Epigenetic regulation of cellular adhesion in cancer

Judith Katto and Ulrich Mahlknecht

Division of Immunotherapy and Gene Therapy, Department of Internal Medicine, Saarland University Medical Center, Kirrbergerstrasse, Building 45.3, D-66421 Homburg/Saar, Germany

*To whom correspondence should be addressed. Tel: +49 (0) 6841 1626157; Fax: +49 (0) 6841 1626418; Email: ulrich.mahlknecht@uks.eu

Epigenetics describes the development and maintenance of stable heritable gene expression patterns, which allow cells to show different phenotypes despite of a commonly shared genetic code. The increasing knowledge in this field during the last decades reveals its importance for many physiological processes like differentiation, embryogenesis and parental imprinting, but also for some diseases such as cancer. Recent data have shown that the complexity of carcinogenesis can no longer be explained solely on the basis of genetic changes, but epigenomic alterations such as changes of the DNA methylation pattern and/or post-translational histone modifications and changes of microRNA expression need to be equally considered. Such epigenetic alterations may cause permanent changes in gene expression patterns and may therefore essentially contribute to some of the known phenotypic characteristics of cancer cells like the loss of growth control, altered intercellular communication and enhanced motility. The two latter may essentially be associated with the downregulation of cellular adhesion molecules, which may therefore be relevant in the context of cancer invasiveness and prognosis. The targeted modification of the epigenome may therefore open new horizons within the increasingly important field of epigenetic therapeutics—particularly in view of the regulation of cellular adhesion with particular attention to tumor cell invasion and metastasis.

Introduction

Epigenetics describes the clonally heritable pattern of expressed genes in association with a specific cellular phenotype without changing the basic genetic information itself (1), which is essential in the regulation of cellular differentiation and development and guarantees the maintenance of tissue- and germ-line-specific phenotypes (2,3). Epigenetics is also relevant for parental imprinting, which leads to the monoallelic expression of genes (4) and X-chromosomal inactivation in women. While a few decades back, information on the epigenome and its functions was scarce, the field of epigenetics is currently booming and increasing knowledge is becoming available every day. Epigenetic regulation of chromatin modification takes place at several different levels of which the methylation and demethylation of promoter DNA and the posttranslational modification of histone proteins through acetylation and deacetylation are most extensively studied. These mechanisms may affect the expression of individual genes either directly through (de)methylation of promoter DNA sequences or modification of the chromatin structure or indirectly through microRNAs which cause a reduction of messenger RNA levels of a whole set of target genes (5,6). Such mechanisms have been reported to be of particular relevance for a number of diseases and most essentially in the pathogenesis of several types of cancer. One of the most interesting potential targets of epigenetic abnormalities in cancer is the change in the expression pattern of cellular adhesion molecules because these molecules play an essential role for cancer development and prognosis by influencing intercellular communication, signaling pathways and cell motility. The aim of this review is to combine recent data about epigenetic modifications in cancer with the influence of such changes on cellular adhesion and the behavior of cancer cells in order to show that epigenetic regulation of adhesion is one of the key contributors to carcinogenesis.

Cancer epigenomics

The genetic concept of mutations alone cannot completely explain the development and behavior of cancer cells (7)—particularly in view of the fact that numerous hematological malignancies appear to be cytogenetically normal (8,9). Epigenetic alterations are gaining increasing attention as potential key players in the pathogenesis of cancer (10). Genetic and epigenetic mechanisms go hand in hand in numerous neoplastic diseases. Every normal cell carries a distinct pattern of epigenomic modifications, which is being established during differentiation and propagated through many cell cycles in order to maintain a distinct function and identity. If these complex patterns are lost or deregulated, biological key features may get out of control and multiple phenotypic changes may take place (11). In this context, cells may lose their tissue-specific identity and function and escape normal regulatory mechanisms (12), which may in turn pave the way for the development of neoplastic disease. Within this process of reprogramming, all epigenetic regulatory components including DNA methylation, histone modification and microRNA expression may be involved (13).

DNA methylation

DNA methylation is the best investigated mechanism of epigenetic regulation and can be found in all species and tissue cell types. It is regulated by a specific group of enzymes, the DNA methyltransferases (DNMTs) of which three main subtypes have been reported in mammals: DNMT1, DNMT3a and DNMT3b (14). These enzymes transfer methyl groups primarily to cytosine residues of CpG dinucleotides. DNA methylation normally leads to the silencing of genomic DNA, which is mediated by two main mechanisms: the direct inhibition of transcription factor binding by the methyl group or recruitment of methyl-binding domain proteins. These methyl-binding domain proteins may recruit other proteins that are involved in gene silencing, such as histone deacetylases (HDACs) (15), histone methyltransferases and transcriptional repressors (16). Accumulations of CpG dinucleotides may be found particularly within highly repetitive regions of the genome (17) and within short CpG-rich stretches at 5’ promoter regions of genes (‘CpG islands’) (18). It has been reported that ~60% of all human promoter regions carry such CpG islands (19). In healthy cells, most of the CpG islands are unmethylated (20) but each cell type has its specific heritable pattern of CpG island methylation, which is established during differentiation and causes a specific configuration of expressed genes. This leads to the silencing of non-coding regions and transposable elements. In contrast, in transformed cells, a genome-wide hypomethylation is frequently being found together with t-site-specific hypermethylation of CpG islands, which are otherwise regularly unmethylated (21). Some research groups have proposed that such a genome-wide hypomethylation may cause increased genomic instability and the reactivation of retrotransposons (22), which are genetic elements that can amplify themselves and make up around 42% of the human genome. These retrotransposons are normally repressed and their reactivation through hypomethylation increases the probability of chromosomal rearrangements and translocation-induced mutations (23,24). In addition, hypomethylation may go along with the reactivation of oncogenes

Abbreviations: AML, acute myeloid leukemia; DNMT, DNA methyltransferases; EMT, epithelial-mesenchymal transition; HDAC, histone deacetylase; SNAIL, snail homolog 1.

© The Author 2011. Published by Oxford University Press. All rights reserved. For Permissions, please email: journals.permissions@oup.com
or growth factors, which are normally being silenced. Also, the t-site
specific hypermethylation and thus silencing of tumor suppressor
genes may promote cancer progression (16). Therefore, in addition
to genetic alterations, both, whole-genome and site-specific methyla-
tion changes constitute important elements in the context of a multi-
step pathway that finally leads to the development of cancer.

Histone modifications

The postranslational modification of histone proteins constitutes an-
other important segment of epigenetic regulation. The covalent postran-
slational modification of N-terminal histone ends through acetylation/deacetylation in association with cancer has been exten-
sively described (7,11) and is achieved primarily through the compet-
ing activities of two families of enzymes, the HDAC and histone
acetyltransferases. In numerous types of cancer, an over expression
of HDAC proteins may go along with global or site-specific patterns
of hypoacetylation, which may silence tumor suppressor genes and
inhibit pathways being involved in cell differentiation (25,26). Indi-
rect effects affecting the expression of HDACs either through the
downregulation of transcription factor activity or through the micro-
RNAs, which may change the expression of complete sets of target
genes via binding of the microRNA to messenger RNAs and inhibi-
tion of translation, are also known to play a role (5). Another key
epigenetic mechanism that regulates gene transcription and which
also involves histone modifications is chromatin remodeling. One
key element that regulates chromatin remodeling is the multimeric
protein complex polycomb repressive complex 2, which consists of
the three core componets EZH1/2, EED and SUZ12 (27). The meth-
yltransferase EZH2 mainly catalyses the dimethylation and trimeth-
ylation of histone H3 lysine 27 (H3K27me3). This methylation pattern is
associated with changes in the chromatin structure and leads to the
stable repression of transcription that is being mediated by the PRC1
complex. This mechanism is essential in the context of early embryonic
development, as well as in the differentiation and maintenance of stem
cell pluripotency (28). The deregulation of these mechanisms can pro-
 mote the development of cellular malignancy as the upregulation of
EZH2 has been observed both for the transition of normal cells to
a neoplastic phenotype as well as in various types of cancer (29,30).
Also, an upregulation of genes that are involved in stem cell mainte-
nance and embryogenesis that are normally repressed by polycomb
repressive complex 2 can be observed in several cancer types (31).
Another key element that regulates chromatin remodeling is the meth-
yltransferase G9a, which catalyzes the methylation of histone H3 lysine
9 (H3K9). This methylation pattern is associated with transcriptional
repression essentially with euchromatic regions and is important in
the context of embryogenesis (32) Its deregulation, however, has also
been associated with the silencing of tumor suppressor genes (33).

Adhesion molecules in cancer

Cell–cell adhesion is one of the key features in the development and
progression of cancer. All mammalian organisms are organized in
tissues and each tissue is characterized by a distinct form, function
and behavior. These properties are substantially based on the pheno-
type of the single cells that constitute the corresponding tissue. If
increasing numbers of cells change their phenotype, tissue organiza-
tion may break down and its function may be impaired. Since full
tissue function is based on the integrity of its single tissue cells, in-
tercellular communication is an absolute necessity. Numerous cellular
events that trigger differentiation, growth, cell cycle and many sig-
naling pathways directly depend on this intercellular communication
(34). Accordingly, alterations of intercellular communication may
disrupt the delicate homeostatic balance of the tissue and subse-
 quently contribute to the development of cancer (35). Cells have
different ways to communicate, including soluble factors like inter-
leukins or growth factors and their corresponding membrane receptors
or cell–cell adhesions. There are constitutive cell–cell connections,
which are important for the development of cell polarity and the
maintenance of tissue organization like cadherin connections are in
epithelial cells (36) and cell–cell contacts that are made only to respond
to distinct signals (34). If normal cells loose their constitutive cell–cell
connections or cell–matrix connections, they go into apoptosis (37).
This specific form of apoptosis is also referred to as "anoikis", which
describes the defense of a cell against transformation. The impairment
of these mechanisms leads to anchorage-independent growth and to
tissue independent apoptosis resistance, which is known to be one of
the key factors of cancer development and metastasis.

The cadherins

The cadherins are among the most important and best examined adhe-
sion molecules, which regulate tissue organization and influence cancer
development and metastasis. The classical cadherins such as E-cadherin,
N-cadherin and vascular endothelial-cadherin are transmembrane pro-
teins with a specific extracellular domain that can bind to cadherins on
other cells but only of the same subtype. The intracellular domain is
highly conserved among different subtypes and interacts with numer-
ous cytoplasmatic proteins, which in turn interact with the cytoskel-
eton and signaling pathways that produce changes in cell motility,
migration, proliferation and shape (38). The catenins are among the
most essential cytoplasmatic proteins that interact directly with the
cadherins, β-catenin binds with high affinity to the intracellular cad-
herin domain and mediates the recruitment of α-catenin, which in turn
directly interacts with actin filaments or with actin-binding proteins.
In this way, cadherins are responsible for the maintenance of cell–cell
contacts within several tissues and are involved in the linkage of cell–
cell junctions to actin containing intracellular filaments. A loss or
decrease of these junctions leads to enhanced motility and allows cells
to disregard their role within organized tissues (39). The cadherins are
also crucial during the course of cellular differentiation and matura-
tion. Because of the high specificity of the extracellular domains of
the cadherins and their preference for homophilic adhesion, they are
able to influence specific cell populations during the course of differ-
entiation and developmental processes (40) as seen for melanocytes
for instance, which are intensively influenced by the presence of
eratinocytes through growth factors and the expression of adhesion
molecules. Cell–cell junctions between melanocytes and keratino-
cyes and cell–matrix adhesions are both significantly involved in
the maintenance and healthy balance between proliferation, differen-
tiation, metastasis and apoptosis of melanocytes. Many studies have
shown that the pattern of expressed adhesion molecules in melanoma
cells differs quite significantly from what is observed in healthy mel-
anocytes (41). Although in melanocytes E-cadherin and desmoglein1
are among the adhesion molecules that are predominantly being ex-
pressed, these are downregulated in most melanoma cells (42). In-
estead of E-cadherin, melanoma cells typically interact with each other
via N-cadherin. This change in the expression of cadherins allows
melanoma cells to segment themselves from other melanocytes and
to uncouple the pathways of tissue organization, which is nor-
mally mediated via E-cadherin (Figure 1). Experiments with trans-
fected melanoma cells on three-dimensional skin models showed an
inhibition of their invasive growth if melanoma cells were overex-
pressing E-cadherin (43,44). This shows the importance of this adhe-
sion molecule for the progression and metastatic potential of skin
cancer.

A disrupted E-cadherin organization has been shown not only for
melanocytes but also for many other cancer types such as breast
cancer and hepatocellular carcinomas (45,46). Breast cancers charac-
teristically exhibit a disrupted E-cadherin organization (47) and miss
tight junctions (48). On the other hand, upregulation of other cell
adhesion molecules like P-cadherins and CD44 have been observed
and are related mostly to poor prognosis in breast cancer (49). This is
believed to be caused by enhanced cell–cell interactions between
cancer cells, which could repress apoptosis (50,51). This means that
the loss of normal cell–cell interactions is not only essential for tumor
invasion but also correlated with tissue independent growth and
survival of cells, which is a hallmark of transformed cells.
The so called epithelial-mesenchymal transition (EMT) is a highly conserved process that regulates tissue morphogenesis and the conversion of cells that exhibit an epithelial phenotype to the mesenchymal phenotype. This model is also being considered to be essential for cell dedifferentiation, during carcinogenesis and cancer cell dissemination from primary tumor sites (52). The loss of E-cadherin, which may be caused by activation of transcriptional repressors, is an event that is highly essential in the context of EMT. This downregulation of E-cadherin is therefore a very critical step in cancer progression since it enables hematogenic and lymphogenic cancer cell dissemination.

### The integrins

The family of integrins renders another example on how alterations of cellular adhesion and the disruption of cell–cell interaction may contribute to the development and progression of cancer. It is postulated that cell adhesion sustains cell survival and represses apoptosis through interaction with growth factors and the extracellular matrix-dependent pathways (41). Although epithelial cell survival in vitro depends on the integrity of their adhesion to an extracellular matrix (37), malignant mammary epithelial cells grow in soft agar with no dependence on cellular adhesion (53) and interestingly, tumor progression models have demonstrated the gradual loss of β1 integrin adhesion dependency as a requirement for cell survival (54). In addition, the correction of such adhesion defects in culture is able to restore tissue organization and the original normal cell behavior (55).

Also, melanoma cells have been reported to upregulate the expression of a number of receptors that belong to the immunoglobulin gene superfamily, including Mel-CAM, α6β1 integrin and activated leukocyte cell adhesion molecule, which are rarely found on the surface of healthy melanocytes (41). Most importantly, it has been postulated that the expression of these adhesion molecules directly correlates with disease progression and tumor metastasis (43). Activated leukocyte cell adhesion molecule, for instance, is expressed on metastatic melanoma cells but absent on non-metastatic cells (56). The expression of α9β1 integrin has been observed to facilitate the extravasation of melanoma cells (57). Cellular adhesion has also been postulated to correlate with the expression of matrix metalloproteinases, which in turn are essential key players in the context of tumor invasion and metastasis.

An upregulation of the leukocyte integrin CD11b has been reported on primary blast cells from acute myeloid leukemia (AML) patients. Although CD11b is normally expressed on monocyteic cells and NK cells, in this instance, it was expressed on highly immature cells lacking all other immunological markers of the former cells (38).

The high expression of CD11b on leukemic blasts was correlated with poor prognosis. Additional factors that have been reported to be of prognostic importance for AML patients are the very late activation antigens (VLA)-4 and -5 and the lymphocyte function-associated antigen-1. They are known to be involved in the retention of hematopoietic progenitor cells and leukemic blast cells within the bone marrow and in the homing of stem cells after transplantation (59,60). It has been suggested that these adhesion molecules are part of a mechanism of resistance to chemotherapeutic agents and that they may be of importance in the mediation of minimal residual disease in AML patients. Experiments in mouse models of minimal residual disease showed a survival rate of 100% if the mice were treated with a combination of the cytotstatic agent cytarabine and a VLA-4-specific antibody. In contrast, cytarabine without the antibody caused a prolongation of life when compared with the untreated control but could not cure the mice (61). Many more examples on the influence of adhesion molecules on the development and progression of cancer may be found in the literature for nearly all cancer types. It is therefore appropriate to say that some of the main characteristics of cancer such as uncontrolled proliferation, the absence of cellular and morphological differentiation, invasion and cell migration to distant organs to form metastases may at least in part be accredited to alterations in adhesion mechanisms.

### Epigenetic regulation of cellular adhesion

All the alterations of cellular adhesiveness that have been observed in cancer so far may be caused by either genetic mutations or alterations of the epigenome. A few decades back genetic mutations were considered to be the leading cause of cancer and enormous effort has been dedicated to the identification of genetic mutations as markers of transformed cells. This has changed since increasing numbers of epigenetic alterations were found in all types of cancer. Today, epigenetic alterations and genetic mutations are acknowledged as different but equally important contributors to the development and progression of cancer (62). This may apply equally to alterations in the adhesion system in the context of cancer, which is similarly regulated at the epigenetic level.

E-cadherin is deregulated in most types of epithelial-derived cancers (63,64). Studies have shown that mutations and loss of heterozygosity are detectable in many of these tumors but cannot be the only mechanism of E-cadherin downregulation (65). In some types of cancer including gastric cancer (66,67), colorectal cancer (68) and leukemia (69,70), hypermethylation even appears to be the leading
mechanism of E-cadherin suppression. Downregulation of E-cadherin caused by hypermethylation of the 5′ CpG island of CDH1 was also observed in breast, prostate and liver cancers (45).

Another mechanism that leads to the downregulation of E-cadherin is the over expression of specific repressing transcription factors. Two of the best examined repressors of E-cadherin expression are the transcription factors snail homolog 1 (SNAIL) and zinc finger E-box binding homeobox 1. SNAIL is known to interact with the E-cadherin promoter and to repress its expression directly (71). Normally, SNAIL is involved in EMT but it is also present in many tumors and carcinoma cell lines and the expression level of SNAIL correlates with low E-cadherin levels and high invasiveness (72). Zinc finger E-box binding homeobox 1 is an inducer of EMT and a strong repressor of E-cadherin expression (73). Zinc finger E-box binding homebox 1 stabilizes EMT and downregulation of E-cadherin by a microRNA-mediated feedforward loop and therefore, its epigenetic activation is very important in promoting the development of cancer and its invasiveness (74).

Hypomethylating mechanisms are not the only involved in the regulation of cellular adhesion in the context of cancer, histone modifications may be just as important. Experiments with different HDAC inhibitors including valproic acid and suberoylanilide hydroxamic acid showed significant downregulation of the adhesion molecule VLA-4 in hematopoietic cells and leukemia cell lines (75). Maeda et al (76) successfully induced an upregulation of the costimulatory/adhesion molecules CD86 and ICAM-1 on AML cells through the inhibition of HDACs. With this treatment, they were convinced to be able to activate leukocytes and to induce an immune reaction against tumor cells.

Conclusions

Strong correlations have been identified between expression patterns of adhesion molecules such as E-cadherin or integrins in association with the clinical prognosis of cancer patients (77). Solid and less invasive tumors generally express higher levels of E-cadherin when compared with widespread and highly invasive tumors. Therefore, these adhesion molecules can be used as prognostic markers in order to assess the metastatic potential of cancer in patients (78). A downregulation of E-cadherin is always associated with undifferentiated cancers and strong invasive tendency and is therefore to be considered as a marker of poor prognosis. But these findings implicate also a chance: if the deregulation of the E-cadherin expression is related to epigenetic changes, these could become a target for epigenetic treatment strategies. Maybe a reduction of the abnormal methylation pattern of E-cadherin could restore the balance of cell adhesion. This could lower the invasive potential of the cancer cells and stop metastasis, which is the most dangerous aspect of cancer. In some in vitro experiments, the invasiveness of malignant cells like melanocytes significantly decreased after restoring the normal balance of the adhesion molecules. It also could bring the cells back to the control of tissue organization and may be restore the responsiveness of the cancer cells to differentiation signals and growth regulators. This raises the hope that similar results could be possible in vivo some day and open new opportunities to cancer patients with poor prognosis. In recent years, first epigenetic modulating agents were tested as cancer drugs in the clinic. To date, the hypomethylating agents 5-azacytidine and 5-aza-2′-deoxycytidine, which are cytidine analogs have been approved in some, but not all countries for the treatment of myelodysplastic syndrome, chronic myelomonocytic leukemia and some forms of AML in adults. For a number of other agents including zebularine and proacaine, it is currently not certain whether they will reach the market because of treatment-associated toxicity. Epigallocatechin gallate is also currently being tested in the context of clinical trials and there is intense research in this field to find new potential agents for epigenetic therapy.

Within the field of HDAC inhibition during recent years, more than 15 HDAC inhibitors have been tested in preclinical and clinical trials (79) including valproic acid, suberoylanilide hydroxamic acid and phenyl butyrate. Especially for leukemia, they show promising results. The downregulation of adhesion molecules such as VLA-4 could on one hand help to reduce minimal residual disease in AML and help to improve peripheral blood stem cell mobilization in donors for stem cell transplantation (75). On the other hand, upregulation of adhesion molecules through HDAC inhibitors could improve stem cell homing after transplantation, which is an essential requirement for the successful engraftment in the context of hematopoietic stem cell transplantation. Another effect that may be ascribed to the influence of HDAC inhibitors on adhesion molecules could be the activation of leukocytes and the induction of tumor immunity through upregulation of lymphocyte function-associated antigen-1 and CD86 molecules in AML patients (76). These examples show the diversity of benefits that may occur in association with epigenetic therapy in the treatment of cancer through modulation of cellular adhesion.

Funding

Deutsche José Carreras Leukämie-Stiftung e. V. (DJCLS R 09/20) to U.M.

Conflict of Interest Statement: None declared.

References

61. J. Katto and U. Mahnknecht
70. Received April 14, 2011; revised June 2, 2011; accepted June 14, 2011.