Introduction to Epigenetics

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Abstract

Epigenetic processes control central genomic functions such as the utilization of genetic information over the course of life. Epigenetic processes are controlled by adding and removing epigenetic modifications on the genes. Epigenetic modifications are added at different molecular levels and form a complex combination of positively and negatively regulating molecular signals. Most of these signals are established directly on the DNA bases or on the proteins that package the DNA, called histones. Modern sequencing methods make it possible to locate these various types of epigenetic modification with precision and to associate their functional significance with a particular gene-specific control. Epigenetic modifications are cell-specific, and their function must therefore be viewed and evaluated in a different way to genetic changes, which are the same in all cells. In epigenetic studies, therefore—unlike genetic analysis—the cell type or (in tissues) the cell composition must always be included in the picture. Cell-type-specific epigenetic patterns can be affected by factors that are endogenous to the organism (ageing, hormonal control) and by those that are exogenous (environment, e.g., metabolism, stress), and they lead

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to persistent changes in cell programming. As a general principle, cell-type-specific epigenetic differences are considerably more stable and more pronounced than changes arising due to exogenous factors. Epigenetic modifications are stably passed on through cell divisions. However, when cell programming changes, they are deleted or their composition is altered (reprogrammed). In human beings, large-scale reprogramming (deletion) of old 'inherited' epigenetic modifications takes place both in gametes and in the embryo shortly after fertilization. For this reason, transmission of 'acquired' epigenetic modifications across generations is possible only to a very limited extent in humans.

1 Basic Principles of Epigenetic Concepts

The word 'epigenetics' means roughly 'above genetics', with undertones of 'in addition to the genome' (see Seitz as well as Schoul, both in this volume). Epigenetics describes mechanisms that lead to changed, heritable structural and activation states of the chromatin[1] without changes to the primary nucleotide sequence[2] (definition by Knippers and Nordheim 2015; see Chap. 20, this volume, for an overview of the molecular aspects). This molecular genetic definition describes some characteristics, but leaves out some aspects of the functional consequences and other possible levels of epigenetic control. In this contribution, therefore, we shall briefly explore some of these additional aspects of epigenetics.

[1]Chromatin is the name given to the entirety of the DNA and protein material in the cell that is stained by alkaline staining. Core elements of chromatin are histone protein complexes, which together with the DNA wrapped around them form the nucleosomes. Almost all the DNA in the chromosomes is organized ('packaged') in nucleosomes. Between nucleosomes lie short regions of free (non-coding) DNA ('spacers'). Chromosome regions that play a part in gene regulation are less densely covered in nucleosomes. Nucleosomes are differently arranged in active and in inactive gene regions; they can also be even more tightly 'packed' in higher-order structures. Such higher-order structures are usually completely inaccessible to gene regulation. Other material that occurs in chromatin includes site-specific RNA molecules and other proteins that are not histones but are important for gene regulation, or control gene regulation in a targeted way.

[2]'Nucleotide sequence' means the sequence of chemical building blocks of DNA (and RNA). DNA forms long chains of linear molecules in which nucleobases and pentoses (sugar) are linked together by phosphates. These molecules are copied by enzymes, and thus the information carried by the molecules is duplicated and transmitted onwards.

The aim of this brief digression is to roughly outline the range of current epigenetic concepts and the differences between them.

Whereas definitions of genetics-oriented epigenetics focus primarily on the aspect of direct heritability via modified DNA bases and chromatin modifications, definitions of epigenetics that are geared more to cell biology, or those that are more purely operational, see it more as a portmanteau word for mechanisms additional to DNA that induce heritable alterations in cellular programming and that can also take place at other levels than DNA. One limitation of definitions based on strict heritability of epigenetic modifications is that even 'classical' epigenetic modifications do not act solely to enable transmission of epigenetic states of DNA and chromosomes: they often also influence other forms of temporary regulation of genomes, such as DNA replication, DNA recombination, short-term base changes (mutations), DNA repair, and transient (non-heritable) gene control. These temporary processes do not in the strict sense lead to stable, heritable phenotypic changes.

In broader definitions the term 'epigenetics' often serves as a kind of overall designation non-genetic heredity at every level; that is, it describes a number of sometimes very different mechanisms whose temporal and heritable components have not in all cases been clearly determined. For example, the passing on of small RNA molecules ('small RNAs') from cell to cell is regarded as epigenetic transmission—even though this is primarily a temporary genetic effect determined by the cell plasma and does not take place in the cell nucleus. In addition, various processes of RNA storage and RNA interference are often referred to together as 'epigenetic'—for some of them this is an accurate description, but for others it is very hard to argue that it is valid. In other interpretations that go still further, even molecular processes in the cell plasma about which little is known, for example the spatial reconfiguration of prions,[3] are cited as examples of epigenetic phenomena (Lewin 1998). Another aspect is 'early embryonic programming', the process by which, under certain circumstances, proteins and RNA molecules passed on with the cell cytoplasm of oocytes and spermatozoa can affect gene expression in the long term. In animal and plant breeding, examples of this are known in the form of (reciprocal) hybrid crossings with differentially strongly expressed traits (Youngson and Whitelaw 2008).

[3]Prions are small glycoproteins (that is, proteins that contain sugar chains), whose physiological function is still unclear. This class of protein became known through pathological (disease-causing) such as those that cause Kreutzfeld–Jakob disease or 'mad cow disease' (bovine spongiform encephalopathy).

The epigenetic processes referred to above make clear how complex are the regulatory aspects that must be considered in relation to the specific biological context in any individual case. In fact, no single, generally valid definition of epigenetics can cover the multiplicity of mechanisms known to science today, some of which go beyond the purely genetic level.

Accordingly, it remains true that, even in the specialist literature, 'epigenetics' continues to be a broadly used term that in many cases inadequately reflects to the systemic processes behind it.

2 Levels of Epigenetic Gene Control

As we have said, epigenetic mechanisms are located at several levels. On the genome, the levels are those of DNA modifications and chromatin. Partly decoupled from the genome there are modifying proteins and non-coding RNA, whose site of action is in the nucleus or the cytoplasm. The common property of all three of these levels of epigenetic mechanism is that they influence the function and regulation of genes in a *long-lasting* but at the same time *reversible* manner.

2.1 DNA Methylation

DNA methylation is added by DNA methyl transferases (DNMTs) to certain building blocks of DNA (bases) in a targeted way. DNA methylation is a chemically very stable covalent modification of certain cytosine bases which can be (indirectly) demonstrated in old DNA. Through its attachment to DNA bases, it serves as a direct signal for a copying procedure performed by the DNA methyltransferase DNMT1 after DNA replication. In this way, DNA methylation can be directly copied and passed on through cell divisions. In a similar way to histone modifications, the cells of our body cell show specific DNA methylation patterns. In the early stages of development, the amount of DNA methylation in the genome is very strongly reduced. Then in the course of development it is restarted in a cell-specific way, and during this process DNA methylation is started in the genome in a very targeted manner. Cytosine building blocks are mostly methylated in the sequence cytosine–guanine (CpG). In neurons and stem cells, methylation is also widely found outside CpGs, but the functional of this "irregular" non-CpG-methylation is still unclear. DNA methylation is recognized as an epigenetic signal by special DNA-binding proteins which translate the epigenetic signal into a function. Depending on its position (site and methylation status), DNA methylation acts as a repressing (often) or activating (less often) epigenetic

signal. In large parts of the genome, DNA methylation serves as a signal to inactivate repetitive DNA structures and 'jumping genes' (transposons). In addition, a number of genes are switched off long-term by DNA methylation.

DNA methylation exists in almost all multicellular organisms except for the classical model organisms in developmental biology *Drosophila melanogaster* (fruit fly) and *Caenorhabditis elegans* (roundworm). In all the organisms in which it is found, DNA methylation has a gene-regulating function. Insects (bees, termites, ants) have highly developed systems for DNA methylation and also for histone modification. Their purpose is to control genes that are important for morphological changes during reproduction, but it is likely that they also have a part in controlling learned and adaptive behaviours (Wang et al. 2006; Maleszka 2008). Observations in bees, for instance, have shown that queens and various other workers differ epigenetically, that queen differentiation is caused by epigenetic changes due to nutritional substances.

In plants, too, DNA methylation plays an essential epigenetic role (Henderson and Jacobsen 2007). A number of heritable, adaptive epigenetic effects are seen in plants that rely on DNA methylation (Hirsch et al. 2012). Plants possess a very highly developed enzymatic system of control over DNA methylation, and some very specialized forms of epigenetic regulation may be seen. It was in plants that researchers showed for the first time that DNA methylation can be actively removed by DNA repair processes (Zheng et al. 2008). Later, similar mechanisms were demonstrated in some vertebrates (zebrafish and *Xenopus*) and in mammals (mouse and human) (Gehring et al. 2009).

In mammals, including humans, DNA methylation can occur in other forms of modification—with highest abundance in stem cells and neurons. Building on 5-methylcytosine, additional modifications catalysed by the Tet enzymes occur in three oxidation states: 5-hydroxy-methylcytosine, 5-formyl-cytosine, and 5-carboxy-cytosine. 5-Hydroxy-methylcytosine (5OH-cytosine) is recognized by special proteins and interpreted differently to simple DNA methylation (e.g., not correctly copied during replication). The higher oxidation steps 5-fluorocytosine and 5-carboxycytosine most likely serve as recognition signals for DNA repair; that is, they are only short-lived and are then removed again from the DNA. There are clear indications that oxidative modifications are important for loss of DNA methylation in early germ cell and embryonic development (Wossidlo et al. 2011; Seisenberger et al. 2013; Arand et al. 2015). The significance of the oxidative forms of DNA methylation that occur especially in gametes, early embryos, stem cells and neurons is currently being investigated in detail. In all these cells types, wide-ranging epigenetic changes are observed during the course of development (stem cells) and ageing (neurons). This suggests that the various forms of DNA

methylation in these cells are used for short-term switches in gene programming. The most recent studies in stem cells indicate that DNA methylation adapts to rapid and extreme changes in culture conditions (e.g. the culture media), and that oxidative modifications play a part in this (Ficz et al. 2013; Habibi et al. 2013; Azad et al. 2013). Similar processes may also be in play in other environmentally induced alterations in somatic cells (e.g. neurons).

2.2 Histone Modifications

The DNA of our genome is packaged in nucleosomes. Nucleosomes are made up of eight histones wound around the roughly 150 bases (the building blocks of DNA). DNA packaged in nucleosomes is not directly accessible to biochemical processes such as gene transcription. Nucleosomes are therefore distributed in a gene-specific way. The histone protein modifications are very important to the strength of the packaging and to nucleosome distribution. These modifications control a series of epigenetic processes (Kubicek et al. 2006). The main entities modified are particular amino acids in the start and end regions ("tails") of histone proteins H3 and H4. Histone modifications are extremely rich in variants: at the present time, a total of 140 histone modifications are known. They are always post-translational modifications,[4] usually of basic polar amino acids such as serine, threonine, lysine and arginine. The modifications are small chemical functional changes in the form of acetylation, methylation, phosphorylation, ubiquitination and SUMOylation (for an overview, see Kouzarides 2007). In functional terms, a distinction can be made between chromatin-opening and chromatin-closing modifications, which respectively promote or inhibit the reading of genes.

Histone modifications are added to histones when the latter are in nucleosomes, at particular locations in the chromosome. The modifications are brought about by special enzymes. Locating and 'deciding' exactly which nucleosomes are to be modified, and in what way they are to be modified, is achieved with the help of other enzymes/proteins (e.g. transcription factors) which position the histone-modifying enzymes in the 'right' place in the chromatin (nucleosome). Histone modification in some cell types follows a very specific sequence and combination on the nucleosomes along the chromosomes. They thus 'encode' the nucleosome packaging, determining which genes are switched on or off. They also mark the regulation sites for the reading (transcription) of the genes and determine

[4]Post-translational modifications are modifications carried out on the 'mature' protein, after translation—of the nucleotide sequence into an amino acid sequence—has been completed.

the speed at which transcription takes place. Other entities important for turning epigenetic modifications into genetic activity are enzymes that can relocate nucleosomes within chromosomes. They are needed in order to release gene-controlling elements from the nucleosomes. To do this, the enzymes read and interpret the local histone modification structure of the nucleosomes. Among others, the so-called polycomb group protein complexes and their antagonists the trithorax complexes are responsible for the exact formation of cell-type-specific histone modifications on the nucleosomes along the gene. These complexes position histone modifications precisely on regulatory segments of the genome and mark these as switched on or off (Whitcomb et al. 2007). Histone-demodifying enzymes can remove these modifications locally or transform them and can thus reverse processes. It is not just the switching on and off of genes that is regulated in this way: RNA splicing and maturation, DNA replication, and DNA repair are all influenced or controlled via histone modifications (Corpet and Almouzni 2009; Varga-Weisz and Becker 2006).

In the course of cell development, gene-specific histone modifications are added and removed in a precisely ordered sequence of events. During development, cell-specific patterns of histone modifications are established in this way, step by step, in every type of cell in the body. Every cell type possesses, in parallel to the DNA methylation described above, its own 'epigenomic signature'. In stem cells, a very specific 'immature' double combination of activating and repressing histone modifications is observed in gene-regulatory regions, allowing these cells to maintain an intermediate epigenetic status which is essential to their ability to retain pluripotency (that is, the quality of stem cells that allows them to differentiate into any kind of cell) (Bernstein et al. 2006; Mikkelsen et al. 2007; Chi and Bernstein 2009). These gene-regulatory regions, which are in an epigenetically neutral waiting condition, react extremely swiftly to exogenous differentiation stimuli; that is, they can quickly switch epigenetically in order to carry out the special tasks of a differentiated cell. Later on in the course of differentiation, increasingly large regions of the genome are then marked by particular histone modifications in such a way that they are permanently closed and only those genes that are necessary to the cell remain switched on.

The modifications of histones can be analysed along the chromosomes using a technique called chromatin immunoprecipitation (ChIP). This involves enriching the modified histone within the nucleosomes with the help of antibodies, each of which binds selectively to one type (and one type only) of modified histone. The DNA of the bound nucleosome fractions is then isolated. High-throughput sequencing of this DNA (ChIP-Seq) allows the determination of the parts of the genome where the nucleosomes with the identified histone modifications were

bound. Since the genome sequence is known, the histone modifications can be 'mapped' along the DNA. These histone modification maps demonstrate that gene-regulatory regions, gene-encoding regions, and segments that lie between the genes, differ markedly in their histone modification patterns. Mapping seven to eight histone modifications appears to be enough to divide up the genome functionally into segments of active genes and gene switches and regions of inactive genes. Together with DNA methylation maps and gene expression data, this provides a wealth of gene- and cell-specific information giving insight into both healthy and diseased cells (Karnik and Meissner 2013). Special electronic mapping aids (known as epigenome browsers) make it possible to analyse complex datasets together and make use of them for gene-specific research. Many examples now exist of how histone modification mapping has provided clues towards a gene-specific, functional interpretation of the molecular causes of disease. Accurate histone modification mapping would therefore provide direct access to the understanding of disease. However ChIP-Seq technologies are of only limited usefulness for diagnostic mass screenings, since it requires large quantities of fresh cells (up to 10^6) for its performance, and the investigation must also be carried out under extremely standardized conditions for sample comparisons to be valid. In the worldwide coordinated analysis of many isolated cell types,[5] it is becoming increasingly clear that some epigenetic information content can be obtained even from the DNA methylation. The patterns of DNA methylation in part follows the distribution of histone modification pattern on histones. Since DNA can be harvested from almost all cells (including frozen ones) in adequate quantities, most epigenomic diagnoses focus on changes in the DNA methylation. The data obtained in this way are subsequently interpreted for functional information using comparisons to histone reference patterns.

All eukaryotes display histone modifications. A large number of enzymes are responsible for adding, removing, and actually recognizing them. Interestingly, species-specific differences occur: the effect of a histone modification or added patterns can vary from organism to organism. Also differences in the molecular interplay between DNA level and histone level are seen. In the extreme case, one level of epigenetic control can even be absent altogether, as is shown by the absence of any DNA methylation in *C. elegans* and several other organisms.

Histone modifications are established at the protein level; that is, they are not located directly on the DNA. Histones are components of nucleosomes, which are duplicated at every cell division and have to be redistributed onto the replicated

[5]See data from the International Human Epigenome Consortium (IHEC): http://ihec-epigenomes.org/.

DNA. How histone modification patterns can be stably inherited on the gene locus during the process of replication (cell division) is a question that has not yet been definitively answered. Some early models and molecular clues exist; these show that nucleosomes remain on their gene locus despite replication and cell division, and that the epigenetic information of the 'old' nucleosome is transferred to the new nucleosome by a kind of copying procedure (see Knippers and Nordheim 2015).

2.3 Epigenetics and 'Non-coding' RNAs

One prominent example of expanded epigenetic control mechanisms is the transcriptional and post-transcriptional "switching-off" or silencing of genes/transcripts by small regulatory RNAs. These epigenetic regulatory mechanisms were first discovered in plants. The subsequent discoveries of RNA-mediated epigenetic regulatory phenomena in almost all organisms—including human beings—increasingly show the close link between RNA-mediated regulatory processes and epigenetic transmission. In particular, small RNAs, such as pirRNAs (in gametes), miRNAs and siRNAs (in all cells), and long non-coding RNAs (lncRNAs) play an important part in establishing or implementing epigenetic processes. RNA is very diverse, not only in its structure, but also in its function. A general distinction should be made here between direct epigenetic effects, indirect intermediary functions, and subsequent implementation functions of non-coding RNAs.

Close interplay between structural and catalytic RNAs and epigenetic modifications is characteristic of many model organisms (yeast, *Drosophila*, *C. elegans*, *M. musculus*, *Arabidopsis thaliana*), but the significance of small RNAs was first identified in connection with expression control and chromatin structures in plants in particular. To put it another way: our conceptual understanding of how small RNAs come into existence and how they operate originates from plant epigenetics (Baulcombe 2004).

The expression of these small RNAs is often regulated in a cell-specific way and controlled via epigenetic modifications (e.g. via promoter methylation or chromatin modifications). Small RNAs have considerable influence on the translation and stability of mRNAs.[6] In addition to that, they assume an important

[6]Messenger RNA (mRNA) is the complementary copy of a coding gene sequence of DNA. This copy transports the information about the gene out of the cell nucleus into the cytoplasm, where it serves as a matrix for biosynthesis of a protein; that is, it is translated into the amino acid sequence of a protein.

function in controlling the formation of heterochromatin by leading histone-modifying and DNA-modifying enzymes to particular target regions such as the centromeres and telomeres, as well as transposable elements.[7] These mechanisms of effect are assumed primarily by special classes of small (si, casi, pi) RNAs. In human beings, close interplay can be demonstrated between the small dsRNAs and gene regulation in the epigenetically controlled (through DNA and histone modification) promoter control of the ribosomal gene cluster.[8] The same is true of imprinting regions.[9] Because of this, piRNAs are centrally important to epigenetic control of gamete development. They lay the foundation for epigenetic silencing of transposable elements (jumping genes) in maturing gametes. Besides the small RNAs, crucial roles in the epigenetic control of gene activity are similarly played by long non-coding RNAs (lincRNAs) such as XIST or AIR and HOTAIR. It turns out that the lincRNA XIST is essential for the silencing of genes on the X-chromosome (gene dosage compensation) in human beings (Clerc and Avner 2006), and in the process it regulates the stable, long-term formation of certain alterations in histone and DNA methylation.

One of the things that can be found in cancer cells is epigenetically misregulated expression of miRNA host transcripts, which results in incorrect regulation of genes targeted by the miRNA. Similar processes are seen in plants and in single-celled organisms (*Paramecium*). This observation requires that the direct association between the expression of small RNAs and other epigenetic gene regulatory cascades should be tested. The functional significance of this observation ranges from direct gene control in the course of development to defence against viruses (inactivation).

At present it is not possible to judge the importance of small and long non-coding RNAs in the control of epigenetic processes in human beings. This is partly because, for reasons that are still incompletely understood, these RNAs occur in such a multiplicity of forms. It is also because their interactions with epigenetic controls at other levels are very diverse. Recent findings show, for example, that a hitherto relatively unknown type of long circular RNAs (circRNAs) play a significant part in how the small RNAs function (circRNAs serve among other things as miRNA 'stores'or 'sponges'). Based on these effects, which

[7]In contrast to euchromatin, heterochromatin is a densely packed, inactive chromatin.

[8]Ribosomal gene clusters are clusters of genes that code for components of ribosomes, the cell's 'translation apparatus'.

[9]Imprinting is the transmission from one generation to the next of DNA and histone modifications that result in silencing of the marked ('imprinted') copies of genes (alleles) from one parent, leading to preferential expression of the alleles from the other parent.

are observed in a very wide variety of model organisms, it may at least be assumed that in humans, too, there is a close interdependence between small RNAs that have structural and enzymatic effects and epigenetic control of the functions of the genome. This is one reason why, in terms of research policy, it will be of absolutely fundamental importance to link together and promote research in these areas, which need to be brought closer together.

3 Epigenomics

Epigenomics (research into the epigenetics of the genome) is one of the youngest branches of epigenetics. The goal of epigenomics is to read, locate, and interpret the complete set of the various levels of epigenetic control of the entire genome. In addition, it aims to compare epigenetic maps between cells and draw conclusions from them about development, disease and ageing.

During development hundreds of special cell types with millions of descendent cells are generated. In this process cells of a given cell type acquire a characteristic epigenetic program. This is referred to as the epigenome (Bernstein et al. 2010). The epigenome of a cell is a direct reflection of the gene activation state of the cell. It encodes the information about how and where gene specific activation switches are located and used in the genome (ENCODE Project Consortium et al. 2012). Epigenome analyses are complex as they combine the layers of DNA methylation patterns, histone modifications and RNA expression. All layers must always be looked at together: that is, the genome needs to be 'scanned' for each modification separately and later combined into the epigenome. Next-generation DNA sequencing techniques have opened up hitherto unknown possibilities to achieve this, enabling the creation of high-resolution epigenome maps of normal and diseased cells. Epigenomics requires complex bioinformatics software for its visualisation and interpretation. The boom in epigenomics research in recent years was triggered by extensive national and international programmes, all united under the auspices of the International Human Epigenome Consortium (IHEC). Even preliminary results testify to the enormous importance of this research direction, which is opening up deep new vistas, hitherto unknown to us, into the basic epigenetic patterns of healthy and diseased cells.

The high-resolution epigenetic mapping techniques allow genetic and epigenetic changes to be analysed simultaneously. In comparative studies, it is increasingly observed that the changes in DNA methylation and in chromatin modifications often co-occur with small genetic variations (base exchanges, insertions, deletions)—that is, that genetics and epigenetics are in fact very often closely interwined.

Epigenomic data provide deep cell-specific information: they explain how the unique genetic programs take effect and is translated in hundreds of cell specific programs. In addition epigenomic data are a rich sourece to explain the function of genetic variants in the human genome. Over the past 20 years, a large number of genome-wide genetic association studies (GWAS) have been carried out for many common diseases—often with somewhat disappointing results. Making use of epigenetic data (DNA methylation and chromatin accessibility) enables a completely new evaluation of these genetic data and offers new functional explanations that make sense of the genetic data.

Epigenomic data convey very deep information about the genetic and epigenetic predispositions of an individual. For this reason, these data need to be handled with great care and discretion, and questions about the ethical and legal aspects of privacy relating to the use of these epigenetic data need to be debated. We do not yet know how to estimate (because of the lack of case numbers and comparators) whether epigenomic data contain permanent traces of personal epigenetic adaptations to life circumstances and thus to lifestyle (e.g. drug abuse, smoking, etc.). It is clear that personal age and cell age can be read from the epigenome. Epigenome mapping will open up new areas of personal diagnosis, and will provide answers to the intensely debated questions of how far the environment leaves long term marks on and influences the function of our genes. In epidemiology, the number of genome-wide epigenetic association studies has therefore increased enormously.

The technical means required for epigenetic diagnosis of personality features already exist. However, our ability to interpret the mass of data is still extremely limited. The complexity of the data creates a mass of sometimes contradictory possible interpretations which require complex computer processing before they can be turned into medically meaningful statements. However, besides these obstacles it is already clear that a personal genetic diagnostic of the future will include epigenetic data. DNA methylation is the level element to analyse. It can be read relatively easily and reliably, genome-wide, using high-throughput technologies—even from small quantities of cells and frozen material, and even indirectly, ‘retrospectively,’ in very old DNA (as recently shown by the reading of Neanderthal DNA).

Genome-wide epigenetic studies also play an increasing role in the diagnostics of human diseases. Epigenetic comparisons of cancer types identified cancer specific signatures (Weisenberger 2014). These signatures provide information about the origin and the prognosis of many cancers. While the causes and influence of epigenetic changes on cancer development are not yet well understood it

becomes more and more clear that the restructuring of genome wide epigenetic program is a hallmark of cancer, causing an uncontrolled biological development.

This feature is accompanied in many cancers by the frequent occurance of mutations in enzymes controlling epigenetic programs. Hence both genetic and epigenetic changes must be taken into consideration for a full understanding of cancer biology and cancer therapies. At present methylation patterns are being increasingly used for development of early cancer recognition tests in body fluids. The first diagnostic products already achieved the leap into clinical use or are at the point of being approved for routine testing.

4 Lessons from Epigenomics

All epigenetic mechanisms serve organisms primarily for differentiated use and control of genetic information. Basic epigenetic mechanisms are found throughout the animal and plant realms, but there they are used (in a way adapted to each organism) for long-term control of genes in the course of development and ageing. In higher organisms and in human beings, the various levels of epigenetic regulation become increasingly complex and specific. Thus, epigenetic mechanisms always need to be viewed in the context of the organism under investigation and of its genome and the research question to be answered. Caution is needed when drawing conclusions from epigenetic processes observed in model organisms and applying them to the human case, and good evidence is mandatory. This is particularly the case for phenomena relating to transgenerational transmission of epigenetically altered gene status or long-term adaptation to changed environmental conditions.

4.1 Epigenetics and Adaptation

Reaction of an individual's genes to environmental stimuli and lifestyle is not a new observation. The individual's basic genetic apparatus also offers a different framework of responses to environmental stimuli. We now often speak in terms of 'epigenetic adaptation'. This term is also associated with neo-Lamarckism and suggests a form of individually programmed adaptation of the organism to altered living conditions. Two elements are often left out of account here. Firstly, epigenetic processes primarily serve to control development and the maintenance of vital functions (health and ageing); that is, epigenetically controlled developmental processes are genetically determined and can only vary within certain limits.

Secondly, there are few indications that environmental influences directly produce targeted epigenetic variation of individual cell programs, rather than that the primary reaction to the environment triggers a secondary epigenetic reaction.

Irrespective of the causality question, the extent of epigenetic adaptability will depend primarily on individual genetic configuration and variation. What is known today indicates that epigenetic processes modulate the genetic range of play—but no new levels of regulation come into existence. Hence, it is always important to ask whether the cause of an observed epigenetic change is to be found at a level above the gene sequence, or whether it is actually coupled to gene variants.

Thus, epigenetic control must be viewed not only as a matter of the switching on or off of genes, but, for many examples of individual variation, as a process of limiting the modulability of genetic information. Epigenetic modifications in effect determine the framework within which genetic information is used. In consequence, it is important to view epigenetic phenomena from a quantitative biological viewpoint.

There are a series of inherited phenomena that are determined epigenetically, such as the part-of-origin imprinting of genes (genomic imprinting) or the silencing of one of the two X chromosomes in women. In both phenomena, the development of the organism is coupled to precisely regulated, fixed epigenetic control in an obligatory manner. Accordingly, epigenetic impairments arising in connection with imprinting and X inactivation lead to serious biological consequences such as syndromal diseases.

4.2 Concepts of Epigenetic Inheritance in Humans

A fundamental characteristic of epigenetics is its heritability; that is, stable transmission or handing on of fixed epigenetic markings that survive cell divisions. In contrast to true mutations, however, epigenetic modifications (‘epimutations’) can be reversed and can be deleted in a targeted way. The heritability of epigenetic modifications (histone modifications and DNA methylation) beyond cell divisions is without doubt a core characteristic of all multicellular organisms. Transmission via the germ line and haploid gametes, on the other hand, cannot be assumed with certainty for all organisms. Except for genomic imprinting, epigenetic modifications in the parent generation in humans are only very sporadically passed on to the children. In much-cited examples of early epigenetics research, the observed epigenetic changes are at least partially coupled to genetic parameters (transmission via the cytoplasm) or even genetic changes (changes in the genome). Despite this, such examples are always being used to develop new concepts of the heritability of

epigenetic adaptation to the environment from one generation to the next. However, neo-Lamarckist adaptive 'epimutation' scenarios of this kind often prove on closer inspection to rely on very sparse data.

With the exception of genomic imprinting, there are so far no clear indications in man for regularly inherited epigenetic effects through the germ line (Heard and Martienssen 2014). Many observations and reports of transgenerational inheritance of metabolic and stress related phenotypes rely on epigenetic interpretations of empirical data collections (health statistics). The reason for a low abundance of transgenerational heritability in humans is probably the extensive epigenetic reprogramming that occurs in the germ line and after fertilization (in early embryogenesis). Spontaneous occurrence of epigenetic errors that cause diseases, such as erroneous deletion of genomic imprints, cannot really be cited as examples of transgenerational inheritance—because they will not occur anew in the next generation. Frequently cited examples of transgenerational effects in animal models include the nutritionally determined (folic acid) change in the 'viable yellow' (fur colour) gene in agouti mice,[10] but closer scrutiny shows that here epigenetic programs are tightly coupled to a genetic mutation and the genetic background of the animals (Whitelaw and Whitelaw 2006). A recent report on a clear inheritance of a metabolic phenotypes in female and male mice strongly points in the direction of epigenetic changes but the exact molecular mechanisms remain unresolved (Huypens et al 2016).

About one fact there is no debate: that early epigenetic modulation of the (inherited) parental genomes by factors from the maternal oocyte cytoplasm can have a long-term effect on the individual epigenetic expression of genes. The presence of small RNAs and certain modifications of proteins that come into contact with the parental chromosomes via the oocyte cytoplasm can have a lasting influence on gene regulation.

In plants, on the other hand, the indications regarding transgenerational epigenetic inheritance can be interpreted much more clearly. Epigenetic inheritance from one generation to another is regarded as confirmed for some plant phenotypes/genes. Some of these phenomena have also been demonstrated at the molecular level (Henderson and Jacobsen 2007). In plants, unlike in animals,

[10]Agouti mice carry a special variant, named 'agouti viable yellow' (Avy), of a gene that determines hair colour. The more intensely methylated this gene is, the darker the hair—and the healthier the mouse. Supplementing the feed of agouti mothers with methylating molecules such as methionine, folic acid and zinc results in more intensely methylated Avy genes in the offspring, even the grandchildren. This experiment is often used as an example of the epigenetically mediated influence of lifestyle on the health of later generations.

deletion of epigenetic modifications in the gametes does not take place. Some acquired epigenetic changes can be retained for generations. Two hundred and fifty years ago Linnaeus and Goethe described a mutant snapdragon (altered flower form) that finally proved to differ from its nearest relative by just one epimutation (Cubas et al. 1999).

4.3 Perspectives in Epigenetic Research

Epigenetics has already made its way into applied biomedicine at many levels. Epigenetic processes play a preeminent role in the production of synthetic and natural stem cells. Epigenetic analysis is increasingly being used to generate personal biomarkers, e.g. for the differential diagnosis and early recognition of cancer. In addition, epigenetic processes offer a way to approach the development of new kinds of epigenetic drug substances. Some of these substances directed against DNA and histone modifications are already being successfully used to treat specific cancers. Many others substances directed against epigenetic modifier enzymes are currently under development.

In preventive health care, psychology and the social sciences, epigenetic mechanisms are already being debated as possible factors influencing personality. This debate, however, rests on very few concrete data. Repeatedly, a few examples, mostly from model organisms, are used to build up lines of argument that relate to empirically derived biological data such as the Dutch famine study or the Överkalix study. The molecular data relating to these studies, however, are either lacking or can only be assessed to a very limited extent. This is the case for a number of empirical studies in which the epigenetic methods employed often do not meet current standards, or the data have been rather boldly interpreted. The molecular changes observed are often very small and, moreover, are usually statistically overstated. Future comparative studies should be based on more solid methodological and statistical foundations.

As a general principle, epigenetic data and their interpretation require very careful handling. It is entirely possible that epigenetic data reflect information about a person's lifestyle. Epigenomic data should therefore be interpreted and evaluated with care in order to prevent stigmatization.

Epigenetics and epigenetic concepts need to be accorded greater value in the current discourse on human biological questions in the natural and social sciences. It is important to keep a sharp eye out for the basic foundations of epigenetic data and the theories derived from them.

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