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Review

The mutator phenotype theory of carcinogenesis and the complex histopathology of tumours: support for the theory from the independent occurrence of nuclear abnormality, loss of specialisation and invasiveness among occasional neoplastic lesions

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Abstract. The mutator phenotype theory of carcinogenesis suggests that genetic instability is an early and essential part of tumour development. This instability provides for substantially random cell-to-cell genomic variation (genomic heterogeneity) to arise among cells of individual tumours. Genetically unstable cells then produce 'successful' clones of cells with the necessary mutations for malignant behaviour. In a previous paper (Bignold L. P., Cell. Mol. Life Sci. 2002; *59:* 950–958), it was pointed out that a population of cells which is heterogeneous for behaviour-related genes may well also be heterogeneous for morphology-related genes. This would result in cellular pleomorphism among cells of individual tumours, and so explain this almost universal characteristic of solid malignancies.

If the concept of random genomic variability applies fully to the histopathology of tumours, then most tumours should show a mixture of neoplastic features, especially nuclear atypia, loss of specialised function (such as loss of production of mucus by glandular cells) and invasiveness. However, occasional lesions might be expected to occur which show these characteristics independently. That is, lesions should exist which exhibit one or two of the three characteristics of neoplasms without the other(s).

This paper identifies, among human tumours, lesions which show independence of these characteristics. Two of the examples discussed are a Bowenoid solar keratosis that shows severe nuclear atypia, but no apparent loss of specialisation and no invasiveness. On the other hand, anaplastic small cell carcinoma of the lung often exhibits marked loss of differentiation, very aggressive invasion and metastasis, but little nuclear pleomorphism.

These examples are considered to provide further support for the importance of the mutator phenotype to the pathogenesis of neoplasia.

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

Introduction: genetic instability and the mutator phenotype theory of carcinogenesis

Mitotic chromosomes were known to be unstable in tumour cells in the 19th century [1, 2]. However, until the middle of the 20th century, genes were not recognised to be a necessary part of the integral structure of chromosomes, and so chromosomal instability was not equated necessarily with any potential enhanced incidence of mutations of transcribable DNA (genetic instability) [2]. In the early 1960s, genetic instability was described as occurring in bacteria [3], and strains of bacteria showing hypermutation rates were referred to as 'mutator strains' as early as 1967 [4].

The application of genetic instability to neoplastic phenomena began at this time. Ono in 1971 [5] and later Nowell in 1976 [6] suggested that mutant clones may arise within a malignant cell population and wax or wane according to local selective pressures. The idea was supported by results of studies of experimental studies of invasion and metastasis using athymic mice [7]. These showed that cell lines could be grown from individual human tumours which had markedly differing (heterogeneous) capacities for invasion and metastasis, implying genetic differences between the cell lines. Also, a basis for tumour dormancy and progression could be provided [8].

The number of mutations in tumour cells was initially thought to be low, and accurate estimations of the numbers of mutations occurring in tumour cells required the development of modern methods of molecular biology. With improved methods of polymerase chain reaction (PCR), up to 11,000 alterations of genes and nontranscribing DNA ('genomic events') per carcinoma cell have been detected [9].

The significance of the high number of mutations has been controversial [10]. However, Loeb [11] has proposed that this genetic instability is not an incidental side effect of the malignant process, but rather a necessary early aspect of the development of tumours. He has applied the term 'mutator phenotype' (previously used in reference to bacteria, see above) to human tumours to emphasise this idea. A more detailed review of theories of carcinogenesis, especially in relation to the histopathology of tumours, is given in [12]).

Genetic instability can account for cellular pleomorphism among cells of individual tumours

The hypermutation of genetic instability is not known to be selective for any part of the genome, and thus not necessarily limited to genes for invasion and metastasis. The present author has argued [12] that if genetic instability can account for variable cell-to-cell behaviour in tumour cells by way of invasion- and metastasis-enhancing mutations, then the phenomenon can also account for the variable cell-to-cell appearances of tumour cells by invoking variable appearance-altering mutations in the cells. A variety of nuclear and cytoplasmic phenomena and structures, which, if their genes were mutant, might alter cellular and nuclear appearances. They include especially the binding of chromatin to nuclear membrane, the possible existence of a nuclear matrix, the functions of nuclear pores and attachments of cytoskeletal structures to the outer nuclear membrane [12]. Further, some of these changes might be exacerbated by histological processing, thus producing the familiar changes of abnormal chromatin patterns in histopathological preparations of tumours [13].

Nuclear abnormalities, loss of specialisation and invasiveness as histopathological categories for assessing randomness of genomic variability

The various histopathological features of tumour cells can be grouped into three categories. The development of each of these probably requires some separate mutational and other pathogenetic mechanisms as follows.

Nucleus-to-nucleus variability of appearance (nuclear atypia, pleomorphism)

This change is almost universal among solid tumours. The appearance of normal nuclei after histological processing is governed by many factors, especially activation status of the cell (e.g. memory vs. activated lymphocyte nuclei), which may act through changes of content of non-histone protein and RNA of the nuclei. Another major factor is the fixative used in histological processing [13].

Also affecting the appearance of tumour cell nuclei are aneuploidy, and abnormal chromosome-chromosome interactions. The latter is manifest in mitosis as chromosomal 'stickiness' [1]. Abnormal non-histone protein patterns may occur in association with nuclear translocation of proteins which are usually located in the cytoplasm. This has been documented as directly associated with mutant nuclear pore proteins [14].

Loss of specialisation

This change (loss of tissue differentiation) is very common among solid tumour cells, and may well result from simple disabling mutations of the specific genes for the relevant metabolic process. For example, disabling mutations of the enzymes for synthesis of keratin may result in a squamous cell carcinoma being poorly differentiated compared with one in which keratin production is preserved.

Invasiveness and colonisation of remote tissues (metastasis)

These phenomena are essential aspects of malignancy. They are not part of 'normal' behaviour of most cell types (e.g. epithelia), but are seen among embryonic cells, and among some adult cell types, such as leukocytes. However, the nuclei of all cells of any particular cell type carry the genes for embryonic differentiation and for other cell differentiation in a repressed state. A common explanation, therefore, of the apparent acquisition of invasive and metastatic capacities by tumour cells is that derepression of the relevant parts of the embryonic and/or other-cell-type genome has occurred in the particular cell type [15]. The mechanism is attractive because cell invasiveness involves active movement on the part of the cell, which in turn requires several specialised cell structures, such as actin filaments to be synthesised and coordinated. Such a complex, organised change of a cell is unlikely to be achieved by any disabling of genes other than of repressor gene(s) for a whole cell process. The notion is compatible with the phenomenon of inactivation of tumour suppressor genes in tumours [16]. Thus in the normal mature epithelial cell, the invasion and metastasis genes of its leukocyte-related and embryonic development-related genome are repressed. In cancer, the repression could be lost by a small number of disabling mutations of the repressor genes, so that invasive and metastatic behaviour emerges.

This notion of derepression of genes in cancer is supported by the expression of foetal proteins by hepatocellular carcinomata [17], and the fact that perhaps some early tumours do not arise from the stem cell region of the relevant tissue, but rather from the terminally differentiating region of the tissue [18, 19] (fig. 1).

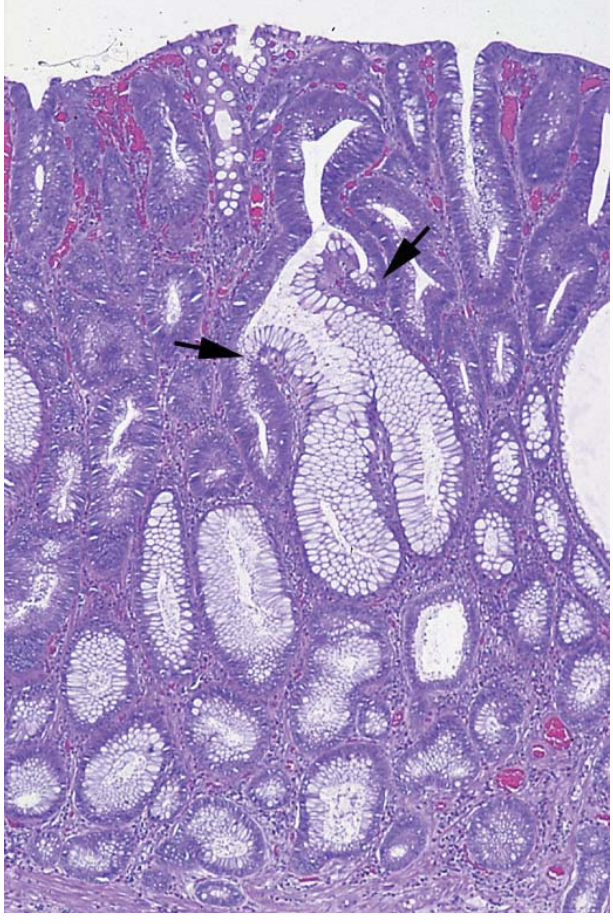

Figure 1. Colonic adenoma showing cytological abnormalities of the epithelium of the upper crypts and surface only, suggesting that the lesion in this case developed from (surface) differentiated cells rather than (basal) stem cells. Arrows indicate junction of atypical surface cells and normal cells lower down in a crypt. Haematoxylin and eosin, ×100 (original magnification).

The rate of cell accumulation cannot be assessed effectively in histopathological sections

A fourth group of cell abnormalities result in cell accumulation. These include those affecting both mitotic rate, cell senescence and, in addition, local nutritional factors. Apart from the visibility of mitoses, this group of factors per se does not affect the histological appearance of tumours because histopathology provides only a 'snapshot-in-time' of the lesions.

Lesions showing independence of nuclear abnormalities, loss of specialisation and invasiveness

Analysis of abnormalities of nuclear appearance, loss of specialisation and invasiveness can be represented as a triaxial diagram (fig. 2). Invasive and metastatic behaviour are represented on the *x*-axis, altered morphology on the *y*-axis, and reduction of specialisation on the *z*-axis. Clinically benign lesions are represented on the left-hand side of the cube, while the most malignant tumours are represented on the right-hand side. Cytologically uniform lesions are at the lower surface of the cube, while pleomorphic lesions are at the upper surface. Specialising lesions occupy the front side, and nonspecialising (undifferentiated, anaplastic) lesions form the rear side. The histopathological features of all tumours fall inside the cube. Some of the uncommon examples exhibiting the effects of each grouping in their extremes are annotated. These extreme examples, both purely and in combination, follow.

Noninvasive cells, showing nuclear uniformity, with normal specialisation

These are histologically normal. However, such cells may harbour genetic instability. Several different, histologically normal epithelia adjacent to carcinomata have been shown to be genetically unstable [20].

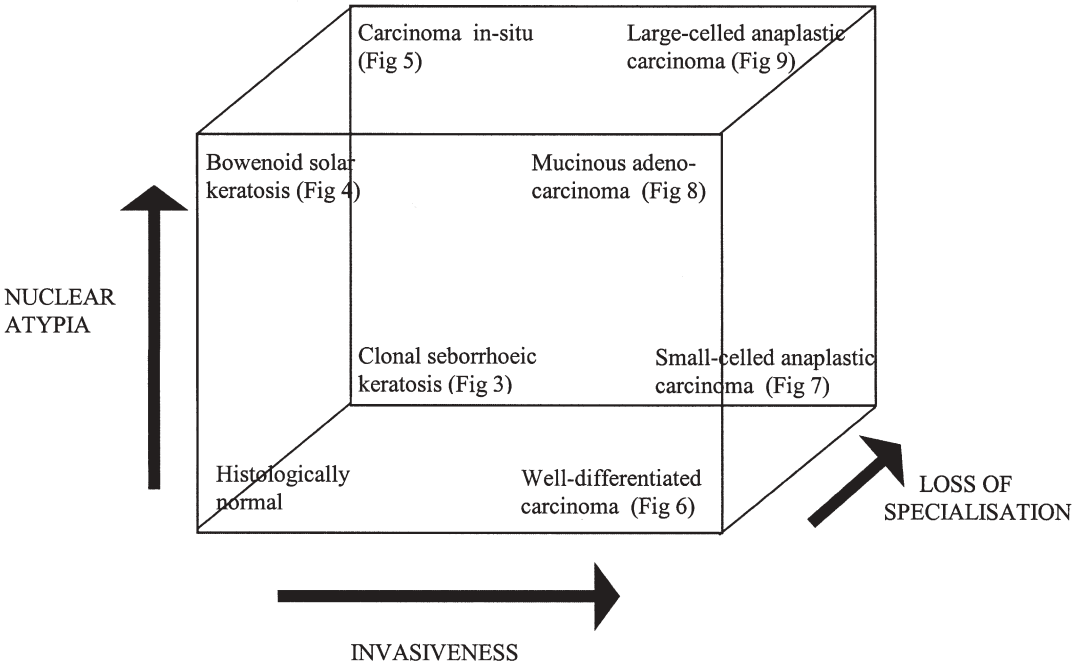

Figure 2. Diagram displaying the histopathological features of tumours in three categories. Invasive behaviour is represented on the *x*-axis, altered nuclear morphology on the *y*-axis, and reduction of specialisation on the *z*-axis. Clinically benign lesion occupy the left hand side of the cube, while the most malignant tumours occupy the right hand side. Cytologically uniform lesions are at the lower surface of the cube, while pleomorphic lesions are at the upper surface. Specialising lesions occupy the front side, and nonspecialising (undifferentiated or anaplastic) lesions form the rear side. The histopathological features of all tumours fall inside the cube. Some of the uncommon examples exhibiting the effects of each grouping in their extremes are annotated in the corners.

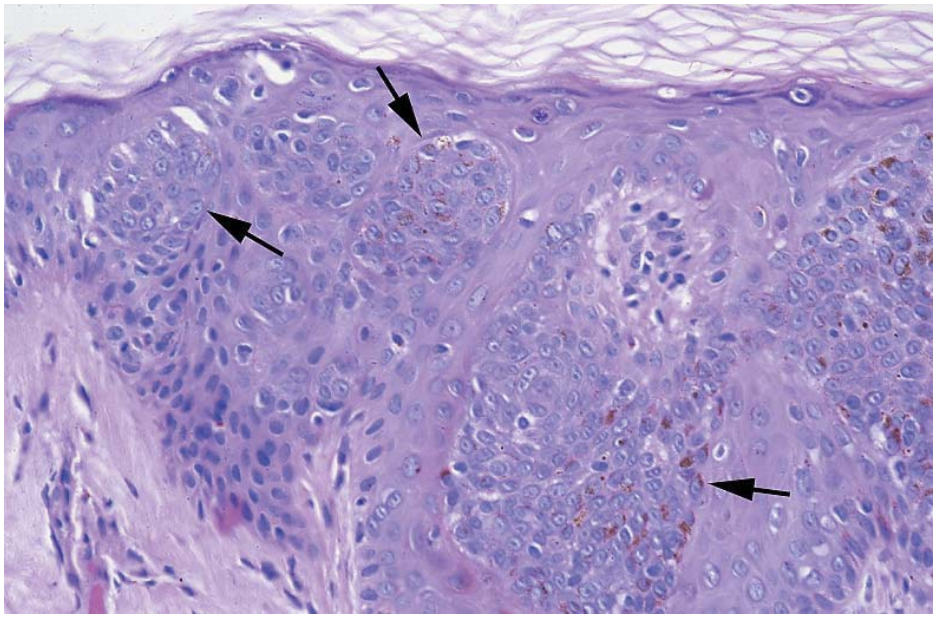

Figure 3. Clonal variant of seborrhoeic keratosis. Loss of keratin production occurs without nuclear atypia or invasiveness. Arrows indicate margins of clones. Haematoxylin and eosin, ×200 (original magnification).

Noninvasive cells which have normal nuclei, but show loss of specialisation (fig. 3)
These changes are seen in lesions such as the clonal form of seborrhoeic keratosis of the skin. The bulk of the tumour is formed by well-demarcated clones which do not themselves form keratin, but have normal appearance of their nuclei and exhibit no invasion. Other skin lesions in this category could include eccrine poroma and hidradenoma simplex. Basaloid adenomas of the salivary glands, and perhaps oncoytomas (which show many mitochondria, but no other specialisation) may be additional examples. Several lesions currently categorized as (idiopathic) hyperplasias fullfil the criteria for this type of lesion. Examples are nonatypical ductal hyperplasias of the breast and basal cell hyperplasia of the prostate.

Noninvasive tumours showing marked nuclear abnormalities but with minimum loss of specialisation (fig. 4)
This category is exemplified by Bowenoid solar keratoses of the skin. In these, the keratin is retained as a 'horn' while the underlying epidermal cells are frequently bizarre, with abnormal mitoses.

Noninvasive tumours showing marked nuclear abnormalities and loss of specialisation (fig. 5)
An example of these lesions is comedo ductal carcinoma in situ (DCIS) of the breast. These cells form no surface phenomena, such as lumina or papillae. The same grouping of abnormalities may be seen in in-situ carcinoma of the colon and stomach.

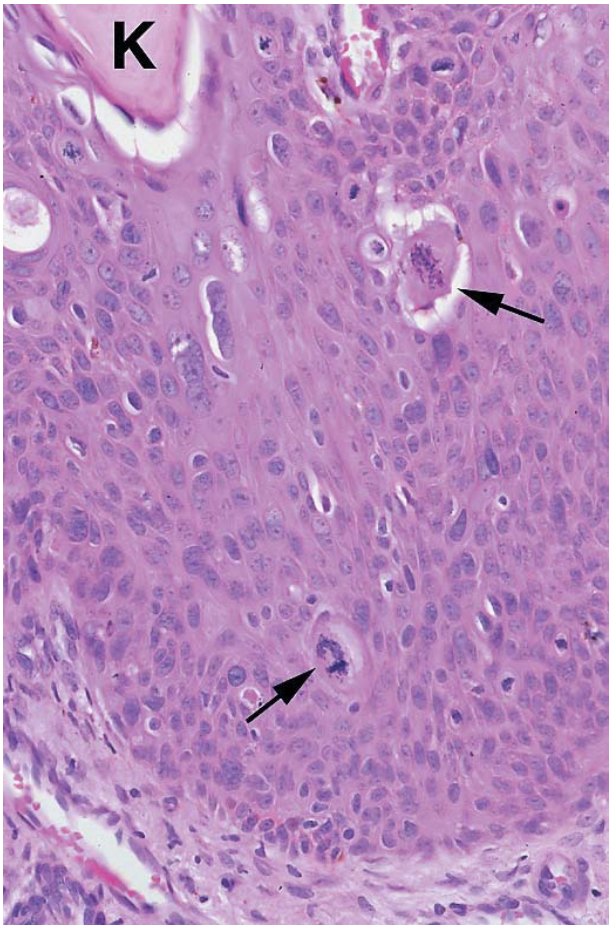

Figure 4. Bowenoid solar keratosis. Severe nuclear pleomorphism is associated with continued keratin production, and lack of invasiveness. K, keratin. Arrows indicate atypical mitoses. Haematoxylin and eosin, ×200 (original magnification).

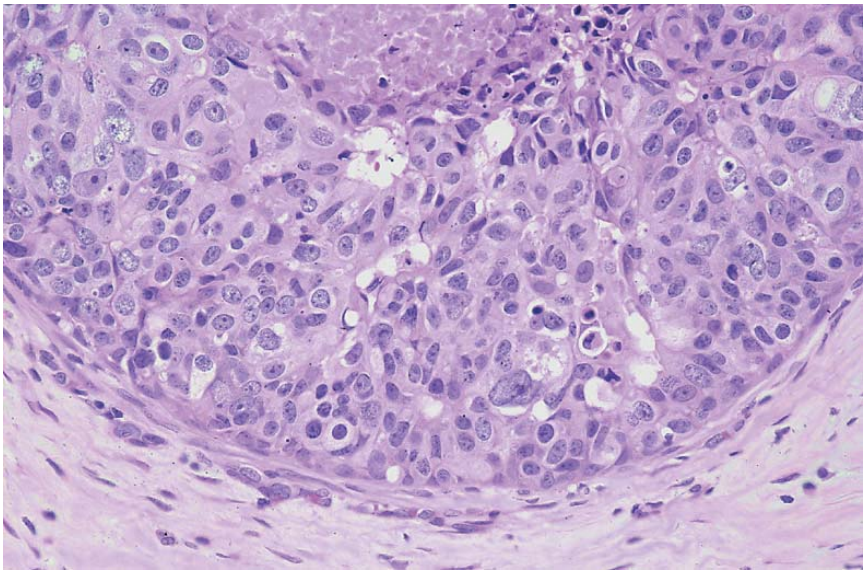

Figure 5. Ductal carcinoma of the breast, comedo type. Marked nuclear pleomorphism is associated with loss of specialisation (there is little formation of luminal surface) and lack of invasiveness. Haematoxylin and eosin, ×200 (original magnification).

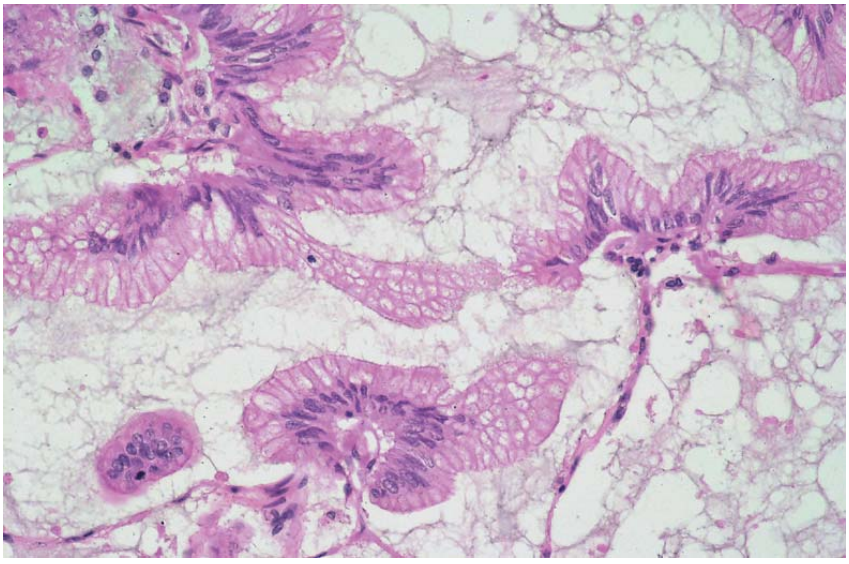

Figure 6. Adenocarcinoma of gall bladder metastatic to lung. The cells, although invasive and metastatic, show little nuclear pleomorphism, and mucus production is preserved. Haematoxylin and eosin, ×200 (original magnification).

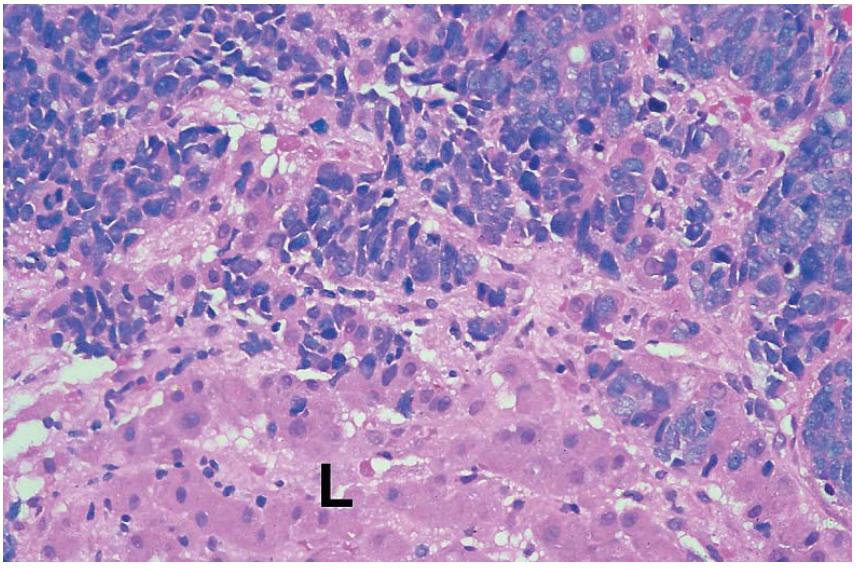

Figure 7. Anaplastic small cell carcinoma of the lung metastatic to the liver. Little nuclear pleomorphism is associated with loss of specialisation and marked invasion. L, liver cells. Haematoxylin and eosin, ×200 (original magnification).

Invasive tumours showing minimal nuclear atypia and minimal loss of specialisation (fig. 6)
These tumours are exemplified by well-differentiated adenocarcinomas of the biliary tree, which can metastasise to the lung and grow in a lepidic pattern and mimic pulmonary adenomatosis. A similar case of pancreatic origin is illustrated in reference [21]. Other examples of the category of tumour are adenoma malignum of the uterine cervix, and follicular carcinoma of thyroid, which can, in metastases, appear very similar to normal thyroid tissue [22].

Invasive tumours showing minimal nuclear abnormalities and marked loss of specialisation (fig. 7)
This category is typified by anaplastic small-cell carcinoma of the lung. In this tumour, the nuclei are regular and are similar to those of lymphoid cells.

Invasive tumours with marked nuclear abnormalities, but with minimum loss of specialisation (fig. 8)
Mucinous (colloid) carcinomas, especially of the colon exhibit these changes. Lakes of mucus surround the invasive, pleomorphic tumour cells. Similar tumours may occur in the lung and breast.

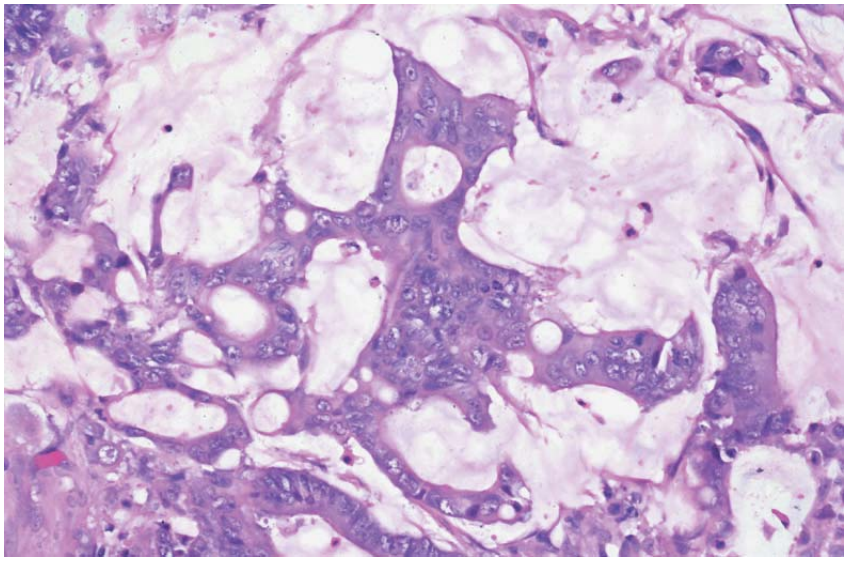

Figure 8. Mucinous carcinoma of the colon. There is marked nuclear pleomorphism and invasive behaviour, but no loss of specialisation. Haematoxylin and eosin, ×200 (original magnification).

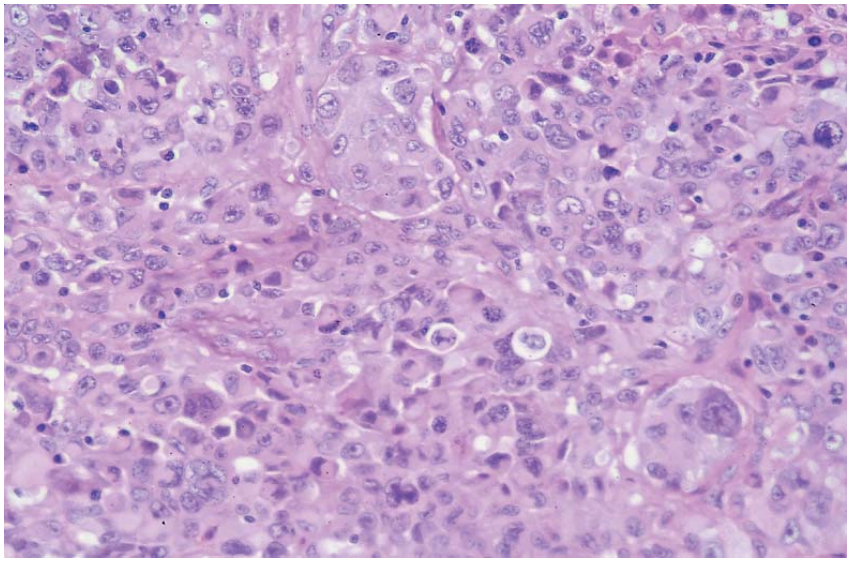

Figure 9. Anaplastic large-celled carcinoma of the bronchus. The nuclei are pleomorphic, there is no evidence of specialisation and the tumour is markedly invasive. Haematoxylin and eosin, ×200 (original magnification).

Invasive tumours with marked cytological abnormalities and complete loss of specialisation (fig. 9)

A typical example of this category is pleomorphic large-cell anaplastic carcinoma of the lung. Similar tumours can arise in many epithelia.

From the above, it is obvious that most tumours fall around the centre of the cube, rather than at its extremes. This is in keeping with the proposed model of randomness of large numbers of mutations and other genomic events which underlie the process, because it is expected that many mutations will be of partial effect.

Factors modifying the effects of random mutation on the histopathological features of tumours

Most solid tumours arise from cells of a particular type (i.e. lineage-committed cells) which are either tissue stem cells or cells in 'transit amplification' phase [19]. Each cell type tends to give rise to tumours resembling the cell of origin, and only in a limited number of histopathological types. For example, epidermis gives rise to well-differentiated tumours which are clinically indolent; bronchial epithelium commonly gives

rise to small-cell anaplastic carcinoma, and its adenocarcinomas are poorly differentiated and clinically aggressive.

Several major categories of mechanisms may be involved in the processes by which only a limited number of morphological types of tumours are derived from each particular cell type, as follows.

Tissue structure

The relative incidences of particular histological types of tumours in a given tissue may be affected by the relative proportions of tissue stem cells and transit-amplifying cells in the normal tissue. Thus epidermis consists mainly of transit-amplifying cells, with stem cells and terminally differentiated cells [19] in the minority. Tumours of the epidermis are mainly well-differentiated and minimally aggressive (above). At the other extreme, bronchial epithelium consists of a single basal layer of cells, which includes stem cells, and the terminally differentiated goblet cells and ciliated cells on the surface. There are few demonstrable transit-amplifying cells, and the tumours of this epithelium are often anaplastic and highly aggressive (above). These relationships may not be coincidental.

Carcinogen-related mechanisms

Most chemical carcinogens are not active in their natural state, but are activated by cell enzymes, such as cytochrome P-450 [23]. They are believed to have their effect by interacting with chromatin, especially DNA, forming 'adducts' 24]. To be effective, the activated chemical carcinogen must avoid detoxification by host enzymatic mechanisms [15] or absorption by non-DNA cell constituents. The susceptibility of a cell type to a particular carcinogen may therefore vary according to both cell-type-dependent metabolic activities, and according to metabolic activities which are specifically associated with a particular stage of specialisation of the cell.

Thus a chemical carcinogen may be able to access the DNA of a basal cell of the epidermis, but be neutralised by the metabolic processes related to keratin production which occur in cells of the spinous layer of the epidermis. A melanocyte adjacent to the basal cell of the epidermis, however, may have metabolic characteristics which render it insusceptible to same the carcinogen ab initio.

In the life of humans, a tissue such as the skin is very likely to be exposed to many differing carcinogens at the same times. Different carcinogens may be activated during different phases of specialisation of the same cell type. One carcinogen may be activated by a precursor (stem) cell and give rise to an undifferentiated tumour type, while another carcinogen might be activated by a more developed cell of the same type, and hence give rise to a more differentiated form of tumour. (Later on, the more differentiated tumour may give rise to a less differentiated version of the tumour by derepression of specialisation genes via genetic instability. This then may be a secondary phenomenon of tumour biology.)

Mutational susceptibility-related mechanisms

Focally variable susceptibility of the human genome to mutation is now well established from population-based studies [25]. Whether or not these results imply that the genetic instability of tumours might be focally variable in all cases is unclear. However, tumour-type-specific chromosomal translocations can frequently be identified in tumour cells by ordinary cytogenetic methods [26]. Furthermore, mutations of the tumour suppressor gene P53 have been noted to vary in their spectra among tumors of breast, large bowel, liver, lung and ovary [27].

These observations provide for the possibility that a cell type may have few 'hot spots' in its repressor genes for embryonic, and other cell genome. Tumours of these cell types might then be less susceptible to mutation of these repressor genes, and exhibit less aggressive behaviour. In contrast, cell types with many hot spots in these repressor genes might exhibit more aggressive behaviour more frequently.

Microenvironmental factors in tumourigenesis

The local environment may affect the survival of mutant cells to a greater or lesser extent. Thus harsh environments such as the lumen of the colon and the stomach, or the surface of the skin may destroy some morphological forms of tumours, which have little self-protective cytoplasm, and especially those with little mucus or keratin production. Conversely, a less harsh internal environment, such as the lumen of the bronchus, may allow less self-protected tumour types, such as small-cell anaplastic carcinoma, to emerge. In an extreme situation, it is possible that more nutritive environments, such as the ducts of the breast, may support a greater diversity of morphologies of tumours.

Conclusion

The complexity of the histopathology of tumours includes variability of nuclear pleomorphism, loss of specialisation, and invasive and metastatic behaviour of tumour cells. This variability, both within the tumours of the same cell type, and across tumours of differing cell types, has defied explanation by conventional theories of carcinogenesis. The mutator phenotype theory provides for considerable cell-to-cell variability of genome of tumours cells in individual tumours, as well as between tumours of different cell types. This genomic variability parallels the histopathologic variability of tumours.

Random, but independent genomic variability among all tumour cell populations would be expected to produce a majority of lesions which show a mixture of nuclear pleomorphism, loss of specialisation, and invasion and metastasis. However, a small number of tumours could be expected to show one or other type of abnormality without the others. The occurrence of examples of tumours which show one category of abnormality in the absence of the others, supports random mutation as a significant phenomenon common to all neoplasms.

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