM was developed by Emmert-Buck and colleagues at the National Cancer Institute (NCI) (*6*). The technique was born of a need to isolate pure populations of tumor, normal, and dysplastic tissues for the Cancer Genome Anatomy Project (CGAP) (http://cgap.nci.nih.gov) (*7*). The most widely used LCM device was developed in collaboration with the NCI and commercially manufactured by Arcturus Engineering (Mountain View, CA). In this version, a microscope is coupled with a laser. After cells of interest are visualized with the microscope, activation of the laser causes fusion of the cells with a thermoplastic transfer film located on the undersurface of a modified Eppendorf cap [see (C)]. Through this translucent cap, one can aim, and "shoot" the laser (thereby fusing) at individual cells or groups of cells. Once all the cells of interest have been fused to the cap surface, the cap can be lifted off the tissue and placed directly onto an Eppendorf tube filled with the appropriate reagents for extraction. Newer versions by Arcturus and MMI (Heidelberg, Germany) allow the researcher to designate areas of interest on a computer screen using the mouse, creating a microdissection map. The software then mechanically guides microscope stage, allowing for a precision that is not possible manually. Whereas manual microdissection required "good" cases—visually distinct populations of cells—for tumor isolation, LCM allows the researcher to choose virtually anything he or she can see under the microscope.

LCM now allows the investigator to ask questions regarding individual cells and the surrounding stromal tissues. Though a considerable number of studies have used this methodology, it is likely that a large number of investigators are struggling with questions regarding the best way to analyze these small samples. In a recent study, Sgroi *et al.* compared cDNA expression profiles from LCM breast cancer samples (*8*). Using LCM, they isolated between 1.7×10^4 and 2.0×10^4 cells from various populations of breast tissue, including cancer. Total RNA was extracted, converted to cDNA, and hybridized to nylon membrane gene arrays containing over 8000 genes. Their work identified genes that were differentially expressed in populations of metastatic and invasive cancers in comparison to normal breast cells. Thus, cDNA analysis combined with LCM represents an important approach to understanding the earliest events in tumor development.

With LCM, limitations in examining individual cells primarily concern the molecular methodologies. For example, in order to perform cDNA expression array analysis on a standard gene chip, one needs approximately 10 to 15 μg of cDNA for the hybridization. This means that, unless thousands of cells are obtained, LCM-isolated samples require an amplification step. Many researchers are working on protocols to take small samples with 50 to 100 ng of RNA and amplify them in a manner that will minimize bias in the transcript profile (*9*).

LCM is being adopted for work in the field of cancer proteomics. A joint venture between the NCI and the Food and Drug Administration (FDA) received widespread publicity for development of a proteomics-based test with the use of a bioinformatic algorithm for ovarian cancer (*10*). Epithelial ovarian cancer kills 16,000 women each year, due in part to late-stage disease detection and the lack of reliable biomarkers for that detection. Alone, CA-125 (*11*), the currently accepted serum marker, lacks the sensitivity for early-stage diagnosis. The test developed by the NCI-FDA team yielded a sensitivity of 100% [95% confidence interval (CI), 93 to 100%], specificity of 95% (95% CI, 87 to 99%), and positive predictive value of 94% (95% CI, 84 to 99%) (*10*). The joint venture is also combining LCM with two-dimensional polyacrylamide gel electrophoresis (2D PAGE) to identify specific proteins that may serve as invasive ovarian cancer–specific biomarkers for early detection and/or new therapeutic targets (*12*). The analysis identified three proteins that were overexpressed in invasive, malignant ovarian tumors in comparison to ovarian tumors of low malignant potential (*12*). This work required isolation of 50,000 cells per tumor, again suggesting that the ability to microdissect has surpassed the molecular methodologies for analysis.

Single-cell microdissection: Uncovering mysteries

Micromanipulation of single cells has enabled researchers to uncover the mysterious origin of the cell responsible for Hodgkin's lymphoma [see (D) of the figure] (*13*–*15*). For over 100 years, the rarity of the characteristic neoplastic cell responsible for the malignant lymphoma, referred to as the Reed-Sternberg cell, kept its identity a mystery. These cells are typically surrounded by a sea of benign inflammatory cells such as B cells, T cells, and macrophages. By performing micromanipulation of the individual Reed-Sternberg cells, researchers were able to create cDNA libraries (*15*) that later were used to further classify this neoplasm (*13*, *14*). On the basis of its gene expression profile, researchers were able to confirm that the Reed-Sternberg cell is derived from B cell lineage. Perhaps the most noted development involving microdissection in the recent past was the transfer of a single nucleus to create Dolly, the first cloned large farm animal (*16*).

These examples of microdissection highlight the incredible power of understanding and using the information of individual cells. As Virchow postulated, the single cell represents the basic unit of disease, or of health. Microdissection is helping to harness emerging technologies so that scientists may better understand disease at its most basic level.

References and Notes

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17. I would like to thank R. Kunkel and L. Schumacher for help in developing the illustrations and A. M. Chinnaiyan, S. Kunkel, and C. G. Kleer for helpful conversations.