Cancer Prevention: Epigenetics Steps Up to the Plate

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The fields both of cancer prevention and epigenetics have matured to a point where their intersection deserves sustained attention. There now is strong evidence for an epigenetic component of early neoplasias, and data on the cancer preventive properties of epigenetic modulation are emerging. With preclinical proof of principle at hand, the challenge is to translate these epigenetic findings into testable clinical hypotheses.

Cancer epigenetics—referring to gene expression patterns that are stable, faithfully transmitted from parent cell to daughter cell after division, largely irreversible, and unrelated to genetic variation or mutations (1)—started as a field of research almost three decades ago with the observation that 5-methylcytosine levels are lower in cancer cells than in normal cells (2). Initially regarded as biological curiosities, epigenetic phenomena have drawn much interest recently because of their importance in determining cellular physiology [such as the stem cell versus committed (determined) cell state] and pathology (such as cancer). Over the past few years, the field has gained center stage in cancer biology through advances in our understanding of how epigenetic changes (perhaps more commonly than genetic changes) are involved in modulating functional pathways that are key to the neoplastic phenotype (3, 4) and by showing the clinical efficacy of targeting epigenetics for the treatment of cancer (5). Epigenetic mechanisms including promoter DNA methylation, histone modifications, and RNA interference are now better understood and relatively easily detected, contributing to the recent explosion of information on these processes. Epigenetic alterations often are involved in the earliest stages of tumor progression, usually precede neoplastic transformation, and have been shown to affect tumor formation even in genetic mouse models of carcinogenesis (6). Therefore, the real clinical home of cancer epigenetics may be prevention, where epigenetics potentially will have its greatest impact.

Dedicated exclusively to cancer prevention, the exciting new journal Cancer Prevention Research provides an excellent home for fleshing out the role of epigenetics in early neoplasia and the practical steps for implementing an epigenetic-based cancer prevention strategy. Epigenetic alterations are promising biomarkers of cancer risk and early neoplasia (7). The next generation of epigenetic-alteration studies should address technical issues (the best methods and markers) and practical translational issues (including positive and negative predictive values in a real-world setting), and Cancer Prevention Research will feature studies of this type. Promising to have an even greater impact, studies of epigenetic modulators in cancer prevention (8) will also be featured prominently in Cancer Prevention Research. Indeed, the current issue of the journal features an exciting report by Yoo et al. (9) of the Peter Jones laboratory showing the potential cancer preventive properties of an oral DNA hypomethylating drug in colon tumorigenesis.

Early Cancer Development as an Epigenetic Disease

To make the case that some (perhaps most) noninherited (sporadic) cancers are epigenetic diseases, one has to postulate (a) a functional role for epigenetic changes, (b) demonstrable positive selection for cells that carry altered epigenomes, and (c) detectable epigenetic changes at the earliest stages of tumor development. All three postulates are now backed by abundant evidence.

All critical pathways in tumor development, including uncontrolled growth, evading apoptosis, and inducing angiogenesis, can be altered via epigenetic loss of key genes (3). It has been shown that half of all tumor-suppressor genes are inactivated in sporadic cancers more often by epigenetic, than by genetic, mechanisms. Several genes with tumor-suppressor properties in mouse models are inactivated exclusively by epigenetic mechanisms in human neoplasia (10, 11). The most compelling evidence for a selective advantage of aberrant epigenetics in cancer comes from studies of tumor-suppressor genes that are mutated in the germ line of patients with familial cancer syndromes. Evidence suggests that, in some sporadic cancers, the tumor-suppressor genes P16, MLH1, VHL, and ECAD carry one allele inactivated by genetic mechanisms and another allele inactivated epigenetically by promoter DNA methylation (6). Such allelic exclusivity in the same tumor subjected to the same exposures most likely is due to an equivalent selective advantage for genetic and epigenetic inactivation. Overall, there are strong data showing that the neoplastic phenotype in many cases is due to epigenetic-based pathway alteration.

The development of accurate measuring technology has allowed researchers to time the process of epigenetic changes within premalignant stages progressing toward cancer. Every study that looked at epigenetic timing has concluded that epigenetic changes occur very early in neoplasia and precede epithelial malignancy (3, 6, 8). For example, in colon and gastric cancer, aberrant DNA methylation can start in normal-appearing mucosa (12), increases in abnormal early lesions (such as aberrant crypt foci in the colon; ref. 13), and is only marginally higher in malignancy than in premalignancy (14). Aberrant methylation in premalignant fields associated with cancer has been seen in many cancer systems including colon (15), esophageal (16), liver (17), lung (18), and stomach (19) cancers. Key genes are inactivated epigenetically in premalignant lesions such as in breast ductal carcinoma in situ (20) and...
prostatic intraepithelial neoplasia (21). Based on these observations, the model depicted in Fig. 1 postulates that aging and exposures result in epigenetically altered cells that are “primed” for neoplastic transformation (22). If this model is verified, epigenetic alterations should have great potential as targets of very early interventions to prevent cancer.

**Tumor Prevention by Epigenetic Modulation**

Directly testing the contribution of epigenetic alterations to cancer development has been difficult because epigenetic carcinogenesis is not easy to model in animals. Human sporadic carcinogenesis takes decades to develop. Mouse models have relied on accelerating genetic changes through engineered strains to approximate the process, but this cannot easily be done to create an epigenetic tumor model because epigenetic reprogramming during early embryogenesis largely erases any induced change (23). A recently reported model of epigenetic tumorigenesis relies on overexpression of the de novo DNA methyltransferase DNMT3b (24). Sporadic tumors did not develop during this study, possibly because the mice were sacrificed at a young age; tumors may have developed over a longer time span, as commonly occurs in many other mouse models. However, DNMT3b clearly accelerated tumor formation in the multiple intestinal neoplasia (MIN) mouse model, in which colon tumors arise as a result of genetic inactivation of APC. As expected, this increased tumorigenesis was associated with increased promoter DNA methylation and provides strong support for a key role of epigenetic changes in early tumorigenesis and the model presented in Fig. 1. In the same MIN mouse model, epigenetic activation of the imprinted gene insulin-like growth factor II (IGFII) also accelerates tumorigenesis (25).

The lack of a purely epigenetic tumor model is clearly a barrier to evaluating the full impact of epigenetic cancer prevention. Nevertheless, the shown interactions between epigenetic changes (hypermethylation) and genetic changes (APC mutations) in a mouse model still allow for testing the epigenetic hypothesis in cancer prevention. In a landmark experiment, inducing hypomethylation by deleting one DNMT1 allele substantially reduced tumor formation in MIN mice (26). This reduction was attributed to a reduction in gene-specific methylation in normal appearing mucosa (27) and provides more support for the aging/methylation/cancer hypothesis portrayed in Fig. 1. In a more dramatic study, the hypomethylating drug 5-aza-2’-deoxycytidine also reduced tumor formation in this model, and a combination of DNMT1 deletion and 5-aza-2’-deoxycytidine almost completely abolished tumor formation. Another study showed the power of hitting two different epigenetic processes/targets, DNA promoter methylation and histone deacetylation, with combined agents in preventing tumors in a mouse model of carcinogen-induced lung cancers (28).

In this issue of *Cancer Prevention Research*, Yoo et. al. report their study, taking this concept one step further by testing zebularine, an oral inhibitor of DNA methylation, in the MIN mouse model (9). Chronic administration of zebularine in drinking water had no discernable toxicity (based on body weight), caused decreased DNA methylation in the target tissue, induced gene expression, and dramatically reduced polyp formation (from 58 to 1). Therefore, oral zebularine was as powerful in tumor prevention as was the combination of DNMT1 deletion and 5-aza-2’-deoxycytidine. It remains to be seen whether zebularine reduces gene-specific hypermethylation (as expected) and whether it affects initiation or progression of polyps in this model, but the reported results clearly suggest that epigenetic modulation may be more powerful in preventing neoplasia than are currently tested clinical strategies such as cyclooxygenase-2 inhibition. Another remarkable finding of Yoo et al. was that zebularine-treated mice had less splenomegaly or anemia than did control mice, suggesting other beneficial effects of hypomethylation induction on age-related phenotypes in this mouse model.

**Fig. 1.** Human sporadic carcinogenesis as an epigenetic disease. In this model, it is hypothesized that human tissue stem cells start life with a relatively uniform epigenetic code (top). Aging and the associated exposures alter epigenetic information in stem cell subsets such that, over time, epigenetic mosaicism develops in patches of cells that have subtly altered gene expression. The sum total of changes in some of these patches favors increased proliferation, reduced apoptosis, and/or altered differentiation, promoting the acquisition of further molecular changes (epigenetic and genetic) that ultimately result in neoplastic transformation.

**Young, healthy individuals: All our cells have normal epigenetic patterns**

**Aging, diet, exposures etc.**

**Some of our cells acquire epigenetic changes which lead to “fields” or “patches” of faulty gene expression. Tissues still look normal.**

**Aging, diet, exposures etc.**

**Cancers start in these abnormal epigenetics fields**
Practical Clinical Issues in Epigenetic Cancer Prevention

Cancer epigenetics has moved from a fringe theory to the forefront of research into molecular cancer pathogenesis. Furthermore, epigenetic-altering drugs are proving to be clinically useful. The two most potent DNA methylation inhibitors, 5-aza-2’-deoxycytidine and 5-azacytidine, are both U.S. Food and Drug Administration approved for the treatment of the myelodysplastic syndrome (5), and histone deacetylase inhibitors show clinical activity in hematologic neoplasms (29). There are substantial barriers, however, in applying this research to clinical cancer prevention, including the limited availability of suitable epigenetic-altering compounds or interventions for cancer prevention. The hypomethylating drugs used for cancer treatment generally are too toxic for prevention (though perhaps not for prevention in very high-risk patients) and have not been tested for potential long-term health effects. Zebularine, described in the Yoo et al. report, is not currently in clinical development partly because of major differences between mice and humans in metabolizing the drug. Several natural compounds and commonly used drugs such as epigallocatechin gallate, selenium, procainamide, and hydralazine are reported to have hypomethylating properties, but these effects are generally weak and not always reproducible (8, 30–32). It is speculated that dietary interventions may alter DNA methylation or histone modifications, but such dietary effects may be greatest during embryogenesis and early development (33). It is not yet known whether dietary intervention in adults is sufficient to alter epigenetic patterns in tissues.

Another clinical prevention barrier, certainly not unique to epigenetics, is the problem of optimum timing. Epigenetic defects occur early (e.g., methylation in normal-appearing mucosa) and may prime epithelia for further progression. Therefore, epigenetic interventions may work at early stages in preventing or reversing the earliest carcinogenic changes, as they have in all of the reported relevant mouse model studies (9, 26–28). The most suitable interventions for preventing early epigenetic changes (Fig. 1, middle) would be lifelong, nontoxic dietary approaches. Testing such approaches in the clinic, however, would require trials of massive size and duration if a cancer end point were used, and there are no validated epigenetic surrogate end points for potentially reducing the sizes and durations of such early-stage epigenetic trials. Epidemiologic studies, however, potentially could address this issue (34). There are only limited data (26) on epigenetic intervention during the later steps of preinvasive carcinogenesis (Fig. 1, bottom), and these data did not indicate that later-step intervention was effective. Research is clearly needed to assess whether epigenetic alterations at this stage are rate-limiting steps of neoplastic progression and whether epigenetic-acting drugs can reverse (rather than prevent) preinvasive neoplasia.

One setting of advanced premalignancy that does show promise for clinical epigenetic intervention is the lung. Investigators showed that methylation markers in the sputum of chronic smokers were associated with a high risk of lung cancer (35). This study raises the intriguing potential for designing a feasible clinical epigenetic strategy for these patients, whose high lung-cancer risk may allow the use of current (rather toxic) agents and certainly would help reduce trial size and duration (36). New, less toxic epigenetic-altering agents also could be tested, potentially targeting the same methylation markers that denote elevated risk in these patients and which could serve as biomarkers of drug activity. Epigenetic-altering agents have shown promise in a murine lung carcinogenesis model (28). The proof of principle of epigenetic activity markers has been provided by work showing that hypomethylation and gene reactivation are associated with response of myelodysplastic syndromes to Food and Drug Administration-approved epigenetic-targeting drugs (5). The sputum methylation risk markers also reflect the broader promise epigenetic markers have shown for clinical cancer risk assessment and early cancer detection (37).

Another interesting, potentially worthwhile clinical approach is based on the observation that chronic inflammation markedly accelerates acquisition of DNA methylation changes (38). Drugs that suppress inflammation, such as cyclooxygenase-2 inhibitors, may act in part by long-term modulation of epigenetics, a concept that needs to be confirmed clinically. It is hoped that the strong data on epigenetic involvement in early carcinogenesis and the potential for epigenetic-based prevention will stimulate research into the development of safe epigenetic modulators (including dietary factors) that could be used regularly over the long term to prevent cancer and other age-related diseases.

Conclusions

Strong data support a role for epigenetics in early neoplasia. There are now equally strong data for epigenetic therapy in cancer prevention, as shown by Yoo et al. (9). Practical applications of the field to human cancer prevention require concerted investigations of epigenetic detection techniques, appropriate tumorigenesis models, sensitive assays to screen for molecules that affect epigenetics, population-based studies of epigenetics, and clinical trials of epigenetic modulators for cancer prevention. These steps will be facilitated by the recent designation of epigenetics as a major initiative of the NIH Roadmap, which provides an “incubator space” for cross-cutting, complex programs that warrant the concerted attention of the entire NIH. Cancer Prevention Research will serve as a welcoming home for these investigations, featuring original research, forums, and commentaries aimed at pushing this young field forward.

Disclosure of Potential Conflicts of Interest

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