Part One

Introduction: The Case for Concern about Mutation and Cancer Susceptibility during Critical Windows of Development and the Opportunity to Translate Toxicology into a Therapeutic Discipline

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What Stressors Cause Cancer and When?

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1.1 Introduction

Translational biomedical research seeks to move laboratory findings based on models *(in silico*, *in vitro,* and *in vivo*) into human clinical trials to more expeditiously develop specific therapeutics, and then back again to the laboratory to inform future discovery [1]. From the background of developmental toxicology, it is well known that toxicant exposures may affect critical events in reproductive development, ranging from early primordial germ cell determination to gonadal differentiation, gametogenesis, external genitalia, or signaling events regulating sexual behavior. Translational genetic toxicology takes advantage of this developmental perspective to assess potential germ line mutagenesis or to study the potential for cancer in the fetus or offspring or the adult as the result of environmental exposures. Translational toxicology must strive to identify applicable therapeutics that can safely and effectively identify and help to mitigate potential harm from natural as well as anthropogenic environmental exposures.

Human exposures to chemicals, physical agents, and social factors are inevitable, thus the human fetus and the adult are subject to exposures and effects that can have lifelong consequences. Particularly, during dynamic developmental intervals described as "critical windows of susceptibility," exposures may have robust and durable effects that drive long-term health outcomes, including metabolism, functional status of organ systems, and cancer risks [2]. These same dynamic developmental intervals should be seen as "critical windows of responsivity" during which favorable/protective interventions should also be highly impactful offering potential durable reduction in

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risks of multiple adverse health outcomes, including cancers. To reduce the lifelong occurrence of preventable cancers, timely protective interventions during "critical windows" should include not only minimization of untoward voluntary exposures and substances of abuse but also active use of protective generally recognized as safe (GRAS) interventions/therapies, including nutritional, dietary supplementation, or well-established/repurposed and/or generally recognized as safe and effective (GRASE) pharmaceutical drugs.

This introductory chapter will promote the elucidation of cell stage, life stage, and lifestyle knowledge of specific cellular and molecular targets of known developmental toxicants, develop a systematic integrated approach to the identification of mutagenic and reproductive toxicants, and discuss sensitive, specific, and predictive animal models, to include minimally invasive surrogate markers, and/or *in vitro* tests to assess reproductive system function during embryonic, postnatal, and adult life. It will argue that integrated testing strategies will be required to account for the many mechanisms associated with development that occur *in vivo*. A key organizing principle used throughout this book is to consider how exposures that incur risk or other exposures/life events that may reduce risk during particular windows of susceptibility/developmental transitions, and thereby impact cancer occurrence.

In consideration of any cause–effect relationship, typically one thinks of the simple questions: Who, what, where, when, and how? Admittedly, "How?" questions are generally the most difficult because that understanding is a synthesis of potentially causal pathways. We aim to consider that the "Who?" and "When?" questions could be seen as people being exposed at different intervals across their respective life spans. Thus, in addition to information regarding what exposures occur that influence cancer occurrence, what is and is not known about exposures to those agents during life span intervals such as childhood, adolescence, across the broader life span, and/or late in life? Assessment of such timing of exposure with cancer outcomes seems to be a critical element if we aim to develop protective interventional strategies. In other words, whether we aim to reduce exposures or advocate protective lifestyle or therapeutic interventions, we must know when those interventions would most effectively impact later cancer outcomes.

Although there are differences between human development and that of laboratory animal models, developmental models have been extremely useful in assessing risks for key human reproductive and developmental processes. Some of these models will be discussed in Chapters 2 and 3. However, such systems have not been fully integrated with models to assess germ line mutagenesis or to study the potential for cancer in the fetus or offspring as the result of environmental exposures. Again, Chapters 2 and 3 will address current proposals for experimental animal test system integration.

To delve into the impact of exposures during "windows of susceptibility/responsivity," we must take into account the unique susceptibilities of the fetus.

Relatively, new information suggests that some widely held notions relevant to fetal exposures are incorrect [3]. Thus, we now know that amniotic fluid can be reabsorbed into the fetal circulation by fetal swallowing as well as via the fetal intramembranous pathway. The latter pathway is thought to be the most important mechanism for the resorption of toxicants, such as ethanol, into the fetal circulation [4]. Together with swallowing, this is a recycling system, through which toxic substances are excreted into the amniotic fluid and reabsorbed into the fetal circulation, thus extending the duration of each exposure [5,6]. This and other information relevant to fetal exposure *in utero* will be discussed in Chapter 8.

1.1.1 General Information about Cancer

Each year the American Cancer Society estimates the number of new cancer cases and deaths that will occur in the United States that year. In 2016, a total of 1,685,210 new cancer cases were expected to be diagnosed and about 595,690 cancer deaths were projected to occur in the United States [7]. Among children up to 14 years of age, an estimated 10,380 new cancer cases were expected to occur in 2016.

Population-based cancer registration began in the United States in 1975. Since then, childhood cancer incidence rates have increased by 0.6% per year. In 2016, 1250 cancer deaths were expected to occur among children. Cancer is the second leading cause of death in children ages 1–14 years, exceeded only by accidents. Childhood cancer death rates declined a total of 66% from 1969 (6.5 per 100,000) to 2012 (2.2 per 100,000). According to the American Society, this was largely due to improvements in treatment and high rates of participation in clinical trials. From 2003 to 2012, the rate of cancer-caused deaths in children declined by 1.3% per year.

Siegel *et al.* [8] reported that during the period 2006–2010, the then most recent 5 years for which there were data, the delay-adjusted cancer incidence rates declined by 0.6% per year in men and were stable in women. At the same time, cancer death rates decreased by 1.8% per year in men and by 1.4% per year in women. The rate of combined cancer deaths per 100,000 populations has declined continuously for two decades, from a peak of 215.1 in 1991 to 171.8 in 2010. The 20% decline during this time period equates to the avoidance of 1,340,400 cancer deaths (952,700 among men and 387,700 among women). Siegel *et al.* reported that the magnitude of the decline in cancer death rates varies substantially by age, race, and sex, with no decline among white women of 80 years of age and older to a 55% decline among black men 40–49 years of age. Remarkably, black men experienced the largest drop within every 10-year age group. The authors noted that progress could be accelerated by applying cancer control knowledge across all segments of the population [8].

While the severity of cancers is often measured in number of deaths, the number of years of life lost (YLL) may be a more appropriate indicator of impact

on society [9]. These authors calculated the YLL of adult cancers in Norway for 2012 and for the prior 15-year period. Their results showed that cancer deaths in Norway in 2012 represented 25.8% of all adult deaths (28.7% in men and 23.1% in women). Cancer deaths represented 35.2% of all YLL, with a 5.0% higher fraction in females than in males (32.8% in men and 37.8% in women) [9].

The etiology of cancer is generally thought to be the product of gene and environmental interactions. Environmental exposures are typically low and to mixtures of constituents that occur indoors and outdoors. Goodson *et al.* hypothesized that low-dose exposures to mixtures of chemicals in the environment may be combining to contribute to environmental carcinogenesis [10]. They reviewed 11 hallmark phenotypes of cancer, with multiple priority target sites for disruption in each area and prototypical chemical disruptors for all targets. Dose–response characterizations and evidence of low-dose effects and cross-hallmark effects for all targets and chemicals were considered. In total, 85 examples of chemicals were reviewed for their actions on key pathways and mechanisms related to carcinogenesis. Although 59% of the chemicals caused low-dose effects, only 15% (13/85) were found to show evidence of a dose–response threshold. No dose–response information was found for the remaining 26% (22/85). The authors speculated that the cumulative effects of individual noncarcinogenic chemicals acting on different pathways in related systems, organs, tissues, and cells could synergize to produce carcinogenic outcomes. They concluded that additional research on carcinogenesis focused on low-dose effects of chemical mixtures needs to be rigorously pursued before the merits of their hypothesis can be further tested [10].

In a published poster abstract, Parkin and Paul [11] estimated the percentage of cancer in the United Kingdom in 2010 resulting from exposure to 14 major life style, dietary, and environmental risk factors. Prevalence and relative risks of exposure to factors, including tobacco smoking, consumption of four different dietary components (fruit and vegetables, meat, fiber, salt) alcohol use, occupation, infections, radiation, hormone use, overweight, physical exercise, and reproductive factors were used to estimate the number of cancers occurring in 2010 attributable to suboptimal exposure levels in the past. These 14 exposures were responsible for 42% of cancer in the United Kingdom in 2010 (males 44%, females 40%). Tobacco smoking was the most important, accounting for about 60,000 new cancers (18.5% of all cancer; 22% in men, 15% in women), with less than 2% being the result of exposure to environmental tobacco smoke. The four dietary components account for 9.4% of cancer (10.7% in men, 7.1% in women). In men, alcohol use (5.1%) and occupational exposures (4.7%) are next in importance and in women, overweight and obesity are next (nearly 7% of cancers). The study is cited because estimates of this kind provide a quantitative assessment of the impact of various exposures. However, they are not synonymous with the fraction of cancers that might reasonably be prevented by modification of exposures. As discussed by the authors, "this requires scenario

modeling, with assumptions on a realistically achievable population distribution of risk factors, and the timescale of change." For example, although 50% of colorectal cancer can be attributed to lifestyle (diet, alcohol, inactivity, and overweight), only about 25% is preventable within a 20-year timescale [11].

Langley *et al.* [12] proposed a new research paradigm, adapted from twenty-first century toxicology that involves the following initiatives:

1) Develop a "big picture" of human disease that integrates extrinsic and intrinsic causes and links environmental sciences with medical research using systems biology.
2) Introduce a disease-centric adverse outcome pathway (AOP) concept, analogous to toxicity AOPs, with the intention of providing a unified framework for describing relevant pathophysiology pathways and networks across multiple biological levels.
3) Create a strong focus on advanced human-specific research (*in vitro*, *ex vivo*, *in vivo*, and *in silico*) in lieu of empirical, animal-based studies.

Langley *et al.* [12] have asserted that integrating data on extrinsic and intrinsic causes of disease using a systems biology (or systems toxicology) approach provides a more comprehensive understanding of human illnesses. Such an approach involves the perturbation of a biological system and the use of molecular expression data gathered through the use of omics technologies to understand the responses that occur at the systems level [13–15].

The AOP concept links exposure, involving chemical structures and molecular initiating events, via a sequence of key events, to an adverse outcome [16]. In a genomic sense, AOPs link external influences (*the exposome*), including drugs, chemicals in consumer products, food, or the environmental media, occupational exposures, infections, behavior, stress, smoking, ageing, nutrition, and radiation exposure to genetic effects (*the genome*), including susceptibility genes, up- and downregulation of genes, germ line and somatic mutations induced by drugs, chemicals and/or radiation, inherited single nucleotide polymorphisms, gene copy number changes, insertions, deletions, exome changes, and the accumulation of DNA damage, as well as epigenetic effects (*the epigenome*), including changes in the localized or global density of DNA methylation; posttranslational modifications of histones; changes in noncoding microRNAs; and changes in chromatin structure, which together alter the regulation of gene expression. Defects in the epigenome can cause disease and may be specific to tissue or cell types. Both genetic and epigenetic effects are then linked to adverse effects at cellular, organ, and individual levels.

According to Langley *et al.,* cellular/organ pathways may locate in immune function, apoptosis, calcium homeostasis, oxidative stress, growth factor signaling, nerve degeneration, and so on. Individual-level effects include embryonic development, disease, and death [12]. We certainly concur with this thinking and applaud the proposed new research paradigm, recognizing that

systems biology and systems toxicology must ultimately be understood at the network level as will be further discussed in Section 1.5.

1.1.2 Stressors and Adaptive Responses

A general reality in biology is that living systems are inevitably subject to external stressors, and a general observation is that these complex biological systems respond by adaptation – if those stresses do not exceed some definable threshold. Such adaptation includes subsequent strengthening of various endogenous responses as well as development of more diversified responses. A semantic point may be made that there is a gradation of meaning where stressors might be seen as positive stimuli on one end of the scale, but potentially harmful or lethal insults on the other end of the scale. Exposures in the early life impact cancer risk across the life span, with some increasing that risk but others reducing it.

1.2 What Stressors Cause Cancer and When?

We must view this question at the cell level, the life stage, and from a lifestyle perspective. We should also ask the question: Is there an adaptive response to modest stress? Regarding development of a cancer-specific translational toxicology therapeutic portfolio; we note that there are biological concepts regarding adaptive responses to modest stressors (adaptive stressors) in contrast to those stressors that exceed one or more bounds of tolerance within which an adaptive response might range.

Our goal is to provide a general overview on windows of susceptibility/responsivity, including maternal and fetal metabolic milieu, childhood cancers and therapies, and transitions into adulthood.

If there is a plausible public health basis to advocate for implementation of certain mitigative risk-reducing interventions, what are the essential ethical considerations to be made for protective "treatments" of the young for prevention of some remotely future disease (cancers) that the individual may or may not otherwise experience? In Chapter 19 we have delved into this and numerous other ethical issues facing the new field of translational toxicology.

In addressing chemical and metabolic exposures of concern, we agree that risks and benefits need to be considered. In this volume, we include natural and anthropogenic substances, both carcinogenic and anticarcinogenic, in the diet with commentary regarding both the good and the bad potential effects of natural chemicals and the evidence supporting each. We note, particularly, the childhood cancers and therapies for those cancers that need to be addressed. Regarding an important window of susceptibility or responsivity, the

peripubertal interval and the well-documented effect of early onset of menarche or breast cancer risk serve as good illustrations that will be discussed.

We note that any number of exposures could have an impact on the risk of cancer occurrence (as well as other diseases), and its indolent or aggressive behavior and progression over time. Chapters 5–8 are devoted to such exposures.

Environmental chemicals and drugs are a source of major concern in human exposure scenarios. We are exposed daily to low levels of literally thousands of industrial and household chemicals in our indoor and outdoor environments. For the most part, these represent involuntary exposures; however, we voluntarily expose ourselves to known human carcinogens in consuming alcoholic beverages and tobacco products. Not only do we expose ourselves, but also our children and even our grandchildren.

We briefly consider smoking relative to transgenerational cancer and other disorders. Thus, Dougan *et al.* [17] have studied grandmaternal smoking during pregnancy and its possible association with overweight status in adolescence. After adjusting for covariates, their findings suggest that the association between maternal smoking and offspring obesity may not persist beyond the first generation. However, grandpaternal smoking may affect the overweight status of the granddaughter, likely through the association between grandpaternal smoking and maternal smoking.

Pagani *et al.* [18] examined the reported behavioral habits of 2055 families by sifting through data from the Quebec Longitudinal Study of Child Development. The investigators looked particularly at levels of household tobacco smoke exposure when their child was between the ages of 1 and 7. They then attempted to ascertain any possible correlations between the level of smoking and measurements of the child's waist circumference and body mass index (BMI) at age 10. Higher amounts of both are known to predict a higher risk of gaining excess weight and developing metabolic disorders, such as diabetes, later on in adulthood.

"By the age of 10, those children who had been intermittently or continuously exposed to tobacco smoke were likely to have waists that were up to three-fifths of an inch wider than their peers. And their BMI scores were likely to be between 0.48 and 0.81 points higher," stated lead author Dr. Linda Pagani, of the University of Montreal, in a press release. "This prospective association is almost as large as the influence of smoking while pregnant. The researchers noted that only occasional smoking exposure was independently associated with excess weight, after controlling for factors like their parent's mental health or income, with a 43 percent greater chance of a child becoming obese or overweight in such a household [18]."

For certain other exposures, the case for transmission of cancer risk to future generations, via both genetic and epigenetic mechanisms, is much stronger. Thus, in a study by Peters *et al.* on parental exposure to solvents and subsequent

brain tumors in their children, parents of 306 cases and 950 controls completed detailed occupational histories. Odds ratios (ORs) and 95% confidence intervals (CIs) were estimated for both maternal and paternal exposure to benzene, other aromatics, aliphatics, and chlorinated solvents in key time periods relative to the birth of their child. Adjustments were made for matching variables, including child's age, sex and state of residence, level of parental education, and occupational exposure to diesel exhaust. Their results demonstrated an increased risk of childhood brain tumors (CBT) with maternal occupational exposures to chlorinated solvents (OR = 8.59, 95% CI 0.94–78.9) any time before birth. Paternal exposure to solvents in the year before conception was also associated with an increased CBT risk mainly attributable to exposure to aromatic solvents: OR = 2.72 (95% CI 0.94–7.86) for benzene and OR = 1.76 (95% CI 1.10–2.82) for other aromatics [19].

The International Agency for Research on Cancer (IARC) has classified 118 agents as known human carcinogens (http://monographs.iarc.fr/ENG/Classification/). IARC considers an additional 75 agents as probable human carcinogens and another 288 agents as possible human carcinogens. Some of these are actually complex mixture of agents. Typically, in order to delineate the relative contribution of its chemical constituents, a mixture must be separated and chemically characterized. Two of the more pervasive complex mixtures of mutagens and carcinogens are combustion emissions and tobacco smoke (including direct, side stream, and environmental exposures).

Combustion emissions resulting from the burning of fossil fuels, in generating electricity, in heating our homes, or in powering our vehicles, represent a substantial contribution to the total human environmental exposure. These emissions include both particulates and products of incomplete combustion that represent the original starting materials (e.g., coal and crude oil). Their combustion yields carbon, sulfur, lead, mercury, and other elements. Fossil fuels can be refined to reduce unwanted constituents, and this has been important in the development of cleaner industries and engine technologies. Even so, oxidized sulfur and nitrogen, elemental products, and volatile organic carbon products (VOCs) are mutagenic, carcinogenic, and otherwise hazardous to human health.

Tobacco smoke (even tobacco vapor) and all tobacco products are human carcinogens. Volatile vapors, nonvolatile compounds, and fine particles are deposited directly into the airways and the pulmonary alveoli. The Food and Drug Administration (FDA) has listed 93 harmful and potentially harmful constituents (HPHCs) of tobacco products and tobacco smoke (Federal Register/Vol. 77, No. 64/Tuesday, April 3, 2012). These constituents account for much of the carcinogenicity and toxicity that is observed in smokers. Other risk factors associated with smoking include hypertension, stroke, atherosclerosis, and myocardial infarction. Smoking also affects reproductive health, causing delay in conception, low birth weight, and advanced menopause.

In addition to xenobiotic chemicals and drugs, human exposures also include both natural and synthetic substances as well as basic nutrition and supplements. For example, the introduction of industrial farming practices in the United States to meet consumer and processed food product requirements for low cost food has come about with significant problems of microbial contamination (from feces) and antibiotic resistance that have not been encountered previously on such a large scale. Thus, infectious exposures and food safety issues are important categories of concern for human exposure, particularly in children who can be frequent consumers, especially of fast foods containing highly processed meats.

Similarly, excessive exposures to the physical agents in the environment, including sunlight, noise pollution, nonionizing radiation, radon gas, and diagnostic medical radiation can be of concern with regard to cancer etiology. Social factors must also be addressed and have been examined more frequently with the evolution of new knowledge in the field of epigenetics, as will be discussed in Chapter 11.

We suggest as an organizing principle, taking a pan-life span view of cancer and to view what causes and prevents cancer in a cumulative incremental way (see Figure 1.1).

This diagram aims to illustrate some of the various factors beginning prior to conception and extending across the subsequent life span that may drive lifetime risk of cancer(s) upward or downward. Some might plausibly have more impact during key developmental windows while others may be

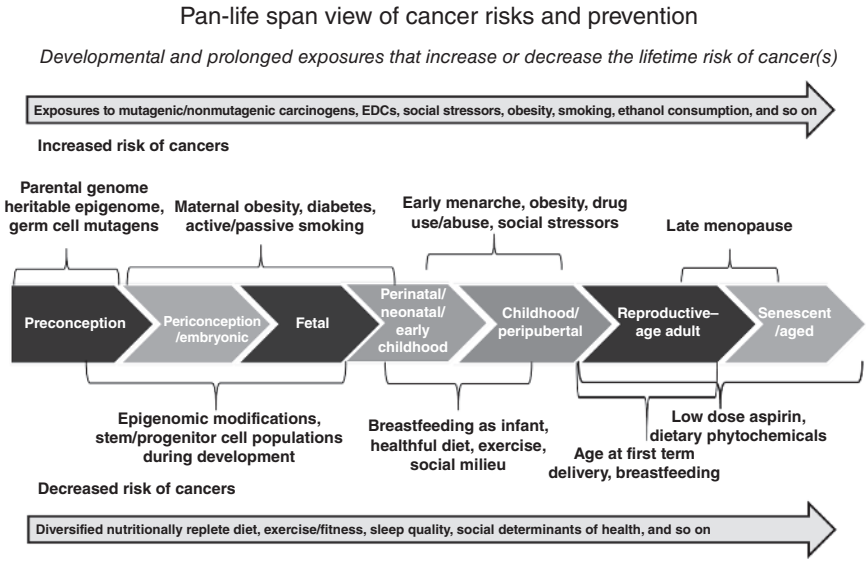

Figure 1.1 A pan-life span view of cancer risks and prevention. (For a color version of this figure, see the color plate section.)

cumulative and rather more subchronic or chronic in terms of either risk or protection. Any number of factors could be important such as biological sex, ethnicity, fitness as an adolescent or teen, assumption of tobacco smoking, discontinuation of tobacco smoking, age at first birth, age of puberty, other behaviors/lifestyle choices. For each cancer or group of cancers, there would be sets of risk factors and risk modifiers (mitigation). Some of these factors are discussed in greater detail in Chapters 9 and 10.

What are some of the considerations that relate lifestyle choices to cancer? A meta-analysis was undertaken by Garcia-Jimenez *et al.* to examine the association between diabetes, obesity, and cancer. Their results indicated that the interplay between hyperglycemia, increase in adipose mass, and inflammation that appears with obesity is critical in both diabetes and cancer, suggesting that obesity may link diabetes and cancer. Indeed, epidemiological evidence positively associates obesity with many site-specific cancers. The associations are strong for endometrial and kidney cancer but weaker for bladder, prostate, and stomach cancers. It may be important to note that highly prevalent lung cancers are inversely associated with obesity. According to Garcia-Jimenez *et al.*, type 2 diabetes (T2D) associates with most cancers that are linked to obesity. T2D represents >90% of diagnosed diabetes; studies that do not distinguish T1D from T2D follow a pattern similar to T2D. Significantly, most site-specific cancers that are positively associated with obesity show an even stronger association with T2D, suggesting that for those cancers T2D exhibits additional contributing factors [20].

What is the molecular basis of these kinds of associations? Genetically and biochemically there are many factors; however, one common denominator is Sirtuin 1 or SIRT1 (a member of the sirtuin family), which is a nicotinamide adenosine dinucleotide (NAD)-dependent deacetylase involved in removing acetyl groups from various proteins. SIRT1 performs a wide variety of additional functions in biological systems. Hubbard and Sinclair have reported that it deacetylates key histone residues involved in the regulation of transcription, including H3-K9, H4-K16, and H1-K26, as well as multiple nonhistone protein targets, including p53, forkhead box protein O1/3 (FOXO1/3), peroxisome proliferator-activated receptor gamma coactivator 1a (PGC-1a), and nuclear factor (NF)-kB. By targeting these proteins, SIRT1 is able to regulate numerous signaling pathways, including DNA repair and apoptosis, muscle and fat differentiation, neurogenesis, mitochondrial biogenesis, glucose and insulin homeostasis, hormone secretion, cell stress responses, and even circadian rhythm. The other sirtuins also play important roles in regulating mitochondrial reactions, glucose and insulin homeostasis, hepatic lipogenesis, DNA damage, telomere maintenance, inflammation, and the response to hypoxia [21].

Sun *et al.* have asserted that the dysregulation of SIRT1 can lead to ageing, diabetes, and cancer [22]. Using a ligand-based virtual screening of 1,444, 880 active compounds from Chinese herbs, they identified 12 compounds as

inhibitors of SIRT1. Three compounds had high affinity for SIRT1 as estimated by a molecular docking software program. Rahman and Islam have recently reviewed the biological functions of SIRT1 in obesity-associated metabolic diseases, adipose tissue, and cancer. In addition, they discuss the involvement of this enzyme in aging, cellular senescence, cardiac aging and stress, prion-mediated neurodegeneration, inflammatory signaling in response to environmental stress, development, and placental cell survival [23].

Another sirtuin is Sir2 or SIRT2, and its homologs are class III histone deacetylases. They are distinguished from class I and class II deacetylases by their requirement for beta-nicotinamide adenine dinucleotide (NAD+) as a cosubstrate [21]. In mammals, there are seven sirtuin homologs (SIRT1–7). SIRT1, SIRT6, and SIRT7 localize primarily to the nucleus; SIRT3, SIRT4, and SIRT5 localize to mitochondria; and SIRT2 localizes to the cytosol [24]. Although sirtuins were originally described as deacetylases, it is now evident that they have broader activity [24]. In addition to deacetylation, SIRT5 possesses desuccinylase and demalonylase activities [24], SIRT4 and SIRT6 are mono-ADP ribosyltransferases [6,24], and SIRT6 can deacylate long-chain fatty acids [25]. Indeed, it has been shown that the ability to catalyze long-chain deacylation is a general feature of mammalian sirtuins, and that in the case of SIRT6, long-chain fatty acids can enhance deacetylase activity [26].

1.2.1 Mutagenic MOAs

The term "mode of action" (MOA) encompasses a sequence of key events and processes beginning with the interaction of a chemical with a cell and proceeding through functional and structural changes that result in cancer. It is well established that mutations in somatic cells play a key early role in cancer initiation and may affect other stages of the carcinogenic process. All cancer cells acquire multiple mutations during carcinogenesis; therefore, mutation induction or acquisition can be key events at some stage in all cancers. Two important considerations in assessing evidence for a mutagenic MOA are (1) *when* the mutation occurs among the events that lead to cancer and (2) *whether* the action of the carcinogen as a mutagen is a key event in its carcinogenic process [27].

Mutagenicity of a chemical or its metabolite is an obligatory early event in a mutagenic MOA for cancer. This is in contrast with other MOAs wherein mutations are acquired subsequent to other key events (e.g., cytotoxicity with regenerative proliferation). With a mutagenic MOA for carcinogenesis, the chemical is expected to interact with DNA early in the process and produce changes in the DNA that are heritable. That a chemical carcinogen can induce mutation in one of a number of mutation assays is not sufficient to conclude that it causes specific tumors by a mutagenic MOA or that mutation is the *only* key event in the pathway to tumor induction. It should be pointed out

that the term "genotoxic" includes all effects on genetic information, whether or not the chemical interacts with the DNA. The term "mutagenic" implies interaction with DNA but not all carcinogenic chemicals that are capable of interacting with DNA will have a mutagenic MOA for cancer.

Yauk *et al.* reported the results of a 2013 International Working Group on Genotoxicity Testing [28]. This report will be discussed in detail in Chapter 2. The workshop key questions and outcomes were as follows: (1) Do genotoxicity and mutagenicity assays in somatic cells predict germ cell effects? Limited data suggest that somatic cell tests detect most germ cell mutagens, but there are strong concerns that dictate caution in drawing conclusions. (2) Should germ cell tests be done, and when? If there is evidence that a chemical or its metabolite(s) will not reach target germ cells or gonadal tissue, it is not necessary to conduct germ cell tests, notwithstanding somatic outcomes. However, it was recommended that negative somatic cell mutagens with clear evidence for gonadal exposure and evidence of toxicity in germ cells could be considered for germ cell mutagenicity testing. (3) What new assays should be implemented and how? There is an immediate need for research on the application of whole genome sequencing in heritable mutation analysis in humans and animals, and integration of germ cell assays with somatic cell genotoxicity tests. Focus should be on environmental exposures that can cause *de novo* mutations, particularly newly recognized types of genomic changes. Mutational events, which may occur by exposure of germ cells during embryonic development, should also be investigated. Finally, where there are indications of germ cell toxicity in repeat dose or reproductive toxicology tests, consideration should be given to leveraging those studies to inform of possible germ cell genotoxicity [28]. Additional information on mutagenic MOAs may be found in Chapter 2.

1.2.1.1 DNA Repair

DNA is subject to damage from environmental and dietary carcinogens, endogenous metabolites, certain anti-inflammatory drugs, and genotoxic chemo therapeutics. The prevention of mutations by DNA repair pathways led to an early appreciation of a role for repair in cancer avoidance. However, the broader role of the DNA damage response (DDR) emerged more slowly [29]. There are multiple DNA repair pathways, with subpathways providing lesion specificity. Nucleotide excision repair removes bulky DNA lesions; DNA nonhomologous end joining and homologous recombination repair DNA double-strand breaks; mismatch repair corrects mismatched base pairs; and base excision repair repairs damaged bases and links to single-strand break repair. Mutations in these pathways increase cancer susceptibility [29].

Cells respond to DNA damage by the activation of complex signaling networks that decide cell fate, promoting DNA repair and survival but also cell death. Whether it is to be cell survival or death depends on factors involved

in DNA damage recognition, and DNA repair and damage tolerance, as well as on factors involved in the activation of apoptosis, necrosis, autophagy, and senescence. The pathways that dictate the fate of the cell also have key roles in cancer initiation and progression. Furthermore, they determine the outcome of cancer chemotherapy with genotoxic drugs. Understanding the molecular basis of these pathways is important not only for gaining insight into carcinogenesis, but also in prescribing successful cancer therapy [30].

DNA damage triggers multiple cellular responses: It activates cell cycle checkpoints that provide time for the cell to repair the damage before it interferes with the replication machinery. Checkpoints prevent progression from G1 to S phase and from G2 to M phase, and an intra-S phase checkpoint regulates fork progression or origin firing. Many tumors have inactivated checkpoint responses. If repair fails or is saturated, the remaining DNA damage impedes replication and transcription, and the activated DDR signal cell death via downstream pathways. Therefore, the ability of a cell to survive DNA damage is proportional to the extent of damage, the repair capacity of the cell, the level of cell proliferation, the status of p53 and key DDR proteins including ataxia-telangiectasia mutated (ATM), ATR, and DNA-PK, the effectiveness of activating DNA repair genes (which is dependent on epigenetic silencing and cellular transcription factors), and the execution of downstream cell death pathways.

There are two DNA damage response signaling pathways: ATM-dependent signaling is activated by double strand breaks; and ataxia telangiectasia and RAD3-related (ATR)-dependent signaling is activated by single-stranded regions of DNA. DDR signaling can activate apoptosis and checkpoint arrest, and can influence DNA repair. Mutations in ATM signaling components confer cancer susceptibility. However, ATR-deficient mice show reduced capacity for tumor formation [29]. Multiple processes function to maintain the accuracy of replication and enhance recovery from replication fork stalling or collapse. Homologous recombination has a key role, and genes involved in this process are commonly mutated in cancers. Several mechanisms prevent DNA rereplication that can cause aneuploidy and subsequently genomic instability. Cancer cells need to maintain telomere length to survive since shortened telomeres lead to senescence. Activation of telomerase or an alternative pathway to maintain telomere length is common in cancers.

DNA repair capacity differs greatly among cell types, with human embryonic stem cells repairing most DNA lesions more effectively than differentiated cell types [31], whereas monocytes and muscle cells are defective in base excision repair [32,33] and some cancers show upregulation of repair, for example, metastatic melanoma [34], or highly variable MGMT repair activity such as in gliomas [35,36]. In simple terms, a low level of DNA damage activates DNA repair (with upregulation of repair genes XPF, XPG, DDB2, XPC, XRCC1, and others), whereas with high levels of DNA damage, repair is saturated, and

unrepaired DNA damage activates one of the death programmes, including apoptosis, regulated necrosis, and autophagy. Apoptosis represents a programmed cell death pathway that functions in some tissues during normal development but also prevents proliferation of damaged cells. Apoptosis can be p53 dependent or independent and p53 is commonly mutated in cancer [29]. It is not well understood how the cell switches between these pathways; however, it appears that the p53 phosphorylation status and antiapoptosis thresholds are key nodes in determining a cell's life or death following DNA damage. ATM and ATR seem to be the main decision makers, informing effectors such as p53 how to proceed. Increased drug resistance of tumors carrying mutations in ATM [37] illustrates the importance of ATM in initiating cell death pathways. Inactivation of p53 in cancer cells can lead to either drug sensitization or resistance, depending on the genotoxic agent employed.

Roos *et al.* [30] have suggested that targeting antiapoptosis proteins and pathways conceivably lowers the threshold for cell death for genotoxic and biological therapies. How specific DNA lesions activate and coordinate the complex interplay between survival and death is of fundamental importance for cancer therapy. The ultimate goal is to protect normal tissue during therapy with genotoxic anticancer drugs while sensitizing cancer cells to die. The protection of normal tissue has far-reaching implications for stem cells and for genome-compromised cells as the former have been shown to activate DNA damage-triggered apoptosis easily, and the elimination of the latter from the healthy cell population is a cancer prevention strategy.

1.2.2 Epigenetic MOAs

Epigenetics is the study of all mechanisms regulating gene transcription and genomic stability maintained throughout cell division, but not including the DNA sequence itself. Environmental epigenetics, also referred to as toxicoepigenetics, investigates the molecular biological processes that potentially link the environment to its impact on disease risk and outcome. This subject is discussed in detail in Chapter 13.

The epigenome modulates gene expression and cellular phenotype via chemical changes in DNA and chromatin that occur without modifying the DNA sequence. The epigenome is highly plastic and reacts to changing external conditions with modifications that can be inherited by daughter cells and across generations. Although this innate plasticity allows for adaptation to a changing environment, it also implies the potential of epigenetic derailment leading to so-called epimutations [38].

To date, DNA methylation is the best-studied epigenetic mechanism in which methyl groups are added to the cytosine base within cytosine–guanine dinucleotides (CpG sites). CpGs tend to be clustered in high-density CpG islands at the promoter of more than half of all genes. Unmethylated CpG

islands are found there in actively transcribed genes, whereas hypermethylation of the promoter results in gene repression. Over the last 5 years, our understanding is that methylation patterns across the gene (so-called intragenic or gene body methylation) may have a role in transcriptional regulation and efficiency. Genome-wide DNA methylation profiling studies support this concept, but whether DNA methylation patterns are a cause or consequence of other regulatory mechanisms is not yet clear. Shenker and Flanagan have examined the evidence for the function of intragenic methylation in gene transcription, its significance in carcinogenesis, and potential use in therapies targeted against DNA methylation [39].

DNA methylation changes have been associated with cancer, infertility, cardiovascular, respiratory, metabolic, immunologic, and neurodegenerative diseases. Experiments in rodents demonstrate that exposure to a variety of chemical stressors, occurring during prenatal or adult life, may induce DNA methylation changes in germ cells, which may be transmitted across generations with phenotypic consequences. A number of human biomonitoring studies show environmentally related DNA methylation changes mainly in blood leukocytes, but there are few studies on possible epigenetic changes induced in the germ line, even though sperm are readily accessible for analysis.

DNA methylation is a life-essential process as it modulates gene expression and drives cell differentiation in multicellular organisms. Synergistically with other epigenetic mechanisms, it allows cells and organisms to adapt to external changes, in a timely manner not matched by mutational mechanisms. Not surprisingly, DNA methylation is sensitive to external stimuli and, in contrast to mutations, is reversible. This duality presents a challenge in establishing possible links between environmental exposure and epigenetic changes that can have a long-lasting impact on cell function and ultimately health. Cancer is a good example of a disease associated with aberrant epigenetics, possibly triggered by environmental exposures.

Epigenetic marks are extensively altered in cancer but they may also change in normal tissues with age, which is the primary risk factor for most cancers. Xu and Taylor performed an epigenome-wide study to identify age-related methylation sites and examine their relationship to cancer and other underlying epigenetic marks. They analyzed DNA in 1006 blood samples from women aged 35–76 years from the Sister Study (http://www.niehs.nih.gov/research/atniehs/labs/epi/studies/sister/) and determined that 7694 (28%) of the 27,578 CpGs assayed were associated with age (false discovery rate, $q < 0.05$). Using independent data sets, they also confirmed 749 "high confidence" age-related CpG (arCpGs) sites in normal blood. Their findings suggest that as cells acquire methylation at age-related sites, they have a lower threshold for malignant transformation and this may explain in part the increase in cancer incidence with age [40].

Evidence exists that erroneous epigenetic marks play prominent roles in Alzheimer's disease, autoimmune diseases such as rheumatoid arthritis, and cardiovascular diseases, among others [38].

It should be noted that interindividual variation in methylation may also be a consequence of DNA sequence polymorphisms that result in methylation quantitative trait loci. Teh *et al.* [41] have investigated the genotypes and DNA methylomes of 237 neonates and found some 1500 punctuate regions of the methylome highly variable across individuals, termed variably methylated regions (VMRs), against a homogeneous background. Their explanation for 75% of VMRs was the interaction of genotype with different *in utero* environments, including maternal smoking, maternal depression, maternal BMI, infant birth weight, gestational age, and birth order. A prevalence of genetic over environmental determinants of interindividual variation of CpGs methylation has been recently reported in large Scottish and Australian cohorts. Finally, age is expected to be a major variable affecting the DNA methylation profiles in different tissues. In fact, recent studies aimed at exploring the importance of epigenetic changes to the ageing process and highlighting age-signatures of DNA methylation.

To fully understand the import of methylation signatures requires query of the human haploid DNA methylome containing approximately 30 million CpGs that exist in a methylated, hydroxymethylated, or unmethylated state. Notwithstanding this challenge, study of environmental epigenetics may be the best way to fully assess the impact of the exposome on human health. Indeed, the hypothesis of prenatal origin of adult-onset diseases is supported by the idea that mammalian tissue differentiation is mainly established during prenatal life, and that fundamental DNA methylation changes occur in the preimplantation embryo and during gonadal differentiation. Epidemiological mother–child cohort studies and maternal exposure assessment are needed to advance science in this area. Although the process of gametogenesis will only be completed after puberty, the bases of reproductive health are founded during prenatal life with primordial germ cell differentiation and gonad development. This requires that multiple exposure windows be considered to assess possible environmental effects on gamete genetic as well as epigenetic integrity [38].

The results of studies in rodents show that DNA methylation in germ cells can be altered by many different exposures during fetal as well as adult life. Limitations of these studies include the fact that more data are available on the male than on the female germ line, and only a few studies were at the whole genome scale, addressed the functional impact of epigenetic changes on gene expression and related cell pathways, and took into consideration dose–effect relationships. Even so, their results establish proof of principle that exogenous stressors may alter DNA methylation at developmentally important imprinted or metabolic genes [38].

Environmental exposure of the human germ line to mutagenic or epimutagenic agents may alter the reproductive capacity of the exposed individual and

may transmit damage to the following generation. Studies in rats and mice have shown that treatment induced not only DNA methylation changes in paternal sperm but also phenotype alterations in offspring. These observations suggest that DNA methylation profiles of gametes are not completely reset after fertilization but can be partly transmitted across generations. While studies have given conflicting results, several authors agree that direct transmission of methylation changes is not the only mechanism through which altered sperm methylation might affect the offspring phenotype and that sustained alterations of transcriptional regulatory networks early in development may likely result from a complex interplay between DNA methylation changes, chromatin modifications, and other epigenetic mechanisms. One implication of epigenetic inheritance systems is that they provide a potential mechanism by which parents could transfer information to their offspring about the environment they experienced [38,42].

From a clinical perspective, DNA methylation and other epigenetic changes in the sperm observed in subfertile patients are also important for reproductive environmental epigenetics because they seem to indicate a functional significance of DNA methylation changes in the male germ line. Thus, there is a need to conduct specific epigenetic analyses on the sperm of men exposed to reproductive toxicants, with the awareness that their PBLs might not be reliable surrogates for the relevant target cells [38].

On the basis of human somatic environmental epigenetics, rodent germ line epigenetic toxicological studies, and knowledge of the most environmentally relevant human reprotoxic agents, a priority list of environmental stressors for future human sperm epigenetic biomonitoring studies might be proposed: (1) dysmetabolism as a consequence of environmental and genetic factors, including their possible interactions, (2) endocrine disrupting compounds, and major lifestyle toxicants like tobacco smoke and alcohol with emphasis on prenatal exposure and mother child cohorts, and (3) prospective, long term, multigeneration follow-up surveys to take into account grandparental effects [38].

What are some of the other consequences of epigenetic inheritance? As already discussed, there is considerable controversy regarding epigenetic inheritance in mammalian gametes. Using *in vitro* fertilization to ensure inheritance exclusively via the gametes, Huypens *et al.* showed that a parental high-fat diet renders offspring more susceptible to developing obesity and diabetes in a sex- and parent of origin-specific mode. The "thrifty genotype" hypothesis postulated that metabolic thrift, the capacity to effectively acquire, store and use energy, is an ancient trait embedded in human genomes [43]. However, the prevalence rates for obesity and type 2 diabetes (T2D) have increased globally over recent decades at a pace that cannot be explained solely by genetic drift. Therefore, Huypens *et al.* experimentally tested whether epigenetic inheritance via gametes by itself could increase an offspring's susceptibility to develop obesity and T2D2. To this end, Huypens and colleagues fed isogenic

C57BL/6NTac mice a calorie-dense high-fat research diet (HFD), a control low-fat research diet, or normal standard chow for a period of 6 weeks. Parental (F0) HFD mice developed obesity, severe glucose intolerance, and fasting hyperinsulinemia. The authors concluded that the epigenetic inheritance of acquired metabolic disorders might contribute to the current obesity and diabetes pandemic [44].

1.2.3 Nongenotoxic Carcinogens, ROS, Obesity, Metabolic, Diet, Environment, Immune, Endocrine MOAs

Nongenotoxic carcinogens are chemicals that cause cancer without directly reacting with DNA. Despite their lack of mutagenicity, nongenotoxic carcinogens can influence the development and progression of cancer through a number of indirect mechanisms that (1) may increase cell proliferation and disrupt cell structures, (2) generate reactive oxygen species (ROS), (3) induce receptor-mediated signaling, (4) alter gene expression or epigenetic programming of cells, and (5) induce inflammation and modulation of the immune response. These diverse and complex secondary mechanisms by which nongenotoxic carcinogens induce neoplasia are often tissue and species specific. They rarely follow low-dose linearity, typically ascribed to genotoxic agents, and thereby they create difficulties for researchers and challenges in human health risk assessment for regulatory agencies. To illustrate the diversity and the complexity of evaluating nongenotoxic mechanisms of carcinogenesis, Chapter 12 examines an estrogenic toxicant and putative carcinogen used widely in a variety of consumer goods. The following introductory information is abstracted from Chapter 12.

A common mode of action for nongenotoxic carcinogens involves receptor-mediated effects. Steroids and xenoestrogens can cause cancer through hormone receptor-mediated interactions, including perturbed hormone balance, increased cell proliferation, and altered gene expression patterns. Estrogenic ligands, such as 17β-estradiol, bind estrogen receptors (ERs) and induce carcinogenicity by altering genomic and nongenomic regulation of transcription. More specifically, binding of estrogenic ligands to estrogen receptors α and β (ERα and ERβ), members of a nuclear receptor super-family, activates these complexes to bind estrogen responsive elements (ERE) in the promoter regions of target genes, thereby regulating their transcription [45]. Gene expression changes can also be induced independent of ERE elements through the interaction of ERα and ERβ with DNA-bound transcription factors [46,47]. Nongenomic signaling can also be induced by estrogenic ligand binding to membrane estrogen receptors or other estrogen binding proteins that induce kinase signaling cascades, such as the mitogen-activated protein kinase (MAPK) pathway [46,47]. Collectively, these alterations induce changes in cell growth, differentiation, motility, and DNA damage response and repair that can

contribute to the development and progression of breast, ovarian, and endometrial cancers [46,48].

Laboratory studies in a number of model systems have confirmed the induction of ROS and DNA damage by oxidative DNA lesions such as 8-oxo-guanine [49–57]. In addition to generating ROS, bisphenol A (BPA) has also been shown to alter the antioxidant balance of cells depleting intracellular glutathione and altering the expression of catalase and superoxide dismutase [50,51,58–60]. Additionally, exposure of mice to BPA during pregnancy and continued exposure of the offspring during infancy has been shown to cause oxidative stress by decreasing antioxidant enzymes and increasing lipid peroxidation, leading to underdevelopment of the testis, brain, and kidneys of the offspring [50,52,61].

Two common ways nongenotoxic carcinogens induce oxidative stress are by generating ROS during their metabolism in the cell and/or by depletion of the antioxidant defense mechanisms in the cell that counterbalance both endogenous and exogenous ROS. BPA primarily induces ROS through the enzymatic (H_2O_2/peroxidase and NADPH/CYP450) and nonenzymatic (peroxynitrite/CO_2 and −OCl/HOCl) formation of BPA phenoxyl radicals [49]. These phenoxyl radicals can then be further converted by NADPH or intracellular glutathione to form superoxide, hydroxyl radicals, and H_2O_2 [49]. Generated ROS can then damage cellular macromolecules and induce DNA strand breaks, purine and pyrimidine lesions, and DNA proteins cross-links.

Recent work has demonstrated that the induction of oxidative stress by BPA induces a number of cellular changes that, when challenged by additional oxidative stress, induce an adaptive response, promoting cell survival [51]. This adaptive response was characterized by an initial compaction of cellular chromatin that prevents the excision of oxidatively induced DNA lesions followed by an up-regulation of DNA repair proteins that increases the repair of oxidatively induced DNA lesions [51]. These results demonstrate that induction of oxidative stress by BPA contributes significantly to its toxicity. These mechanisms need to be evaluated more thoroughly to understand the role they play in addition to the endocrine disrupting properties of BPA.

In addition to the induction of oxidative stress, inflammation and modulation of the immune response are also important mechanisms of action for some nongenotoxic carcinogens. The role that chemicals and chemical mixtures have on the cells of the human immune system is an emerging research area in environmental toxicology. Thompson *et al.* have reviewed the role that the innate immune cells and inflammatory responses play in tumorigenesis. Their focus is on the molecules and pathways that have been mechanistically linked with tumor-associated inflammation in the context of chemically induced disturbances in immune function as co-factors in carcinogenesis. Specifically, they consider the evidence linking environmental toxicant exposures with perturbation in the balance between pro- and anti-inflammatory responses.

Reported effects of bisphenol A, atrazine, phthalates, and other common toxicants on molecular and cellular targets involved in tumor-associated inflammation (e.g., cyclooxygenase/prostaglandin E2, nuclear factor kappa B, nitric oxide synthesis, cytokines, and chemokines) are presented as examples of chemically mediated target molecule perturbations relevant to cancer. Commentary is presented on areas of additional research required for development and integration of systems biology approaches to the study of environmental exposures and cancer causation [62].

The combination of inflammation and modulation of the immune response can lead to increases in the expression of growth factors and cytokines that ensure survival, while inducing inflammation and altering the immune response. Prenatal exposure of mice to BPA promoted the production of TH2 cytokines and was associated with a decrease in T regulatory CD4+ CD25+ cells [63]. Perinatal exposure to BPA also promoted the production of proinflammatory mediators through the dysregulation of mast cells [64]. Other links to mast cell degranulation, lymphocyte proliferation, and antibody response have also been reported [65,66]. Whether these inflammation and immune changes induced by BPA directly influence the progression and development of cancer has not been examined and the effects of these changes on allergic responses and asthma have not been conclusively verified [64,66].

Chronic inflammation is associated with an increased risk of cancer, and impairment of immune response, whether through immunosuppression or impaired surveillance, can contribute to tumor promotion [67,68]. Given the association of inflammation with cancer and the importance of immune surveillance in the removal of precancerous cells, more work is necessary to determine how BPA is influencing these responses. However, the robust responses of IL-6 and TNFα observed in a number of studies indicate that it may play an important role. Population studies support a role for BPA-induced inflammation, with an increase in C-reactive protein (CRP) levels observed in postmenopausal women [57], increase in IL-6 and CRP observed in premenopausal women with polycystic ovary syndrome [69], and increased levels of IL-6 and TNF-α observed in males [70].

Is BPA a transplacental carcinogen? Based on a recent study in mice it appears to be. Weinhouse *et al.* [71] explored the effects of exposure to BPA during gestation and lactation on adult incidence of hepatic tumors in mice. Isogenic mice were perinatally exposed to BPA through maternal diets containing one of four environmentally relevant doses (0, 50 ng, 50 μg, or 50 mg of BPA per kg diet) and approximately one male and one female per litter were followed until 10 months of age. Animals were tested for known risk factors for hepatocellular carcinoma, including bacterial and viral infections. Hepatic tumors were observed in exposed 10-month mice; 23% of offspring presented with hepatic tumors or preneoplastic lesions. A statistically significant dose–response relationship was observed, with an odds ratio for neoplastic and

preneoplastic lesions of 7.23 (95% CI: 3.23, 16.17) for mice exposed to 50 mg BPA per kg diet compared with unexposed controls. The authors concluded that early disease onset, the absence of bacterial or viral infection, and a lack of characteristic sexual dimorphism in tumor incidence support a nonclassical etiology. This is the first report of a statistically significant association between BPA exposure *in utero* and frank tumors in any organ. The results clearly link early life exposure to BPA with the development of hepatic tumors in rodents, with potential implications for human health and disease [71].

In a clinical investigation, Tarapore *et al.* examined the association between urinary BPA levels and prostate cancer and assessed the effects of BPA on induction of centrosome abnormalities as an underlying mechanism promoting prostate carcinogenesis. Their study, involving 60 urology patients, found higher levels of urinary BPA (creatinine-adjusted) in prostate cancer patients (5.74 mg/g [95% CI; 2.63, 12.51]) than in nonprostate cancer patients (1.43 mg/g [95% CI; 0.70, 2.88]) ($p = 0.012$). These findings suggest that urinary BPA level is an independent prognostic marker in prostate cancer and that BPA exposure may lower serum PSA levels in prostate cancer patients. Moreover, disruption of the centrosome duplication cycle by low-dose BPA may contribute to neoplastic cell transformation in the prostate [72].

Are there windows of susceptibility for epigenetic effects and are there opportunities for intervention? Day *et al.* showed that early puberty timing is associated with higher risks for type 2 diabetes and cardiovascular disease in women; it therefore represents a potential target for early preventive interventions. They characterized the range of diseases and other adverse health outcomes associated with early or late puberty timing in men and women in the very large UK Biobank study. Recalled puberty timing and past/current diseases were self-reported by questionnaire. Analyses were limited to individuals of White ethnicity (250,037 women; 197,714 men) and to disease outcomes with at least 500 cases (~0·2% prevalence) with careful correction for multiple testing (corrected threshold $P < 7.48 \times 10^{-5}$). In models adjusted for socioeconomic position and adiposity/body composition variables, both in women and men separately, earlier puberty timing was associated with higher risks for angina, hypertension and T2D. Futhermore, compared to the median/average group, earlier or later puberty timing in women or men was associated with higher risks for 48 adverse outcomes, across a range of cancers, cardiometabolic, gynaecological/obstetric, gastrointestinal, musculoskeletal, and neurocognitive categories. Notably, both early and late menarche was associated with higher risks for early natural menopause in women. In conclusion, puberty timing in both men and women appears to have a profound impact on later state of health [73].

Given that epigenetic transmission is across generations, what is the look-back period and what kinds of exposures must be considered? To begin to answer this question, Cohn *et al.* hypothesized that *in utero* exposure to DDT is associated with an increased risk of breast cancer. What is the extent of concern

for epigenetic effects from prior exposures? Many women were heavily exposed *in utero* during widespread DDT use in the 1960s. Cohn *et al.* designed a case-control study (involving $n = 118$ breast cancer cases, diagnosed by age 52 years and 354 controls matched on birth year) nested in a prospective 54-year follow-up of 9300 daughters in the Child Health and Development Studies pregnancy cohort. This study links measured DDT exposure *in utero* to risk of breast cancer. The primary participants were Kaiser Foundation Health Plan mothers who had received obstetric care in Alameda County, California, from 1959 to 1967; their adult daughters participated in the study. The daughters' breast cancer diagnosed by age 52 years (as of 2012) was the main outcome measured. The results showed that maternal *o,p*-DDT levels predicted daughters' breast cancer (odds ratio fourth quartile versus first = 3.7, 95% confidence interval 1.5–9.0). Lipids, weight, race, age, and breast cancer history did not explain the findings. Additional experimental studies are essential to confirm these results and discover causal mechanisms. The findings support classification of DDT as an endocrine disruptor, a predictor of breast cancer, and a marker of high risk for breast cancer [74].

Costello *et al.* studied the association between dietary patterns and risk of breast cancer in Spanish women, stratifying by menopausal status and tumor subtype, to compare the results with those of Alternate Healthy Index (AHEI) and Alternate Mediterranean Diet score (aMED). Costello *et al.* recruited 1017 incident breast cancer (BC) cases and 1017 matched healthy controls of similar age (±5 years) without a history of breast cancer. Adherence to the Western dietary pattern was related to higher risk of breast cancer (OR for the top versus the bottom quartile 1.46 (95% CI 1.06–2.01)), especially in premenopausal women (OR = 1.75; 95% CI 1.14–2.67). In contrast, the Mediterranean pattern was related to a lower risk (OR for the top quartile versus the bottom quartile 0.56 (95% CI 0.40–0.79)). While the deleterious effect of the Western diet was similarly observed in all tumor subtypes, the protective effect of the Mediterranean diet was stronger for triple-negative tumors (OR = 0.32; 95% CI 0.15–0.66 and P heterogeneity = 0.04). The results confirmed the harmful effect of a Western diet on breast cancer risk and provided evidence for the overall preventive benefits of a diet rich in fruits, vegetables, legumes, oily fish, and vegetable oils, particularly with triple-negative breast cancer [75].

Ferris *et al.* first used generalized equations to estimate a population average effect across all families ($n = 389$ cases, $n = 5643$ controls) followed by conditional logistic regression in order to examine within-family differences in a subset with at least two sisters discordant on ovarian cancer status ($n = 109$ cases, $n = 149$ unaffected sister controls). In the generalized estimation model, there was a reduced risk of ovarian cancer for ever use of oral contraceptives compared with never use (OR = 0.58, 95% CI: 0.37, 0.91), and in the conditional logistic model there was a similar inverse association, although it was not statistically significant (OR = 0.52, 95% CI: 0.23, 1.17). Ferris *et al.* examined this

association by BRCA1/2 status and observed a statistically significant reduced risk in gene noncarriers only. They observed a decreased risk of ovarian cancer with oral contraceptive use, supporting that this association observed in unrelated women extends to related women at higher risk [76].

1.2.4 Tumor Microenvironment MOAs

Potentially carcinogenic compounds may cause cancer through direct DNA damage or through multiple indirect cellular or physiological effects. As we have seen, the identification and investigation of these varied effects involves work in endocrinology, genetics, epigenetics, medicine, environmental health, toxicology, pharmacology, and oncology. Disruptive chemicals may contribute to multiple stages of tumor development via effects on the tumor microenvironment. The tumor microenvironment consists of complex interactions among blood vessels that feed the tumor, the extracellular matrix that provides structural and biochemical support, signaling molecules that send messages, and soluble factors, such as cytokines. It also consists of many types of host effector cells, including multipotent stromal cells/mesenchymal stem cells, fibroblasts, endothelial cell precursors, antigen presenting cells, lymphocytes, and innate immune cells.

Carcinogens can influence the tumor microenvironment through effects on epithelial cells, the most common origin of cancer, as well as on stromal cells, extracellular matrix components, and immune cells. Casey *et al.* have reviewed how environmental exposures can perturb the tumor microenvironment. They suggest a role for disrupting chemicals, such as nickel chloride, bisphenol A, butyltins, methylmercury, and paraquat, as well as more traditional carcinogens such as radiation, and pharmaceuticals, such as diabetes medications, in the disruption of the tumor microenvironment. Further studies interrogating the role of chemicals and their mixtures in dose-dependent effects on the tumor microenvironment could have important general mechanistic implications for the etiology and prevention of tumorigenesis [77].

Are certain cell types more sensitive to toxicants or more replaceable following damage than others? Recent work in the field of stem cell biology suggests that there is no single adult tissue stem cell hierarchy, and that self-renewal and repair requirements are unique to different tissues. Thus, stem cells may be uni- or multipotent and can exist in quiescent or actively dividing states. Activated "professional" stem cells may coexist with facultative stem cells, which are more specialized daughter cells that revert to a stem cell state under specific tissue damage conditions. Visvader and Clevers discuss stem cell strategies as observed in three solid mammalian tissues: the intestine, mammary gland, and skeletal muscle.

It is becoming increasingly clear that multiple stem cell types with different tissue renewal capacity can reside within the same tissue. Both transplantation

and lineage tracing assays have proved to be invaluable in dissecting stem cell compartments. The small intestine contains multipotent stem cells that are actively cycling to restore all cells within the crypt–villus unit. Adding an apparent layer of complexity, both uni- and bipotent epithelial stem cells appear to reside in the mammary epithelial tree. Functional diversity within a stem cell compartment is presumably established through the complex interplay between intrinsic and extrinsic signals [78]. Chapter 14 explores MOAs related to the tumor microenvironment and Chapter 15 explores MOAs related to dysregulated metabolism.

1.3 Relevance of Circulating Cancer Markers

Chapter 16 is devoted to the topic of this section. Developments in genomic techniques have provided substantial insight into the genetic complexity of malignant tumors. As will be discussed, there is increasing evidence that solid tumors encompass subpopulations of cells with distinct genomic alterations, that is, intratumor heterogeneity. Fisher *et al.* have asserted that intratumor heterogeneity is likely to have implications for cancer therapeutics and biomarker discovery, particularly in the era of targeted treatment. Evidence for a relationship between intratumoral heterogeneity and clinical outcome is emerging. The processes that exacerbate intratumoral heterogeneity, both iatrogenic and tumor specific, are likely to increase with the development and implementation of advanced sequencing technologies, and adaptation of clinical trial design to include comprehensive tissue collection protocols [79].

While there is accumulating evidence for substantial genetic diversity both within and between many common solid tumors, less is known about how such diversity is generated or its impact upon clinical outcomes such as response or resistance to anticancer therapies and the natural history of the disease. Tumor heterogeneity conceivably may impede the identification of predictive biomarkers, and the quest for personalized, or even curative treatment, and is an area of cancer research worthy of intensive and collaborative effort [79].

The identification of circulating tumor cells (CTC) in blood has emerged as one of the most intense areas of cancer research. CTC detection and enumeration can serve as a "liquid biopsy" and as an early marker of response to systemic therapy. The clinical relevance of CTCs as a prognostic factor is well established both in metastatic and early-stage breast cancer patients. The molecular characterization of CTC in breast cancer patients has a convincing potential to enable individualized targeted treatment and to spare these patients unnecessary and ineffective therapies. The elimination or decrease of CTCs following treatment is associated with improved clinical outcomes [80]. In addition, molecular characterization of single CTC holds considerable promise for predictive biomarker assessment enabling scientists to explore CTC heterogeneity. The application of reliable

single CTC isolation together with CTC molecular characterization using advanced next-generation sequencing technologies is opening new frontiers in the management of cancer patients [81].

Peeters *et al.* explored potential differences in the detection and prognostic significance of CTCs in metastatic breast cancer (MBC) based on immunohistochemical subtypes of breast cancer. They used the enumeration of CTCs with the EpCAM-based CellSearch system to determine the prognostic significance of these CTCs in patients with MBC. The EpCAM-based CTCs detected were not associated with any of the immunohistochemical subtypes of breast cancer in patients before first-line treatment; however, potentially clinically relevant differences were observed at very high CTC counts. Their results suggested a lower prognostic significance of CTC evaluation in HER2-positive patients with MBC [82].

Nygaard *et al.* examined the potential prognostic value of circulating cell-free DNA (cfDNA) in malignant disease. The level of cfDNA increases with malignancy but the biological mechanism is not fully understood. In a prospective biomarker trial in 53 patients with advanced non-small cell lung cancer (NSCLC), Nygaard *et al.* used positron emission tomography (PET) to examine the correlation between cfDNA and total tumor burden. There were no correlations between cfDNA and MTV ($r \leq 0.1$) or TLG ($r \leq 0.1$); however, cfDNA 475th percentile was correlated with shorter OS ($P \leq 0.02$) as confirmed by multivariate analysis. MTV 4the median was associated with a significantly shorter OS ($P \leq 0.02$). Nygaard *et al.* concluded that there was no significant difference in OS according to TLG ($P \leq 0.08$); thus, cell-free DNA may not be a simple measure of tumor burden, but seems to reflect more complex mechanisms of tumor biology, making it attractive as an independent prognostic marker [83].

Primarily, due to drug resistance, metastatic cancer patients face a prognosis largely affected by treatment failure. Circulating tumor-specific microRNAs (miRNAs) are promising biomarkers of tumor presence and recurrence, especially for diseases whose best chance of successful treatment requires early diagnosis and timely surgery of an already malignant but not yet invasive tumor such as colorectal cancer (CRC). Chemotherapy could miss CTCs, particularly a subpopulation of more aggressive stem-like CTCs characterized by multidrug resistance. Therefore, Gazzaniga *et al.* investigated the prognostic value of drug resistance and stemness markers in CTCs derived from 40 metastatic colorectal cancer patients that had been treated with oxaliplatin (L-OHP) and 5-fluoruracil (5-FU). Their results support the idea that isolating survivin and MRP5 CTCs may help in the selection of metastatic colorectal cancer patients resistant to standard 5-FU and L-OHP-based chemotherapy and for which alternative regimens may be appropriate [84].

MicroRNAs (miRNAs) are small (22 nt), tissue-based regulatory RNAs that are frequently dysregulated in cancer and have shown promise in cancer

classification and prognosis. Mitchell *et al.* [85] showed that such miRNAs are present in human plasma in a remarkably stable form that is protected from endogenous RNase activity. miRNAs originating from human prostate cancer xenografts enter the circulation, are readily measured in plasma, and can be used to easily distinguish xenografted mice from controls. This concept also applies to human cancer, where serum levels of miR-141 (a miRNA expressed in prostate cancer) can distinguish patients with prostate cancer from healthy controls.

Expression levels of miRNAs found to be differentially expressed in tumor versus normal colon tissues were investigated by Zanutto *et al.* [86]. They employed quantitative real-time PCR using plasma from CRC patients and from healthy donors and confirmed their results in independent case control series. Validated miRNAs were also measured in patients following surgery. Zanutto *et al.* identified four miRNAs differentially expressed between the compared groups. Two of these, miR-21 and miR-378, were validated and miR-378 expression was observed to decrease in nonrelapsed patients 4–6 months after surgery. Hemolysis did not influence the ability of miR-378 to discriminate CRC patients from healthy individuals. Zanutto *et al.* concluded that analysis of miRNA expression in plasma samples represents a useful noninvasive tool to assess the presence of CRC as well as tumor-free status at follow-up. They also concluded that plasma levels of miR-378 could be used to discriminate CRC patients from healthy individuals, irrespective of hemolysis [86].

Wang *et al.* [87] have reported that the circulating miRNAs, miR-17-5p, and miR-20a (miR-17-5p/20a) are elevated in the plasma of gastric cancer patients. However, the clinical significance of the circulating levels of these miRNAs, their prognostic predictive power, and their application in monitoring the effectiveness of chemotherapy remains unclear. To this end, Wang *et al.* measured plasma miR-17-5p/20a levels in unpaired preoperative ($n = 65$), postoperative ($n = 16$), and relapse ($n = 6$) groups of gastric cancer patients. Their results suggest that the levels of circulating miR-17-5p/20a may be a promising noninvasive molecular marker for pathological progression of gastric cancer, as well as prognosis and monitoring of results of chemotherapy.

Lu *et al.* [88] reported that nasopharyngeal carcinoma (NPC) has a distinctive geographic distribution and is characterized by its strong tendency of metastasis. They examined the microarray expression profiles of miRNAs in plasma samples of NPC patients to explore their clinical significance in disease development and progression. Their research identified 33 differentially expressed miRNAs between NPC patients and healthy volunteers and reported that plasma miR-9 may serve as a useful biomarker to predict NPC metastasis and to monitor tumor dynamics.

Shapira *et al.* [89] generated comprehensive miRNA profiles on presurgical plasma samples from 42 women with confirmed serous epithelial ovarian cancer, 36 women diagnosed with a benign neoplasm, and 23 comparable

age-matched women with no known pelvic mass. Twenty-two miRNAs were differentially expressed between healthy controls and the ovarian cancer group ($P < 0.05$) and six miRNA subset distinguished presurgical plasma from benign and ovarian cancer patients. Significant differences in miRNA profiles in presurgical plasma were observed in women diagnosed with ovarian cancer who had overall short survival when compared to women with overall long survival ($P < 0.05$). The preliminary data supported the utility of circulating plasma miRNAs to distinguish women with ovarian cancer from those with a benign mass and to distinguish women likely to benefit from currently available treatment for serous epithelial ovarian cancer from those who may not [89].

The science of using tumor molecular profiles to select clinical trial participants or to optimize therapy for individual patients is still in its infancy. However, the potential importance of methods that can integrate molecular, histopathological, and clinical information into a synergistic understanding of tumor progression cannot be overstated. While the possibilities are exciting, significant challenges remain before they can be effectively implemented with a strong evidence base and in a widely available and cost-effective manner [90].

1.4 Potential Cancer Translational Toxicology Therapies

Integrative medicine, as defined by Block *et al.* [91], is an approach to health and healing that "makes use of all appropriate therapeutic approaches, health care professionals, and disciplines to achieve optimal health and healing [91]." An integrative medicine intervention for cancer patients typically includes nutritional counseling, biobehavioral strategies, promotion of physical activity, and dietary supplements (including herbs, nutraceuticals, and phytochemicals). A comprehensive intervention of this type may contribute uniquely to improved cancer outcomes through its impact on a variety of relevant molecular targets, including effects on multiple cancer hallmarks [92,93]. Hallmarks that may be particularly affected include genetic instability, tumor-promoting inflammation, deregulated metabolism, and immune system evasion. Block *et al.* [91] characterize these hallmarks as metabolic since they are susceptible to manipulation by diet, exercise, and supplementation. Research on such comprehensive integrative approaches can contribute to the development of systems of multitargeted treatment regimens and help clarify the combined effect of these approaches on cancer outcomes [91].

Potential cancer translational toxicology therapies are discussed in Chapter 18. Examples of potential components of integrated therapies include incorporation of exercise into prenatal care that yields improvements in offspring metabolic disorders, cancer, and cardiovascular health and disease risk [94], and use of vitamin supplementation among middle-aged women, which is associated with substantial reduction in risk of all invasive cancers combined [95].

Maternal behaviors during pregnancy have been reported to impact offspring health in adulthood. Blaize *et al.* [94] explored the hypothesis that exercise during pregnancy can protect against chronic disease susceptibility in the offspring. To date, research has demonstrated that improvements in metabolic outcomes, cardiovascular risk, and cancer can occur in response to maternal exercise during pregnancy, including improvements in offspring metabolic disorders, cancer, and cardiovascular health and disease risk. Thus, overall, the current body of work supports the recommendations for exercise during pregnancy set by the ACOG and DHHS. Health care providers can use these data to educate pregnant mothers on the benefits of exercise during pregnancy for their offspring and encourage them to incorporate exercise into their prenatal care [94].

Regarding the use of vitamin supplementation among middle-aged women, higher serum 25-hydroxyvitamin D [25(OH)D] concentrations have been associated with a lower risk of multiple cancer types. McDonnell *et al.*[95] investigated whether the inverse association between 25(OH)D and cancer risk could be replicated, and if a 25(OH)D response region could be identified among women aged 55 years and older across a broad range of 25(OH)D concentrations. Age-adjusted cancer incidence was studied across a combined cohort ($N = 2304$) with 840 cases per 100,000 person-years (1020 per 100,000 person-years in the Lappe cohort and 722 per 100,000 person-years in the Grassroots Health cohort). Indeed, incidence was lower at higher concentrations of 25(OH)D. Women with 25(OH)D concentrations equal to or greater than 40 ng/ml had a 67% lower risk of cancer than women with concentrations <20 ng/ml (HR = 0.33, 95% CI = 0.12–0.90). In conclusion, 25(OH)D concentrations equal to or greater than 40 ng/ml were associated with substantial reduction in risk of all invasive cancers combined [95].

Gynecologic cancers constitute the fourth most common cancer type in women. Phytochemicals are a broad class of natural compounds derived from plants, a number of which exhibit useful bioactive effects toward these pathways. High-throughput screening methods, rational modification, and developments in regulatory policies will accelerate the development of novel therapeutics based on these compounds, which will likely improve overall survival and quality of life for patients [96].

Although growing evidence from trials and population-based studies has supported a protective role for dietary flavonoids in relation to risk of certain chronic diseases, the underlying mechanisms remain unclear. In particular, the impact of different dietary flavonoid subclasses on risk of epithelial ovarian cancer is also unclear with limited previous studies that have focused on only a few compounds. Some studies have focused on individual inflammatory biomarkers, but because of the limited specificity of any individual marker, an assessment of a combination of biomarkers may be more informative.

Cassidy *et al.* [97] prospectively examined associations between habitual flavonoid subclass intake and risk of ovarian cancer. They followed 171,940

Nurses' Health Study and Nurses' Health Study II participants to examine associations between intakes of total flavonoids and their subclasses (flavanones, flavonols, anthocyanins, flavan-3-ols, flavones, and polymeric flavonoids) and risk of ovarian cancer. Intake was calculated from validated food frequency questionnaires collected every 4 years. Participants in the highest quintiles of flavonol and flavanone intakes had modestly lower risk of ovarian cancer than that of participants in the lowest quintile, although the *P*-trend was not significant (HRs: 0.76 (95% CI: 0.59, 0.98; *P*-trend = 0.11) and 0.79 (95% CI: 0.63, 1.00; *P*-trend = 0.26), respectively). The authors concluded that higher intakes of flavonols, flavanones, and black tea (polyphenol) consumption may be associated with lower risk of ovarian cancer, but that additional prospective studies are required to confirm their findings [97].

1.4.1 Well-Established/Repurposed Pharmaceuticals

Drug repurposing (also known as repositioning) is the application of known drugs and compounds for new clinical indications. Colesevelam is an example of drug repurposing. Colesevelam was developed as an adjunct to diet and exercise to reduce elevated low-density lipoprotein cholesterol (LDL-C) in patients with primary hyperlipidemia. It has also gained approval to improve glycemic control in adults with type 2 diabetes mellitus. For other well-established drugs, such as aspirin, the number of indications has expanded based on continuing research, drug use, and experience.

Aspirin or acetylsalicylic acid (ASA) is a classic, nonsteroidal anti-inflammatory drug (NSAID) that is widely used to relieve minor aches and pains and to reduce fever. Aspirin is certainly one of the most established/repurposed pharmaceuticals and there is a significant body of epidemiological evidence demonstrating that regular aspirin use is associated with a decreased incidence of developing cancer. Langley *et al.* [98] have reported that aspirin has several mechanisms of action, independent of its inhibition of the enzyme cyclooxygenase (Cox) that may contribute to its anticancer effect. Thus, aspirin also influences cellular processes, such as apoptosis and angiogenesis that are crucial for the development and growth of malignancies. Evidence suggests that these effects can occur through Cox-independent pathways which places into question the rationale of focusing on Cox-2 inhibition alone as an anticancer strategy [98].

Randomized studies with aspirin primarily designed to prevent cardiovascular disease have demonstrated a reduction in cancer deaths with long-term follow-up. Concerns about toxicity, particularly haemorrhage, have limited the use of aspirin in cancer prevention. However, recent epidemiological evidence demonstrating that regular aspirin use after a diagnosis of cancer improves outcomes suggesting that it may have a role as an adjuvant, where the risk–benefit ratio will be different [98].

Regular aspirin use is associated with reduced risk of several malignancies. Epidemiologic studies analyzing aspirin, nonaspirin NSAIDs, and acetaminophen use and ovarian cancer risk have been inconclusive. Trabert *et al.* [99] reported analyses of pooled data from 12 population-based case–control studies of ovarian cancer, including 7776 case patients and 11843 control subjects accrued between 1992 and 2007. They found that aspirin use was associated with a reduced risk of ovarian cancer (OR = 0.91; 95% confidence interval (CI) = 0.84–0.99). Similar but not statistically significant results were obtained for nonaspirin NSAIDs, but there was no association with acetaminophen. In seven studies with frequency data, the reduced risk was strongest among daily aspirin users (OR = 0.80; 95% CI = 0.67–0.96). In three studies with dose information, the reduced risk was strongest among users of low dose (<100 mg) aspirin (OR = 0.66; 95% CI = 0.53–0.83), whereas for nonaspirin NSAIDs, the reduced risk was strongest for high dose (≥500 mg) usage (OR = 0.76; 95% CI = 0.64–0.91). In summary, aspirin use was associated with a reduced risk of ovarian cancer, especially among daily users of low-dose aspirin. These findings suggest that the 81 mg/day aspirin regimen proven to protect against cardiovascular events and several cancers could reduce the risk of ovarian cancer 20–34% depending on frequency and dose of use [99].

Epidemiological studies and other experimental studies suggest that ASA use reduces the risk of different cancers, including BC, and may be used as a chemopreventive agent against BC and other cancers. These studies have raised the tempting possibility that ASA could serve as a preventive medicine for BC. However, lack of in-depth knowledge of the mechanism of action of ASA reshapes the debate of risk and benefit of using ASA in prevention of BC. Studies by Maity *et al.* [100], using *in vitro* and *in vivo* tumor xenograft models, show a strong beneficial effect of ASA in the prevention of breast carcinogenesis. ASA not only prevents breast tumor cell growth *in vitro* and tumor growth in nude mice xenograft model through the induction of apoptosis, but also significantly reduces the self-renewal capacity and growth of breast tumor-initiating cells (BTICs)/breast cancer stem cells (BCSCs) and delays the formation of a palpable tumor. Moreover, ASA regulates other pathophysiological events in breast carcinogenesis, such as reprogramming the mesenchymal to epithelial transition (MET) and delaying *in vitro* migration in BC cells. The tumor growth inhibitory and reprogramming roles of ASA could be mediated through inhibition of TGF-β/SMAD4 signaling pathway that is associated with growth, motility, invasion, and metastasis in advanced BCs. Collectively, ASA has a therapeutic or preventive potential by attacking possible target, such as TGF-β, in breast carcinogenesis [100].

After skin cancer, prostate cancer is the most common cancer among men and it can often be treated successfully. More than 2 million men in the United States are prostate cancer survivors (American Cancer Society). The first description of the benefits of surgical castration in the treatment of prostate

cancer occurred 70 years ago. Despite advances in medical therapy (e.g., cabazitaxel, enzalutamide, abiraterone), androgen deprivation therapy (ADT) remains the cornerstone of treatment for advanced prostate cancer. The costs of ADT have risen dramatically, with uncertain survival benefits and substantial associated risks. At the same time, increasing numbers of men are undergoing prostate specific antigen (PSA) testing and prostate cancer is being diagnosed earlier [101].

Clinical studies of potent novel agents have shown survival benefits even in advanced disease, and with more aggressive treatment, men are remaining on ADT for much longer than might have been originally anticipated. The evidence is good that treatment of advanced prostate cancer by ADT results in improvements in symptoms in men with end-stage disease but, there is weak evidence for improvement in survival, except when ADT is combined with radical local treatment, particularly radiotherapy. As new agents enter clinical practice, a comprehensive research strategy is essential to optimize benefits while minimizing harm. According to Bourke *et al.* [101], it is only a matter of time before they will be considered in earlier disease, possibly as a new form of maximal androgen blockade.

Breast cancer is one of the most commonly diagnosed cancers in women. Fabian *et al.* [102] have reported that high intake ratios of the marine omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) relative to the omega-6 arachidonic acid reduced risk of breast cancer compared to those with low ratios in some but not all case–control and cohort studies. Plausible mechanisms include reduction in proinflammatory lipid derivatives, inhibition of nuclear factor-κB-induced cytokine production, and decreased growth factor receptor signaling as a result of alteration in membrane lipid rafts [102].

The research team at the Comparative Toxicogenomics Database (CTD) and a group of safety researchers at Pfizer developed a collaboration to text mine and manually review a collection of 88,629 articles relating over 1200 pharmaceutical drugs to their potential involvement in cardiovascular, neurological, renal, and hepatic toxicity. The CTD curates chemical–gene–disease interactions, and these detailed, contextualized, high-quality annotations curated from the past 70 years of scientific literature are now fully integrated with public CTD and phenotype interactions can be downloaded. Importantly, this curation can be leveraged for information about toxicity important to drug safety and enable researchers to develop testable hypotheses for drug–disease events. The availability of these curated chemical–gene–disease interactions will help facilitate development of new mechanistic screening assays for pharmaceutical compounds. This partnership demonstrates the value of public/private research data sharing and collaboration and the complementary needs of the pharmaceutical and environmental health science research communities (http://ctdbase.org/) [103].

With the recognition that histone deacetylases (HDACs) are a key component of the epigenetic machinery regulating gene expression, and behave as

oncogenes in several cancer types, there has been major interest in development of histone deacetylase inhibitors (HDACi) as anticancer drugs. A recent review by Ceccacci and Minucci [104] discusses results of the new research regarding the role of HDACs in cancer and the effect of HDACi on tumor cells, emphasizing haematological malignancies, particularly acute myeloid leukemia. HDACs can have opposite roles at various stages of tumor progression and in different subpopulations of cancer stem cells, which emphasizes the importance of investigating these attributes in conjunction with the clinical use of HDACi in cancer therapy.

Ceccacci and Minucci [104] reported that Pan-HDACi have given promising results in a small group of patients with selected haematological diseases, but their use individually has not been satisfactory. The difference in sensitivity to HDACi cannot be attributed to a single cause, making it difficult to envision a simple approach to patient stratification. This field deserves further study and remains a promising therapeutic avenue if selective HDACi prove to be more effective in the clinic with fewer side effects.

Studies in murine models of leukemia suggest that it is necessary to consider not only the differences among different classes of HDACs but also how the same molecules may act in "time" and "space." Ceccacci and Minucci have proposed a systematic study of the effects of HDACi and other epidrugs on the stem cell compartment versus the rest of tumor cells to devise treatment schemes to combine drugs targeting the different tumor cell subpopulations. Thus, the combination of epidrugs with DNA-damaging chemotherapeutic drugs, or proteasome inhibitors, has already shown promising results [104].

1.4.2 GRAS/GRASE, Diet, and Nutraceuticals

To reduce the occurrence of preventable cancers, we believe that timely protective interventions during "critical windows" should include not only minimization of hazardous voluntary exposures and substances of abuse but also the active use of protective GRAS interventions/therapies, including nutritional, dietary supplementation, including nutraceuticals, and/or well-established/repurposed pharmaceutical drugs. Repurposed pharmaceutical drugs must be safe and effective (GRASE) for the intended application. A drug is not considered a new drug by the USFDA *only* when it is generally recognized as safe and effective (GRASE). At a minimum, the general acceptance of a product as GRASE must be supported by the same quality and quantity of scientific and/or clinical data necessary to support the approval of a New Drug Application [105].

Diet clearly plays a major role in cancer risk. Lifestyle factors, including diet, have long been recognized as potentially important determinants of both susceptibility to and survival with many types of cancer. For details on diet factors in cancer risk and voluntary exposures – natural herbals, supplements,

and substances of abuse, refer to Chapters 5 and 6, respectively. Chapter 10 deals with dietary/supplemental interventions and personal dietary preferences for cancer reduction.

In this section we will address primarily the protective role of natural substances in the diet. For example, the association between coffee intake, tea intake, and cancer has been extensively studied, but associations are not established for many cancers. Certain other dietary agents, such as natural products, have been reported to show anticancer effects or potential beneficial effects for other diseases via a MOA that is plausibly relevant to reduction of cancer risk(s). However, the underlying mechanisms of these substances in human cancer often remain unclear. We have attempted to group studies according to putative mechanisms.

1.4.2.1 Suppression of Cell Proliferation and Induction of Cell Death

Epidemiological analysis has demonstrated a negative or inverse correlation between green tea consumption and the risk of non-Hodgkin lymphoma and prostate cancer. Recent studies show that epigallocatechin-3-gallate (EGCG), the major green tea polyphenol, suppresses the proliferation of cancer cells and induces cell death without adversely affecting normal cells. Several molecular mechanisms have been suggested to be responsible for this effect. Thus, the 67-kDa laminin receptor (67LR) was recently identified as the sensing molecule for EGCG [106]. This receptor overexpresses in cancer cells and plays a crucial role in the selective toxicity of EGCG.

Kumazoe *et al*. [106] focused on the molecular mechanism of EGCG and developing a novel strategy to amplify its effect. They identified 67LR as the sensing molecule of EGCG, and also revealed the downstream mechanism of EGCG-induced cell death and growth inhibition. Their studies demonstrated that 67LR acts as the cancer-overexpressed death receptor that induces the apoptotic signaling pathway. Their findings revealed that 67LR could be an attractive target for cancer chemotherapy and provide a rationale for the clinical value of EGCG as a 67LR-targeting drug. Based on the putative molecule mechanisms of EGCG-induced anticancer effect, PDE5, SET, and SphK1 act as resistant factors against EGCG, and also provided a novel strategy to amplify its anticancer effect [106].

Hashibe *et al.* [107] reported that previous investigations are not consistent on whether caffeine may be the source of possible associations between coffee and cancer risk. In the prostate, lung, colorectal, and ovarian cancer screening trial, of the 97,334 eligible individuals, 10,399 developed cancer. Cancers observed were 145 head and neck, 99 oesophageal, 136 stomach, 1137 lung, 1703 breast, 257 endometrial, 162 ovarian, 3037 prostate, 318 kidney, 398 bladder, 103 gliomas, and 106 thyroid. Hashibe *et al.* [107] reported that mean coffee intake was higher in lower education groups, among current smokers, among heavier and longer duration smokers, and among heavier alcohol drinkers. However, coffee intake

was not associated with the risk of all cancers combined (RR = 1.00, 95% confidence interval (CI) = 0.96–1.05). Tea drinking was associated with a slightly decreased risk of cancer overall (RR = 0.95, 95% CI = 0.94–0.96 for 1+ cups per day versus <1 cup per day). For endometrial cancer, a definite decreased risk was observed for coffee intake (RR = 0.69, 95% CI = 0.52–0.91 for greater than or equal to 2 cups per day). Caffeine intake from either substance was not associated with cancer risk in a dose–response manner. Hashibe *et al.* concluded that they observed a decreased risk of endometrial cancer for coffee intake, and a decreased risk of cancer overall with tea intake [107].

Plant food-derived polyphenolic compounds as a group are low in toxicity, and many of them have been proven to modulate key factors in cancer drug resistance, making them good candidates for reversing cancer resistance. Wang *et al.* [108] analyzed the combination effect of two chemopreventive polyphenols, curcumin (Cur) and EGCG, in reversing resistant breast cancer. Their results showed that EGCG significantly enhanced the growth inhibition and apoptosis in both doxorubicin (DOX)-sensitive and doxorubicin (DOX)-resistant MCF-7 cells induced by Cur. They believe that the mechanism may be related to the further activation of caspase-dependent apoptotic signaling pathways and the enhanced cellular incorporation of Cur by inhibiting the P-glycoprotein (P-gp) pump. They also suggested that Cur and EGCG in combination could enhance the toxicity of DOX, increasing the intracellular level of DOX in resistant MCF-7 cells. Their findings with the combination of Cur and EGCG encourage the treatment of human breast cancer resistance by combining two low-toxic chemotherapeutic agents from diet [108].

1.4.2.2 Anti-Inflammatory Effects: Insights from Various Diseases

The purpose of a study by McFarlin *et al.* [109] was to determine the effects of oral Cur supplementation (Longvida® 400 mg/days) on muscle and related activities, muscle soreness following exercise, creatine kinase, and inflammatory cytokines (TNF-α, IL-6, IL-8, IL-10) following eccentric-only dual-leg press exercise (EMID). Subjects ($N = 28$) were randomized to either Cur (400 mg/day) or placebo (rice flour), and were given supplements two days before to four days after EMID. The study demonstrated that Cur supplementation reduced biological inflammation but *not* quadriceps muscle soreness during recovery after EIMD. The authors suggested that observed improvements in biological inflammation may translate to faster recovery and greater functional capacity during subsequent exercise sessions, and that the next step would be to evaluate further the efficacy of an inflammatory clinical disease model [109].

In a cross-sectional analysis of 2375 Framingham Heart Study Offspring Cohort participants, Cassidy *et al.* [110] used an inflammation score (IS) to integrate 12 individual inflammatory biomarkers associated with intake of different flavonoid classes. Intakes of total flavonoids and their classes (anthocyanins, flavonols, flavanones, flavan-3-ols, polymers, and flavones) were calculated from validated

food frequency questionnaires. Individual inflammatory biomarkers were ranked, standardized, and summed to derive an overall IS (and subgroup scores of functionally related biomarkers). Their results remained significant after adjustment for physical activity, and vitamin C and fruit and vegetable intakes: Higher anthocyanin intake was inversely associated with all biomarker subgroups, whereas higher flavonol intake was associated with lower cytokine and oxidative stress biomarker concentrations. The authors concluded that these findings suggest that an anti-inflammatory effect may be a key component underlying the reduction in risk of certain chronic diseases associated with higher intakes of anthocyanins and flavonols [110].

During the past 15 years, there has been a substantial increase in interest in triterpenes (members of phytosterol family) due to their cholesterol lowering properties. Saleem [111] reported at least 25 clinical studies, 20 patents, and 10 major commercial triterpene-based products currently being sold worldwide. Lupeol, also known as Fagarsterol, is a triterpene found in vegetable oils, white cabbage, green pepper, strawberry, olive, mangoes, and grape. In the West, humans consume an average of 250 mg of triterpenes per day. The review by Saleem [111] provides a detailed account of preclinical studies conducted to determine the utility of Lupeol as a therapeutic and chemopreventive agent for the treatment of inflammation and cancer. These studies suggest that it is a multitarget agent with major anti-inflammatory potential involving key molecular pathways that include nuclear factor kappa B (NFκB), cFLIP, Fas, Kras, phosphatidylinositol-3-kinase (PI3K)/Akt, and Wnt/β-catenin in a variety of cells. Importantly, therapeutic effective doses of Lupenol exhibit no toxicity to normal cells and tissues [111].

Autism spectrum disorders (ASDs) have been associated with brain inflammation as indicated by microglia activation, as well as brain expression and increased plasma levels of interleukin-6 (IL-6) and tumor necrosis factor (TNF). Tsilioni *et al.* [112] reported that serum levels of IL-6 and TNF were elevated (61.95 ± 94.76 pg/ml and 313.8 ± 444.3 pg/ml, respectively) in the same cohort of patients with elevated serum levels of corticotropin-releasing hormone (CRH) and neurotensin (NT), while IL-9, IL-31, and IL-33 were not different from controls. The elevated CRH and NT levels did *not* change after treatment with a dietary formulation containing luteolin. The natural flavonoid luteolin has antioxidant, anti-inflammatory, mast cell blocking, and neuroprotective effects. In the study by Tsilioni *et al.* [112], the mean serum IL-6 and TNF levels decreased significantly ($P = 0.036$ and $P = 0.015$, respectively) at the end of the treatment period (26 weeks) as compared with levels at the beginning; these decreases were strongly associated with children whose behavior improved the most after luteolin formulation treatment. The results obtained indicate that there are distinct subgroups of children within the ASDs that may be identifiable through serum levels of IL-6 and TNF and that these cytokines may constitute distinct prognostic markers for the beneficial effect of the luteolin formulation [112].

The aim of a study by Dawson *et al.* [113] was to assess whether omega-3 polyunsaturated fatty acid supplementation alone or in combination with folic acid and B-group vitamins is effective in lowering homocysteine. Lowering homocysteine levels with folic acid and B-vitamins could interfere with cognitive decline and Alzheimer's. The Medline Ovid, Embase, and Cochrane databases were searched for randomized-controlled trial studies that intervened with omega-3 supplementation (with or without folic acid) and measured changes in homocysteine concentration. A total of 3267 participants completed 21 trials. Across all trials, omega-3 supplementation was effective in lowering homocysteine by an average of 1.18 μmol/l (95%CI: (−1.89, −0.48), $p = 0.001$). The average homocysteine lowering effect was greater when omega-3 supplementation was combined with folic acid and B-group vitamins (−1.37 μmol/l, 95%CI: (−2.38, −0.36), $p < 0.01$) compared to omega-3 supplementation alone (−1.09 μmol/l 95%CI: (−2.04, −0.13), $p = 0.03$). Omega-3 polyunsaturated fatty acid supplementation was associated with a modest reduction in homocysteine. The authors concluded that for the purpose of reducing homocysteine, a combination of omega-3s (0.2–6 g/day), folic acid (150–2500 μg/day), and vitamins B6 and B12 might be more effective than omega-3 supplementation alone [113].

1.4.2.3 Upregulation of Tumor Suppressor MicroRNAs

Hagiwara *et al.* [114] found that resveratrol exerts an anticancer effect by upregulating tumor suppressor microRNAs (miRNAs). In further study, they aimed to identify new dietary products that have the same ability to activate tumor suppressor miRNAs and therefore may serve as novel tools for the prevention and treatment of human cancers. They have described the generation and use of an original screening system based on a luciferase-based reporter vector for monitoring miR-200c tumor suppressor activity.

By screening a library containing 139 natural substances, three natural compounds – enoxolone, magnolol, and palmatine chloride – were identified as being capable of inducing miR-200c expression in breast cancer cells at 10 μM. Moreover, these molecules suppressed the invasiveness of breast cancer cells *in vitro*. Next, they identified a molecular pathway by which the increased expression of miR-200c induced by natural substances led to ZEB1 inhibition and E-cadherin induction. These results indicate that their method may be a valuable tool for identification of natural molecules that exhibit tumor suppressor activity in human cancer mediated through miRNA activation [114].

1.4.2.4 Regulation of Oxidative Stress

In their review paper, Gorrini *et al.* [115] discuss the controversial role of reactive oxygen species (ROS) in tumor development and in response to anticancer therapies, and the idea that targeting the antioxidant capacity of tumor cells can have a positive therapeutic impact [115]. As has been discussed

earlier, the regulation of oxidative stress is an important factor in both tumor development and response to anticancer therapies. Many signaling pathways that are linked to tumorigenesis can also regulate the metabolism of reactive oxygen species (ROS) through direct or indirect mechanisms. High ROS levels are generally detrimental to cells, and the redox status of cancer cells typically differs from that of normal cells. Because of metabolic and signaling aberrations, cancer cells exhibit elevated ROS levels. The observation that this is balanced by increased antioxidant capacity suggests that high ROS levels may constitute a barrier to tumorigenesis. However, ROS can also promote tumor formation by inducing DNA mutations and prooncogenic signaling pathways. These contradictory effects have important implications for potential anticancer strategies that aim to modulate levels of ROS [115].

Antioxidants are widely used to protect cells from damage induced by ROS. Sayin *et al.* [116] have asserted that the concept that antioxidants can help fight cancer is widely held in the general population and promoted by the food supplement industry, although clinical trials have reported inconsistent results. The authors show that supplementing the diets of mouse models of B-RAF- and K-RAS-induced lung cancer with the antioxidants N-acetylcysteine (NAC) and vitamin E markedly increases tumor progression and reduces survival. Furthermore, RNA sequencing revealed that NAC and vitamin E produce highly coordinated changes in tumor transcriptome profiles that are dominated by reduced expression of endogenous antioxidant genes. NAC and vitamin E also increase tumor cell proliferation by reducing ROS, DNA damage, and p53 expression in mouse and human lung tumor cells. The inactivation of p53 increases tumor growth to a similar degree as antioxidants and abolishes the antioxidant effect. Thus, antioxidants accelerate tumor growth by disrupting the ROS-p53 axis. Because somatic mutations in p53 occur late in tumor progression, antioxidants may accelerate the growth of early tumors or precancerous lesions in certain high-risk populations, such as smokers and patients with chronic obstructive pulmonary disease, who receive NAC to relieve mucus production [116].

Some trials show that antioxidants actually increase cancer risk. The study in mice by Sayin *et al*. reported that antioxidants accelerate the progression of primary lung tumors; however, little is known about the impact of antioxidant supplementation on the progression of other types of cancer, including malignant melanoma. Le Gal *et al.* [117] show that administration of NAC increases lymph node metastases in a mouse model of malignant melanoma but does not alter the number and size of the primary tumors. These results demonstrate that antioxidants and the glutathione system play a previously unrecognized role in the progression of malignant melanoma [117].

1.4.2.5 Activation of Signal Transduction Pathways

Lupeol is a pharmacologically active triterpenoid found in white cabbage, green pepper, strawberry, olive, mangoes, and grapes. Siveen *et al.* [118] evaluated the

effect of lupeol on the STAT3 signaling cascade and its regulated functional responses in HCC cells. The constitutive activation of STAT3, a signal transducer and activator of transcription signaling, has been linked with survival, proliferation, and angiogenesis in a wide variety of malignancies, including hepatocellular carcinoma (HCC). Lupeol effectively suppressed constitutive activation of STAT3 phosphorylation at the tyrosine 705 residue in a dose- and time-dependent manner. The phosphorylation of Janus-activated kinases (JAKs) 1 and 2 and the protooncogene tyrosine–protein kinase, Src, was also suppressed by lupeol. Thus, lupeol exhibited its potential anticancer effects in HCC through the downregulation of the STAT3-induced prosurvival signaling cascade [118].

Ascorbate, at millimolar concentrations, acts as a pro-oxidant, induces DNA damage and depleted cellular adenosine triphosphate (ATP), activates the ataxia-telangiectasia mutated (ATM)/adenosine monophosphate-activated protein kinase (AMPK) pathway, and results in mTOR (mammalian target of rapamycin) inhibition and death in ovarian cancer cells. The Akt/mammalian target of rapamycin (mTOR) signaling pathway serves as a critical regulator of cellular growth, proliferation, and survival. Akt aberrant activation has been implicated in carcinogenesis and anticancer therapy resistance. The combination of parenteral ascorbate with the chemotherapeutic agents carboplatin and paclitaxel synergistically inhibited ovarian cancer in mouse models and reduced chemotherapy-associated toxicity in patients with ovarian cancer. On the basis of its potential benefit and minimal toxicity, Ma *et al.* [119] recommended further study of intravenous ascorbate in combination with standard chemotherapy in larger clinical trials [119].

Piperlongumine (PL), a natural alkaloid present in the fruit of the Long pepper, is known to exhibit notable anticancer effects. Makhov *et al.* [120] investigated the impact of PL on Akt/mTOR signaling. Makhov *et al.* [120] examined Akt/mTOR signaling in cancer cells of various origins including prostate, kidney, and breast after PL treatment. They demonstrated for the first time that PL effectively inhibits phosphorylation of Akt target proteins in all tested cells. Makhov *et al.* then investigated the efficacy of *in vivo* treatment with PL and the autophagy inhibitor, chloroquine (CQ), using a mouse xenograft tumor model. The downregulation of Akt downstream signaling resulted in a decrease of mTORC1 activity and autophagy stimulation. Using the autophagy inhibitor, CQ, the level of PL-induced cellular death was significantly increased. Combination treatment with PL and CQ demonstrated a substantial antitumor effect in the xenograft mouse model. Their data suggest therapeutic opportunities to mediate cancer cellular death using PL, offering a new paradigm for both prevention and treatment of malignancy [120].

1.4.2.6 Mitigating Inherited Deleterious Mutations

Studies have shown that 3,3′-diindolylmethane (DIM) can upregulate BRCA1 expression in breast cancer cells. Haplo-insufficiency may

contribute to the development of breast cancer among women with a BRCA1 mutation. Thus, interventions that enhance BRCA1 expression may represent avenues for prevention. However, this has yet to be demonstrated *in vivo*. Kotsopoulos *et al.* [121] performed a study to evaluate the ability of orally administered DIM to upregulate BRCA1 mRNA expression in white blood cells. Eighteen women were enrolled in the study. Under the tested conditions, oral DIM was associated with an increase in BRCA1 mRNA expression in women having a BRCA1 mutation. Thus, the possibility exists of mitigating the effect of an inherited deleterious BRCA1 mutation by increasing the physiologic expression of the gene and normalizing protein levels. This approach represents a clinically important paradigm shift in the prevention strategies available to these high-risk women. Kotsopoulos *et al.* concluded that future studies with a larger sample size and higher doses of DIM are warranted [121].

Nucleostemin is a GTPase residing in the nucleolus that is considered to be an important cancer stem/progenitor cell marker protein due to its high expression levels in breast cancer stem cells and its role in tumor initiation of human mammary tumor cells. Tin *et al.* [122] proposed that nucleostemin might represent a promising therapeutic target for breast cancer. They used a new breast cancer cell line, 10AT-Her2, which is highly enriched in cells having a stem/progenitor cell-like character. 10AT-Her2 cells display a CD44+/CD24-/low phenotype with high levels of the cancer stem/progenitor cell marker protein nucleostemin, as well as active aldehyde dehydrogenase-1 (ALDH-1). 10AT-Her2 cells are highly sensitive to the antiproliferative apoptotic effects of indole-3-carbinol (I3C).

I3C is a natural anticancer indole carbinol found in cruciferous vegetables of the Brassica genus, such as broccoli and cabbage. I3C promotes the interaction of nucleostemin with MDM2 (murine double mutant 2), an inhibitor of the p53 tumor suppressor, and disrupts the MDM2 interaction with p53. I3C also induces nucleostemin to sequester MDM2 in the nucleolus compartment, thereby freeing p53 to mediate its apoptotic activity. Small interfering RNA knockdown studies of nucleostemin demonstrated functionally that nucleostemin is required for I3C to trigger its cellular antiproliferative responses, to inhibit tumorsphere formation, and to disrupt MDM2–p53 protein–protein interactions. In addition, expression of an I3C-resistant form of elastase, the only known target protein for I3C, prevented I3C antiproliferative responses in cells and in tumor xenografts *in vivo*, as well as disrupting the I3C-stimulated nucleostemin–MDM2 interactions. The results of Tin *et al.* [122] provide the first evidence that a natural anticancer compound mediates its cellular and *in vivo* tumor antiproliferative responses by selectively stimulating cellular interactions of the stem/progenitor cell marker nucleostemin with MDM2, freeing p53 to trigger its apoptotic response. Furthermore, their studies provide a new mechanistic template that can potentially be exploited for the

development of therapeutic strategies targeted at cancer stem/progenitor cells [122].

1.4.2.7 Mitigating Adverse Epigenetic States

While epigenetic drugs are being studied in cancer therapeutics, potential intentional use of epigenome modifying compounds to prevent cancers later in life raises scientific and ethical questions, some of which are now being openly considered in the neurosciences. Drugs that can reverse epigenetic states and alter behavior have been discussed from an ethical perspective by Szyf [123]. His thesis is that epigenetic drugs could be used not only in diseases such as dementia, Alzheimer disease, schizophrenia or major depression, for which ethical issues may be easier to address, but also within the spectrum of "normal" behaviors. In an experimental rodent model, an adult behavioral phenotype of anxiousness and hyperstress triggered by poor maternal care in early life was reversed with the histone deacetylase inhibitor trichostatin A7. Szyf [123] asks, "Could we justify using epigenetic drugs to alter phenotypes that are epigenetically controlled and might be socially disruptive but are not a disease *per se*? Would we use such approaches preventively to 'improve' behavioural phenotypes? Is it ethical to use epigenetic drugs to prevent 'criminality' in people or groups of people who display epigenetic marks of aggression or people who have already committed aggressive acts? What will be the regulatory limits for the use of epigenetic drugs for behavioural modifications of antisocial phenotypes? The possibility of epigenetic behavioural modifications raises the spectre of 'social engineering'. Who should have the authority to prescribe behaviour-modifying drugs for cases in which public security or goods are involved? What are the roles of parents and legal courts in such decisions when children are involved? What should the rules be for consenting adults? [123]."

It is widely accepted that performance-enhancing drugs are banned during athletic competitions. It is also clear that competitions that require cognitive skills affect every human and have a dramatically higher impact on human life course than entertainment sports. Szyf asks, "If epigenetic drugs could indeed enhance cognitive abilities in healthy people, should they be used? Would the use of 'epigenetic cognitive enhancement' drugs be considered unethical during exams and competition for jobs, grants and promotions? Do epigenetic cognitive enhancement drugs introduce an unfair element to the regular competition between humans for resources and rank?" Critically important is the possibility that a transient treatment with an epigenetic drug could result in long-lasting effects on cognitive skills. It would, in this case, be ineffective to police the use of drugs at the time of the competition. Furthermore, access to epigenetic cognitive enhancement drugs might enhance the gap that already exists between poor and rich communities within rich countries, as well as between rich and poor countries [123].

These and other ethical issues in translational toxicology are discussed in Chapter 19.

1.4.2.8 Paradigm for Study of Cancer Chemoprevention

Cancer chemoprevention involves the chronic administration of a synthetic, natural, or biological agent to reduce or delay the occurrence of malignancy. The potential value of this approach has been demonstrated with trials in breast, prostate, and colon cancer. The paradigm for developing new chemopreventive agents has changed markedly in the last decade and now involves extensive preclinical mechanistic evaluation of agents before clinical trials are instituted and a focus on defining biomarkers of activity that can be used as early predictors of efficacy. A mini-review by Steward and Brown [124] summarizes the current status of the field of chemoprevention and highlights new developments. Table 1.1 lists some potential mechanisms of chemoprevention. Some potential molecular targets for chemopreventive agents are shown in Table 1.2.

Progress in development of clinical chemopreventive agents has proceeded using a similar model to new drug development in cancer therapy, with sequential phase I, II, and III studies (see Figure 1.2).

Phase I studies have the primary aim of determining safety and pharmacokinetics such that a dose and regimen that is well tolerated by participants can

Table 1.1 Potential mechanisms of chemoprevention.

Mechanisms of tumor-blocking agents
Scavenging of free radicals
Antioxidant activity
Induction of phase II drug-metabolizing enzymes
Inhibition of phase I drug-metabolizing enzymes
Induction of DNA repair
Blockade of carcinogen uptake
Mechanisms of tumor-suppressing agents
Alteration in gene expression
Inhibition of cell proliferation, clonal expansion
Induction of terminal differentiation, senescence
Induction of apoptosis in preneoplastic lesions
Modulation of signal transduction

Source: Reproduced from Ref. [124] with permission of Nature Publishing Group.

Table 1.2 Selected molecular targets of potential chemopreventive agents (effects may be tissue and cell specific as well as dose dependent).

Gene expression	Transcription factors	Protein kinases	Enzymes	Others
Chemokines	NF-κB	IκBα		
Kinase	FTPase	ICAM-1		
Cyclin D1	AP-1	EGFR	Xanthine oxidase	VCAM-1
MMP9	Egr-1	HER2	Heme oxygenase	ELAM-1
COX2	STAT1	AKT	uPA	TF
5-LOX	STAT3	JAK2	GST	Bcl-2
iNOS	STAT5	TYK2	GSH-px	Bcl-χ
IL-12	PPAR-γ	JNK		P53
TNF	EpRE	PKC		
IL-6	CBP	Src		MDR
IL-8	β-catenin	PKA		Telomerase
				Cyclin D1

AP-1: activator protein 1; CBP: CREB-binding protein; COX2: cyclooxygenase 2; EGFR: epidermal growth factor receptor; Egr-1: early growth response protein 1; ELAM-1: endothelial-leukocyte adhesion molecule 1; EpRE: energy per resource element; GSH: glutathione; GST: glutathione-S-transferase; HER2: human epidermal growth factor receptor 2; ICAM-1: intercellular adhesion molecule 1; IL: interleukin; iNOS: inducible nitric oxide synthase; JAK2: janus kinase 2; JNK: c-Jun N-terminal kinases; MDR: multidrug resistance; MMP9: matrix metallopeptidase 9; NF-kB: nuclear factor-kB; PKA: protein kinase A; PKC: protein kinase C; PPARg: peroxisome proliferator-activated receptor-g; STAT: signal transducer and activator of transcription; TF tissue factor; TNF: tumour necrosis factor; uPA: urokinase-type plasminogen activator; VCAM-1: vascular cell adhesion molecule 1. *Source*: Reproduced from Ref. [124] with permission of Nature Publishing Group.

be defined. Some phase I studies may incorporate preliminary assessments of potential biomarkers of effect. Exposure is usually relatively short (up to 3 months). Choosing a starting dose and schedule is extremely difficult and may be guided by preclinical studies. Dose conversions can be used, which seek to achieve plasma concentrations in humans that should be safe and may approximate dose levels producing a biological effect in preclinical models. PK data from phase I studies provide the actual levels that are achieved in humans, and these can be refined in preclinical models that explore possible mechanisms of effect at clinically achievable concentrations. Studies may utilize existing drugs, such as aspirin, for which there is already extensive human data, and rapid progress to phase III trials can be contemplated in this situation.

Phase II trials typically follow, utilizing the optimal dose determined previously, with the aim of exploring in relatively few patients the impact of exposure on a selected biological endpoint. When potential biomarkers of effect are available, these can also be examined in the few patients. Phase II trials may

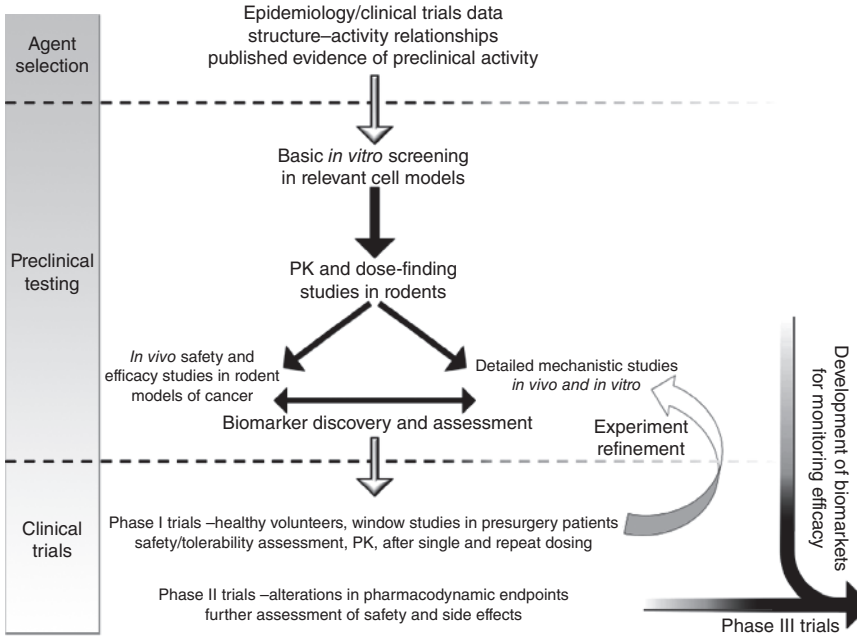

Figure 1.2 Stages in the preclinical and clinical development of potential chemoprevention agents. *Source:* Reproduced from Ref. [124] with permission of Nature Publishing Group.

incorporate a placebo (phase IIb) to better define subtle side effects and tolerability, and also to more accurately measure biological effects.

Phase III trials typically involve thousands of participants over a long period of time. Normally, there is randomization between the agent under investigation and a placebo. In chemoprevention trials, modification of a clinically relevant value, which is usually the incidence of malignancy, is the standard endpoint. Such trials may take many years and involve huge costs.

Given the time required for development of chemopreventive agents, recent interest has focused on phase 0 trials. These employ very low doses of the experimental agent and utilize new methodologies and technologies to study pharmacokinetics at a dose that minimize any risk of toxicity. It is anticipated that this approach will provide information to help determine a rational dosage regimen for future studies and will lead to early delineation of agents that have unfavorable bioavailability, metabolism, or distribution [124]. For detailed study on cancer prevention, please refer to Chapter 4.

Systems pharmacology is the name that is increasingly being used for the new systems-based approach that is being used to understand drug actions and for drug discovery. Systems pharmacology will take into account genomic variations

and molecular complexity in defining physiological and pathophysiological responses at the tissue, organ, and organism levels.

Systems-level analysis can be a powerful driver for understanding drug action. One can envisage three kinds of new knowledge coming from such analyses [125]:

- First is the identification of unanticipated adverse events that each drug might not produce on its own. Identification and prediction of such adverse effects could prove useful to guide physicians regarding which medicines can be coprescribed.
- The second kind of knowledge is the opposite of the first: identification of unanticipated beneficial effects by drug combinations, such as mitigation of side effects. This type of knowledge might lead to repurposing of approved drugs if their efficacy in suppressing adverse events could be established in rigorous clinical trials.
- The third kind of knowledge, which is the most forward-looking, is that network biology can be used for the discovery of new drugs. Network analysis can provide a rational basis for identifying targets, which, when modulated together by drug combinations, might be distinctively efficacious in treating complex diseases.

Combination therapy based on network biology could become efficacious for the treatment of progressive diseases, such as type 2 diabetes, kidney disease, congestive heart failure and, of course, many cancers. While the necessary knowledge is not yet available, the path forward can be readily seen. Large databases, such as FAERs, can provide empirical knowledge of good and bad outcomes associated with combination therapies in humans. As large amounts of genomic and molecular data are integrated with clinical data when electronic medical records become more widely used and molecular characterization of patients becomes more standardized, it will probably generate a wealth of systems-level information to analyze and generate hypotheses. These hypotheses might help with the design of studies to better understand the progression of diseases, and design new drugs or repurpose existing drugs that, in combination, are more effective for treating complex diseases.

For breast and ovarian cancer, steps have been taken to create a risk prediction model incorporating several of the known risk factors for computations. The Breast and Ovarian Analysis of Disease Incidence and Carrier Estimation Algorithm (BOADICEA) is a risk prediction model that is used to compute probabilities of carrying mutations in the high-risk breast and ovarian cancer susceptibility genes BRCA1 and BRCA2, and to estimate the future risks of developing breast or ovarian cancer. Lee *et al.* [126] have described updates to the BOADICEA model that extend its capabilities, make it easier to use in a clinical setting, and yield more accurate predictions:

(1) updates to the statistical model to include cancer incidences from multiple populations; (2) updates to the distributions of tumor pathology characteristics using new data on BRCA1 and BRCA2 mutation carriers and women with breast cancer from the general population; (3) improvements to the computational efficiency of the algorithm so that risk calculations now run substantially faster; and (4) updates to the model's web interface to accommodate these new features and to make it easier to use in a clinical setting. Lee *et al.* [126] present results derived using the updated model, and demonstrate that the changes have a significant impact on risk predictions. All updates have been implemented in a new version of the BOADICEA web interface that is now available for general use: http://ccge.medschl.cam.ac.uk/boadicea/ [126].

1.5 Modeling and the Future

The integration of transcriptomic, metabolomic, epigenomic, and proteomic profiling technologies has helped to build the foundation of systems biology [13,14] and systems toxicology [15].

Systems biology has been used for several years across different scientific areas of biological research to uncover the complex interactions occurring in living organisms. Applications of systems concepts at the mammalian genome level are quite challenging, and new complimentary computational/experimental techniques are being introduced. Most recent work applying modern systems biology techniques has been conducted on bacteria, yeast, mouse, and human genomes. The systems biology view that complex networks underlie many diseases is being increasingly recognized and demonstrated for heart disease, kidney disease, diabetes, metabolic diseases, and cancers. To cast systems of interacting entities as networks is useful because it allows the use of graph theory, a branch of mathematics that analyses how complex systems are organized and how such organization enables system-level functions. Chapter 17 describes the omics technologies upon which translational toxicology modeling and the future of the field will depend.

Figure 1.3 illustrates the sequence of events between initial exposure to a toxicant and final disease outcome (left to right). Note that genetic susceptibility (red dot) influences every level of toxicological analysis. After exposure, the ADME (absorption, distribution, metabolism, and excretion) systems of the body control local concentrations of a chemical stressor in various body compartments. This is affected by genetics through the involvement of specific alleles encoding various transporters and xenobiotic-metabolizing enzymes among others.

Mathematical models, including exposure models, physiologically based pharmacokinetic (PBPK), and biologically based dose response (BBDR) models can be used to approximate the relevant processes. PBPK models are a set of differential equations structured to provide a time course of a chemical's

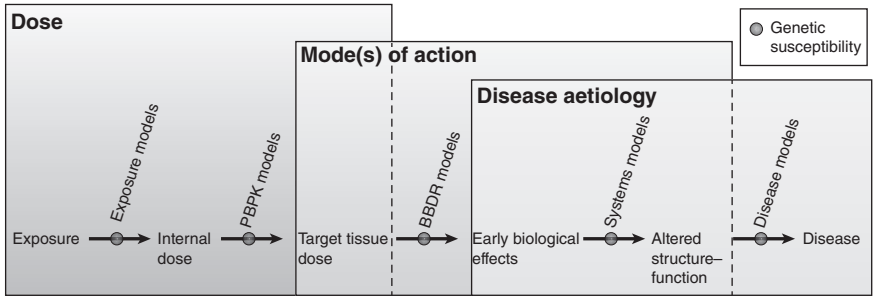

Figure 1.3 Computational models on the continuum between exposure and disease. *Source:* Reproduced from Ref. [15] with permission of Nature Publishing Group. (For a color version of this figure, see the color plate section.)

mass–balance disposition (wherein all inputs, outputs, and changes in total mass of the chemical are accounted for) in preselected anatomical compartments. BBDR models are dose–response models that are based on underlying biological processes. Once the target tissue is exposed to a local stressor, the cells respond and adapt, or undergo a toxic response; this process can be modeled with systems toxicology approaches. Finally, the disease outcome itself can be mimicked by genetic or chemically induced models of particular diseases. The colored boxes show the type of toxicologically relevant information that can be obtained from each set of models [15].

Figure 1.4 attempts to provide a framework for systems toxicology. This figure indicates the paths from the initial observation (rat in upper left) to an integrated toxicogenomics knowledgebase (blue cylinder), and so to systems toxicology (bottom right). The -omics data stream is shown by the clockwise path from rat to knowledgebase; and the "traditional" toxicology approach is shown in the anticlockwise path. The knowledgebase will integrate both data streams, along with literature-based knowledge; and by virtue of iterative modeling, will lead to a systems toxicology understanding. The framework involves "phenotypic anchoring" (to toxicological endpoints and study design information) and "sequence anchoring" (to genomes) of multidomain molecular expression datasets in the context of conventional indices of toxicology, and the iterative biological modeling of the resulting data [15].

Mathematical modeling in systems biology uses both bottom-up and top-down approaches to assemble information from all levels of biological pathways that coordinate physiological processes.

A top-down data driven approach integrates experimental data from various "omics" technologies. In a top-down approach, metabolic network reconstructions are performed using "omics" data (e.g., transcriptomics, proteomics) generated through DNA microarrays, RNA-Seq, or other modern high-throughput genomic techniques via appropriate statistical and bioinformatics methodologies. The top-down approach solves the problems through a large

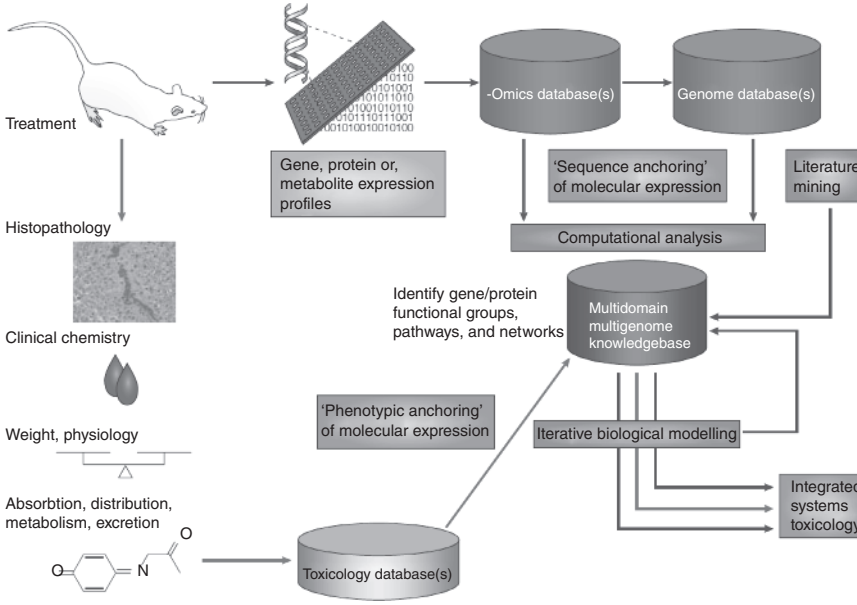

Figure 1.4 A framework for systems toxicology [15]. *Source:* Reproduced from Ref. [15] with permission of Nature Publishing Group. (For a color version of this figure, see the color plate section.)

number of entities. This approach does not emphasize the microscopic entities explicitly, but estimate their behavior at the macroscopic level, exemplified by ordinary differential equations (ODE) and partial differential equations (PDE). The ODE and PDE-based models are all population based, and the spatiality and topology that depend on individual interactions are, in general, ignored.

A model-based bottom-up approach depends upon a given model structure with kinetic parameters chosen such that an experimental observation can be reproduced quantitatively or qualitatively. A bottom-up approach typically encompasses draft reconstruction, manual curation, network reconstruction through mathematical methods, and validation of these models through literature analysis (i.e., bibliomics). The bottom-up approach is based on the synthesis of a complex from the activities on a lower system level; it emphasizes the microscopic level. This approach requires greater computational power in order to simulate a large number of significant entities in real world. From the model built by this approach, we can observe the interactions between entities specifically and study how they contribute to the emergence of global property. Cellular automata and (multi) agent-based methods are the most used bottom-up ones [127].

In order to ascertain the potential for exposures in early life to provide cancer-protective benefits across the life span, these two modeling approached must be unified in order to guide selection and assessment of plausible

protective interventions [125]. Mathematical models are frequently used to elucidate cellular design principles in order to understand complex biochemical networks preferably by using both approaches that can lead to a consistent description of cellular and molecular dynamics [128,129].

A third modeling theme in cancer that has its roots in developmental biology relates to stem cell division (proliferation) and whether cellular "bad luck" is primarily extrinsic or intrinsic in origin.

Recent research has highlighted a strong correlation between tissue-specific cancer risk and the lifetime number of tissue-specific stem cell divisions. Whether such correlation implies a high unavoidable intrinsic cancer risk has become a key public health debate with the dissemination of the "bad luck" hypothesis. Xu and Taylor [40] provide evidence that intrinsic risk factors contribute only modestly (less than ~10–30% of lifetime risk) to cancer development. First, they demonstrate that the correlation between stem cell division and cancer risk does not distinguish between the effects of intrinsic and extrinsic factors. They then show that intrinsic risk is better estimated by the lower bound risk controlling for total stem cell divisions. Finally, they show that the rates of endogenous mutation accumulation by intrinsic processes are not sufficient to account for the observed cancer risks. Collectively, they conclude that cancer risk is heavily influenced by extrinsic factors. These results are important for strategizing cancer prevention, research, and public health [40].

In contrast, Tomasetti and Vogelstein [130] point out that some tissue types give rise to human cancers millions of times more often than other tissue types. Although this has been recognized for more than a century, it has never been explained. They show that the lifetime risk of cancers of many different types is strongly correlated (0.81) with the total number of divisions of the normal self-renewing cells maintaining that tissue's homeostasis. These results suggest that only a third of the variation in cancer risk among tissues is attributable to environmental factors or inherited predispositions. The majority is due to "bad luck," that is, random mutations arising during DNA replication in normal, noncancerous stem cells. This is important not only for understanding the disease but also for designing strategies to limit the mortality it causes [130].

Our aim for the future is to promote the following objectives for future animal and human studies: (1) elucidate specific cellular and molecular targets of known toxicants; (2) design a systematic approach to the identification of mutagenic and developmental toxicants; (3) develop sensitive, specific, and predictive animal models, to include minimally invasive surrogate markers, and/or *in vitro* tests to assess function of cancer control systems during embryonic, postnatal, and adult life. While we will not be able to accomplish each of these objectives with our collective efforts on our previous book [131] or on this one, perhaps we can begin to lay the necessary foundations.

As for future protective developmental interventions, integrated testing strategies need to systematically account for the many mechanisms associated

with developmental events that occur *in vivo*. In order to apply the translational concept to mitigate environmentally induced toxicity, we are guided by the modest number of established and accepted therapeutics used primarily for fetal benefit and the limited number of dietary or supplemental interventions that have proven to be beneficial to or protective of the adult. These established or potential therapeutic interventions suggest that early steps in testing or implementing translational toxicology therapies during the *in utero* and early neonatal period will likely derive from GRAS options.

If we are to translate environmental health discoveries into safe and effective interventions, we must assert and characterize valid, applicable therapies, such as GRAS treatments, and eventually GRASE and other "ethical pharmaceuticals" for the protective care of highly vulnerable young patients. Since toxicology has repeatedly demonstrated that the fetus and child is more susceptible to adverse exposures than the adult, we believe we can create a safe and efficacious environmental health portfolio of interventional options to improve human health that include both reduction/avoidance of exposure and specific preventative/mitigative/restorative therapeutics.

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