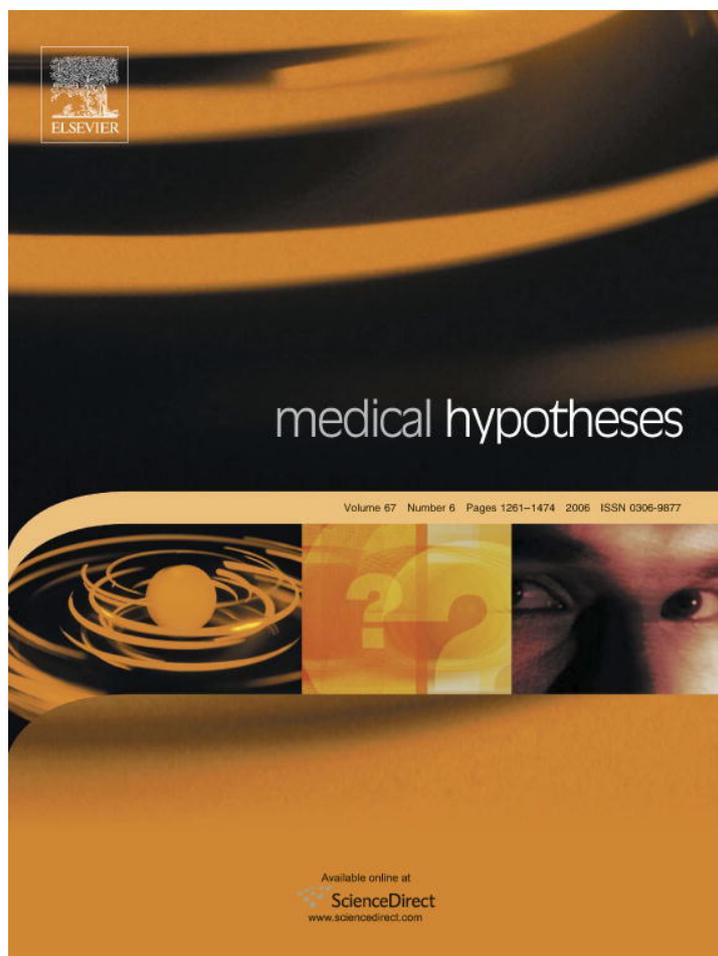


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Recursive causality in evolution: A model for epigenetic mechanisms in cancer development

A. Haslberger ^{a,c}, F. Varga ^b, H. Karlic ^{a,*}

^a Ludwig Boltzmann Institute for Leukemia Research and Hematology, Hanusch Hospital, Heinrich Collinstrasse 30, A-1140 Vienna, Austria

^b Ludwig Boltzmann Institute of Osteology at the Hanusch Hospital of WGKK and AUVA Trauma Centre Meidling, 4th Medical Department, Hanusch Hospital, Vienna, Austria

^c Vienna Ecology Center, University of Vienna, Austria

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Summary Interactions between adaptative and selective processes are illustrated in the model of recursive causality as defined in Rupert Riedl's systems theory of evolution. One of the main features of this theory also termed as theory of evolving complexity is the centrality of the notion of 'recursive' or 'feedback' causality – 'the idea that every biological effect in living systems, in some way, feeds back to its own cause'. Our hypothesis is that "recursive" or "feedback" causality provides a model for explaining the consequences of interacting genetic and epigenetic mechanisms which are known to play a key role in development of cancer. Epigenetics includes any process that alters gene activity without changes of the DNA sequence. The most important epigenetic mechanisms are DNA-methylation and chromatin remodeling. Hypomethylation of so-called oncogenes and hypermethylation of tumor suppressor genes appear to be critical determinants of cancer. Folic acid, vitamin B12 and other nutrients influence the function of enzymes that participate in various methylation processes by affecting the supply of methyl groups into a variety of molecules which may be directly or indirectly associated with cancerogenesis. We present an example from our own studies by showing that vitamin D3 has the potential to de-methylate the osteocalcin-promoter in MG63 osteosarcoma cells. Consequently, a stimulation of osteocalcin synthesis can be observed. The above mentioned enzymes also play a role in development and differentiation of cells and organisms and thus illustrate the close association between evolutionary and developmental mechanisms. This enabled new ways to understand the interaction between the genome and environment and may improve biomedical concepts including environmental health aspects where epigenetic and genetic modifications are closely associated. Recent observations showed that methylated nucleotides in the gene promoter may serve as a target for solar UV-induced mutations of the p53 tumor suppressor gene. This illustrates the close interaction of genetic and epigenetic mechanisms in cancerogenesis resulting from changes in transcriptional regulation and its contribution to a phenotype at the micro- or macroevolutionary level. Above-mentioned interactions of genetic and epigenetic mechanisms in oncogenesis defy explanation by plain linear causality, things like the continuing adaptability of complex systems. They can be explained by the concept of recursive causality and has introduced molecular biology into the realm of cognition science and systems theory: based

* Corresponding author. Tel.: +43 69919241457; fax: +43 19143214.
E-mail address: heidrun.karlic@meduniwien.ac.at (H. Karlic).

on the notion of so-called feedback- or recursive causality a model for epigenetic mechanisms with relevance for oncology and biomedicine is provided.

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Introduction

The interactions between adaptative and selective processes are illustrated in the model of recursive causality (Fig. 1) as defined in systems theory in evolution [1]. New evidences underline why this is important for understanding the consequences of genetic and epigenetic mechanisms. The term "epigenetics" includes any process that alters gene activity without changes in of the DNA sequence. Literally, the word means "in addition to changes in the genetic sequence". Alleles of the genes containing epigenetic marks are termed epi-

alleles. Epigenetic templates that control gene expression are transmitted to daughter cells independently of DNA sequence through mitosis and/or meiosis.

The interaction of both genetic and epigenetic mechanisms illustrates the complex nature of evolution which implies both the cellular, organismal and environmental aspects. It is generally believed that an improved understanding of epigenetic mechanisms will result in improved concepts of biomedicine and ecology [2]. However, such an improved understanding of epigenetics obviously needs considerations of evolutionary biology,

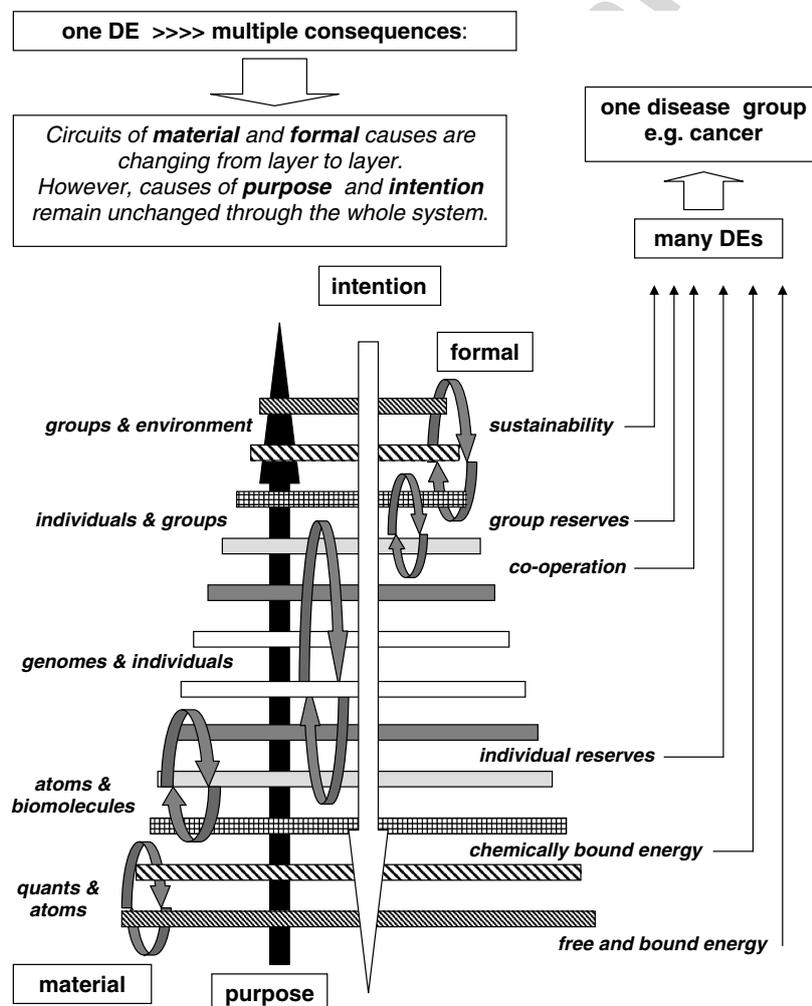


Figure 1 In the model of recursive causality the four types of causes are positioned within the multi-layered system of an organism and its environment. Circuits of material and formal causes are changing from layer to layer. Final (purpose) and efficiency (energetic) causes however, go straight and unchanged through the whole multi-layered system in Riedl's concept. DE, disturbing event.

where it appears that there is a close interrelationship between philosophical and biological theories. Evolutionary theory not only became the whole conceptual edifice in biology, but also much of the agenda of modern philosophy [3]. The important work of philosophers of biology on the units and levels of selection and evolution can be regarded as a contribution to evolutionary biology [4,5]. In his classic *Adaptation and Natural Selection: A Critique of Some Current Evolutionary Thought* (1966), George Williams [6] showed definitively that our understanding of adaptation is a central concept of evolutionary theory which must not be gene-centered in the sense of DNA sequences. The scientific achievement of Rupert Riedl's "systems theory of evolution" was a synthesis of biological and philosophical approaches [7] which fit to evolutionary concepts at the level of organisms and their body plans. Here we show that this model also illustrates the development of malignancy.

Epigenetic mechanisms in development of malignancy

When malignant diseases are initiated, both genetic and epigenetic mechanisms of altered gene expression often go hand in hand; not surprisingly, biallelic inactivation of a given tumor suppressor gene may occur via a combination of mutational and epigenetic events and is entirely consistent with the Knudson two-hit hypothesis of tumorigenesis [8,9]. Other reviews [10–12] summarize recent developments within the field of cellular evolution in tumors, highlighting its association with the transcriptional effects in a variety of human cancers.

Epigenetic changes are the most common alterations in human cancer, but it has been difficult to sort out cause and effect from studies of human tumors [10,13,14]. The main types of epigenetic modifications are DNA-methylation and histone modification. These changes in chromatin are now at the forefront of research in the field of oncogenesis, both as mechanisms of malignant development and as prognostic indicators of cancer risk. Leukemia, due to the defects in cellular differentiation associated with the disease, has important connections to epigenetic gene regulation. Hypomethylation appears to be a critical determinant of cancer, affecting chromosomal stability and specific gene targets [13,15–20]. In addition, hypomethylation is a mechanism of drug, toxin, and viral effects in cancer. In addition to gene amplification, hypomethylation of the multidrug-

resistance gene MDR1 correlates with increased expression and drug resistance in acute myelogenous leukemia [18]. In vivo studies of rats fed with methyl donor (e.g., folate) deficient diets showed that overexpression of genes such as c-myc, c-fos and c-ha-ras in liver-derived RNA correlated with hypomethylation of DNA. Interestingly, not all cytosines were re-methylated following methyl donor repleting diets which are known for their genetic effects [21]. Thus DNA-methylation defects can be irreversible after prolonged diets low in methyl donors [22].

A recent publication shows that methyl CpG may serve as a mutational target for solar UV-induced mutations of the p53 tumor suppressor gene in skin cancer [23]. In addition to the above mentioned various effects of hypomethylation [10], aberrant promoter hypermethylation that is associated with inappropriate gene silencing of tumor suppressor genes may affect virtually every step in tumor progression [24].

Folic acid, vitamin B12 and other nutrients [21,25] influence the function of enzymes that participate in various methylation processes by affecting the supply of methyl groups into a variety of molecules which may be directly or indirectly associated with ageing and cancerogenesis. Our tissue culture experiment (Fig. 2) shows that human osteosarcoma cells (MG63) confirm previous data from rat osteosarcoma [26] by showing the relationship between Vitamin D3-associated de-methylation of the osteocalcin (OCN) – promoter and transcriptional stimulation of the OCN gene.

Thus the role of coding and non-coding DNA is at least of equal importance especially regarding feedback mechanisms between RNA and DNA,

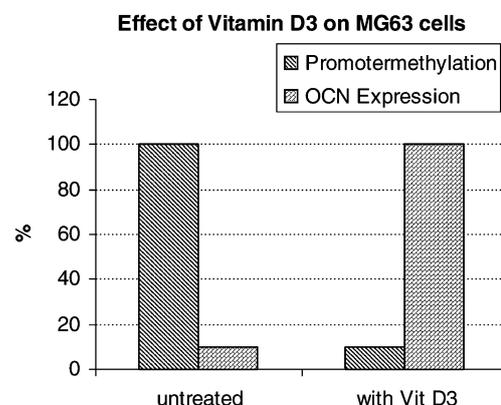


Figure 2 The demethylating effect of Vitamin D3 on the osteocalcin (OCN) promoter is associated with transcriptional activation of OCN in the MG63 osteosarcoma cell line.

which might provide the basis for epigenetic modifications. However, it has to be mentioned that such changes are rather random. As methylases could also work in a feedback mechanism, it appears possible that this model could explain non-random mRNA-directed epigenetic changes resulting from adaptatory processes to lifestyle and environment.

Treatments, such as irradiation and chemotherapy, and compounds, such as environmental toxins, pose a threat to the integrity of the genome. Studies have shown that these agents can result in genetic or developmental defects in the offspring or F1 generation from an exposed gestating mother. The ability of an external agent to induce a transgenerational effect requires stable chromosomal alterations or an epigenetic phenomenon such as DNA-methylation [27]. The term “transgenerational” [28] refers to a germ line transmission to multiple generations, minimally to the F2 generation. Transgenerational effects of irradiation were the first to be identified through transmission of DNA mutations in the germ line to multiple generations [29], often associated with tumor formation. Chemotherapeutic treatments [30] and environmental toxins such as endocrine disruptors [31] can cause effects in the F1 generation, but they have not been shown to affect the F2 generation. Environmental factors can induce an epigenetic transgenerational phenotype through an apparent reprogramming of the male germ line [28]. Future toxicology studies will be needed to test this model for assessment of cancer-risk on animal and possibly also human populations.

Evolutionary developmental biology (Evo-Devo)

As mentioned above, the black box between genotype and phenotype has gained some light at the cellular level in biomedicine, but there is still a large area of research to be done in so-called Evo-Devo. Following the definition from Hall [32] evolutionary developmental biology (Evo-Devo) as a discipline is concerned, among other things, with discovering and understanding the role of changes in developmental mechanisms in the evolutionary origin of aspects of the phenotype. Changes in the timing or positioning of an aspect of development in a descendant relative to an ancestor (heterochrony and heterotopy) were two evolutionary developmental mechanisms identified by Ernst Haeckel in the 1870s. Many more have since been identified, in large part because of our enhanced understanding of development and because new

mechanisms emerge as development proceeds: the transfer from maternal to zygotic genomic control; cell-to-cell interactions; cell differentiation and cell migration; embryonic inductions; functional interactions at the tissue and organ levels; growth. Within these emergent processes, gene networks and gene cascades (genetic modules) link the genotype with morphogenetic units (cellular modules, namely germ layers, embryonic fields or cellular condensations), while epigenetic processes such as embryonic inductions, tissue interactions and functional integration, link morphogenetic units to the phenotype.

Evolutionary developmental mechanisms also include interactions between individuals of the same species, individuals of different species, and species and their biotic and/or abiotic environment. Such interactions link ecological communities. Importantly, there is little to distinguish the causality that underlies these interactions from that which underlies inductive interactions within embryos. Embryological studies published in the mid 1980s [33,34] referred to conditioning of the maternal and paternal genomes during gametogenesis, such that a specific parental allele is more abundantly (or exclusively) expressed in the offspring. This process was termed “imprinting” (see Table 1).

Table 1 A sample of evolutionary developmental mechanisms at various levels of biological hierarchy

Level	Mechanisms
<i>Molecular:</i> Gene Transcriptome Proteome	Regulation, networks, interactions, genome size, epigenetic processes (methylation, imprinting, chromosome inactivation), transcriptional regulation, signal transduction, metabolism...
Cell	Division, migration, condensation, differentiation, interaction, patterning, morphogenesis
Tissue, organ	Modularity, segmentation, embryonic inductions, epithelial-mesenchymal interactions, growth
Organism	Ontogenetic re-patterning, genetic assimilation, phenotypic plasticity, polymorphism, functional morphology

Imprinting results from the unequal expression of the maternal or paternal allele of a small number of genes in the genome (about 50 imprinted genes have been identified). This allele-specific transcriptional repression is achieved for most genes by differential methylation of promoter-associated CpG islands of nearby imprinted control regions. This methylation mark is established during gamete development. Loss of imprinting (LOI), leading to pathological biallelic expression of a gene insulin like growth factor 2 (*IGF2*) was discovered in Wilms tumours [35,36]. These observations were the first to indicate a gatekeeper role for epigenetic alterations in cancer. The effect of this epigenetic lesion is silencing of *H19*, a gene that encodes an abundant spliced but non-translated RNA, and a reciprocal increase in expression of *IGF2* [37,38]. The roughly twofold increase in effective *IGF2* gene dosage is now considered the most likely explanation for the associated tumor susceptibility, although *H19* RNA is growth suppressive in some cancer cell lines [39] (see Table 2).

A growing body of evidence suggests that changes in transcriptional regulation play an important role for the evolution of development. At a microevolutionary level, all the necessary conditions are present: populations harbor abundant genetic variation for differences in transcription profiles, a substantial fraction of these variants can influence organismal phenotype, and some variants have fitness consequences and are subject to natural selection. At a macroevolutionary level,

the evidence is less direct but strongly suggestive: specific differences in anatomy and gene expression are often correlated, while comparisons of transcription profiles among distantly related taxa point to extensive evolutionary changes in regulatory gene networks. Understanding how transcriptional regulatory systems evolve, and what contributions these changes have made to the evolution of phenotype, represents a major challenge for Evo-Devo [40].

Although genes (in the sense of information units) have specific phenotypic consequences in a given species, this functional relationship can clearly change during the course of evolution. Many cases of evolutionary dissociations between homologous genes and homologous morphological features are now known. These dissociations have interesting and important implications for understanding the genetic basis for evolutionary changes [41,42], some of them may also be applied to developmental mechanisms of malignancies and tumor progression.

Systems theory of evolution

One of the main features of systems theory of evolution also termed as theory of evolving complexity [1,7] is the centrality of the notion of 'recursive' or 'feedback' causality – 'the idea that every biological effect in living systems, in some way, feeds back to its own cause' (see also Fig. 1). This concept of recursive causality can explain phenomena like the above-mentioned interactions of genetic and epigenetic mechanisms in oncogenesis that defy explanation by plain linear causality, things like the continuing adaptability of complex systems.

In addition, all kinds of developmental constraints, and macroevolutionary phenomena like parallel evolution, orthogenesis, and typogenesis fit to this model. An increasing number of data underline an inclusion of the concept of feedback causality within biomedicine. Riedl even considers it as vital, in the long run, for our own survival as a species [1,7,43]. Apparently, explicit awareness of the causal complexity of biosystems does not prevent one from monocausal – or in this case: duocausal – thinking in the cultural-historical domain.

Systems theory of evolution emphasizes the role of functional and developmental integration in limiting and enabling adaptive evolution by natural selection. The main objective of this theory is to account for the observed patterns of morphological evolution, such as the conservation of body plans

Table 2 Emergent units and process between genotype and phenotype and the basis of evolutionary developmental mechanisms operating within each (*)

Functional units	Basis of evo-devo mechanism
Genotype	Genes (in the sense of information-units)
Genetic modules	Gene networks, gene cascades
Morphogenetic units	Cell condensations
Epigenetic processes	Tissue interactions, functional integration
Phenotype	Inter- and intra-individual/ species and ecological/ environmental interactions

(*) Units of evolutionary developmental mechanisms such as gene networks/cascades cross over between units in the hierarchy.

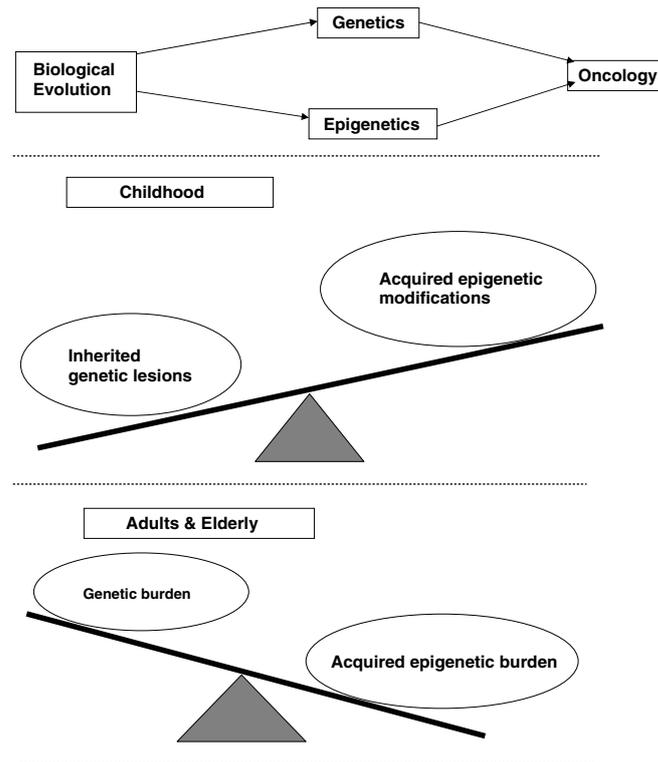


Figure 3 The balance between genetic and epigenetic impacts in development of malignancy is changing from childhood to later age. Whereas the majority of childhood tumors are associated with an inherited genetic or epigenetic (e.g., imprinted) burden, this balance shifts in favor of acquired epigenetic and genetic hits in tumors of adults and elderly.

but fits also to concepts of cellular and molecular evolution. Riedl thought it necessary to contextualize natural selection with the organismal boundary conditions of adaptation. In Riedl's view development is the most important factor besides natural selection in shaping the pattern and processes of molecular, cellular and morphological evolution. Epigenetics may be considered as a shaping mechanism in this model.

Conclusion

Certain patterns of evolutionary change, like body plans and innovations at the cellular and molecular levels, are not properly accounted for by the theory of natural selection alone, but require us to look at the developmental boundary conditions under which natural variation and selection take place. As tumors arise from aberrant cells, it appears easy to imagine, how the genetic-epigenetic mix-up drives the evolution of malignancy. The relative weight of these boundary conditions is changing during lifetime (Fig. 3). Epigenetic mechanisms appear as a machinery of recursive feedback which

determines the average rate of change of characters at all levels from molecules to cells, organisms and their environment.

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