A Molecular Classification for Precancerous Lesions
Report of an EDRN Working Group

Meeting Summary
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Introduction

On February 1-2, 2001, the Early Detection Research Network (EDRN) of the National Cancer Institute (NCI) sponsored a workshop to explore the possibility of developing a molecular classification for precancerous lesions. The Working Group was called to discuss the clinical implications of a molecular classification for these lesions and to formulate recommendations for subsequent research. Because of their importance for prevention and early diagnosis, the EDRN recognizes that these lesions are key for any research program in early cancer detection.

The basic objectives of the EDRN are to lay the groundwork for a national program in early detection research, to establish a link between laboratory research and clinical application, and to set in motion collaborative approaches to early detection research. The EDRN Steering Committee established a Working Group on the Molecular Taxonomy of Preneoplastic Lesions on April 11, 2000, with the objective of developing a systematic classification, using advances in molecular biology in order to provide the most clinically predictive system for classifying neoplastic lesions. The Working Group members were specifically asked to provide advice and suggestions on whether this effort to begin developing a molecular classification for the precursor lesions of cancer should proceed, and if so, how to proceed. The Working Group noted that these lesions will become more significant in the future as greater emphasis is placed on early detection and screening of cancer.

As discussed by the Working Group, a molcular classification should serve the same clinical needs as a histologic classification, although with more precision and accuracy. It

should define lesions likely to progress, identify lesions susceptible to interventions, provide targets for early detection and chemoprevention, and expand our understanding of etiology. A molecular classification would separate lesions that are truly neoplastic from those that are only reactive. For instance, familial C-cell hyperplasia of the thyroid gland has now been shown to be neoplastic by molecular techniques and should no longer be considered a hyperplastic lesion. Recent work supports the concept that pancreatic intraepithelial neoplasia is a neoplastic lesion. A molecular classification could also have an effect on prevention research because it could change our understanding of the time frame a tumor development, which now relies on rates of histologic and clinical progression.

Since malignant transformation is thought to result from an accumulation of irreversible genetic changes over time, a molecular classification of precancerous lesions would likely be closer to the basic mechanisms of cancer and closer to the cause than a molecular classification based only on invasive cancer. Molecular changes occurring early in the neoplastic process are more likely to be the most fundamental alterations occurring in cancer. Although specimens are often difficult to obtain for study, technically it may be easier to develop a molecular classification of precancerous lesions, since there may be less cellular heterogeneity, fewer genetic abnormalities, and theoretically less intraneoplastic and interneoplastic diversity than with invasive cancers.

The Group concluded that insufficient information exists to institute a molecular classification for the precancerous lesion at this time. However, the World Health Organization (WHO) in its series *Pathology and Genetics of Tumours* is currently adding molecular features to conventional histologic parameters in order to provide additional information to the histological classifications. The Working Group noted that adopting the WHO histological classifications as a working framework was an option for the EDRN to use as a starting point in developing a molecular classification. For the classification of lymphomas and leukemias, the identification of the molecular pathogenetics of a tumor has long been instrumental in disease definition.

Challenges

While the Working Group recognized the advantages of a molecular classification for the precancerous lesions, it rapidly identified a number of challenges that needed to be addressed in order to develop a taxonomy. Some of these challenges are related to the current operational structure of medical practice while others relate to the need for research and consensus development.

- Some of the challenges simply relate to anatomy. In some anatomic sites the precancerous lesions are not accessible because they occur in deep-seated organs, for instance in the pancreas or in a lung. For this reason, these lesions are rarely found and usually not available for study. In contrast, superficial sites, for instance the uterine cervix or skin, there are no practice cost-effective clinical tools for the detection of precancerous lesions I the deep-seated organs. Moreover, there are no specific markers for the identification of incipient neoplastic lesion in any site.

- Since these lesions are usually obtained by endoscopic or needle biopsy, they are usually small often millimeters in size. As a result they are immediately processed in formalin and embedded in paraffin for complete histologic study. Fresh frozen unfixed precancerous lesions are generally not available for research. Precancerous lesions usually are recognized only microscopically.
- There has been a lack of uniform terminology for the precancerous and non-invasive lesions. Reasons for this lack relate in part to changing concepts about the biology of these lesions, subjective interpretation of criteria, heterogeneity of the neoplastic cell population, less than optimal interobserver reproducibility, and even changes in treatment. Very often descriptive terms applied to these lesions contain a mixture of diagnostic and prognostic meanings. In the colon, for example, the term "high grade dysplasia" has replaced "carcinoma in situ" as the preferred diagnostic term. In a number of sites the term "high grade intraepithelial neoplasia" has gained popularity and has been applied to lesions occurring in the prostate, vulva, pancreas, and breast among others. Changes in terminology can alter our concepts of the natural history and even the management of these lesions.
- While not a specific barrier, there may be biological variation usually referred to as heterogeneity that may pose a challenge. It is possible that morphologically similar lesions may have different genetic mutations. It was noted that with invasive tumors, not all mutations are found across all cancers and similarly not all mutations may be found across the precancerous lesions within the same site. Investigators have already identified multiple potential genetic pathways or circuits through which cancer can develop. Analysis has revealed that cancer represents many heterogeneous diseases.
- Many lesions considered precancerous do not progress to invasive cancer. Some may even regress. Thus, lesions that are destined to progress must be separated from those not likely to progress, which is often not possible based on histologic features alone.
- Ethical concerns, issues of anonymity, and issues of informed consent present challenges, although these can usually be dealt with at the institutional level. Tissue samples most likely will have to be anonymized, making it difficult to trace what happens to the patient over time. The NCI's tumor bank does not contain follow-up data, and NCI-sponsored tissue banks do not collect or store precancerous lesions.
- Many institutions are discarding specimens after a certain amount of time or are requiring that they be sent back to the original institution because of storage and perceived legal constraints (e.g. custody issues and fear of litigation).

Recommendations

In order to address the challenges listed above, the Working Group offered a number of recommendations that in the future might serve as a basis for the development of a molecular classification for the precancerous lesions. While most of the recommendations were general, some specifically apply to the EDRN, since the Network is in a position, because of its research direction, to play a leading role in the development of a molecular classification for these lesions.

General

The methods for obtaining and processing tissue need to change in order to meet the needs of the new molecular technology. In particular fresh or frozen tissues need to be processed promptly and correctly so as to permit DNA, RNA and protein analyses. Ideally, molecular characteristics should be determined in fresh tissue before it has been fixed for processing. However, since it is doubtful whether tissue fixation and processing methods will change soon, research should be directed at finding new fixatives and methods of tissue processing so that specimens can be evaluated both histologically and analyzed for intact DNA, RNA, and protein.

Methods should be established for collecting, storing, and retrieving tissue specimen blocks. These area a valuable resource for the study and development of a molecular classification for the precursor lesions.

There needs to be a standardization and validation of technology as there are multiple institutions involved in research and in drawing conclusions. Results will need to be confirmed independently.

Specific

For these recommendations the Working Group suggested that EDRN should play a leading role in the development of a molecular classification, because of its interest in early detection and research in the field.

- Because of the consistent lack of a common diagnostic terminology, which is a major impediment to classification, agreement on the terminology and criteria for the precancerous lesions in all major sites should be sought. Small groups of experts with an interest in early lesions should be appointed to standardize the terminology and criteria at the more common cancer sites. The result of this work would then serve as the basis or framework for subsequent developments in classification.
- Following the work of the experts, examples of the precancerous lesions along with the terminology should be annotated in detail on the Interent with images and clinical and pathological features as a reference so that pathologists, molecular biologists, and others can become familiar with the specific terminology and histology. As a result, all involved in early detection research will have access to common terminology and criteria. While this approach would not solve all issues in terminology, it should at least provide a standard histologic framework or reference on which molecular changes can be attached. It would also provide a common terminology for an EDRN centralized database in precancerous lesions. As mentioned, initially adopting the WHO classification as a working framework was an option for the EDRN.
- Through the Internet, the images (lesions) can be associated with specific precancerous entries in the gene and protein databases. As a result, molecular patterns can be related to specific histologic lesion in different anatomic sites.
- Because these lesions are often rare, the EDRN should promote inter-institutional collaborative projects among investigators who are in a position to share ideas and resources. Perhaps a decentralized infrastructure for collecting, preserving, shipping, and sharing specimens and data should be created by NCI along the lines of the EDRN.

- Finally, the group observed that a molecular classification must be consistent with the logic of informatics and computer searching and retrieval. At some level, the semantics of representing the molecular information associated with premalignant conditions must be discussed. As determinations are made regarding what will be accepted semantically, that information needs to be represented in a form that can be manipulated by computers.

Conclusion

The Working Group concluded that the recommendations were reasonable and offered an initial approach to a molecular classification of the precancerous lesions. Such an approach seems cost-effective and takes advantage of the research programs and databases already sponsored by the NCI.