Can Molecular Markers Now Be Used for Early Diagnosis of Malignancy?

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Most of the presently available cancer markers are neither specific for malignancy nor allow early diagnosis. However, the recent elucidation of the molecular events occurring during carcinogenesis may provide new markers that are likely to be both specific for cancer and sensitive for early disease. The key molecules undergoing alterations during carcinogenesis are the cellular oncogenes and suppressor genes. Alterations in these genes can be detected in cells shed from malignant and premalignant lesions. Thus, mutant p53 genes have been found in urine from patients with bladder cancer, mutant ras genes in stools from patients with colorectal and pancreatic cancers, and both mutant p53 and ras genes in sputum from patients with lung cancer. These findings show that the genetic alterations in cancer can be detected in fluids or secretions that had contact with the malignant tissue. The preliminary studies, however, had small numbers of both patients and controls and used time-consuming, labor-intensive, and expensive assays. For routine applications, these assays must be simplified, automated, and tested for sensitivity, specificity, and predictive value.

Indexing Terms: cancer/premalignant alterations/c-oncogenes/suppressor genes

Although many of the currently used tumor markers are helpful in the follow-up of patients with diagnosed cancers, few are of value for early diagnosis of malignancy. Many of the traditionally used markers are increased only in blood from patients with metastatic cancers. For example, carcinoembryonic antigen (CEA) is generally increased only in patients with advanced colorectal cancer (1), and CA 15-3 in patients with metastatic breast cancer (2). In addition, none of the presently used markers is specific for malignancy, as all can be increased in benign disorders.

We now know that the primary alterations in most human cancers are found in two groups of genes known as c-oncogenes and suppressor genes (3, 4). These alterations involve changes in both gene structure and gene expression. In several different situations, these abnormalities are found in premalignant disorders (Table 1). Genes or their protein products, which are altered in preneoplastic lesions, could provide a new generation of markers that may allow the early diagnosis of cancer. Furthermore, unlike our present markers, these structurally altered molecules (DNA or its protein product) should be specific for malignant or premalignant conditions. The aim of this short review is to describe how these altered or mutant molecules are beginning to be exploited for the early and noninvasive diagnosis of cancer. Comprehensive reviews on the genes altered in malignancy and a preliminary discussion of the diagnostic applications of these abnormalities are found in refs. 13 and 14.

Detection of Gene Mutations

Bladder Cancer

In one of the first attempts to use a noninvasive approach to detect mutant genes, Sidransky et al. (15) investigated urine from patients with bladder cancer for p53 mutations. Using PCR and oligomer-specific hybridization, these authors reported the presence of identical p53 gene mutations in urine and bladder tumor tissue of three patients. In bladder tumor tissue, p53 gene mutations were found in 11 of 18 (61%) of samples. Later, Haliassos et al. (16) found mutant H-ras genes in 10 of 21 (47.6%) urine specimens from patients with bladder cancer. In the bladder tumor specimens a mutant ras gene was present in 14 cases (66.6%).

H-ras mutations in urine sediments were found to be more sensitive than cytology in detecting low-grade (i.e., grades 1 and 2) bladder cancer, whereas cytology was more sensitive than the mutant gene test in identifying carcinoma in situ (75% vs 31%) (17). The combined results from the two tests substantially increased tumor detection rate, leading to identification of cancer in 60% of the patients.

Table 1. List of some c-oncogenes and suppressor genes that can be activated early* in the development of cancers.

<table>
<thead>
<tr>
<th>Cancer</th>
<th>Genes</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colorectal</td>
<td>APC, ras, DCC, p53</td>
<td>5</td>
</tr>
<tr>
<td>Stomach (intestinal type)</td>
<td>tpr-met, ras, p53</td>
<td>6</td>
</tr>
<tr>
<td>Esophagus</td>
<td>p53</td>
<td>7</td>
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<tr>
<td>Oral</td>
<td>p53</td>
<td>8</td>
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<tr>
<td>Lung</td>
<td>p53</td>
<td>9</td>
</tr>
<tr>
<td>Endometrial</td>
<td>ras</td>
<td>10</td>
</tr>
<tr>
<td>Breast</td>
<td>ras, c-erbB-2, p53</td>
<td>11,12</td>
</tr>
</tbody>
</table>

* Early is defined here as before invasion of the basement membrane. APC, adenomatous polyposis coli; DCC, deleted in colon cancer; tpr, translocated promoter region.
The potential of mutant p53 genes for the early diagnosis of bladder cancer was recently illustrated by the case history of Hubert Humphrey, Vice President of the US, 1963–69 (18). In 1967, Humphrey developed hematuria. Visual cystoscopy of his bladder was consistent with chronic proliferative cystitis, and a biopsy showed a focus of dysplastic change in the transitional epithelium. Urinary cytology was inconclusive and a definite diagnosis was not established. Humphrey was thus followed up with regular cystoscopy. Although in situ cancer was diagnosed in 1969, Humphrey remained asymptomatic until 1973, when he presented with borderline malignancy. In 1976, infiltrating cancer of the bladder with lymph node metastasis was diagnosed and the patient died in 1978. In 1994, researchers reported that Humphrey’s urine from 1967 contained the same p53 gene mutation as was detected in the primary bladder cancer removed in 1976. These findings suggest that the bladder cancer or at least a preneoplastic lesion was present in 1967. Had this information been known then, it is possible that more aggressive treatment such as potentially life-saving surgery would have been carried out earlier (18).

Colorectal Cancer

Mutated c-oncogenes have also been found in stools from patients with gastrointestinal cancers. In one study, the stools of nine patients with colorectal tumors containing a mutant K-ras gene were investigated (19). In eight of nine of these patients, ras mutations were detected in cellular DNA purified from the stools. One of the tumors that gave rise to a mutant gene in the stools was a small adenoma, 1.3 cm³.

Although in this study the correlation between the presence of mutant ras genes in the tumor and in the stools was good, only nine of 24 randomly selected tumors had mutant ras genes. Thus, the sensitivity for tumor detection was only 37.5% for the study as a whole. In other reports, however, mutant ras genes were found in ~50% of colorectal cancers and in ~60% of adenomas >1 cm in diameter (5). Mutant ras genes were found in <10% of adenomas <1 cm in diameter (5).

In another study, mutant K-ras genes have been detected before the development of colorectal cancer in subjects who were at high risk for this disease. Applying the technique of enriched PCR to colonic effluent samples, Tobi et al. (20) found mutations in codon 12 of the K-ras gene in seven of 39 (18%) subjects with either a family history of colorectal cancer, patients with adenomatous polyps, or patients with previously resected colorectal cancer. In one case, effluent was found to contain a mutant ras allele 4 years before the patient was diagnosed with colorectal cancer (20).

Pancreatic Cancer

Although mutant ras genes are only found in ~50% of colorectal cancers, alterations in this gene are found in 75–100% of pancreatic carcinomas (21). As with colorectal cancer discussed above, stools have also been used to assess the frequency of ras mutations in pancreatic lesions. In one study Caldas et al. (22) found K-ras gene mutations in stools from 6 of 11 patients with pancreatic adenocarcinoma and from one of three patients with chronic pancreatitis.

In another study involving pancreatic cancer, K-ras gene mutations were found in DNA purified from pancreatic juices in all of six patients with pancreatic adenocarcinomas and in one case of intraductal papillary neoplasm of the pancreas (23). In two of six further cases of pancreatic carcinoma, mutant ras genes were detected in circulating metastatic cells from peripheral blood (23). In this study, mutant ras genes were not present in the pancreatic juice from three control patients (two patients with chronic pancreatitis and one with choledocholithiasis) or in peripheral blood of two patients with insulinomas.

Mutant ras genes have also been found in either serum or plasma from three patients with pancreatic cancer (24). The presence of these mutant genes were found by both PCR with allele-specific primers and direct DNA sequencing. Whether these circulating DNA molecules were derived directly from tumor or metastatic cells in the blood is unclear.

Although ras gene mutations are present in the vast majority of pancreatic adenocarcinomas, mutations can also be found in certain benign pancreatic lesions. Thus, mutations in the K-ras gene has been reported in three of five ductal cyst adenomas (25), 10 of 16 mucous cell hyperplasias (26), and one case of benign hyperplasia associated with intraductal papillary neoplasm (27). Whether these benign pancreatic diseases are precursors of a malignant state is presently unclear. These findings, however, must be taken into consideration if ras gene analysis is to be used as a diagnostic test for pancreatic cancer.

Lung Cancer

Mutations in ras and p53 genes have also been exploited for the detection of lung cancer. Mao et al. (28) found mutations in either one of these genes in 10 of 15 primary lung carcinomas. In 8 of the 10 patients, the identical mutation identified in the primary cancer was also detected in sputum samples taken before the clinical diagnosis. In one patient, cancer cells were found in the sputum at least 1 year before the clinical diagnosis.

Gene Amplification and Activation

Breast Cancer

Unlike ras and p53, the c-erbB-2 gene is rarely mutated in human cancer. Instead, this gene is usually activated by gene amplification and overexpression (29). Overexpression of c-erbB-2 protein has been found in 40–70% of patients with ductal in situ carcinoma of the breast and in 15–30% of patients with invasive breast cancer (12, 30). Inaji et al. (31) therefore looked for concentrations of c-erbB-2 protein in nipple discharge from patients with both benign and malignant...
lesions of the breast. High amounts of c-erbB-2 were found in six of nine patients with breast cancer, including three of six with nonpalpable disease. Furthermore, two of eight patients with intraductal papillarity and one of two patients with borderline lesions had high amounts of c-erbB-2 protein. However, one of 19 patients with fibrocystic disease of the breast also had high concentrations of c-erbB-2. Combining c-erbB-2 with CEA, all patients with cancer had a positive result but only two of 19 patients with benign disease were positive by the combination test.

Recently, Takei et al. found high concentrations of basic fibroblast growth factor (bFGF) in serum from 25 of 35 (71%) patients with early-stage or stage 1 breast cancer (32). Concentrations of bFGF fell significantly after surgical resection of the tumor, suggesting that the breast tumors were the origin of the growth factor. In this study, bFGF was not assayed in sera from patients with benign breast disease or other benign disorders.

Comment

The reports described above show the potential of activated c-oncogenes and suppressor genes to diagnose cancer in its early stages and even at a preneoplastic level. However, all of the above-mentioned studies are preliminary, with small numbers of both cancer patients and controls. Furthermore, the technology used to detect activated c-oncogenes and suppressor genes is time-consuming, labor-intensive, and expensive. In its present form it cannot be applied in routine diagnostic laboratories. In the above studies, no data were supplied on the reproducibility of the procedures, the proportion of samples from which it was not possible to obtain suitable DNA, or the costs of the assays. Although mutant c-oncogenes and suppressor genes may detect some early cancers, one must remember that, with few exceptions, e.g., mutant ras in pancreatic cancers, c-oncogenes and suppressor genes are only altered in a proportion of cancers. This proportion rarely exceeds 60% of a given type of cancer. Thus, although a particular mutant gene has the potential to diagnose an early cancer, an unacceptable number of tumors will be missed. Combining a number of altered genes, however (e.g., mutant ras, p53, and APC in colonic cancer), should enhance the diagnostic yield.

The diagnostic yield might also be increased by looking for circulating antibodies against structurally altered gene products. To date, only antibodies against p53 have been found (33). However, it is possible that antibodies are also produced against other mutated proteins such as ras. The advantage of antibodies is that they can be measured in serum with relatively simple and quantitative immunoassays. Present data, however, suggest that serum antibodies, at least against p53, are present in <15% of patients with the common cancers (33).

Up until now most work relating to the early diagnosis of cancer has focused on the c-oncogene ras and the tumor suppressor gene p53. A particular problem with using mutant p53 as a disease marker is the large number of different mutations found in this gene (4). Although these mutations are mostly found in the conserved exons (i.e., exons 5, 6, 7, 8), alterations can also occur in other exons. Identification of all these mutations in small numbers of exfoliated cells will be most time consuming and tedious. For example, detection of 80% of p53 mutant genes in lung cancer required 100 specific oligomeric probes (34). In studies reporting the presence of altered p53 mutant genes in cells shed from tumors, the specific mutation in the primary cancer tissue was already known. This undoubtedly simplified detection of mutations in the cells released from the primary tumor. If detection of mutant p53 genes in exfoliated cells is to be used as a noninvasive screening test, information on the mutation status of the tumor tissue would not be available.

In contrast to p53, ras gene mutations in naturally occurring tumors seem to be confined to only three codons (codons 12, 13, and 61) (35). Of these three, codon 12 is by far the most frequently altered. Thus, in pancreatic cancers, about all the ras gene mutations detected are in this codon, whereas ~80% are confined to codon 12 in neoplastic and preneoplastic colorectal lesions (35).

On the positive side, we now for the first time have the prospect of having assays for the early diagnosis of several different malignancies as well as specific assays for cancer and precancerous lesions. These new markers should also be able to provide prognostic information and be of use in monitoring patients with diagnosed malignancies. One of the first clinical applications of c-oncogenes and suppressor genes was to provide new prognostic markers in different cancers (36). In regard to monitoring patients, molecular markers are providing very sensitive tests for the early detection of recurrent disease in certain hematological malignancies (37). The presence of specific mutant genes in tumors may be used to predict response to certain forms of treatment. Thus, the response to therapy of fibrosarcomas in immunocompromised mice depended on the status of the p53 gene (38). Tumors containing mutations in this gene were more resistant to both chemotherapy and gamma irradiation than tumors without these mutations.

Clearly, detection of altered c-oncogenes and suppressor genes have several potential applications in cancer diagnosis and management. The assays described above should be simplified and applied to larger numbers of patients. The results obtained should then be compared with existing assays with regards to diagnostic sensitivity, specificity, and predictive value. The ultimate test will be to see if these difficult and expensive tests can really save lives.

References

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