



Shifting paradigms in cancerogenesis: from genetics to epigenetics & back

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This review paper is intended to bring together common features of two important new approaches to cancerogenesis. The first is the epigenetic progenitor model of human cancer origin, proposed by Feinberg AP, Ohlsson R & Henikoff S (2), and the second is idea of cancer phenotype hallmarks proposed by Hanahan D & Weinberg RA (6). Both models are briefly described and arguments that these two models are actually explaining the same process from two perspectives are put forward.

The second half of the 20th century was a period marked by great advancement in medicine due to substantial investments in biomedical science. Although acquired knowledge did not have immediate impact on our understanding of development of solid malignomas, each subsequent decade has revealed new, more complex and well-founded concepts, both in basic medical sciences and in clinical practice.

Gradual progress in understanding the origins of malignant neoplasms and their development

An overview of how our ideas on the causes of spontaneous development of malignant tumours in the human body evolved is presented in Table 1 (based on ref. 1 and textbook sources). It is obvious that we have been fascinated by DNA mutations that cause cancers for a long time. Numerous data have been collected on development of malignomas after being exposed to ionising radiation, some chemicals, and certain viruses. Furthermore, mutations of certain genes that were passed down within families have been identified as risk factors for development of breast or ovarian cancer, or cancer of the colon. However, it has not been possible to identify mutations significant for establishing links between alcohol consumption and cancer development, as well as for some other factors that were undoubtedly associated with cancer development according to epidemiological studies. It has been equally impossible to find clear explanation for the fact that out of all hormones, only sex hormones have epidemiologically important links with development of malignomas in their target tissues (breast cancer, endometrial cancer, prostate cancer). Finally, it has not been possible to determine how tobacco smoke causes mutations that result in malignant tumours of head and neck as well as of respiratory tract, the link sometimes ironically called "the smoking gun".

The first decade of the 21st century has brought a new approach to this complex issue. After careful analysis of numerous data obtained from research conducted on animal models as well as on human

TABLE 1

Historical perspective of paradigm shifting in our understanding of cancerogenesis (based on ref. 1 and textbook sources).

Decade	Paradigm	Postulates of the prevailing paradigm
1940-9	tumor stem cells	cancer spreading depends on the minority of tumor stem cells, while all other cells are not able to initiate new cancer sites
1950-9	two-hit hypotheses	individuals can inherit a gene mutation that make susceptible to a certain cancer type that usually occur earlier than in individuals without that mutation
1960-9	chromosome aberrations	cancerogenesis is often accompanied with chromosome aberrations in tumor cells
1970-9	oncogenes and tumor suppressor genes	activation or mutation of certain viral and human genes lead to cancer, some of them are considered to be oncogenes while others act as tumor suppressor genes
1980-9	altered activity of cell oncogenes	malignomas result from the altered balance of activities of oncogenes and tumor suppressor genes in tumor cells
1990-9	gene mutations behind familiar cancers	familiar cancers are explained as inherited mutations of a certain tumor suppressor genes directly linked to that tumor type
2000-9	concept of cancer hallmarks	seemingly different cancer types share common hallmarks of almost universal cancer phenotype that allows cancer spreading
	carcinogenesis as combination of epigenetic and genetic changes	altered expression of tumor associated genes is probably a combination of epigenetic and genetic changes
	tumor growth and dissemination through continuous self-seeding of cancer cells	solid tumors growth due to continuous self-seeding of tumor cells, most of them perish in circulation, some are reintroduced in existing tumor sites and some initiate new sites.

malignomas, both sporadic and inherited, researchers have proposed new models of cancer development. These new models define phenotypic features common to a great majority of cancer cells. Simultaneously, other researchers combine single-gene defects and epigenetic modifications of gene expression in determining mechanisms that can statistically predict development of cancer in the average person's lifetime. Similarly, a new model of tumour growth by self-seeding of tumour cells assumes that continuous seeding of tumour cells explains essential development characteristics of primary and metastatic cancer sites.

Genome and cellular phenotype

Genome is the entirety of an organism's hereditary information, contained both in chromosomal and in mitochondrial DNA. It is beyond any doubt that structure and function of a live cell is governed by the genome, whereby its expression varies from cell to cell. Specific characteristics in structure and function of a single cell, despite the same or extremely similar common heritage, can be regarded as cellular phenotype.

The term non-coding DNA denotes the part of the genome that is not organised into genes responsible for protein synthesis. It is also important to notice that non-coding DNA is a part of the genome in multi-cellular organisms, and as such it most probably reflects their anatomical or functional complexity. The functional complexity also applies to the function of a cell in time, which means that a cell should contain information to enable the optimising of the cell's function in variable environmental conditions.

Cellular phenotype and epigenetics

The research described previously is closely linked to epigenetics (1, 2, 3, 4). Cellular genetics studies passing down the information stored in DNA sequence (genes and non-coding DNA), while epigenetics studies heritable changes in gene expression, which defines cellular phenotype. These changes are then passed down to the next cell generation through cell division despite the fact that the underlying DNA sequence has not been altered.

Epigenetic mechanisms are classified into three groups of mechanism that change gene expression: messenger RNA (mRNA) marking, DNA marking and chromatin condensation (Table 2). It is evident that mRNA marking is probably the mechanism of transient epigenetic modulation of the cell functions, while DNA marking and chromatin condensation are more permanent changes that define cell structure and function and are passed down through mitosis to the next generation of cells. Immediately after fertilisation of an ovum, methylation of the genome in the zygote is decreased to a minimum (complete dedifferentiation of the zygote), which indicates that histone epigenetics can be the way to pass down epigenetic information from ancestors to a new organism, despite DNA demethylation.

Regulation of gene expression in most genes on autosomal chromosomes comes down to two possibilities: one allele is more expressed, or, the result is a mixed phenotype of both alleles. There is a set of almost 100 genes where activation of the maternal allele is necessary for their optimal function, while for some other genes the activation of the paternal alleles is important. Epigenetic process that enables activation of the suitable allele is so

TABLE 2

Basic epigenetic mechanisms that determine cell shape and function.

Features	DNA changes	mRNA changes	Chromatin changes
Substrata	DNA	mRNA	histone configuration
Mechanism	methylation		histone deacetylation
Results of alteration	hyper_	reduced expression of certain genes	
	hypo_	increased expression of certain genes, often in benign tumors	
Regulatory control	the exact control mechanism is still not determined, possibly linked to the non coding DNA		
Mitotic division of somatic cells	inherited – these changes control cell differentiation	probably not inherited since mRNA is not stable it temporarily changes cell function	probably inherited since life conditions of parents can influence health risks in their offsprings
Conception	not inherited – conception diminish DNA methylation and lead to a new omnipotent cell		

called gene imprinting. It is generally considered that thus optimal co-operation between maternal mitochondrial genome and maternal mitochondrial genes that are part of the nuclear DNA is achieved. Defects in imprinting can alter the phenotype of the cell, number of progenitor cells or their susceptibility to stimuli and thus significantly increase the risk for development of malignomas. This has been noticed in IGF2 gene, which is normally activated only by paternal allele, while the activation of both alleles is associated with Wilms' tumour in children.

Comparison of a mechanism and consequences of mutations and epigenetic alterations

Mutations are consequences of random damage to DNA caused by mutagen agents, so they are destructive in their nature and they disturb the highly organised structure of the cell genome. The process of cells differentiation in a tissue shows that a series of epigenetic changes amazingly govern programmed alterations in the structure and function of body cells. Epigenetics also governs all the other permanent or transient phenotypic adjustments of an organism to environmental factors. It can be assumed that epigenetics is important not only for development and growth of an organism, but also for the whole process of alterations in the phenotype of a cell and the whole body, including ageing as the final result of accumulated epigenetic changes in somatic cells.

Gene mutations, regardless of the mechanism and the level of damage they cause (point mutations, deletions, frame shift mutations, translocations, etc.), are likely to result in cell death. The situation is reverse in epigenetics. It is highly unlikely that cell death can be caused by any single epigenetic change, although a large number of epigenetic modifications lead to cell apoptosis.

Non-repaired gene mutations that did not result in cell death are always passed to daughter cells by divisions. If these cells are gametes, mutation is passed down to next generations via fertilisation, which means that a permanent modification in DNA sequence has occurred. Epigenetic changes in form of DNA marking are passed

down to daughter cells via mitosis, which is probably the principal way of cell differentiation, and thus these changes can be considered stable as long as this particular cell line exists.

Immediately after fertilisation of an ovum DNA demethylation of the new genome occurs and thus the majority of, if not all, epigenetic marks in DNA are lost. It has not been clearly determined whether phenotypic changes are permanent and hereditary in the other two groups of epigenetic changes (histone and RNA modifications). However, it is likely that RNA modifications are only transient alterations in cell structure and function and that they are not hereditary. In histone modifications as well as in some other epigenetic changes inheritance via fertilisation cannot be excluded since there are documented cases of passing down the epigenetic modulation of metabolism to progeny.

Genetics and epigenetics in development of malignomas

It is important to emphasize that most diagnostic methods commonly used in oncology practice measure various characteristics of cancerous phenotypes that reflect the changed gene expression. This is the result of mutations as well as of epigenetic changes of both genes and of non-coding DNA.

As it has been proved in most well understood cancers, it takes from 5 to 7 or more mutations for a person to develop cancer, which is highly unlikely to occur during the lifetime of a cell line due to inherent randomness of mutations that result in cell death. Unlike this, epigenetics very precisely controls gene expression changes that are passed down via mitosis, despite its still unclear regulation of complex sequences in cell differentiation changes. This is exactly what makes epigenetics an ideal and probably the only reliable mechanism to define any cellular phenotype, including the cancerous.

In 2006, A. P. Feinberg, R. Ohlsson and S. Henikoff (2) suggested that epigenetics and genetics need to be combined to achieve better understanding of cancer development, which had tremendous influence on basic

TABLE 3

Model of the epigenetic progenitor origin of human cancer (based on ref. 2 and 3).

Phases of cancerogenesis	Involved genes	Alteration	Consequences
Epigenetic changes of one healthy tissue stem or progenitor cell	tumor progenitor genes	epigenetic changes during mitosis due to external factors (nutrients, microenvironment etc.)	results in a clone of cells with altered expression of tumor associated genes, possible benign tumors
The key mutation of one tumor associated gene	one of the genes that regulate the cell cycle	mutation starts continuous cell division of cells with previous epigenetic features of the cancer phenotype	the resulting malignant clone lead to the primary tumor growth
Genetic & epigenetic instability of cancer cells	DNA repair genes and epigenetic correction genes	continuous cell divisions introduce new mutations and altered epigenetic coding change cell differentiation	clinically evident polyclonal malignant solid tumor

oncology research and development of epigenetic diagnostics and medications. A simplified cause-and-result series of epigenetic changes that results in progenitor cells cancer (3) is presented in Table 3.

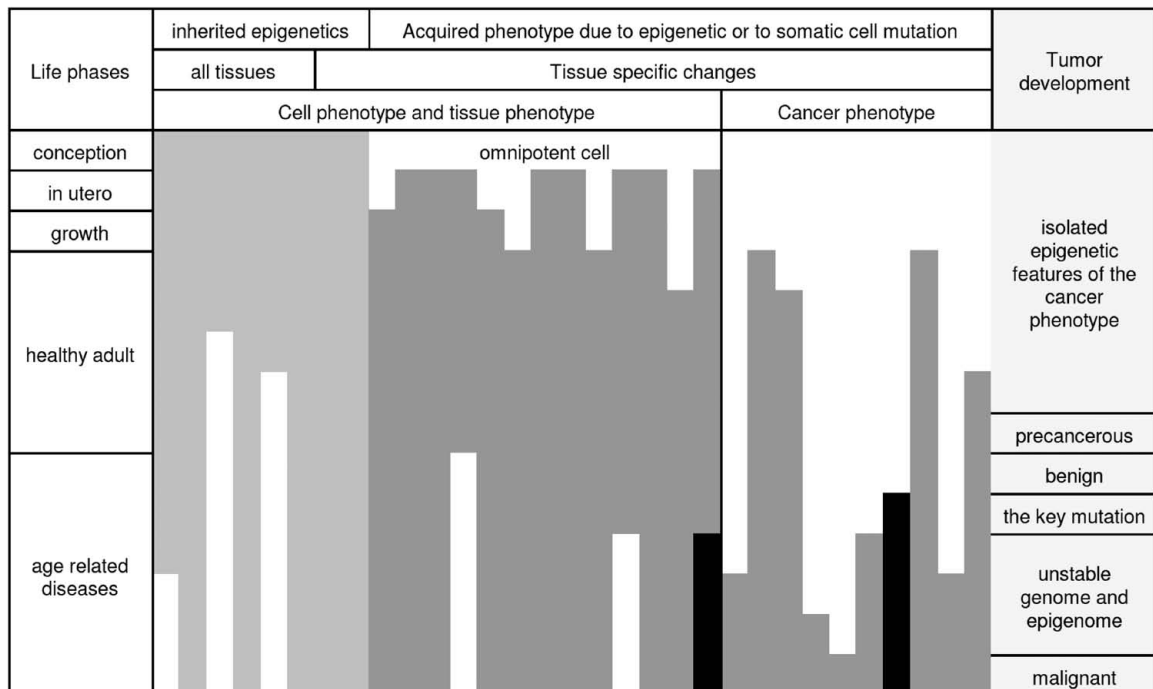
Epigenetic model of solid tumours development is grounded in the concept that exposure to repetitive injuries, stress, and deficiency in a diet can cause epigenetic changes in the group of stem cells of a tissue. Cellular phenotype is affected by pre-modifications in this pre-malignant phase and in considerably large number of cells in such a way that a mutation of the gene governing

the cell division occurs, and the new cells acquire the ability to form a primary cancer site through frequent divisions.

One example of epigenetic change in cancerous phenotype is induction of neoangiogenesis, which is an important precondition for growth of any malignant tumour. If in healthy progenitor cells the VEGF secretion is epigenetically increased due to various unfavourable environmental factors, malignantly altered progenitor cell will ensure undisturbed tumour growth through fast division and secretion of VEGF. Epigenetic alterations in

TABLE 4

A schematic presentation of presumed roles of gene mutations and epigenetic changes in the occurrence of malignant tumors and chronic diseases. Epigenetic features are partially inherited and acquired through the lifetime through tissue differentiation and reparation. Cancer phenotype is gradually assembled from epigenetic changes (grey areas), resulting in precancerous lesions or in benign tumors. The key mutation (shown as a black area) initiates the malignant clone that will develop in a tumor. Chronic, age related diseases can come from accumulated inherited and acquired epigenetic changes, but also due to loss of some important epigenetic coding (shown as white areas).



progenitor cells may and may not be manifested by development of benign tumours in the phase prior to the key mutation that results in a malignant clone. If the mutation of the gene for cell division does not occur, benign neoplasms do not change. The first cancer cell is produced only after key mutation has taken place, which will result in exponential growth of this malignant cell.

According to this model the key mutation is a change in DNA that will either result in permanent reduction in the function of the important tumour suppressor gene or stimulate the function of an oncogene. A clone of rapidly multiplying cells that do not have essential epigenetic features of the cancerous phenotype (such as VEGF expression) is not likely to develop into primary cancer site since it is limited by normal energy metabolism, not reduced histocompatibility expression, adhesivity, preserved contact inhibition or insufficient angiogenesis.

Development of clinically relevant primary tumours is expected to originate only from progenitor cells that have developed important characteristics of cancerous phenotype prior to malignant transformation. Key mutation can be detected in cells of most well-researched sporadic tumours. It is not necessarily always the same gene, it is sufficient that mutation has occurred in an important gene and expression of other genes with malignant phenotype can be altered by epigenetic mechanisms, if not by gene mutation. What makes this key mutation a necessity is its irreversibility. In other words, if all 5, 7 or 9 necessary genes were not epigenetically modified, the changes would be potentially reversible and thus there might be a little, but real probability for the disease auto-inhibition, due to simple loss of some epigenetic marks in a dominant tumour clone. This theoretical possibility in described model could explain extremely rare cases of favourable outcome of undoubtedly confirmed malignant diseases that have gone into remission in the phase of rapid dissemination.

In epigenetic model of research the authors focus on tissue stem cells as cells from which solid tumours originate. It is not rare that cancer cells contain almost all normal features of the tissue and in histological classification they are described as well differentiated malignomas. This fact requires further analysis to explain when the cancer cells achieved this level of differentiation. In theory there are several possible explanations.

In phase 3 of the epigenetic model, after rapid mitoses that follow as a consequence of the key mutation, increased plasticity to epigenetic changes and new mutations occurs. In this way, a stem cell that has undergone malignant changes can go through incomplete differentiation after relatively small number of divisions, thus achieving the stage that will be found in histological examination of the tumour tissue sample acquired by biopsy.

The next logical question regarding this model is whether this combined epigenetic model with at least one mutation that causes rapid multiplication of cells can be grounded in basic research and clinical practice (5). From the available amount of evidence on the role of

epigenetics in cancer development, three examples will be presented. The first one is the case of a successfully cloned mouse from the genome of the mouse's melanoma. It confirms that almost everything in this cell line of melanoma cells was epigenetically changed so that resetting of DNA methylation in the fertilised cell created the whole animal, admittedly susceptible to melanoma development (its genome probably still contains the key mutation). The second important finding is the epidemiological parameter showing that the incidence of breast cancer has significantly dropped in various countries within two years after reducing hormone replacement therapy. This drop in breast cancer incidence in a short period of time undoubtedly shows that giving oestrogen to menopausal women temporarily and reversibly increased the risk for breast cancer development and also that the reason could not be found in rapid accumulation of mutations but only in some epigenetic effect that started to disappear as soon as oestrogen therapy was stopped. The third example is the fact that breast cancer incidence within a family is almost always linked to BRCA1 and BRCA2 gene mutation, while in the tissue samples from sporadic breast cancers BRCA1 gene mutation is extremely rare. Instead, many cases of BRCA1 methylations were documented, which meant that this gene was epigenetically inactivated in sporadic cancers.

To sum up, in simple words, all solid tumours that have reached certain clinically significant size have developed cancerous phenotype mainly due to epigenetics, but probably have a minimum of one mutation of a gene that regulates cell division. This model predicts that there is a set of key mutations of genes in cell cycle, cell immortality and apoptosis that has to be searched for in all tumours, since it is probably common in tumours of various organs and is potentially significant for their treatment.

This approach suggests that accumulation of epigenetic changes in progenitor tissue cells leads to various chronic illnesses, impaired tissue regeneration, ageing and death (presentation is given in Table 4). Mutation of the gene responsible for division of progenitor cell would in this case be the trigger that would change the disease development from chronic illnesses linked to ageing to development of a cancerous clone. If this proves to be accurate, it is possible that active prevention methods will be found for a wide spectre of epigenetic diseases, including both malignomas and chronic illnesses of older age.

Cancerous phenotype characteristics common to various types of tumours

Accumulated knowledge on genomes of spontaneously developed human tumours supports the idea that various tumours share some characteristics crucial for development of a neoplasm that can grow into a malignant tumour and metastasize. In their article "The Hallmarks of Cancer" (6) D. Hanahan and R. Weinberg have initially proposed six characteristics, or 'hallmarks', and in 2011 added another four (7).

TABLE 5

Cancer cell phenotype common to the various solid cancers.

Cancer cell phenotype	Possible mechanisms		Examples and consequences
	genetic	epigenetic	
Self-sufficiency in growth signals	YES	YES	Autocrine stimulation – growth self-inflicting with growth factors stimulating the self receptors (EGF, PDGF, c-KIT). Growth without receptor stimulation as a consequence of oncogene activation.
Insensitivity to growth-inhibitory signals	?	YES	Loss of contact inhibition, possible through autocrine stimulation. Loss of respond to the proliferative inhibitors (TGF- β , CDK).
Evasion of apoptosis	YES	?	Inactivation of P53 tumor suppressor gene or activation of anti-apoptotic genes (overexpression of the BCL2 protein).
Sustained angiogenesis	?	YES	Angiopoetin and VEGF – cancer cells and inflammatory cells (macrophages) induce angiogenesis.
Limitless replicative potential	YES	?	Up-regulation of the enzyme telomerase. Alternative lengthening of telomeres. Stem cell concept – self-renewal capacity.
Invasion and metastasis	?	YES	Dissociation of cells: down-regulation of E-cadherin and other adhesion molecules expression. Degradation of the basement membrane and interstitial connective tissue: proteases secretion (metalloproteinases, cathepsin D, urokinase plasminogen activator). Metastasis oncogenes (SNAIL, TWIST): epithelial-to-mesenchymal transition – development of a promigratory phenotype.
Metabolic alterations	?	YES	Growth advantage in the hypoxic tumor microenvironment: Warburg effect or aerobic glycolysis. Changes in specialized functions: hepatocellular cancer cells produce less albumins; plasmacytoma cells can not fuse light and heavy immunoglobulin chains.
Protection from the immune system surveillance	?	YES	Stromal desmoplastic reaction (fibroblast growth and division stimulation protects tumor from the mechanical and immune host reactions).
Genomic instability with chromosomal abnormalities	YES	?	Hyperchromatic nuclei of the cancer cells: more DNA than the normal cell (triploidy, tetraploidy, aneuploidy). Structural chromosomal alterations (deletions, translocations, izochromosome forming, etc). Nuclein acids replication instability (mutations of the DNA-repair proteins: mismatch repair, nucleotide excision repair, and recombination repair – HNPCC syndrome). Amplification of the proto-oncogenes and deletion of the tumor suppressor genes (loss of heterozygosity – LOH).
Immune reaction to the tumor growth	?	YES	Lymphocytes, NK-cells, plasma-cells and macrophages through tumor-specific antigens and tumor-associated antigens (AFP, CEA).

These characteristics are described in Table 5. In an attempt to fuse these two new concepts in the research of solid tumours, epigenetic/genetic model and cancerous phenotype features, Table 5 presents ten characteristics of cancerous phenotype as well as assumed genetic and epigenetic mechanisms for their development.

Epigenetics and treatment of malignant and non-malignant diseases

There are numerous medications that can have influence on epigenetic changes (5). Research of epigenetic diagnostics, epigenetic medications and their effects is among the most researched areas in the field of biomedicine in the last ten years. It is necessary to distinguish two separate areas.

The first one includes epigenetic medications used in treatment of haematologic disorders. DNA methylation

in myelodysplastic syndrome is reduced by azacitadine and decitabine, while vorinostat and romidepsin act on histone changes and are used in treatment of cutaneous T-cell lymphomas. Simultaneous application of two epigenetic drugs is being researched in patients with non-microcellular bronchial cancer (combination of 5-azacitidine, a DNA methylation inhibitor and the histone deacetylase inhibitor entinostat).

The second, potentially much larger area is application of medications that modulate epigenetics in prevention of malignomas and other chronic diseases that are partially or mostly of epigenetic pathogenesis (8). It can be illustrated by the fact that many effects of statine that have been confirmed today (slowing the progression of atherosclerosis by activating endothelial progenitor cells, lower incidence of cancer, etc.) are considered to be epigenetic effects of this group of medications whose main effect is hypolipemic.

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