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Host Susceptibility to Cancer Progression

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It is widely accepted that the rate of cancer progression varies between individuals and that this variation exceeds random expectation. This variation is apparent to the clinician who observes patients with similar disease relapse at different intervals after primary treatment. In biological terms, tumors of the same histological type are observed to grow at different rates in different patients. To a large extent, tumor recurrence can be predicted, but much of the variance remains unexplained. Of the factors that help explain variation in prognosis, interest to date has focused on the tumor itself (its size and grade), the proportion of cells observed to be undergoing cell division, and the presence or absence of a host of tumor markers. Several classes of prognostic markers have a genetic basis and are the result of somatic mutation, including gene amplification, chromosome rearrangement, point mutation, and allelic loss (loss of heterozygosity [LOH]).

Rates of recurrence can also be predicted by the type and intensity of treatment, including the extent of surgery performed, the presence of positive tumor margins after surgery, and the use of radiotherapy and chemotherapy. In addition, lifestyle factors such as obesity, diet, smoking, and inadequate social support may also influence recurrence rates and, ultimately, survival, in patients with established cancer.

A fourth class of prognostic factors is related to host variation—that is, allelic variation of genes that may influence the course of cancer. There has been much progress in the past decade in the identification of genes that, when mutated, predispose to cancer and lead to familial clustering. Much less is known about individual variation in genes that influence the prognosis of cancer, once established. It is reasonable to speculate that inherited factors may complement those factors listed above and may help predict long-term survival.

Cancer progression is influenced both by cell growth rates (a function of cell division and apoptosis) and by the ability of the tumor to evolve through a complex metastatic pathway. There are several classes of candidate genes for host susceptibility to tumor progression. These include genes that influence cancer susceptibility, genes that are mutated somatically in tumors, and genes that influence critical steps in metastasis.

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Cancer Susceptibility and Outcome

It is natural to begin by asking whether the genes that predispose to cancer may also influence the rate of progression of cancer, once established. Several clinical studies have addressed the question of whether the natural history of cancer differs when it occurs in a carrier of a predisposing cancer mutation. For example, several studies compare the natural histories of hereditary and nonhereditary breast and ovarian cancer. Rubin et al. (1996) reported a very favorable prognosis for women with advanced-stage ovarian cancer who carry a *BRCA1* mutation. They estimated the 10-year survival rate to be 42% for stage III and stage IV ovarian cancers—a much better rate than expected. This information is important for clinical decisions regarding surgical prevention and early detection. For example, if it were known that ovarian tumors that develop in a woman with a *BRCA1* mutation were likely to be of high grade and to behave aggressively, then preventive oophorectomy would be a more rational choice than periodic ultrasound screening. Unfortunately, later studies of patients with hereditary ovarian cancer were unable to confirm the findings of improved prognosis (Brunet et al. 1997; Johannsson et al. 1998).

Much of the discrepancy in the estimated recurrence rates among studies of hereditary breast and ovarian cancer appears to arise from methodological differences. It is very difficult to obtain unbiased relative risks of survival for hereditary versus nonhereditary cancers by examining clinic records. Individuals at elevated risk of cancer are often under close surveillance, and their tumors may be detected at an earlier stage. Lead-time bias may result in a spuriously elevated survival rate. Other problems arise because of the difficulty that genetic epidemiologists face in obtaining vital status and mutation status independently in an unselected group of patients. This is because cancer patients who are referred to a

genetic-counseling program are more likely to undergo genetic testing and to have their mutation status known than are patients who are not referred or who have died. Even when tumor specimens are available for deceased patients, it is technically demanding to perform genotyping for large genes such as *BRCA1* and *BRCA2*. It is comparatively easier to test the proband (who is alive), but including the proband in the study will result in enhanced survival estimates. Even when the proband is excluded from the study, the problem of ascertainment bias is not entirely eliminated, because not all families with hereditary cancer in a population will be ascertained with equal probability. Once a family is ascertained, all affected relatives are usually invited to participate. For example, if each living woman with hereditary breast cancer in a population were equally likely to be referred for genetic assessment, then a family with many living affected women would be more likely to be referred than would a family with only a single living affected member. *BRCA1* analyses are not done in the absence of a living index patient. It is insufficient to exclude the proband to correct for bias of this type.

Nevertheless, increasing numbers of reports are published about survival in hereditary cancer syndromes. Because of the difficulties inherent in using clinic populations for estimating survival, alternate methods are desirable. Ideally, one would like to ascertain incident cases of cancer in a well-defined population; obtain information on mutation status, grade, stage, and other relevant prognostic factors; and follow the patients for an extended period of time. Unbiased survival estimates for the hereditary and nonhereditary subgroups could then be constructed and adjusted for stage and grade. This method is attractive but requires $\geqslant$10 years of follow-up.

An alternate method is to use historical cases and archived tumor specimens. For example, one might ascertain mutation status on an unselected sample of breast specimens in a hospital tumor bank and then compare survival for women with and without mutations. This method is ideal when there is a population with a common founding mutation and a permissive scientific environment. The study design requires the investigator to perform anonymous genetic testing on archived material and to link mutation results with clinical data. It is important that policy makers who wish to restrict anonymous genetic testing are made aware of the critical clinical information that may be lost. This approach is now feasible for several populations, including Icelanders, French Canadians, and Ashkenazi Jews. A small number of recurrent *BRCA1* or *BRCA2* mutations have been identified in each of these populations. Information from these test populations might be generalized to a wider group, but allele-specific differences may also prove significant.

Approximately 2% of all Jewish women and ~12% of Jewish women with breast cancer carry mutations in *BRCA1* or *BRCA2*. Using archived tumor DNA, Foulkes et al. (1997) found the prognosis of breast cancer in Jewish women with *BRCA1* mutations to be worse than that of Jewish women without mutations. The difference was particularly marked for women with lymph node–negative cancer (Foulkes et al. 1998); the 5-year survival rate for the mutation carriers with node-negative disease was 58%, compared with a 94% survival rate for the noncarriers ($P < .01$). It will be necessary to confirm these observations in a larger number of patients and in patients with other mutations. Previous studies based on clinic patients failed to discriminate between hereditary and nonhereditary cases (Marcus et al. 1996; Johannson et al. 1997; Verhoog et al. 1998), possibly because of methodological limitations.

It has long been speculated that patients with familial colon cancer have a favorable prognosis. Hereditary nonpolyposis colon cancer (HNPCC) is often associated with the replication error–repair (RER) phenotype and a germ-line mutation in one of the DNA mismatch–repair genes. Anecdotal reports of patients who survive a first colon cancer only to develop independent tumors in the colon or elsewhere are supported by more systemic research. A study from Finland found that colon cancers in individuals with *MLH1* mutations had a 5-year survival rate of 65%, compared with 44% for patients with sporadic tumors (Sankila et al. 1996). Watson et al. (in press) show that patients from HNPCC families with advanced-stage colon cancer are less likely to present with liver metastases than are patients from a tumor registry with cancer of equivalent stage.

Several explanations have been offered to explain the favorable course of familial colon cancer. It has been proposed that the genetic instability that is the hallmark of hereditary colon cancer leads to the production and cell-surface expression of diverse abnormal proteins that could evoke an effective immune response. In support of this theory is the observation of a characteristic lymphoid infiltration of colon tumors in HNPCC, which is known to be a favorable prognostic marker (Graham and Appelman 1990). Others suggest that a very high somatic mutation rate may result in derangement of critical metabolic processes, thereby enhancing cell death (Shibata et al. 1994). The study by Watson et al. (in press) suggests that the ability of emergent tumor emboli to form liver metastases may be impaired. This might be due to the reduced expression of relevant cell-surface molecules that are required for cell adhesion and growth in the hepatic environment. However, there is also a reduced frequency of p53 overexpression in RER-positive colon cancers (Kim et al. 1994).

It is interesting that, despite the slow progression of established colon cancer in HNPCC families, the rate of

premalignant change appears to be enhanced. Most hereditary colon cancers (like sporadic cancers) occur in the context of a preexisting adenomatous polyp (Jass and Stewart 1992). Despite the very high risk of cancer in HNPCC, the frequency of such polyps in the colons of carriers is only modestly increased. This suggests that high risk of colon cancer in the syndrome is the result of a rapid transformation of a benign polyp into an invasive cancer.

The gene mutations that underlie hereditary ovarian, breast, and colon cancer syndromes are rare and will account for only a small amount of the variation in recurrence rates in a population of cancer patients. Potentially of more importance are frequent polymorphic alleles, even if the genes exert a relatively modest effect. Genes in this class include the human leukocyte antigens (HLA), the cytochrome P450 genes, and the gene for the androgen receptor. Melanoma patients with stage I or stage II disease face a greater risk of recurrence if they carry the HLA class II allele DQB1*0301 (Lee et al. 1996). The androgen-receptor gene contains a polymorphic CAG-repeat sequence that ranges in length from 8 to 31 repeat units. Short repeat lengths are associated with high transcriptional activity of the androgen receptor. The length of the polymorphic tract is correlated with prostate cancer risk; however, the association appears to be restricted to tumors of high grade or advanced stage. In the Physician's Health Study, Giovanucci et al. (1997) found that repeat lengths of <19 units were associated with a doubling of the risk of cancer that had spread beyond the prostate. In contrast, the risk of cancers confined to the gland was not measurably increased. These data suggest that the androgen-receptor polymorphisms affect the rate of metastatic progression from localized disease, but direct evidence of this hypothesis is still lacking.

Heritable Variation in Genes That Are Altered as Tumors Progress

A second class of genes that may contribute to host susceptibility to progression is the genes found to be somatically mutated in tumor tissue. The best known of these include oncogenes (e.g., ERBB2, N-myc, HRAS, and KRAS) and tumor-suppressor genes (e.g., p53, RB, NF2, and VHL). In some tumors, these genes—when mutated, overexpressed, or amplified—are also prognostic markers. Many of the tumor-suppressor genes are also susceptibility genes. To date, there is very little evidence that allelic variants of genes in this class are related to tumor progression, but few have been studied. Inactivating p53 mutations (or p53 overexpression) is an adverse prognostic feature for a range of tumor types. One might expect therefore that tumors arising in the context of an inherited p53 mutation (the Li-Fraumeni syndrome) would be functionally inactivated for p53 and would have a poor prognosis. To my knowledge, these experiments have not been done. A VNTR polymorphism near the HRAS1 proto-oncogene has been associated with susceptibility to a range of cancer types, both sporadic (Krontiris et al. 1993) and inherited (Phelan et al. 1996), but the locus has not been studied with regard to cancer progression.

Possibly the best candidate genes for tumor progression are those that confer a mutator phenotype on the individual and thereby increase the somatic mutation rate at other loci. Cancer cell lines with microsatellite instability (typical of HNPCC) have an increased frequency of mutations in the TGF-β receptor (Markowitz et al. 1995). The I1307K allele of the APC gene is present in 6% of the Ashkenazi population and has recently been associated with an increased risk of colon cancer (Laken et al. 1997). Tumors with mutations in the I1307K allele of the APC gene show somatic mutations at adjacent sequences, leading to the speculation that the APC gene polymorphism is associated with hypermutability (Laken et al. 1997). The observation that the I1307K allele is also associated with an increased risk of breast cancer (M. Redston and W. D. Foulkes, unpublished data) suggests the possibility that the somatic mutation rate at other loci may also be increased. Defects in genes for DNA-repair syndromes, such as xeroderma pigmenostum, Bloom syndrome, or ataxia-telangiectasia, are associated with increased numbers of somatic mutations in nonmalignant cells. It is not known whether cancer patients, either as homozygotes or heterozygotes, with mutations or functional polymorphisms of genes associated with DNA repair face an increased rate of relapse. Shen et al. (1998) have documented genetic polymorphisms in several genes of this class, including *XRCC1, XRCC3,* and *XPD* and *XPF,* as a prelude to association studies of cancer susceptibility and progression.

Although compelling, the hypothesis that a mutator phenotype is an adverse prognostic feature is probably simplistic. It may be that the rate-limiting steps in early carcinogenesis are mutation related but that later steps in progression depend on epigenetic phenomena. Most cancers contain a large number of genetic mutations, LOH events, and over- and underexpression of proteins. Many of these changes, individually or in combination, are also related to progression. Emerging evidence suggests that the individual changes are not independent and that, within a tumor type, there may be characteristic gene combinations. For example, if there are 30 genes that may be mutated or otherwise abnormal in a tumor of a particular type (e.g., breast), then there are $>10^9$ potential subtypes, each defined by the specific genes involved. In reality, the actual number of subtypes is probably only a small fraction of this. Each subtype may have a characteristic prognosis, and the overall

range in recurrence risks may be large. If the pattern of genetic changes associated with a hereditary cancer (i.e., one with an inherited mutation of one of the relevant genes) differs systematically from that of the corresponding nonhereditary cancer, then the prognosis may vary accordingly.

Genes Controlling Metastatic Spread and Tumor Dormancy

There are abundant candidates for host susceptibility at the level of tumor metastasis, including genes that control for cell adhesion, invasion, and angiogenesis and apoptosis. The malignant cell must overcome cell-cell adhesion to invade surrounding tissues or to form distant metastases. E-cadherin is an important adhesion molecule in normal epithelial cells, but it is often lost in their malignant counterparts. Perl et al. (1998) found, by using a transgenic mouse model, that the loss of E-cadherin expression could result in the transition from a well-differentiated pancreatic islet-cell adenoma to frank carcinoma. It remains to be established whether heritable variation in the gene for E-cadherin or in other molecules of this class is relevant for the rate of progression from benign to malignant tumors in humans.

The phenomenon of tumor dormancy is of particular interest as it relates directly to relapse and prognosis. For several tumor types, including breast and melanoma, the tumor growth rate is not exponential, and the rate of clinical recurrence cannot be predicted entirely from the rate of cell division of incipient metastases. Rather, it appears that tumor cells may undergo a period of dormancy followed by rapid growth and relapse. Dormancy is not a state of rest, as the name implies, but reflects a balance between cell division and apoptotic cell death (Holmgren et al. 1995). Experimental data suggest that angiogenesis plays a critical role in reactivation, and a host of positive and negative regulators of angiogenesis have been discovered. It will be of interest to see whether there are functional variants of the genes involved in the regulation of angiogenesis and, if so, whether these also predict relapse.

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