PERIODICUM BIOLOGORUM
VOL. 112, No 4, 411–418, 2010

UDC 57:61
CODEN PDBIAD
ISSN 0031-5362

Overview

Epigenomics – A Bird's Eye Perspective on the Genome

KORALJKA GALL TROŠELJ
RENATA NOVAK KUJUNDŽIĆ

Laboratory of Epigenomics
Division of Molecular Medicine
Ruđer Bošković Institute
Bijenička 54, 10 000 Zagreb, Croatia

Correspondence:
Koraljka Gall Trošelj
Laboratory of Epigenomics
Division of Molecular Medicine
Ruđer Bošković Institute
Bijenička 54, 10 000 Zagreb, Croatia
E-mail: troselj@irb.hr

Received September 30, 2010.

Abstract

The constant interplay between DNA methylation, histone modifications and small interfering RNAs – including micro-RNA – provides the basis for establishing a cellular epigenomic network. Its occurrence depends on various endogenic and exogenic factors and is specific to a given type of cell and during a precise period of time. Thus, an epigenomic network is where phenomena known for a long time as »tissue and time specific gene expression« – both necessary for proper cell functioning – are manifested. A cancer cell is an excellent example of disturbed epigenomic network. Because of the reversible nature of the epigenomic process, the network can be partially or fully restored by the use of epigenomic drugs, to date, the inhibitors of DNA methylation and histone deacetylases. The possible intervention on the epigenome offers a very powerful tool in different fileds, including understanding disease pathophysiology, its treatment and, very significantly, its prevention.

INTRODUCTION

For some time now, most of us were convinced that susceptibility to disease primarily depended on inherited information given through a DNA genetic code. Accordingly, many efforts were made to establish functional links between changes in the DNA linear sequence (i.e., mutations, gene fusions resulting in chimeric proteins, gene amplifications resulting in gene disregulated expression) and specific disease phenotypes. This approach provided answers to a considerable number of questions, including the discovery of genes which, if mutated, caused a disease like cystic fibrosis. However, we were still left with many unsanswered and persistent questions based on the lack of knowledge because of complexity of DNA and protein interactions.

The term »Epigenetics« has been coined in order to cover different phenomena included in controlling the functional state of DNA. It is defined as heritable changes in gene expression which are independent of the primary DNA sequence but also stable, long-term alterations in the transcriptional potential of a cell which are not necessarily heritable. So defined, epigentics includes all mechanisms needed for controlling gene activity at different levels: transcriptionally, post-transcriptionally, at the level of protein translation and in post-translational protein modifications. All these processes work in a well-orchestraded manner, making a unique epigenome network that is specific for a given cell during a given period of time. This network represents a specific »mark« of the integrative signals arising from both endogenous and en-

vironmental signals. This »mark« directs phenomena known for some time as »tissue specific« and »time specific« expression.

By utilizing its epigenome, in order to adapt to these signals, through turning genes on and off, a cell adapts to such changes by removing and adding DNA methylation marks or through altering the histone proteins. These molecular processes are crucial for an epigenomic response. *Per contra*, deregulation of the epigenetic mechanism of »marking«, is associated with a variety of human diseases.

Major epigenetic mechanisms

Major epigenetic mechanisms include DNA methylation, covalent post-translational modifications of histone proteins (methylation, acetylation, phosphorylation, sumoylation) and RNA-mediated gene silencing. These three major epigenetic molecular mechanisms are closely linked because they create their own network of signals which re-amplify each other and lead to regulating different cellular processes. They are also a very important part of a specific response to environmental mutagens (or »epimutagenes«, in this context).

DNA methylation and gene transcription

Mammalian genomes are dominated by methylated DNA and approximately 56% of human genes contain CG-rich regions in their promoter regions *(1)*. These sequences, known as CpG islands (defined as regions with more than 500 base pairs and a GC content greater than 55%), which are non-randomly distributed in mammal genomes, are present in 1–2% of the genome. Typically, they are unmethylated in normal, healthy cells, except those assocated with imprinted genes, X-chromosome inactivation and transposable elements *(2)*. DNA methylation, as a phenomenon, has been studied primarily in the context of gene transcription. Indeed, the very sensitive and precise process of cytosine methylation, represents an essential mechanism for normal development in all species, shedding some light on »time and organ specific expression«.

DNA methylation itself represents the covalent addition of a methyl group to the five-carbon position of the cytosine base in CpG nucleotides. This biological process is not only extremely important in development, but also in disease pathophysiology – of which cancer represents a very important part. Its importance has been proved in experimental models which showed that differently active transgenes (active and silent, depending on their methylation status), keep the pattern of their activity (consequential to the pattern of their methylation status) during as many as 100 divisions *(3)*. The network responsible for exact »copying of methylation pattern«, from generation to generation, depends on two types of DNA methyltransferases (DNMTs).

DNA methyltransferases DNMT3A and DNMT3B have *de novo* methylation activity and, as such, they establish DNA methylation patterns during early development *(4)*. These patterns are then propagated through the activity of DNMT1 – the enzyme maintaining methylation with extreme fidelity *(5)*. Without any of these three enzymes, life would not be possible as their targeted disruption results in lethality (during or after embryonic development) accompanied by global DNA demethylation, as shown in mice models *(6)*.

As already noted, DNA methylation represses inappropriate expression of endogenous transposons which are involved in parental-specific silencing of one allele of imprinted genes. However, during the process of malignant transformation, the CpG islands, positioned in promoter regions of genes crucial for transformation, become hypermethylated. The consequence of this molecular event is the abnormal silencing of tumor suppressor genes (TSG) and other cancer-associated genes. This phenomenon is frequently related to increased level of DNMT1, as shown in different malignant tumors (colon, lung, breast, liver, stomach, acute and chronic myelogenous leukemia) *(7–12)*.

Very recent findings show the important role of the transcription factor Sp1 overexpression and/or p53 gene alteration in inducing DNMT1 protein expression in lung cancer. As shown in the clinical model, patients with altered p53 and overexpressed Sp1, followed by overexpressed DNMT1, have an increased risk of hypermethylation of multiple TSG promoters *(13)*. This finding is the first mechanicistic proof of functional link between p53 alterations and methylation status in the cancer cell.

However, it is still not clear whether DNA hypermethylation represses gene transcription directly or indirectly. There are two major proposed models of gene silencing related to promoter methylation *(14)*. The first model proposes that the methyl group of the ^{5}meC »extends« into the major groove of DNA, inducing conformational change. As a consequence, transcription factors cannot bind to their CpG-recognition sites. The second model is based on MeCPs (methyl cytosine binding proteins) function. As indicated by their name, these proteins bind to methylated CpG islands through their methyl-CpG-binding domains (MBD), creating a steric barrier to access by transcription factors. Additional proposed mechanisms by which CpG methylation represses gene transcription are related to RNA polymerase elongation, impairment of RNA-polymerase loading and interferrance with RNA polymerase initiation that involves localized histone acetylation.

Although these explanations seem to be relatively easily understood, actually, the process is very complex and dependent on many molecular players. DNA methylation occurs in a complex chromatin structure and is influenced by many factors, among which the modified histone tails represent a very important contributing facor.

Histones and histone modifications

Linear DNA, consisting of 147 base pairs, is wrapped by one octameric complex composed of two molecules of each of the four histones (H2A, H2B, H3, H4) and forming an array of nucleosomes. The amino termini of histones, histone tails, contain different post-translational modifications (lysine and arginine methylation, lysine acetylation, serine and threonine phosphorylation and lysine sumoylation, ubiquitinylation and ADP ribosylation) (Figure 1). Diversity of these modifications gave rise to the »histone code« hypothesis, proposed in 2000 *(15)*. The code itself has been linked to chromatine structure and gene function, mediating the transcriptional state of a cell *(16)*.

The nomenclature of any histone modifications takes the name of the histone, adds the single letter amino acid abbreviations (e.g. K for lysine), the position of amino acid in the protein, the specific type of modification and, if appropriate, the number of modifications. For example H3K27me3 denotes three methyl groups in H3, on the 27th lysine from the N-terminal end of the protein.

What seems to be clear is that histone acetylation is able to prevent DNA methylation through inhibiting the access of DNA methylases and facilitating the binding of transcription factors. Generally, acetylation of lysine residues on histones H3 and H4, contributes to the formation of an open chromatin structure which indicates transcriptional activity. In other words, global histone acetylation correlates with general transcriptional activity *(17)*. So, it is not surprising that administering histone deacetylase (HDAC) inhibitor, supporting histones in their acetylated form, also influences the methylation status of the promoters. These were exhibited in prostate cancer tissues where *RASSF*1 was a model gene, based on previous data showing its inactivation in prostate cancer. The results obtained with 131 samples of prostate cancer and 65 samples of benign prostate hipertrophy (BPH) were: a) inactivation of *RASSF*1 transcription related to aberrant promoter methylation in 74% of cancers and 18.5% of BPH; b) methylation frequency being higher in high stage/high Glisson sum samples; c) unmethylated promoters were enriched in acetylated histones and H3K4m2 (dimethylation of lysine, the fourth amino acid in the histone 3 (H3) structure); d) the reversal of histone code on hypermethylated promoter after administering DNA methylation inhibitor, but not TSA (trichostatin, HDAC inhibtor). The final piece of the puzzle, surprisingly conclusive, showed that reduced histone acetylation, or H3K4 dimethylation, joined with increased H3K9m2 (dimethylation of lysine, the ninth amino acid in H3 structure) plays a crucial role in *RASSF*1 silencing in this investigatory model *(18)*. From this and some other scientific reports, it is clear that the subtle communication between histone acetylation and DNA methylation is an enormously important interaction, whose rules and basic principles have yet to be discerned *(19)*.

In addition to histone modification *via* acetylation, another post-translational modification – methylation, has also been extensively studied. Generally, high levels of histone acetylation and H3K4 mono-, di- and trimethylation are present in promotores of active genes *(20, 21)*. On the other hand, trimethylation of H3K27 (H3K27m3) is related to gene silencing.

Many epigenetic questions have been answered through research on embryonic stem cells.

Lessons from embryonic stem cells

It was proved that expression of Oct-4, transcription factor essential for maintainance of pluripotency and specific for these cells, depends on high levels of H3K9ac and H3K4m3 and low level of H3K27m3. After only eight days of differentiation, H3K27m3 increases while and H3K4m3, and H3K9ac dramatically decreases (33--fold and 4.7-fold reduction). This picture seems to be very clear, almost »black and white« in its occurance. This process is mediated through a repressor complex that contains histone methyltransferase G9 and enzymes with histone deacetylase activity. As the next step, DNMT3A and DNMT3B become recruited through the ankyrin motif of G9, *de novo* DNA methylation occurs and, as a consequence, promoter region becomes hypermetyhlated *(22)* and the whole region becomes »locked« – no transcription. It has to be noted, however, that it is still unclear which modification, histone acetylation or CpG methylation, makes the primary signal by which gene expression is determined.

It is very interesting that H3K4m3 and H3K27m3, as two functionally opposite histone modifications, co-localize in so-called »bivalent domains« in the promoter region of approximately 2000 developmentally regulated »to be expressed« loci, in undifferentiated mouse and human embryonic stem cells *(23, 24)*. These two opposing modifications keep developmental regulators silenced,

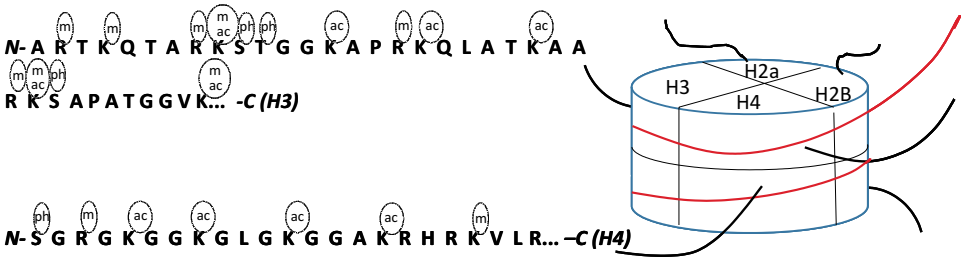

Figure 1. *Some histone tail post-translational modifications; Histones H3 and H4.*

while keeping them poised for alternate fates *(25)*. The differentiation is a trigger for these promoter marks to to become »univalent«: induced genes become enriched in H3K4me3 and lose H3K27me3 (as recently shown for the human brachyury T locus during mesoderm induction). Non-induced genes keep their H3K27me3 mark and lose H3K4me3 *(26)*.

This process is dependent on the presence of components of Polycomb group proteins (PcG) forming large complexes of which the two Polycomb Repressive Complexes (PRC1 and PRC2) are best characterized *(25)*. The PRC2 core complex contains four proteins: the catytic subunit – Enhancer of Zeste (EZH2), Suppressor of Zeste (SUZ12), Embryonic Ectoderm Development (EED) and the histone binding proteins RbAp46/RbAP48. So far, among these four members of the complex, EZH2, which is a methyltransferase, seems to get the highest level of attentionin in the field of cancer research. This interest is primarily related to specific silencing of TSGs as a consequence of lysine (tri)methylation (H3K27me3), which is directed by EZH2. However, PcG mediated gene repression represents a very complicated cascade of events triggered by the recruitment of PRC2 to target promoters and with H3K27 trimethylation representing the final consequenc of this process. Additionaly, PcG targeting and silencing predispose target genes to DNA methylation, as shown in different cancer types *(27, 28)*. This is in accord with previous observation on PRC2 interactions with DNMT1 and DNMT3B. Although, as recently shown, the PcG mediated suppression may occur independent of DNA methylation status *(29)*.

One of the first published reports on EZH2 in cancer clearly showed its significance in the progression of prostate cancer. It was the most significantly upregulated gene in metastatic prostate cancer as compared to localized prostate cancer *(30)*. Its overexpression led to silencing more than 100 genes. Five years later, in 2007, PRC2 repression signature, consisting of 14 repressed EZH2 genes, has been described as a tool for predicting prostate cancer patient outcome *(31)*. Even more important, based on profiled 14 gene candidates (CTSG – cathepsin G, CXCL12 – chemokine (C-X-C motif) ligand 12 (stromal cell-derived factor 1), DARC – Duffy blood group, chemokine receptor, EPHB6 – EPH receptor B6, FST – follistatin, ITGB2 – integrin, beta 2 (complement component 3 receptor 3 and 4 subunit), KRT17 – keratin 17, NCKAP1L – NCK-associated protein 1-like, PRKG1 – protein kinase, cGMP-dependent, type I, PTGER3 – prostaglandin E receptor 3 (subtype EP3), RLN1 – relaxin 1, SNCA – synuclein, alpha (non A4 component of amyloid precursor), SOCS2 – suppressor of cytokine signaling 2 and WNT2 – wingless-type MMTV integration site family member 2) the functional link related to PRC--repression signature surfaced: PRC2-related repressive marks known to control stem cell pluripotency and differentiation are also critical for cancer progression *(31, 32)*. Some of these genes were found to be significantly downregulated in metastases (when compared to the primary tumor) in some other tumor types such as breast – WNT2 and DARC *(33, 34)*, non-small cell lung cancer – EPHB6 *(35)* and small cell lung cancer *(36)*.

The most recent findings on EZH2 indicate that in myelodysplastic/myeloproliferative neoplasms and myelofibrosis, it may act as a tumor suppressor gene *(37, 38)*.

Epigenomic Drugs

Epigenomic changes in somatic cells, triggered by environmental factors, can be reversed by the use of epigenetic drugs. This therapy also can influence inherited epigenetic marks. The basis for this approach is reversing the epigenetic process, which is very different from conventional chemotherapy which kills cancer cells as well as all dividing cells. Reversing the epigenetic process appears to be very promising and the hope is that »reversion« will be very significant in the diagnosis and treatment of major human diseases (diabetes, cardiopulmonary diseases, Rett syndrome and some other neurological disorders, autoimmune diseases and cancer), as well as the consequences of aging. Particularly in cancer, missteps in epigenetic programming have been directly implicated and reversibility of changes point to epigenetics as a key discipline for developing targeted chemoprevention.

There are two major types of epigenetic drugs: DNA methylation inhibitors and histone deacetylation (HDAC) inhibitors. The first group is also known as »demethylators«, which act as substitutes for cytosine residues during cell division. The consequences of their incorporation into DNA, in lieu of cytosines, is the blocking and binding of DNMTs, causing depletion of overly active methylator enzymes. There are two FDA-approved demethylating, cytidine derivative, drugs: Vidaza™ (5-azacytidine), which was approved in May, 2004; and Dacogen™ (5-aza-2'-deoxycytidine, decitabine), which is a deoxyribose analog of 5-azacytidine, approved in May, 2006. These two drugs are chemically related to each other. However, while 5-azacytidine contains ribose sugar ring, decytabine contains deoxyribose. Accordingly, 5-azacytidine incorporates largely into RNA. The first target for both drugs is Myelodysplastic Syndrome (MDS). The antineoplastic effects caused by Vidaza™ and Dacogen™ are related to DNA hypomethylation (restoration of gene activity necessary for differentiation) and a direct cytotoxic effect on abnormal, rapidly dividing hematopoietic cells in the bone marrow which became unresponsive to normal growth control mechanisms. Non-proliferating cells are relatively insensitive to Vidaza™.

There are many scientific reports of abnormal histone acetylation in tumor cells, in favor of deacetylation. Hence, the rationale for introducing HDAC inhibitors in the clinic would be to inhibit histone deacetylase, prevent histone deacetylation and consequential tight DNA wrapping around histones. The HDAC inhibitors contribute to the formation of open, euchromatin structure, through indirectly »keeping« the acetyl group attached to the lysine of the histone tail and decreasing its positive charge. As a consequence, the interaction between lysine

and DNA decreases *(39)*. In cancer therapy, the application of HDAC would results in recovering otherwise silent TSGs.

However, the problem here is selectivity, as HDAC inhibitors would inevitably modulate other acetylation signaling events and »hide« the mechanism of action (targeted HDAC inhibition vs. incidental interference with off-target pathways) needed for a targeted approach (pure therapeutic effect). One should keep in mind that HDACs are a family of at least 11 isozymes, organized in multisubunit complexes that are not yet well understood *(40)*. Accordingly, any kind of intervention with HDAC inhibitors may result in side effects that can be extremely serious. These days, more than 80 clinical trials are underway, testing more than ten different substances from this group of inhibitors, in both solid and hematopoietic malignancies *(41)*.

Vorinostat (suberoylanilide hydroxamic acid; SAHA; Zolinza™), whose approval was granted by FDA in October, 2006 for the treatment of cutaneous manifestations of cutaneous T-cell lymphoma (CTCL) in patients with progressive, persistent, or recurrent disease, acts as pan-inhibitor, without selectivity. It is not surprising that the immediate goal must be selectivity achievement, in this field. This approach has been pursued by some companies.

MethylGene's CEO, Donald Corcoran, claims that their HDAC inhibiting compound MGCD0103 in clinical trials all around the world, represents: »… a rationally designed, potent and selective for specific HDAC isoforms« drug. Its novel isotype-selective histone deacetylase inhibition has been well documented through antitumor activity *in vitro* and *in vivo (42)* and it is the first HDAC inhibitor with proved microtubule destabilizing activity *(43)*. Indeed, there are many exciting results based on a very good therapeutic responses by cancer patients suffering from Hodgkin's lymphoma, acute myeloid leukemia and MDS.

This has been also the case with Novartis' panobinostat – LBH589, whose mechanism of molecular action has been studied in great detail. This substance has shown very good results in treating multiple myeloma in combination with melfalan and doxorubicin, *in vitro and in vivo (44)*. It was also shown that LBH589 induces expression of DNA damage response genes and apoptosis in Ph^- acute lymphoblastic leukemia cells. It also inhibits the activity of aromatase gene in breast cancer cells, which makes it a very promising candidate in treating hormone dependent breast cancer *(45, 46)*.

There are many different compounds inhibiting histone deacetylase (phenylbutyrate, valproic acid, romidepsin, trichostatin A). However, currently, we are still unclear which modification, histone acetylation or CpG methylation, makes the primary signal by which gene expression is determined. In any event, hypermethylated CpG islands of tumor suppressor genes are commonly associated with hypoacetylated histone. One reason for this phenomenon is ability of methylated promoters to recruit, through a group of proteins with methyl DNA binding domain, a protein complex containing histone deacetylase and histone methyltransferase *(47)*. Hence, it is not surprising that a combination of agents from these two groups (demethylators and HDAC inhibitors) showed very promising results, in *vitro*. It was shown that the sequential treatment of cancer cells with 5-azacytosine analogs and, subsequently, an HDAC inhibitor results in stronger re-expression of methylated TSGs than either agent alone *(48)*. These data prompted several clinical trials with the combination approach. Some of results are very promising *(49, 50)*.

With the number of these optimistic reports dramatically increasing, one could really hope that these epigenomic approaches represent a new era in cancer chemoprevention and treatment because of the reversibility of epigenome changes.

Micro-RNA (mi-RNA; miR)

A very powerful epigenetic modulator of epigenome is micro-RNA (mi-RNA). These short (~22 nt long), non-coding, single stranded RNA molecules are found both in plants and animals *(51)*. As of August, 2010, there are 940 confidently identified mi-RNA genes in humans with this number constantly increasing (miRBase; University of Manchester, Manchester UK) *(52, 53)*. These molecules negatively regulate gene activity post-transcriptionally, depending on the complementarity level between the taget (mRNA) and mi-RNA itself. Perfect or near perfect binding of mi-RNA, depending on fully complementary or nearly perfect complementary, respectively, induces a RNA-mediated interferance (RNAi) pathway, as commonly found in plants. More common pathway in humans is based on mi-RNA binding to imperfectly complementary sites within the 3' untranslated regions (3'UTR) of their mRNA targets, through a RISC (RNA Induced-Silencing) complex, which affects the target gene activity at the level of translation. In this situation, target recognition depends mainly on »seed« sequence which is seven nucleotides long and corresponds to mi-RNA nucleotides 2–8. However, this is not the only parameter affecting the binding and there are several additional features of site context strongly influenzing the binding *(53)*. Mi-RNA can also affect mRNA stability. As a consequence, mRNA levels are not commonly affected, but protein levels are reduced. It has been estimated that each mi-RNA is predicted to target hundreds of different mRNAs, thus influencing key cellular regulatory mechanisms.

mi-RNA transcription is directed by RNA Pol II, through the process generating the molecule known as pri-micro-RNA. This molecule, after being processed by two enzymes, Drosha (ribonuclease type III) and Pasha (DGCR8) into ~70 nucleotide long pre-micro-RNA must be exported into cytoplasm by Exportin 5. Additional processing is necessary for creating a mi-RNA: mi-RNA duplex, which must be excised from the pre-mi-RNA hairpain by the enzyme Dicer (ribonuclease type III). Finally, the mi-RNA:mi-RNA incorporates into the

miRISC complex which retains the mature, single-stranded mi-RNA which binds to the complementary sites in the mRNA target (Figure 2).

The most recent finding shows that Dicer does not need to be always included in mi-RNA processing. In mice, the presence of mature miR-451 does not depend on Dicer, but on Ago2 (Argonaute Protein 2, Eif2cs) *(54)*.

There is growing data on mi-RNA expression profiles in cancer. Specific miRNA signatures were shown to be informative for tumor classification and clinical outcome in some tumor types *(55)*. Although there are several mi-RNAs that are upregulated in different malignancies (like miR-21, miR-191, and miR-17-5p), the global picture of cancer shows global mi-RNA downregulation *(56)*. The reason, as shown *in vitro* and *in vivo*, in breast cancer patients, may well be the increased expression of mi-R-103/107 family which binds, as determined through computational analysis, to eight sights in Dicer's 3'UTR mRNA. Based on what was previously said about mi-RNA processing, it is clear that miR-103/107 overexpression leads to inhibition of mi-RNA biogenesis, through downregulation of Dicer. Specifically related to the miR-103/107 family in breast cancer, if overexpressed, they promote metastasis and epithelial-to mesenchymal transition *(57)*. This is only one example showing how an only 22 nucletides long molecule can reshape and modify the epigenome.

Another example of strong epigenome reshaping influenced through only one micro-RNA comes from studies related to miR-101. This molecule, as shown in prostate and some other carcinoma types, targets EZH2, previously mentioned histone methyltransferase that tri-methylates H3K27 and consequentially leads to gene silencing. If abundantly present, miR-101 acts as a tumor suppressor molecule, keeping the protein level of EZH2 low. The cells in which it is lost (mostly through deletion of the part of the chromosomes where it is located; 1p31.3, 9p24) show high level of both EZH2 and H3K27m3. As a consequence, a decrease in the activity of various PRC2 target genes occurs, as shown for: ADRB2, DAB2IP, CIITA, RUNX3, CDH1 and WNT1 *(58)*.

Based on this limited set of data it becomes clear that manipulating mi-RNA expression level may serve as a good therapeutic goal. This possibility has just begun to be explored in HCV infected monkeys. In addition to its organotropism, the HCV also shows a certain »mi-RNA--tropism« directed to a liver-specific miR-122. So far, it has been shown that miR-122 and HCV RNA form a complex to maintain viral abundance *(59)*. Intravenous injection of HCV-infected chimpanzees with locked nucleic acids (SPC3649, a 15 nt long molecule complementary to the 5' of miR-122) sequestering and inactivating miR-122, results in dramatic decrease of HCV yield *(60)*. The treatment in monkeys was not associated with toxicities and there is no data on the safety of its usage in humans. One can also doubt not only the selectivity of the treatment itself (as it surely affects more than one mi-RNA), but also selectivity related to a broad spectrum of targets for a given mi-RNA (according to miRanda database, miR-122 potentially targets 1,958 mRNAs).

The need for caution is of extreme importance in this area because one should understand the role of any mi-RNA in the pathogenic process, which is and will be extremely difficult taks.

As a part of this complex picture are several studies showing dietary modulation of mi-RNA expression. The expectation is that some cancer-preventive effects, related to well known dietary compounds, may be explained through their influence on mi-RNA expression. To date, the results from only a few studies were published with the following compounds investigated: genistein, folate, retinoic acid, curcumin and some others *(61)*.

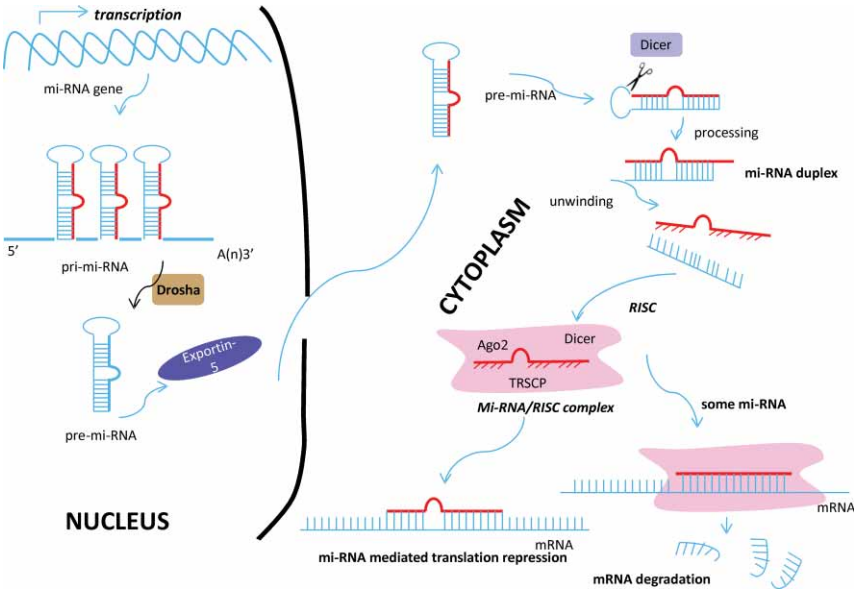

Figure 2. *Simplified scheme of mi-RNA processing.*

This mini-review can only provide some highlights of epigenetics, with every molecule and process described constituting its own mini-universe. Each of them, and others which could not be included, may represent a promising potential therapeutic target, not only in the field of cancer treatment, but also in all areas of human pathology.

REFERENCES

1. ANTEQUERA F, BIRD A 1993 Number of CpG islands and genes in human and mouse. *Proc Natl Acad Sci USA 90*: 11995–11999
2. JAENISCH R, BIRD A 2003 Epigenetic regulation of gene expression: how the genome integrate intrinsic and environmental signals. *Nat Genet 33 (Suppl)*: 245–254
3. SCHÜBELER D, LORINCZ M C, CIMBORA D M, TELLING A, FENG Y Q, BOUHASSIRA E E, GROUDINE M 2000 Genomic targeting of methylated DNA: influence of methylation on transcription, replication, chromatin structure, and histone acetylation. *Mol Cell Biol 20*: 9103–9112
4. REIK W, KELSEY G, WALTER J 1999 Dissecting *de novo* methylation. *Nat Genet 23*: 380–382
5. BIRD A 2002 DNA methylation patters and epigenetic memory. *Genes Dev 16*: 6–21
6. LI E, BESTOR R, JAENISCH R 1992 Targeted mutation of the DNA methyltransferase gene results in embryonic lethality. *Cell 69*: 915–926
7. DE MARZO A M, MARCHI V L, YANG E S, VEERASWAMY R, LIN X, NELSON W G 1999 Abnormal regulation of DNA methyltransferase expression during colorectal carcinogenesis. *Cancer Res 59*:3855–3860
8. LIN R K, HSU H S, CHANG J W, CHEN C Y, CHEN J T, WANG Y C 2007 Alteration of DNA methyltransferases contributes to 5'CpG methylation and poor prognosis in lung cancer. *Lung Cancer 55*: 205–213
9. GIRAULT I, TOZLU S, LIDEREAU R, BIECHE I 2003 Expression analysis of DNA methyltransferases 1, 3A, and 3B in sporadic breast carcinomas. *Clin Cancer Res 9*: 4415–4422
10. SAITO Y, KANAI Y, NAKAGAWA T, SAKAMOTO M, SAITO H, ISHII H, HIROHASHI S 2003 Increased protein expression of DNA methyltransferase (DNMT) 1 is significantly correlated with the malignant potential and poor prognosis of human hepatocellular carcinomas. *Int J Cancer 105*: 527–532
11. ETOH T, KANAI Y, USHIJIMA S, NAKAGAWA T, NAKANISHI Y, SASAKO M, KITANO S, HIROHASHI S 2004 Increased DNA methyltransferase 1 (DNMT1) protein expression correlates significantly with poorer tumor differentiation and frequent DNA hypermethylation of multiple CpG islands in gastric cancers. *Am J Pathol 164*: 689–699
12. MIZUNO S, CHIJIWA T, OKAMURA T, AKASHI K, FUKUMAKI Y, NIHO Y, SASAKI H 2001 Expression of DNA methyltransferases DNMT1, 3A, and 3B in normal hematopoiesis and in acute and chronic myelogenous leukemia. *Blood 97*: 1172–1179
13. LIN R K, WU C Y, CHANG J W, JUAN L J, HSU H S, CHEN C Y, LU Y Y, TANG Y A, YANG Y C, YANG P C, WANG Y C 2010 Dysregulation of p53/Sp1 control leads to DNA methyltransferase-1 overexpression in lung cancer. *Cancer Res 70*: 5807–5817
14. SINGAL R, GINDER G D 1999 DNA methylation. *Blood 93*: 4059–4070
15. STRAHL B D, ALLIS C D 2000 The language of covalent histone modifications. Nature 403: 41–45
16. TURNER B M 2002 Cellular memory and the histone code. *Cell 111*: 285–291
17. GOTTESFELD J M, FORBES D J 1997 Mitotic repression of the transcriptional machinery. *Trends Biochem Sci 22*: 197–202
18. KAWAMOTO K, OKINO S T, PLACE R F, URAKAMI S, HIRATA H, KIKUNO N, KAWAKAMI T, TANAKA Y, POOKOT D, CHEN Z, MAJID S, ENOKIDA H, NAKAGAWA M, DAHIYA R 2007 Epigenetic modifications of RASSF1A gene through chromatin remodeling in prostate cancer. *Clin Cancer Res 13*: 2541–2548
19. VAISSIÈRE T, SAWAN C, HERCEG Z 2008 Epigenetic interplay between histone modifications and DNA methylation in gene silencing. *Mutat Res 659*: 40–48
20. ROH T Y, CUDDAPAH S, CUI K, ZHAO K 2006 The genomic landscape of histone modifications in human T cells. *Proc Natl Acad Sci U S A 103*: 15782–15787
21. BARSKI A, CUDDAPAH S, CUI K, ROH TY, SCHONES DE, WANG Z, WEI G, CHEPELEV I, ZHAO K 2007 High-resolution profiling of histone methylations in the human genome. *Cell 129*: 823–837
22. EPSZTEJN-LITMAN S, FELDMAN N, ABU-REMAILEH M, SHUFARO Y, GERSON A, UEDA J, DEPLUS R, FUKS F, SHINKAI Y, CEDAR H, BERGMAN Y 2008 *De novo* DNA methylation promoted by G9a prevents reprogramming of embryonically silenced genes. *Nat Struct Mol Biol 15*: 1176–1183
23. BERNSTEIN B E, MIKKELSN T S, XIE X, KAMAL M, HUEBERT D J, CUFF J, FRY B, MEISSNER A, WERNIG M, PLATH K, JAENISCH R, WAGSCHAL A, FEIL R, SCHREIBER S L, LANDER E S 2006 A bivalent chromatin structure marks key developmental genes in embryonic stem cells. *Cell 125*: 315–326
24. MIKKELSEN T S, KU M, JAFFE D B, ISSAC B, LIEBERMAN E, GIANNOUKOS G, ALVAREZ P, BROCKMAN W, KIM T K, KOCHE R P, LEE W, MENDENHALL E M, O'DONOVAN A, PRESSER A, RUSS C, XIE X, MEISSNER A, WERNIG M, JAENISCH R, NUSBAUM C, LANDER E S, BERNSTEIN B E 2007 Genome-wide maps of chromatin state in pluripotent and lineage-committed cells. *Nature 448*: 553–560
25. KU M, KOCHE R P, RHEINBAY E, MENDENHALL E M, ENDOH M, MIKKELSN T S, PRESSER A, NUSBAUM C, XIE X, CHI A S, MAZHAR A, KASIF S, PTASZEK L M, COWAN C A, LANDER E S, KOSEKI H, BERNSTEIN B E 2008 Genomewide analysis of PRC1 and PRC2 occupancy identifies two classes of bivalent domains. *Plos Genet 4*: e10000242
26. GOLOB J L, PAIGE S L, MUSKHELI V, PABON L, MURRY CE 2008 Chromatin remodeling during mouse and human embryonic stem cell differentiation. *Dev Dyn 237*: 1389–1398
27. SCHLESINGER Y, STRAUSSMAN R, KESHET I, FARKASH S, HECHT M, ZIMMERMAN J, EDEN E, YAKHINI Z, BEN-SHUSHAN E, REUBINOFF BE, BERGMAN Y, SIMON I, CEDAR H 2007 Polycomb-mediated methylation on Lys27 of histone H3 pre-marks genes for *de novo* methylation in cancer. *Nat Genet 39*:232-236
28. WIDSCHWENDTER M, FIEGL H, EGLE D, MUELLER-HOLZNER E, SPIZZO G, MARTH C, WEISENBERGERDJ, CAMPAN M, YOUNG J, JP, JACOBS I, LAIRD PW 2007 Epigenetic stem cell signature in cancer. *Nat Genet 39*:157-158
29. KONDO Y, SHEN L, CHENG A S, AHMED S, BOUMBER Y, CHARO C, YAMOCHI T, URANO T, FURUKAWA K, KWABI-ADDO B, GOLD D L, SEKIDO Y, HUANG T H, ISSA J P 2008 Gene silencing in cancer by histone H3 lysine 27 trimethylation independent of promoter DNA methylation. *Nat Genet 40*: 741–750
30. VARAMBALLY S, DHANASEKARAN S M, ZHOU M, BARRETTE T R, KUMAR-SINHA C, SANDA M G, GHOSH D, PIENTA K J, SEWALT R G, OTTE A P, RUBIN M A, CHINNAIYAN A M 2002 The polycomb group protein EZH2 is involved in progression of prostate cancer. *Nature 419*: 624–629
31. YU J, RHODES DR, TOMLINS SA, CAO X, CHEN G, MEHRA R, WANG X, GHOSH D, SHAH R B, VARAMBALLY S, PIENTA K J, CHINNAIYAN A M 2007 A polycomb repression signature in metastatic prostate cancer predicts cancer outcome. *Cancer Res 67*: 10657–10663
32. LEE TI, JENNER RG, BOYER LA, GUENTHER MG, LEVINE SS, KUMAR RM, CHEVALIER B, JOHNSTONE SE, COLE MF, ISONO K, KOSEKI H, FUCHIKAMI T, ABE K, MURRAY HL, ZUCKER JP, YUAN B, BELL GW, HERBOLSHEIMER E, HANNETT NM, SUN K, ODOM DT, OTTE AP, VOLKERT TL, BARTEL DP, MELTON DA, GIFFORD DK, JAENISCH R, YOUNG RA 2006 Control of developmental regulators by Polycomb in human embryonic stem cells. *Cell 125*:301-313
33. ELLSWORTH R E, SEEBACH J, FIELD L A, HECKMAN C, KANE J, HOOKE J A, LOVE B, SHRIVER C D 2009 A gene expression signature that defines breast cancer metastases. *Clin Exp Metastasis 26*: 205–213
34. WANG J, OU Z L, HOU Y F, LUO J M, SHEN Z Z, DING J, SHAO Z M 2006 Enhanced expression of Duffy antigen receptor for chemokines by breast cancer cells attenuates growth and metastasis potential. *Oncogene 25*: 7201–7211
35. YU J, BULK E, JI P, HASCHER A, TANG M, METZGER R, MARRA A, SERVE H, BERDEL W E, WIEWROTH R, KOS-

CHMIEDER S, MÜLLER-TIDOW C 2010 The EPHB6 receptor tyrosine kinase is a metastasis suppressor that is frequently silenced by promoter DNA hypermethylation in non-small cell lung cancer. *Clin Cancer Res 16*: 2275–2283

36. OGINO H, YANO S, KAKIUCHI S, MUGURUMA H, IKUTA K, HANIBUCHI M, UEHARA H, TSUCHIDA K, SUGINO H, SONE S 2008 Follistatin suppresses the production of experimental multiple-organ metastasis by small cell lung cancer cells in natural killer cell-depleted SCID mice. *Clin Cancer Res 143*:660–667
37. NIKOLSKI G, LANGEMEIJER SM, KUIPER R P, KNOPS R, MASSOP M, TÖNNISSEN E R, VAN DER HEIJDEN A, SCHEELE T N, VANDENBERGHE P, DE WITTE T, VAN DER REIJDEN B A, JANSEN J H 2010 Somatic mutations of the histone methyltransferase gene EZH2 in myelodysplastic syndromes. *Nat Genet 42*: 665–667
38. ERNEST T, CHASE A J, SCORE J, HIDALGO-CURTIS C E, BRYANT C, JONES A V, WAGHORN K, ZOI K, ROSS F M, REITER A, HOCHHAUS A, DREXLER H G, DUNCOMBE A, CERVANTES F, OSCIER D, BOULTWOOD J, GRAND F H, CROSS N C 2010 Inactivating mutations of the histone methyltransferase gene EZH2 in myeloid disorders. *Nat Genet 42*: 722–726
39. GREGORY PD, WAGNER K, HÖRZ W 2001 Histone acetylation and chromatin remodeling. *Exp Cell Res 265*:195-202
40. GRAY SG, EKSTRÖM TJ 2001 The human histone deacetylase family. *Exp Cell Res 262*:75-83
41. TAN J, CANG S, MA Y, PETRILLO R L, LIU D 2010 Novel histone deacetylase inhibitors in clinical trials as anti-cancer agents. *J Hematol Oncol 3*: 5
42. FOURNEL M, BONFILS C, HOU Y, YAN PT, TRACHY-BOURGET MC, KALITA A, LIU J, LU AH, ZHOU NZ, ROBERT MF, GILLESPIE J, WANG JJ, STE-CROIX H, RAHIL J, LEFEBVRE S, MORADEI O, DELORME D, MACLEOD AR, BESTERMAN JM, LI Z 2008 MGCD0103, a novel isotype-selective histone deacetylase inhibitor, has broad spectrum antitumor activity *in vitro* and *in vivo*. *Mol Cancer Ther 4*: 759-768
43. CHIA K, BEAMISH H, JAFFERI K, GABRIELLI B 2010. The histone deacetylase inhibitor MGCD0103 has both deacetylase and microtubule inhibitory activity. *Mol Pharmacol 78*: 436–443
44. SANCHEZ E, SHEN J, STEINBERG J, LI M, , BONAVIDA B, CHEN H, LI Z W, BERENSON J R 2010 The histone deacetylase inhibitor LBH589 enhances the anti-myeloma effects of chemotherapy in vitro and in vivo. *Leuk Res (in press)*.
45. SCUTO A, KIRSCHBAUM M, KOWOLIK C, KRETZNER L, JUHASZ A, ATADJA P, PULLARKAT V, BHATIA R, FORMAN S, YEN Y, JOVE R 2008 *Blood 111*: 5093–5100
46. CHEN S, YE J, KIJIMA I, EVANS D 2010 The HDAC inhibitor LBH589 (panobinostat) is an inhibitory modulator of aromatase gene expression. *Proc Natl Acad Sci USA 107*:11032–11037
47. NAN X, NG H H, JOHNSON C A, LAHERTY C D, TURNER B M, EISENMAN R N, BIRD A 1998 Transcriptional repression by the methyl-CpG-binding protein MeCP2 involves a histone deacetylase complex. *Nature 393*: 386–389
48. CAMERON EE, BACHMAN KE, MYÖHÄNEN S, HERMAN JG, BAYLIN SB 1999 Synergy of demethylation and histone deacetylase inhibition in the re-expression of genes silenced in cancer. *Nature Gen 21*:103-107
49. GRIFFITHS E A, GORE S D 2008 DNA methyltransferase and histone deacetylase inhibitors in the treatment of myelodysplastic syndromes. *Semin Hematol 45*: 23–30
50. JAIN N, ROSSI A, GARCIA-MANERO G 2009 Epigenetic therapy of leukemia: an update. *Int J Biochem Cell Biol 41*: 72–80
51. BARTEL D P 2009 MicroRNAs: Target recognition and regulatory functions. *Cell 136*: 215–233
52. GRIFFITHS-JONES S, SAINI H K, VAN DONGEN S, ENRIGHT A J 2008 miRBase: tools for microRNA genomics. *Nucleic Acids Res 36*: D154–588
53. GRIMSON A, FARH K K, JOHNSTON W K, GARRETT-ENGELE P, LIM L P, BARTEL D P 2007 MicroRNA targeting specificity in mammals: determinants beyond seed pairing. *Mol Cell 27*: 91–105
54. CHELOUFI S, DOS SANTOS C O, CHONG M M, HANNON G J 2010 A dicer-independent miRNA biogenesis pathway that requires Ago catalysis. *Nature 465*: 584–589
55. VENTURA A, JACKS T, 2009 MicroRNAs and cancer: short RNAs go a long way. *Cell 136*: 586–591
56. VOLINIA S, CALIN G A, LIU C G, AMBS S, CIMMINO A, PETROCCA F, VISONE R, IORIO M, ROLDO C, FERRACIN M, PRUEITT R L, YANAIHARA N, LANZA G, SCARPA A, VECCHIONE A, NEGRINI M, HARRIS C C, CROCE C M 2006 A microRNA expression signature of human solid tumors defines cancer gene targets. *Proc Natl Acad Sci U S A 103*: 2257–2261
57. MARTELLO G, ROSATO A, FERRARI F, MANFRIN A, CORDENONSI M, DUPONT S, ENZO E, GUZZARDO V, RONDINA M, SPRUCE T, PARENTI A R, DAIDONE M G, BICCIATO S, PICCOLO S 2010 A MicroRNA targeting dicer for metastasis control. *Cell 141*: 1195–2207
58. VARAMBALLY S, CAO Q, MANI R S, SHANKAR S, WANG X, ATEEQ B, LAXMAN B, CAO X, JING X, RAMNARAYANAN K, BRENNER J C, YU J, KIM J H, HAN B, TAN P, KUMAR-SINHA C, LONIGRO R J, PALANISAMY N, MAHER C A, CHINNAIYAN A M 2008 Genomic loss of microRNA-101 leads to overexpression of histone methyltransferase EZH2 in cancer. *Science 322*: 1695–1699
59. JOPLING C L, YI M, LANCASTER A M, LEMON S M, SARNOW P 2005 Modulation of hepatitis C virus RNA abundance by a liver-specific MicroRNA. *Science 309*: 577–1581
60. LANFORD R E, HILDEBRANDT-ERIKSEN E S, PETRI A, PERSSON R, LINDOW M, MUNK M E, KAUPPINEN S, ØRUM H 2010 Therapeutic silencing of microRNA-122 in primates with chronic hepatitis C virus infection. *Science 327*: 198–201
61. DAVIS C D, ROSS S A 2008 Evidence for dietary regulation of microRNA expression in cancer cells. *Nutr Rev 66*: 477–482