those sensitive groups that are likely to bear disproportionate risk, thus reducing the incidence of cancer and avoiding environmental inequities (*50*).

In modeling risk distribution and identifying susceptible populations, molecular epidemiology can be a useful tool, provided that the biomarkers are adequately validated and study designs are sound (*1*, *49*). Biomarkers can also contribute to risk assessment by providing dose-response data for extrapolation from laboratory animals to humans, by elucidating mechanisms in human carcinogenesis, and by serving as intermediate endpoints for monitoring the effectiveness of interventions.

Cancer is largely a preventable disease. Molecular epidemiology has contributed to the growing awareness of the importance of relatively common genetic and acquired susceptibility factors in modulating risks from environmental carcinogens. To make greater strides in preventing cancer, we need public health strategies that reflect this knowledge.

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Recent Advances in Chemoprevention of Cancer

Waun Ki Hong* and Michael B. Sporn

Chemoprevention is the use of pharmacologic or natural agents that inhibit the development of invasive cancer either by blocking the DNA damage that initiates carcinogenesis or by arresting or reversing the progression of premalignant cells in which such damage has already occurred. Recent advances in our understanding of the mechanisms of carcinogenesis have led to the synthesis of new drugs that can inhibit tumor development in experimental animals by selective action on specific molecular targets, such as the estrogen, androgen, and retinoid receptors or inducible cyclooxygenase. Several of these agents (including tamoxifen, 13-*cis*-retinoic acid, retinyl palmitate, and an acyclic retinoid) are clinically effective in preventing the development of cancer, particularly in patients who are at high risk for developing second primary tumors after surgical removal of the initial tumor.

In spite of immense efforts to improve treatment and find cures for advanced disease, overall mortality rates for most forms of epithelial cancer have not declined in the past 25 years. The prognosis for a patient with metastatic carcinoma of the lung, colon, breast, or prostate (four of the most common and lethal forms of cancer, which together account for more than half of all deaths from cancer in the United States) remains dismal (*1*). A current scientific view indicates that damage to numerous regulatory genes ultimately results in the development of invasive and metastatic cancer, which is the culmination of the chronic disease process, carcinogenesis. The natural history of carcinogenesis and cancer provides a strong rationale for a preventive

approach to the control of this disease and leads one to consider the possibility of active pharmacological intervention to arrest or reverse the process of carcinogenesis before invasion and metastasis occur (*2*). Such intervention is called chemoprevention.

The clinical practice of chemoprevention is still in its infancy, although there is a wealth of data documenting its effectiveness in experimental animals. As genetic testing has the potential to identify large numbers of people who are at increased risk for the development of invasive cancer, preventive strategies are becoming increasingly important. Here, we will summarize several recent advances, both basic and clinical, that justify optimism that chemoprevention will be an effective approach for the control of human cancer.

Basic Aspects of Chemoprevention

Carcinogenesis as an aberrancy of differentiation. Altered states of cell and tissue differentiation are characteristic of premalignant lesions long before they become invasive and metastatic. This pathology of differentiation (dysplasia) offers a defined target for pharmacological intervention, because in some circumstances, it is possible to reverse the abnormal differentiation with a hormonelike agent that is essentially noncytotoxic. Two other approaches to the control of preneoplastic lesions are to block their expansion with nontoxic agents that suppress cell replication or to induce an apoptotic state in cells that ordinarily would be programmed to die (as in colon epithelium) but that may have undergone carcinogenic mutations that provide extended life-span. These three mechanisms provide the basis for the use of most of the chemopreventive agents that are currently in experimental or clinical use. Another approach is to use blocking agents that prevent metabolic activation of carcinogens or their subsequent binding to DNA, but this approach has limited application when genetic damage already exists. The intriguing possibility of using antiangiogenic agents to prevent the progression of advanced, premalignant, noninvasive lesions to frankly invasive lesions remains to be exploited.

The maintenance of normal epithelial differentiation thus represents a primary goal for the pharmacology of chemoprevention. However, it should be emphasized that epithelial cells do not exist in a contextual vacuum. Their differentiation is determined by their reciprocal interactions with their underlying stromal (mesenchymal) cells, and it has been postulated that failure of these reciprocal interactions may play an important role in carcinogenic progression. Thus, in an organ such as the prostate, the underlying mesenchyme regulates epithelial proliferation, ductal morphogenesis, and the expression of prostate-specific secretory proteins, and, conversely, the prostatic epithelium induces smooth muscle cell differentiation in its underlying stroma (Fig. 1). It has been suggested that, during carcinogenesis, the normal reciprocal paracrine cross-talk between these two compartments (mediated by various cytokines) breaks down, leading to dedifferentiation of both epithelium and mesenchyme (*3*).

Molecular targets for chemopreventive agents. Although many chemopreventive agents have been developed empirically in the past, recent advances in the molecular biology of carcinogenesis suggest that it will be possible to develop new and better agents on a more mechanistic basis. The most striking example is in colon cancer, which is considered a paradigm for understanding the role of multiple genetic lesions in tumorigenesis (*4*, *5*). From the perspective of chemoprevention, the recent discovery that overexpression of the gene for inducible cyclooxygenase (COX-2), a key enzyme for the formation of prostaglandins from arachidonic acid, is an early and central event in colon carcinogenesis now provides an important target for drug development (*5*). Mice with defects in the *Apc* (adenomatous polyposis coli) gene develop large numbers of intestinal adenomatous polyps at a young age, and marked increases in COX-2 enzyme concentrations have been found in early polyps in these animals (*6*). Interestingly, this increase in COX-2 concentrations occurs in the stromal rather than in the epithelial components of the adenomas (*6*). The functional relevance of COX-2 to intestinal tumorigenesis was demonstrated by the analyses of mice genetically deficient in both COX-2 and *Apc*; in comparison with mice defective in *Apc* alone, the double mutants had a marked diminution in the number of intestinal polyps (*6*). Consistent with the animal findings, increased amounts of COX-2 messenger RNA and protein have been observed in many primary human colon cancers and colon cancer cell lines (*5*). The mechanisms by which an increase in COX-2 activity promotes tumor formation are not entirely clear, although suppression of apoptosis appears to be contributory (*5*, *7*).

For clinical chemoprevention, the next step will be the use of drugs that selectively inhibit COX-2, that is, drugs free of the serious side effects, such as gastrointestinal ulceration and bleeding, that can be caused by inhibition of the related constitutive enzyme COX-1. Although a voluminous literature exists on the ability of nonselective COX inhibitors to block experimental colon carcinogenesis (*5*, *6*) and one such agent, sulindac, has even been used clinically to suppress colon adenoma formation (*8*), the side effects of these nonselective agents preclude their widespread use for

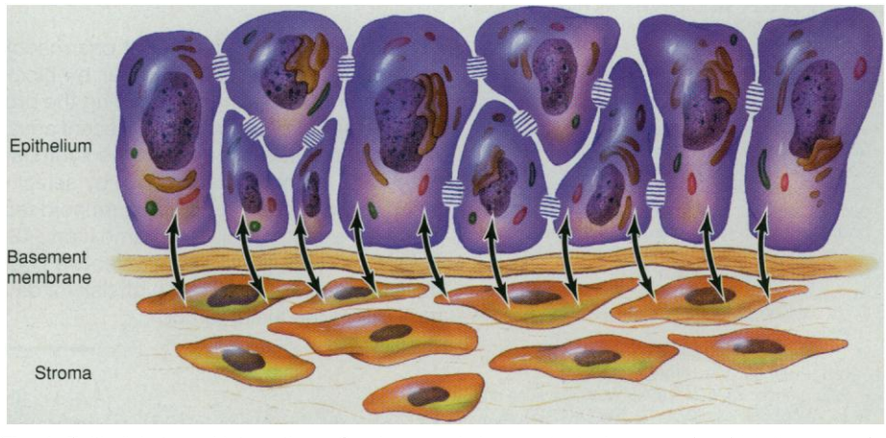

Fig. 1. Epithelial-stromal interactions. Carcinogenesis is a contextual process in which there may be defective communication between the epithelium and its underlying stroma. Some chemopreventive agents may exert their primary action on stromal, rather than epithelial, target cells (paracrine action). Stromal cells may also be targets for gene damage that contributes to carcinogenesis. Reciprocal interactions between epithelium and stroma regulate cell differentiation in the prostate and other organs. During colon carcinogenesis, increased COX-2 concentrations occur first in stromal, rather than epithelial, cells; the use of inhibitors of COX-2 to prevent colon cancer would initially be directed at such stromal cells. The double-headed arrows indicate that paracrine communication, mediated by cytokines and other regulatory molecules, is bidirectional.

W. K. Hong is in the Department of Thoracic/Head and Neck Medical Oncology, University of Texas M. D. Anderson Cancer Center, 1515 Holcombe Boulevard, Box 80, Houston, TX 77030, USA. M. B. Sporn is in the Department of Pharmacology and Norris Cotton Cancer Center, Dartmouth Medical School, 7650 Remsen, Hanover, NH 03755, USA.

*To whom correspondence should be addressed. E-mail: whong@notes.mdacc.tmc.edu

chemoprevention in large numbers of people. The recent synthesis of safer, selective COX-2 enzyme inhibitors, such as MF-tricyclic (Fig. 2), and the successful use of MF-tricyclic to inhibit polyp formation in mice with an *Apc* defect (*6*) are major new developments with potentially far-reaching implications for chemoprevention of human colon cancer. Clinical trials with the selective COX-2 inhibitors will be of critical importance in validating the practicality of the entire chemopreventive approach to the control of human cancer.

In these colon cancer studies, the identification of a molecular target (COX-2) was of major importance in directing the development of a whole new class of pharmacologic agents. This approach is applicable to other forms of cancer as well. In the case of breast cancer, the estrogen receptor is an important target. Estrogen has long been known to be a promoting factor for mammary carcinogenesis, and antagonism of the action of its receptor in the breast is an important experimental and clinical approach to breast cancer prevention. The practical problem is how to inhibit estrogen in the breast without losing its beneficial agonistic effects on bone, the brain, and the cardiovascular system.

The development of selective estrogen receptor modulators (SERMs) may help to achieve this goal. The aim is to design new agents that will function as estrogen agonists in the tissues in which estrogen is beneficial but that will function as antagonists in sites where estrogen may promote carcinogenesis, such as the breast, uterus, or ovary (9). Although the estrogen analog tamoxifen comes close to fulfilling these criteria and has been shown to suppress mammary carcinogenesis in both animals and women, it has the undesirable effect of being estrogenic in the uterus, which may increase the risk of endometrial carcinoma (9). A new generation of SERMs, exemplified by the benzothiophene estrogen analog raloxifene (*10*) and its more potent experimental congener LY353381 (*11*) (Fig. 2), now offers the potential for chemoprevention of breast cancer without the uterine risk of tamoxifen: These agents are highly active in preventing breast cancer in experimental animals (*11–13*) but do not promote the growth of uterine epithelium (*10, 11*). As an estrogenic agent that maintains bone mass in postmenopausal women, raloxifene has been used clinically to prevent osteoporosis (*10*); however, at present, there are not enough data to evaluate its clinical efficacy in preventing breast cancer.

The question of how an estrogen analog can have such varied actions in different target organs is of fundamental importance, and there are several possible answers. Recently, it was shown that an estrogen analog such as raloxifene can regulate gene transcription at DNA nucleotide sequences other than the well-known estrogen response element on genes regulated by estrogen; a new "raloxifene response element," with a distinct nucleotide sequence, has been identified and is believed to mediate the tissue-specific effects of raloxifene (*14*). Furthermore, a second estrogen receptor (ERβ) has been identified (*15*), and the expression patterns of this new receptor and the classical receptor (ERα) are distinct (*16*). For example, ERβ is expressed in much higher amounts than ERα in rat prostate (*16*); recent studies in rats have shown that tamoxifen, which binds to both estrogen receptors, can prevent prostate cancer (*17*). The estrogenic or antiestrogenic response may thus be mediated by two distinct receptors that can act at more than one site on responsive genes.

In the case of prostate cancer, improvements in cancer-screening methods have led to increased detection of early lesions that do not require surgery, so there is an urgent need for chemoprevention. Because prostate carcinogenesis is driven by androgen, the androgen receptor is an ideal molecular target for chemoprevention (*18*). Several new pharmacological approaches to androgen deprivation are being pursued clinically or considered for development. These new approaches include the use of 5-α-reductase inhibitors (this enzyme is responsible for the conversion of testosterone to dihydrotestosterone, which has a higher affinity for the androgen receptor), such as finasteride, and nonsteroidal antiandrogens that block androgen action competitively at the receptor level, such as flutamide (*18*). Unfortunately, prostate cancer is not easily studied in animal models, so it has been difficult to test the efficacy of these agents in inhibiting tumorigenesis. In addition to androgen receptor modulation, the use of retinoids (*17, 19*) and estrogen analogs (*17*) is an alternative approach to chemoprevention of prostate cancer that has some experimental basis.

The nuclear receptors for retinoids are additional key molecular targets for new chemopreventive agents. There are six well-defined retinoid receptors, all of which are transcription factors that regulate specific genes with specific response elements (*20*), and it is now possible to synthesize new retinoids that are specific ligands for these receptors. Retinoids are required for proper differentiation of lung and upper airway epithelium, and loss of expression of retinoic acid receptor–β (RAR-β) is characteristic of many lung cancers (*21*) and many premalignant lesions of the oral epithelium (*22*). In the latter case, administration of 13-*cis*-retinoic acid can restore the expression of RAR-β, as well as reverse the development of the lesions (*22*). The retinoid X receptors, known as RXRs, are presently the target of intensive efforts to develop selective ligands. The three RXRs form heterodimers with many other nuclear receptors, including the RARs, the thyroid receptor, the vitamin D receptor, and a large group of other nuclear receptors known as orphans, for which the ligand is not yet known (*20*). Selective ligands such as LG100268 (Fig. 2) and its congener Targretin bind tightly to the three RXRs (*23*) but have essentially no affinity for the three RARs.

After it was shown that 9-*cis*-retinoic acid (an agonist for all six retinoid receptors) prevented experimental breast cancer (*13*), these RXR-selective ligands were also tested in rats and found to be highly efficacious, notably without the classic pattern of retinoid toxicity (skin dryness, cheilitis, hypertriglyceridemia, and conjunctivitis) seen with retinoids that bind to RARs (*24*). Because this toxicity has been a major concern in the clinical use of retinoids for cancer prevention, the discovery of these RXR-selective ligands represents an important advance. The mechanisms through which retinoids suppress carcinogenesis are complex. A very large number of genes

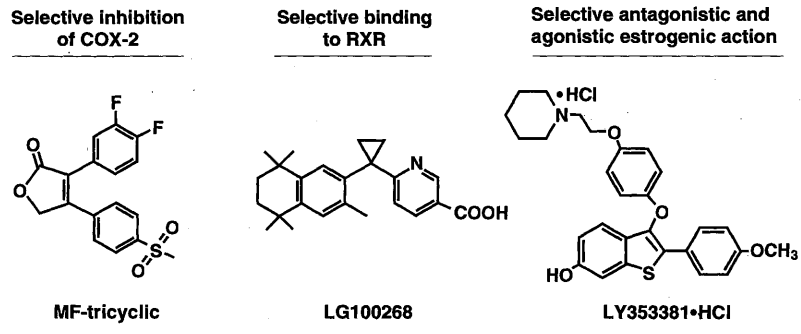

Fig. 2. Structures of new chemopreventive agents that are selective for specific molecular targets. In experimental animals, MF-tricyclic has been used to prevent intestinal tumors, and LY353381 and LG100268 have been used to prevent breast cancers.

involved in differentiation and proliferation have retinoid response elements (*20, 25*), and retinoids play a key role in the regulation of cytokines and the extracellular matrix (*25*). All of these processes are dysregulated during carcinogenesis, and it is unlikely that a single locus of action will be found for these highly pleiotropic molecules.

Clinical Aspects of Chemoprevention

Uniqueness of chemoprevention trials. The ultimate clinical goal of chemoprevention studies is reduction in cancer incidence, and the "gold standard" of chemoprevention trials is the large, long-term, randomized study in which cancer incidence is the end point (*26*). However, such studies are extremely costly, in that they may require thousands of patients who are studied for many years before useful data are obtained. In addition to their expense, the size and length of these studies inevitably lead to problems with recruitment, motivation, and compliance. Thus, because of these intrinsic difficulties, chemoprevention trials, especially those for the primary prevention of initial cancers, have a unique set of requirements if they are to succeed. In the planning of clinical trials for primary prevention of cancer, it is important to select agents for which there is a strong mechanistic or experimental basis for inhibition of carcinogenesis. Indeed, the failures that have characterized some of the large-scale, expensive prevention trials of the past can be attributed to a lack of a strong mechanistic and experimental rationale for the selection of the chemopreventive agents that were used. Epidemiological data alone provide a poor basis for the design of a major trial for the primary prevention of cancer in the general population (*26, 27*).

Importance of secondary prevention trials. When possible, trials of agents for primary prevention should be preceded by extensive clinical evaluation of their efficacy and practical utility for secondary prevention of cancer (the reversal or arrest of progression of premalignant lesions or the prevention of second primary tumors in patients cured of an initial cancer) in specific epithelial target sites. Without such data to support a more ambitious primary prevention trial, there is a high likelihood that large sums of money will be expended with little return. In contrast, secondary prevention trials have yielded the most valuable data in this field and have been more cost-effective, because the very nature of secondary prevention trials ensures that the burden of carcinogenesis on the patients is high and that some meaningful end point can be measured in a reasonable time frame in a smaller number of patients. Such data can then be used to plan more difficult and expensive studies in primary prevention.

Thus, within recent years, agents such as 13-*cis*-retinoic acid, difluoromethylornithine, all-*trans*-retinoic acid, fenretinide, and etretinate have been shown to be effective in arresting or reversing premalignant lesions such as bronchial metaplasia, oral leukoplakia, uterine cervical dysplasia, and actinic keratoses, and an inhibitor of COX-1 and COX-2, sulindac, caused a marked diminution in colon adenomas (*8, 26, 28–30*). The large number of former smokers with persistent bronchial metaplasia (characterized by molecular and genetic abnormalities that can be objectively scored) is a particularly important set of patients for evaluating chemopreventive intervention with retinoids because (i) these individuals still have a high risk for developing lung cancer in spite of their smoking cessation; (ii) bronchial metaplasia in former smokers is a classic example of "field carcinogenesis," a phenomenon characterized by the development of multiple premalignant foci of independent origin in areas repeatedly exposed to carcinogens (as in exposure of the airway to cigarette smoke), which requires a field-wide approach to prevention; (iii) the loss of expression of the retinoid receptor RAR-β has been shown to contribute to lung carcinogenesis; and (iv) it is possible that retinoids can reverse lung carcinogenesis by restoring retinoid signaling pathways, especially in the absence of continued smoking exposure (*21, 31*).

Prevention of second primary tumors in patients who have undergone surgery for removal of a first primary tumor provides an even more meaningful end point, as these subjects may be at an exceptionally high risk for a new cancer. Effective secondary prevention has been achieved with tamoxifen for breast cancer (39% reduction in contralateral second primary tumors in 30,000 women from 40 randomized adjuvant trials); 13-*cis*-retinoic acid—but not etretinate—for head and neck cancer (in 103 patients randomized to 13-*cis*-retinoic acid or a placebo, 14% of 13-*cis*-retinoic acid–treated patients developed second primary tumors compared with 31% of patients receiving the placebo, with a median follow-up of 54 months); retinyl palmitate for lung cancer (in 307 patients randomized to retinyl palmitate or a placebo, 8.6% of retinyl palmitate–treated patients developed second primary tumors compared with 16% of patients receiving the placebo, with a median follow-up of 46 months); and a new acyclic retinoid for liver cancer (in 89 patients randomized to acyclic retinoid or a placebo, 27% of acyclic retinoid–treated patients developed recurrent or second primary tumors compared with 49% of patients receiving the placebo, with a median follow-up of 38 months). In all four conditions, the development of a second primary cancer is associated with an ominous prognosis, and the development of effective chemoprevention has been of major importance (*32–35*). Studies of this nature provide a strong rationale for further evaluation of agents for primary prevention. Two major secondary prevention trials of the use of 13-*cis*-retinoic acid for aerodigestive cancers (head and neck, and lung) are currently in progress in the United States.

Primary prevention. In contrast to these important achievements in secondary prevention, thus far, there are few compelling data that would justify the widespread use of pharmacologic agents for primary chemoprevention. On the basis of epidemiologic data, several major trials of β-carotene for primary chemoprevention, particularly of lung cancer, were initiated in the mid-1980s, but no benefit was found in recent analyses of the data (*36*). Notably, these trials were relatively devoid of any mechanistic or experimental basis; that is, there is little evidence that β-carotene can prevent lung cancer in animals, nor is there a strong experimental basis in cell or molecular biology studies that would argue for its use as an agent to arrest or reverse the progression of preneoplastic lesions.

Two major primary chemoprevention trials are currently in progress in the United States (*26*). On the basis of its ability to prevent second primary breast cancers, as well as extensive molecular, cellular, and animal data indicating that it acts as an effective estrogen antagonist in the breast, tamoxifen is now being evaluated for primary prevention of breast cancer in women. The tamoxifen study, for which enrollment was opened in 1992 and completed earlier this year, has a total of 13,200 participants. All women aged 60 and over were eligible, as well as women between the ages of 35 and 59 who were found to have at least the risk level of a 60-year-old woman. This randomized, placebo-controlled, prospective study, with cancer as an end point, is a landmark primary prevention trial, because there is already an immense amount of data on tamoxifen in terms of its other benefits (lowering of serum cholesterol and maintenance of bone mass) and its undesirable properties (formation of blood clots and uterine carcinogenicity). The results of this trial are expected to be analyzed in late 1999 and will have an immense impact on the future of chemoprevention. The second major trial involves the use of finasteride in 18,867 men aged 55 and over who are at risk for development of prostate cancer. This study was

activated in 1993, and accrual of participants was completed in 1996. As with the tamoxifen trial, the finasteride trial is a classic phase III, randomized, placebo-controlled, prospective study with cancer as an end point. The results of this trial are expected to be available within 5 to 6 years.

Biomarkers and intermediate end points. Biomarkers and intermediate end points of carcinogenesis are predictors of potential cancer occurrence. Biomarkers can be considered "signposts" that carcinogenic tissue damage has occurred. For example, in many epithelial tissues, an early biomarker indicating the probability of cancer development is increased cell proliferation with accompanying DNA aneuploidy. Other biomarkers include genetic and epigenetic alterations such as loss of heterozygosity, p53 mutations, increased expression of the epidermal growth factor receptor, and genomic instability (*26, 27, 31*).

Biomarkers and intermediate end points are important to chemoprevention for two reasons. First, as predictors of increased risk, they help to identify individuals who are likely to develop cancer and for whom higher risk interventions may be justified. Second, they can be used to measure the efficacy of chemopreventive treatment in a relatively short period of time. If biomarkers and intermediate end points are validated as predictors for certain cancers, they can provide a scientific tool for the design of more efficient and cost-effective chemoprevention trials. Intervention trials in which biomarkers and intermediate end points are used, rather than those requiring the development of cancer as an end point, will be completed in a shorter time and will require fewer patients.

Combination chemoprevention. Cancer chemoprevention in high-risk cohorts is still at an early stage of development, but it is already recognized that prevention by a single agent will be limited by both toxicity and potency. The concomitant use of multiple agents with different mechanisms of action is an exciting new field of investigation. The combination of a promoter of differentiation, an antiproliferative agent, and an inducer of apoptosis would be particularly appropriate for the treatment of advanced premalignant lesions. Several such combination trials are in progress, including the use of 13-*cis*-retinoic acid and α-tocopherol for the reversal of bronchial metaplasia. The combined use of these two agents plus interferon-α has recently shown promising results in patients with advanced laryngeal dysplasia (*37*). The combination of a retinoid (such as fenretinide) and an estrogen antagonist (such as tamoxifen) has been highly effective in preventing experimental breast cancer and is now being evaluated clinically for this purpose.

Conclusion

The continuing magnitude and severity of the cancer problem make it imperative to develop a preventive approach to this disease. As advances in the molecular and cellular biology of carcinogenesis continue, specific targets for preventive intervention are being identified, and effective new chemopreventive agents are being synthesized and tested. Clinical trials, particularly for the prevention of second primary cancers, have already validated the concept of chemoprevention. In the future, clinical chemoprevention will require further development of trials based on a mechanistic understanding of carcinogenesis. As inflammatory mediators, such as prostaglandins and nitric oxide, are increasingly shown to have important roles in the pathogenesis of many other chronic degenerative diseases as well as cancer, the development of effective chemoprevention for cancer should also have important benefits in the prevention and treatment of neurodegenerative and cardiovascular diseases as well.

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