

WHAT PATHOLOGY EXAMINATION OF YOUR ESOPHAGEAL BIOPSY CAN TELL YOUR DOCTOR ABOUT THE HEALTH OF YOUR ESOPHAGUS

Sydney D. Finkelstein, MD

Adjunct Professor of Pathology, Drexel University

Co-Founder and Chief Scientific Officer

RedPath Integrated Pathology, Inc.

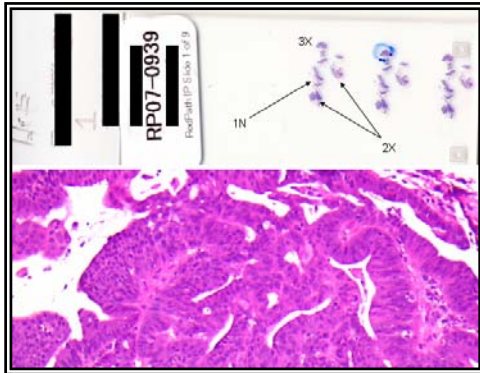


Figure A: *Top:* Multiple biopsies of the esophagus are prepared for microscopic examination by being placed on a microscope slide. The arrows point to specific sites within the biopsies that are most important for diagnosis. Note that the pathologist has indicated in blue ink one specific site labeled 3X which is of particular concern. *Bottom:* Observed at higher power, there is evidence of cancer present at the site marked 3X.

TRADITIONAL MICROSCOPIC EVALUATION AND DIAGNOSIS OF BARRETT'S ESOPHAGUS and ESOPHAGEAL CANCER

The diagnosis of Barrett's esophagus is performed by the pathologist viewing your biopsy at high magnification under the microscope (*Figure A*). In Barrett's esophagus the inner lining of the organ undergoes a conversion from normal squamous cells to a lining composed of cells that resemble those that compose the inner lining of the small intestine (*Figure B*). The evaluation does not stop there. The Barrett's esophageal lining is then carefully evaluated for changes that indicate a transition to cancer is occurring. This is a very important part of the pathology evaluation because Barrett's esophagus has a much higher tendency than normal esophageal squamous mucosa to pursue transition from normal to cancer.

The transition from Barrett's esophagus to cancer is a process which does not take place at one point in time but it occurs through a series of progressive changes affecting the lining cells over an extended period of time, typically several years. During this time, the Barrett's esophagus lining cells

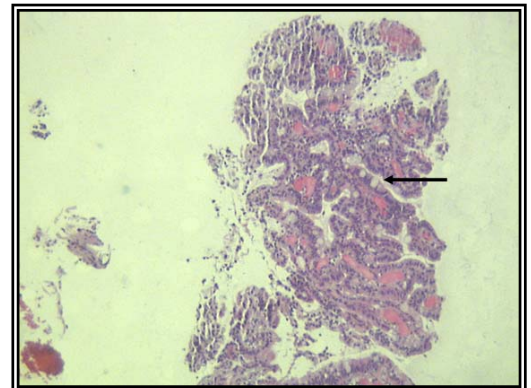


Figure B: While the resemblance to small intestine is not perfect, the lining of the lower esophagus has been converted to one that simulates a more acid resistant pattern seen in the small intestine. Mucin is produced by a goblet cell which is not normally found in the esophagus but which is present in the small intestine. While cancer is not present, there are cellular changes in this Barrett's esophagus that indicate transition towards cancer.

continue to turnover being replaced by successive populations of cells that approach those that are present in cancer (*Figure C*). During this phase, which is referred to as dysplasia, the cells are not yet fully cancer cells but at the same time they are not normal either. The transition from normal cells to cancer cells is a continuum starting with normal cells and ending, over a period of time, with cells that are cancerous. It is important not only to detect the presence of Barrett's esophagus but, when it is present, to detect whether changes are occurring that indicate that the process is heading towards becoming cancer. The specific form of treatment that is applied will be determined by this information derived from examining the biopsy specimens.

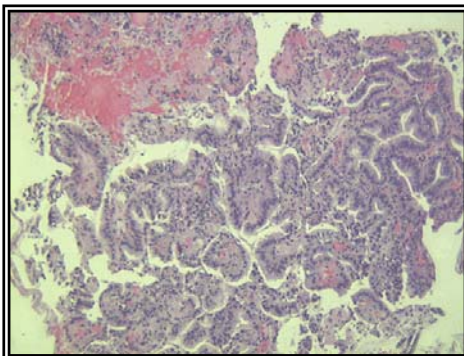


Figure C: Over time changes have taken place in the Barrett's esophagus lining cells that bring them closer to becoming cancer.

Not all patients with Barrett's esophagus undergo this progressive transition to cancer. In many patients, the cells resemble normal appearing small intestine lining cells and remain in that state indefinitely never changing to become cancer.

In a significant proportion of patients with Barrett's esophagus, however, the cells begin the process of changing into cancer cells. The rate at which this progression occurs can be quite variable from months to many years. In those Barrett's esophagus patients that undergo the transition in the direction of becoming cancer, the transition is generally slow at first, gaining speed as the Barrett's lining cells approach cancer. But this is not

always predictable and the rate of progression can vary from one person to another. The cells can progress to a particular stage of precancerous dysplasia and then remain fixed at that stage for an extended period of time. We do not fully understand what accounts for the variability in Barrett's dysplasia progression but it is well known that each patient pursues his or her own unique and personalized pattern of disease evolution. Molecular methods applied to tissue biopsy samples now exist where they have not in the past to enable the gastroenterologist and the pathologist to be able to detect and characterize this progression at the individual patient level affording personalized medicine.

The conversion from normal appearing cells to cancer is a continuum. The ends of the continuum - normal and cancer cells - are relatively easy to recognize, and pathologists are generally very confident when faced with diagnosing the polar ends of the Barrett's esophagus spectrum. Barrett's cells along the continuum, however, can be very difficult to precisely define and pathologists generally differ with each other on the exact placement of individual cases. In practice, two phases of the precancerous dysplasia continuum are recognized and referred to as low grade dysplasia and high grade dysplasia. Low grade dysplasia (LGD) refers to Barrett's esophagus cells along the continuum that more closely resemble normal cells than cancer cells. Conversely, high grade dysplasia (HGD) refers to Barrett's esophagus cells along the continuum that more closely resemble cancer cells than normal cells (*Figure D*). It is important to recognize the difference between LGD versus HGD because each is managed in different ways reflecting the relative risk that cancer will occur if left untreated. LGD is handled in a relatively gentle fashion ensuring that the side effects of treatment are minimized. HGD is treated relatively more aggressively, accepting moderate side effects in order to avert the development of cancer.

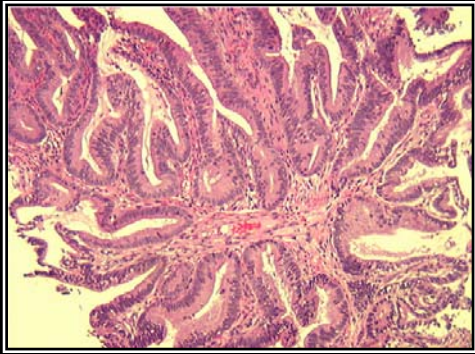


Figure D: The Barrett's lining cells more closely resemble cancer cells than they do the normal small intestinal cells from which they have been derived. This is referred to as high grade dysplasia.

Pathology evaluation of the esophageal biopsy addresses all these important aspects of the biology of Barrett's esophagus. Your physician wants to understand your Barrett's esophagus to the best extent possible so that treatment can be optimal for you. The more information your treatment physician has about the unique form of Barrett's esophagus that you have, the better and more confident your doctor will be in managing this disease. The pathologist plays a critical role in providing your physician information on 1) whether Barrett's esophagus is present, 2) whether dysplasia is present raising a concern that cancer may occur at a later point, 3) where along the continuum from normal to cancer Barrett's dysplasia has reached at the time that the biopsies are taken and 4) how rapidly the transition to cancer is taking place when Barrett's dysplasia is found to be present.

PERSONALIZED DIAGNOSIS OF BARRETT'S ESOPHAGUS

	NORM	PRE	PRE	PRE	POST	POST
	ESOPH	ESO	ESO	ESO	ESO	ESO
	ESOPH	AREA 1	AREA 2	AREA 3	AREA 1	AREA 2
1p						
1p						
3p		48%	54%	62%	76%	69%
3p						
5q						
5q						
9p		50%	44%	78%	69%	63%
9p						
10q						
10q					60%	66%
17p		83%	78%	94%	56%	63%
17p						
17q						
18q		66%	72%	74%		
21q						

Figure E: In addition to performing microscopic examination, there now are means to detect and characterize the mutational changes that are present at specific microscopic locations in Barrett's esophagus. Here, molecular analysis has been performed before and after treatment has been applied.

No two patients with Barrett's esophagus are alike. Barrett's patients differ in the extent of the esophagus lining that is converted into small intestinal appearing cells. In some cases, only a short segment is affected while in other cases a large portion of the lower esophagus experiences this change. Patients differ with respect to whether they will undergo dysplasia or not. Most patients with Barrett's esophagus never develop dysplasia but some do. Patients developing dysplasia are of particular concern since they are the ones at risk for going on to cancer. And those patients with dysplasia will differ in how rapidly and consistently they will progress through dysplasia to cancer. It is possible for dysplasia, especially LGD, to even undergo regression which is highly desirable. Patients can transition from HGD down to LGD and from LGD down to normal appearing Barrett's type small intestinal mucosa lacking evidence of dysplasia. Your doctor managing your Barrett's esophagus is constantly aware of the unique personalized aspects of the disease. Thus each patient

receives treatment that is appropriate for the unique pattern of Barrett's esophagus that is present in that individual.

Recognizing the personalized quality of Barrett's esophagus includes the pathology evaluation of the biopsies that are taken. Each specimen is examined under the microscope to see if the Barrett's type intestinalized mucosa is present. The extent of such changes in the different biopsy samples of tissue is noted. Then the pathologist looks for the important evidence of dysplasia, indicating that the patient may be part of the subset of patients with Barrett's esophagus that are transitioning in the direction that ultimately may lead them to cancer. Recognizing the earliest such changes is very important and the pathologist now has powerful tools to assist in the effort: microscopic examination and sensitive molecular genetic methods which will be described in more detail below (Figure E).

Having detected the presence of Barrett's esophageal dysplasia, the pathologist then must determine how far along the spectrum a particular patient has progressed in the transition from normal appearing small intestinal lining cells to cancer cells. Because the changes are a continuum, it can be challenging to define whether a given person is within low grade dysplasia (LGD) or high grade dysplasia (HGD). Even within these two stages of dysplasia development, an individual patient at a particular point in time may find himself or herself closer to one end or the other. The pathologist now is empowered with state of the art methods to define dysplasia progression as accurately and objectively as possible using microscopic examination combined with molecular analysis (Figure F). The result of the pathology evaluation is a personalized diagnosis and characterization of Barrett's esophagus that will guide the most appropriate treatment and enable biopsy specimens taken over time to be compared to each other in a sensitive, reliable and highly informative manner.

Personalized diagnosis and treatment of cancer and precancer stages has emerged as a fundamental and valuable part of patient management. In the past, all patients classified as having the same disease process tended to be treated in the same way. It has been clear that despite having the same disease as determined solely by how the tissue looks under the microscope, patients apparently classified as one homogeneous group in fact can behave in widely differing fashions. The advent of the modern molecular era of gene discovery and DNA research has challenged longstanding concepts and led to diagnosis and treatment being adapted to the individual patient disease process. This is especially true for Barrett's esophagus which may look the same from one person to another but which is individually unique.

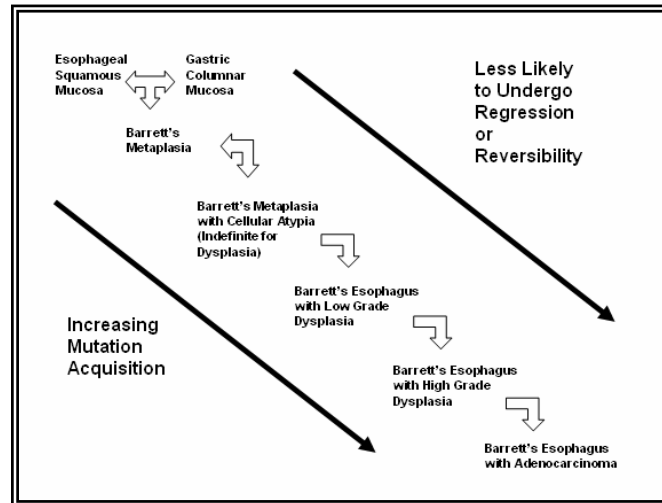


Figure F: This diagram highlights the transition from Barrett's esophagus to carcinoma. In concert with the cellular evolution, there is progressive accumulation of mutations.

THE ROLE OF MOLECULAR ANALYSIS TO COMPLEMENT MICROSCOPIC EXAMINATION OF BARRETT'S ESOPHAGUS SPECIMENS

There is much we need to discover about the forces that cause Barrett's esophagus to occur and what drives the development and progression into cancer for a subset of patients with the disease. Also, there is more to be learned about what makes one Barrett's patient different from another because the disease is not the same in every affected person. One concept that has been proven to be true is that Barrett's esophagus dysplasia has a genetic basis with multiple mutations acquired over time causing the lining cells to become progressively more altered until cancer is reached. Most Barrett's esophagus is not inherited but arises and progresses by the acquisition of mutational damage to the DNA of the lining cells. This process occurs as multiple events over time. When mutations are not inherited but arise in otherwise normal cells they are called *somatic* mutations. Somatic mutations are not present at birth and are not passed on from parent to child. Barrett's esophageal dysplasia and the cancer that can evolve from dysplasia are caused by the cells acquiring somatic mutations in a stepwise fashion over time (Figure F).

The relationship between somatic mutation acquisition and Barrett's dysplasia has been well studied over the past decade. The amount of mutations present in Barrett's lining cells correlates with the degree of dysplasia and provides an objective and quantitative means to define the extent of progression along the continuum of dysplasia. High grade dysplasia has more total mutations than low grade dysplasia cells (Figure F). Serial biopsies of Barrett's

Cancer cells differ from the normal cells from which they were derived, by a number of microscopically visible changes (*Figure I*). In cancer, most of the visible changes are found in the nucleus of the cell with enlargement, irregularity in shape and texture, and prominent increase in mitotic or replicative activity (*Figure J*). Pathologists search for such changes in all tissues that are examined under the microscope. When the alterations in the cells are extreme, then the diagnosis of cancer is made. When the changes are less intense, the diagnosis of precancerous dysplasia is applied. Two levels of precancerous dysplasia are generally recognized. Low grade dysplasia (LGD) is used when the changes associated with cancer are mild and overall the appearance of the Barrett's cells is more like normal with only slight deviation. High grade dysplasia (HGD) is invoked when the cells more closely resemble cancer cells but have not attained the full complement of alteration seen in that condition. The distinction between metaplasia, LGD, HGD and cancer is very important because the patient will be managed in different ways depending upon these phases of Barrett's esophagus.

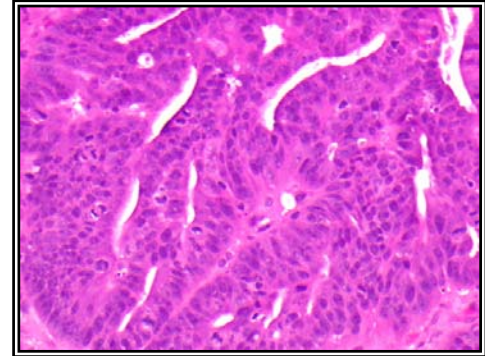


Figure I: Fully developed cancer cells differ from their normal counterparts by cellular and architectural features.

One of the main challenges in microscopic examination of Barrett's esophagus lies in the inability to objectively and reliably discriminate between the important stages of metaplasia, LGD, HGD and cancer. These states exist as a continuum and it can be very difficult to draw a sharp line between the different categories. Moreover, pathologists can differ among themselves in their interpretation of Barrett's esophagus. In a landmark paper published almost 20

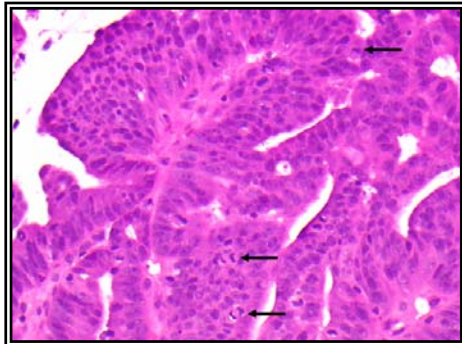


Figure J: Cancer cells show abundant mitosis indicating very active growth.

years ago, and subsequently confirmed in numerous further studies, it was found that a panel of nationally recognized experts in Barrett's esophagus differed among themselves up to 30% of the time when attempting to diagnose the stage of Barrett's dysplasia. This called into question the accuracy of Barrett's esophagus diagnosis even when the same microscopic slides are shown to a group of experienced Barrett's esophagus pathologists.

One conclusion that arose from these studies was a suggested classification of Barrett's esophagus that pathologists use so that there can be greater uniformity in the diagnosis of this condition. The classification recognizes a distinct microscopic category of changes in the Barrett's cells called "indefinite for dysplasia". Pathologists were

encouraged to make an affirmative diagnosis of "indefinite for dysplasia" when the changes were found to lie between two distinct states such as metaplasia versus LGD (*Figure K*). The problem with this approach is that it added even greater indecision to an already problematic classification that was subject to observer variation. Now, not only were pathologists differing with respect to the major categories of Barrett's dysplasia, but they were disagreeing on what precisely constituted "indefinite for dysplasia".

The recent availability of molecular genetic information through efforts such as the Human Genome Project and associated cancer genome database development has extended the diagnosis and characterization of Barrett's esophagus to the molecular level. Barrett's esophagus is a precancerous state leading to esophageal cancer and its basis is largely due to molecular changes in the form of mutational damage to DNA of the Barrett's lining cells. The microscopic appearance of the cell results from the aggregate effects of DNA damage occurring at the molecular level. Cell appearance is an end result of genetic damage while mutational change to DNA both precedes and is the cause of precancer and cancer formation. The power of molecular analysis lies in its ability to detect those critical changes within the cell that eventually manifest themselves in the appearance of Barrett's esophagus and dysplasia. Molecular analysis deals with causation of Barrett's esophagus while microscopic examination deals with the consequences of events which lead to cancer formation. It is only logical and practical that the addition of molecular analysis to microscopic evaluation will lead to a more accurate, reliable and more effective diagnosis and characterization of Barrett's esophagus.

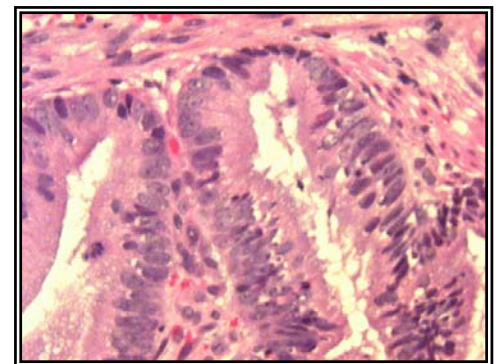


Figure K: Here is a commonly encountered situation where the microscopic features fall between the presence or absence of dysplasia. Using microscopic analysis alone, this distinction can be very subjective. Using molecular methods, the distinction can be made objectively.

WHAT HAS MOLECULAR ANALYSIS TAUGHT US ABOUT BARRETT'S ESOPHAGUS

We have learned a great deal by applying molecular analysis to conditions such as Barrett's esophagus and there remains a great deal more to discover. Even at this time, several insights have been clearly established. Cancer requires multiple DNA mutations to be acquired over time which act together to create a state of uncontrolled cell growth. Precancer states such as Barrett's esophagus contain lesser numbers of mutations associated with partial loss of growth control. It is possible to diagnose and characterize dysplasia on the basis of the cumulative amount and pattern of acquired DNA mutations. It is also feasible to separate dysplasia from the normal state and from cancer on this basis and to discriminate between low grade and high grade dysplasia based on the mutational profile of accumulated mutations (*Figure F*). The advantage of an integrated molecular and microscopic approach to Barrett's esophagus is the objective and quantitative manner in which the disease can be diagnosed and characterized.

A very important attribute of integrated molecular analysis lies in its capacity to detect mutation acquisition before those changes can be seen through the microscope. This makes sense because mutations cause dysplasia while microscopic change follows the effects of DNA damage to the affected cell. In essence, mutational damage is one step ahead of microscopic cellular changes and a diagnostic approach that utilizes molecular analysis has the capacity for earlier and more accurate detection of Barrett's dysplasia.

Because microscopic examination is subjective, the usual recourse pathologists adopt when faced with indecision in the microscopic diagnosis of Barrett's esophagus is to seek consensus opinions from colleagues and outside consultant pathologists. It is not uncommon for a group of pathologists to differ among themselves with respect to the diagnosis, which does not resolve the issue. In any event, consensus opinions can be quite variable even among experienced pathologists. The integrated molecular pathology approach provides an objective and quantitative means to address the limitations of microscopic evaluation by providing a unique mutational fingerprint of collective DNA damage. In doing so the variability between the different stages of Barrett's esophagus is greatly reduced and the "indefinite for dysplasia" diagnosis can be eliminated.

In addition to preceding the microscopic appearance of dysplasia, molecular analysis has also taught us that the mutational damage of Barrett's dysplasia is reversible with regression of the cells that have acquired DNA damage. This concept is important to understand because regression and elimination of Barrett's esophagus is the ultimate objective of all therapies for this disease. DNA mutations are themselves irreversible meaning that once a Barrett's lining cell acquires DNA damage, that structural damage to the DNA molecule cannot be reversed. The driving force for acquiring mutational change is to allow the cell to grow more rapidly, likely in response to stress injury to the cells from reflux of gastric acid. Dysplastic cells with mutations have the advantage of growing more actively than their normal cell counterparts. If the stress is reduced or eliminated, as in the case of medical therapy to reduce gastric acid production by other means, the temporary advantage conferred by DNA mutations becomes disadvantageous for many other reasons. There is a cost to the cell for the benefit of more rapid growth. Early in the process, when the number of acquired mutations is relatively low, the mutated cells in the unstressed environment can regress and die off due to the disadvantages that mutational changes bring. However, if the extent and degree of mutation acquisition advances too far, the lack of growth control can cause the cells not to regress but to continue to gain further mutations eventually ending up in cancer. This inexorable progression towards cancer occurs even if the initial stresses are alleviated.

Here then is the power that integrated molecular analysis brings to traditional microscopic analysis of Barrett's esophagus. The appearance of the cells through a microscope are subjective in nature, and problematic for interpretation by pathologists (*Figure J*). Given the objective and quantitative information derived from molecular analysis on the most representative tissue sites within biopsies from a patient with Barrett's esophagus, the treating physician can now understand exactly how advanced the process of dysplasia has become and can use the mutational marker information to test the efficacy of measures to antagonize dysplasia progression meant to help the esophagus to heal itself. In the same way that molecular changes precede overt microscopic evidence of microscopic cellular change, molecular changes persist longer than microscopic change when the stress to induce mutation acquisition is lessened. By using integrated molecular and microscopic information, the status of each individual patient's Barrett's esophagus can be detailed enabling the treating physician to offer the optimal treatment at any point in time.

THE ROLE OF INTEGRATED MOLECULAR PATHOLOGY ANALYSIS IN ADVANCED BARRETT'S ESOPHAGEAL DYSPLASIA

One of many important goals in managing patients who have Barrett's esophagus is to identify advanced precancer at a stage prior to full cancer development, i.e. at the stage of high grade dysplasia (HGD). When significant amounts of mutational damage have already been acquired by Barrett's esophagus lining cells, dysplasia will continue to progress into cancer at which point the cells will have the full capacity to invade surrounding tissues and spread to other parts of the body. Because Barrett's esophagus is common in the general population and most patients with low grade dysplasia (LGD) will not progress to more serious levels of dysplasia and cancer, treating the lower levels of Barrett's dysplasia with aggressive measures is not warranted. That would represent over treatment. However HGD is a more serious condition and merits more active intervention designed to eliminate the abnormal precancerous cells before they have had a chance to reach full blown cancer.

Mucosal ablation of HGD represents a powerful and effective means to intercede at the stage of HGD and avert the development of cancer. In the past, when this option did not exist, patients with HGD were advised to have their esophagus resected (removed). This procedure is highly invasive and associated with significant post-operative complications. This very demanding form of therapy was justified based on the desire to interrupt the development of cancer and the lack of suitable alternatives. In recent years, less invasive options have become available in the form of mucosal ablation by endoscopic techniques that do not require major surgery. The technique removes the inner lining of Barrett's esophageal dysplasia allowing healthy tissue to grow back and eliminate all dysplastic lining tissue. This has permitted many patients to preserve their esophagus and achieve complete cure of HGD without risking major operative complications.

The challenge in mucosal ablation, which is done by highly skilled and experience physicians, is to ensure that the entire abnormal, highly dysplastic Barrett's esophageal lining cells are removed. When done successfully, the regenerating lining will return without evidence of dysplasia. Unfortunately, microscopic biopsies of the regenerating esophageal lining cannot be reliably assessed due to the very active cell proliferation and ongoing inflammation. Pathologists relying only upon the microscopic appearance very often cannot provide a definitive discrimination between healthy nondysplastic regenerating cells versus persistence of highly dysplastic Barrett's esophageal lining cells. In these circumstances, the general approach is to rebiopsy after an interval in the hope that a definitive diagnosis can be reached. In some cases where HGD cells are left behind, further progression to cancer can take place before a definitive microscopic diagnosis is reached.

This occurrence is highly undesirable and can be avoided by integrated molecular pathology evaluation. The pre-ablation biopsies of Barrett's HGD reveal their unique mutational profile of DNA mutational damage. The post-ablation biopsy material can then be analyzed in the same way. Three outcomes are possible. The best is the return of lining cells free of any mutations which definitely affirms that the ablation procedure has accomplished its purpose. The second possibility is the return of lining cells with the same mutational profile seen before the procedure. The third possibility is repair of the HGD lining with cells showing a lesser amount of mutational damage. Because the molecular analysis is performed using objective laboratory techniques, the limitation of subjective microscopic evaluation and the presence of inflammation are not interfering factors. Integrated molecular pathology analysis permits an earlier and more definitive diagnosis of the success of mucosal ablation so that further measures can be appropriately applied.

PATHOLOGY EVALUATION OF ESOPHAGEAL CANCER

Integrated molecular pathology analysis is not restricted to precancerous dysplasia but can enable a better and more comprehensive analysis of esophageal cancer when it is present. When esophageal cancer is found, the most valuable step in the analysis is determining how aggressive the tumor is with respect to its capacity for invasive growth and spread. Currently this is determined by the pathologist documenting how deeply invasive the cancer has spread at the primary site of its formation in the esophagus. The depth of invasion forms the basis of primary cancer staging. Esophageal cancer limited to the mucosa, or inner lining, is classified as stage T1S. This is the earliest stage of cancer and is associated with the best prognosis for disease free and overall survival. Esophageal cancer that has invaded immediately beneath the lining is termed T1 and has the next best prognosis. T2 is designated for esophageal cancer that has invaded into but not through the muscular wall of the esophagus. Cancer invasion beyond the muscular wall is the most advanced and is referred to as T3. The most aggressive stage of esophageal

cancer is called T4, applied to patients in whom the cancer has directly invaded beyond the esophagus into adjacent structures such as the trachea, pericardial sac or lung.

This traditional pathologic staging of esophageal cancer has proven valuable and generally accurate for predicting the outcomes for groups of patients organized into different stages of tumor invasion. However, traditional pathologic staging has several drawbacks. First, it can only be effectively applied when the cancer bearing esophagus has been resected and is available in its entirety for microscopic evaluation. This precludes the most effective use of preoperative oncologic therapy. Second, for similar reasons, pathologic staging cannot be used on biopsy specimens of esophageal cancer when such information would be of enormous value in planning optimal treatment. Third, traditional pathologic staging works most effectively when statistically applied to large groups of patients. When applied to individual patients, considerable variability is seen and predictive analysis tends to break down. For each stage of esophageal cancer extension, there are patients with good, intermediate and poor outcomes. In general, the prognosis is better for more limited invasion and worse for more extensive invasion. There are significant numbers of patients who show relatively good and relatively poor outcomes despite the average behavior expected for the specific stage of disease. Traditional pathologic staging, while useful when applied in a general sense, is not personalized to the unique pattern of biological aggressiveness present in an individual patient. As a result, treatment, which typically is given uniformly to all patients according to pathologic stage of esophageal cancer, is not tailored to the intrinsic nature of the cancer and its responsiveness to certain forms of chemotherapy and other therapies.

By including molecular analysis with microscopic evaluation, it is possible to gather more discriminating information of value to the doctors treating an individual patient's esophageal cancer. Aggressive forms of esophageal cancer, better suited to more intensive treatment, detected at an early pathologic stage can be distinguished from intrinsically indolent forms of esophageal cancer that can be managed in a lesser intensive fashion. In this way, cancer therapy can be better suited to the unique biological aspects of each cancer offering patients the opportunity to gain the most from their therapy and avoid unwanted side effects.