Spring 2017 – Epigenetics and Systems Biology
Lecture Outline (Epigenetics and Disease Etiology)
Michael K. Skinner – Biol 476/576
Weeks 13 and 14 (April 4 & 11)

Epigenetics and Disease Etiology

- Epigenetics and Disease Etiology Introduction
- Epigenetic Disease
- Environmental Epigenetics and Disease
- Epigenetics and Cancer
- Epigenetics and Neuroscience
- Epigenetics and Metabolic Syndrome
- Epigenetic Therapy Development
- Epigenetic Transgenerational Inheritance of Disease

Required Reading

Wolkenhauer and Green (2013) The search for organizing principles as a cure against reductionism in systems medicine. FEBS J. 280(23):5938-48.

Books (Reserve in Library)

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MINIREVIEW

The search for organizing principles as a cure against reductionism in systems medicine

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Biological complexity has forced scientists to develop highly reductive approaches, with an ever-increasing degree of specialization. As a consequence, research projects have become fragmented, and their results strongly dependent on the experimental context. The general research question, that originally motivated these projects, appears to have been forgotten in many highly specialized research programmes. We here investigate the prospects for use of an old regulative ideal from systems theory to describe the organization of cellular systems 'in general' by identifying key concepts, challenges and strategies to pursue the search for organizing principles. We argue that there is no tension between the complexity of biological systems and the search for organizing principles. On the contrary, it is the complexity of organisms and the current level of techniques and knowledge that urge us to renew the search for organizing principles in order to meet the challenges that are arise from reductive approaches in systems medicine. Reductive approaches, as important and inevitable as they are, should be complemented by an integrative strategy that de-contextualizes through abstractions, and thereby generalizes results.

Introduction

Cell-biological systems are difficult to study because they are complex in several ways [1]. One aspect of biological complexity that is particularly important to systems medicine is multi-levelness: the structural and functional organization of the human body into organ systems and tissues composed of cells. From molecules to organs, levels are inter-related and inter-dependent, so that the organism is able to conserve and adapt the integrity of its structural and functional organization against a back-drop of continuous changes within the organism and its environment. This capacity, whether it is described as 'autoconservation' [2], 'functional stability' [3], 'evolvability' or 'robustness' [4–6], is a consequence of non-linear spatio-temporal intra- and inter-cellular interactions. To understand disease-relevant cellular processes, we therefore require methodologies that allow us to study non-linear spatio-temporal systems with multiple levels of structural and functional organization.

The most recent decades of research in the life sciences have been largely driven by development of new technologies, which have brought about unprecedented insights into the structural organization of cells [7,8]. Together with these technological developments, a form of reductionism, i.e. studying higher-level phenomena by analysing the lower levels, has been established [9]. While some aspects of this 'zooming in' are a pragmatic and indispensible response to biological complexity, we here demonstrate the negative side-effects of molecule-, pathway- and cell-centred approaches.

The emergence of systems biology is connected to the limitations of molecule-centred approaches [10]. Systems biology has shifted the focus from

identification and characterization of molecular components towards an understanding of networks and functional activity. As a consequence, dynamic systems theory has played an increasingly important role in understanding cellular processes [11,12]. We argue that, for the transition from systems biology to systems medicine, a further shift of perspective has to occur: re-focusing our attention away from pathway-centred approaches to an understanding of complex multi-level systems. Looking at the developments from biochemistry to systems biology, it becomes quite apparent that reductive approaches are rather limited when it comes to answering questions in systems medicine [13]. In systems medicine, our understanding of cellular functions must be integrated across multiple levels of structural and functional organization: from cells to tissues and organs to whole organisms, and from cell functions (growth, proliferation, differentiation and apoptosis) to the physiology of organs or the human body [14]. Multi-levelness is a hallmark of disease-relevant processes, which challenges conventional dynamic systems theory [15,16]. Here we provide an example from cancer research that demonstrates the limitations of pathway- and cell-centred approaches.

Our goal in this review is to evaluate, from a personal and necessarily biased perspective, reductive approaches and their limitations in answering questions at the tissue and organ level by conducting experiments at the molecular and cell level. We first consider how biological complexity challenges experimentalists and modellers alike, and then look at how the associated difficulties have led to specialization, fragmentation and the contextualization of knowledge. Following a discussion of reductive approaches and their negative consequences (in our view), we suggest possible future directions for research in systems medicine. In particular, we argue that the search for organizing principles may serve as a cure against the side-effects of reductive approaches in systems medicine.

While not essential to our arguments, here we understand systems biology as the science that studies how biological function emerges from interactions between the components of living systems, and how these emergent properties constrain the behaviour of these components. In practice, systems biology is an inter-disciplinary approach by which biological questions are addressed by integrating experiments in iterative cycles with mathematical and computational analysis. Systems medicine should be understood as application of the systems biology approach to disease-focused or clinically relevant research problems. A research challenge arising from systems medicine, that is discussed in detail here, is the fact that, for many diseases, it is necessary to study and model complex systems from the molecular to the organ level.

Reductionism and specialization

In studying networks rather than individual molecular components, some proponents of systems biology have considered systems biology a 'holistic approach' [17–19]. This unfortunate misconception ignores the fact that technological advances have continued to enforce reductive approaches, along with increasing levels of specialization. Ten years ago, the focus on pathways rather than single molecules may have been seen to be a more comprehensive approach, but even today we are still far down the reductive route, with the current dominance of pathway-centred approaches to understand disease phenomena. Reductive strategies are indeed an indispensible response to biological complexity, but, as we discuss here, they have negative side-effects. One such side-effect is over-specialization, which, in the current practice of systems biology, means that the choice of experimental and modelling strategies is more frequently guided and limited by personal and practical considerations than by the need to validate a general hypothesis that underlies the research project. The approaches chosen are frequently linked to decisions based on pragmatic considerations of the associated efforts in terms of time and costs required for experiments. For example, in research on metastasis, many projects are focused on single molecules or small pathways, frequently using specific cell lines. There is a mismatch between the research goal (understanding mechanisms underlying metastasis in humans) and the highly specialized projects, whose results are only valid in a narrowly defined context. There is an obvious need for integration of results from individual research projects and a need for generalization (de-contextualization) of results.

Below, we describe several reductive strategies used in biological and biomedical research. We first emphasize how the use of model organisms and the development of new experimental technologies provide key resources for biomedical research, but also require a high degree of specialization that may lead to fragmentation. Next, we indicate the difficulties arising from pathway-centred approaches and mechanistic modelling. Finally, we discuss the limitation of cell-centred approaches in cancer research.

The use of model organisms is one response to biological complexity, allowing us to study a complex organism by using another one that is either simpler or easier to handle in experiments. An example is yeast studies in cancer research, motivated by questions related to the

cell cycle and its consequences for carcinogenesis or tumor progression [20]. The experimental focus on a particular model organism, the decision to perform cell line *in vitro* experiments or the availability of a suitable *in vivo* model are our first examples of a common reductive approach, which also imply a disciplinary specialization with separate conferences and journals. However, research on model organisms also provides de-contextualized insights. A basic assumption in using model organisms or cell lines is that, while details may differ, there are some generalizable principles at work. We believe that the relationship between reductive choices, inevitable and successful as they are, and the generalization of results obtained, requires more attention from scientists, philosophers of science and funding bodies. For reductive approaches to succeed, they must be complemented by integrative strategies. We argue that these integrative strategies also require higher levels of abstraction than most biological and biomedical researchers currently feel comfortable with, and this requires further mathematical research.

What have been heralded as revolutionary advances in molecular and cell biology are largely due to technological developments, allowing us to study molecules and cells in greater detail and more comprehensively. The costs and the specialist expertise required to perform experiments with state-of-the-art measurement devices have meant that only one or a selection of technologies are used in any one study for most research projects. Whether the choice is microscopy, proteomics, transcriptomics or deep sequencing, their use requires a high degree of specialization. 'Omics' technologies are frequently tied to a focus on a particular class of subcellular processes, i.e. gene regulation (e.g. transcriptomics), signal transduction (e.g. proteomics) or metabolism (e.g. metabolomics). Again, a disciplinary fragmentation, with specialized conferences and journals, may be observed. Furthermore, another enforcement of scientific specialization is linked to the focus on a particular cell function, such as cell growth, proliferation, differentiation and apoptosis. It is quite obvious, albeit not generally appreciated, that, for application of systems biology approaches in biomedical research, there is not only a need for computational tools that enable integration of data from heterogeneous sources, but also a need for radically new methodologies that enable generalization of context-dependent experimental results.

Our next example of a reductive strategy is the focus on selected pathways or networks. Pathways are frequently defined by practical considerations, meaning that only a relatively small number of molecules are considered in experiments. However, for most disease-relevant processes, these pathways are sub-systems of a larger whole. Rational criteria to identify modules or sub-systems are largely lacking. In practice, one is usually forced to define a boundary for the network as it is investigated experimentally. If this pathway is one of several that contribute to a particular cell function, for example, the notion of 'cross-talk' between pathways has been used. However, for most pathways that interact, this notion of cross-talk raises questions about the conceptual and experimental isolation of the two systems. In order to use the experimental results related to a specific pathway in a wider context (e.g. studying the Jak–Stat signalling pathway to investigate cell differentiation), we require new methodological and conceptual frameworks to de-contextualize and generalize. A similar situation occurs when studies at the cellular level (looking at single cells, cell cultures and single pathways) need to be related to tissue-level phenomena and the physiology of an organ. We believe that the problem of generalization through de-contextualization and the integration of experimental results requires more attention and research, as otherwise the currently favoured pathway-centred approaches will be of limited value.

Systems biology is largely defined as an inter-disciplinary approach that combines experiments with mathematical and computational modelling. Like experimentalists, who are often not free to choose any technology they want, most modellers are not really free to choose a conceptual framework for modelling. Despite the development of user-friendly tools that guide the modelling and simulation of biological systems, the construction of a model and its parameterization requires expert knowledge. Although the choice of an appropriate approach should in principle be guided by the question under consideration alone, more often, practical considerations and personal choices are decisive. Similar to the efforts required to perform experiments, the construction and analysis of a model may be challenging, requiring a high degree of specialization and experience. For example, non-linear ordinary differential equations are the most frequently used framework, but, for larger numbers of variables, parameterization and analysis of these models is difficult. Dynamic systems theory is particularly intuitive if systems can be reduced to a few variables. For systems with only two variables, and for systems that are linearized around a steady state, the theory is most powerful and well developed. It is therefore not surprising that some case studies are selected to fit the tools, rather than the other way round. In contrast to differential equation models, agent-based simulation models handle many variables and represent spatial

aspects more easily, but the 'model' is programmed, lacking the desirable simplicity of representation. Also, stochastic approaches, even if the most appropriate, are often avoided because they require a deeper understanding of the maths by the modeller. The choice of an appropriate modelling formalism, the construction of the model, the estimation of parameter values and subsequent exploration of the model through simulation and formal analysis are aspects of a craft that requires specialization. Tailoring a model around a particular question, making various assumptions and simplifications along the way, will unfortunately also make it context-dependent.

The creation of large collections of information from experiments using various experimental models and employing a wide range of technologies and methodologies requires integrative strategies through which fragmented information may be put together [13,21,22]. A pragmatic, computational way forward is to support integration of information through visualization of information in data management systems or data warehouses. However, this would only be a partial contribution to what is the actual scientific challenge: how can we, from large collections of information, extract principles, understood as robust generalizations, independent of the experimental context of any particular study? Take, for example, our understanding of cell functions, say apoptosis, for which numerous studies, using different technologies and experimental models (e.g. cell lines, genetic mouse models), have provided pieces of a puzzle that give us deeper insights into apoptosis in the context of carcinogenesis. Many experiments in molecular and cell biology are however valid only within a well and often narrowly defined experimental context, determined by the choice of technology and the biological model. Furthermore, most mathematical models are constructed to answer specific questions, and, while the assumptions made may be valid in this particular context, it is difficult if not impossible to merge models for complex multi-level systems. An important challenge for systems medicine is thus the integration and decontextualization of results, to put the pieces of a puzzle together.

A survey of review articles focusing on epithelial cell renewal in the context of colon cancer uncovers numerous speculations about the theories and (explanatory) models that may be formulated as organizing principles, including the 'unitarian hypothesis' of monoclonal conversion, the 'single stem cell hypothesis' or the 'stem cell niche hypothesis' in the context of niche succession, the 'hierarchical model' compared to the 'stochastic model' for niche homeostasis, the 'somatic mutation theory' versus 'tissue field organization theory' to explain carcinogenesis, or the 'top-down' versus 'bottom-up' hypothesis of clonal expansion linked to early growth of adenomas, or cancer progression being discussed in terms of the 'cancer stem cell model' versus the 'clonal evolution model' versus the 'interconversion model'. What this selection exemplifies is that the formulation of such principles and arguments for or against them are developed in exceptionally well-written review articles in biological journals: leading experts integrate knowledge by interpreting collections of fragmented pieces of information. Very often, the experimental studies are about cellular processes, but the results are interpreted with respect to consequences at the tissue level. What we propose is not simply to support this integrative process through data management and visualization tools. In addition, the search for organizing principles should be supported by systems theoretic approaches, specifically new forms of mathematical modelling to formalize cross-level relationships from molecules and cells to tissues and organs.

Our argument here is that a review of current practice leads us to the proposition that, if you want to understand a tissue, you need to study it as a whole! Interestingly, this argument mirrors an aspect in the transition from biochemistry to systems biology. In 1986, Kacser, commenting on whole–part relationships in metabolism, wrote 'to understand the whole, one must study the whole' [21]. Here, however, we reach an apparent contradiction because we also argue that reductive approaches, focusing on pathways and cells, are inevitable in the light of biological complexity and the experimental/technical challenges. How then may we escape the reductive cul-de-sac? One avenue is to 'up-scale' experiments and models, to incrementally increase the number of molecular components and pathways to be looked at. However, we have come to the conclusion that it is necessary to try to complement such reductive strategies by novel approaches that provide higher levels of abstraction, using systems theory. Abstraction in mathematical modelling allows us to link evidence and knowledge of the subcellular domain or cell level with the tissue and whole-organ level. A conceptual framework that provides a straightforward generalization of mechanistic models and that has been considered elsewhere is mathematical general systems theory [22,23]. An interesting problem that arises in this context is transition of a mechanistic model as an 'ontological' description of a biochemical and biophysical reality to a mathematical representation of what we know about the biological system – an 'epistemological' version of logical possi-

bilities that link evidence [24]. The move to higher levels of abstraction poses a number of challenges. For example, abstraction implies generalization, which in turn implies a lack of specificity – the more abstract the representation becomes, the less predictive the models are about a specific experimental context. In our view, this aspect is in fact showing the way forward: reductive approaches that 'zoom in' on cellular mechanisms in the context of human medicine ought to be complemented by a search for general organizing principles at higher levels of structural and functional organization in tissues and organs.

Below, we identify the challenges specific to systems medicine, leading up to a proposal for a way forward that addresses the complexity of disease-relevant processes. We argue that, despite its limitations, modelling is essential not only for systems biology and systems medicine, but for science in general. In our view, the response to biological complexity should not only be a reductive one. To go forward, there is also a need to strategically focus on the development of approaches that 'zoom out' to help us understand multi-level systems. Addressing experimentalists and modellers alike, we wish to proclaim that, to study disease-relevant processes in tissues, one should also study tissues through an active search for organizing principles.

Consequences for systems medicine

Many diseases represent problems of tissue organization: changes in the structure and function of a tissue may be the results of changes within cells (e.g. mutations), leading to cellular malfunction, but, simultaneously, tissue organization may also induce changes within cells (e.g. through epigenetic mechanisms). It therefore appears obvious that we require methodologies to investigate systems across multiple levels of functional and structural organization.

Cancer research is an example that illustrates the problems arising from reductive approaches, fragmentation and the dependency of results on a particular technological and/or experimental context. Hanahan and Weinberg's review 'The hallmarks of cancer' [25] may serve as a classification of research efforts. Most cancer projects focus on a particular cancer and on either carcinogenesis, tumour progression, or metastization and invasion. These high-level/tissue-level phenomena provide the motivation and background for the projects, but, in practice, the highly specialized research in most projects actually does not address such general questions directly. Instead, the current practice is rather 'pathway-centred', where most projects ask a very specific question, related to a specific pathway, say the Jak–Stat pathway or an MAPK pathway, or concentrate on the role of a particular molecule, say p53 or E2F1 [26]. The 'zooming in' on molecular components has been very important and has generated enormous amounts of valuable information. The work on a particular molecule, say p53, is argued to be justified on the basis of its role in a cellular process, like DNA damage response. This focus on a particular molecule leads to definition of a network of molecules linked to p53, small enough to be experimentally tractable. However, as the cancer biologist Lazebnik notes: 'the mystery of what the tumour suppressor p53 actually does seems only to deepen as the number of publications about this protein rises above 23 000 [27]. In this famous and provocative paper, Lazebnik asks whether biologists can meet two challenges described as analogous: fixing a radio and developing a general characterization of apoptosis. He comes to the conclusion that the strategy of biologists would fail in both cases, as this most likely would be to crush the radio down to all its components and analyse these, just as much of medical research has been a search for a miracle target whose malfunction is thought to explain the investigated disease. If no such master gene exists that can explain cancer, Lazebnik argues, the status of research is like the Chinese proverb alluding to the search for a cat in darkness that is not even there.

It appears that we have become so preoccupied with molecular details that we have forgotten to ask how all the research results relate to answering the big (higher-level) questions. We believe that, for some disease-related phenomena, we are failing to see the wood for the trees. It is paradoxical that most cancer research projects are motivated by a far more general research question that is largely ignored in the execution of these research programmes. The pragmatic reductionism that focuses on particular molecules and pathways creates a fundamental problem. The focus on a particular molecule or pathway may be justified by researchers on the basis of its relevance for an important cellular process (e.g. DNA repair), which in turn is associated to some cell function (e.g. apoptosis), that is then linked to some disease-relevant process (e.g. carcinogenesis). However, starting with a high-level phenomenon, say angiogenesis, one may easily identify a large number of molecules and pathways that are relevant. Therefore, how may any single project, motivated by a higher-level process but limited to a particular experimental context, provide any meaningful contribution? In our view, the current practice is not sustainable, and requires re-thinking of

how we go about answering bio-medically relevant questions in molecular and cell biology.

Systems biology emerged from a shift of focus, away from identification of cellular components and their molecular characterization towards an understanding of functional activity [28,29]. For systems medicine, it will be of utmost importance to move on from pathway-centred approaches. Rather than starting with subcellular mechanisms and models thereof, before generalizing these to the level of cell functions and their role in phenomena at the tissue level, we wish to promote an alternative route that starts with a hypothesized general principle about tissue organization, to then identify and investigate cellular functions and subcellular processes in an effort to validate the original hypothesis.

We believe that such a search for organizing principles is happening but is mostly hidden in a few review articles and left to the inspiration of a few scientists. Cancer research is an area in which review articles play a particularly important role due to the above-mentioned flood of information about individual molecular components. Exceptionally good review articles not only gather and list information in a summarized form, but the authors try to organize the information to speculate about the larger picture into which the pieces of the puzzle may fit. Take, for example, the highly cited review article 'The hallmarks of cancer' by Hanahan and Weinberg [25]. Looking at a quarter of a century of rapid advances in cancer research, the authors argue that rather than 'adding further layers of complexity to a scientific literature that is already complex beyond measure', the search for the origin and treatment of cancer will not only be driven by developments at the technical level 'but ultimately, the more fundamental challenge will be conceptual'. In 2000, Hanahan and Weinberg foresaw 'cancer research developing into a logical science, where the complexities of the disease, described in the laboratory and clinic, will become understandable in terms of a small number of underlying principles' [25]. In their seminal review article, Hanahan and Weinberg 'suggest that the vast catalog of cancer cell genotypes is a manifestation of six essential alterations in cell physiology that collectively dictate malignant growth' which 'are shared in common by most and perhaps all types of human tumors'. They refer to the functional capabilities that cancers acquire during their development as 'hallmarks of cancer'. A hallmark of cancer is here understood to be a generalization in the sense that it may be acquired by various cellular mechanisms. Hanahan and Weinberg's hallmarks therefore take us some way towards the search for organizing principles as an epistemological tool.

As discussed further below, organs and tissues are multi-level systems manifesting both 'regressive determination' and 'progressive determination': the whole (organ or tissue) is the product of the parts (tissue or cells, respectively), but the parts in turn depend upon the whole for their own functioning and existence. Karsenti's initial definition of self-organization implied that understanding of functions in living systems implied an understanding of (self) organization [30]. This also implies that we should focus on principles rather than on single molecules or pathways alone. In our view, the current practices in cancer systems biology require re-thinking. The technological advances that have enabled us to 'zoom in' should be complemented by methodologies that allow us to 'zoom out': the microscope of molecular and cell biology should be complemented by the 'macroscope' of systems theory.

Multi-levelness and the search for organizing principles

Living systems, from organisms to organs, tissues and cells are phenomena of organized complexity [31] whose relationships and properties are largely determined by their function as a whole. The tissues of our human body are self-organizing systems: every cell owes its presence to the action of all its surrounding cells, and also exists for the sake of the others. The whole (tissue) and its parts (cells) reciprocally determine functioning of each other. For instance, the pacemaker rhythm of the heart is not only caused by the activity of the ion channels at the molecular level, but is also dependent on the functioning of the organ, and even the body, as a whole. The systems biologist Denis Noble elegantly demonstrated the importance of such downward determination in simulations of the heart rhythm, where feedback from cell voltage was removed and fluctuations in ion current ceased [32,33]. To understand such phenomena in multi-level systems, it is not only important to understand molecular mechanisms but also to understand the organizational maintenance of the system at higher levels.

The human body provides the prototypical example of a multi-level system, where molecules, cells, tissues and organs are sub-systems of physiological systems (e.g. the cardiovascular system, the digestive system, the immune system etc.) The human body is thus structurally organized into spatio-temporal scales and functionally organized into behavioural levels (Fig. 1). A characteristic of the system, as a whole, is its functional stability against a back-drop of continuously changing and perturbed sub-systems [3].

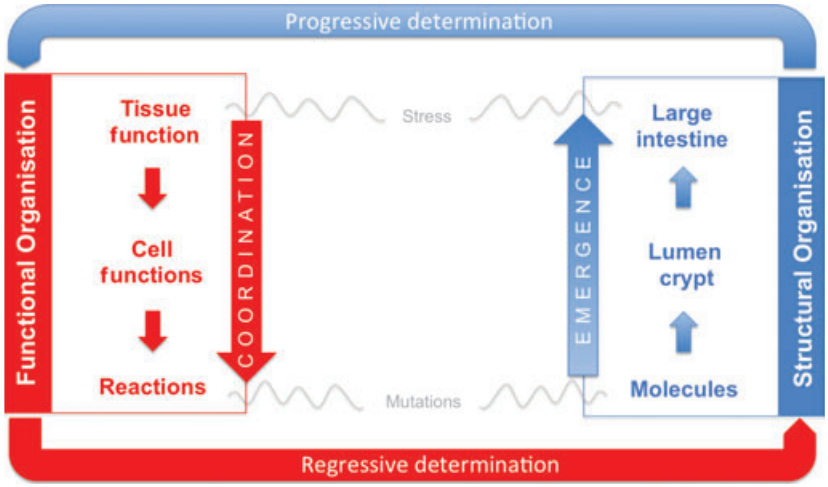

Fig. 1. Structural and functional (self) organization of tissues using the intestinal colon as an example.

Take, for example, the large intestine (colon) of the digestive system, which is also a common site for carcinogenesis. The inner lining of the colon is organized into millions of crypts [34,35]. The base of the crypts form a niche and micro-environment for a small number of stem cells that continuously renew the epithelial layer in order to maintain the physiological function of the colon (nutrient absorption) and to repair or avoid possibly detrimental effects from mechanical or chemo-toxic stress, which may lead to the formation of neoplasms and possibly carcinomas. The structural organization of the crypt emerges 'bottom-up', and its function is maintained through division and differentiation of stem cells. At the same time, the behaviour of these stem cells is coordinated by higher-level phenomena resulting from the need for tissue maintenance and repair. In the more general framework of multi-level systems with reciprocal and simultaneous cross-level determination, levels are inter-dependent but not necessarily causally linked [36]. Here, intra-level relationships may be conventional causal interactions, such as the mechanisms realized through subcellular biochemical networks, where causality is understood as a principle of explanation of change, not changes of things, but changes of states, represented with mechanistic models from dynamical systems theory. Inter-level relationships, on the other hand, constitute an interdependence in which levels are allowed a degree of autonomy [35,37]. The fact that levels are inter-dependent, but not necessarily causally linked, challenges the current practice of reductive approaches in experimentation and modelling. While systems approaches have been quite successful in describing mechanisms underlying intra-level relationships or 'causal interactions', we are in need of new ideas when it comes to understanding inter-level relationships. Below, we argue that mathematical general systems theory is one possible conceptual framework that abstracts conventional dynamical models and thus provides a basis for generalization from mechanistic models.

Let us consider an example from cancer research, where the need for identification and understanding of cross-level principles is of crucial importance. This example continues our discussion about the negative side-effects of reductive approaches. A widely accepted view on cancer is that it is a cell-based disease [38]. With cancer research following closely the developments in molecular and cell biology, pathway- and cell-centred (reductive) approaches have enforced the view that sporadic cancers are initiated and largely driven by accumulation of mutations in what may then be called a 'cancer cell' that loses control over its proliferation. Hanahan and Weinberg state that, 'By simplifying the nature of cancer – portraying it as a cell-autonomous process intrinsic to the cancer cell – these experimental models have turned their back on a central biological reality of tumor formation *in vivo*: cancer development depends upon changes in the heterotypic interactions between incipient tumor cells and their normal neighbors' [25]. Soto and Sonnenschein [39], who refer to the cell-centred view of carcinogenesis as the 'somatic mutation theory', have proposed an appealing alternative theory that considers cancer to be a problem of tissue organization. A key premise to their 'tissue field organization theory' is that 'carcinogenesis takes place at the tissue level of biological organization, as does normal morphogenesis'. Here cancer is not a cell-based phenomenon but a tissue-based phenomenon, comparable to organogenesis during early development. A startling conclusion is that

the genetic instability of tumours is likely to be a consequence, not a cause, of cancer. As new deep-sequencing technologies are pushing forward the reductionist agenda, we here call for a reflection about the original questions at tissue level, and ask whether the technology-driven reductionism should not be complemented by an equally well supported research programme into new, integrative and abstract methodologies. The purchase of technologies that dig deeper into the molecular details of a tumour sample is the seemingly more comfortable route. However, if cancer is a problem of tissue organization rather than of single cells, new experimental designs will be required. For modelling, the outlook is as challenging as it is exciting: if cancer is a problem of tissue organization, reciprocal interactions between cells and their environment come into focus, and ordinary differential equations are no longer sufficient to capture the spatial coupling of biochemical and biophysical/mechanical interactions. As discussed below, modelling complex systems across multiple scales of spatial and temporal organization may take two routes.

From multi-scale to multi-level systems analysis

How does one study multi-level systems, i.e. investigate, the functioning at higher levels of tissue organization? One possibility, proposed by several large-scale research projects such as the Virtual Physiological Human Project [14,40] or the Human Brain Project [41–43] is to simulate organs in physical and chemical detail, bottom-up, from molecules to organs. However, the attempt to meet biological complexity with a complexity of models that include ever increasing details seems somewhat to be analogous to Lewis Carroll's and Jorge Borge's fictions, where the art of cartography attains such perfection that the maps become as detailed and as big as the countries they represent. These maps are abandoned as useless, not because of the lack of precision, but because of their exact accuracy [44,45]. Similarly, it has been argued that the way forward in the biological and biomedical sciences is not to try to include all details and to add further levels of complexity to models and the scientific literature, but rather to develop approaches that zoom out and focus on key aspects of the phenomena studied [46–48].

An alternative response to the complexity of tissues and organs is to abstract away from the biophysical and biochemical details. The basis for such generalization of mechanistic models into more abstract representations is mathematical general systems theory [23]. While more abstract, and therefore less specific about a particular system, these approaches provide a framework to formulate and identify organizing principles [24,35,37]. An example of what such a theory should deliver is a formal framework to represent tissue organization, which may then be used to decide between the alternative theories of carcinogenesis discussed above.

The focus here on organizing principles is a re-introduction of an old regulative ideal in systems sciences dating back to Bertalanffy's ideals for a general systems theory [49], to Rashevsky and Rosen's notion of optimality principles [50–52], and to Savageau's so-called demand theory for gene expression, which exemplify design principles in biochemical systems theory [53,54]. The prospects of a more theoretically grounded biology searching for general and perhaps even law-like principles of living systems has been the issue of long debate in philosophy of biology [55–57]. However, the search for organizing principles need not rest on the widely criticized optimality approach [37,58,59], but is here understood as robust generalizations that account for the general behaviour of a class of (often different) systems. This strategy is not an attempt to reduce away biological complexity with abstract approaches. Our proposed focus on organizing principles is not an alternative to bottom-up approaches, or mechanistic modelling; it is a complementary approach. For that matter, it is also reductionist, but in a different sense. Every model or scientific theory is a reduction of something complex to something simpler [47]. The search for organizing principles is a matter of reducing the number of details and the amount of context-dependent information for the sake of the generality achieved through abstraction. This ideal is not in opposition to finding biological mechanisms but rather has a different aim, namely to find out how a class of systems works in principle.

In recent years, interest in general principles underpinning the organization of biological systems has intensified, and we expect this to continue. Efforts in network modeling have led to the discovery of general topological aspects and shared functional constraints of various networks [54,60–63]. Evolutionary systems biology has initiated the search for evolutionary design principles that demonstrate general features of evolving networks [59]. Furthermore, attempts to develop abstract cell models and explore the potential of category theory and mathematical general systems theory have recently been initiated [35,37,64–68]. As these approaches address questions at a higher level of abstraction, the relationships between theoretical models and experimental practices will be an important

point of discussion in future biology and medicine [69]. Another example from our own work is the study of epithelial cell renewal in the context of colon cancer [35]. Using simple-order relationships to link the division of stem cells in their niche to the fate of the crypt, we formulated a theorem that shows how the fate of the tissue is determined by a single lineage. The approach does not use any numbers to characterize the system, but analyses what is logically possible 'in principle' [24]. In such approaches, the definition of (and assumptions about) variables and the subsequent formulation of the theorem create an argument about an organizing principle relating to a tissue. To identify or suggest a principle is to generalize a phenomenon from particular instances, to de-contextualize it, for example, generalizing it beyond a specific experimental context. We believe that, if the gap between systems theory and mainstream biology can be bridged through more research in this direction, theoretical models may be of high practical value because they address fundamental properties of the system under consideration.

In summary, we here considered the transition from systems biology to systems medicine by personal reflection upon the developments that took us from biochemistry and molecular biology to systems biology. We noted that advances in molecular and cell biology were largely technology-driven, leading to high degrees of specialization and a reduction of the validity of results to the specific experimental context. In the context of many diseases, which cross multiple levels of structural and functional organization, reductive approaches and conventional dynamic systems theory are limited in facilitating identification of general principles underlying these diseases. Another contribution of our analysis is the proposal for a strategy that promotes integrative approaches and the search for organizing principles. While new technologies are widely welcome and their development is well supported, we hope that our analysis contributes to a better appreciation of the development of new and abstract methodologies. We firmly believe that systems medicine not only requires new means of measuring things, but also new ways of thinking.

Conclusions

A review of the current practice of molecular and cell biology reveals negative side-effects of technology-driven reductive approaches. Although much has been learned about molecular components and subcellular processes, these sub-systems are part of a larger whole that is frequently ignored when it comes to understanding tissue- and organ-level questions. Many diseases are a problem of tissue organization, and require us to integrate our knowledge from the molecular level all the way up to the tissue and organ level. Multi-levelness is a hallmark of biological complexity, and, in our view, is the final frontier and the greatest hurdle in the success of systems medicine. In our analysis, pathway- and cell-centred approaches have severe limitations when it comes to understanding disease-relevant multi-level systems. As a consequence, we believe that the future of systems medicine will rely not only on technologies, but will also require a strategic focus on the development of new methodologies. Our analysis has revealed a need for generalization through abstraction, and we proposed the search for organizing principles as a cure against negative side-effects of reductive approaches. To this end, we suggest systems theory as systems medicine's next stethoscope.

The search for organizing principles is not only of theoretical value but of high relevance for solving practical problems. The ideal of general principles has a long history [49,50,70–72], but is still not fully appreciated [24,35,37,66]. The focus on general principles enables a shift away from molecule- and cell-centred studies and from what Robert Rosen called 'thinghood properties', towards an understanding of 'systemhood similarities' [57]. Organizing principles do not provide fine-grained causal explanations of biological mechanisms. Their epistemic value lies elsewhere; as higher-level abstractions, organizing principles may facilitate transfer of methods across disciplinary boundaries, and development of what Bertalanffy called 'in principle explanations' [49]. These are coarse-grained descriptions of the behaviour of a system that may be seen as templates for how such a system can be investigated. Organizing principles thus signify an epistemological framework for understanding complex phenomena. The formal framework of mathematical general systems theory forces us to be precise about our assumptions, and helps us to check the logical consistency of the argument made about a biology system [24,35]. Understood this way, they are not fruitful despite their abstract and often idealized nature, but because of it.

We believe that the limitations of reductive approaches will be particularly detrimental to progress in systems medicine. We provided an example from cancer research, demonstrating that many phenomena at the level of tissues and organs cannot be reduced to cellular events. Tissue organization, the tissue's structure and function are emergent properties that reciprocally determine the behaviour of the cells that make up the tissue. Cancer provides an example of a problem of tissue organization, and we argue that if one wants to

study tissues, one has to study tissues as a whole and not only focus on single pathways and single cells.

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Spring 2017 – Epigenetics and Systems Biology
Lecture Outline (Epigenetics and Disease Etiology)
Michael K. Skinner – Biol 476/576
Weeks 13 and 14 (April 4 & 11)

Epigenetics and Disease Etiology

- Epigenetics and Disease Etiology Introduction
- Epigenetic Disease
- Environmental Epigenetics and Disease
- Epigenetics and Cancer
- Epigenetics and Neuroscience
- Epigenetics and Metabolic Syndrome
- Epigenetic Therapy Development
- Epigenetic Transgenerational Inheritance of Disease

Required Reading

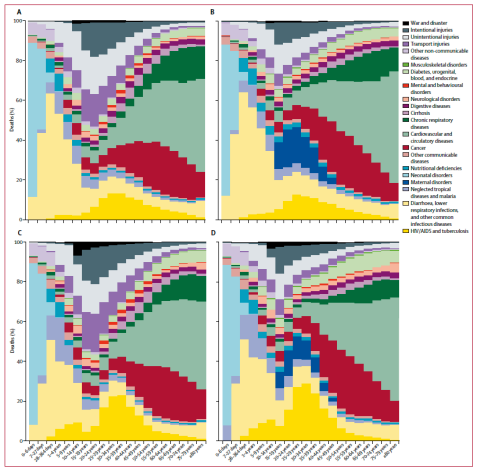
Wolkenhauer and Green (2013) The search for organizing principles as a cure against reductionism in systems medicine. FEBS J. 280(23):5938-48.

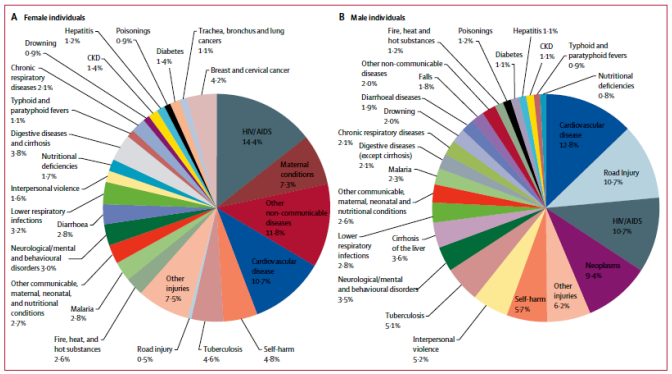
Books (Reserve in Library)

Haslberger, Alexander G, and Sabine Gressler. Epigenetics and Human Health: Linking Hereditary, Environmental, and Nutritional Aspects. Weinheim: Wiley-VCH, 2010. (e-book)

General Medicine

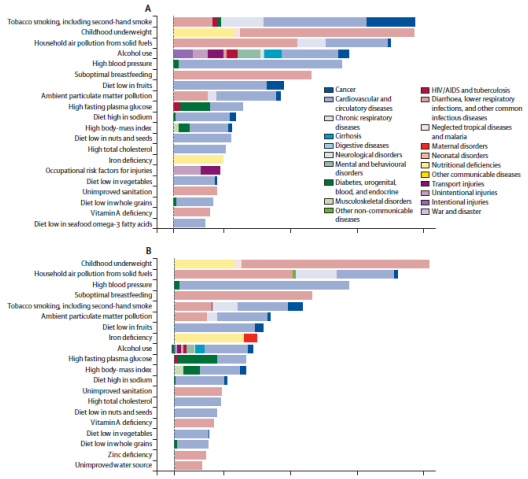
Lozano R, et al. (2012) Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet. 380(9859):2095-128.

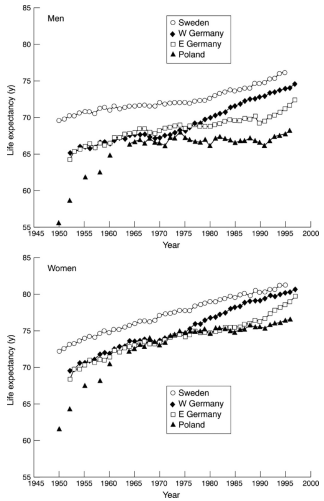


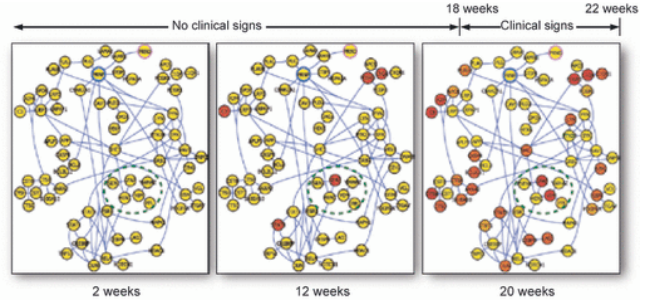


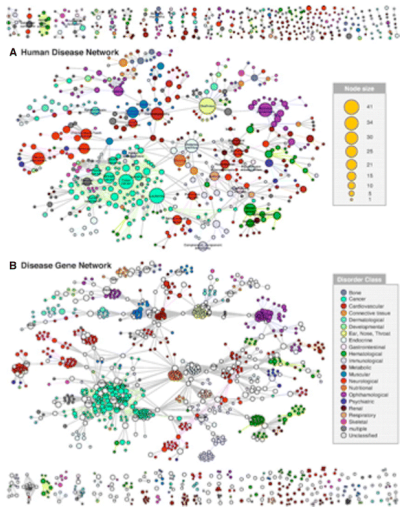
	Disability-adjusted life-years (%)
Physiological risk factors	
High blood pressure	53%
High total cholesterol	29%
High body-mass index	23%
High fasting plasma glucose	16%
Alcohol use	33%
Tobacco smoking, including second-hand smoke	31%
Dietary risk factors and physical inactivity	
Diet low in nuts and seeds	40%
Physical inactivity and low physical activity	31%
Diet low in fruits	30%
Diet low in seafood omega-3 fatty acids	22%
Diet low in whole grains	17%
Diet high in sodium	17%
Diet high in processed meat	13%
Diet low in vegetables	12%
Diet low in fibre	11%
Diet low in polyunsaturated fatty acids	9%
Diet high in trans fatty acids	9%
Diet high in sugar-sweetened beverages	2%
Air pollution	
Ambient particulate matter pollution	22%
Household air pollution from solid fuels	18%
Other environmental risks	
Lead exposure	4%

Table 2: **Proportion of ischaemic heart disease disability-adjusted life-years attributable to individual risk factors, worldwide, 2010**



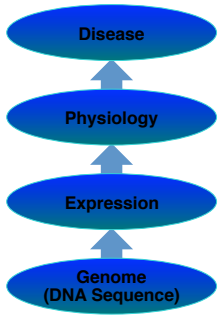






Human disease-omes. The human disease network (**a**) and the disease gene network (**b**), taken from the original article by Goh et al. [137] published in the Proceedings of the National Academy of Sciences and reproduced here with permission

Genetic Determinism

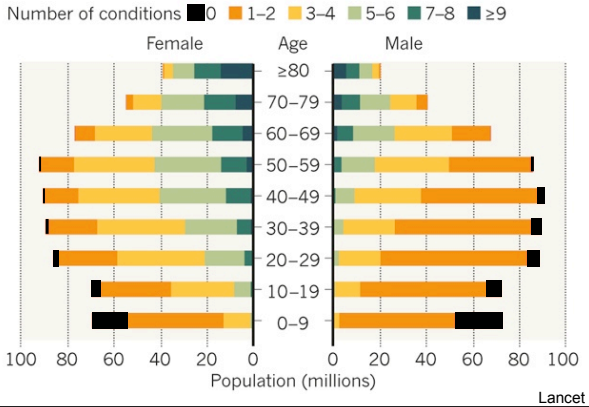


Environmental Impact on Biology

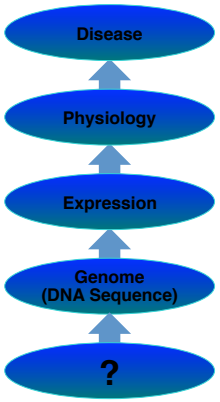
- Regional Disease Frequencies
- Low Frequency of Genetic Component of Disease (GWAS)
- Increases In Disease Frequencies
- Identical Twins and Variable Disease Frequency
- Environmental Exposures and Disease
- Evolutionary Regional Differences and Rapid Induction

GROWING SICKNESS

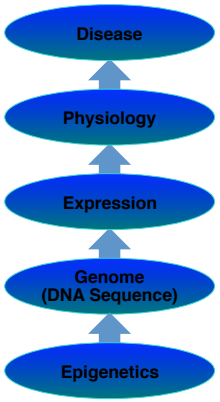
Although people are living longer, they are also living with more chronic conditions, as seen here in data for the developed world.



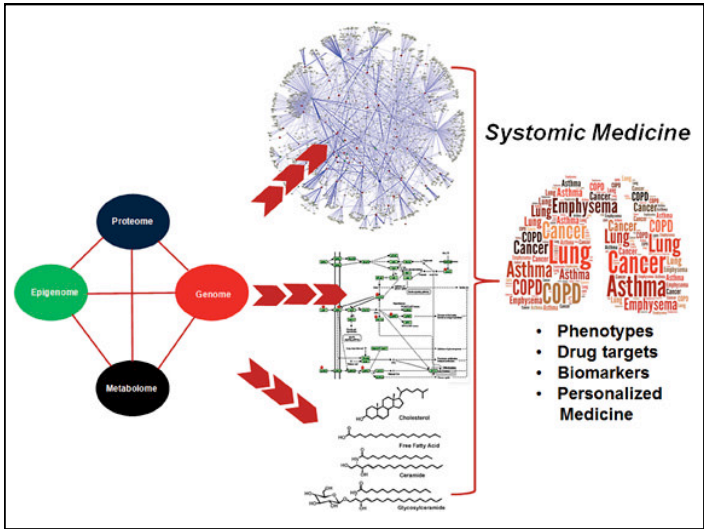
Lancet (2015)

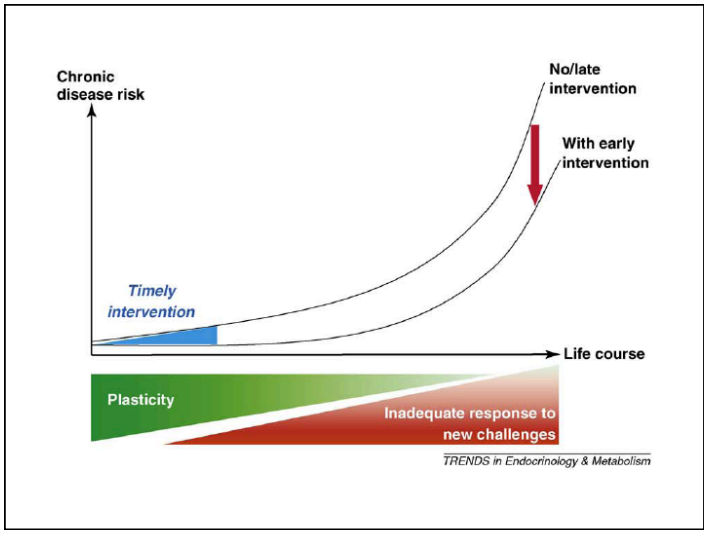


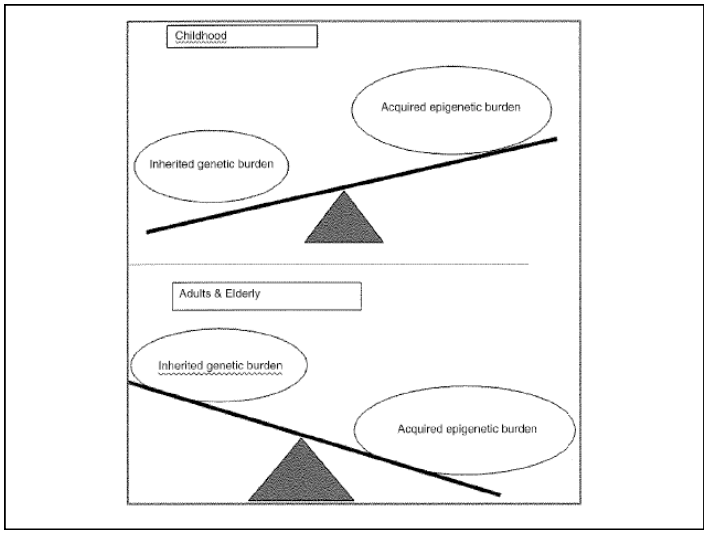
Epigenetic Solutions to Genetic Determinism Failures

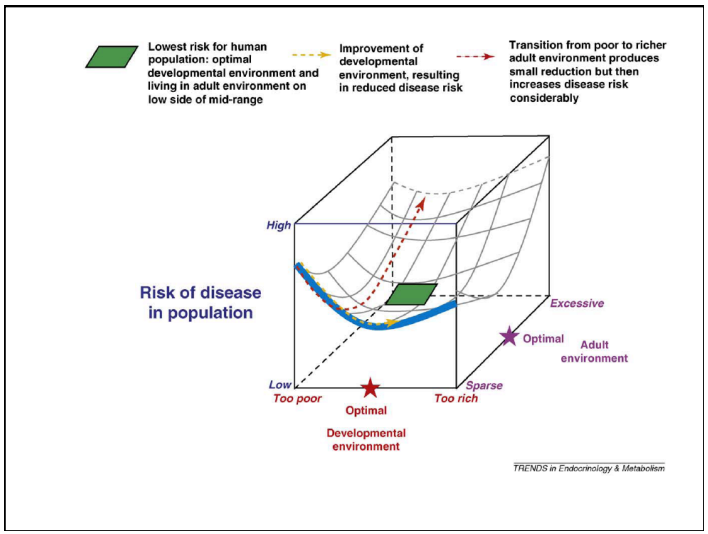


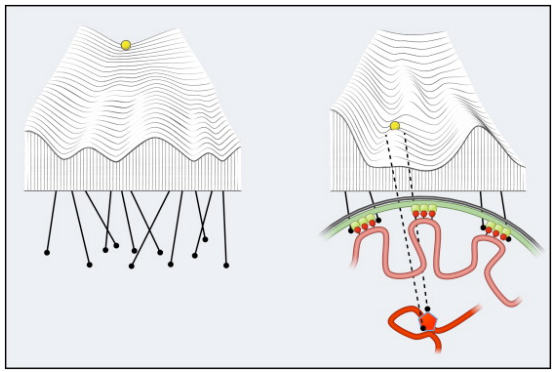
Epigenetics and Disease Etiology











Regulated Noise in a Dynamic Epigenetic LandscapeOn the left is a depiction of the classical Waddington representation of canalization, in which the ball rolling down the hill is directed into one of multiple valleys as a consistent endpoint, despite perturbation that might occur on the way. Waddington suggested a deterministic model with genes (small black circles below) pulling on the landscape from below to direct these endpoints. Changes in the landscape would arise by mutations in the genes. On the right, we suggest that modulation of the effects of noise is regulated during development and in response to external cues, which affects the contour of the epigenetic landscape itself. During differentiation, as the ball rolls down the hill, nuclear structure changes in a metastable manner through, for example, structures such as LOCKs and methylated blocks, thus changing the steepness of the valleys. At the same time, new chromosomal interactions could increase localized variability in ways that were not possible at the ground state—in this case, changing the landscape to open an alternative pathway to diversity (new bifurcation shown below the ball). The other shapes represent chromatin modifications (red circles), lamin proteins (green), and chromosome interactome mediators (red pentagon).

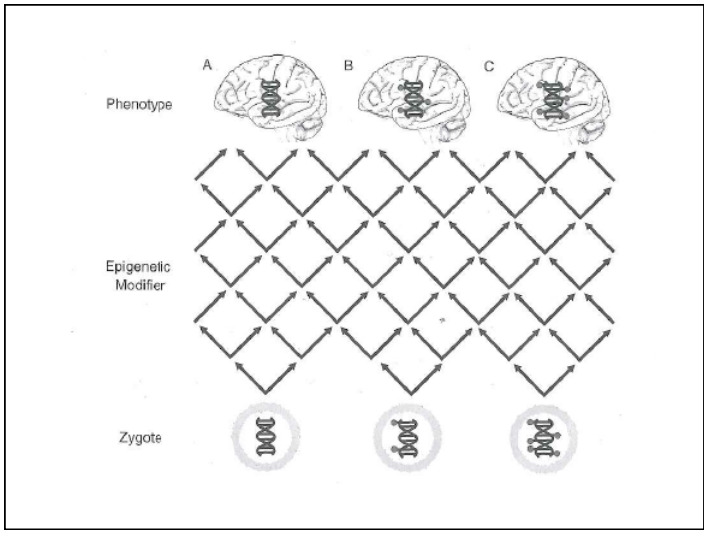
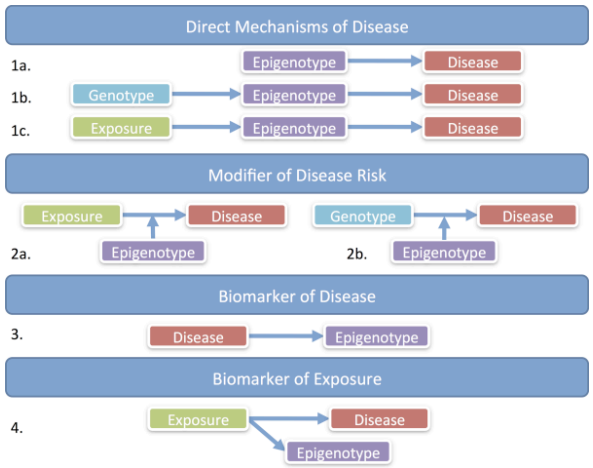
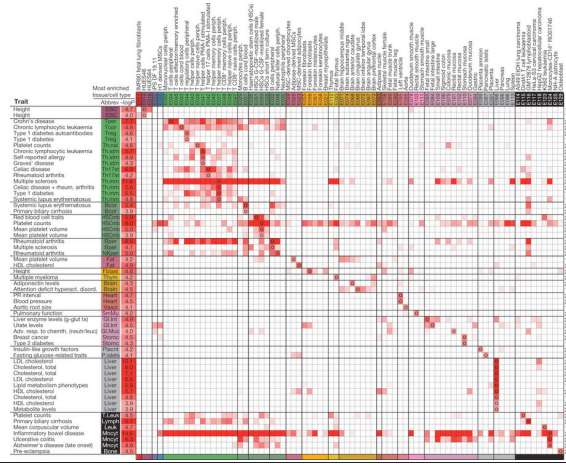


Table 1 How epigenomics is transforming the search for genetic causes of common human disease

Epigenome anatomy	Possible disease link	New approach to common disease search
Environmentally driven epigenetic variation	Epigenome changes in absence of sequence variant	Methylome arrays, capture bisulfite sequencing, chromatin immunoprecipitation with sequencing
Regulatory site or expression	Noncoding RNAs	RNA sequencing and methods above
Key disease sequences unlinked to target genes	Intra- and interchromosomal interactions	Chromatin network mapping
Regulatory sequence distant from gene	Coregulated gene clusters	Genome-scale methylation, chromatin mapping
Sequence-defined methylation	Sequence variants controlling epigenome	Linked GWAS and epigenome studies
New class of VMRs	Sequence variants controlling epigenomic variance	New statistics for reexamining and integrating GWAS
Domain disruption, anchoring proteins	LOCKs and LADs	Native chromatin whole-genome analysis



Integrative analysis of 111 reference human epigenomes.
Nature. 2015 Feb 19;518(7539):317-30
Roadmap Epigenomics Consortium, Kundaje A, et al.



Environmental Health and Long Non-coding RNAs.
Karlsson O, Baccarelli AA.
Curr Environ Health Rep. 2016 Sep;3(3):178-87.

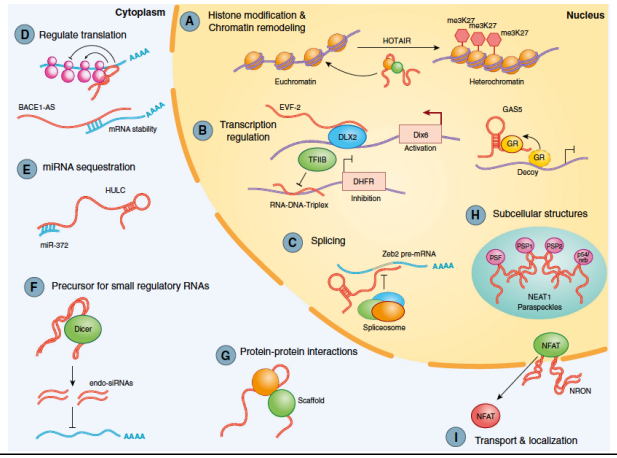


Table 1 LncRNAS: examples of biological functions and associations with human disease

Disease	lncRNA	Status[a]	Molecular mechanisms/role in disease	Ref.
Colorectal cancer (CRC)	PINT	↓	PINT acts as a tumor suppressor that reduces cell proliferation by regulating the expression of genes involved in p53 signaling via a PRC2-dependent mechanism.	[59]
Liver tumor	HULC	↑	HULC act as a molecular sponge that can bind and inhibit the function a number of miRNA, including the tumor suppressor miR-372.	[48, 60]
Breast, uterus, ovary tumors	SRA	↑	SRA forms ribonucleoprotein complexes with a number of nuclear receptors generally acting to stimulate transcriptional activation. SRA is a potential biomarker of steroid-dependent tumors	[61, 62]
Breast, colorectal tumors, prostate cancer, etc	HOTAIR	↑	HOTAIR function as a molecular scaffold to link and target PRC2 and LSD1, leading to chromatin remodeling via H3K27 methylation and H3K4 demethylation and silencing genes implicated in inhibiting cancer progression/metastasis.	[29, 63, 64]
Breast tumor, type 2 diabetes	GAS5	↓	GAS5 act as a decoy and competes for binding to the DNA-binding domain of the glucocorticoid receptor. GAS5 expression induces growth arrest and apoptosis. Decreased serum levels of GAS5 has been associated with diabetes	[65–67]
Cancer, type 2 diabetes, coronary artery disease, myocardial infarction	ANRIL	–	Several SNPs in the ANRIL locus on chromosome 9p are involved in coronary artery disease, diabetes and cancer. ANRIL binds PRC1/PRC2 and regulate the tumor suppressors CDKN2A/B. However, the clear role in the pathogenesis of these conditions is yet to be understood	[68–73]
Myocardial infarction, diabetic retinopathy, schizophrenia	MIAT or GOMAFU	–	MIAT is involved in pathological angiogenesis and is suggested as a predictor of myocardial infarction. MIAT forms ribonucleoprotein complex with three splicing proteins, SRSF1, SF-1, and QKI. Downregulation of MIAT leads to alternative splicing, suggesting a lncRNA-driven mode of splicing-defect pathogenesis.	[58, 74–77]
Alzheimer's disease	BACE1-AS	↑	BACE1-AS increases BACE1 mRNA stability leading to accelerated amyloid β42 accumulation	[78]
Autism spectrum disorder	MSNP1AS	↑	MSNP1AS regulates the moesin protein, regulator of synapse development and function, by stabilizing moesin mRNA. This mechanism may causally connect SNP variants in the MSNP1AS locus to autism spectrum disorder pathogenesis.	[79, 80]

[a]↓ downregulated, ↑ upregulated

LncRNAs are important regulators of physiological and pathological responses. Their role and functions have been mostly studied in tumorigenesis but dysregulation of lncRNAs is not only associated with several types of cancers but a variety of human diseases

PINT p53-induced non-coding transcript, *HULC* highly upregulated in liver cancer, *SRA* steroid receptor RNA activator, *HOTAIR* HOX transcript antisense RNA, *GAS5* growth arrest-specific 5, *ANRIL* antisense non-coding RNA in the INK4 locus, *MIAT* myocardial infarction associated transcript, *GOMAFU* spotted pattern in Japanese, *BACE1* beta-site APP-cleaving enzyme 1, *BACE1-AS* BACE1 antisense RNA, *MSNP1AS* moesin pseudogene 1 antisense RNA

Epigenetic Diseases

Table 1. Selected disorders of genomic imprinting

Disorder	Gene	Comments	Gene(s) involved
Prader-Willi syndrome	deletion, UPD, imprint defect	15q11-q13	snoRNAs and other (?)
Angelman syndrome	deletion, UPD, imprint defect, point mutation, duplication[a]	15q11-q13	*UBE3A*
Beckwith-Wiedemann syndrome	imprint defect, UPD, 11p15.5 duplication, translocation point mutation	11p15.5	*IGF2, CDKN1C*
Silver-Russell syndrome	UPD, duplication translocation, inversion	7p11.2	several candidates in the region
	epimutation	11p15.5	biallelic expression of H19 and decrease of IGF2
Pseudohypoparathyroidism	point mutation, imprint defect, UPD	20q13.2	*GNAS1*

[a]Maternal duplications, trisomy, and tetrasomy for this region cause autism and other developmental abnormalities.

Table 2. Selected genetic disorders affecting chromatin structure in *trans*

Disorder	Gene	Comments
Rubinstein-Taybi syndrome	*CREBBP, EP300*	
Rett syndrome	*MECP2*	loss of function as well as duplication causes a broad spectrum of phenotypes
α-Thalassemia and X-linked mental retardation	*ATRX*	somatic mutations cause α-thalassemia and myelodysplastic syndrome
ICF Syndrome	*DNMT3B*	
Schimke immuno-osseous dysplasia	*SMARCAL1*	
Mental retardation	*MTHFR*	

Table 1
Epigenetics and human diseases

Gene/protein	Disease
DNA methylation system	
MeCP2	Rett syndrome
MBD2	Colon cancer antigen
MBD4	Tumors with microsatellite instability
DNMT3b	ICF syndrome
Epigenetic regulation of genes	
FMR-1	Fragile X mental retardation
IGF2	Wilms' tumor
Imprinted genes	Prader–Willi & Angelman syndromes, Beckwith–Wiedemann syndrome
Tumor suppressor genes	Many tumors
X-Inactivation center	Functional disomy of X-linked genes
Histone acetylation system	
CBP	Rubinstein–Taybi syndrome
p300	Gastric cancer, colon cancer, brain tumor
MOZ-CBP	Acute myelocytic leukemia
MLL-CBP	Leukemias
Histone modification	
Phosphorylation defect of histone H3	Coffin–Lowry syndrome
Chromatin remodeling system	
Mi2	Autoantibody in dermatomyositis
MTA1	Metastatic potential of cancer
hSNF5/Ini-1	Rhabdoid tumor
BRG1	Tumors
ATRX	α-Thalassemia/mental retardation syndrome, X-linked
Transcriptional control	
PML-RARα	Acute promyelocytic leukemia

Lv J, et al. (2012) DiseaseMeth: a human disease methylation database. Nucleic Acids Res. 2012 Jan; 40(Database issue):D1030-5.

A screen shot of the DiseaseMeth search results for the gene RASSF1. The default view generated by the search tool is shown. Clicking the 'Fetch gene-centric information of all genes' button in the toolbar displays the gene-centric results, where the gene ID, gene Name, methylation level (from 0% to 100%), the number of relevant data in the database, and the significance of the methylation difference between disease and normal data sets for the genes are shown. In addition, the relevant reference links are also included in the overview panel. Concurrent searching of multiple genes is supported. In the gene-centric panel, a link (Visualization) is available to display the epigenomic data in the genomic context. There is also a 'Visualize a selected gene' button in the toolbar in the default view that does the same task. The whole of the search results can be downloaded by clicking the 'Download all' button in the toolbar.

The emerging role of epigenetics in rheumatic diseases.
Rheumatology (Oxford). 2014 Mar;53(3):406-14.
Gay S, Wilson AG.

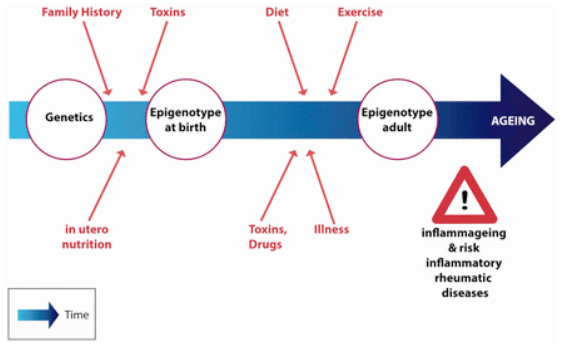
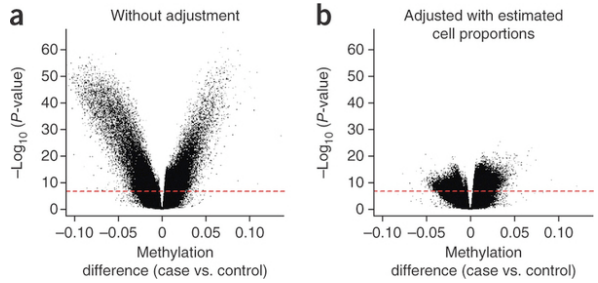


TABLE 1 Epigenetic alterations in common rheumatic diseases

Disease	Cell type	Epigenetic difference from control	Reference
RA	RASF	↓DNA methylation of cell adhesion and migration genes	[35, 92]
		↑Histone acetylation and HDAC1 expression	[39]
	Peripheral blood mononuclear cells	↓IL-6 methylation	[7]
	CD4 T cells	↓CD40 methylation	[43]
OA	Chondrocytes	↓Leptin, MMP-9, MMP-13, IL-1β and ADMSTS-4 methylation	[45, 46, 93]
SLE	T cells	↓DNA methylation and DNMT1 expression	[41, 53]
SSc	Dermal fibroblast	↑DNA methylation and DNMT1 expression	[58]

Liu Y, et al. (2013) Epigenome-wide association data implicate DNA methylation as an intermediary of genetic risk in rheumatoid arthritis. Nat Biotechnol. ;31(2):142-7.



Epigenetics in ocular diseases.
Curr Genomics. 2013 May;14(3):166-72.
Liu MM, Chan CC, Tuo J.

Table 1. DNA Methylation and Histone Modifications in Ocular Diseases.

Gene	Modification	Study Population	Tissue	Effect/significance	Reference
IL17RC	Hypomethylation of promoter region	AMD patients	Peripheral blood mononuclear cells	Increased frequency of IL-17RC$^+$CD14$^+$ mononuclear cells in peripheral blood	[16]
GSTM1 and *GSTM5*	Hypermethylation of promoter region	AMD patients	RPE/choroid and neurosensory retina	Decreased mRNA and protein levels of GSTM1 and GSTM5	[17]
CRYAA	Hypermethylation of CpG island at -856 to -640	Age-related cataract patients	Lens epithelial cells	Decreased mRNA and protein levels of CRYAA	[18]
TGM2	Hypermethylation of CpG sites at -268, -32, -29 bp	Pterygium patients	Pterygium tissue	Decreased mRNA and protein levels of TGM2	[19]
MMP2	Hypomethylation of CpG sites at +484 and +602 bp	Pterygium patients	Pterygium tissue	Increased mRNA and protein levels of MMP2	[19]
CD24	Hypomethylation of CpG sites at -809, -762, -631, -629 bp	Pterygium patients	Pterygium tissue	Increased mRNA and protein levels of CD24	[19]
MSH6, *CD44*, *PAX5*, *ATA5*, *TP53*, *VHL*, *GSTP1*, *GMT*, *RB1*, and *CDKN2*	Hypermethylation of promoter regions	Retinoblastoma patients	Formalin-fixed paraffin-embedded retinoblastoma tissue	Epigenetic dysregulation of tumor suppressors	[20]
CXCR4	Hypermethylation of CpG site in promoter region	Balb/c NOD SCID mice	LS174T human colon adenocarcinoma cells injected into anterior chamber	Ocular microenvironment can regulate promoter methylation and expression of *CXCR4*	[21]
Sod2	Increased H4K20me3 and H3K9ac at promoter and enhancer regions	Streptozotocin-induced diabetic rat	Retina	Decreased *Sod2* expression	[22]

Table 2. miRNA Signatures in Ocular Diseases.

miRNA	Modification	Study Population	Tissue	Effect/significance	Reference
miR-96, miR-182, miR-183	Expression downregulated	Multiple mouse models of retinitis pigmentosa: rho-/-, Δ307-rds, rds-/-, P347S-Rhodopsin	Retina	These miRNAs are part of a sensory-organ specific cluster and are normally highly expressed in retina	[39, 40]
miR-1, miR-133, miR-142	Expression upregulated	Multiple mouse models of retinitis pigmentosa: rho-/-, Δ307-rds, rds-/-, P347S-Rhodopsin	Retina	Unknown	[39, 40]
let-7	Expression upregulated	Age-related cataract patients	Lens epithelial cells	Increased expression correlated with cataract severity	[41]
miR-142-5p, miR-21	Expression upregulated	Mouse model of experimental autoimmune uveoretinitis	Eye	miR-21 targets IL-12p35	[46]
miR-182	Expression downregulated	Mouse model of experimental autoimmune uveoretinitis	Eye	miR-182 may target IL-17A	[46]
miR-23, miR-27	Locked nucleic acid-modified anti-miRNA (LNA-anti-miR) mediated knockdown	Mouse model of laser-induced choroidal neovascularization	Eye after intravitreal injection of LNA-anti-miRs	Knockdown suppresses development of retinal vasculature, protects against laser-induced choroidal neovascularization	[53]
miR-106a, miR-146, miR-181, miR-199a, miR-214, miR-424, miR-451	Expression upregulated	Mouse model of ischemia-induced retinal neovascularization	Retina	Unknown	[54]
miR-31, miR-150, miR-184	Expression downregulated	Mouse model of ischemia-induced retinal neovascularization	Retina	Intraocular injection of pre-miR-31, 150, or 184 reduces ischemia induced retinal or laser induced CNV	[54]
miR-200b	Expression downregulated	Streptozotocin-induced diabetic rat and human diabetic retinopathy patients	Retina	miR-200b mimic prevents diabetes-induced increase in VEGF	[56]
miR-17/92	Expression upregulated	Human and murine retinoblastoma	Retinoblastoma tissue	Overexpression in Rb/p107 double knockout mouse accelerates retinoblastoma development	[58]

Advances in lupus genetics and epigenetics.
Curr Opin Rheumatol. 2014 Sep;26(5):482-92.
Deng Y, Tsao BP.

Table 2. MicroRNA dysregulation in systemic lupus erythematosus

Function	miRNA	Expression in SLE patients	Target gene	Reference
Hyperactivation of type I IFN pathway				
	miR-146a	Downregulated in PBMCs	*IRAK1, TRAF6, IRF5, STAT1*	[96]
Aberrant cyto/chemokines release				
	miR-125a	Downregulated in PBMCs	*KLF13*	[97]
	miR-23b	Downregulated in kidney tissue	*TAB2, TAB3, CHUK*	[98]
	miR-21	Upregulated in $CD4^+$ T cells	*PDCD4*	[99]
	miR-31	Downregulated in $CD4^+$ T cells	*RHOA*	[100]
DNA hypomethylation				
	miR-126	Upregulated in $CD4^+$ T cells	*DNMT1*	[101]
	miR-21	Upregulated in $CD4^+$ T cells	*RASGRP1*	[102]
	miR-148a	Upregulated in $CD4^+$ T cells	*DNMT1*	[102]

CHUK, conserved helix-loop-helix ubiquitous kinase; *DNMT1*, DNA methyltransferase 1; *IRAK1*, interleukin-1 receptor associated kinase 1; *IRF5*, interferon regulatory factor 5; *KLF13*, Kruppel-like factor 13; PBMCs, peripheral blood mononuclear cells; *PDCD4*, programmed cell death 4; *RASGRP1*, RAS guanyl releasing protein 1; *RHOA*, ras homolog family member A; *STAT1*, signal transducer and activator of transcription 1; *TAB2*, TGF-β activated kinase1/MAP3K7 binding protein 2; *TAB3*, TGF-β activated kinase1/MAP3K7 binding protein 3; *TRAF6*, tumor necrosis factor receptor-associated factor 6.

Update on epigenetics in allergic disease.
J Allergy Clin Immunol. 2015 Jan;135(1):15-24
Harb H, Renz H.

TABLE III. Examples of environmental exposure on clinical phenotype mediated through epigenetic modifications: current examples

Effector	Epigenetic regulation	Clinical phenotype	Genes (cell type)	References
Allergens (OVA)	Histone deacetylation	AA, COPD	*LAT* ($CD4^+$)	48
	Histone acetylation	AA	*PDE4E* ($CD4^+$)	
			ACLS3 ($CD4^+$)	
Microbes/farm environment	DNA methylation	AA	*RAD50* (PBMC)	50,51
			IL13 (PBMC)	
			IL4 (PBMC)	
			IFNG ($CD4^+$)	
Tobacco smoke	DNA methylation	COPD	*GSTM1/GSTP* (macrophages)	61-63
	Histone acetylation	COPD	*TNF* (macrophages)	
	Histone deacetylation	COPD		
Diesel exhaust/polycyclic aromatic hydrocarbons	Histone deacetylation	COPD, AA	*FOXP3* ($CD4^+$)	4,60,73,75
	DNA methylation	A	*IFNG* ($CD4^+$)	
			FOXP3 ($CD4^+$)	
			ACSL3 ($CD4^+$)	
Folic acid	DNA methylation	AA	*ZFP57* ($CD4^+$)	83,84
	Histone Acetylation	AA		
Fish oil	Histone deacetylation	Cell-culture analysis	*IL6* (macrophages)	91,92
			TNF (macrophages)	
Lifestyle (obesity)	DNA methylation	AA	*CCL5, IL2RA,* and *TBX21* (PBMC)	100
Stress	DNA methylation	AA	*ADCYAP1R1* (PBMC)	102

A, Nonallergic asthma; *AA*, allergic asthma; *COPD*, chronic obstructive pulmonary disease; *LAT*, linker for activation of T cells; *TBX21*, T-box transcription factor.

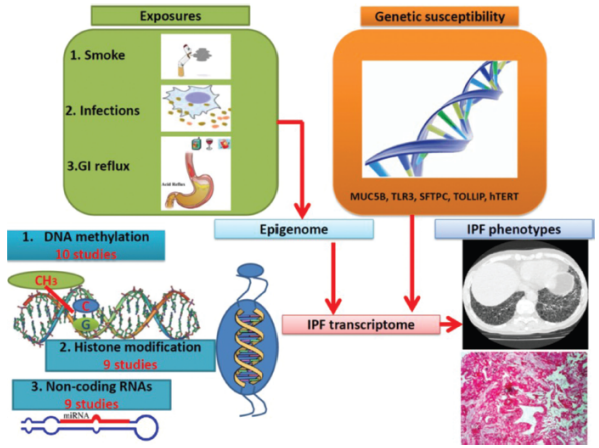
Epigenetics in immune-mediated pulmonary diseases.
Clin Rev Allergy Immunol. 2013 Dec;45(3):314-30.
Liu Y, Li H, Xiao T, Lu Q.

Table 1 Impaired DNA methylation in immune-mediated pulmonary diseases

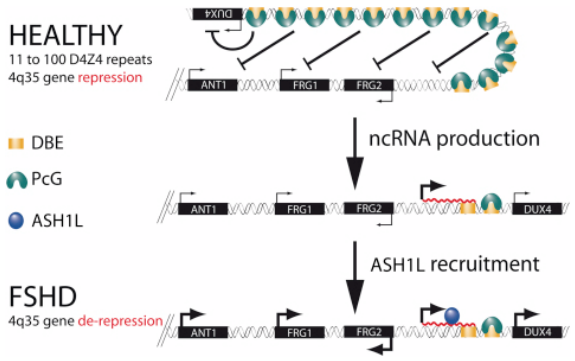
Disease	Tissue/cells	Gene/molecules	Methylation status	Expression level	Function	Contribution to the pathogenesis of disease	References
Asthma	Human blood or saliva	ADRB2	↑	↓	Beta-adrenergic response	Asthma severity, nocturnal asthma, airway hyperresponsiveness, lung function	[41]
	Human peripheral blood mononuclear cell	ZPBP2	↓	↑	Influence gene expression levels in the 17q12-q21 region	The development of childhood-onset asthma	[43]
	Distal airway tissue from mouse model	IL-4	↓	↑	Th2 cell differentiation	Th2-driven inflammation	[44]
	Human cord blood	IL-2 (site 1)	↑	↓	Response to virus infection	Asthma exacerbation via an alteration of the response to rhinovirus	[48]
	CD4+ T cells from mouse models	IFNG	↑	↓	Th1 cytokine(IFN-γ) expression	Th1/Th2 polarization, dominant Th2 phenotype	[36]
Idiopathic pulmonary fibrosis (IPF)	Fibroblasts from lung biopsy specimens of patients with IPF and lung of mouse model	PTGER2	↑	↓	Antifibrotic mediator	Increase the PGE2 resistance of fibroblasts	[80]
	Fibroblasts from patients with IPF	Thy-1	↑	↓	Cell–cell and cell–matrix interactions and regulates intracellular signaling pathways	Promote myofibroblastic differentiation of lung fibroblasts	[81]
	IPF lung tissue	STK17B, STK3 HIST1H2AH	↓	↑	Apoptosis and nucleosome formation	ND	[79]
Silicosis	Primary macrophages and fibrocytes from rats model	Genomic DNA of cFb	↓	↑	Activation of fibroblasts	Fibrosis	[138]
	Serum from the patients with silicosis	MGMT p16INK4a RASSF1A DAPK RARb	↑	↓	Tumor suppressor genes	Promote the tumorigenesis in lung	[139]
Sarcoidosis	Peripheral blood leukocytes from sarcoidosis patients	Subtelomere	↓	↑	Accessory peptide factors	Accelerated telomere shortening	[132]

(↓) decreased, (↑) increased, *PGE2* prostaglandin E2, *ND* not determined

Epigenetics in idiopathic pulmonary fibrosis.
Biochem Cell Biol. 2015 Jan 13:1-12.
Tzouvelekis A, Kaminski N.



Cabianca DS, et al. (2012) A long ncRNA links copy number variation to a polycomb/trithorax epigenetic switch in FSHD muscular dystrophy. Cell. 11;149(4):819-31.



Grace AA, Roden DM. (2012) Systems biology and cardiac arrhythmias. Lancet. 27;380(9852):1498-508.

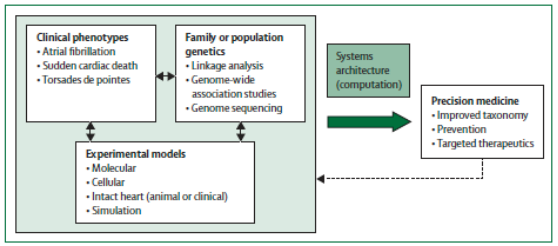


***Figure 1:* A systems approach to analysis of arrhythmia susceptibility**
A major objective of a systems approach to arrhythmia susceptibility is to develop a new taxonomy that is clinically useful and that integrates multiparametric data. Clinical and genetic inputs are used in conjunction with appropriately designed mechanistic studies in clinical and model systems. Iterative computer-based modelling provides an architectural framework that leads to improved taxonomy and allows for tailoring of medical treatment (precision medicine).

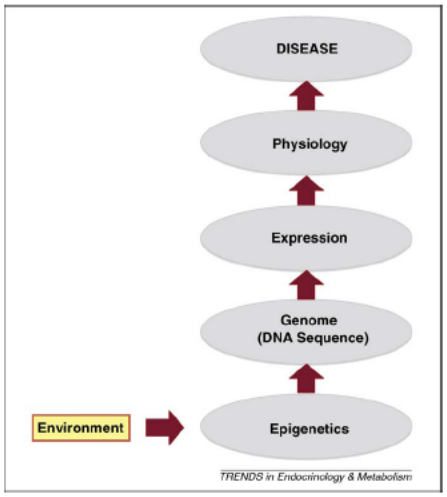
Baccarelli A, Ghosh S. (2012) Environmental exposures, epigenetics and cardiovascular disease. Curr Opin Clin Nutr Metab Care. 15(4):323-9.

Table 1. MicroRNAs in response to different environmental exposures and relation to cardiovascular disease

Exposure	miRNA/miRNA regulatory gene	Change/effect of	Target/function	CVD relevance	References
PM, carbon black	Dicer polymorphism rs13078	Minor allele A	miRNA biogenesis	Correlated with higher serum sICAM-1 and sVCAM-1 levels	[26]
	GEMIN 4 polymorphism rs1062923	Minor allele C	miRNA biogenesis	Higher sVCAM-1 levels	
Air pollution, metal pollutants	miR 222	Increased in peripheral blood	cKit, p57 (Kip2)	Induce vascular smooth muscle cell growth, angiogenesis [27]; reduction in eNOS, vasoconstriction [25]	[24]
	miR 21		Phosphatase PTEN, PI3 Kinase pathway	Prevents cardiomyocyte apoptosis in MI [28]	
Aluminum	miR 146a	Increased, in-vitro experimental model	NF-κB dependent, oxidoreductive pathway, ErbB pathway	Cardiomyocyte apoptosis cardiac hypertrophy [29]	[30]
Bisphenol A	miR 146a	Increased in placental cells			[31]
Alcohol	miR 199a	Increased in liver sinusoidal endothelial cells	Hypoxia Inducible Factor HIF-1 α, Sirtuin 1	Prevents hypoxia injury	[32]

CVD, cardiovascular disease; HIF1, hypoxia inducible factor 1; ICAM-1, intercellular adhesion molecule 1; PTEN, phosphatase and tensin homolog; VCAM-1, vascular cell adhesion molecule 1.

Epigenetics and Disease (Environmental Epigenetics)



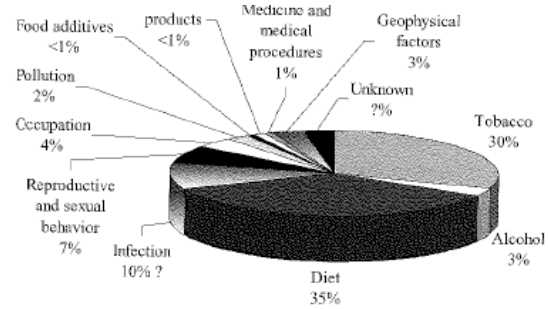


Figure 9.1 Proportions of cancer deaths attributed to different factors (according to Doll and Peto [2]).

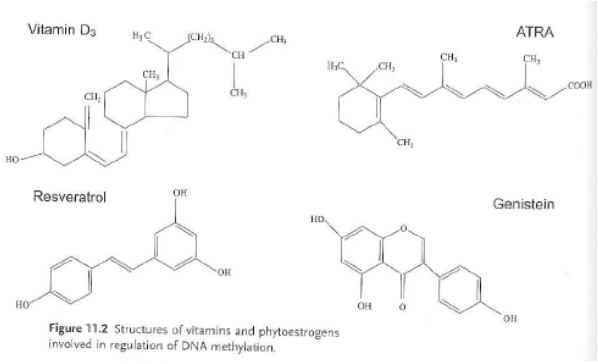
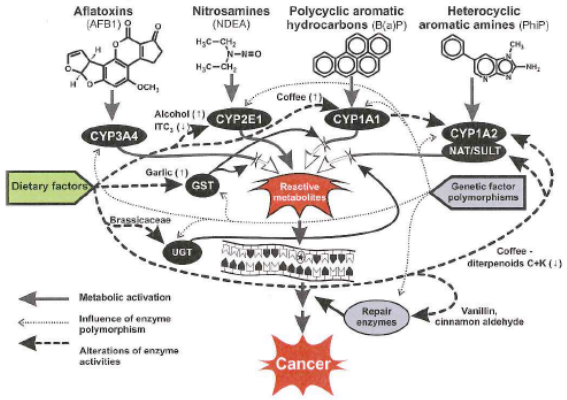


Figure 11.2 Structures of vitamins and phytoestrogens involved in regulation of DNA methylation.

Table 1. Common endocrine disruptors and their actions

Endocrine disruptor	Effect	Reference
DDT	Reproductive failure	[110]
Phytoestrogens (e.g. genistein)	Impaired fertility, reproductive effects, breast cancer protection	[15,16]
DES	Vaginal cancer in humans	[111–113]
	Developmental toxicity in hamsters	
Dicofol	Abnormal ovarian follicles, high plasma estrogen levels	[114]
BPA	Prostate cancer	[14,115]
Aflatoxin	Liver cancer	[17]
Cadmium	Lung cancer, reproductive problems	[18]
Heterocyclic amines	Cancer of colon, stomach and breast	[19]
Arsenic	Liver cancer	[21]
Dioxins (TCDD)	Mammary tumor	[116]
Vinclozolin	Impaired male fertility	[33]
Methoxychlor	Impaired male fertility	[117]
Phthalates	Impairs male reproductive tract and testis	[13]

TCDD, 2,3,7,8-Tetrachlorodibenzo-p-dioxin .



Endocrine disruption of the epigenome: a breast cancer link.
Endocr Relat Cancer. 2014 Mar 12;21(2):T33-55.
Knower KC, et al.

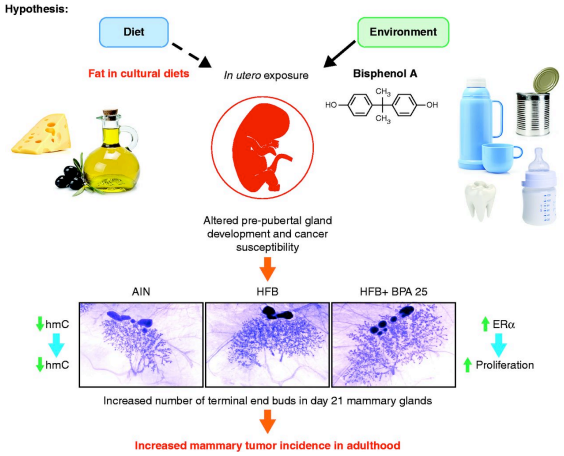


Table 1 Summary of the epigenetic effects mediated by EDCs in breast cancer

Endocrine disruptor	Route of exposure	Breast epigenetic effect	Epigenetic effect: other tissues	References
Bisphenol A (BPA)	Plastics, dental sealants, epoxy resins, thermal paper	Altered methylation of *LAMP3, BRCA1, CCNA1, CDKN2A, TAB1S1, TNFRSF10C* and *TNFRSF10D* Upregulation of *EZH2* Unique miRNA signature	Increase in *DNMT* activity in brain and testis	Doherty *et al.* (2010), Weng *et al.* (2010), Doshi *et al.* (2011), Qin *et al.* (2012), Tilghman *et al.* (2012) and Kundakovic *et al.* (2013)
Phytoestrogens	Plant-derived xenoestrogens (e.g. soy, tomatoes and red wine)	Demethylation of *BRCA1, BRCA2, GSTP1, RARβ2, CCND2* Repression of *DNMT* activity	miRNA changes in cancers of prostate, pancreas and ovaries	King-Batoon *et al.* (2008), Li *et al.* (2009), Qin *et al.* (2009), Paluszczak *et al.* (2010) and Bosviel *et al.* (2012)
Diethylstilbestrol (DES)	Prescribed drug (discontinued 1970s)	Increase in H3 trimethylation by upregulation of *EZH2* expression	Methylation pattern of *Hox* genes, *Fos* and *Nsbp1* different in mouse uterus and endometrium *Dnmt1* expression increased in mouse uterus	Li *et al.* (2003), Tang *et al.* (2008), Bromer *et al.* (2009), Sato *et al.* (2009) and Doherty *et al.* (2010)
2,3,7,8-tetrachloridibenzo-*p*-dioxin (TCDD)	Combustion and manufacture of chemicals	Hypermethylation of *BRCA1*	Transgenerational effects on sperm methylome Methylation-induced silencing of *p53* and *p16INK4a* in keratonites Increased *DNMT* activity in mouse embryos	Ray & Swanson (2004), Wu *et al.* (2004), Papoutsis *et al.* (2010) and Manikkam *et al.* (2012)
Polychlorinated biphenyls (PCBs)	Coolants and heat-transfer agents		Increased abundance of SAM and *DNMT*, leading to increased methylation in rat liver cells H4K16Ac post-translational histone modifications reduced	Fraga *et al.* (2005) and Desaulniers *et al.* (2009)
Polycyclic aromatic hydrocarbons (PAHs)	Incomplete combustion, including wood, cigarettes, coal and crude oil	Forms DNA adducts near methylation sites in breast epithelium and breast milk Alters DNA methylation and histone modification patterns		Li *et al.* (1996), Gorlewska-Roberts *et al.* (2002), Thompson *et al.* (2002), Bradley *et al.* (2007) and Sadikovic *et al.* (2007, 2008)
Perfluorooctanoic acid (PFOA)	Chemical in surfactants, waterproofing, insulating agents and dental products		Inverse correlation between *in utero* exposure and cord blood methylation *GSTP* hypermethylated in normal liver cells, leukemia, prostate and liver cancer cells	Zhong *et al.* (2002), Nakayama *et al.* (2006), Guerrero-Preston *et al.* (2010), Karius *et al.* (2011) and Tian *et al.* (2012)
DDT and DDE	Insecticides	Distinct miRNA signature in MCF-7 cells	Reduced expression of *Dnmt1* in rat hypothalamus	Shutoh *et al.* (2009) and Tilghman *et al.* (2012)
Vinclozolin	Pesticide		Germ line epigenetic alterations	Anway *et al.* (2006)

Interindividual Variability in Stress Susceptibility: A Role for Epigenetic Mechanisms in PTSD.
Front Psychiatry. 2013 Jun 26;4:60.
Zovkic IB, et al.

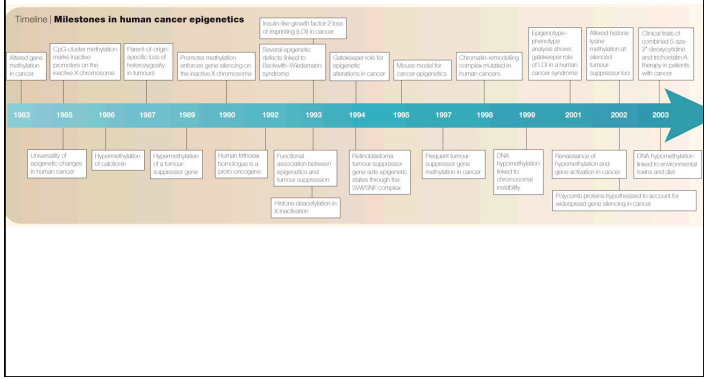
Table 1 | A summary of epigenetic modifications reported in rodent models of fear conditioning.

Epigenetic modification measured	Gene	Brain region	Effect	Reference
MEMORY CONSOLIDATION (30 min–2 h AFTER FEAR CONDITIONING)				
H3 acetylation	Global	CA1	↑	Chwang et al. (2006), Levenson et al. (2004), Miller et al. (2008)
	Bdnf IV promoter	CA1	↑	Lubin et al. (2008)
		Hippocampus	↑	Takei et al. (2011)
	Homer 1 promoter	Hippocampus	↑	Mahan et al. (2012)
	Global	Lateral amygdala	↑	Monsey et al. (2011), Maddox et al. (2013)
H3 phosphorylation	Global	CA1	↑	Chwang et al. (2006)
H3 phosphoacetylation	Global	CA1	↑	Chwang et al. (2006)
H3K9me2	Global	Entorhinal cortex	↑	Gupta-Agarwal et al. (2012)
H3K4me3	Global	CA1	↑	Gupta et al. (2010), Gupta-Agarwal et al. (2012)
	zif268 promoter	CA1	↑	Gupta et al. (2010)
	BDNF I	CA1	↑	Gupta et al. (2010)
	Homer 1 promoter	Amygdala	↓	Mahan et al. (2012)
DNA methylation	PP1	CA1	↑	Miller and Sweatt (2007)
	Reelin		↓	Miller and Sweatt (2007)
	Bdnf		↓	Lubin et al. (2008)
	zif268		↑	Gupta et al. (2010)
MEMORY MAINTENANCE (7–30 DAYS)				
DNA methylation	Calcineurin	PFC	↑	Miller et al. (2010)

Table 2 | A summary of epigenetic modifications in human and animal models of PTSD.

Species/model	Gene(s) of interest	Major findings	Reference
CANDIDATE-GENE STUDIES			
Human	*ADCYAP1, ADCYAP1R1*	PTSD symptoms correlated with *Adcyap1r1* locus in women	Ressler et al. (2011)
Rat – predator odor + social instability	*Bdnf*	↑ Exon IV methylation in dorsal DG and CA1, ↓ exon IV methylation in ventral CA3, ↓ exon IV mRNA in both dorsal and ventral CA1	Roth et al. (2011)
Human	*SLC6A4*	Controlling for genotype, *SLC6A4* methylation modified the effect of PTEs on PTSD. ↓ *SLC6A4* promoter methylation associated with ↑ PTSD risk; ↑ *SLC6A4* promoter methylation was protective against PTSD	Koenen et al. (2011)
Human	*SLC6A3*	↑ *SLC6A3* promoter methylation associated with ↑ risk of lifetime PTSD in 9R allele carriers	Chang et al. (2012)
Human	*COMT*	*COMT* Met/Met genotype interacted with CpG methylation in mediating impaired fear inhibition in PTSD patients	Norrholm et al. (2013)
Human	*FKBP5*	GC exposure was associated with ↑ *FKBP5* GRE demethylation and ↑ *FKBP5* expression in carriers of the risk compared with the protective allele	Klengel et al. (2013)
GENOME-WIDE/LARGE SCALE STUDIES			
Human	Genes involved in immunity, neurogenesis, the startle response, *DNMT3B*, *DNMT3L*, imprinted genes: *NDN, MAGEL2, ATP10A*	PTSD was associated with: (1) ↑ methylation of *DNMT3B*, ↓methylation of *DNMT3L*; (2) degulated methylation of genes involved in Prader-Willi and Angelman syndromes; (3) methylation profiles suggesting upregulation of immune-related genes and downregulation of genes involved in neurogenesis and the startle response	Uddin et al. (2010)
Rat – predator odor	*Dlgap2, Dlt3, Pkcη, Rps6kb2*	Of the four differentially methylated genes identified, *Dlgap2* was associated with a change in mRNA expression. ↓ intragenic methylation associated with ↓ hippocampal mRNA expression	Chertkow-Deutsher et al. (2010)
Human	*TPR, CLEC9A, APC5, ANXA2, TLR8, BDNF, CXCL1,* immune-related genes	PTSD associated with: (1) ↓ methylation of *TPR* and *ANXA2* and ↑ methylation of *CLEC9A, APC5, TLR8* in PTSD; (2) ↑ methylation of BDNF and CXCL1; (3) 19 of 54 of the differentially methylated immune-related genes examined in Uddin et al. (2010)	Smith et al. (2011)
OTHER			
Human	33 loci previously associated with PTSD	Only *MAN2C1* showed evidence of interaction with PTE no. in PTSD risk: ↑ *MAN2C1* methylation interacted with ↑ no. of PTEs to ↑ PTSD risk	Uddin et al. (2011)
Human	Repetitive elements: *LINE-1, Alu*	In US military service members recently deployed to Afghanistan or Iraq: LINE-1 was hypomethylated in PTSD cases vs. control post-deployment. *Alu* was hypermethylated in PTSD cases vs. control pre-deployment	Rusiecki et al. (2012)

Epigenetics and Disease (Cancer)



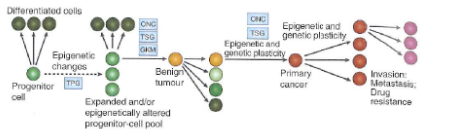
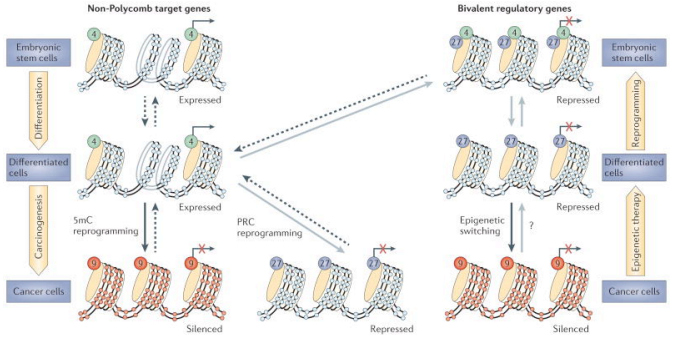
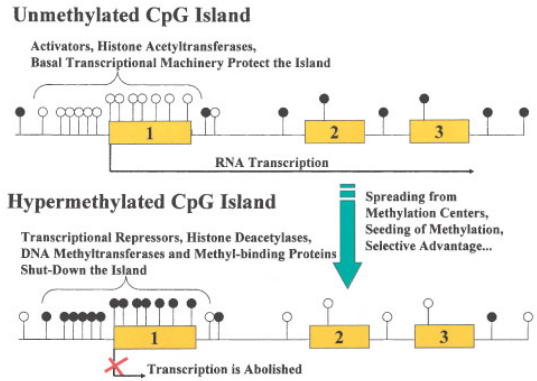


Figure 16.3 The epigenetic progenitor model of cancer. According to this model, cancer arises in three steps. First is an epigenetic alteration of stem/progenitor cells within a given tissue, which is mediated by aberrant regulation of tumor-progenitor genes (TPG) within the stem cells themselves, due to the influence of the stromal compartment, or environmental damage. Second is a gatekeeper mutation (GKM) (tumor-suppressor gene (TSG) in solid tumors, and rearrangement of oncogene (ONC) in leukemia and lymphoma). Although these GKMs are themselves monoclonal, the expanded or altered progenitor compartment increases the risk of cancer when such a mutation occurs and the frequency of subsequent primary tumors (shown as separately arising tumors). Third is genetic and epigenetic instability, which leads to increased tumor evolution. Note that many of the properties of advanced tumors (invasion, metastasis and drug resistance) are inherent properties of the progenitor cells that give rise to the primary tumor and do not require other mutations (highlighting the importance of epigenetic factors in tumor progression). Reprinted by permission from Macmillan Publishers Ltd: *Nat Rev Genet* (2006), **7**, 21–33, copyright 2006.



Modes of abnormal gene silencing in cancer
The currently suggested routes to abnormally silenced genes in cancer are shown. Genes that are active in cells throughout development and adult cell renewal initially have active promoter chromatin that is characterized by the presence of the histone modification, H3K4me (indicated by green circles and dashed arrows), and a lack of DNA methylation (indicated by pale blue circles). Genes that become silenced (indicated by a red X) can do so either by the acquisition of DNA methylation (indicated by red circles) and the presence of the repressive mark, H3K9me (indicated by orange circles and black arrows), or by the presence of Polycomb-mediated repressive chromatin (PRC) marks, H3K27me (purple circles and grey arrows). DNA methylation and H3K9me marks during tumour progression are shown. The wide yellow arrows at the sides of the figure depict movements that link stem and progenitor cells and differentiated cells and which can be impeded by epigenetic abnormalities in cancer or which can be corrected by epigenetic therapy.



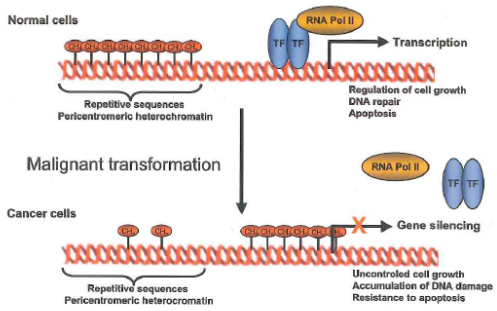
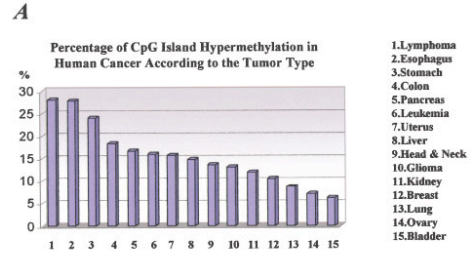


FIGURE 2.1 Repetitive DNA sequences and a typical CpG island of a tumor suppressor gene are shown for a normal and a tumor cell. The presence of dense hypermethylation completely changes the molecular environment.

Table 3. Some examples of tumor suppressor genes silenced by DNA hypermethylation in cancer

Cancer type	Tumor suppressor gene	Refs
Retinoblastoma	*pRb*	72
Breast cancer	*BRCA1*	73
Colorectal carcinoma	*MLH1, APC*	56,74
Melanoma	*p16INKK4a*	75
Haematological neoplasia	*p15INKK4b*	76
Renal carcinoma	*VHL*	77



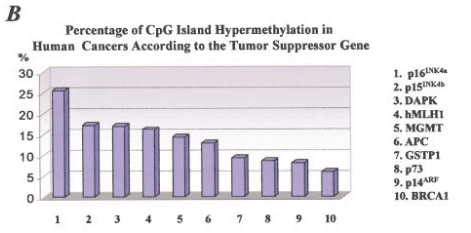


Table 1 A selected list of genes that undergo CpG island hypermethylation in human cancer

Gene	Function	Location	Tumor profile	Consequences
$p16^{INK4a}$	Cyclin-dependent kinase inhibitor	9p21	Multiple types	Entrance in cell cycle
$p14^{ARF}$	MDM2 inhibitor	9p21	Colon, stomach, kidney	Degradation of p53
$p15^{INK4b}$	Cyclin-dependent kinase inhibitor	9p21	Leukemia	Entrance in cell cycle
hMLH1	DNA mismatch repair	3p21.3	Colon, endometrium, stomach	Frameshift mutations
MGMT	DNA repair of O6-alkyl-guanine	10q26	Multiple types	Mutations, chemosentivity
GSTP1	Conjugation to glutathione	11q13	Prostate, breast, kidney	Adduct accumulation?
BRCA1	DNA repair, transcription	17q21	Breast, ovary	Double-strand breaks?
p73	p53 homolog	1p36	Lymphoma	Unknown
LKB1/STK11	Serine/threonine kinase	19p13.3	Colon, breast, lung	Unknown
ER	Estrogen receptor	6q25.1	Breast	Hormone insensitivity
PR	Progesterone receptor	11q22	Breast	Hormone insensitivity
AR	Androgen receptor	Xq11	Prostate	Hormone insensitivity
PRLR	Prolactin receptor	5p13-p12	Breast	Hormone insensitivity
RARβ2	Retinoic acid receptor β2	3p24	Colon, lung, head, and neck	Vitamin insensitivity?
RASSF1A	Ras effector homolog	3p21.3	Multiple types	Unknown
NORE1A	Ras effector homolog	1q32	Lung	Unknown
VHL	Ubiquitin ligase component	3p25	Kidney, heman-gioblastoma	Loss of hypoxic response?
Rb	Cell cycle inhibitor	13q14	Retinoblastoma	Entrance in cell cycle
THBS-1	Thrombospondin-1, antiangiogenic	15q15	Glioma	Neovascularization

(Continued)

Table 1 Genomic features of differentially methylated regions in colon cancer

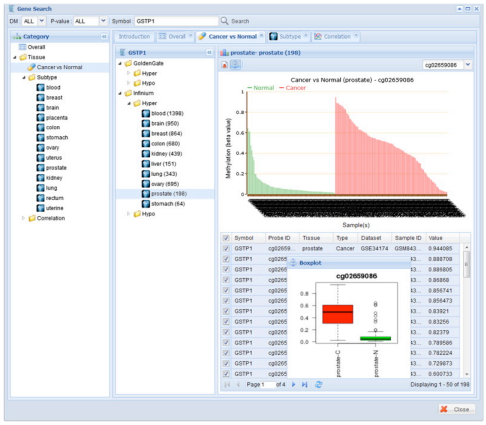
	N	No. of CpG	Genomic size	Median size (bp)	Overlap with islands	Overlap with shores	Overlap with Refseq mRNA TSS
Normal genome (reference)	N/A	28.2M	3.10 Gb	N/A	27.7K	55.4K	36,983
Hypomethylated blocks	13,540	16.2M	1.95 Gb	39,412	17.6%	26.8%	10,453
Hypermethylated blocks	2,871	485K	35.8 Mb	9,213	13.4%	36.4%	976
Hypomethylated small DMRs	4,315	59.5K	2.91 Mb	401	2.2%	51.0%	1,708
Novel hypomethylated	448	8.35K	367 kb	658	2.9%	19.9%	30
Shift of methylation boundary	1,516	17.5K	741 kb	261	2.1%	92.8%	1,313
Other	2,351	33.7K	1.80 MB	479	2.1%	29.9%	368
Hypermethylated small DMRs	5,810	403K	6.14 Mb	820	67.2%	17.0%	3,068
Loss of boundary[a]	1,756	165K	2.36 Mb	1,159	80.9%	3.4%	1,091
Shift of methylation boundary	1,774	96.3K	1.40 Mb	502	60.3%	33.0%	1,027
Other	2,280	142K	2.38 MB	769	62.2%	15.1%	983

[a]As described in the text, loss of boundary DMRs were associated with increase of methylation in the CpG island and a decrease of methylation in the adjacent shore. We score these as a single event and classify them here since there are more CpGs in the islands than in the shores. N/A, not applicable, as only ref genome assembly hg19 was used.

Table I. Genes frequently methylated in haematopoietic malignancies.

Acute myeloid leukaemia	*p15, E-cadherin, SOCS-1, p73, DAPK1, HIC1, RARβ2, ER*
Myelodysplastic syndromes	*p15, E-cadherin, calcitonin, HIC1, and ER*
Acute lymphoid leukaemia	*E-cadherin, p16, p15, p73, DAPK1, MGMT*
Lymphoma	*DAPK1, p73, p16, MGMT, GSTP1, RARβ2, CRBP1*
Multiple myeloma	*p15, p16, SOCS-1, E-cadherin, p73, DAPK1, PF4*

Baek SJ, Yang S, Kang TW, Park SM, Kim YS, Kim SY. (2013) MENT: Methylation and expression database of normal and tumor tissues. Gene. 2013 Apr 10;518(1):194-200.



A screenshot showing the 'Cancer vs Normal' search result for GSTP1. The patterns of DNA methylation of GSTP1 in each tissue are shown. Here, GSTP1 methylation in normal and tumor prostate tissues is shown as an example. Users can apply different cutoffs for DM (differential methylation) and p-value to select tissues meeting the criteria.

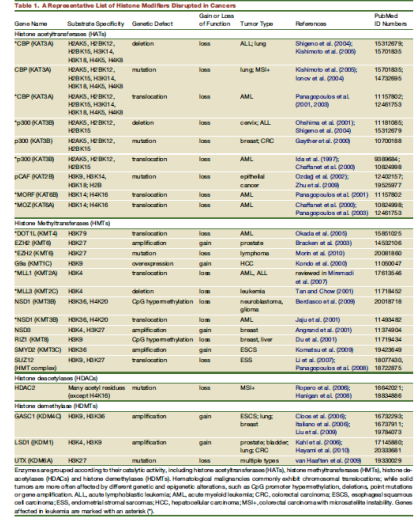
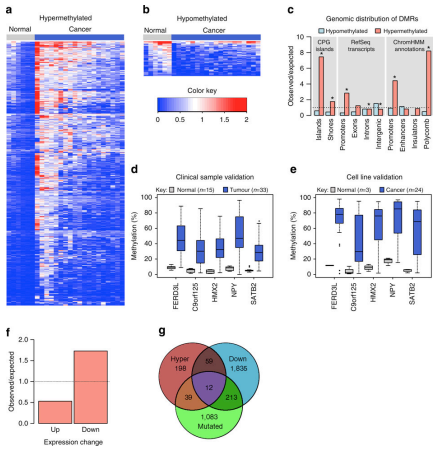


Table 1. A Representative List of Histone Modifiers Disrupted in Cancers

Gene Name	Substrate Specificity	Genetic Defect	Gain or Loss of Function	Tumor Type	References	PubMed ID Numbers
Histone acetyltransferases (HATs)						
*CBP (KAT3A)	H3AK9, H3BK12, H3BK15, H3K14, H3K18, H4K5, H4K8	deletion	loss	ALL; lung	Shigeno et al. (2004); Kishimoto et al. (2005)	15312679; 15701835
CBP (KAT3A)	H3AK9, H3BK12, H3BK15, H3K14, H3K18, H4K5, H4K8	mutation	loss	lung; MSI+	Kishimoto et al. (2005); Ionov et al. (2004)	15701835; 14732895
*CBP (KAT3A)	H3AK9, H3BK12, H3BK15, H3K14, H3K18, H4K5, H4K8	translocation	loss	AML	Panagopoulos et al. (2001, 2002)	11157802; 12481753
*p300 (KAT3B)	H3AK9, H3BK12, H3BK15	deletion	loss	cervix; ALL	Chekima et al. (2001); Shigeno et al. (2004)	11181085; 15312679
p300 (KAT3B)	H3AK9, H3BK12, H3BK15	mutation	loss	breast; CRC	Gayther et al. (2000)	10700188
*p300 (KAT3B)	H3AK9, H3BK12, H3BK15	translocation	loss	AML	Ida et al. (1997); Chetcharet et al. (2000)	9389684; 10824958
pCAF (KAT2B)	H3K9, H3K14, H4K8,16,H3B	mutation	loss	epithelial cancer	Ozdag et al. (2002); Zhu et al. (2009)	12432157; 19525977
*MORF (KAT6B)	H3K14; H4K16	translocation	loss	AML	Panagopoulos et al. (2001)	11157802
*MOZ (KAT6A)	H3K14; H4K16	translocation	loss	AML	Chetcharet et al. (2000); Panagopoulos et al. (2002)	10824958; 12481753
Histone Methyltransferases (HMTs)						
*DOT1L (KMT4)	H3K79	translocation	loss	AML	Okada et al. (2005)	15851025
EZH2 (KMT6)	H3K27	amplification	gain	prostate	Bracken et al. (2003)	14532106
*EZH2 (KMT6)	H3K27	mutation	loss	lymphoma	Morin et al. (2010)	20081860
G9a (KMT1C)	H3K9	overexpression	gain	HCC	Kondo et al. (2008)	11050047
*MLL1 (KMT2A)	H3K4	translocation	loss	AML, ALL	reviewed in Milenadi et al. (2007)	17618346
*MLL3 (KMT2C)	H3K4	deletion	loss	leukemia	Tan and Chow (2001)	11719452
NSD1 (KMT3B)	H3K36, H4K20	CpG hypermethylation	loss	neuroblastoma, glioma	Berdasco et al. (2009)	20018718
*NSD1 (KMT3B)	H3K36, H4K20	translocation	loss	AML	Jaju et al. (2001)	11489452
NSD3	H3K4, H3K27	amplification	gain	breast	Angrand et al. (2001)	11374894
RIZ1 (KMT8)	H3K9	CpG hypermethylation	loss	breast, liver	Du et al. (2001)	11719434
SMYD2 (KMT3C)	H3K36	amplification	gain	ESCS	Komatsu et al. (2003)	19423649
SUZ12 (HMT complex)	H3K9, H3K27	translocation	loss	ESS	Li et al. (2007); Panagopoulos et al. (2008)	18077430, 18723973
Histone deacetylases (HDACs)						
HDAC2	Many acetyl residues (except H4K16)	mutation	loss	MSI+	Ropero et al. (2006); Hanigan et al. (2006)	16642021; 16934868
Histone demethylase (HDMTs)						
GASC1 (KDM4C)	H3K9, H3K36	amplification	gain	ESCS; lung; breast	Cloos et al. (2006); Italiano et al. (2006); Liu et al. (2009)	16732293; 16737911; 19794073
LSD1 (KDM1)	H3K4, H3K9	amplification	gain	prostate; bladder; lung; CRC	Kahl et al. (2006); Hayami et al. (2010)	17145880; 20393981
UTX (KDM6A)	H3K27	mutation	loss	multiple types	van Haaften et al. (2009)	19330029

Enzymes are grouped according to their catalytic activity, including histone acetyltransferases (HATs), histone methyltransferases (HMTs), histone deacetylases (HDACs) and histone demethylases (HDMTs). Hematological malignancies commonly exhibit chromosomal translocations; while solid tumors are more often affected by different genetic and epigenetic alterations, such as CpG promoter hypermethylation, deletions, point mutations or gene amplification. ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; CRC, colorectal carcinoma; ESCS, esophageal squamous cell carcinoma; ESS, endometrial stromal sarcomas; HCC, hepatocellular carcinoma; MSI+, colorectal carcinoma with microsatellite instability. Genes affected in leukemia are marked with an asterisk (*).

Methylome sequencing in triple-negative breast cancer reveals distinct methylation clusters with prognostic value.
Nat Commun. 2015 Feb 2;6:5899.
Stirzaker C, et al.



Global regulation of H2A.Z localization by the INO80 chromatin-remodeling enzyme is essential for genome integrity.

Papamichos-Chronakis M, Watanabe S, Rando OJ, Peterson CL.

Cell. 2011 Jan 21;144(2):200-13.

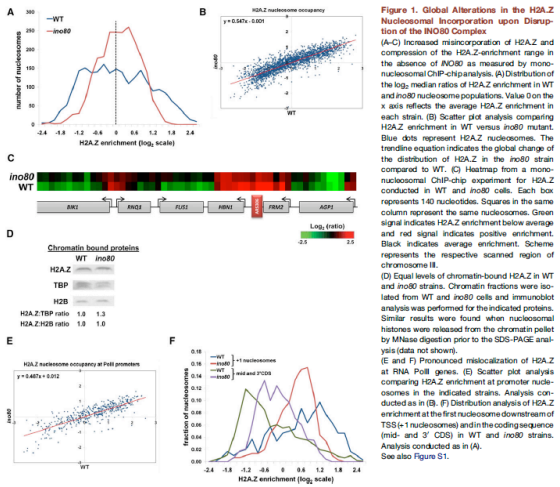


Figure 1. Global Alterations in the H2A.Z Nucleosomal Incorporation upon Disruption of the INO80 Complex

(A–C) Increased misincorporation of H2A.Z and compression of the H2A.Z-enrichment range in the absence of *INO80* as measured by mono-nucleosomal ChIP-chip analysis. (A) Distribution of the $\log_2$ median ratios of H2A.Z enrichment in WT and *ino80* nucleosome populations. Value 0 on the x axis reflects the average H2A.Z enrichment in each strain. (B) Scatter plot analysis comparing H2A.Z enrichment in WT versus *ino80* mutant. Blue dots represent H2A.Z nucleosomes. The trendline equation indicates the global change of the distribution of H2A.Z in the *ino80* strain compared to WT. (C) Heatmap from a mono-nucleosomal ChIP-chip experiment for H2A.Z conducted in WT and *ino80* cells. Each box represents 140 nucleotides. Squares in the same column represent the same nucleosomes. Green signal indicates H2A.Z enrichment below average and red signal indicates positive enrichment. Black indicates average enrichment. Scheme represents the respective scanned region of chromosome III.

(D) Equal levels of chromatin-bound H2A.Z in WT and *ino80* strains. Chromatin fractions were isolated from WT and *ino80* cells and immunoblot analysis was performed for the indicated proteins. Similar results were found when nucleosomal histones were released from the chromatin pellet by MNase digestion prior to the SDS-PAGE analysis (data not shown).

(E and F) Pronounced mislocalization of H2A.Z at RNA PolII genes. (E) Scatter plot analysis comparing H2A.Z enrichment at promoter nucleosomes in the indicated strains. Analysis conducted as in (B). (F) Distribution analysis of H2A.Z enrichment at the first nucleosome downstream of TSS (+1 nucleosomes) and in the coding sequence (mid- and 3′ CDS) in WT and *ino80* strains. Analysis conducted as in (A).

See also Figure S1.

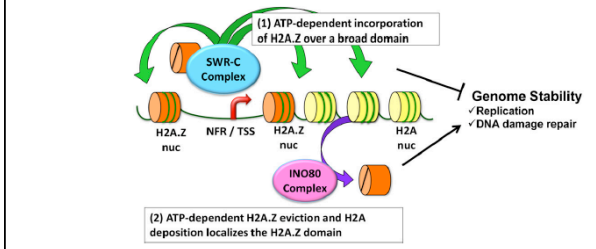
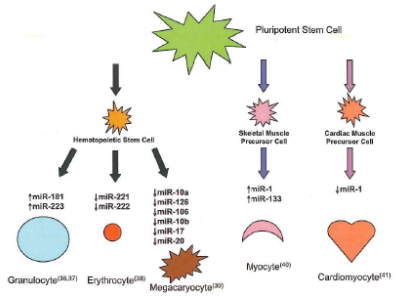


Figure 7. Proposed Model for the Role of the INO80 Chromatin-Remodeling Complex in Establishing the Proper Chromatin Localization of H2A.Z

In this model, INO80 performs a boundary function by removing H2A.Z that is deposited distal to the targeted SWR-C enzyme. See text for details.



Examples of microRNA involved in differentiation

Kunej T, Godnic I, Ferdin J, Horvat S, Dovc P, Calin GA. (2011) Epigenetic regulation of microRNAs in cancer: an integrated review of literature. Mutat Res. 1;717(1-2):77-84.

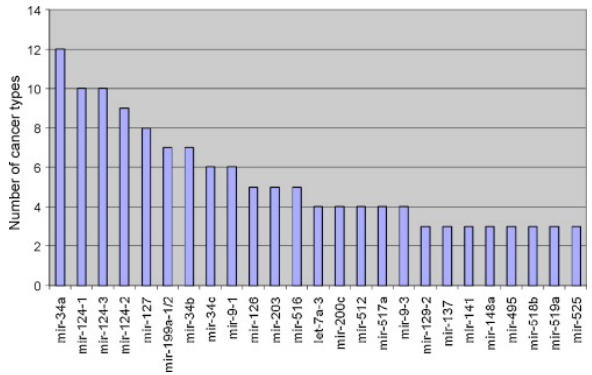


Table 1 Published epigenetically regulated MiRNAs in cancer

miRNA	Treatment	Expression change	Target	Cancer type	Reference
hsa-miR-127	Aza-CdR, PBA	Upregulation	Bcl-6	T24 cells (bladder cancer)	Saito et al. 2006
hsa-miR-124	DNMT1/DNMT3b double KO	Upregulation	Cyclin D kinase 6	HCT116 cells (colon cancer)	Lujambio et al. 2007
hsa-let-7a-3	DMNT1/DNMT3b double KO, Aza-CdR	Downregulation	RAS?	HCT116, A549 (lung cancer)	Brueckner et al. 2007
hsa-let-7a-3	Aza-CdR	Downregulation	IGF-II?	Ovarian cancer samples	Lu et al. 2007
hsa-miR-9	Aza-CdR	Upregulation	?	Various breast cancer cell lines	Lehmann et al. 2008
hsa-miR-370	IL-6, Aza-CdR	Downregulation	MAP3K8	MzChA-1, KMCH-1 (cholangiocarcinoma)	Meng et al. 2008
hsa-miR-223	Aza-CdR	Upregulation	NFI-A, MEF2C	SKNO-1 (acute myeloid leukemia)	Fazi et al. 2007
hsa-miR-342	–	Downregulation	?	Colon cancer patient samples	Grady et al. 2008
hsa-miR-34b/34c	DNMT1/DNMT3b double KO, Aza-CdR	Upregulation	p53 network	HCT116 cells, DLD-1, RKO (all colon cancer)	Toyota et al. 2008

Aza-CdR = 5-Aza-2′-deoxycytidine (decitabine); PBA = 4-phenylbutyric acid

TABLE 7.1
Oncogenic and Suppressor microRNAs

Putative Function	miRNA	Location	Chromosomal Rearrangements	Expression in Cancer	Molecular Consequences*	Suggested References
	let-7 family	Various	LOH in lung cancers	Reduced expression in lung	let-7 regulates RAS oncogene expression in lung tumors	[54], [90], [91]
Suppressors	miR-16-1/miR-15a cluster	13q14.3, intron 4 of DLEU2	Deletion of 13q143 band or LOH in hematopoietic and solid cancers	Downregulated in the majority of B-CLLs and in the majority of DLBCLs	Exogenous restoration in leukemia cells induces apoptosis by directly reducing levels of anti-apoptotic BCL2	[62], [82], [88]
	miR-124a-1	8p23.1 intergenic		Downregulated in various solid cancers	Activation of cyclin D kinase 6 oncogene and phosphorylation of retinoblastoma suppressor gene	[97]
	miR-127	14q32.31	LOH in solid cancers	Reduced expression in prostate and bladder cancers	Translational downregulation of the transcriptional repressor BCL6	[96]
	miR-145/ miR-143 cluster	5q32, intergenic	Deletion of 5q32 band or LOH in MDS (5q- syndrome)	Reduced expression in colon adenomas and carcinomas and in breast cancers	Unknown	[56], [94]
Oncogenic	miR-17/92 cluster	13q31.3, intron 3 C13orf25	AMPLIF in follicular lymphomas	Overexpressed in malignant lymphomas and lung cancers	The miRNAs cluster, but not the host C13orf25 gene, enhances cell proliferation	[71], [73]
	miR-21	7q23.2 3′UTR VMP1	AMPLIF in neuroblastoma and breast, colon, and lung cancers	Elevated levels in glioblastomas, breast, colon, lung, pancreas, prostate, and stomach cancers, and cholangiocarcinomas	Increased apoptotic cell death after knockdown in glioblastoma cells;miR-21 modulated gemcitabine-induced apoptosis by PTEN	[53], [56], [59]
	miR-155	21q21.3, exon 3 of ncRNA BIC	Not reported	High expression in pediatric BL, in Hodgkin's, primary mediastinal and DLBCL lymphomas; overexpressed in breast, colon, and lung cancers	Unknown	[53], [54], [56], [61], [62], [64]
	miR-372, miR-373	19q13.42, intergenic	Not reported	High expression in testicular germ cell tumors	Proliferation of p53 wild-type cancer cells sensible to DNA-damaging agents	[81]

Note: * Molecular consequences with proven cancer relevance, as reported by the references in the last column, were presented; B-CLL: B-cell chronic lymphocytic leukemia; BIC: noncoding RNA gene; BL: Burkitt lymphoma; DLBCL: diffuse large B-cell lymphoma; DLEU2: noncoding RNA gene; MDS: myelodysplastic syndrome; VMP1: vacuole membrane protein 1. Modified with permission from Ref. 47.

Cancer Genome Atlas Research Network. Identification of a CpG island methylator phenotype that defines a distinct subgroup of glioma.

Noushmehr H, Weisenberger DJ, Diefes K, Phillips HS, Pujara K, Berman BP, Pan F, Pelloski CE, Sulman EP, Bhat KP, Verhaak RG, Hoadley KA, Hayes DN, Perou CM, Schmidt HK, Ding L, Wilson RK, Van Den Berg D, Shen H, Bengtsson H, Neuvial P, Cope LM, Buckley J, Herman JG, Baylin SB, Laird PW, Aldape K;

Cancer Cell. 2010 May 18;17(5):510-22.

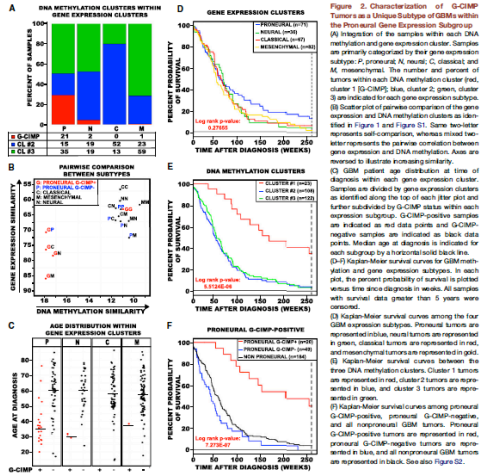


Figure 2. Characterization of G-CIMP Tumors as a Unique Subtype of GBMs within the Pro-neural Gene Expression Subgroup

(A) Integration of the samples within each DNA methylation and gene expression cluster. Samples are primarily categorized by their gene expression subtype: P, proneural; N, neural; C, classical; and M, mesenchymal. The number and percent of tumors within each DNA methylation cluster (red, cluster 1 [G-CIMP]; blue, cluster 2; green, cluster 3) are indicated for each gene expression subtype.

(B) Scatter plot of pairwise comparison of the gene expression and DNA methylation clusters as identified in Figure 1 and Figure S1. Same two-letter represents self-comparison, whereas mixed two-letter represents the pairwise correlation between gene expression and DNA methylation. Axes are reversed to illustrate increasing similarity.

(C) GBM patient age distribution at time of diagnosis within each gene expression cluster. Samples are divided by gene expression clusters as identified along the top of each jitter plot and further subdivided by G-CIMP status within each expression subgroup. G-CIMP-positive samples are indicated as red data points and G-CIMP-negative samples are indicated as black data points. Median age at diagnosis is indicated for each subgroup by a horizontal solid black line.

(D–F) Kaplan-Meier survival curves for GBM methylation and gene expression subtypes. In each plot, the percent probability of survival is plotted versus time since diagnosis in weeks. All samples with survival data greater than 5 years were censored.

(D) Kaplan-Meier survival curves among the four GBM expression subtypes. Proneural tumors are represented in blue, neural tumors are represented in green, classical tumors are represented in red, and mesenchymal tumors are represented in gold.

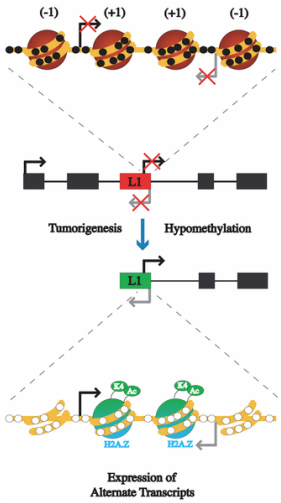
(E) Kaplan-Meier survival curves between the three DNA methylation clusters. Cluster 1 tumors are represented in red, cluster 2 tumors are represented in blue, and cluster 3 tumors are represented in green.

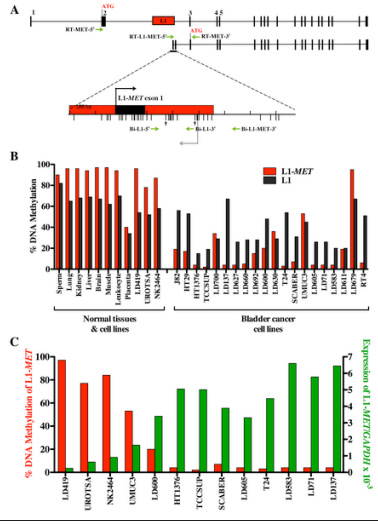
(F) Kaplan-Meier survival curves among proneural G-CIMP-positive, proneural G-CIMP-negative, and all nonproneural GBM tumors. Proneural G-CIMP-positive tumors are represented in red, proneural G-CIMP-negative tumors are represented in blue, and all nonproneural GBM tumors are represented in black. See also Figure S2.

Hypomethylation of a LINE-1 promoter activates an alternate transcript of the MET oncogene in bladders with cancer.

Wolff EM, Byun HM, Han HF, Sharma S, Nichols PW, Siegmund KD, Yang AS, Jones PA, Liang G.

PLoS Genet. 2010 Apr 22;6(4):e1000917.





The genomic complexity of primary human prostate cancer.

Berger MF, Lawrence MS, Demichelis F, Drier Y, Cibulskis K, Sivachenko AY, Sboner A, Esgueva R, Pflueger D, Sougnez C, Onofrio R, Carter SL, Park K, Habegger L, Ambrogio L, Fennell T, Parkin M, Saksena G, Voet D, Ramos AH, Pugh TJ, Wilkinson J, Fisher S, Winckler W, Mahan S, Ardlie K, Baldwin J, Simons JW, Kitabayashi N, MacDonald TY, Kantoff PW, Chin L, Gabriel SB, Gerstein MB, Golub TR, Meyerson M, Tewari A, Lander ES, Getz G, Rubin MA, Garraway LA.

Nature. 2011 Feb 10;470(7333):214-20.

Table 1 | Landscape of somatic alterations in primary human prostate cancers

	Tumour						
	PR-0508	PR-0581*	PR-1701*	PR-1783	PR-2832*	PR-3027	PR-3043
Tumour bases sequenced	97.8×10^9	93.9×10^9	110×10^9	90.9×10^9	106×10^9	93.6×10^9	94.9×10^9
Normal bases sequenced	96.7×10^9	57.8×10^9	108×10^9	92.3×10^9	103×10^9	87.8×10^9	96.6×10^9
Tumour haploid coverage	31.8	30.5	35.8	29.5	34.4	30.4	30.8
Normal haploid coverage	31.4	18.8	34.9	30.0	33.4	28.5	31.4
Callable fraction	0.84	0.83	0.87	0.82	0.84	0.84	0.85
Estimated tumour purity†	0.73	0.60	0.49	0.75	0.59	0.74	0.68
All point mutations (high confidence)	3,898 (1,447)	3,829 (1,430)	3,866 (1,936)	4,503 (2,227)	3,465 (1,831)	5,865 (2,452)	3,192 (1,713)
Non-silent coding mutations (high confidence)	16 (5)	20 (3)	24 (9)	32 (20)	13 (7)	43 (16)	14 (10)
Mutation rate per Mb	0.7	0.7	0.8	1.0	0.8	1.2	0.7
Rearrangements	53	67	90	213	133	156	43

*Harbours *TMPRSS2–ERG* gene fusion
†Estimated from SNP array-derived allele specific copy number levels using the ABSOLUTE algorithm (Supplementary Methods).

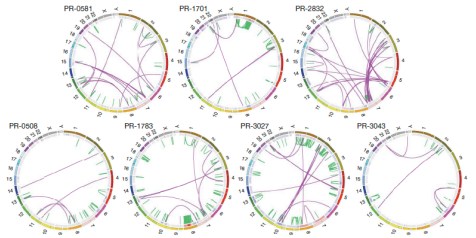


Figure 1 | Graphical representation of seven prostate cancer genomes. Each Circos plot[11] depicts the genomic location in the outer ring and chromosomal copy number in the inner ring (red, copy gain; blue, copy loss). Interchromosomal translocations and intrachromosomal rearrangements are shown in purple and green, respectively. Genomes are organized according to the presence (top row) or absence (bottom row) of the *TMPRSS2–ERG* gene fusion.

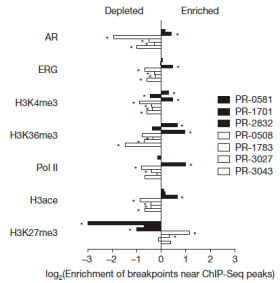


Figure 3 | Association between rearrangement breakpoints and genome-wide transcriptional/histone marks in prostate cancer. ChIP-seq binding peaks were defined previously for the *TMPRSS2–ERG* positive (ERG-positive) prostate cancer cell line VCaP[35]. For each genome, enrichment of breakpoints within 50 kb of each set of binding peaks was determined relative to a coverage-matched simulated background (see Methods). *TMPRSS2–ERG*-positive prostate tumours are in black; ETS fusion-negative prostate tumours are in white. Enrichment is displayed as the ratio of the observed breakpoint rate to the background rate near each indicated set of ChIP-seq peaks. Rearrangements in ETS fusion-negative tumours are depleted near marks of active transcription (AR, ERG, H3K4me3, H3K36me3, Pol II and H3ace) and enriched near marks of closed chromatin (H3K27me3). *P*-values were calculated according to the binomial distribution and are displayed in Supplementary Fig. 5 and Supplementary Table 6. *Significant associations passing a false discovery rate cut-off of 5%.

Decrypting ENCODEd epigenetic marks of human tRN-A-RS genes in normal, stem and cancer cell lines.
Mitra S, Samadder A, Das P, Das S, Dasgupta M, Chakrabarti J.
J Biomol Struct Dyn. 2016 Oct 6:1-13.

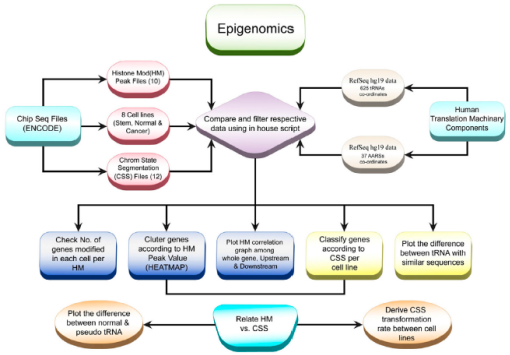


Figure 1. Pipeline of work.
Notes: Epigenetic files of genes retrieved from ENCODE to analyse HMs and CSs in cell lines, CS alteration across cell lines, how HM distributions correlate to CS, the differences between tRNAs and pseudo tRNAs. tRNAs in a cell with identical/similar sequences may have vastly different HMs and CS.

Biomarkers of genome instability and cancer epigenetics.
Reis AH, Vargas FR, Lemos B.
Tumour Biol. 2016 Oct;37(10):13029-13038.

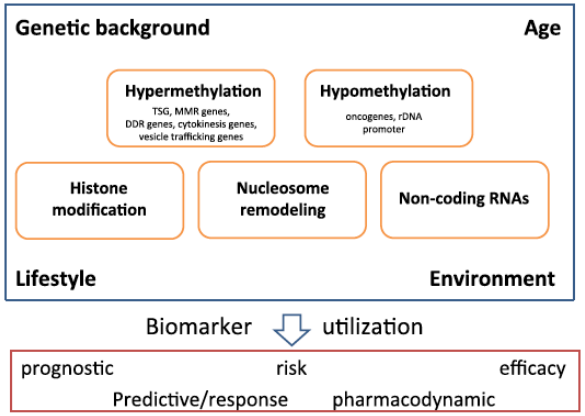
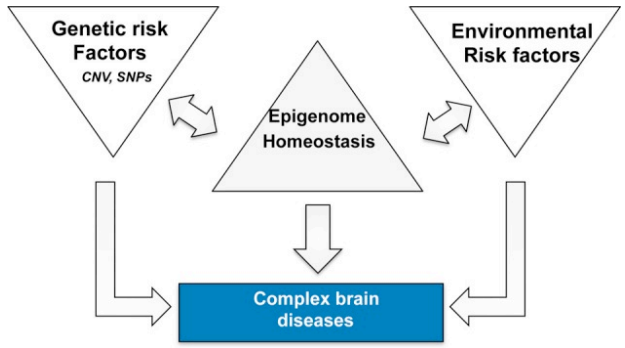


Table 1 Biomarkers of genome instability and epigenetic events involved in carcinogenesis discussed in this review

Type	Source	Consequence
Genomic instability	- Single nucleotide polymorphisms - Copy number variation - Short tandem repeats - Chromosomal translocations - Chromosome aneuploidy	Genetic lesions of a variety of sizes and qualities are a prominent source of genetic diversity within tumors
Risk alleles	- Alleles in a variety of regions of the genome	Increase the risk of cancer and are modified by the presence of genetic susceptibility variants at multiple loci
DNA methylation	- Hypomethylation of repetitive DNA elements - Gene-specific hypermethylation of CpG islands - Hypomethylation of ribosomal genes	Altered regulation of gene expression, chromatin structure, genome stability, and allelic expression
Histone modification	- Post-translational modifications like methylation, ubiquitylation, acetylation, sumoylation, phosphorylation, homocysteinylation, crotonylation, and glucosylation	Altered regulation of chromatin structure with impact on gene expression, chromatin structure, genome stability, and allelic expression
Nucleosome remodeling	- ATP-dependent nucleosome remodeling like SWI/SNF complexes	Altered regulation of chromatin structure with impact on gene expression, chromatin structure, genome stability, and allelic expression
Non-coding RNAs	- Ribosomal RNAs - Micro-RNAs - Small nucleolar RNAs - Transfer RNAs - Long non-coding RNAs - Small interfering RNAs - Piwi-interacting RNAs - Small nuclear RNAs	Altered translation and degradation of target mRNAs, impact on chromatin structure, chromatin remodeling, transcription, and post-transcriptional processing

Epigenetics and Disease
(Neuroscience)

Histone-acetylation: a link between Alzheimer's disease and post-traumatic stress disorder?
Front Neurosci. 2014 Jun 24;8:160.
Bahari-Javan S, Sananbenesi F, Fischer A.

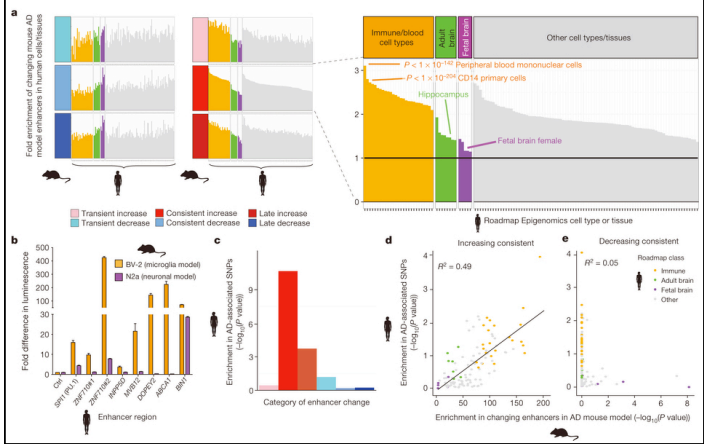


Environmental factor(s)	Associated with
Alzheimer's disease	
Metals (iron, copper, zinc, mercury, aluminum)	Increased risk, inconclusive results
Pesticides	Increased risk
Solvents	Increased risk, inconclusive results
Electromagnetic fields	Increased risk, inconclusive results
Caloric restriction	Protection
Antioxidants	Protection
Mediterranean diet, fruit and vegetables	Protection
Fish and omega-3 fatty acids	Protection
Traumatic brain injuries	Increased risk
Infections and inflammation	Increased risk
Parkinson's disease	
Metals (iron, copper, manganese, lead)	Increased risk, conflicting results
Rural environment (pesticides, herbicides)	Increased risk
Tobacco smoking	Protection
Caffeine (coffee and tea drinking)	Protection
Fruit and vegetables, legumes, nuts	Protection
Fish	Protection
Head injuries with loss of consciousness	Increased risk
Amyotrophic lateral sclerosis	
Metals (lead)	Increased risk
Pesticides and insecticides	Increased risk
Electromagnetic fields	Increased risk
Some sports (soccer, football)	Increased risk
Head injuries	Increased risk
Tobacco smoking	Increased risk, in women

Genetic variant(s)	Associated with
Alzheimer's disease	
*APOE*ε4	Increased risk
SORL1 variants	Increased risk
ACE Intron 16 (ins/del)	Increased risk
ACE rs1800764, rs4291, rs4343	Decreased risk
CTBNBL1 rs4845378	Decreased risk
CST3 5′UTR-157, 5′UTR-72	Increased risk
CST3 A25T	Increased risk
ESR1 PvuII, XbaI	Increased risk
GAPDHS rs12984928, rs4806173	Decreased risk
IDE rs2251101	Decreased risk
MTHFR A1298C	Decreased risk
NCSTR 139 intron 16	Increased risk
PRPN M129V	Decreased risk
PSEN1 rs165932	Decreased risk
TF P570S	Increased risk
TFAM rs2306604	Decreased risk
TNF rs4647198 (-1031)	Increased risk
GOLPH2 rs10868366[c], rs7019244[c]	Decreased risk
Rs 9886784 (Chromosome 9)	Increased risk
Rs 10519262[d]	Increased risk
Parkinson's disease	
SNCA Rep1	Increased risk
LRRK2 G2385R	Increased risk[b]
MAPT H1 haplotype	Increased risk
DCTN1 S139Y	Decreased risk[c]
GSTM1 null genotype	Increased risk[d1]
GSTP1 variants	Increased risk[d2]
CYP2D6 variants	Increased risk[d3]
FAM 79B Rs 1000291[a]	Increased risk
UNC5C Rs 2241743[a]	Increased risk
Rs 8018626 (Chromosome 11)[a]	Increased risk
Amyotrophic lateral sclerosis	
APEI D148E	Increased risk[a]
ANG G110G	Increased risk[a]
hOGG1 Ser326Cys	Increased risk in males
VEGF variants	Inconclusive results
HFE H63D	Increased risk
SMN1 variable copy number	Increased risk
DPP6 variant[a]	Increased risk

a From WGA studies.
b Only in Asiatic populations.
c Conflicting results.
d In combination with environmental factors (^{d1} = solvents, ^{d2} = pesticides and herbicides, ^{d3} = pesticides, tobacco smoking).

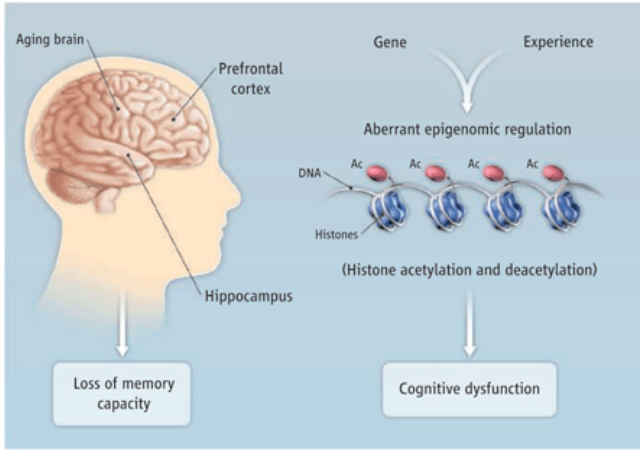
Conserved epigenomic signals in mice and humans reveal immune basis of Alzheimer's disease.
Nature. 2015 Feb 19;518(7539):365-9.
Gjoneska E, et al.

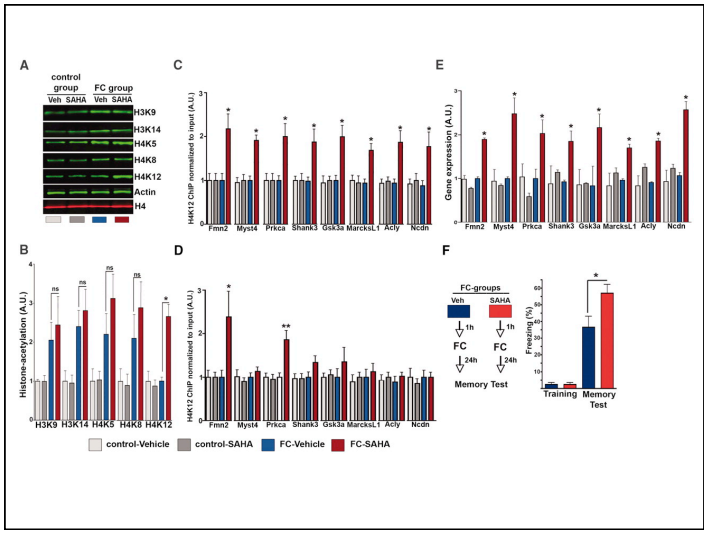


Altered histone acetylation is associated with age-dependent memory impairment in mice.

Peleg S, Sananbenesi F, Zovoilis A, Burkhardt S, Bahari-Javan S, Agis-Balboa RC, Cota P, Wittnam JL, Gogol-Doering A, Opitz L, Salinas-Riester G, Dettenhofer M, Kang H, Farinelli L, Chen W, Fischer A.

Science. 2010 May 7;328(5979):753-6.





Global methylation profiling of lymphoblastoid cell lines reveals epigenetic contributions to autism spectrum disorders and a novel autism candidate gene, RORA, whose protein product is reduced in autistic brain.

Nguyen A, Rauch TA, Pfeifer GP, Hu VW.

FASEB J. 2010 Aug;24(8):3036-51. Epub 2010 Apr 7.

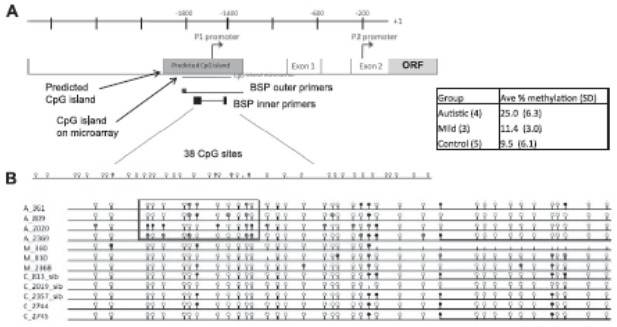
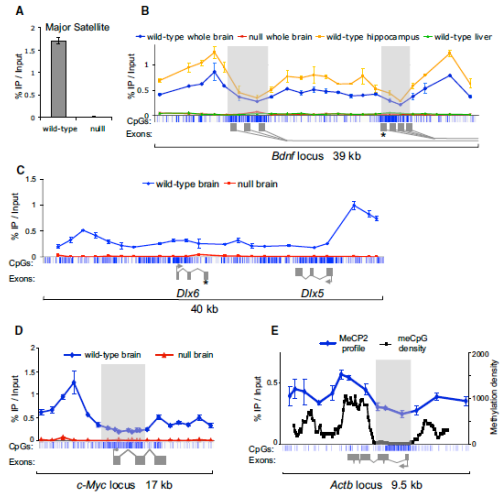


Figure 2. *A*) Bisulfite sequencing of the BCL-2 P1 promoter region. *B*). Bisulfite sequencing was performed across 38 CpG sites from genomic DNA isolated from LCLs of discordant MZ twins. Autistic co-twins are designated by A_ preceding the blood identifier number (*e.g.*, A_809); undiagnosed co-twins are designated by M_ preceding the blood number (*e.g.*, M_810). Control (C) individuals include unaffected siblings (*e.g.*, C_813), one normal monozygotic twin pair (C_2744 and C_2745). A pair of siblings, one autistic (A_2020) and one unaffected (C_2019), was also included in this analysis. Inset: average percentage methylation with sd across all 38 CpG sites for each group of samples.

Neuronal MeCP2 is expressed at near histone-octamer levels and globally alters the chromatin state.

Skene PJ, Illingworth RS, Webb S, Kerr AR, James KD, Turner DJ, Andrews R, Bird AP.

Mol Cell. 2010 Feb 26;37(4):457-68.



Beyond transcription factors: the role of chromatin modifying enzymes in regulating transcription required for memory.

Barrett RM, Wood MA.

Learn Mem. 2008 Jun 26;15(7):460-7.

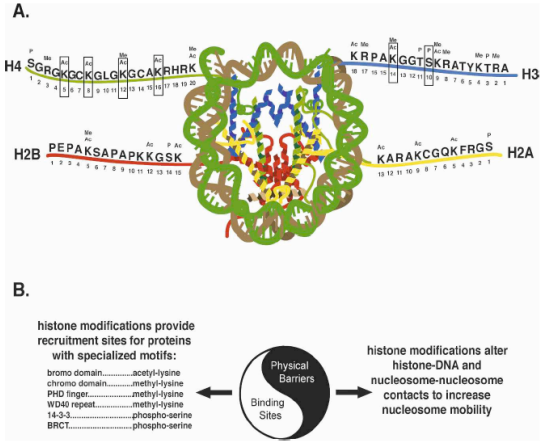


Table 1. Types of memory deficits observed in genetically modified mice and memory enhancements generated by HDAC inhibition

Histone acetyltransferases (HATs) and memory[a]

Mutation	Memory/Plasticity Impairment	Reference
Dominant-negative truncated CBP	Cued fear conditioning Passive avoidance Novel object recognition	Oike et al. 1999 Bourtchouladze et al. 2003
CBP knockout	Contextual fear conditioning Novel object recognition Cued fear conditioning (trend only) L-LTP	Alarcon et al. 2004
CBP^{HAT}	Novel object recognition Morris water maze	Korzus et al. 2004
$CBP^{KIX/KIX}$	Novel object recognition Contextual fear conditioning	Wood et al. 2006
CBPΔ1	Morris water maze Contextual fear conditioning L-LTP generated by: 1 train E-LTP + D1 agonist	Wood et al. 2005
p300Δ1	Novel object recognition	Oliveira et al. 2007
	Novel object recognition Contextual fear conditioning	Oliveira et al. 2007
PCAF knockout	Morris water maze Inhibitory avoidance Novel object recognition	Maurice et al. 2008

Histone modifications and memory[c]

Location	Functional group/relation to memory	Reference
H3 S10	Phosphate; ↑ in response to fear conditioning/hippocampal slice stimulation	Chwang et al. 2006, 2007
H3 K14	Acetyl; ↑ in response to fear conditioning/hippocampal slice stimulation	
H3 K14	Acetyl; ↑ in response to treatment with 5-HT in aplysia	Guan et al. 2002
H4 K8	Acetyl; ↑ in response to treatment with 5-HT in aplysia	
H4 K8	Acetyl; ↓ correlates with long-term depression	
H3 K14	Acetyl; ↑ in response to fear conditioning	Levenson et al. 2004
H4 K5/8/12/16	Acetyl; ↑ in response to latent inhibition training	
H3 K14	Acetyl; ↑ in response to fear conditioning + TSA	Vecsey et al. 2007
H4 K5/8/12/16	Acetyl; ↑ in response to fear conditioning + TSA	

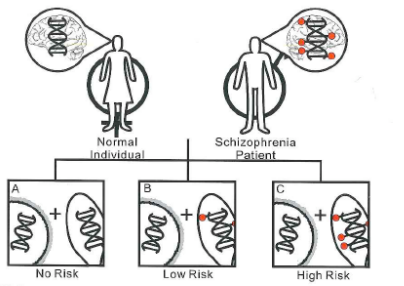
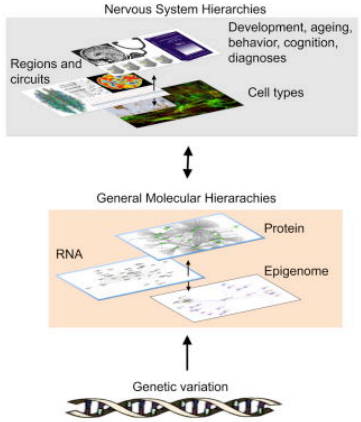


FIGURE 11.1 Partial meiotic epigenetic stability. In this hypothetical family, the father is affected with schizophrenia and has an epimutation on the gene predisposing to schizophrenia. (*A*) The epimutation is completely erased in the father's germline and the offspring has no disease. (*B*) There is partial erasure of epimutation, which results in a higher risk of developing schizophrenia. (*C*) The epimutation is meiotically stable; in which case, the offspring has a high chance of developing schizophrenia.

Systems biology and gene networks in neurodevelopmental and neurodegenerative disorders.
Parikshak NN, Gandal MJ, Geschwind DH.
Nat Rev Genet. 2015 Aug;16(8):441-58.

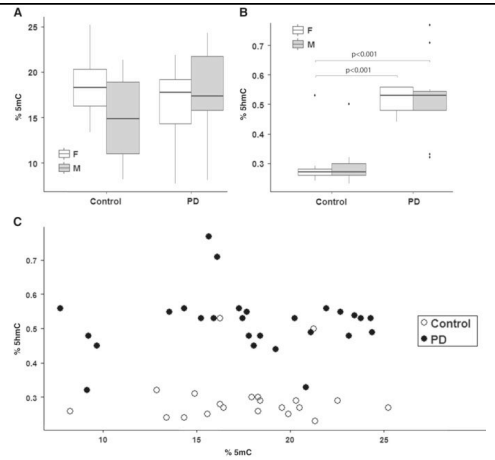


Elevated 5hmC levels characterize DNA of the cerebellum in Parkinson's disease

Reinhard Stöger[1], Paula J. Scaife[2], Freya Shephard[2] and Lisa Chakrabarti[2]

5-methylcytosine and the oxidation product 5-hydroxymethylcytosine are two prominent epigenetic variants of the cytosine base in nuclear DNA of mammalian brains. We measured levels of 5-methylcytosine and 5-hydroxymethylcytosine by enzyme-linked immunosorbent assay in DNA from post-mortem cerebella of individuals with Parkinson's disease and age-matched controls. 5-methylcytosine levels showed no significant differences between Parkinson's disease and control DNA sample sets. In contrast, median 5-hydroxymethylcytosine levels were almost twice as high ($p < 0.001$) in both male and female Parkinson's disease individuals compared with controls. The distinct epigenetic profile identified in cerebellar DNA of Parkinson's disease patients raises the question whether elevated 5-hydroxymethylcytosine levels are a driver or a consequence of Parkinson's disease.

npj Parkinsons Disease (2017)3:6; doi:10.1038/s41531-017-0007-3



Quantitation of 5mC and 5hmC in DNA of the cerebellum from age-matched control (n = 27; 11 females, 16 males) and PD individuals (n = 36; 12 females (F), 24 males (M)). Data are presented as box-and-whisker plots and indicate the full range, interquartile range, and median value in each group. Error bars represent SEM (a, b). The scatter plot depicts 5hmC and 5mC levels for individuals of the control and PD group (c)

Epigenetics and Disease (Metabolic Syndrome and Complex Disease)

Epigenetics in adipose tissue, obesity, weight loss, and diabetes.
Adv Nutr. 2014 Jan 1;5(1):71-81.
Martínez JA, et al.

TABLE 1 Examples of nutritional factors having beneficial metabolic effects that are regulated by epigenetic mechanisms[1]

Nutritional factor	Metabolic disorder	Epigenetic mechanisms	Reference
Methyl donors			
Betaine	Insulin resistance, liver steatosis	Histone and DNA methylation	(13)
Choline	Liver steatosis	Histone and DNA methylation	(14)
Folate	Insulin resistance, adiposity	DNA methylation	(15)
Methionine	Insulin resistance, obesity	Histone and DNA methylation	(15)
Vitamin B-12	Insulin resistance, obesity	DNA methylation	(15)
Phytochemicals			
Curcumin	Inflammation, obesity	Histone acetylation, DNA methylation, and microRNA	(16)
Epigallocatechin 3-gallate	Obesity, insulin resistance, liver steatosis	Histone acetylation and DNA methylation	(17)
Genistein	Obesity	Histone acetylation and DNA methylation	(18)
Resveratrol	Obesity, liver steatosis	Histone acetylation	(19)
Sulforaphane	Adipocyte differentiation	Histone acetylation	(20)
Fatty acids			
Butyrate and other SCFAs	Insulin resistance, inflammation	Histone acetylation and propionylation	(21)

[1] Based on (12).

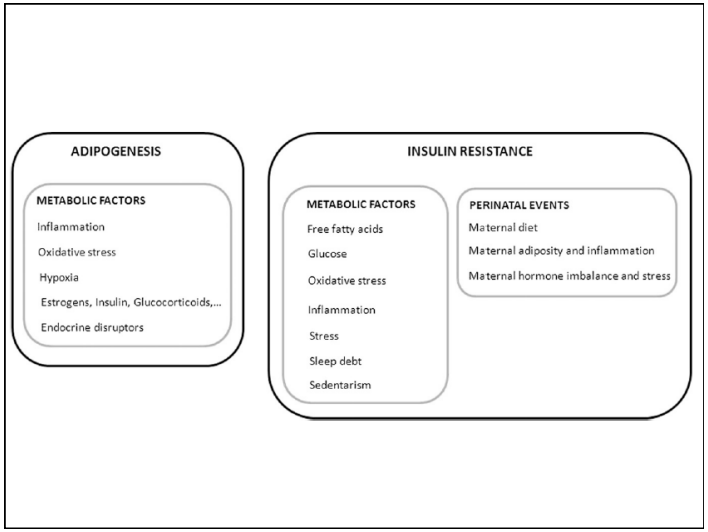


TABLE 2 Examples of metabolic processes related to obesity and type 2 diabetes that are regulated by genes whose expression is controlled by epigenetic mechanisms

Metabolic process	Gene symbol	Common gene name	Epigenetic mechanism	Reference
Adipogenesis	*CEBPA*	CCAAT/enhancer binding protein (C/EBP) α	Histone acetylation and methylation	(24)
	PPARA	Peroxisome proliferator-activated receptor α	DNA methylation	(25)
Appetite regulation	*LEP*	Leptin	DNA methylation	(26)
	MC4R	Melanocortin 4 receptor	DNA methylation	(27)
	NPY	Neuropeptide Y	DNA methylation	(28)
	POMC	Proopiomelanocortin	DNA methylation and histone acetylation and methylation	(28)
Body weight homeostasis	*FTO*	Fat mass and obesity associated	DNA methylation	(29)
Glucose homeostasis	*ADIPOQ*	Adiponectin	DNA methylation and histone acetylation	(30)
	GLUT4	Insulin-responsive glucose transporter 4	DNA methylation and histone acetylation	(31)
	INS	Insulin	DNA methylation and histone acetylation	(32)
Hypoxia	*HIF1A*	Hypoxia inducible factor 1	DNA methylation and histone acetylation and methylation	(33)
Inflammation	*IFNG*	Interferon γ	DNA methylation	(34)
	TNF	Tumor necrosis factor α	DNA methylation	(35)
Lipid storage	*FASN*	Fatty acid synthase	DNA methylation	(36)
Stress	*NR3C1*	Glucocorticoid receptor	Histone acetylation	(37)
Thermogenesis	*UCP1*	Uncoupling protein 1	DNA methylation	(38)

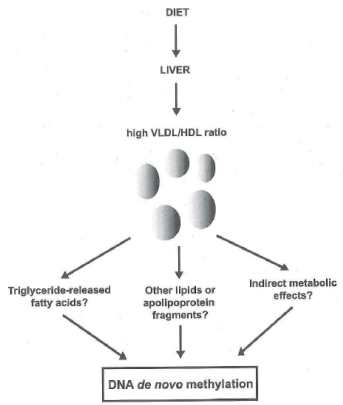


FIGURE 12.3 Possible alternative or concomitant mechanisms mediating aberrant DNA methylation by triglyceride-rich VLDL particles.

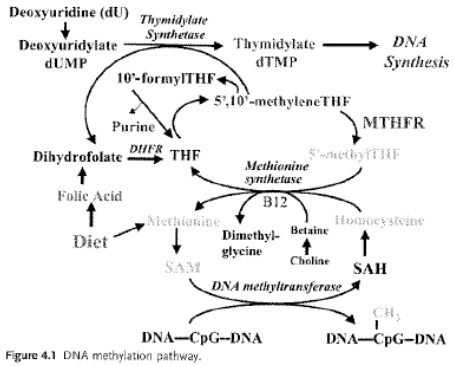
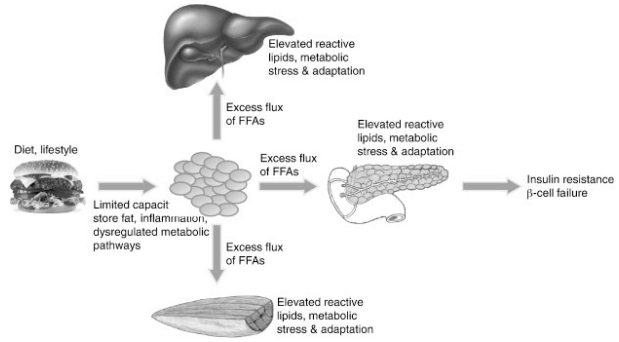


Figure 4.1 DNA methylation pathway.

Nutrition, epigenetics, and developmental plasticity: implications for understanding human disease.

Burdge GC, Lillycrop KA.

Annu Rev Nutr. 2010 Aug 21;30:315-39.



Schematic illustration of the integrative framework of lipotoxicity in the context of metabolic syndrome. An important component of this framework is "adipose tissue expandability" [62], which affects the adipose tissue metabolism and flux of free fatty acids (FFAs) from the adipose tissue to peripheral tissues. When the capacity of adipose tissue is reached, the excess FFAs as precursors of reactive lipids such as ceramides may in excess amounts interfere with the tissue-specific metabolic and signaling networks.

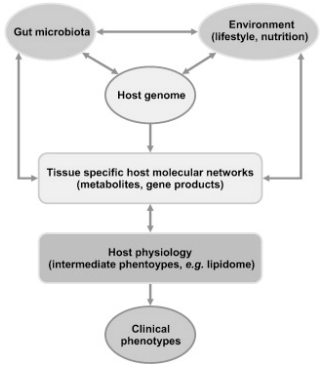


Fig. 2. Hierarchy of dependencies of clinical phenotypes on genetic and environmental factors.

Table 1 Top-ranked molecular disease pathways related to the metabolic syndrome, determined by gene enrichment analysis using the ToppFun application

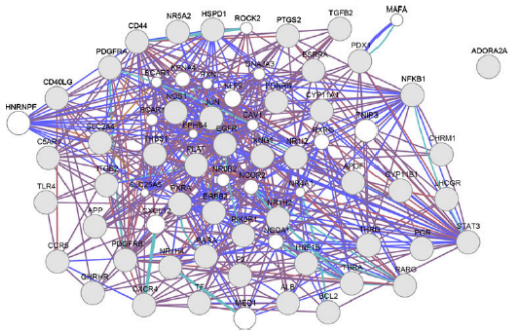
Pathway	PATHWAY ID	Source	*P*-value	Terms in query[a]	Terms in genome[b]
Statin pathway	Statin_pathway_pharmgkb	MSigDB	5.14e-16	16	18
PPAR signaling pathway	hsa03320	KEGG pathway	5.56e-12	25	70
Lipoprotein metabolic	pw:0000482	Pathway ontology	6.62e-12	12	13
Nuclear receptors in lipid metabolism and toxicity	H_nuclearrspathway	CGAP BioCarta	1.91e-10	17	34
Adipocytokine signaling pathway	hsa04920	KEGG pathway	1.85e-09	22	67
Neuroactive ligand-receptor interaction	hsa04080	KEGG pathway	3.72e-08	42	256
Altered lipoprotein metabolism	pw:0000484	Pathway ontology	1.42e-06	7	7
GPCRDB class a rhodopsin-like	gpcrdb_class_a_rhodopsin_like	MSigDB	1.88e-06	32	183
Reverse cholesterol transport	pw:0000498	Pathway ontology	3.03e-06	8	10
ACE inhibitor pathway	ace_inhibitor_pathway_pharmgkb	MSigDB	1.08e-05	7	8
Visceral fat deposits and the metabolic syndrome	h_vobesitypathway	CGAP BioCarta	1.08e-05	7	8
Obesity pathway	vobesitypathway	MSigDB	1.08e-05	7	8
γ-Hexachlorocyclohexane degradation	map00361	GenMAPP	1.21e-05	12	29
Tryptophan metabolism	tryptophan_metabolism	MSigDB	2.16e-05	16	56
Leptin system	pw:0000363	Pathway ontology	3.03e-05	8	12

ACE—angiotensin-converting enzyme; CGAP—Cancer Genome Anatomy Project; GenMAPP—Gene Map Annotator and Pathway Profiler; GPCRDB—G protein-coupled receptor database; KEGG—Kyoto Encyclopedia of Genes and Genomes; MSigDB—Molecular Signatures Database; pharmgkb—The Pharmacogenomics Knowledge Base; PPAR—peroxisome proliferator-activated receptors

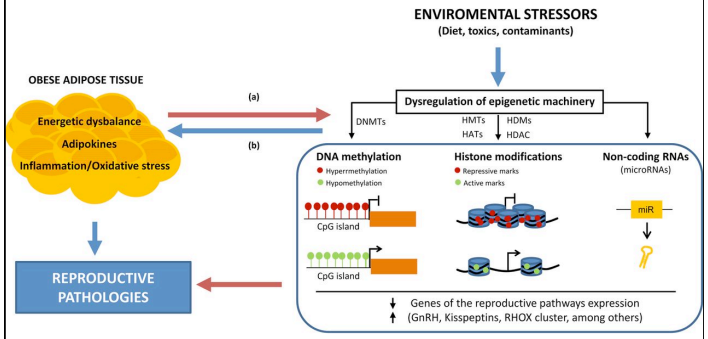
[a] The number of genes in the training sets belonging to that pathway

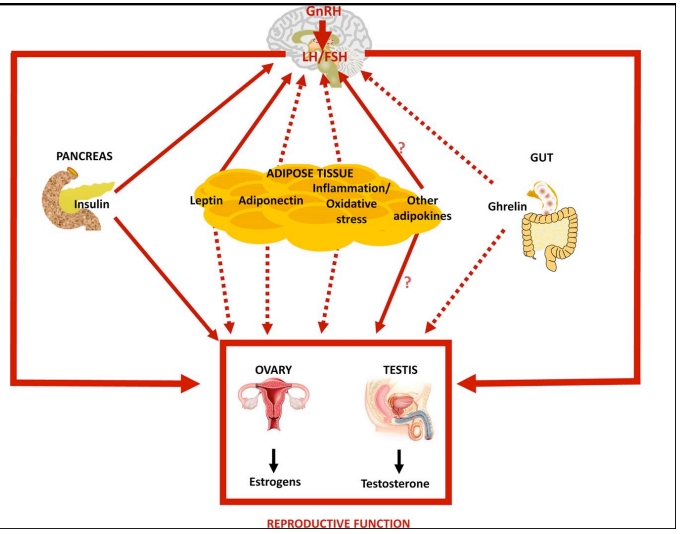
[b] Similar genes according to the Toppfun application

Fig. 1 Graph obtained from the Genemania application (genemania.org [37]) for 48 of the 50 genes (*gray circles*) described in Table 2 (*RXRB* and *LRP1* were not recognized by the software) and 20 predicted related genes taken as an example for the sake of simplicity (*white circles*); the size of these circles corresponds to their probability of belonging to the networks assigned by the program. Of these genes, 49.74% of their products are predicted to have physical interactions (*blue lines*), 34.73% are co-expressed (*purple lines*), 12.13% are co-localized (*bordeaux lines*), 2.33% belong to the same pathways (*teal lines*) and 1.07% are predicted to have direct interactions by small-scale datasets (*tan lines*)



Obesity and the reproductive system disorders: epigenetics as a potential bridge.
Hum Reprod Update. 2015 Mar;21(2):249-261.
Crujeiras AB, Casanueva FF.





Early nutrition, epigenetics, and cardiovascular disease.
Loche E, Ozanne SE.
Curr Opin Lipidol. 2016 Oct;27(5):449-58.

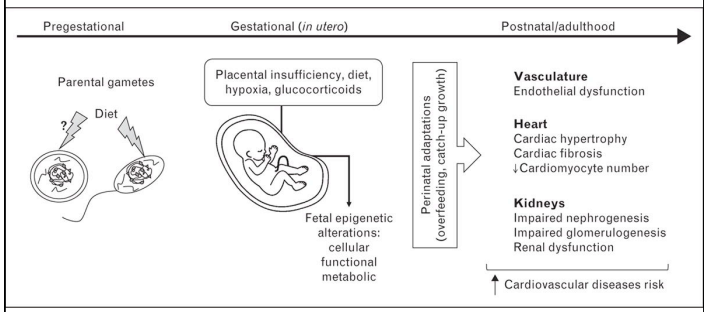


Table 1. Experimental evidence of the effects of maternal under and overnutrition on the offspring cardiovascular system

Maternal diet	Species	Timing of exposure	Cardiovascular outcome	Sex studied	Reference
High fat	Mouse	*In utero* and lactation	Hypertension	M and F	[44]
	Mouse	*In utero* and lactation	Hyperglycemia, insulin resistance, obesity, and hypertension	F	[45]
	Rat	*In utero* and lactation	Increased lipid peroxidation and evidence of mitochondrial dysfunction	Not available	[46]
	Rat	*In utero* and lactation	Vascular dysfunction	Not available	[47]
	Rat	*In utero* and lactation	High SBP and DBP, abnormal vascular function, reduced endothelium-dependent relaxation	M and F	[48–52]
	Rat	*In utero*	Cardiac vulnerability to ischemic injury in adult male offspring	M and F	[53▪]
	Rat	*In utero* and lactation	Increased blood pressure, insulin resistance, dyslipidemia, obesity, and mesenteric artery endothelial dysfunction in adult offspring	M and F	[54]
	Sheep	*In utero*	Fibrosis and collagen deposition	M and F	[55]
	Sheep	*In utero*	Impaired cardiac insulin signaling and impaired left ventricular-developed pressure in response to high workload stress.	M and F	[56]
	Sheep	*In utero*	Myofibril hypertrophy and fascicular disarray	M and F	[57]
	Japanese macaque	*In utero* and lactation	Vascular dysfunction manifested as depressed endothelium-dependent vasodilatation and thickened intima wall	not available	[58]
High fat/high sugar (obesogenic)	Mouse	*In utero* and lactation	Hypertension, cardiac hypertrophy, and cardiac dysfunction *ex vivo*	M	[59▪,60–62]
Caloric restriction	Mouse	*In utero* and lactation	Increase in SBP, perivascular fibrosis of the coronary artery, cardiomegaly, and cardiomyocyte hypertrophy	M	[63,64]
	Rat	*In utero* and lactation	Endothelial dysfunction	M	[65]
	Rat	*In utero*	Elevated blood pressure	M and F	[66]
	Rat	*In utero* and lactation	Persistent hypertension and endothelial dysfunction across F1–F3 offspring	M	[67]
	Rat	*In utero*	Reduced heart weight and cardiomyocytes number at birth	F	[68]
	Rat	*In utero*	Pathological cardiac remodeling, diastolic dysfunction, altered Ca^{2+} handling properties in isolated cardiomyocytes	M and F	[69▪,70]
	Rat	*In utero*	Hypertension and reduced number of glomeruli	M	[71]
	Sheep	Gestation and/or lactation	Hypertension and impaired glomerulogenesis	M	[72]
	Sheep	*In utero*	Left and right ventricular cardiac hypertrophy (fetus and adult offspring)	F	[73,74]
Low protein	Mouse	*In utero* and lactation	Elevated offspring SBP	M and F	[75]
	Mouse	*In utero* and lactation	Cardiac hypertrophy	M	[76]
	Mouse	*In utero* and lactation	Hypertension and vascular dysfunction	M	[77]
	Rat	*In utero* and lactation	Reduced cardiac β-adrenergic responsiveness	M	[78]
	Rat	*In utero* and lactation	Increase in the cardiovascular sympathetic tone	M	[79]
	Rat	*In utero*	Higher SBP at 4 weeks of age	M and F	[80]
	Rat	*In utero* and lactation	Increased oxidative stress	Not available	[81]
	Rat	*In utero*	Increased SBP, impaired recovery of left ventricular developed pressure after ischemia (Langendorff)	M and F	[82]
	Rat	*In utero*	Hypertension and renal dysfunction	M and F	[83]
	Goat	Late gestation	Reduced heart and body weight at birth	M	[84]
Low protein and postnatal catch-up growth	Rat	*In utero*	Cardiac DNA damage and oxidative stress	M	[24,25]

F, female; M, male.

Developmental Origins of Common Disease: Epigenetic Contributions to Obesity
Kappil M, Wright RO, Sanders AP.
Annu Rev Genomics Hum Genet. 2016 Aug 31;17:177-92.

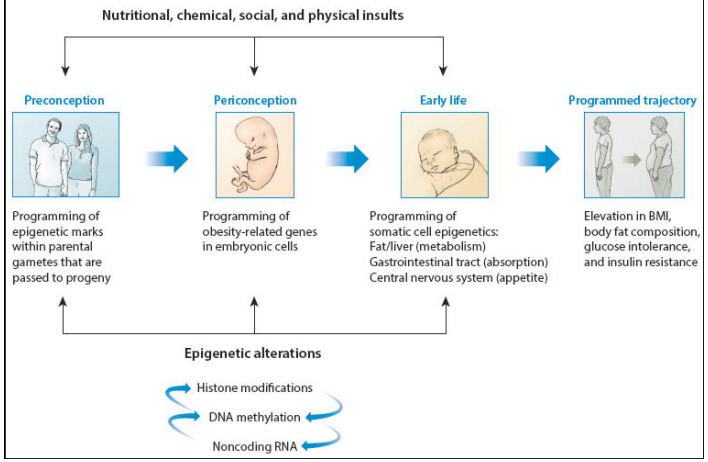


Table 1 Recent studies of epigenetic mechanisms linking intrauterine environment to later onset of obesity

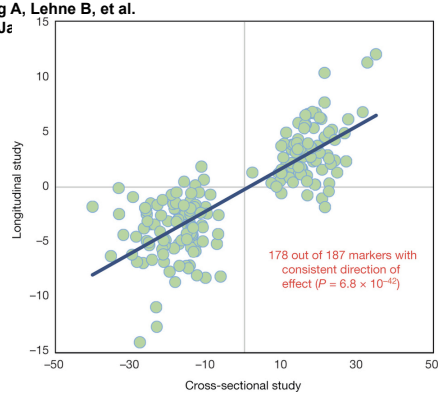
Intrauterine environmental agent	Epigenetic locus	Epigenetic assessment	Biospecimen	Outcome measure	Model	Reference
Parental obesity	*CCDC12, MCOLN3*	DNA methylation	Cord blood	Child BMI	Human	31
	RXRA, eNOS, SOD1	DNA methylation	Cord blood	Adiposity at age 9	Human	15
	Zfp423, Cebp-β, Pparγ	DNA methylation and expression	Fat	Body weight and fat mass	Rat	6
	Zfp423	DNA methylation, expression, and histone modifications	Fat	Adipogenic potential of fetal tissue	Mouse	43
	let-7g, miR-381, miR-376, *Tnfrsf4, Fit, Tnfa, Il6, Tlr4, Pparγ, Cebp-α*	miRNA and mRNA	Musde and mesenchymal stem cell line	Adipogenic potential of fetal tissue	Sheep	41
	Tlr1, Tlr2, Lat, Dnmt1, Dnmt3a/b	DNA methylation	Fat	F1–F2 body weight, adipocyte size, and metabolic dysfunction	Mouse	11
	Pgc-1α	DNA methylation and expression	Muscle	Fat mass	Mouse	21
	miR-503, miR-456b-5p, miR-542-3p, miR-652	miRNA	Sperm	Metabolic dysfunction (glucose intolerance and insulin sensitivity)	Mouse	26
	Hnrgr, Lx	DNA methylation	Liver and oocytes	F1–F2 body weight, WAT weight, and metabolic dysfunction	Mouse	40
Dietary supplement	Global methylation	DNA methylation	Fat	Body weight and fat mass	Mouse	19
IUGR	*Pgc-1α*	DNA methylation and expression	Muscle	Fat mass and metabolic dysfunction	Rat	44
	Igf2	DNA methylation and expression	Fat	Fat mass and metabolic dysfunction	Rat	8
	Igf1	DNA methylation	Liver	F1–F2 body weight, fat mass, and metabolic dysfunction	Rat	16
PAHs	*Pparγ, Cebp-β, Cox-2, Fas, Adipoq*	DNA methylation and expression	Fat	F1–F2 weight gain and fat mass	Mouse	42
BPA	*Igf2*	DNA methylation and expression	F2 embryos	F1–F2 weight gain, fat mass, and metabolic dysfunction	Mouse	35
DDT	*Tubb3, Carm1, Slc4a4*	DNA methylation	Sperm	F3 body weight and fat mass	Rat	34
Methoxychlor	37 DMRs	DNA methylation	Sperm	F3 obesity incidence	Rat	24
JP-8	33 DMRs	DNA methylation	Sperm	F3 body weight and fat mass	Rat	36

Abbreviations: BMI, body mass index; BPA, bisphenol A; DDT, dichlorodiphenyltrichloroethane; DMR, differentially methylated region; F1, F2, and F3, first, second, and third filial generation, respectively; IUGR, intrauterine growth restriction; miRNA, microRNA; PAH, polycyclic aromatic hydrocarbon; WAT, white adipose tissue.

Epigenome-wide association study of body mass index, and the adverse outcomes of adiposity

Wahl S, Drong A, Lehne B, et al.

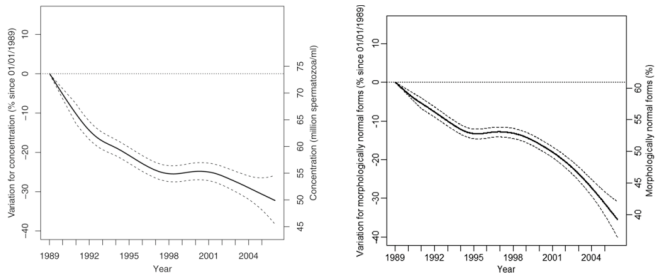
Nature. 2017 Ja



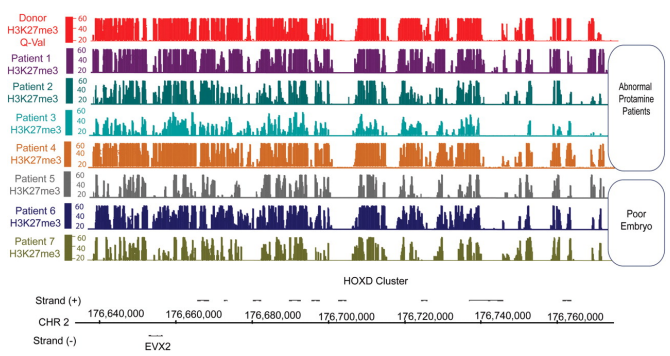
Relationship between DNA methylation in blood and BMI amongst 1,435 participants of the KORA S4/F4 population cohort. Cross-sectional results (x axis) are for the relationship between methylation in blood and BMI at each of the 187 sentinel CpG sites in the baseline samples; longitudinal results are for the relationship between change in methylation (in blood) and change in BMI after 7 year follow-up. Units for both axes are kg m−2 change in BMI per unit increase in methylation (scale 0–1, in which 1 represents 100% methylation).

Male Infertility

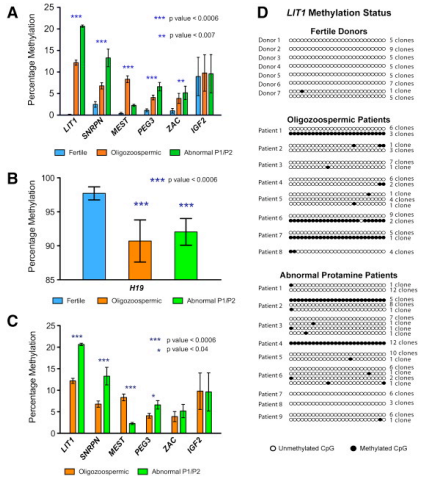
Rolland M, Le Moal J, Wagner V, Royère D, De Mouzon J. (2012) Decline in semen concentration and morphology in a sample of 26,609 men close to general population between 1989 and 2005 in France. Hum Reprod. 28(2):462-70.



Hammoud SS, et al. (2011) Genome-wide analysis identifies changes in histone retention and epigenetic modifications at developmental and imprinted gene loci in the sperm of infertile men. Hum Reprod. 26(9):2558-69.

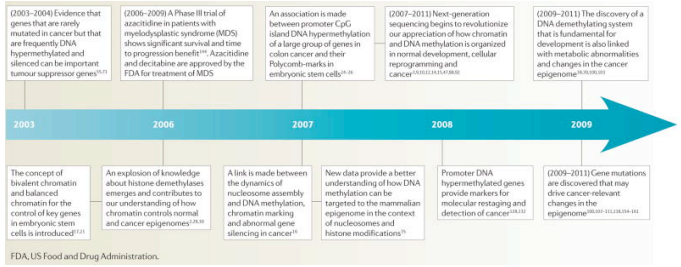


Hammoud SS, et al. (2010) Alterations in sperm DNA methylation patterns at imprinted loci in two classes of infertility. Fertil Steril. 94(5):1728-33.



Epigenetics and Disease
(Epigenetic Therapy Development)

Baylin SB, Jones PA. (2011) A decade of exploring the cancer epigenome - biological and translational implications. Nat Rev Cancer. 23;11(10):726-34.



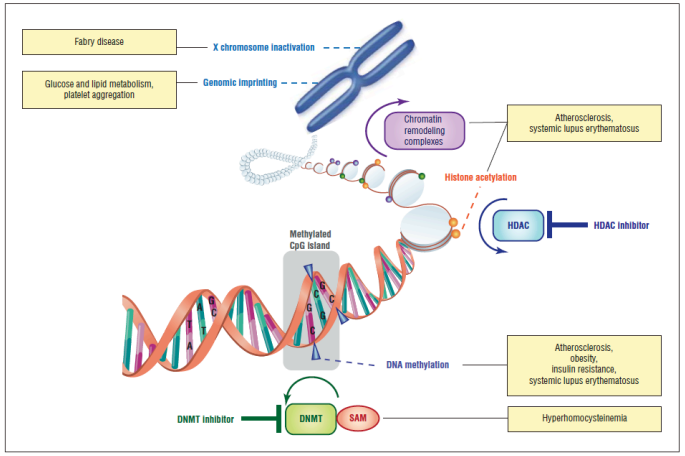
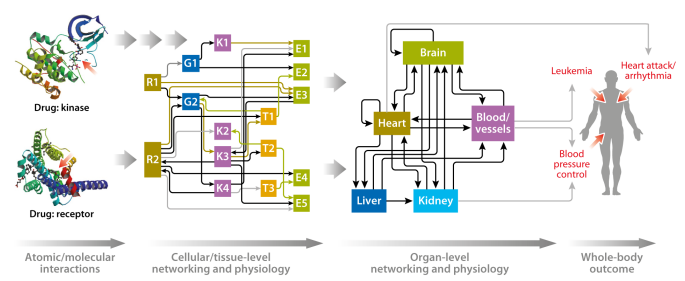


Table 1. Chromatin Modifications, Readers, and Their Function

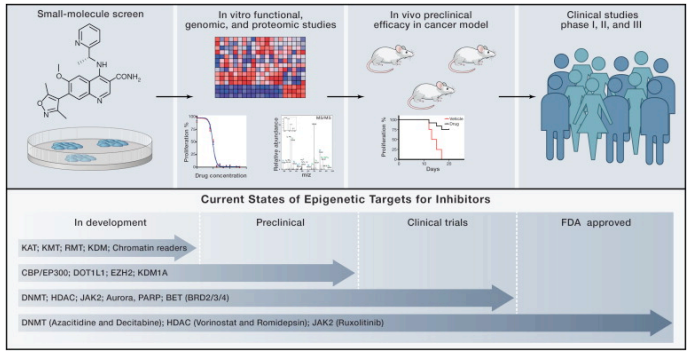
Chromatin Modification	Nomenclature	Chromatin-Reader Motif	Attributed Function
DNA Modifications			
5-methylcytosine	5mC	MBD domain	transcription
5-hydroxymethylcytosine	5hmC	unknown	transcription
5-formylcytosine	5fC	unknown	unknown
5-carboxylcytosine	5caC	unknown	unknown
Histone Modifications			
Acetylation	K-ac	BromodomainTandem, PHD fingers	transcription, repair, replication, and condensation
Methylation (lysine)	K-me1, K-me2, K-me3	Chromodomain, Tudor domain, MBT domain, PWWP domain, PHD fingers, WD40/β propeller	transcription and repair
Methylation (arginine)	R-me1, R-me2s, R-me2a	Tudor domain	transcription
Phosphorylation (serine and threonine)	S-ph, T-ph	14-3-3, BRCT	transcription, repair, and condensation
Phosphorylation (tyrosine)	Y-ph	SH2[a]	transcription and repair
Ubiquitylation	K-ub	UIM, IUIM	transcription and repair
Sumoylation	K-su	SIM[a]	transcription and repair
ADP ribosylation	E-ar	Macro domain, PBZ domain	transcription and repair
Deimination	R→Cit	unknown	transcription and decondensation
Proline isomerisation	P-cis⇔P-trans	unknown	transcription
Crotonylation	K-cr	unknown	transcription
Propionylation	K-pr	unknown	unknown
Butyrylation	K-bu	unknown	unknown
Formylation	K-fo	unknown	unknown
Hyroxylation	Y-oh	unknown	unknown
O-GlcNAcylation (serine and threonine)	S-GlcNAc; T-GlcNAc	unknown	transcription

Modifications: me1, monomethylation; me2, dimethylation; me3, trimethylation; me2s, symmetrical dimethylation; me2a, asymmetrical dimethylation; and Cit, citrulline. Reader domains: MBD, methyl-CpG-binding domain; PHD, plant homeodomain; MBT, malignant brain tumor domain; PWWP, proline-tryptophan-tryptophan-proline domain; BRCT, BRCA1 C terminus domain; UIM, ubiquitin interaction motif; IUIM, inverted ubiquitin interaction motif; SIM, sumo interaction motif; and PBZ, poly ADP-ribose binding zinc finger.

[a]These are established binding modules for the posttranslational modification; however, binding to modified histones has not been firmly established.



Zhao S, Iyengar R. 2012.
Annu. Rev. Pharmacol. Toxicol. 52:505–21

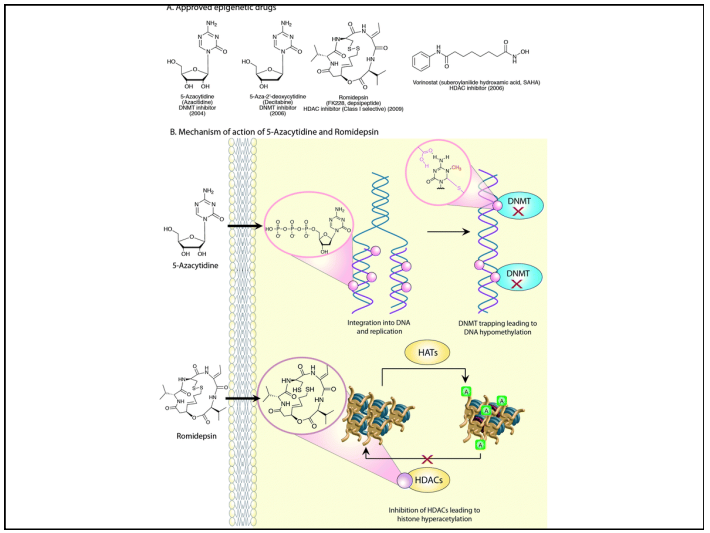


Epigenetic Inhibitors as Cancer TherapiesThis schematic depicts the process for epigenetic drug development and the current status of various epigenetic therapies. Candidate small molecules are first tested in vitro in malignant cell lines for specificity and phenotypic response. These may, in the first instance, assess the inhibition of proliferation, induction of apoptosis, or cell-cycle arrest. These phenotypic assays are often coupled to genomic and proteomic methods to identify potential molecular mechanisms for the observed response. Inhibitors that demonstrate potential in vitro are then tested in vivo in animal models of cancer to ascertain whether they may provide therapeutic benefit in terms of survival. Animal studies also provide valuable information regarding the toxicity and pharmacokinetic properties of the drug. Based on these preclinical studies, candidate molecules may be taken forward into the clinical setting. When new drugs prove beneficial in well-conducted clinical trials, they are approved for routine clinical use by regulatory authorities such as the FDA. KAT, histone lysine acetyltransferase; KMT, histone lysine methyltransferase; RMT, histone arginine methyltransferase; and PARP, poly ADP ribose polymerase.

Table 2. Contemporary Epigenetic Therapeutic Approaches for Stroke

Epigenetic Mechanisms of Action	Agents	Relevance to Stroke
Inhibition of DNMT enzyme activity	5-Azacytidine	Treatment with an inhibitor of DNA methylation reduces the extent of ischemic injury following MCAO
	5-Aza-2-deoxycytidine (or decitabine), zebularine, and MG98	Mice with reduced levels of DNMT1 exhibit significantly smaller infarcts following MCAO, compared with control animals
Inhibition of HDAC enzyme activity	Trichostatin A	Neuroprotective mechanisms affected by HDAC inhibition include the critical cellular processes that control growth and viability and stress responses
	Suberoylanilide hydroxamic acid, sodium butyrate, sodium 4-phenylbutyrate, valproic acid, and curcumin	Paradigm for the restoration of impaired neural network connections and the recovery of lost neurological functions, including learning and memory

Abbreviations: DNMT, DNA methyltransferase; HDAC, histone deacetylase; MCAO, middle cerebral artery occlusion.



Histone deacetylase inhibitors for cancer therapy.

Kim TY, Bang YJ, Robertson KD.

Epigenetics. 2006 Jan-Mar;1(1):14-23.

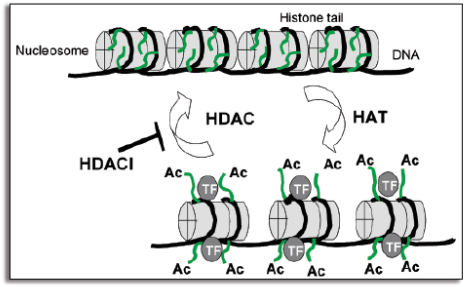
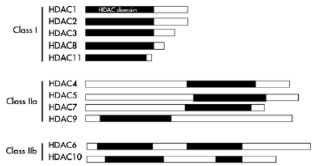


Figure 1. Regulation of chromatin condensation and gene transcription by histone acetylation and deacetylation. The N terminal tails of the core histones contain positively charged lysine residues. With histone acetyltransferase activity, or inhibition of HDAC activity by HDACIs, nucleosomal histones become hyperacetylated (Ac) and the DNA that is tightly wrapped around them becomes more accessible to transcription factors (TF).

Table 1 **Classification of mammalian histone deacetylases (HDACs)**

	Class I	Class IIa	Class IIb	Class III
Yeast HDAC	RPD3	HDA1	HDA1	SIR2
Human HDAC	HDAC1–3, 8, 11	HDAC4, 5, 7, 9	HDAC6, 10	SIRT1–7
Distribution	Ubiquitous	Brain, heart, SM*	Testis, liver, kidney	Unknown
Localization	Nuclear	Nuclear/cytoplasmic	Mostly cytoplasmic	Nuclear
Target substrates	Histones, p53, NF-κB	Histones	Histones, Tubulin HSP	Histones, Tubulin p53, TAF
Protein complexes	NuRD, SIN3			
Co-repressor complexes	N-CoR, SMRT	N-CoR, SMRT		
Interacting proteins	RB, p53, MyoD, NF-κB, SP11, BRCA1, DNMT1, DNMT3A-B, MBD2-3, MECP2, ATM	MEF2 MEF2	Tubulin, HSP Tubulin, HSP	p53 p53
Co-factor	Zn	Zn	Zn	NAD+
Inhibitor sensitivity	S**	S	S	NT***

*, smooth muscle; **, sensitive; ***, not tested; HD domain, histone deacetylase catalytic domain.



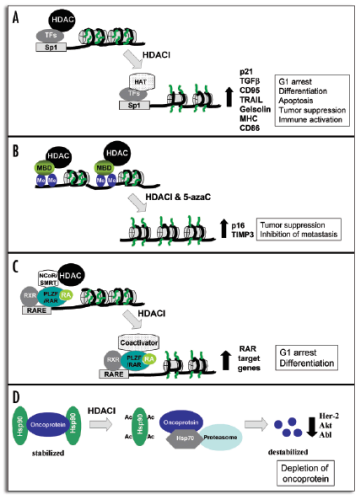


Table 2 **Overview of histone deacetylase inhibitors and their properties**

Structural class	Drugs	Concentration	HDAC Inhibition Isotope	Reversibility	Clincial trials
Short-chain fatty acids	Sodium butyrate	μM	I, IIa	R	I/II
	Valproic acid	mM	I, IIa	R	I/II
Epoxides	Depudecin	mM		IR	
	Trapoxin	μM	I, IIa	IR	
Cyclic tetrapeptides	Apicidin	nM		R	
	Depsipeptide	nM	I (HDAC1, 2)	R	I/II
Hydroxamic acids	TSA	nM	I, IIa, IIb	R	
	SAHA	nM	I, IIa, IIb	R	I/II
	Oxamflatin	μM		R	
	Scriptaid	μM		R	
	Pyroxamide	μM		R	II
	LAQ824	nM	I, IIa, IIb	R	I
	LBH589	nM		R	I
	PXD101	μM	I, IIa, IIb	R	I
Benzamides	MS-275	μM	I (HDAC1, 3)	R	I/II
	CI-994	*		R	I
HybridsCHAP	nM	I, IIa (HDAC1, 4)	R		
	SK-7068	nM	I (HDAC1, 2)	R	

*indirect effect; R:reversible; IR:irreversible

Table 3 **Tumor-associated proteins whose expression is altered by HDACI treatment**

Upregulation of gene expression

Cell cycle inhibitory gene	p21, p16, p27
Tumor suppression gene	p53, VHL, p107, gelsolin, IGFBP-3
Differentiation game	RARα, TGFβ1
Apoptotic gene	CD95, CD95l, TRAIL, DR4, DR5, Bak, Bax, Bim
Immune Activation	MHC-I, MHC-II, CD86

Downregulation of gene expression

Cell cycle gene	cyclin D1, cyclin A, TS
Antiapoptotic gene	bcl2, bcl-XL
Angiogenic factor	HIF1α, VEGF, IL2, IL10

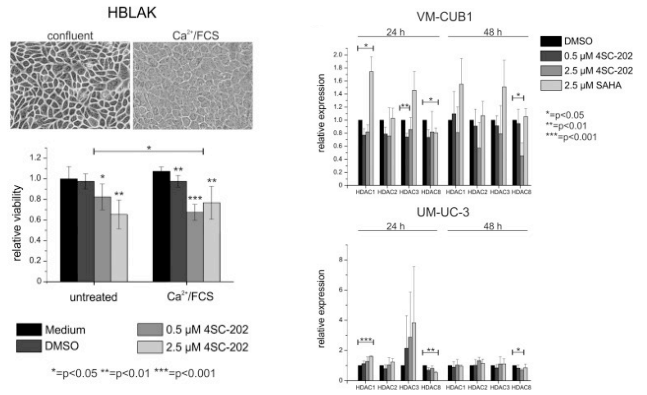
Downregulation of protein expression

EGFR	Flt-3
ErbB2	Akt
Abl	Raf-1

Evaluation of the Therapeutic Potential of the Novel Isotype Specific HDAC Inhibitor 4SC-202 in Urothelial Carcinoma Cell Lines.

Pinkerneil M, Hoffmann MJ, Kohlhof H, Schulz WA, Niegisch G.

Target Oncol. 2016 Dec;11(6):783-798.



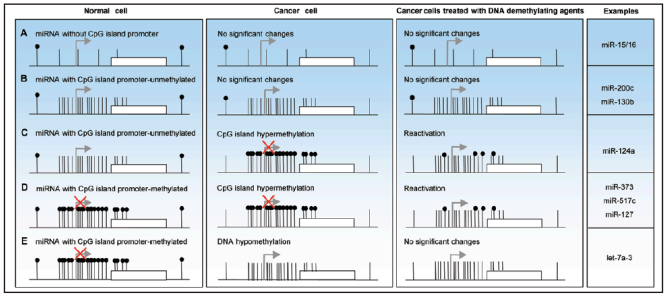
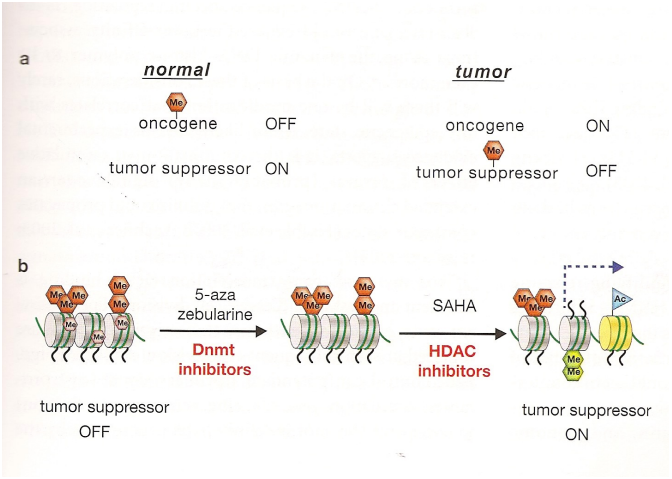
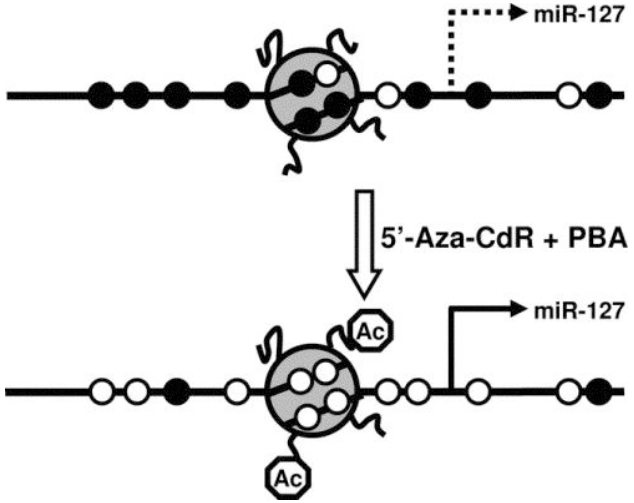
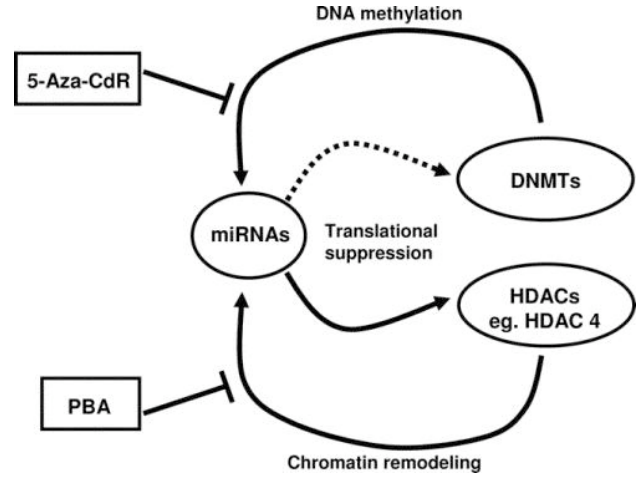


Figure 2. Repertory of different DNA methylation patterns of miRNAs in normal and caner cells. (A) miRNAs that are not located in a CpG island might not be greatly influenced by DNA methylation changes. (B) miRNAs located in a CpG island that is not methylated either in normal or in cancer cells. (C) Cancer-specific methylation. Tumor suppressor miRNAs, like miR-124a, are unmethylated in normal cells but may undergo methylation in cancer cells. These miRNAs are susceptible to demethylation by DNA demethylating agents, leading to miRNA expression and downregulation of oncogenic targets. (D) Tissue-specific methylation unchanged in cancer cells. The CpG island of the miRNA is methylated in both normal and tumoral cells, such as miR-127. As with cancer-specific methylated miRNAs, these miRNAs can be reactivated by epigenetic drugs. (E) Tissue-specific methylation lost in cancer cells that might be related to oncogenic activity.







Modes of action of the DNA methyltransferase inhibitors azacytidine and decitabine.

Stresemann C, Lyko F.

Int J Cancer. 2008 Jul 1;123(1):8-13.

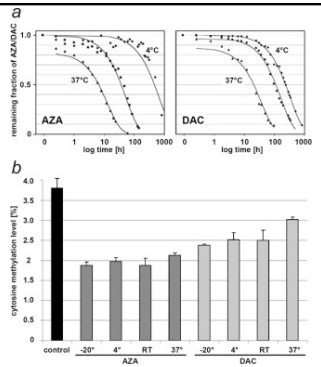


Figure 1. Chemical stability of neutral azacytidine and decitabine solutions. (a) Temperature-dependent decomposition of azacytidine (AZA) and decitabine (DAC). Compounds were dissolved in neutral 0.9% NaCl solutions, stored at 4, 20 and 37°C, respectively, and snap-fozen in liquid nitrogen at the time points indicated. Samples were then diluted to 0.45 mg/mL and mixed with adenine as an internal standard (400 μM final concentration). Analyses were performed on a Beckman Coulter capillary electrophoresis system (MDQ Molecular Characterization System) with UV detection at 254 nm. Separation occurred in an untreated fused-silica column of 60 cm (effective length 50 cm) in a 10 mM phosphate buffer system, pH 7.0, with 150 mM SDS. Analyses were performed at 25 kV and a capillary temperature of 25°C. (b) Pharmacological potency of stored azacytidine and decitabine solutions in inhibiting DNA methylation. Genomic cytosine methylation levels were analyzed by capillary electrophoresis.20 Drug solutions were dissolved in neutral aqueous buffer and stored under the conditions indicated for 24 hr. HCT116 cells were treated with 2.0 μM azacytidine (AZA) or 0.5 μM decitabine (DAC). A significant reduction in pharmacological potency could only be observed after storage of decitabine at 37°C.

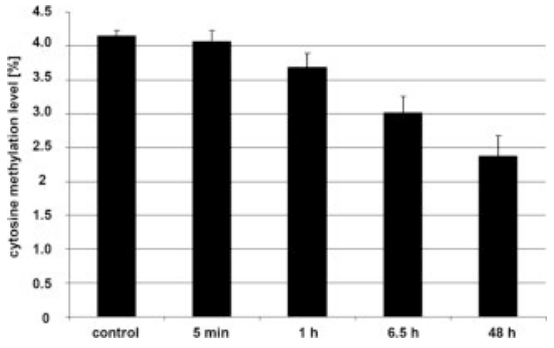


Figure 2. Azacytidine-induced DNA demethylation requires extended drug exposure. Global methylation analysis was performed by capillary electrophoresis,20 after treatment of HCT116 cells with 2 μM azacytidine. Cells were incubated in drug-containing medium for the time indicated. The medium was then exchanged for drug-free medium and cells were grown for a total of 48 hr.

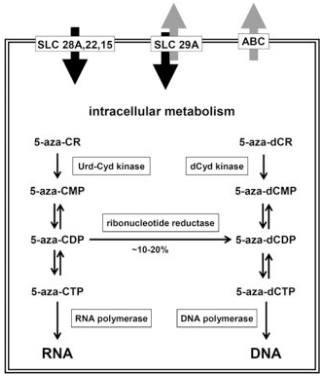


Figure 3. Membrane transport and intracellular metabolism of azanucleosides. Four candidate transporter protein families (black and gray arrows) are believed to mediate the transport of nucleosides and nucleoside metabolites across the cell membrane (double line). After cellular uptake, azacytidine (5-aza-CR) and decitabine (5-aza-dCR) are modified by different metabolic pathways. It is assumed that 80–90% of azacytidine is incorporated into RNA, because ribonucleotide reductase limits the conversion of 5-aza-ribonucleotides to 5-aza-deoxyribonucleotides.

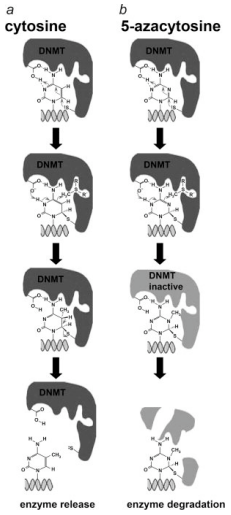
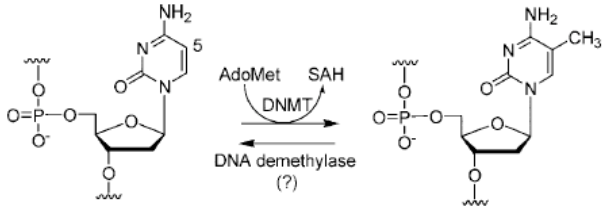


Figure 4. Trapping mechanism of azacytosine. (a) A nucleophilic attack of the protein-thiol group (from a catalytic cysteine residue of the DNA methyltransferase enzyme, DNMT) at the C6 position of cytosine drives the subsequent transfer of the methyl group from the methyl donor S-adenosyl-L-methionine. The transfer proceeds through a covalent complex at position C6 between the DNA and the DNMT protein. The complex is resolved through a β-elimination reaction resulting in the release of the active DNA methyltransferase enzyme. (b) Mechanism-based inhibition of DNMTs by azacytosine-containing DNA. The covalent complex at C6 cannot be resolved through β-elimination, because of the presence of a nitrogen atom at position 5. Covalently trapped DNMTs are degraded, resulting in the depletion of cellular DNMTs.

Chemical regulation of epigenetic modifications: opportunities for new cancer therapy.

Zheng YG, Wu J, Chen Z, Goodman M.

Med Res Rev. 2008 Sep;28(5):645-87.



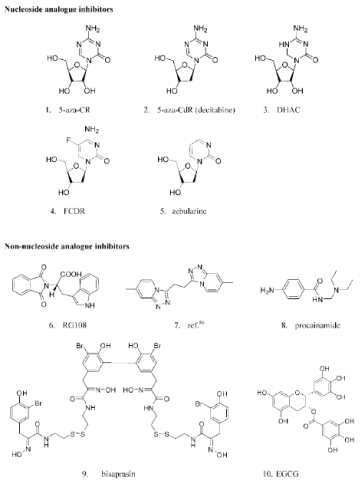
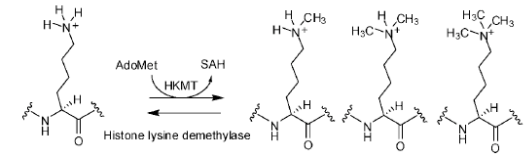


Figure 1. Structures of DNMT inhibitors.[84]



Scheme 2. Histone lysine methylation and demethylation. The methylation is typically catalyzed by a HKMT enzyme containing a SET domain. The inverse demethylation is catalyzed by different demethylases such as LSD1 and JmjC proteins.

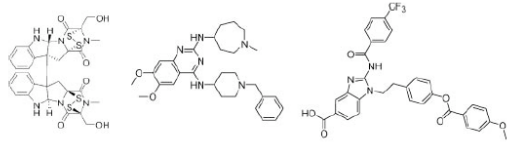
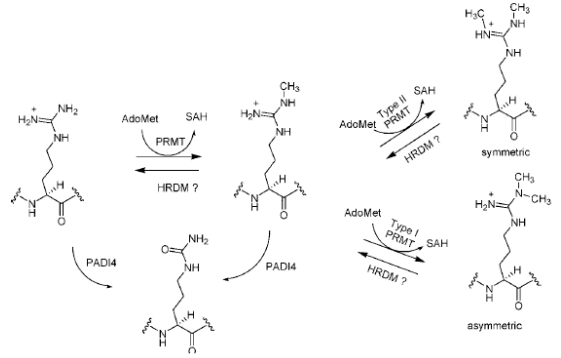
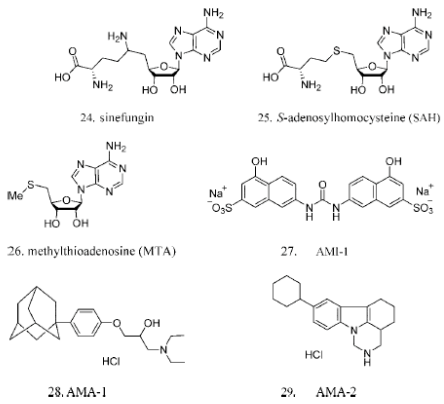


Figure 2. Structures of histone lysine methylation inhibitors.

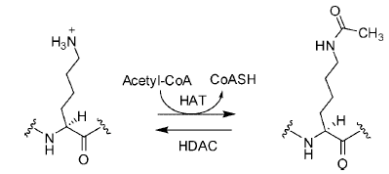


Scheme 3. Histone arginine methylation. The methylation reaction is catalyzed by a PRMT enzyme. No histone arginine demethylases (HRDMs) have been reported. The arginine and monomethylarginine can be deiminated by PADI4 to a citrulline residue.



24. sinefungin
25. *S*-adenosylhomocysteine (SAH)
26. methylthioadenosine (MTA)
27. AMI-1
28. AMA-1
29. AMA-2

Figure 4. Structures of PRMT modulators.



Scheme 4. Histone acetylation and deacetylation. The acetylation reaction is catalyzed by a HAT enzyme. The inverse deacetylation is catalyzed by HDAC.

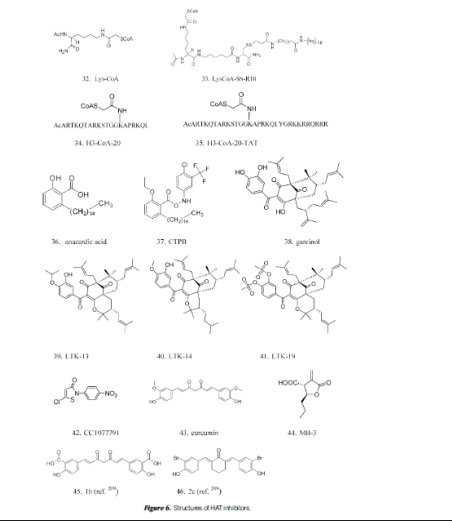
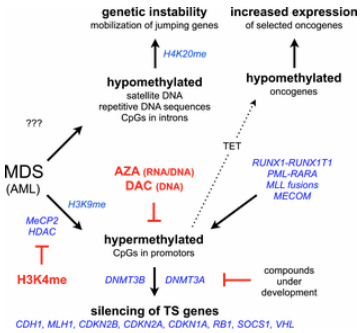


Figure 6. Structures of HAT inhibitors.

Scholz B, Marschalek R. (2012) Epigenetics and blood disorders. Br J Haematol.158(3):307-22.



Rationale for using AZA, DAC in MDS patients. Myelodysplastic syndrome (MDS) and presumably also acute myeloid leukaemia (AML) patients exhibit aberrant DNA methylation patterns. These were, e.g., hypermethylated CpG island located in promoter regions of transcribed genes that cause a shut-down of gene transcription, or hypomethylated repetitive DNA elements or satellite DNA which confer genetic instability. Treatment with 2-azacytidine (AZA) or decitabine (DAC) helps to remove aberrant DNA methylation patterns on promoter regions of distinct genes, mostly tumour suppressor genes (TS). Of interest, many known gene fusions associated with AML also produce wrong DNA methylation patterns. CpG: CG dinucleotides of CpG islands; MeCP2: methyl-CpG binding protein 2.

Epigenetic targets for novel therapies of lung diseases.
Pharmacol Ther. 2015 Mar;147C:91-110.
Comer BS, et al.

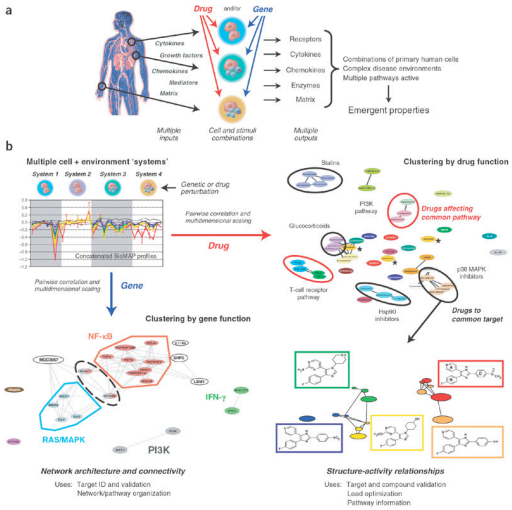
Table 1
Epigenetic modifiers of lung disease in preclinical studies.

Mechanism	Disease/model	Drug	References
DNMT inhibitors	Asthma, OVA mouse	5-Azacytidine	Wu et al., 2013b
	IPF, bleomycin mouse model	Decitabine	Dakhlallah et al., 2013
	IPF fibroblasts	Decitabine	Huang et al., 2010
Histone acetyltransferase inhibitors	Lung cancer	C646	Gao et al., 2013
HDAC inhibitors	Asthma, OVA mouse	Trichostatin A	Banerjee et al., 2012
	Airway smooth muscle	OSU-HDAC-44	Li et al., 2014a
	IPF fibroblasts	LBH589 and SAHA	Coward et al., 2009
	IPF fibroblasts	Trichostatin A	Huang et al., 2013
	IPF, bleomycin mouse model	SAHA	Sanders et al., 2014
	IPF fibroblasts	SAHA	Zhang et al., 2013a
HDAC upregulation	COPD, elastase mouse model	Quercetin	Ganesan et al., 2010
	COPD	Theophylline	Cosio et al., 2004
Histone methyltransferase inhibitors	IPF fibroblasts	BIX-01294 and 3-Deazaneplanocin	Coward et al., 2010, 2014
Bromodomain protein inhibitor	IPF fibroblasts	JQ1	Filippakopoulos, et al., 2010

Systems biology in drug discovery.

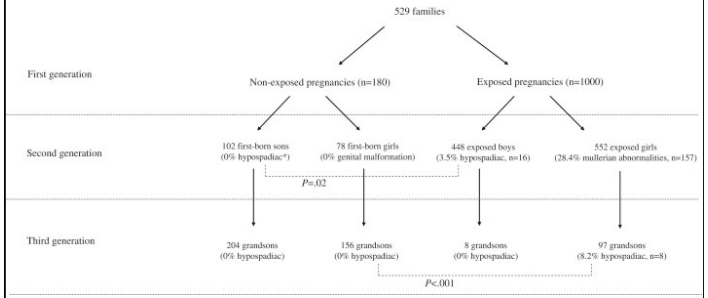
Butcher EC, Berg EL, Kunkel EJ.

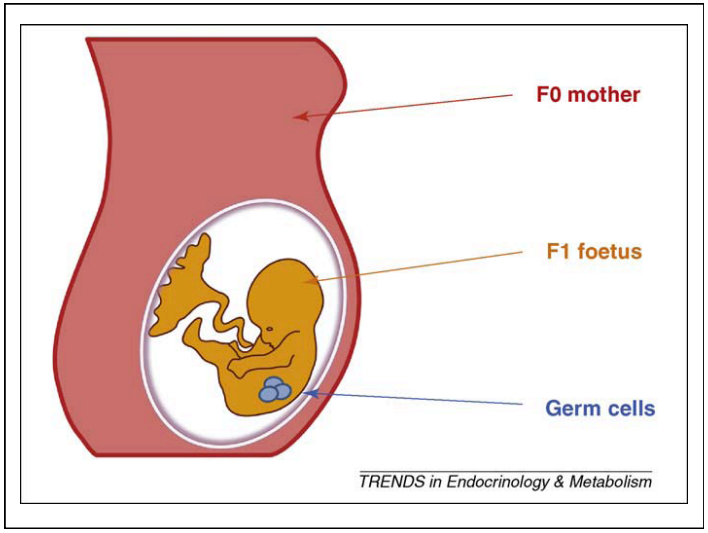
Nat Biotechnol. 2004 Oct;22(10):1253-9.

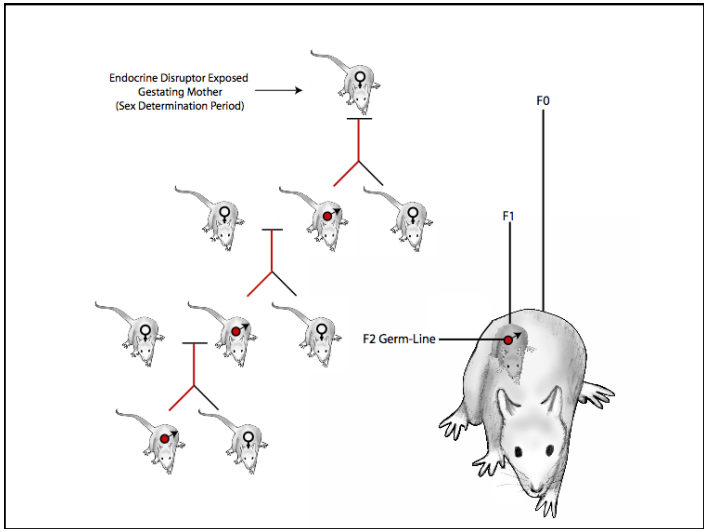


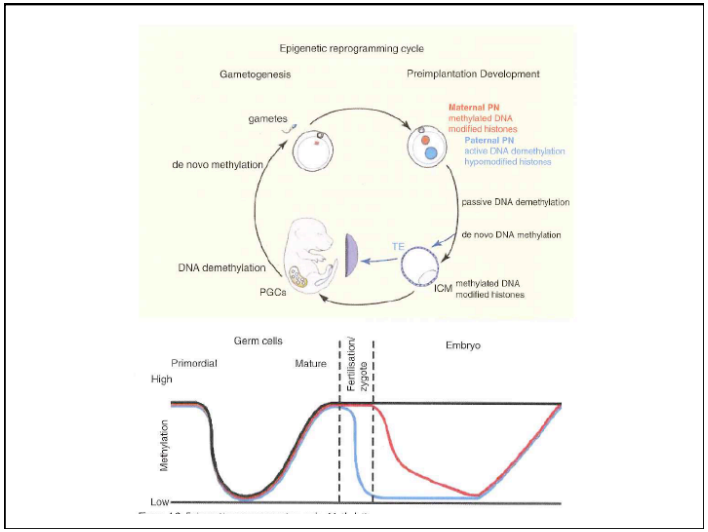
Epigenetics and Disease (Epigenetic Transgenerational Inheritance)

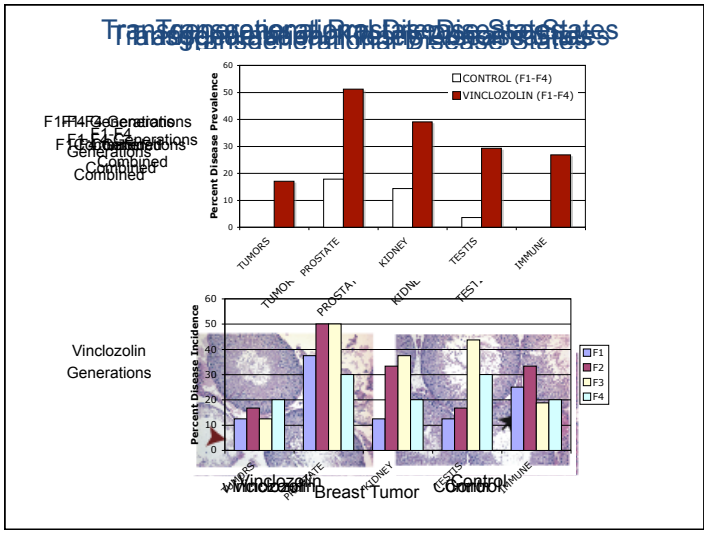
Kalfa N, Paris F, Soyer-Gobillard MO, Daures JP, Sultan C. (2011) Prevalence of hypospadias in grandsons of women exposed to diethylstilbestrol during pregnancy: a multigenerational national cohort study. Fertil Steril. 30;95(8):2574-7.



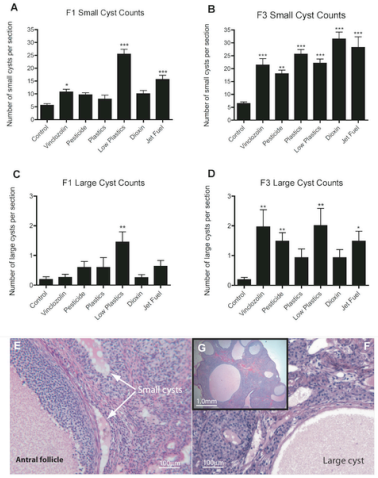




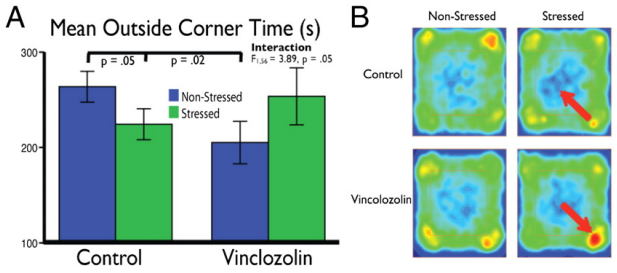




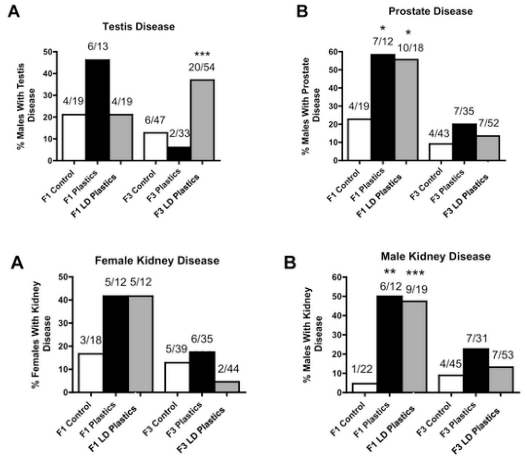
Nilsson E, Larsen G, Manikkam M, Guerrero-Bosagna C, Savenkova MI, Skinner MK. (2012) Environmentally induced epigenetic transgenerational inheritance of ovarian disease. PLoS One. 7(5):e36129.

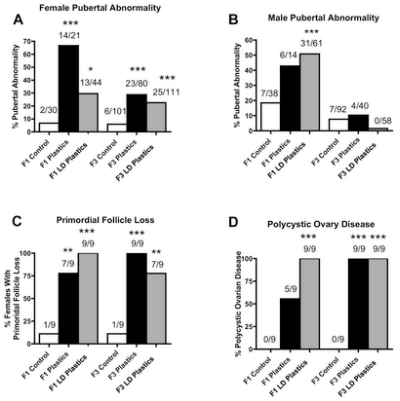


Crews D, Gillette R, Scarpino SV, Manikkam M, Savenkova MI, Skinner MK. (2012) Epigenetic transgenerational inheritance of altered stress responses. Proc Natl Acad Sci U S A. 5;109(23):9143-8.

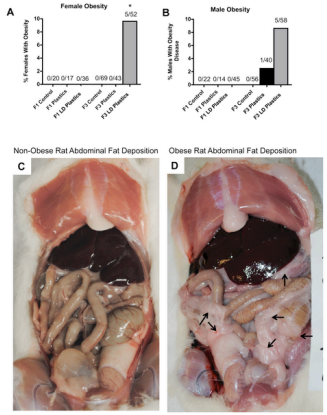


Manikkam M, Tracey R, Guerrero-Bosagna C, Skinner MK. (2013) Plastics derived endocrine disruptors (BPA, DEHP and DBP) induce epigenetic transgenerational inheritance of obesity, reproductive disease and sperm epimutations. PLoS One. 2013;8(1):e55387.



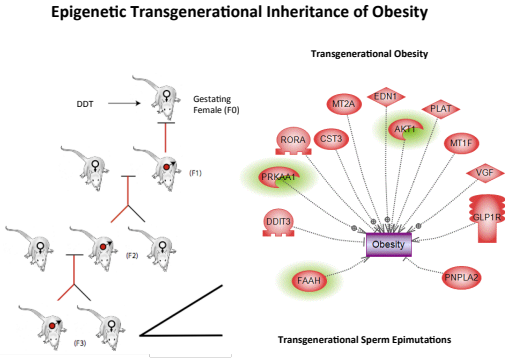


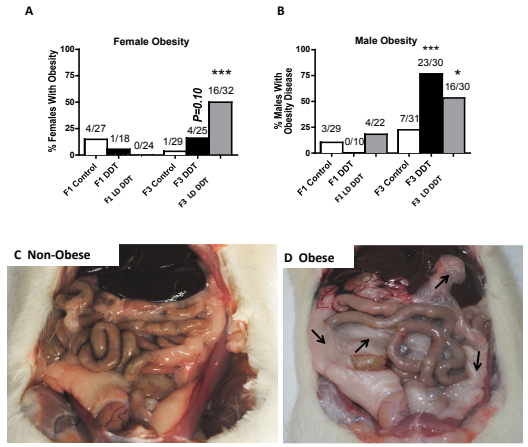
Pubertal abnormalities, primordial follicle loss, polycystic ovary disease and tumor development from control, plastics, or lower dose
(LD) plastics (BPA, DEHP and DBP) lineages. Percentages of females (panel A) and males (panel B) with pubertal abnormalities, or those females with primordial follicle loss (panel C) or polycystic ovary disease (panel D). The actual number of diseased rats/total number of rats in each group are shown above the respective bar graphs (* P<0.05; ** P<0.01; *** P<0.001).



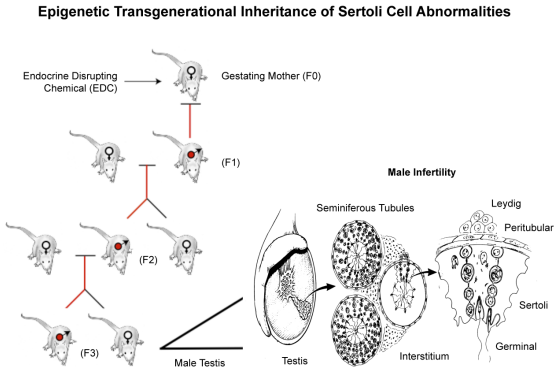
Obesity developed in control, plastics, or lower dose
(LD) plastics (BPA, DEHP and DBP) lineages. Percentages of females (panel A) and males (panel B) with obesity in F1 and F3 generations are presented. The actual number of diseased rats/total number of rats in each group are shown above the respective bar graphs (* P<0.05). Abdominal fat deposition in F3 generation 1yr old rats from non-obese (C) and obese (D) animals. Pink colored fat deposition over most organs noted in panel B (arrows).

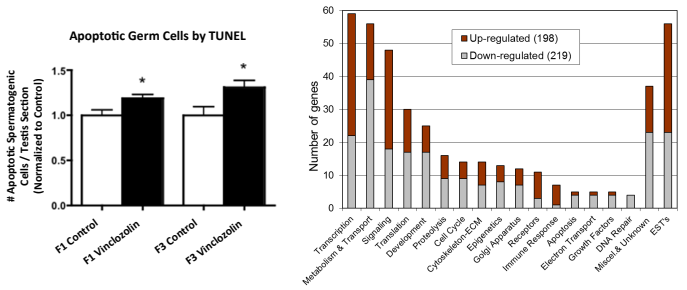
Michael K. Skinner, Mohan Manikkam, Rebecca Tracey, Eric Nilsson, Md. M. Haque and Carlos Guerrero-Bosagna (2013) Ancestral DDT Exposures Promote Epigenetic Transgenerational Inheritance of Obesity and Reproductive Disease (Submitted).

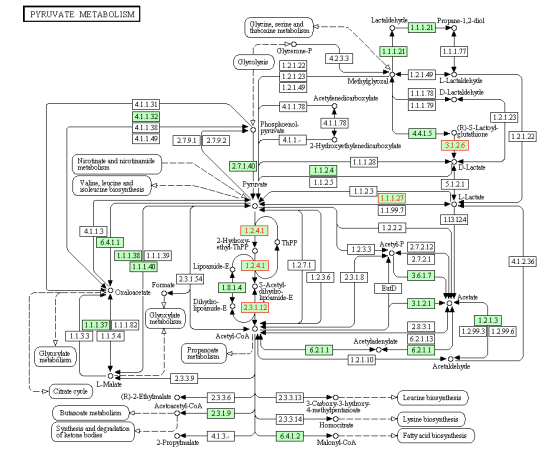


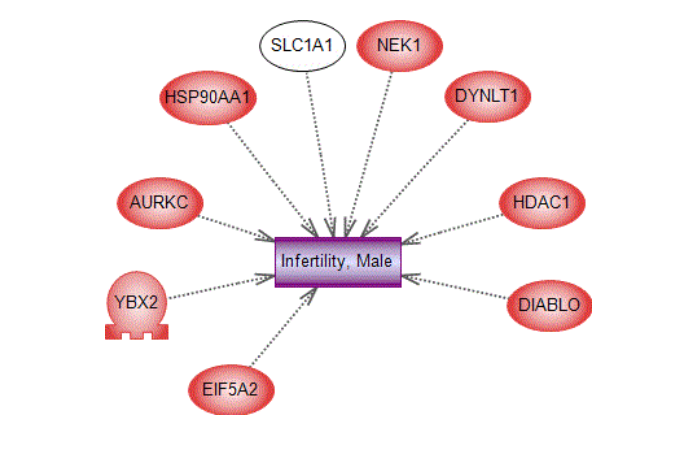


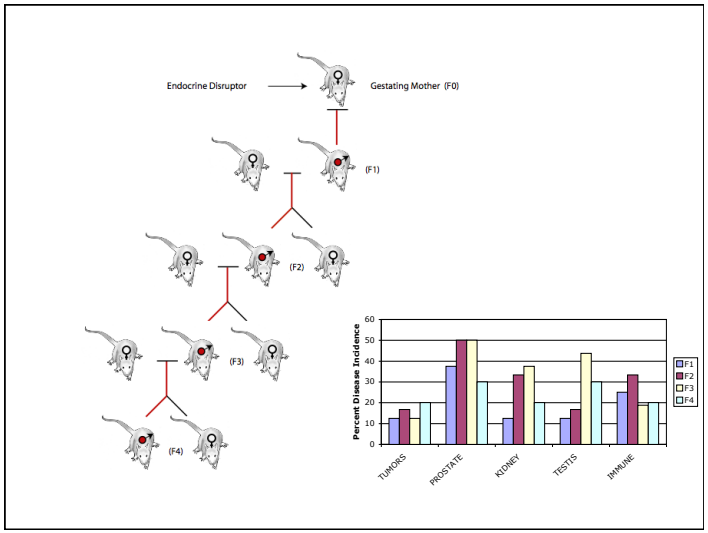
Guerrero-Bosagna C, Savenkova M, Haque Md. M, Sadler-Riggleman I, and Skinner MK (2013) Environmentally Induced Epigenetic Transgenerational Inheritance of Altered Sertoli Cell Transcriptome and Epigenome: Molecular Etiology of Male Infertility. PLoS ONE (In Press).

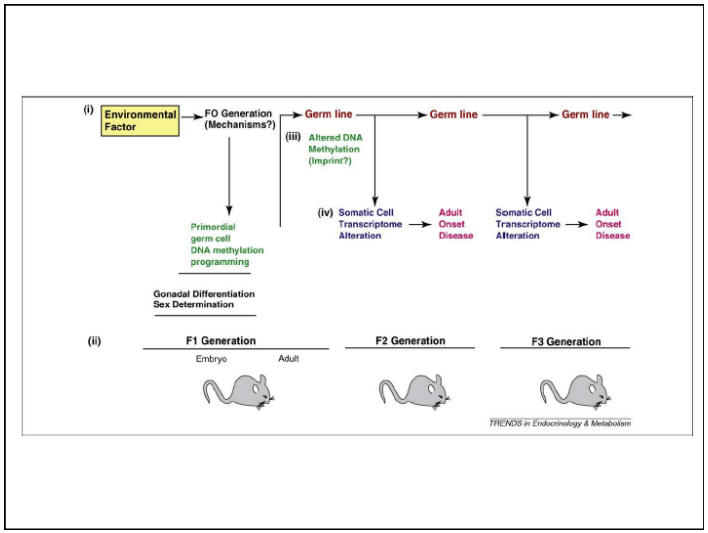












Transgenerational Disease Etiology

- •Spermatogenic Defect (>90%)
- •Male infertility (complete ~10%, severe 20%)
- •Kidney disease (~30-40%)
- •Prostate disease (~50%)
- •Increase in mammary tumor formation (~10-20%)
- •Behavior (Mate Preference,Anxiety&Stress)(>90%)
- •Pre-eclampsia-like during late pregnancy (~10%)
- •Premature Ovarian Failure POF (>90%)
- •Ovarian Polycystic Ovarian Disease (>90%)
- •Female Premature Pubertal Onset (>90%)
- •Obesity (~10-50%)

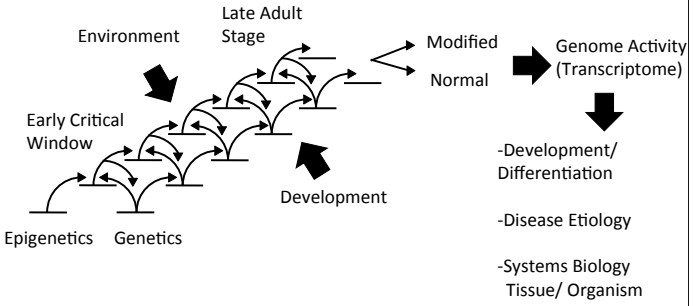
ENVIRONMENTALLY INDUCED EPIGENETIC TRANSGENERATIONAL INHERITANCE

Environmental Toxicants

Vinclozolin (Agricultural Fungicide)	Permethrin & DEET (Insect Repellants)
Methoxychlor (Agricultural Pesticide)	DDT (Pesticide)
Dioxin/TCDD (Industrial Contaminant)	Tributyltin (Industrial Toxicant & Biocide)
Plastic Compounds (BPA & Phthalates)	Hydrocarbons (Jet Fuel)

Other Types Exposures

Nutrition (High Fat or Caloric Restriction)	Smoking & Alcohol
Temperature & Drought (Plant Health & Flowering)	Stress (Behavioral)



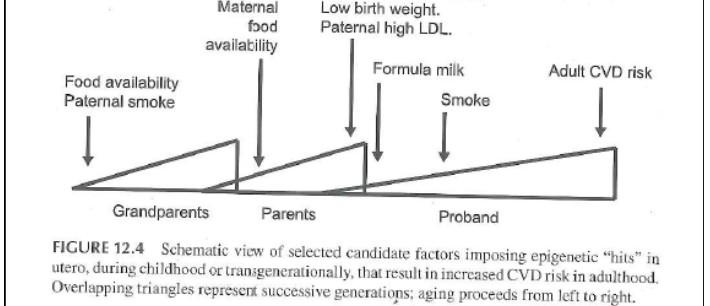


FIGURE 12.4 Schematic view of selected candidate factors imposing epigenetic "hits" in utero, during childhood or transgenerationally, that result in increased CVD risk in adulthood. Overlapping triangles represent successive generations; aging proceeds from left to right.