Hypothesis/Commentary

Hypoxia-Inducible Factor 1 as a Possible Target for Cancer Chemoprevention

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Abstract

Despite the intense cancer research carried out in the last 30 years, cancer therapy has not managed to decrease cancer mortality. We need new strategies to control a disease that kills over six million people worldwide every year. It is accepted that cancer chemoprevention (the use of chemicals to prevent, stop, or reverse the process of carcinogenesis) is an essential approach to controlling cancer; yet, the clinical usefulness of this strategy is very limited. Successful implementation of cancer chemoprevention depends on a mechanistic understanding of the carcinogenesis process. Our knowledge about this process is still limited and may therefore be preventing cancer chemoprevention from becoming a widely used anticancer tool. This report discusses recent evidence that suggests that the activation of the hypoxia-inducible factor 1 (HIF-1) is a key event in carcinogenesis and may therefore represent a key target for cancer chemoprevention. Based on an understanding of the mechanisms responsible for HIF-1 activation, possible general strategies for targeting HIF-1 are proposed. Successful implementation of these strategies might turn the great promise of cancer chemoprevention into a fundamental tool for reducing the burden of this disease. (Cancer Epidemiol Biomarkers Prev 2006;15(12):2332–5)

Introduction

During the last several decades, surgery, radiotherapy, and chemotherapy have been the useful yet unsatisfactory tools for decreasing cancer mortality. Although chemotherapy in the advanced stages of disease has been highly successful for the treatment of relatively rare cancers, the prognosis for patients with invasive and metastatic disease at the common epithelial sites remains poor (1). Because conventional strategies are not expected to increase in efficiency in the near future (1, 2), we need other approaches to decrease cancer incidence and mortality, which have virtually remained the same for the last 30 years (3, 4). We are becoming aware that the process of carcinogenesis can take decades to complete, providing time and opportunity to intervene to prevent this process either before the clinical appearance of cancer or at its earliest stages. Thus, evidence suggests that in addition to lifestyle factors and vaccines, chemopreventive interventions hold the greatest promise for reducing cancer incidence and mortality (1, 5, 6). Cancer chemoprevention is the use of chemicals to prevent, stop, or reverse the process of carcinogenesis. It is now accepted that cancer chemoprevention is not only an essential approach to controlling cancer but also a quality-of-life issue (1). It makes more sense to prevent cancer at its earliest stages by using low-toxic chemicals than to wait until the disease has reached its final stages, where it becomes necessary to use more toxic drugs.

Despite the great promise of cancer chemoprevention, this strategy is currently restricted to some areas of the head and neck, breast, and colon carcinogenesis (1). It is important to note that successful implementation of chemoprevention depends on a mechanistic understanding of carcinogenesis (1) and that, regretfully, we still do not fully understand the carcinogenesis process. Most researchers consider cancer to be a genetic disease caused by the acquisition of multiple mutations in key genes that control cell proliferation, cell death, and genetic stability (7). This is called the ''somatic mutation theory of cancer'' and has been the prevalent hypothesis to explain carcinogenesis over the last several decades. This theory, however, is increasingly being challenged by growing experimental evidence that contradicts or cannot be explained by this hypothesis (8-12). For instance, this theory cannot explain metastasis (7), the key process of carcinogenesis that distinguishes a benign tumor from a malignant cancer.

According to the most accepted theory, oncogenic activation and the inactivation of tumor suppressor genes are key events in carcinogenesis. Oncogenic activation would produce increased synthesis of growth factors, increased expression of growth factor receptors, and inappropriate activation of downstream signal transduction pathways and nuclear transcription factors. Oncogenic activation would therefore stimulate cell proliferation. Tumor suppressor genes, on the other hand, would code for proteins that normally act as checkpoints for cell proliferation or cell death. Because tumor suppressor genes code for proteins that can restrain cell proliferation, the loss of such proteins would allow a cell to grow and divide in an uncontrolled fashion. Therefore, this theory suggests that cancer chemoprevention can be achieved by using chemicals to decrease oncogenic proteins or increase tumor suppressor proteins. Several years ago, it was believed that cancer could be explained by modifications in several of these cancer-related genes. To date, >100 oncogenes and >30 tumor suppressor genes have been identified. In addition to these high numbers, it has been observed that different types of cancers, and even the same cancer type from different individuals, have different genetic alterations. Furthermore, some of the most commonly altered cancer genes have oddly inconsistent effects. For instance, it has been observed that the

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much-studied oncogenes *c-fos* and *c-erbb3* are less active (not activated) in some tumors than they are in nearby normal tissues. Likewise, the tumor suppressor gene *RB* has been shown to be hyperactive (not disabled) in some colon cancers, and it seems to protect these tumors from apoptosis (7, 9, 10). Chemoprevention strategies aimed at targeting specific oncogenic or tumor suppressor proteins might therefore result in unpredictable or negative outcomes. In other words, our limited knowledge about the process of carcinogenesis may be preventing cancer chemoprevention from becoming a widely used anticancer tool.

Hypoxia-Inducible Factor 1 as a Key Target for Cancer Chemoprevention

Hypoxia-inducible factor 1 (HIF-1) is a heterodimeric transcription factor that consists of a constitutively expressed HIF-1β subunit and a HIF-1α subunit, the expression of which is highly regulated (13). Recent experimental evidence suggests that HIF-1 is a key player in carcinogenesis. Interest in the role of HIF-1 in cancer has grown exponentially over the last two decades, as this factor activates the transcription of many genes that code for proteins involved in several pathways intimately related to cancer (13-15). A growing body of evidence suggests that the most important oncogenic and tumor suppressor gene pathways are connected with the HIF-1 pathway (7). Vogelstein and Kinzler have recently reviewed our current knowledge about cancer genes and have shown that the most relevant cancer genes can be put into several cancer-related pathways; these pathways include the receptor tyrosine kinase pathway, the RB pathway, the p53 pathway, the apoptosis pathway, the activated protein C pathway, the GLI pathway, the SMAD pathway, and the HIF-1 pathway (7). Interestingly, all these cancer gene pathways seem to culminate in HIF-1 activation (7). It is not surprising, therefore, that HIF-1 overexpression has been observed in the most common human cancers and has already been associated with increased patient mortality in several cancer types (13, 16-20). For instance, Zhong et al. identified increased HIF-1 expression (relative to adjacent normal tissue) in 13 tumor types, including lung, prostate, breast, and colon carcinoma, which are the leading causes of U.S. cancer mortality. HIF-1 was also overexpressed in preneoplastic and premalignant lesions, such as colonic adenoma, breast ductal carcinoma *in situ*, and prostate intraepithelial neoplasia. These data show that overexpression of HIF-1 may occur very early in carcinogenesis, before histologic evidence of angiogenesis or invasion (16), and suggest that HIF-1 might be a biomarker of carcinogenesis and a suitable target for cancer chemoprevention.

It is widely accepted, even by those who challenge the somatic mutation theory of cancer, that all invasive cancers must develop several acquired capabilities: increased proliferation (caused, in part, by resistance to growth inhibition and independence from mitogenic stimulation), decreased apoptosis, cellular immortalization, invasion/metastasis, and increased angiogenesis (21, 22). There is evidence that HIF-1 activation may explain all these hallmarks of cancer (Fig. 1). Thus, it is recognized that HIF-1 overexpression produces apoptosis resistance, invasion/metastasis, and increased angiogenesis (13). Recent reports support that HIF-1 activation may also produce cellular immortalization by activating telomerase (23, 24). Furthermore, it is accepted that HIF-1 activation plays a key role in the maintenance of the glycolytic phenotype (13, 14, 25), and that glycolysis activation plays an important role in cancer development, as it favors invasion/metastasis (26), angiogenesis (26), and proliferation (20, 27). Indeed, established biochemical evidence suggests that glycolysis is necessary for sustained cell proliferation, as cell proliferation requires the synthesis of new molecules, and as glycolysis provides most of the building blocks required for the synthesis of these molecules (27).

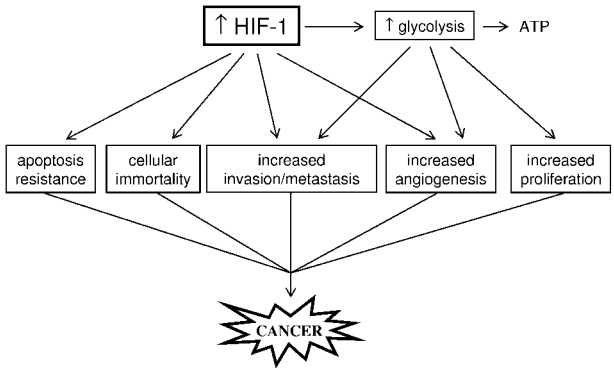

Figure 1. Key role of HIF-1 activation in carcinogenesis. The activation of HIF-1 may explain or favor the acquisition of all the hallmarks of cancer and be important for keeping adequate ATP levels in cancer cells.

In addition to favoring the acquisition of several hallmarks of cancer, the activation of glycolysis seems to be critical for cancer cells to generate energy. The Nobel laureate Otto Warburg proposed several decades ago that cancer was caused by a defect in respiration (oxidative phosphorylation). This defect would produce a deficient ATP generation through oxidative phosphorylation that would be compensated by the activation of glycolysis (28). Although his theory has been rejected by most researchers, it is now well accepted that cancer cells have increased glycolytic rates (26). Furthermore, recent experiments have suggested that cancer cells depend on glycolysis for ATP generation, and that cancer cells' dependence on glycolytic energy progressively increases as malignant transformation occurs (29, 30). Because HIF-1 activation plays a key role in the activation of glycolysis (13, 14, 25), and because glycolysis seems necessary for cancer cells to keep adequate ATP levels (29, 30), it is reasonable to think that HIF-1 activation is important for keeping ATP levels in cancer cells. Accordingly, it has been reported that hypoxia-induced glycolysis (Pasteur effect) is mediated by HIF-1 activation, and that ATP levels are dramatically reduced during hypoxia in the absence of HIF-1 (31).

In brief, the most important cancer-related gene pathways seem to culminate in HIF-1 activation, and HIF-1 activation may explain all the hallmarks of cancer and be necessary for keeping adequate ATP levels in cancer cells. Furthermore, HIF-1 overexpression is observed in the most common cancer types and has been associated with increased patient mortality in several cancer types. This suggests that HIF-1 plays a key role in carcinogenesis and may therefore represent a key target for cancer chemoprevention.

Targeting HIF-1 for Cancer Chemoprevention

Preclinical studies have already shown that inhibition of HIF-1 activity has marked effects on tumor growth (13). HIF-1 is considered a potential target for cancer therapy, and, recently, many efforts to develop new HIF-1-targeting agents have been made by both academic and pharmaceutical industry laboratories (13, 20, 32-34). Thus, it has been found that several Food and Drug Administration–approved anticancer drugs (e.g., topotecan, imatinib mesylate, trastuzumab, NS398, celecoxib, and ibuprofen) inhibit HIF-1 activity (20). Several natural products (e.g., resveratrol, genistein, apigenin, and berberin) have also been found to inhibit the activity of this transcription

factor (20, 33). Because HIF-1 seems to have an important function in carcinogenesis, HIF-1 inhibitors may be considered a source of potential cancer chemopreventive agents. It is important to note, however, that the use of HIF-1 inhibitors in cancer chemoprevention might be associated with toxicity. An excessive inhibition of HIF-1 may produce adverse effects, as HIF-1 regulates many cellular processes under physiologic conditions (14, 25). For instance, an agent that inhibits HIF-1 directly may prevent normal cells from responding to a situation of hypoxia; this may be associated with toxicity. Toxicity can be acceptable in preventing cancer in people with precancerous lesions or who are at high risk for developing cancer. Thus, tamoxifen is used in the chemoprevention of specific breast cancers, although it increases the rate of endometrial cancer and uterine sarcoma (6). However, any degree of toxicity is unacceptable in preventing cancer in healthy people who may never develop the disease. Therefore, although HIF-1 inhibitors may represent a useful source of chemopreventive agents, the potential toxicity associated with these agents should be considered carefully, especially when chemopreventive interventions are aimed at preventing cancer in healthy populations.

In addition to targeting HIF-1 directly with HIF-1 inhibitors, HIF-1 can be targeted by identifying a/the key cellular event responsible for HIF-1 activation and developing strategies to prevent such activation. It has been proposed that cancers have increased HIF-1 because of intratumoral hypoxia and genetic alterations that activate oncogenes and inactivate tumor suppressor genes (13, 20). Unfortunately, intratumoral hypoxia is difficult to modulate, and, as suggested before, the genetic alterations responsible for HIF-1 activation may be too irregular and numerous to target.

A recent report has proposed a new model to explain HIF-1 activation under both physiologic conditions and cancer. This report discusses evidence that suggests that, although hypoxia and genetic alteration can activate HIF-1, the key cellular event involved in the activation of HIF-1 is an alteration in oxygen metabolism (dysoxia; ref. 15). This model shows that this alteration in O_2 metabolism activates HIF-1 by increasing the cellular levels of hydrogen peroxide (H_2O_2) and by activating glycolysis. Therefore, in addition to targeting HIF-1 directly with HIF-1 inhibitors, HIF-1 activation may be inhibited by preventing or decreasing excessive cellular levels of H_2O_2 and an excessive activation of glycolysis. These general strategies may reduce the possible toxicity associated with a direct inhibition of HIF-1 induced by HIF-1 inhibitors. For instance, it is known that cancer cells have increased rates of glycolysis (26), and that the accumulation of glucose metabolites keeps high levels of HIF-1 in cancer cells (35, 36). This suggests that a chemopreventive strategy aimed at preventing an excessive activation of glycolysis may reduce the levels of HIF-1 and might therefore produce anticancer effects. Interestingly, this strategy would allow normal cells to activate HIF-1 in response to a situation of hypoxia, as hypoxia induction of HIF-1 mainly occurs via H_2O_2 (37, 38).

Inhibition of HIF-1 may fit into current strategies for cancer chemoprevention. For instance, it is considered that angiogenesis is a potential biomarker and target in cancer chemoprevention, and that the vascular endothelial growth factor (VEGF) is a key angiogenic factor (39). Because several angiogenic genes (including *VEGF*) are transcriptionally activated by HIF-1 (13), the inhibition of HIF-1 may produce angiogenesis inhibition. As mentioned above, there is evidence that H_2O_2 is a key activator of HIF-1 (15). A reduction in the cellular levels of H_2O_2 would reduce HIF-1 activation and may therefore inhibit angiogenesis. This is in accordance with experimental data that have shown that overexpression of the H_2O_2-detoxifying enzyme catalase can reduce VEGF expression and inhibit angiogenesis (40).

In conclusion, cancer statistics remind us that new strategies are necessary for controlling this disease. Although it is accepted that cancer chemoprevention holds great promise for reducing cancer incidence and mortality, the clinical usefulness of this strategy is very limited. This restricted use may be due to our still limited knowledge of the carcinogenesis process. The present report has discussed that the activation of the transcription factor HIF-1 is a key event in carcinogenesis and may therefore represent a key target for the development of cancer chemopreventive strategies. Based on an understanding of the mechanisms responsible for HIF-1 activation, possible general strategies for targeting HIF-1 have been proposed. Successful implementation of these chemopreventive strategies may arrest or reverse the carcinogenesis process and might therefore turn the great promise of cancer chemoprevention into an essential anticancer tool.

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