associated with human cancer. In general, the potential significance of epigenetic dysfunction in human malignancies is illustrated by the fact that the LOI and loss of X-chromosome inactivation occur at much higher frequency compared to genetic mutations. With the exception of the IGF2/H19, an extensively imprinted locus whose deregulation is implicated in human malignancies, much remains unknown. The IGF2/H19 locus has been studied in childhood tumors such as Wilms tumor and overgrowth syndromes such as the Beckwith–Wiedemann syndrome. The strong support for a gatekeeper role for LOI of IGF2 also in Wilms tumor has come from the studies showing that Beckwith–Wiedemann syndrome, a prenatal overgrowth disorder, predisposes to various embryonal tumors including Wilms tumor. Consistent with an important role of LOI of IGF2 in cancers of adults, recent studies have demonstrated that epimutation of IGF2/H19 locus is a common epigenetic event in adults and is associated with fivefold increased incidence of colorectal cancer. These studies argue that LOI may be a frequent mechanism by which epigenetic alterations predisposes to the development of cancer. In Chapter 6, Eamonn Maher and Derek Lim discuss recent advances on imprinting and LOI and mechanistic insights into developmental syndromes and human malignancies.

B. Epigenetic codes in stem cells and cancer stem cells

Stem cells constitute a minority of cell population in adult tissues, yet they play the key role in the development and tissue homeostasis. The main properties of stem cells are the self-renewal, essential for maintenance of the stem cell pool, and the ability to differentiate in different lineages required for the integrity and function of tissues. Given their special properties, stem cells are tightly regulated by multiple genes and gene networks. This control prevents the shift in the balance between self-renewal and differentiation. Deregulation of epigenetic information (encoded in DNA methylation, histone modification patterns, and noncoding RNAs) in cells with pluripotent potential may alter defining properties of stem cells, self-renewal, and differentiation potential, leading to cancer initiation and progression (Feinberg *et al.*, 2006; Shukla *et al.*, 2008). Histone modifications appear to play the key role in establishing and maintaining distinct gene expression patterns and consequently pluripotent state and differentiation fates of stem cells. Given that all cell types and many cancers are derived from pluripotent cells, a better understanding of epigenetic mechanisms controlling pluripotency and reprograming will greatly impact on many areas of modern biology and will help to tailor efficient therapeutic strategies (Feinberg *et al.*, 2006). In Chapter 7, Yasuhiro Yamada and Akira Watanabe review current knowledge on stem cells and cancer stem cells, discuss epigenetic mechanisms that regulate "stemness" and pluripotency, and speculate how these new findings impact cancer therapeutics.

C. Constitutional epimutations and cancer susceptibility

In addition to the germ line mutations that represent a high risk of developing specific cancer, accumulating evidence argues that epigenetic changes can also occur constitutionally to confer a risk of developing particular types of human cancer. These epigenetic aberrations are known as "constitutional epimutations" that alter gene activity to confer a phenotype without change within the DNA code of the affected gene (Cubas *et al.*, 1999). Constitutional epimutations are characterized by promoter methylation and transcriptional silencing of a single allele of the gene in normal somatic tissues. Example of constitutional epimutations is particularly well characterized in Lynch syndrome, an autosomal dominant cancer susceptibility syndrome characterized by the early development of several human malignancies, including cancer of colorectum and uterus (Lynch, 1999). Recent studies have indicated that epimutations are associated with distinct patterns of inheritance depending on the underlying mechanisms (Hitchins and Ward, 2007; Hitchins *et al.*, 2007). In Chapter 8, Megan Hitchins describes "the journey of discovery of epimutations" and reviews different types of epimutations, with a focus of human disease phenotypes, notably cancer. The author also discusses potential mechanisms underlying epimutations and describes how these correlate with the observed patterns of inheritance.

IV. CANCER EPIGENOME—ZOOMING IN ON GENOME-WIDE SCALE

Tumor development is a complex multistep process and human cancers are characterized by profound abnormalities in the genome and the epigenome. Such abnormalities include genetic changes (mutations, chromosomal rearrangements) and epigenetic changes, including aberrant DNA methylation and histone modifications, both of which are believed to be triggered by exposure to environmental, dietary, and lifestyle factors. Therefore, in addition to genetic analysis, a comprehensive epigenetic profiling of cancer genomes is essential in identifying causative changes involved in cancer development and progression, regardless whether these changes are inherited or acquired during the life.

A. Cancer epigenome

In comparison with the genome, the epigenome is believed to be more complex and highly dynamic, which may underlay and/or reflect different functional states in time and space. This dynamics is governed by reversible modifications of genomic DNA (CpG methylation) and core histone proteins (histone modifications). Emerging technologies for detection of epigenetic changes and recent progress in the field of epigenomics promise to rapidly advance the capacity to

address important issues in cancer research. Such tools are already in use to characterize tumor samples in high-throughput settings. While the analysis of cancer genomes is well underway as part of multiple national and international efforts, the analysis of cancer epigenomes is still at an early stage (American Association for Cancer Research Human Epigenome Task Force, 2008; Jones and Martienssen, 2005). However, recent technological advances that now allow cancer epigenetics to be studied genome-wide have already begun to provide both biological insight in the process of cancer development and critical information for new avenues in translational research (Lister *et al.*, 2009). In Chapter 9, Stephan Beck and colleagues review recent progress in high-resolution and genome-wide analyses on DNA methylation and its implications for cancer research.

B. Identification of driver DNA methylation changes

Human cancer genomes exhibit widespread epigenetic alterations, among which changes in DNA methylation patterns are particularly well documented. This includes global loss of methyl-cytosine (caused by DNA hypomethylation of repetitive sequences) and unscheduled hypermethylation of CpG islands in the promoter of a wide range of genes. In recent years, the analysis of DNA methylation patterns in cancer has progressed from single gene studies, which focused on potentially important candidate genes, to a more global analysis in which large number or nearly all gene promoters are analyzed (Hernandez-Vargas *et al.*, 2010; Martinez *et al.*, 2009; Rauch *et al.*, 2008). In Chapter 10, Gerd Pfeifer and Satish Kalari give an overview of these genome-scale methylation-profiling techniques and summarize the key information obtained with these approaches. The current knowledge on the specificity of methylation aberrations in cancer at a genome-wide level is covered. The authors also discuss how to identify those DNA methylation changes that are important for the processes of cancer initiation, progression, or metastasis (driver methylation changes) as well as challenges associated with distinguishing these from methylation changes that are merely passenger events during cancer development and progression.

C. Epigenetic drivers and genetic passengers in cancer

Genetic changes and aneuploidy are associated with alterations in DNA sequence, and they are a hallmark of the malignant process. Similarly, epigenetic alterations are universally present in human cancer and result in heritable changes in gene expression over many cell generations, leading to functional consequences equivalent to those induced by genetic alterations. Intriguingly, accumulating evidence suggests that epigenetic changes may precede and

provoke genetic changes (Sawan *et al.*, 2008). Epigenetic alterations may occur early in tumor development and may trigger a spectrum of genetic alterations such as mutations and chromosomal aberrations. There are different pathways by which disruption of epigenetic states (DNA methylation, histone modifications, and noncoding RNAs) either individually or in combination may trigger genetic changes. In this scenario, epigenetic events are primary events while genetic changes (such as mutations) may simply be a consequence of disrupted epigenetic states. Aberrant epigenetic events affect multiple genes and cellular pathways in a nonrandom fashion and this can predispose to induction and accumulation of genetic changes in the course of tumor initiation and progression. Chapter 11 (by Minoru Toyota and Hiromu Suzuki) addresses how these considerations may be important for better understanding of the process of tumorigenesis and molecular events underlying the acquisition of drug resistance, as well as the development of novel strategies for cancer therapy.

V. EPIGENETICS AND ENVIRONMENTAL FACTORS—WHERE YOUR GENES MEET THE ENVIRONMENT

A. Epigenetic alterations induced by dietary and other environmental factors

An important role of dietary, lifestyle, and environmental factors in the development of a wide variety of cancers is well supported by both epidemiological and laboratory-based studies (Herceg, 2007). Environmental and dietary factors known to play important roles in the etiology of human cancer include chemical carcinogens, dietary toxins (such as aflatoxin B1), and physical carcinogens (UV and ionizing radiation), whereas tobacco smoking, alcohol abuse, and excess exposure to sunlight are lifestyle factors known to contribute to human cancer. Epigenetic mechanisms are believed to play a critical role in response to both endogenous stimuli and exogenous (environmental) factors. These mechanisms are thus physiological tools used by cells to establish and maintain gene expression patterns that are appropriate for specific environmental cues. It is believed that epigenetic mechanisms in animals also play important roles in the adaptation and response to environmental exposures. In many instances, however, clear-cut causal relationship between epigenetic states and environmental factors proved to be difficult to establish. This stems from the fact that environmental factors are likely to induce subtle changes, which are often cumulative, thus quantitative manifestation of phenotypic traits may occur after repetitive exposure over a long period of time. For these reasons, epidemiology proved to be incapable of identifying complex environmental factors and dietary regimes that induce and/or promote tumor development by triggering epigenetic changes.

This was in part due to the lack of epidemiological and laboratory-based studies that addressed the role of epigenetic changes induced by environment and nutrition with sufficient statistical power. In Chapter 1, Vol. 71, John Mathers, Gordon Strathdee, and Caroline Relton review recent studies implicating epigenetic changes induced by environment, diet, and lifestyle in human cancer. The authors also argue that epigenetic marks represent attractive candidates for the development of surrogate endpoints that could be used in dietary or lifestyle intervention studies for cancer prevention.

B. Induction of epigenetic changes by chronic inflammation

Infectious agents including viruses, such as human papillomavirus (HPV), Epstein–Barr virus (EBV), and human hepatitis virus (HBV), and bacteria such as *Helicobacter pylori* may also alter expression of host genes via an epigenetic strategy. Epigenetic mechanisms including DNA methylation and chromatin modifications are known to regulate viral gene expression. It has been shown that methylation of integrated HPV-associated primary cancers and cervical cancer cell lines inhibits the transcription of most viral genes. Interestingly, CpG methylation appears to correlate with HPV pathogenesis, suggesting that methylation of HPV DNA is implicated in the development and progression of cervical cancer. EBV genomes are also subject to host cell-dependent epigenetic modifications including DNA methylation, binding of regulatory proteins and histone modifications, and different EBV latency types are associated with distinct viral epigenotypes. Associations between EBV and HBV infection and promoter hypermethylation of several genes were commonly found in several cancers including hepatocellular, gastric, and nasopharyngeal carcinoma. These studies provide strong evidence that infectious agents may employ epigenetic strategies to deregulate cellular processes and promote tumorigenesis; however, underlying mechanisms are poorly understood. In Chapter 2, Vol. 71, Toshikazu Ushijima reviews current knowledge on inducers of epigenetic changes with a focus on chronic inflammation induced by bacterial and viral infections. The authors also discuss molecular mechanisms underlying aberrant epigenetic states leading to the development of cancer.

C. Maternal diet, early life exposure, and epigenetic processes

Several studies have reported that diet may influence epigenetic patterns and this could explain many diet-associated disorders (Gluckman *et al.*, 2008). During early development, both paternal and maternal genomes undergo a striking epigenetic reprograming, most notably through DNA demethylation immediately after fertilization. After implantation, methylation patterns are reestablished via *de novo* methylation (Reik, 2007). This epigenetic reprograming during early development must be a well-tuned process since it is an attempt to establish a

configuration of the genome that can respond to changing needs of the early life development. How maternal diet may affect the phenotype of the offspring by epigenetic mechanisms and how this early life exposure may modulate susceptibility to cancer and other diseases in childhood and later life are discussed by Kent Thornburg and collaborators (Chapter 3, Vol. 71).

D. Folate, one carbon metabolism, and DNA methylation in cancer

Folate is a methyl donor that plays an essential role in DNA synthesis and biological methylation reactions, including DNA methylation. Folate deficiency may be implicated in the development of genomic DNA hypomethylation, which is an early epigenetic event found in many cancers. Numerous studies employing *in vitro* systems, animal models, and human interventional studies have tested this hypothesis. While numerous technical challenges remain in this important field of research, changes in folate intake appear to be capable of modulating DNA methylation levels in the human colonic mucosa and this may potentially alter colorectal cancer (CRC) risk. In Chapter 4, Vol. 71 (by Robyn Ward and Jia Liu), the impact of folate intake and one carbon metabolism on cancer susceptibility is discussed. The authors also discuss existing evidence on folate and its relationship to DNA methylation using CRC as an example. The evidence from animal, human, and *in vitro* studies on the effects of folate deficiency and supplementation on epigenetic states including DNA methylation and histone modifications is provided.

VI. EPIGENETIC CHANGES IN CANCER—MARKERS IN TRACKING CANCER CELLS

Epigenetic changes (e.g., promoter-specific hypermethylation) occur early and at high frequency in different human malignancies; this feature combined with high sensitivity and specificity of detection may be exploited in the area of molecular diagnostics and cancer risk assessment. As a result, the research on epigenetic changes as potential biomarkers is in full swing. Chapters 5–7, Vol. 71 review advantages of epigenetic biomarkers for cancer pathophysiology, such as response to therapeutics, prognosis, and occurrence of metastasis, over those involving genomic alterations and gene expression changes, and describe current examples.

A. Epigenetic biomarkers for cancer pathophysiology

In recent years, the development of high-throughput and genome-wide analytic methods has opened the possibility of identifying simultaneously multiple changes in gene expression as well as epigenetic alterations affecting the

epigenome of cancer cells. The main question raised by such studies is to determine which alterations, or combinations thereof, can be interpreted as reliable biomarkers for providing information about the carcinogenesis process. This assessment should be done from the viewpoint of the suspected, primary role of such alterations in the initial steps of tumorigenesis. For example, the molecular events that occur in early stage of cancers or in precursor lesions are more likely to have a direct influence on cancer occurrence and progression than those that accumulate at later stage of cancer development. Among the latter, many alterations may be considered as "passengers" that represent a mere consequence of the highly disturbed genomic and epigenomic instability that accompanies the progression of many cancers. Chapter 5, Vol. 71 (by Dajun Deng and colleagues) discusses the challenge of incorporating current knowledge in epigenetics and epigenomics to identify new biomarkers that may be useful in the early detection and treatment of cancer.

B. Detection of epigenetic changes in body fluids

Tumor-derived cell-free circulating DNA isolated from the plasma and serum of individuals with cancer has been shown to contain cancer-associated alterations. While the origin and possible function of this free circulating DNA is not fully understood, it represents an attractive target for biomarker discovery. In addition to genetic changes (mutations, microsatellite alterations), plasma DNA from individuals with tumors was shown to harbor epigenetic changes, namely alterations in DNA methylation at CpG sites in the promoter regions of a wide range of tumor suppressor genes and other cancer-associated genes. Epigenetic changes are tumor-specific and thus have the potential to serve as highly specific biomarkers. In addition, DNA methylation changes appear early in tumor development and can be found in virtually every type of human cancer, thus they can provide particularly attractive markers with broad application in diagnostics and risk assessment. The development of epigenetic markers for cancer-bearing individuals could similarly enhance the management of their disease. In Chapter 6, Vol. 71, Triantafillos Liloglou, and John Fields summarize recent progress in the development and application of new assays for the detection of DNA methylation changes in body fluids with both time- and cost-effectiveness.

C. Epigenetic biomarkers for cancer risk assessment

Epigenetic events are shown to influence virtually each steps in tumor development; therefore, understanding epigenetic changes associated with cancer onset, progression, and metastasis are fundamental to improving our abilities to successfully treat and prevent cancer. Epigenetic alterations in comparison with genetic changes are typically acquired in a gradual manner. These features offer

an enormous potential for prevention strategies. Based on quantitative estimates over two-thirds of the cancer incidence accounted for by environmental and dietary factors, therefore the majority of cancers are potentially avoidable.

VII. EPIGENETIC DRUGS ON THE RISE—WAKING UP SLEEPING BEAUTY

The interest in the epigenetics of cancer is strongly augmented by the recent realization that epigenetic changes can be exploited as a powerful tool in the clinic and as a novel approach in cancer treatment. A distinguishing feature of epigenetic changes in comparison with genetic changes is that they are reversible; therefore, aberrant DNA methylation, histone acetylation, and methylation are attractive targets for therapeutic intervention. Many efforts and resources have been mobilized in the development of different therapeutic approaches that are known as "epigenetic therapies." The ubiquity of epigenetic changes in many malignancies and other significant human diseases has triggered an impressive quest for the development of "epigenetic drugs" and epigenetic therapies. A number of agents have been subjected to an intensive investigation, many of which have been found capable of altering epigenetic states including DNA methylation patterns and histone modification states. Chapters 12 and 13, Vol. 70, give an overview of recent development and opportunities in the field of epigenetic therapy of cancer.

A. DNA demethylating and coupling therapies

Different approaches are directed to modify DNA methylation states in cancer cells and are based on specific properties of various chemical agents affecting the activity of the enzymes involved in the establishment and maintenance of DNA methylation. Among these agents, demethylating agents (inhibitors of DNA methyltransferases) are the most extensively studied epigenetic agents. These include 5-azacytidine (5-aza-CR) and 5-aza-2-deoxycytidine (5-aza-CdR), both of which efficiently inhibit DNMTs and lower DNA methylation levels in a variety of cancer cell lines, leading to reactivation of gene expression (Egger *et al.*, 2004). 5-aza-CR and 5-aza-CdR are nucleoside analogs of cytosine and the mechanism by which they inhibit DNA methylation involves incorporation of these molecules at the position of cytosine during DNA replication. This event results in trapping and inactivation of DNA methyltransferase; therefore, event transient treatment of cells with demethylating agents can lead to a long lasting demethylation effect (Egger *et al.*, 2004). In a similar manner, pseudoisocytosine (also known as zebularine) can induce efficient demethylation (Marquez *et al.*, 2005). Numerous studies showed that these agents can efficiently reactivate the

expression of aberrantly silenced genes in a variety of cancer cells. In Chapter 12, Vol. 70, Zdenko Herceg gives a brief overview of epigenetic therapy, and detailed explanation on demethylation therapy, such as mode of action, clinical indication, dose adjustment, and assessment of adverse effects as well as the principles of combinatorial therapies that couple DNA methylation inhibitors with HDAC inhibitors as well as coupling therapies.

B. Histone modification therapy of cancer

Similar to DNA methylation changes, aberrant histone acetylation and methylation are attractive targets for the epigenetic therapy. Indeed, a number of drugs that are capable of altering levels or patterns of histone modifications have been discovered, and many of these drugs are now in clinical trials. Inhibitors of histone deacetylases (HDACs) turned out to be effective against specific human cancers. For example, HDAC inhibitor vorinostat has already been approved by FDA. Other HDAC inhibitors of various chemical structures are currently in clinical trials. Drugs targeting histone methylation are also under development. In Chapter 13, Severio Minucci and colleagues summarize how HDAC inhibitors work, and describe clinical effects of vorinostat and other drugs for which clinical effects are available. The status of development of other drugs targeting different histone modifications is also covered.

VIII. EPIGENETICS AND CANCER PREVENTION—PROTECT YOUR EPIGENOME

A. Epigenetics in molecular epidemiology

Recent progress in epigenomics and emergence of powerful technologies for the detection of epigenetic changes in high-throughput settings holds promise to advance our capacity to evaluate the contribution of epigenetic changes induced by the environmental epimutagens to human cancer. These powerful tools are beginning to be applied to large population-based and case-control studies which offer some of the most exciting opportunities to study the contribution of epigenetic events to specific human cancers. Chapter 7, Vol. 71 (by Yasuhito Yuasa) discusses the application of epigenetics to molecular epidemiology, such as assessment of exposure to environmental epimutagens by epigenetic markers. It also covers different considerations relevant to molecular epidemiology studies, regarding the adoption of stringent criteria for the design, conduct, and evaluation of studies in which epigenetic markers are applied.

B. Epigenetic cancer prevention

A plethora of studies points to a fundamental role of epigenetic changes in cancer development and progression. Accumulation of aberrant epigenetic changes is observed even in histologically normal predisposed tissues, indicating that induction of epigenetic alterations occurs in very early stages of human carcinogenesis. In animal models, genomic hypomethylation suppresses intestinal tumorigenesis and treatment with 5-aza-dC delays androgen-independent disease in prostate cancer model. These observations led to the realization that epigenetic changes have tremendous potential in the prevention of cancer. Reversibility and gradual acquisition of epigenetic alterations are key features that offer an enormous potential for prevention strategies. Based on quantitative estimates over two-thirds of the cancer incidence accounted for by environmental and dietary factors, therefore the majority of cancers are potentially avoidable. In Chapter 8, Vol. 71, Jia Chen reviews current knowledge on the application of epigenetics in the development of novel strategies for cancer prevention.

IX. EPIGENETICS DATABASES AND COMPUTATIONAL METHODOLOGIES—RESOURCE AND TOOLBOX FOR COMMUNITY

The field of epigenetics has seen surge of interest with the recent technical advances that allow robust, quantitative, and genome-wide studies of epigenetic alterations. This resulted in an explosion of different types of epigenetic data. The list of genes altered by epigenetic mechanisms is rapidly expanding (several hundreds of genes have been reported to be modified by epigenetic mechanisms to date) and with the Human Epigenome Project in preparation, a more comprehensive epigenetic landscape of the cancer epigenome will be available. Despite the fact that progress in identification of different types of epigenetic changes and the genes altered through epigenetic deregulation has been remarkably rapid, much remains unknown. Even for the insiders, the comparison of experimental data and the extraction of trends represent a difficult task due to the fact that experimental strategies, techniques used, and data processing differ considerably between the studies. The data on epigenetic changes are heterogeneous and often range from the global measurement of specific modification to the exact and quantitative detection of epigenetic change or pattern. Furthermore, publication standards for data processing and presentation of epigenetic alterations have not yet been established. In his Chapter 9, Vol. 71, Maté Ongenaert overviews different tools including databases available in the field of cancer epigenetics. How the combined expertise of researchers in different fields may be applied to provide better tools and resources for the scientific community in the field of cancer epigenetics is also discussed.

X. CLOSING REMARKS

Both the scientific and medical communities now recognize that epigenetic changes lie in the heart of several important human diseases, most notably cancer. Epigenetic events have been associated with virtually every step of tumor development and progression, and epigenetic alterations are believed to occur early in tumor development and may precede the malignant process (Belinsky, 2004; Laird, 2003; Nephew and Huang, 2003). Therefore, epigenetic deregulation can be exploited as a powerful tool in the clinic and as novel approach to early diagnosis, prediction of clinical outcome, and risk assessment (Belinsky, 2004; Egger *et al.*, 2004; Fraga *et al.*, 2005; Laird, 2003; Seligson *et al.*, 2005). An important distinction between epigenetic and genetic alterations is intrinsic reversibility of the former, making cancer-associated changes in DNA methylation, histone modifications, and expression of noncoding RNAs particularly attractive targets for the epigenetic therapy (Egger *et al.*, 2004; Feinberg and Tycko, 2004; Jones and Baylin, 2007). Another distinguishing feature of epigenetic changes is that they arise in a gradual manner, leading to a progressive silencing of specific genes. This represents an exciting opportunity that can also be exploited in the development of novel strategies for the modulation of the susceptibility to diseases and prevention.

The race is on to find efficient drugs and therapeutic strategies that can reverse epigenetic changes and unscheduled gene silencing. A number of drugs that are capable of altering levels or patterns of DNA methylation or histone modifications have been discovered, and many of these drugs are now in clinical trials. However, despite the promise of epigenetic therapy, several concerns need to be addressed before it can be fully exploited in clinics. There is a need to develop target-specific DNMT inhibitors and isotype-selective HDAC inhibitors to minimize the toxicity associated with these drugs. Early clinical trials with demethylating agents showed relatively strong cytotoxic effect and were not well tolerated. However, these drugs were used at relatively high doses, and more recent studies with significantly lower doses showed encouraging results with relatively mild cytotoxicity. Therefore, combinatorial therapies that target different epigenetic mechanisms such as DNA methylation inhibitors with HDAC inhibitors may prove particularly efficient. Recent studies including clinical trials should give an answer on important questions regarding dosing schedules, routes of administration, and combination regimens.

The intrinsic reversibility of epigenetic alterations represents an exciting opportunity not only for cancer therapy but also for the development of novel strategies for cancer prevention. It is hoped that it may become feasible to select appropriate combinations of epigenetic drugs to revert or block the functional consequences of these alterations in early pre-neoplastic lesions. By targeting specifically and simultaneously multiple pathways based on epigenetic

signatures, epigenetic intervention may confer a greater therapeutic or preventive efficacy, while having less side effects than conventional cytotoxic drugs. Reversibility of epigenetic events offers a unique opportunity for chemoprevention or diet-based intervention that targets critical epigenetic pathways. However, because diet-induced epigenetic modulation varies during different developmental periods and early life, it is critical to consider the issue of "window of vulnerability" that may directly impact the timing of effective strategies for cancer prevention. For example, dietary effects may be the greatest during embryogenesis and early development (Waterland and Jirtle, 2003). Therefore, further studies are needed to test whether dietary intervention in adult population could result in sufficient and sustaining restoration of epigenetic patterns in target tissues. Most of the studies aiming to investigate the role of diet on epigenetic states have focused on one carbon metabolism intermediates and have been carried out in animal models. Therefore, more comprehensive studies need to be carried out, and should focus on other dietary components and effects of dietary regimes in humans. A particular attention should be given to early life exposure and epigenetic reprograming during "the window of vulnerability" and their effect on the susceptibility to diseases in later life. Intriguingly, epigenetic changes seem to affect not only the health of the exposed individual but also the future generations. Therefore, further studies are required to substantiate the observations that human germ line could "capture" information about the ancestral environment through epigenetic states and pass it to the next generation.

The first human methylome at single base resolution for human embryonic stem cells and fetal fibroblasts was reported in 2009 (Lister *et al.*, 2009), and this study highlighted that comprehensive methylome analysis is highly informative and holds considerable significance for the proposed stem cell origin of human cancer. Despite tremendous progress in recent years, the field of cancer epigenomics is still at an early stage and no comprehensive analysis of a cancer epigenome has been performed. However, several studies aiming to profile entire cancer methylome at single base resolution are well underway. Therefore, near future is likely to bring comprehensive epigenetic landscapes in different cancer types that will be the great leap forward in our understanding of mechanisms of cancer development and progression and pave the way for comprehensive functional studies.

Remarkable advances in epigenomics and emergence of powerful technologies allows the detection of epigenetic changes in high-throughput and genome-wide settings. The advent and rapid development of massively parallel sequencing technologies (next generation and next–next generation sequencing) has dramatically accelerated cancer research and opened up new perspectives. This holds promise to advance our capacity to elucidate mechanisms

underlying tumor development and progression and to evaluate the contribution of epigenetic changes induced by the environmental, dietary, and lifestyle epimutagens to human cancer (Fig. 1.1). In particular, it will also help in elucidating the role of epigenetic changes induced by bacterial and viral agents and chronic inflammation in several common human cancers. These powerful tools are beginning to be applied to large population-based and case-control studies. Large cohort and case-control studies offer some of the most exciting opportunities to study the contribution of epigenetic events induced by the diet and environment to human cancer. Such examples are the European Prospective Investigation into Cancer and Nutrition (EPIC), a large prospective cohort study designated to investigate the relationship between diet, various lifestyles and the incidence of cancer in a number of European countries (Riboli and Kaaks, 1997), and the International Childhood Cancer Cohort Consortium (I4C) (Brown *et al.*, 2007). However, the application of epigenetic markers to epidemiological studies requires careful considerations in the design of such studies. Sample size should be estimated based on power calculations which depend on the background rate of the marker (e.g., among controls) and the expected strength of the association (e.g., difference in the markers between cases—or exposed—and controls). Different considerations relevant to molecular epidemiology studies, regarding the adoption of stringent criteria for the design, conduct, and analysis of studies in which epigenetic markers are applied, should be taken into account.

While deregulation of epigenetic mechanisms is primarily studied in the context of cancer biology which is the focus of this book, epigenetic changes have been also implicated in several developmental syndromes (Egger *et al.*, 2004; Feinberg and Tycko, 2004; Jiang *et al.*, 2004), diabetes, obesity, cardiovascular diseases (Maier and Olek, 2002; McKinsey and Olson, 2004), and neurological disorders (Urdinguio *et al.*, 2009). Therefore, basic epigenetic concepts and mechanisms reviewed in this book will be of interest for those working on other complex human diseases and in related fields of modern biology. In conclusion, the epigenetic research is a field in full swing and the near future is likely to bring long-awaited answers that will help in better understanding how tumors develop and progress, and provide important information for the development of novel and efficient strategies for cancer control, a major public health priority in the 21st century. We believe that this book will spread awareness of opportunities and challenges that epigenetics may offer.

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2 Induction of Epigenetic Alterations by Chronic Inflammation and Its Significance on Carcinogenesis

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ABSTRACT

Chronic inflammation is deeply involved in development of human cancers, such as gastric and liver cancers. Induction of cell proliferation, production of reactive oxygen species, and direct stimulation of epithelial cells by inflammation-inducing factors have been considered as mechanisms involved. Inflammation-related cancers are known for their multiple occurrences, and aberrant DNA methylation is known to be present even in noncancerous tissues. Importantly, for some cancers, the degree of accumulation has been demonstrated to be correlated with risk of developing cancers. This indicates that inflammation induces aberrant epigenetic alterations in a tissue early in the process of carcinogenesis, and accumulation of such alterations forms "an epigenetic field for cancerization." This also suggests that inhibition of induction of epigenetic alterations and removal of the accumulated alterations are novel approaches to cancer prevention. Disturbances in cytokine and chemokine signals and induction of cell proliferations are important mechanisms of how inflammation induces aberrant DNA methylation. Aberrant DNA methylation is induced in specific genes, and gene expression levels, the presence of RNA polymerase II (active or stalled), and trimethylation of H3K4 are involved in the specificity. Expression of DNA methyltransferases (DNMTs) is not necessarily induced by inflammation, and local imbalance between DNMTs and factors that protect genes from DNA methylation seems to be important.

I. INTRODUCTION

Chronic inflammation is deeply involved in development and progression of human cancers, contributing up to 25% of them (Hussain and Harris, 2007). As mechanisms of how chronic inflammation induces irreversible genetic/epigenetic alterations, acceleration of cell proliferation and production of reactive oxygen species (ROS) have been mainly considered. At the same time, as involvement of epigenetic alterations in development and progression of cancers became apparent, induction of epigenetic alterations has joined the mechanisms of how chronic inflammation induces cancers.

In this chapter, we will describe the relationship between inflammation and cancers before the epigenetic era, epigenetic alterations induced by chronic inflammations, and how epigenetic alterations are induced.

II. TRADITIONAL VIEWS ON HOW INFLAMMATION LEADS TO CANCERS

Specific types of inflammation are closely associated with cancer development and progression, and the association had been attributed mainly to induction of cell proliferation and mutations.

A. Types of inflammation associated with cancer development and progression

Epidemiological data demonstrate a close connection between specific types of chronic inflammation and cancer (Hussain and Harris, 2007). Hepatitis due to infection of hepatitis B and C viruses is responsible for the majority of hepatocellular carcinomas (Gomaa *et al.*, 2008). Inflammation mainly involving the intrahepatic biliary tract, induced by the liver fluke, a parasite, elevates risk of cholangiocarcinoma (Shin *et al.*, 2010). Chronic gastritis induced by *Helicobacter pylori* infection is the major risk factor of human gastric cancers with hazard ratios of 2.2–21 (Ekstrom *et al.*, 2001).

Exposure to chemicals can also induce chronic inflammation and cancers. Reflux of gastric acids to the esophagus can lead to reflux esophagitis associated with metaplasia (Barrett's esophagus), and the esophagitis is associated with increased risk of esophageal cancers with hazard ratios of 2.2–10.6 (Solaymani-Dodaran *et al.*, 2004). Inhalation of asbestos fibers causes chronic lung and pleural inflammation, and is a definite inducer of mesotheliomas (Bianchi and Bianchi, 2007). Chronic inflammation due to some immunological defects, such as ulcerative colitis (UC) and Crohn's disease, is associated with increased risk of colon cancers (UC, 5.7-fold; Crohn's disease, 2.5-fold; Ekbom *et al.*, 1990a,b). It is also noteworthy that these inflammation-related cancers are known for their multiple occurrences (Cotran *et al.*, 1989; Choi and Zelig, 1994; Nakajima *et al.*, 2006b), suggesting irreversible genetic/epigenetic alterations are accumulated in normal-appearing tissues exposed to these kinds of inflammation.

On the other hand, there are other types of inflammation that are not associated with cancers, such as asthmatic bronchitis, rheumatoid arthritis, and atopic dermatitis.

B. Traditionally known molecular mechanisms of how chronic inflammation leads to cancers

Acute inflammation is induced upon infection of a tissue with a microorganism, in which neutrophils infiltrate to eliminate the microorganism and damaged cells. If the elimination fails, the acute inflammation will make a transition into a

chronic phase, in which lymphocytes and macrophages will dominate (Cotran *et al.*, 1989). During the acute and chronic phases of inflammation, strong cell proliferation is induced. Not only compensatory cell proliferation in response to severe tissue damage but also inflammatory cytokines (e.g., IL1B and TNF) and prostanoids (e.g., prostaglandin E2) induce cell proliferation and inhibit apoptosis (Castellone *et al.*, 2005; Kim *et al.*, 2005). Accelerated cell proliferation leads to increased spontaneous mutations even if the mutation rate is not affected, and some investigators suggest that abnormally increased cell proliferation may be accompanied by increased mutation rates (Tomatis, 1993). Besides the accelerated cell proliferation, production of ROS by infiltrating inflammatory cells leads to DNA strand breaks and production of 8-hydroxyguanine that eventually leads to G to T transversions (Federico *et al.*, 2007). Peroxydation of proteins and lipids by ROS also leads to accelerated cell proliferation due to cellular damage (Federico *et al.*, 2007).

Direct stimulation of epithelial cells by inflammation-related factors is also considered to be a mechanism of how specific types of inflammation promote carcinogenesis. For instance, CagA protein directly injected by *H. pylori* affects polarity and junctions of epithelial cells via perturbation of PAK1/MAPK kinase signals (Saadat *et al.*, 2007). Physical stimulus by asbestos leads to upregulation of specific transcription factors in epithelial cells (Fig. 2.1) (Heintz *et al.*, 1993).

III. EPIGENETIC ALTERATION INDUCED BY INFLAMMATION AND ITS SIGNIFICANCE ON CARCINOGENESIS

In addition to mutations, epigenetic alterations are now recognized to be induced by chronic inflammation, and the induction of epigenetic alterations is an important mechanism of how inflammation leads to carcinogenesis.

A. Aberrant DNA methylation induced by inflammation

1. Association between inflammation and aberrant DNA methylation

In normal cells, most CpG islands (CGIs) are kept unmethylated (Yamashita *et al.*, 2009). In contrast, in noncancerous tissues exposed to inflammation, aberrant DNA methylation can be detected (Table 2.1). Such an association was first identified in colonic mucosae of patients with UC (Hsieh *et al.*, 1998). Methylation of promoter CGI of *CDKN2A* (*p16*) was detected in 12.7% of UC samples without dysplasia, and the incidence increased to 70% in UC samples with dysplasia and to 100% in those with carcinomas. Methylation involved other CGIs, such as a promoter CGI (*MYOD1*) and gene body CGIs [*CDKN2A*

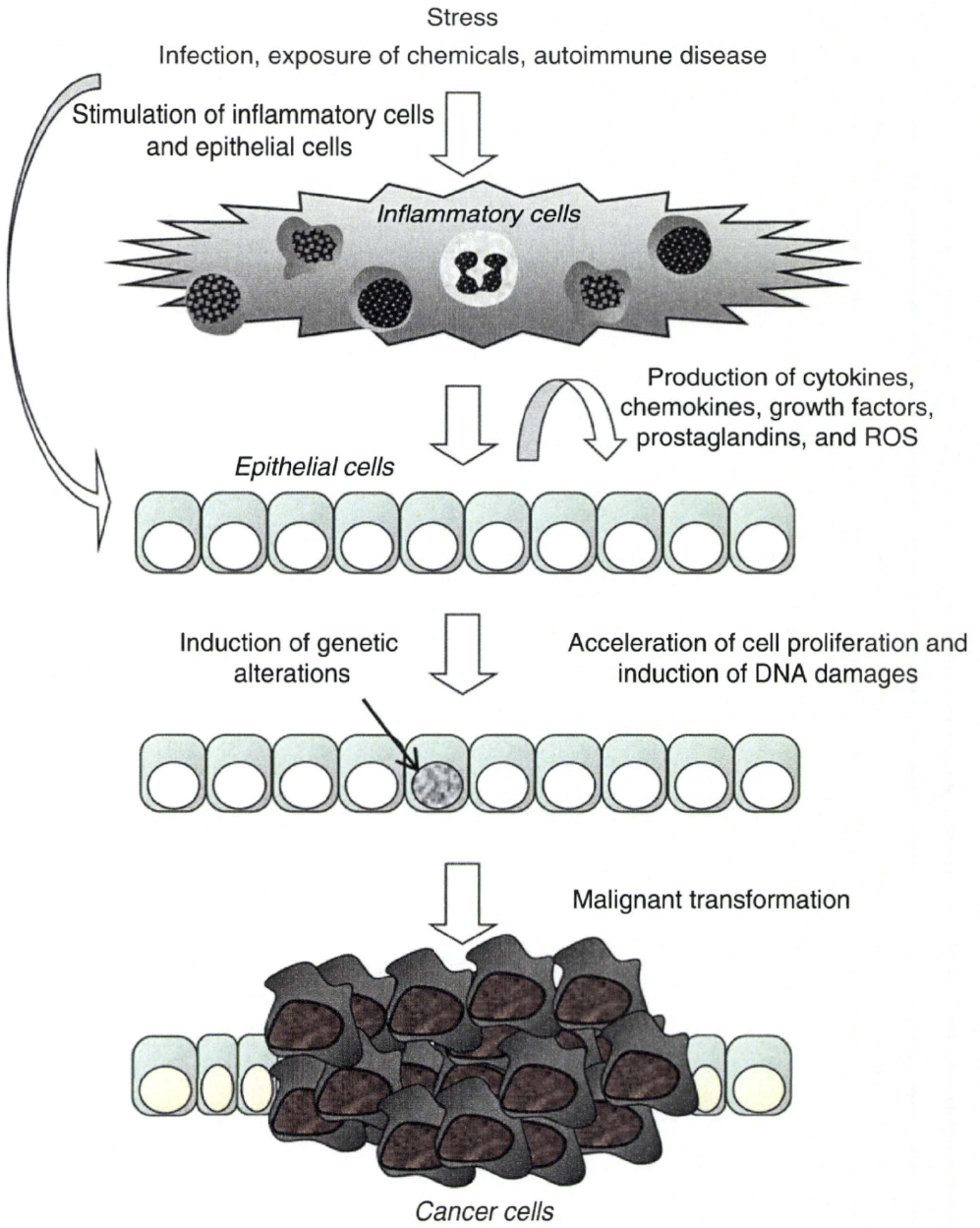

Figure 2.1. Schematic representation of a traditional carcinogenic scenario during inflammation. Stresses stimulate inflammatory cells and epithelial cells to produce inflammation-related factors, such as cytokines, chemokines, growth factors, prostaglandins, and ROS. These factors induce DNA damage in the epithelial cells directly or accelerate cell proliferation, both of which lead to induction of genetic alterations. If such genetic alterations are induced in critical genes, for example, tumor-suppressor genes and oncogenes, multistep carcinogenesis is promoted.