Oncogene Mutations as Intermediate Markers

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Abstract   Tumors arise through a series of genetic changes which include activation of protooncogenes and inactivation of tumor suppressor genes. It is now possible to identify rare cells containing genetic mutations in an excess background of normal cells. Theoretically, the identification of a clonal population of cells sharing an early genetic marker for malignant transformation would lead to valuable intermediate endpoints and could diagnose premalignant lesions amenable to chemoprevention. Ideally, these genetic changes would be specific point mutations that occur early in the tumor cascade, prior to the development of a clinically significant tumor. To identify these markers, precise histopathologic and genetic tumor models must be described. Early candidate markers include p53 point mutations in squamous cell carcinoma of the aerodigestive tract.   © 1993 Wiley-Liss, Inc.

Key words: Tumor models, oncogenic mutations, molecular markers, p53

Oncogenes are very specific markers for cancer [1,2]. Mutations within protooncogenes or tumor suppressor genes lead to tumor progression and uncontrolled growth. Cells that acquire these mutations retain them as they progress through clonal evolution [3,4]. Identification of these specific changes can lead to novel diagnostic strategies, since their detection is synonymous with the identification of cancer. A novel assay was recently developed that allows identification of rare cells containing gene mutations in a large excess background of normal cells [5]. Based on amplification of DNA by the polymerase chain reaction (PCR), this assay detects specific gene mutations within cytologic samples. These cytologic samples are obtained from easily accessible bodily fluids which bathe the organs of interest. Detection of such genetic changes may allow early diagnosis of cancer and provide important intermediate markers for chemoprevention.

TUMOR PROGRESSION MODELS

To use oncogenes as specific markers, it is important to understand their specific order of progression for a given tumor system. For example, a clear histopathologic description of progression has allowed identification of the specific genetic steps involved in both initiation and progression of colorectal cancer [6,7]. In order to apply the strategy used in developing the colorectal model to other tumors, histopathologic progression must be generally well understood. Subsequently, various genetic changes can be sought at different stages of transformation. These genetic changes often involve specific point mutations in both tumor suppressor genes and protooncogenes. Additionally, amplifications and large deletions are sometimes seen at both the cytogenetic and molecular levels. Areas of chromosomal loss can be confirmed by establishing loss of highly polymorphic markers

on various chromosomal arms [7]. Polymorphic markers are inherited in Mendelian fashion; when one specific marker is lost, it is often referred to as "loss of heterozygosity." Based on Knudson's hypothesis of a "two hit" model [8], these areas of allelic loss in cancer cells are thought to represent inactivation of a tumor suppressor gene at that locus [9]. Areas of loss help identify the specific loci where tumor suppressor genes reside, pointing to an alteration in the remaining allele that leads to complete inactivation of the target tumor suppressor gene. This has been well established for both retinoblastoma (RB) and p53 as well as other tumor suppressor genes [9]. By obtaining specimens from a specific tumor type in a well-arranged histopathologic order and carefully analyzing them for point mutations or allelic loss, a tumor progression model can be established based on genetic changes. The colorectal tumor progression model continues to be the paradigm for all tumor progression [6]. An excellent subset of tumors can be obtained within different histologic stages that establishes genetic changes in a common order. It is important to remember that these changes have a general order but can be variable. The accumulation of these genetic changes, not the specific order, leads to tumor progression.

TUMORS OF THE AERODIGESTIVE TRACT

Most tumors of the upper aerodigestive tract are squamous cell carcinomas. A histopathologic order of progression may exist from non-neoplastic lesions such as metaplasia and dysplasia, through carcinoma *in situ* (CIS), to invasive carcinomas. While the "early" lesions may develop into invasive carcinoma, the propensity to progress does not appear to be as reliable as in the colon adenoma-to-carcinoma progression model [10,11]. Nevertheless, early preneoplastic lesions like dysplasia (clinically, leukoplakia or erythroplakia) and CIS can be used to define a general histopathologic progression model within the upper aerodigestive tract. The specific genetic changes from lesions of each stage can then be characterized. At the present time, few genetic alterations have been well-described in this tumor type with the exception of the involvement of p53 gene mutations [12,13] and overexpression [14,15]. While amplification of

specific sequences within chromosome 11 are occasionally seen [16,17], no common genetic changes other than p53 have been well-described in primary tumors.

p53 gene mutations are the most common alterations seen in epithelial tumors [18]. In most tumor types this genetic change appears to arise in transition from more benign lesions to aggressive carcinomas. This is exemplified in progression from adenoma to carcinoma of the colon, superficial to invasive bladder carcinoma, low grade to high grade astrocytomas, and low grade to high grade leukemias, as well as lymphomas. There is increasing evidence that in most tumor types p53 overexpression is often a consequence of p53 mutation. Additionally, p53 overexpression has been demonstrated in dysplastic and early lesions of the esophagus [19,20], skin [21] and lung [22]. Furthermore, some p53 gene mutations have been described within these lesions [19,20,22]. Although suggestive, p53 overexpression is not synonymous with p53 gene mutation [23]. We are only now beginning to understand other mechanisms by which wild-type p53 protein may accumulate [24].

The recent identification of p53 gene mutations in several "early" dysplastic lesions of the aerodigestive tract raises several questions. What is the true incidence of p53 gene mutations in early lesions? Will p53 mutation be an intermediate event in head and neck cancer or a late event, as in many other epithelial tumors? If mutation of the p53 gene occurs "early" in aerodigestive tract tumors, it may serve as an important marker for the use of chemopreventive agents.

GENE MUTATIONS AS MARKERS

The recent development of a novel assay demonstrates the ability to identify rare cells containing gene mutations in an excess of normal cells [5,25]. This assay is based on PCR, in which small quantities of DNA derived from cytologic samples can be amplified to obtain a sufficient amount for diagnostic studies. A subsequent cloning step into phage allows separation of individual PCR copies of the target gene. Hybridization with oligomeric probes specific for mutant gene sequences then allows the specific identification of gene mutations as well as the

quantitation of the relative abundance of these mutations (and therefore of the specific malignant cells). This has been successfully demonstrated for p53 [25] in urine cytologic samples of patients with bladder cancer and for the *ras* gene [5] in stool samples of patients with colorectal cancers. This method appears to offer a novel and unique approach to detect specific gene mutations which indicate the presence of cancer. Extending this methodology to other cytologic samples such as bile, sputum, saliva, and cervical scrapings seems assured. Furthermore, the identification of rare clonal populations of tumor cells raises the possibility of using the identified specific point mutations as intermediate biomarkers.

THE ROLE OF MOLECULAR MARKERS IN CHEMOPREVENTION

Ideally, if mutations of a gene such as p53 are *valid intermediate* endpoint markers in aerodigestive cancers, patients undergoing chemoprevention could be periodically tested for the presence of these markers. Paramount to this potential application is the importance of p53 as an early rather than a late event in squamous cell carcinoma tumorigenesis in the aerodigestive tract. If other tumor suppressor genes and protooncogenes are identified in cancers of the aerodigestive tract, it seems probable that at least one of these markers will be early enough to be used as an intermediate endpoint. Patients may be monitored for the development of a rare population of clonal cells indicative of a pre-clinical transformation event. This may provide valuable information about the need for chemoprevention prior to the development of clinical cancer. Additionally, it may provide the opportunity to change chemoprevention to another regimen before the development of clinical tumors. Eventually, these highly specific markers may be used for initial identification and eradication of pre-invasive tumors. (As markers for cancer, they assure extreme specificity.) The sensitivity of particular assays will need to be evaluated over the course of time using proven epidemiologic methods. However, this approach promises an exciting new method of determining the presence of cancer cells prior to the development of clinical neoplasms. The use of assays for the presence of p53 mutations

holds great potential for early diagnosis of neoplastic lesions of the upper aerodigestive tract and for monitoring the efficacy of chemopreventive therapeutic regimens.

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