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Review

# The mutator phenotype theory can explain the complex morphology and behaviour of cancers

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**Abstract.** Almost all solid malignancies exhibit complex cytological and architectural abnormalities, which vary from cell to cell and area to area within the same tumour, and between tumours of the same type. The degrees of these abnormalities do not correlate perfectly with the biological behaviour (especially growth rate and metastatic potential) among the various tumour types. These features of tumours have long been considered to invalidate simple mutational or 'abnormal gene expression' (epigenetic) theories of carcinogenesis. The 'mutator phenotype/clonal selection' hypothesis is based on the now well-established phenomenon of genetic instability of cancer cells, and proposes that this instability is an essential requirement for the development of tumours, and not an irrelevant side-effect of some other process. This paper argues that this hypothesis can provide a satisfactory explanation for the diverse histological and biological features of solid malignancies. Further, because virtually all solid tumours are histologically abnormal, genetic instability is likely to be essential for the malignant process. The concepts of mutator phenotype and clonal selection are therefore supported.

**Key words.** Cancer; histopathology; carcinogenesis; genetic instability; mutator.

## Introduction

Most of the major current theories of cancer emerged in the period 1870–1910, in association with current methods for histological tissue fixation, sectioning and staining. Studies using these methods demonstrated that tumours in any particular organ are usually composed of cells which resemble one cell type normally present in that organ and, if not, they have usually spread there (directly or by metastasis) from another organ [1]. Initially, tumour cells were thought to develop from abnormal local proliferations of embryonically immature 'left-over' cells, or 'cell rests' [1–4]. Tumours were later shown to arise from proliferations of cells of adult tissues [3]. Von Hansemann in 1902 considered that the variations of appearance between tumour cells resemble the transitions of appearance of cells during differentiation of embryonic and adult tissues. He therefore thought that malignancy is a disturbance of these processes and described the gradations of the histological appearances of tumours in terms of 'dedifferentiation' and 'anaplasia' [3, 4]. As an approximate rule, the greatest degrees of 'dedifferentiation' were noted as being associated with the most aggressive behaviour of tumours, but that numerous exceptions occurred. 'Differentiation' and 'anaplasia' are still in use today, and the concept of a disorder of differentiation is among the 'epigenetic' theories of cancer.

Also in pre-1900 biology, mitoses were recognised as a part of the phenomenon of cell division, and meiosis as the basis of gametogenesis for sexual reproduction [5]. Their full significance, however, only became apparent with the rediscovery of genes by Bateson in 1902. With respect to mitosis, it was quickly realised that the precise

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duplication and equal allocation of chromosomes to daughter cells corresponded to the equal duplicate transfer of genetic material required for daughter cells to be identical to the parent cell [5]. The possibility that cancer might be caused by a somatic mutation in a reproducing non-embryonic [somatic] cell type was proposed as early as 1907 by Boveri [4, 6].

A genetic basis for malignancy was supported by the finding by Muller in the 1920s that carcinogenic radiations are also mutagenic [7]. Subsequently, a similar correlation between carcinogenicity and mutagenicity was discovered for chemical carcinogens [7] and, since then, only a few 'non-genotoxic' exceptions [8] have been established.

For the next several decades, genetic theories were strongly advocated by authors including Little [9] and Lockhart-Mummery [10] as the basis of cancer. Others, such as Foulds [11] supported epigenetic theories, using the term 'differential utilisation of the genome'. As described below, no one theory gained universal acceptance because they were unable to explain common morphological phenomena of cancer.

## Morphologic objections to simple epigenetic and genetic theories of cancer

From the outset, most morphological pathologists did not accept that simple genetic change can be the major pathogenetic mechanism of cancer. This was because most then-known genetic mutations are relatively rare single events, which lead to a single, permanent abnormality of the genome which is replicated exactly in all progeny, and is not associated with any greater likelihood of further mutation. The argument was advanced in detail by Willis [4], and remains current [12].

Some of the particular morphological features which are not explained include:

1) the cell-to-cell variation of cytological features (fig. 1) and area-to-area variation of architectural features within the same tumour (fig. 2).

2) the great degrees of architectural and cytological abnormality which vary in a continuous spectrum between cases of tumours of the same cell type, and across the tumour types, but which correlate imperfectly with formation of metastases in the individual patient. Two examples of tumours which are exceptions to the approximate rule that loss of differentiation is associated with worse clinical behaviour are provided (fig. 3).

3) the frequent smooth transitions (i.e. without stepwise morphological changes) from normal to malignant changes which are frequently seen at the margins of tumours, especially those of epithelia.

4) such phenomena as tumour dormancy (cases in which rapidly growing metastases appear many years after the primary has been removed) and tumour progression (local recurrences and metastases showing more aggressive appearances and behaviour patterns than the primary).

5) that in experimental cutaneous carcinogenesis, tumours appear only after long periods (months to years) of time, and almost always in a 'field' of lesser abnormalities, where there is a continuous spectrum from normal to malignant.

Willis [4] concluded that any single-mutation theory of cancer was unlikely because:

> '… in any given class, the individual tumours show every possible gradation of behaviour … and to explain this in terms of gene mutation we would have to suppose almost as many mutations as there are cancers.'

This view has been influential, and authors of current textbooks of pathology address the pathogenesis of cancer with caution. The Oxford Textbook of Pathology (1992) [13] suggests only 'a broad agreement that ... the basic alteration in cancer cells must be a modification in the structure or expression of the genetic material of the cell'. Rubin and Farber (1994) [14] state that '... the causes of most cancers are not identified, and the mechanisms of carcinogenesis remain obscure …'. Walter and Talbot (1996) [15] conclude that '… the fundamental change in the (cancer) cells is not known.'

## Tumor cell heterogeneity and its basis of genetic instability

In the period 1900–1960, the cell in the proliferating fraction of each individual tumour were assumed to be alike, with the remainder of the tumour cell population consisting of variably 'differentiating' post-proliferative cells. The abnormality involving differentiation might be either arrest of the maturation of stem cells, or dedifferentiation of mature cells which retain their ability to proliferate [16].

However, detailed comparisons of cell lines taken from the same primary tumours showed that there are often marked differences in the metastatic capabilities of these proliferating cells. This was confirmed in comparisons of studies of tumour transplantability to athymic mice [17, 18], in which differential immunogenicity of the cell lines in the recipient host can be excluded as a basis for differential metastatic potential. For this phenomenon, the term 'tumour heterogeneity' [19–21] was introduced. Further support for the concept came from documentation of heterogeneity of in vitro functions including motility [22], adhesiveness and enzyme production [23–25] among cell lines from the same primary tumours. Even expression of markers of differentiation, for example, in breast cancer cell lines from one tumour of the breast were shown to be heterogeneous [26, 27].

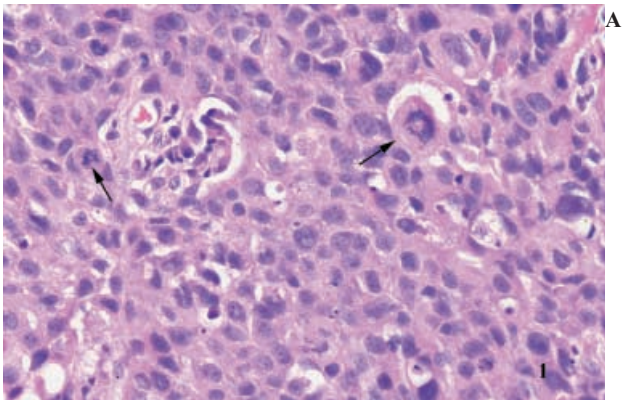


Figure 1. Variation of size and shape of adjacent cells forming a tumour. Note also abnormal mitoses (arrows). From a case of high-grade transitional cell carcinoma of bladder (H&E, ×400).

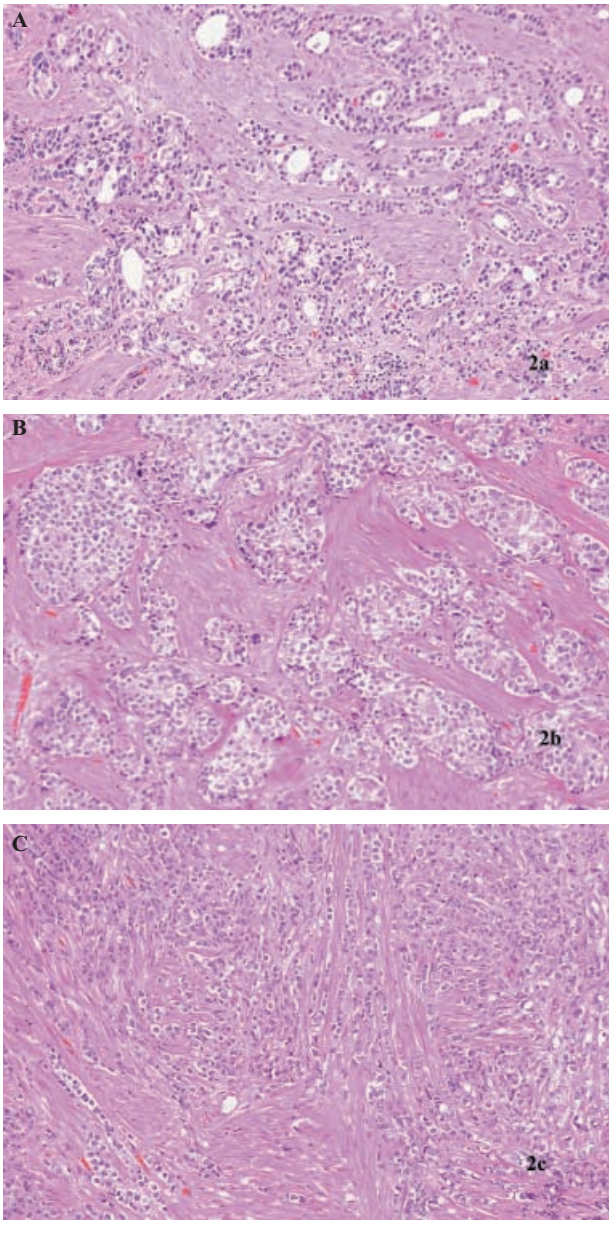


Figure 2. Variation in structures formed by cancer cells of the same case of adenocarcinoma of prostate (H&E, ×400). (*A*) Carcinoma mainly in tubular structures with lumina. (*B*) Carcinoma mainly as well-circumscribed groups, but without lumina. (*C*) Carcinoma mainly as cords and single cells between smooth muscle fibres.

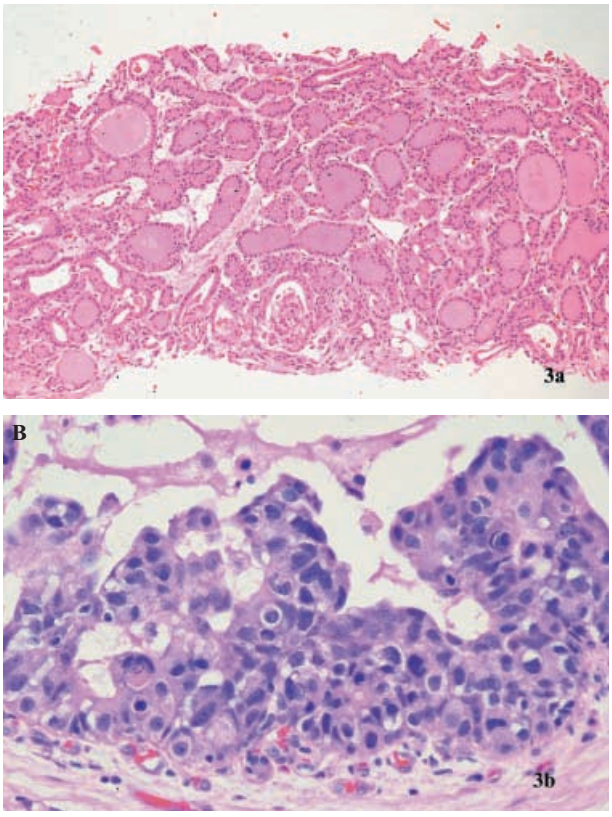


Figure 3. Examples of tumours which are exceptions to the approximate rule of greater degrees of histological abnormality being associated with worse behaviour. (*A*) Histologically almost normal tumour which has metastasised to bone from a primary carcinoma of the thyroid gland. Note the regular follicular structures, and slight pleomorphism of the tumour cells (bone biopsy, H&E, ×400) [a similar case is illustrated in ref. 106]. (*B*) Morphologically highly abnormal cells in a lesion which is not invasive. Note the nuclear and cytoplasmic pleomorphism, but the absence of invasion of the basement membrane. From a case of in situ ductal carcinoma of the breast (high grade) (H&E, ×400) [other cases of this lesion are illustrated in ref 107].

As has been indicated above, tumour cell behavioural heterogeneity could lie either in the control mechanisms for expression of the relevant genes (epigenetic mechanisms) or the tumour cell genes themselves (heterogenous mutations). However, only since the 1980s has it been possible to quantify overall genetic variability in human tumour cells. Many forms of DNA damage (e.g. gene deletion, point mutation, frameshift mutation) are present in tumour cells [28] in addition to those associated with chromosomal abnormalities. Individual tumour cells have recently been reported to include thousands of genomic events [29, 30].

To achieve these large numbers of mutations, the rate of mutation in tumours is much greater than any 'background' mutation rate in any normal tissue, and the term 'genetic instability' was applied, especially in relation to tumour progression [31, 32]. The whole process of genetic instability takes many cell divisions, and hence many months to years to develop. This is in keeping with the known 'latent period' of production of malignancy observed in both experimental and clinical studies.

Numerous mechanisms for genetic instability have been documented. The first recognised were those of mutation-induced deactivation of DNA repair mechanisms which lead to an increased incidence of cutaneous tumours in the xeroderma pigmentosa syndrome [33]. Currently, mutations which result in defects of DNA repair mechanisms are thought to be a major mechanism underlying genetic instability of tumours [34].

Other possible mechanisms of hypermutability include damage to genes of DNA helicase [35], of chromosome stabilisation [36], of chromosome segregation during mitosis [35, 37, 38], of the mitotic spindle checkpoint [39] and of nucleotide excision repair [40, 41]. Alternatively, influences on epigenetic gene-stabilising mechanisms, such as DNA methylation [42], interactions with spindle proteins [43] and telomerase dysfunction [44–46] have been proposed as mechanisms of genetic instability.

## Mutator phenotype clonal selection theory of the pathogenesis of malignancy

Since its discovery, many authors have identified genetic instability as another example of the chaotic effects rather than as an essential part of the malignancy process [47].

However, to emphasise the excessive and accelerated rate of accumulation of mutations in individual tumour cells, and based on previous work by Nowell [31], Loeb and colleagues [37, 48–50] used the term 'mutator phenotype' (from the nomenclature of bacterial mutants with increased mutation rates) [51]. According to this theory, carcinogenic agents cause primary damage to the mechanisms responsible for fidelity of genetic replication in reproducing cells, so that somatic mutations in tumour cells accumulate at a much faster rate than is possible at the 'normal' spontaneous somatic mutation rate. The resulting progeny are cell biologically diverse, with many daughter cells probably being non-viable. Randomly, however, cells form which have differing combinations of mutations, in variable degrees, necessary for excessive growth, invasion, metastasis and other malignant features. These cells self-select into expanding clones. The precise combinations and intensities of the various pro-malignant cell biological changes in the clone(s) determine the behaviour of the particular tumour in each individual patient.

## Mechanisms of morphological abnormalities of neoplastic cells in terms of genetic instability and the mutator phenotype theory

Obviously once a high random mutation rate develops in a proliferating population of cells, then all aspects of the metabolism become susceptible to enhancement or reduction in random combinations on a cell-by-cell basis. In tumours, all molecules involved in the structure, function and behaviour (including interaction with adjacent cells) of the cells are then susceptible to this variability. The following does not deal with obviously injurious mutations to the genes of well-established cell structures ('housekeeping' genes), e.g. for the plasma membrane, signal receptors and enzymes of metabolism. Nor does it deal with factors increasing the mitotic rate ('growth factors') as these are discussed elsewhere [52–54]. Rather, it discusses some of the more topical, and less well understood structures, in terms of mutation-based explanations of cellular pleomorphism of tumours.

## Chromatin, chromosomes and nuclei

Traditionally, the interphase nucleus has been considered a bag containing randomly mixed, partly unfolded chromosomal material [55]. Several contrary ideas have been suggested.

### Binding of chromatin to the nuclear membrane

There is now strong evidence for in vivo binding of chromatin to the nuclear membrane. First, the phenomenon is observable in some living non-mammalian cells [56]. Second, the Barr body is a folded X chromosome, usually bound to the inner aspect of the cell membrane, in a constant relative position in cells according to cell type [56].

Third, lamins have been identified as one particular family of intranuclear fibrous proteins [57] which are particularly located in the submembranous region of the nucleus (also referred to as the 'nuclear lamina'). Lamins are thought to have a structural role, giving the periphery of the nucleus mechanical strength [58] and continuity with the cytoskeleton [59]. Lamins are known to bind chromatin and

certain DNA sequences and so may have an additional role in the control of gene expression [60].
Whether or not the centromeres (those parts of the chromosomes to which the spindle attaches in metaphase) are particularly associated with the nuclear membrane is controversial, being supported by some [61] but not other [62] authors.
In malignancy, excessive sites for chromatin-nuclear membrane binding (centromeric or otherwise) are likely to influence nuclear size and shape. Chromosomes with two or more binding sites might produce focal intranuclear bands which draw opposite sides of the nucleus inwards, the binding site being at the apex of the invaginated parts [this phenomenon is illustrated in ref 63].

**Chromosomal domains**
Evidence for the existence of specific, mutually exclusive chromosomal volumes (domains) in the nucleus, rather than the chromatin of the chromosomes being randomly intermixed, has been obtained in the last 20 years using the technique of fluorescence in situ hybridisation [64]. Such studies have shown that, in interphase, the unfolded chromosomes occupy discrete (non-overlapping) domains which collectively fill the entire interior of the nucleus [65–68].
In malignancy, variable chromosome domain size due to chromosomal lesions, especially aneuploidy, is likely to contribute to variations in nuclear size and shape [63].

**Do the domains of chromosomes occupy the same relative intranuclear positions in all cells of the same type?**
An additional proposal has been that the chromosomes in their domains are in the same relative position in the nuclei of all cells of a particular cell type [69]. This is supported by the constancy of location of the Barr body in the cells of particular tissues. However, for other chromosomes and cell types, the suggestion is controversial [70].
Whether malignancy might require some rearrangement of the sites of specific chromosome-nuclear membrane attachments is not known.

**Nuclear matrix and the nuclear skeleton/scaffold**
Characterisation of the non-histone proteins of the nucleus in the search for 'transcription factors' and other gene-expression-controlling proteins has identified relatively insoluble types of nuclear proteins which are sometimes referred to as nuclear 'matrix' proteins [71–73]. These, together with nuclear membrane proteins (especially lamins, see above) and the nucleolar matrix proteins have been suggested to form a nuclear 'scaffold' [61]. The function of the matrix or scaffold has been suggested to be an internal skeleton, to which transcription-regulating proteins and/or DNA itself might become attached, so that gene transcription may be controlled [71–73]. Not all authors agree [74].
Nickerson [75] has speculated that nuclear matrix abnormalities might contribute to the pathogenesis of malignancy by reducing the fidelity of gene replication and the control of gene expression. The abnormalities might also contribute to abnormal shape.

**Nuclear membrane permeability and pores**
Nuclear membrane permeability has long been known to be limited and selective, and controlled by pores [76–78]. Molecules up to 20–40 kDa diffuse passively through pores, while larger molecules, including nuclear proteins, and RNAs require specific active mechanisms for import or export. Nuclear pores are complexes of 'nucleoporins', of which there are 50–100 different types in vertebrates.
Among malignancies, nuclear pore function has been little studied [79–81], and little is known of the possibility of cell-to-cell variation of nuclear pore function among pleomorphic nuclei of cancer cells. If nuclear pore permeability were to vary from cell to cell among cancer cells, then non-specific diffusion of cytoplasmic materials into the nucleus may variably alter nuclear appearances. Abnormal nuclear pore function in a cell might affect overall nuclear size. Similarly, abnormal egress of nuclear contents into the cytoplasm might affect RNA transcription and other cytoplasmic functions.

**Mitoses**
Morphological lesions of chromosomes in malignancy have been well described since the 19th century [1–3] and in current texts [63, 82, 83]. Variable cell-to-cell mutations of the genes for the proteins associated with mitosis could obviously give rise to these abnormalities. For example, abnormal spindle proteins [84] could lead to separation of chromosomes from the spindle. Loss of DNA-binding proteins including histones [85] could lead to mechanical weakness of chromosomes and their fragmentation, and excess proteins involved in DNA condensation could lead to chromosomal stickiness [86] and other abnormalities.
Of note is that a wide variety of toxins [87] can cause chromosomal aberrations, and these might act by direct impairment of a chromosomal protein, mimicking the possible genetically derived aberrations occurring in tumours. This supports the idea of abnormal nucleoproteins having a role in abnormal mitoses.

**Cytoplasmic volume**
The cytoplasmic size which a cell exhibits at any particular time in its life cycle (mitotic formation, development,

senescence) depends on the net balance of synthesis over catabolism of space-occupying material over its life span (development and senescence), modified by reduction of cytoplasm by further mitosis. As a result, the largest cells are likely to be those with the highest net positive balance of synthesis, which have the most prolonged life span before senescence, and which do not undergo mitosis (mitosis without cytokinesis results in multinucleate giant cells). Mutation of cell cycle regulators, such as cyclins and cyclin-dependent kinases [88] may be particularly relevant.

**Cytoplasmic shape: the cytoskeleton**

Mechanisms governing cell shape largely involve the cytoskeletal proteins actin, tubulin and vimentin [89, 90]. These are attached to the submembranous 'cortex', or the internal surface of the plasma membrane itself. Angulations of cell outline could occur by way of abnormal attachments at both ends of shorter-than-normal fibres over longer-than-normal spans of membrane.

Balloonings of cell outline might be due to loss of attachment of the fibres to the relevant part of the membrane.

**Gap junctions**

Injections of small molecules into the cytoplasm of individual cells have been shown to 'leak' into adjacent cells according to size, through structures identifiable in electron micrographs as gaps in the contiguous plasma membranes. Normal gap junctions are regulated by the content of their unique proteins ('connexins') and have a permeability limit of 1–2 kDa [91]. Their function is thought to relate to control of the behaviour of adjacent cells [92–94], for example, when cells growing to contact with each other cease multiplying ('contact inhibition' [95]).

Cancer cells in culture frequently exhibit reduced proliferation and other aggressive features when grown in contact with normal cells [11]. The basis for these supposed 'normalising' intercellular controlling influences may be movement of small, controlling molecules through gap junctions [96–98]. Malignancy is usually associated with decreased connexin expression, and loss of gap junction density [99]. Fentiman et al. [100] described gap junctions between breast cancer cells and different cell types ('non-selective communication') in coculture. Clearly, genetic instability could cause cell-by-cell variation of these functions in individual tumours.

**Gradual changes of morphology at the margins of tumours**

The gradual change of malignant to benign histological appearances at the margins of tumours could be due to either the intermixing of benign and malignant populations of cells, as Azzopardi [101] suggests occurs in ductal lesions of the breast, or to variable cell-cell communications via gap junctions (see above).

**Differentiation**

Both embyrological and histological differentiation involve alteration of appearance and function of cells through their life span. The process probably relies on activation and inactivation of groups of genes in the correct order, but the 'master switch' genes are poorly understood [102]. The means by which the ordering of the gene activation occurs is unknown, but is almost certainly chemical, and may involve Id proteins [103]. Any of these mechanisms may be liable to mutational interference [104].

Tumours are characterised by variable degrees of incomplete differentiation-related change of appearance. This has been discussed in terms of dedifferentiation, or stem cell arrest [16]. Either process could be affected by genetic instability in terms of randomly variable modifications of the genes required for the relevant steps. However, the appearances might be explicable in clonal terms, in that a highly proliferative clone, although capable of differentiation, might be denied the opportunity to express the differentiation genes by nutrient or other pressure of the proliferating cells.

Reverse differentiation (e.g. the production of alphafoetoprotein by hepatocarcinoma cells arising in an adult) is an uncommon phenomenon, and presumably results in derepression of embryonic genes [105].

## Conclusions

The cell-to-cell variability of appearances and behaviour of cancer cells has prevented acceptance of all previous theories of the pathogenesis of malignancy of solid tumours. Genetic instability, however, provides a mechanism by which cell-to-cell variability can occur, through mutation of the genes for the large number of morphology-affecting proteins which are now known to exist.

Because cell-to-cell variability is virtually always observed in solid tumour malignancy, the cause of the variability is likely to be necessary for malignancy. If genetic instability is the cause of variable morphology, then genetic instability is likely to be necessary for malignancy. This last idea, already described as the 'mutator phenotype theory', can be considered strongly supported as the major pathogenetic mechanism of malignancy.

1 Ewing J. (1928) Neoplastic diseases, 3rd edn, Saunders, Philadelphia
2 Cohnheim J. (1889) Lectures in General Pathology, trans. McKee A. B., London, New Sydenham Society
3 Rather L. J. (1978) The Genesis of Cancer: A Study in the History of Ideas, Johns Hopkins University Press, Baltimore
4 Willis R. A. (1948) Pathology of Tumours, Butterworths, London

5 Ford C. E. (1970) Pathological consequences of chromosomal abnormality. In: General Pathology, 4th edn, p. 590, Florey H. W. (ed), Lloyd Luke, London
6 Burdette W. J. (1955) The significance of mutation in relation to the origin of tumours: a review. Cancer Res. **15:** 201–226
7 Berenblum I. (1958) The study of tumours in animals. In: General Pathology, 2nd edn, pp. 513–549, Florey H. W. (ed.), Lloyd Luke, London
8 Williams G. M. and Whysner J. (1996) Epigenetic carcinogens: evaluation and risk assessment. Exp. Toxicol. Pathol. **48:** 189–195
9 Little C. C. (1922-3) The relation of genetics to the problems of cancer research. Harvey Lectures **17:** 65–68
10 Lockhart-Mummery J. P. (1934) The Origin of Cancer, Churchill, London
11 Foulds L. (1969) Neoplastic Development, Academic Press, London
12 Iversen O. H. (1995) Of mice and men: a critical reappraisal of the two-stage theory of carcinogenesis. Crit. Rev. Oncog. **6:** 3357–3405
13 McGee J. O'D., Isacson P. G. and Wright N. A. (eds) (1992) Oxford Textbook of Pathology, vol. 1, p. 636, Oxford University Press, Oxford
14 Rubin E. and Farber J. L. (eds) (1994). Pathology, 2nd edn, p. 144, Lippincott, Philadelphia
15 Walter J. B. and Talbot I. C. (eds) (1996) General Pathology, p. 530, Churchill Livingstone, Edinburgh
16 Sell S. (1993) Cellular origin of cancer: dedifferentiation or stem cell maturation arrest? Environ. Health Perspect. **101** (suppl 5)**:** 15–26
17 Carney D. N. (1986) Recent advances in the biology of small cell lung cancer. Chest **89:** 253S–257S
18 Fidler I. J. (1986) Rationale and methods for the use of nude mice to study the biology and therapy of human cancer metastasis. Cancer Metastasis Rev. **5:** 29–49
19 Brattain M. G, Levine A. E., Chakrabarty S., Yeoman L. C., Willson J. K. and Long B. (1984) Heterogeneity of human colon carcinoma. Cancer Metastasis Rev. **3:** 177–191
20 Heppner G. H. (1984) Tumour heterogeneity. Cancer Res. **44:** 2259–2265
21 Heppner G. H. and Miller F. R. (1998) The cellular basis of tumour progression. Int. Rev. Cytol. **177:** 1–56
22 Schor S. L., Grey A. M., Picardo M., Schor A. M., Howell A., Ellis I. et al. (1991) Heterogeneity amongst fibroblasts in the production of migration stimulating factor (MSF): implications for cancer pathogenesis. EXS **59:** 127–146
23 Walker R. A., Jones J. L., Chappell S., Walsh T. and Shaw J.A. (1997) Molecular pathology of breast cancer and its application to clinical management. Cancer Metastasis Rev. **16:** 5–27
24 Beckmann M. W., Niederacher D., Schnurch H. G., Gusterson B. A. and Bender H. G. (1997) Multistep carcinogenesis of breast cancer and tumour heterogeneity. J. Mol. Med. **75:** 429–439
25 Dabelsteen E. (1996) Cell surface carbohydrates as prognostic markers in human carcinomas. J. Pathol. **179:** 358–369
26 Warnes T. W. and Smith A. (1987) Tumour markers in diagnosis and management. Baillieres Clin. Gastroenterol. **1:** 63–89
27 Underwood J. C. (1983) Oestrogen receptors in human breast cancer: review of histopathological correlations and critique of histochemical methods. Diagn. Histopathol. **6:** 1–22
28 Jackson A. L. and Loeb L. A. (1988) On the origin of multiple mutations in human cancers. Semin. Cancer Biol. **8:** 421–429
29 Stoler D. L., Chen N., Basik M., Kahlenberg M. S., Rodriguez-Bigas M. A., Petrelli N. J. et al. (1999) The onset and extent of genomic instability in sporadic colorectal tumor progression. Proc. Natl. Acad. Sci. USA **96:** 15121–15126
30 Boland C. R. and Ricciardiello L. (1999) How many mutations does it take to make a tumor? Proc. Natl. Acad. Sci. USA **96:** 14675–14677
31 Nowell P. C. (1976) The clonal evolution of tumor cell populations. Science **194:** 23–28
32 Hill R. P. (1990) Tumor progression: potential role of unstable genomic changes. Cancer Metastasis Rev. **9:** 137–147
33 Cleaver J. E. (1994) It was a very good year for DNA repair. Cell **76:** 1–4
34 Perkins A. S. and Stern D. F. (1997) Molecular biology of cancer: oncogenes. In: Cancer Principles and Practice of Oncology, 5th edn, DeVita V. T., Hellman S. and Rosenberg S. A. (eds), pp. 79–102, Lippincott-Raven, Philadelphia
35 Miyagawa K. (1998) Genetic instabilty and cancer. Int. J. Haematol. **67:** 3–14
36 Tarapore P. and Fukasawa K. (2000) p53 mutation and mitotic infidelity. Cancer Invest. **18:** 148–155
37 Loeb L. A. (1998) Cancer cells exhibit a mutator phenotype. Adv. Cancer Res. **72:** 25–26
38 Holloway S. L., Poruthu J. and Scata K. (1999) Chromosome segregation and cancer. Exp. Cell Res. **253:** 308–314
39 Cahill D. P., Lengauer C., Yu J., Riggins G. J., Willson J. K., Markowitz S. D. et al. (1998) Mutations of mitotic checkpoint genes in human cancers. Nature **392:** 300–303
40 Canitrot Y., Hoffmann J. S., Calsou P., Hayakawa H., Salles B. and Cazaux C. (2000) Nucleotide excision repair DNA synthesis by excess DNA polymerase beta: a potential source of genetic instability in cancer cells. FASEB J. **14:** 1765–1774
41 Friedberg E. C. (2000) Rous-Whipple Award Lecture. Nucleotide excision repair and cancer predisposition: a journey from man to yeast to mice. Am. J. Pathol. **157:** 693–701
42 Jones P. A. and Laird P. W. (1999) Cancer epigenetics comes of age. Nat. Genet. **21:** 163–167
43 Duesberg P., Rasnick D., Li R., Winters L., Rausch C. and Hehlmann R. (1999) How aneuploidy may cause cancer and genetic instability. Anticancer Res. **19:** 4887–4906
44 Dhaene K., Van Marck E. and Parwaresch R. (2000) Telomeres, telomerase and cancer: an up-date. Virchows Arch. **437:** 1–16
45 Artandi S. E., Chang S., Lee S. L., Alson S., Gottlieb G. J., Chin L. et al. (2000) Telomere dysfunction promotes non-reciprocal translocations and epithelial cancers in mice. Nature **406:** 641–645
46 Artandi S. E. and DePinho R. A. (2000) Mice without telomerase: what can they teach us about human cancer? Nat. Med. **6:** 852–855
47 Prehn R.T. (1994) Cancers beget mutations versus mutations beget cancers. Cancer Res. **54:** 5296–5300
48 Cheng K. C. and Loeb L. A. (1993) Genomic instability and tumor progression: mechanistic considerations. Adv. Cancer Res. **60:** 121–156
49 Jackson A. L. and Loeb L. A. (1988) On the origin of multiple mutations in human cancers. Semin. Cancer Biol. **8:** 421–429
50 Loeb K. R. and Loeb L. A. (2000) Significance of multiple mutations in cancer. Carcinogenesis **21** 379–385
51 Hoess R. H. and Herman R.K. (1975) Isolation and characterization of mutator strains of *Escherichia coli* K-12. J. Bacteriol. **122:** 474–484
52 Mendelsohn J. and Lippman M. E. (1993) Principles of molecular cell biology of cancer: growth factors. In: Cancer Principles and Practice of Oncology, 4th edn, DeVita V. T., Hellman S., Rosenberg S. A. (eds), pp. 114–133, Lippincott, Philadelphia
53 Wells A. (2000) Tumor invasion: role of growth factor-induced cell motility. Adv. Cancer Res. **78:** 31–101
54 Favoni R.E. and Cupis A. de (2000) The role of polypeptide growth factors in human carcinomas: new targets for a novel pharmacological approach. Pharmacol. Rev. **52:** 179–206

55 Weiss L. (1983) Histology, Cell and Tissue Biology, 4th edn, p. 25, Elsevier, New York
56 Mirsky A. E. and Osawa S. (1961) The interphase nucleus. In: The Cell: Biochemistry, Physiology, Morphology, vol. 2; pp. 677–770, Brachet J. and Mirsky A. E. (eds), Academic Press, New York
57 Gruenbaum Y., Wilson K. L., Harel A., Goldberg M. and Cohen M. (2000) Nuclear lamins – structural proteins with fundamental functions. J. Struct. Biol. **129:** 313–323
58 Stuurman N., Heins S. and Aebi U. (1998) Nuclear lamins: their structure, assemby and interactions. J. Struct. Biol **122:** 42–66
59 Bosman F. T. (1999) The nuclear matrix in pathology. Virchows Arch. **435:** 391–399
60 Hutchinson C. J., Alvares-Reyes M. and Vaughabn O. A. (2001) Lamins in disease: why do ubiquitous nuclear envelope proteins give rise to tissue-specific disease phenotypes. J. Cell Sci.**114:** 9–19
61 Haaf T. and Schmid M. (1991) Chromosome topology in mammalian interphase nuclei. Exp. Cell Res. **92:** 325–332
62 Santos S. J., Singh N. P. and Natarajan A. T. (1997) Fluorescence in situ hybridization with comets. Exp. Cell Res. **232:** 407–411
63 Bibbo M. (ed) (1991) Comprehensive Cytopathology, pp. 1–13, pp. 474, Saunders, Philadelphia
64 Albertson D. G., Fishpool R. M. and Birchall P. S. (1995) Fluorescence in situ hybridization for the detection of DNA and RNA. Methods Cell Biol. **48:** 339–364
65 Alberts B., Bray D., Lewis J., Raff M., Roberts K. and Watson I. D. (eds) (1989) Molecular Biology of the Cell, pp. 483–504, Garland, New York
66 Spector D. L. (1993) Macromolecular domains within the cell nucleus. Annu. Rev. Cell Biol. **9:** 265–315
67 Cremer T., Kreth G., Koester H., Fink R. H., Heintzmann R., Cremer M. et al. (2000) Chromosome territories, interchromatin domain compartment, and nuclear matrix: an integrated view of the functional nuclear architecture. Crit. Rev. Eukaryot. Gene Expr. **10:** 179–212
68 Visser A. E, Jaunin F., Fakan S. and Aten J. A. (2000) High resolution analysis of interphase chromosome domains. J. Cell Sci. **113:** 2585–2593
69 Nagele R. G., Freeman T., McMorrow L., Thomson Z., Kitson-Wind K. and Lee H. Y. (1999) Chromosomes exhibit preferential positioning in nuclei of quiescent human cells. J. Cell Sci. **112:** 525–535
70 Santos S. J., Singh N. P and Natarajan A. T. (1997) Fluorescence in situ hybridization with comets. Exp. Cell Res. **232:** 407–411
71 Stuurman N., Meijne A. M., Pol A. J. van der, Jong L. de, Driel R.van and Renswoude J. van (1990) The nuclear matrix from cells of different origin: evidence for a common set of matrix proteins. J. Biol Chem. **265:** 5460–5465
72 Nelson W. G., Pienta K. J., Barrack E. R. and Coffey D. S. (1986) The role of the nuclear matrix in the organization and function of DNA. Annu. Rev. Biophys. Biophys. Chem. **15:** 457–475
73 Bosman F. T. (1999) The nuclear matrix in pathology. Virchows Arch. **435:** 391–399
74 Pederson T. (2000) Half a century of 'the nuclear matrix'. Mol. Biol. Cell. **11:** 799–805
75 Nickerson A. (1998) Nuclear dreams: the malignant alteration of nuclear architecture. J. Cell Biochem. **70:** 172–180
76 Bagley S., Goldberg M. W., Cronshaw J. M., Rutherford S. and Allen T. D. (2000) The nuclear pore complex. J. Cell Sci. **113:** 3885–3886
77 Macara I. G. (2001) Transport into and out of the nucleus. Microbiol. Mol. Biol. Rev. **65:** 570–594
78 Vasu S. K. and Forbes D. J. (2001) Nuclear pores and nuclear assembly. Curr. Opin. Cell Biol. **13:** 363–375
79 Hood J. K. and Silver P. A. (1993) Diverse nuclear transport pathways regulate cell proliferation and oncogenesis. Biochim. Biophys. Acta **1471:** M31–M41
80 Martinez N., Alonso A., Moragues M. D., Ponton J. and Schneider J. (1999) The nuclear pore complex protein Nu88 is overexpressed in tumour cells. Cancer Res. **59:** 5408–5411
81 Sugie S., Yoshimi N., Tanaka T., Mori H. and Williams G. M. (1994) Alterations of nuclear pores in preneoplastic and neoplastic rat liver lesions induced by 2-acetylaminofluorene. Carcinogenesis **15:** 95–98
82 Hiem S. and Mitelamn F. (1995) Cancer cytogenetics, 2nd edn, Wiley-Liss, New York
83 Ruddon R. W. (1995) Cancer Biology, 3rd edn, Oxford University Press, New York
84 Duensing S. and Munger K. (2001) Centrosome abnormalities, genomic instability and carcinogenic progression. Biochim. Biophys. Acta **1471:** M81–M88
85 Archer S. Y. and Hodin R. A. (1999) Histone acetylation and cancer. Curr. Opin. Genet. Dev. **9:** 171–174
86 Gaulden M. E. (1987) Hypothesis: some mutagens directly alter specific chromosomal proteins (DNA topoisomerase II and peripheral proteins) to produce chromosome stickiness, which causes chromosome aberrations. Mutagenesis **2:** 357–365
87 Fiskesjo G. (1988) The Allium test – an alternative in environmental studies: the relative toxicity of metal ions. Mutat. Res. **197:** 243–260
88 Cordon-Carlo C. (1995) Mutations of cell cycle regulators: biological and clinical implications for human neoplasia. Am. J. Pathol. **147** 545–560
89 Pawlak G. and Helfman D. M. (2001) Cytoskeletal changes in cell transformation and tumorigenesis. Curr. Opin. Genet. Dev. **11:** 41–47
90 Holth L. T., Chadee D. N., Spencer V. A., Samuel S. K., Safneck J. R. and Davie J. R. (1998) Chromatin, nuclear matrix and the cytoskeleton: role of cell structure in neoplastic transformation. Int. J. Oncol. **13:** 827–837
91 Hertzberg E. L., Lawrence T. S. and Gilula N. B. (1981) Gap junctional communication. Annu. Rev. Physiol. **43:** 479–491
92 Unwin P. N. (1987) Gap junction structure and the control of cell-to-cell communication. Ciba Found. Symp. **125:** 78–91
93 Klaunig J. E. and Ruch R. J. (1990) Role of inhibition of intercellular communication in carcinogenesis. Lab. Invest. **62:** 135–146
94 Bertram J. S. (2000) Cellular communication via gap junctions. Sci. Med. **March/April:** 18–27
95 Goldberg G. S., Bechberger J. F., Tajima Y., Merritt M., Omori Y., Gawinowicz M. A. et al. (2000) Connexin43 suppresses MFG-E8 while inducing contact growth inhibition of glioma cells. Cancer Res. **60:** 6018–6026
96 Yamasaki H. (1991) Aberrant expression and function of gap junctions during carcinogenesis. Environ. Health Perspect. **93:**191–197
97 Ruch R. J. (1994) The role of gap junctional intercellular communication in neoplasia. Ann. Clin. Lab. Sci. **24:** 216–231
98 Trosko J. E. and Ruch R. J. (1998) Cell-cell communication in carcinogenesis. Front. Biosci. **3:** D208–D236
99 Weinstein R. S. and Pauli B. U. (1987) Cell junctions and the biological behaviour of cancer. Ciba Found. Symp. **125:** 240–260
100 Fentiman I. S., Hurst J., Ceriani R.L. and Taylor-Papadimitriou J. (1979) Junctional intercellular communication pattern of cultured human breast cancer cells. Cancer Res. **39:** 4739–4743
101 Azzopardi J. G. (1979) Problems in Breast Pathology, Saunders, London
102 Boucamp P. (1995) Transdifferentiation induced by gene transfer. Semin. Cell Biol. **6:** 157–163

103 Lasorella A., Uo T. and Iavarone A. (2001) Id proteins at the cross-road of development and cancer. Oncogene **20:** 8326–8333
104 Bos J. L. and Kreijl C. F. van (1992) Genes and gene products that regulate proliferation and differentiation: critical targets in carcinogenesis. IARC Sci. Publ. **116:** 57–65
105 Sell S., Becker F. F., Leffert H. L. and Watabe L. (1976) Expression of an oncodevelopmental gene product (alpha-fetoprotein) during fetal development and adult oncogenesis. Cancer Res. **36:** 4239–4249
106 Rosai J., Carcangui M. L. and Delellis R. A. (1992) Tumours of the Thyroid Gland, p 61, Armed Forces Institute of Pathology, Washington
107 Rosen P. P. and Oberman H. A. (1993) Tumours of the Mammary Gland, p 148–149. Armed Forces Institute of Pathology, Washington