EPILOGUE

Moshe Szyf

Advances made in the last decade have firmly established the critical role of the epigenome in orchestrating the complex and dynamic gene expression program of multicellular organisms such as humans. The epigenome is composed of two distinctly different layers of information, chromatin and DNA methylation. While chromatin is associated with the genome and serves to package its different regions in either tight or open structures, DNA methylation is part of the chemical covalent structure of the DNA. DNA methylation is therefore believed to be a fixed component of the epigenome and to be a consistent and stable signal of gene inactivation. These two layers of information are tightly correlated. DNA methylation is characteristic of inactive regions of the genome that are packaged in tight chromatin, whereas hypomethylated DNA is found in open and active chromatin structures. Recent advances in understanding the relation between chromatin and DNA methylation have provided some insights into the mechanisms that tie these processes to each other. It is clear that DNA methylation has to be understood within its chromatin context and aberrations in DNA methylation must be understood in relation to changes in chromatin structure and in the proteins that remodel chromatin. Cancer is a disease of foiled programming of gene expression and could be therefore considered an epigenomic disease. Aberrations in either one or both chromatin structure and DNA methylation have been found by many studies to be a persistent hallmark of cancer. An understanding of DNA methylation changes and their diagnostic and therapeutic implications in cancer could only be achieved if they are analyzed in the context of the chromatin.

The chapters of this book unravel multiple meeting points between DNA methylation and cancer therapy. Each of these points has distinct implications for cancer therapy. A first example relates to the diagnostic potential of DNA methylation in cancer. Notwithstanding the causal role of DNA methylation in cancer, it is well established that distinct DNA methylation patterns characterize many tumors when compared with their noncancerous-paired tissue. Several of the changes in DNA methylation observed in cancer are easy to explain since they include hypermethylation of tumor suppressor genes, which marks these genes for inactivation. Similarly, methylation of repair genes, adhesion proteins and angiogenesis inhibitors confer a selective advantage upon cancer cells. Such changes in DNA methylation are consistent with a causal role for DNA methylation in cancer and could provide a clear mechanism. However, not all changes in methylation necessarily relate to a clear biological function. Paradoxically, in addition to DNA hypermethylation of specific genes, it is well established that global hypomethylation of repetitive sequences as well as of genes that promote metastasis is characteristic of many tumors as discussed earlier in the book. Whereas resolving this paradox is critical for our understanding of the mechanisms responsible for alterations in DNA methylation in cancer as well as their therapeutic potential, the diagnostic value of methylation changes is independent of these questions. The diagnostic value of DNA methylation markers is a function of their correlation with tumorigenic states and not their mechanism of action. Thus, it is possible to take advantage of the unique DNA methylation profiles of tumors without understanding their function.

The main issue that will hopefully be resolved in the near future is whether we could utilize specific DNA methylation profiles for early diagnosis of cancer, classification of tumor grades, and predicting their susceptibility to specific therapies. Up to recently, a small number of genes,

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which were selected for analysis by a candidate gene approach, were shown to be altered by DNA methylation. This limited and biased repertoire was insufficient for methylation profiling of a broad range of tumors and for classification of cancers. Some of the candidate genes are methylated only in a subset of tumors and cannot serve as markers for comprehensive diagnostic tests. The prediction however is that all cancers exhibit cancer-type and grade-characteristic DNA methylation profiles that would be unraveled once a broad range of methylation markers are defined. This book discusses a number of whole genome approaches for methylation profiling. These whole genome approaches will hopefully lead to a comprehensive directory of methylation markers. As a consequence, this might provide diagnostic methylation tools that will increase the precision of early diagnosis as well as result in a more accurate classification of cancers.

Although the aberrations in DNA methylation in cancer might have important diagnostic value irrespective of the mechanism causing them, it is essential to understand how these paradoxical patterns of methylation are generated in cancer and whether they have a causal role in tumorigenesis. Answering these questions has evidently important implications on any potential use of DNA methylation therapeutics in cancer. Without understanding how these changes in methylation come about, it would be impossible to truly determine their role in tumorigenesis. In the absence of a comprehensive understanding of the role of methylation changes in the mechanisms of tumor generation and progression, it is hard to take full advantage of the therapeutic potential of the DNA methylation machinery. It is imperative that future studies will be directed at these cardinal questions.

An important issue that needs to be resolved is whether aberrant DNA methyltransferase expression can stimulate tumorigenesis independent of DNA methylation. DNA methyltransferases are multifunctional proteins, which are involved in suppression of gene expression and DNA replication in addition to their DNA methylating activity. It is essential that the specific functions of DNA methyltransferases that lead to tumorigenesis be identified. This will allow us to direct therapies at these functions specifically. It is also critical to determine whether hypomethylation plays a causal role in cancer and what is the mechanism involved. As discussed earlier in this book, DNA methyltransferase inhibitors are tested in clinical trials for their anticancer activity. If hypomethylation of DNA can play a causal role in tumorigenesis by stimulating metastasis as previously proposed, hypomethylating agents should be used with extreme care. Other agents that inhibit the tumor promoting activity of DNA methyltransferase 1 in the absence of global hypomethylation should thus be used.

Recent studies discussed in this book suggest that our whole understanding of the DNA methylation machinery must be redirected in light of the putative involvement of DNA demethylases and chromatin structure in shaping DNA methylation patterns. Our traditional understanding of the DNA methylation pattern has been that the pattern is laid down during development and is then fixed and maintained by a semiconservative DNA methyltransferase throughout life, which copies the DNA methylation pattern as directed by the template state of methylation. This model fails to explain how methylation patterns change in somatic cells once they are transformed. Using this model, it is even more difficult to explain how it is possible to have both DNA hypermethylation and demethylation occurring simultaneously in the same cancer cell. It has originally been proposed that increased DNA methyltransferase results in increased DNA methylation. However, there is no strong data to suggest that regional hypermethylation of CG islands correlates with the levels of the DNA methyltransferases. In addition, if an increase in DNA methyltransferase activity is responsible for the changes in DNA methylation in cancer cells, how is it possible to have global hypomethylation in the presence of high levels of DNA methyltransferase activity? It is clear that our long-established understanding of the maintenance of DNA methylation patterns in somatic cells lacks a number of key players.

Two very recent advances might unveil a new understanding of DNA methylation patterns in general and particularly in cancer. These advances raise the prospect that the DN A methy-

lation pattern is in a dynamic steady state in somatic cells, and that a relative change in the factors that maintain this dynamic steady state in cancer can result in alteration of DNA methylation. First, is the realization that chromatin structure might have a serious impact on DNA methylation patterns, and since chromatin structure is dynamic, DNA methylation might be dynamic as well? Second, is the discovery of demethylase enzymes that reverse DNA methylation patterns in a replication independent manner, thus introducing a novel understanding of DNA methylation pattern as a balance of two reversible reactions, DNA methylation and demethylation. The access of demethylases to methylated DNA is gated by chromatin structure as discussed in the chapter by Szyf et al. Local changes in chromatin structure that alter accessibility to demethylase can explain how regional hypermethylation is generated in the presence of high levels of DNA demethylase. On the other hand, a global increase in demethylase activity might explain global demethylation in cancer. Future research must delineate how chromatin structure fashions DNA methylation patterns in cancer and identify the key factors that alter the accessibility of DNA to either DNA methyltransferases or demethylases. These factors might unfold into important cancer drug targets.

Understanding the role of other factors in altering chromatin and DNA methylation patterns in cancer will help us address the issue of whether DNA methylation patterns *per se* play a causal role in tumorigenesis, as currently believed, or whether these changes are merely fingerprints of other important alterations. Addressing this question is obviously critical for DNA methylation based anticancer therapy. It is essential to define the goal of therapy as either reversing DNA methylation patterns or as interfering with the factors that cause these changes in DNA methylation. In the latter case, DNA methylation is a surrogate marker of other more significant events. A future understanding of DNA methylation pattern changes and their relevance to cancer will require a complete different perception of DNA methylation as a reversible and dynamic state, which is in an interactive relation with other components of chromatin.

Using pharmacological or genetic knock down of the different components of the DNA methylation machinery, it is possible to determine that a certain protein plays a causal role in cancer and is therefore an anticancer drug target. This could be accomplished in absence of a full understanding of the mechanisms involved. A long list of data from tissue culture, animal and clinical trials supports the hypothesis that DNA methyltransferase1 (DNMT1) is critical for cancer. Recent data also suggests that MBD2/demethylase is critical for cancer. However, these proteins play different roles in cancer. DNMT1 is important for cell growth and possibly initiation of DNA replication, while MBD2/demethylase is not required for normal cell growth. The fact that different proteins of the DNA methylation machinery are required for distinct processes involved in tumorigenesis raises the hope that in the future we will be able to accurately target specific cellular functions critical for cancer using these agents. Agents that affect tumorigenesis without affecting the cell cycle are especially attractive, since they should not have side effects on dividing normal tissue which is common to most anticancer drugs that target cell growth functions.

The role of hypomethylation in cancer has been neglected for some time. The chapter by Ehrlich and colleagues provides an incentive to revisit the therapeutic implications of these observations. In addition to the cautionary note raised above on the potential untoward effects of demethylating agents, it is possible that inhibitors of hypomethylation would also be anticancer and antimetastatic agents. The chapter by Szyf et al discusses the therapeutic implications of hypermethylating agents. The discovery of demethylases such as MBD2/demethylase, which is highly expressed in some tumors, raises the possibility that inhibition of hypomethylation could be accomplished by inhibiting demethylases. However, our understanding of demethylases and their regulation and deregulation in cancer is rudimentary. It is therefore important to characterize the demethylases that are highly expressed in cancers and are involved in tumorigenesis. It is also critical to delineate the specific tumorigenic factors, which are regulated by these demethylases. Understanding the molecular machineries which contain demethylases and determining the factors that guide their specificity is obviously highly

significant for any attempt to design therapeutic agents targeting demethylases. Although this area of DNA methylation is in its infancy, it is conceivable that in the near future demethylases would become important targets for anticancer agents.

Another important issue that is unresolved and requires further attention is the relation between the level of methyl promoting agents such as folates in diets, DNA methylation, and cancer. We must understand how diets affect the levels of both the methyl donor S-adenosylmethionine (AdoMet), and S-adenosylhomocysteine, the product of the DNA methylation reaction, in target tissues. It is also important to determine the mechanisms through which AdoMet levels affect DNA methylation levels. AdoMet was originally proposed to stimulate the DNA methyltransferase reaction, but if the DNA methylation pattern is dynamic, then methylation-promoting and methylation-deficient diets might alter both sides of the DNA methylation equilibrium. Recent data from our laboratory suggests that AdoMet inhibits demethylase activity. It is important to determine whether AdoMet inhibits the specific demethylase activity responsible for the global hypomethylation in cancer. If hypomethylation plays a causal role in cancer progression or metastasis, and if it is possible to inhibit it by modulating dietary intake of methyl promoting agents, nutrition might emerge to play an important role in DNA methylation based anticancer therapy and prevention. The possibility that the deleterious effects of global hypomethylation could be modulated by diet is extremely attractive. In addition, pharmacological agents that mimic the activity of AdoMet and folates might then be developed to reverse global hypomethylation and its putative effects on tumor progression.

In summary, while a long list of data reviewed in this book has established many links between DNA methylation and cancer therapy and diagnostics, many questions remain to be resolved. We hope that the chapters of this book will inspire the reader to get involved in studying the remaining issues discussed here. Unraveling of these issues promises to unfold into new modalities of cancer diagnosis and cancer therapy, as well as a better understanding of the mechanisms involved in cancer and of the basic rules that guide and maintain epigenomic gene regulation in somatic cells.