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Is cancer really a 'local' cellular clonal disease?

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Summary Cancer is not simply the result of specific genetic alterations in key regulatory genes, but rather a complex multistep process involving selection of a clonal population of cells. To accumulate three, or often as many as seven, specific mutations in a single cell without incurring a significant number of additional mutations that might lead to cell lethality requires a large number of target cells, some mutagenic activity acting on those target cells for a variable period of time, and efficient selection strategies, which may be to some extent tissue-specific. A number of 'protective' intracellular regulatory circuits might be present in proliferating cells deliberately to protect against carcinogenesis. If it does require some seven sequential carcinogenic 'genetic hits' in a single cellular clone for a malignant tumor to develop, it is mathematically more likely to occur in a tissue with a high background of genetic alterations in neighboring cellular clones, than in a tissue with a low background of such alterations, or with no detectable carcinogenic mutations at all. In this context, the old 'field cancerization' theory by Slaughter and the more recent 'multistep carcinogenesis' model by Fearon and Vogelstein can come together in a single model: 'multistep field cancerization'. This simple conclusion, and our ability to measure 'background carcinogenesis' in different parts of the body, might allow early detection of cancer risk, and eventually help us to develop suitable therapeutic strategies to delay or suppress the carcinogenic process. Molecular technologies are just beginning to be sufficiently sensitive to start testing the hypothesis.

RELATIVELY OLD VIEWS ON CARCINOGENESIS

For a long time the concept of 'lineage' has been central to all our ideas about cancer. According to this concept, cancer starts as a local disease, in a given cell clone that, for reasons which remained unclear until some 20 years ago, could multiply faster than normal and displace its neighbors: families of cells can emerge that increase in numbers at the expense of their neighbors. Described in these terms, the evolution of a cancer can be viewed as the result of Darwinian selection among competing populations of dividing cells. The tissues of the body normally preserve their initial fine mosaicism even in old age, indicating that under normal circumstances there is 'harmony' in tissues, equilibrium between cell death and cell proliferation, balanced regulatory exchanges between stroma and epithelial cells, and little or no competition between adjacent cells. However, according to this old view that remains widely accepted today, one of the earliest steps in the sequence leading to cancer is the emergence of families that are able to displace their neighbors (1–3). No doubt this is partly the result of some 'intrinsic change' in the cells (and mutations were suspected long before oncogenes and tumor suppressor genes were discovered), that enable them to compete for territory, but the whole process can presumably be accelerated by anything that causes cell death creating an opportunity for competition to occur. As a result, the normal equilibrium is lost and a 'tumor' gradually develops.

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These 'intrinsic changes' in the cells could induce cells to multiply without the usual restraints, e.g., they divide more frequently or are subject to less cell loss, but nevertheless keep within their normal territory and do not invade the surrounding tissues, thereby forming benign tumors. Alternatively, if these 'intrinsic changes' somehow damage the normal regulatory networks that maintain 'territoriality' and the normal cells acquire the ability to spread to alien sites, locally or to distant regions, then the tumors formed are called 'cancers' because of their malignant behavior.

Therefore, monoclonality is generally considered the hallmark of tumors, but situations exist in which clonality is not unequivocally associated with malignancy. Clonal markers are useful for the diagnosis or follow up of disease progression for both solid and hematologic tumors. Modern methods of clonality determination include X-chromosome inactivation (in females), immunoglobulin and T-cell receptor gene rearrangement analysis, specific chromosomal translocations or deletions. Benign condition (e.g., benign monoclonal gammopathy), and some pre-malignant conditions (e.g., lymphomatoid granulomatosis, lymphomatoid papulosis, Langerhans cell histiocytosis, lymphoepithelial proliferations associated with Sjögren's disease, etc.) may show monoclonal rearrangement without necessarily developing malignancy after prolonged follow-up (4,5).

Regarding the nature of these 'intrinsic changes' that lead to cancer, several decades ago there was much debate as to whether their nature was 'genetic', secondary to mutations or chromosomal alterations, or 'epigenetic', due to abnormalities in gene expression without an underlying genetic lesion. Thus, although most known human carcinogens were proven to be mutagens, it was postulated that some cancers for which no cause had been discovered could be partly due to agents that were not mutagens, but acted instead by provoking cell division and errors in gene expression.

MORE MODERN VIEWS ON CARCINOGENESIS

However, most of the evidence was clearly in favor of a 'genetic' cause. Cancer incidence rises sharply with age and various models had been proposed to account for this (*6–7*). These models shared the view that a cancer cell arises as the end result of a series of steps that have occurred at some time in the life of the patient. They often postulated that each cell has several genes that independently restrain it from forming an ever-expanding family of cells, and that a cancer arises when a cell is created in which each of those genes has been inactivated by a separate, independent mutation. Logically, the probability of any particular one of our cells having a mutation in a particular gene will increase in direct proportion to our age.

In 1958, Armitage and Doll (8) had even calculated that for some of the common solid tumors the logarithm of cancer incidence should be linearly related to the logarithm of our age, and that, if such an interpretation were literally true, then we could deduce from the slope of the death rate from cancer of the large intestine in relation to age plotted logarithmically, that about six mutations are needed to produce a cancer of the large intestine. This guess is extraordinary if one thinks that more or less the same conclusion was reached, based on molecular genetic knowledge, by Fearon and Vogelstein in their classic work on colorectal tumorigenesis (*9*). The 'multistep mutations' theory of cancer was also supported by other lines of epidemiological evidence (10,11), and by the 'initiation and promotion models of carcinogenesis'. A characteristic feature of most forms of carcinogenesis is the long period that elapses between initial application of the carcinogen and the time the first cancers appear. It is necessary to apply coal tar repeatedly to the skin of a mouse for several months before any tumors are detectable. Similarly, most common human cancers can take 3 to 30 years or more to develop.

The chemical carcinogenesis experimental models also helped to identify at least two classes of carcinogenic compounds: the initiators and the promoters. For example, if a group of mice are fed a small amount of the carcinogen dimethylbenzanthracene (DMBA) this produces widespread irreversible alterations (presumably mutations) in the cells of each mouse. Subsequent irritation of the skin by painting it twice a week with croton oil (the 'promoter') eventually results in the local appearance of tumors. These tumors will appear even if croton oil is not started until 16 weeks after the DMBA feeding, but no tumors arise if either DMBA or croton oil is given alone or if the order of the treatments is reversed. In several aspects, estrogens (for breast cancer) and testosterone (for prostate cancer) have also been regarded as potential tumor promoters.

Other insights into the 'genetic' nature of tumorigenesis came from studies on viral carcinogenesis (12–14) and from seminal observations in the uncommon retinal cancers in children (15).

The discovery of tumor oncogenes and tumor suppressor genes almost 20 years ago opened the way to the molecular epidemiology of cancer (16–20). Thus, it soon became apparent that in general more than one somatic mutational event was needed for malignant transformation.

The possible exception being the uncommon hereditary retinoblastomas, already described by the 'two-hits model' proposed by Knudson (15). Then it was also

found that certain carcinogens are linked to selective (though not entirely 'specific' mutational events. For example, molecular linkage between exposure to carcinogens and cancer types have been described for p53 mutational spectra of hepatocellular carcinoma, skin cancers and lung cancer (19). Fearon and Vogelstein (9) proposed a molecular model for colorectal carcinogenesis in 1990 based on the sequential accumulation of genetic events in key regulatory genes along the sequence adenoma to carcinoma.

More recently, in 1997, Kinzler and Vogelstein (21) proposed the concept of two different types of carcinogenic genetic events: those involving 'gatekeeper' or 'caretaker' genes characterized by their control of net cellular proliferation or maintenance of genomic integrity, respectively. Examples of gatekeeper genes include APC and β-catenin in colon epithelium, Rb in retinal epithelial cells, NF1 in Schwann cells, and VHL in kidney cells. Thus, it is proposed that an alteration in APC leads to a derangement of the cellular proliferation pathway that is important for maintaining a constant cell population, at least in colonic cells. The identification of other gatekeeper genes is expected, and some may be genes crucial to morphogenetic events of specific tissues. Unlike gatekeeper genes, caretaker genes generally maintain genomic stability and are not involved directly in the initiation of the neoplastic process, but their mutations enhance the probability of mutations in other genes, including those in the gatekeeper class. Because multiple mutations are found in cancer cells, the existence of a 'mutator phenotype' was suggested by Loeb in 1991 (22,23) as an important step in tumor development, and candidate mutator genes are involved in multiple cellular functions needed for maintaining genetic stability, such as DNA repair, DNA replication, chromosomal segregation, cell cycle control and apoptosis.

Finally, some individuals may be predisposed to cancer because of inherited mutations of some key genes that may confer a familial predisposition to cancer. This has attracted considerable attention in recent times, particularly in relationship to breast cancer and colon cancer susceptibility genes (24,25).

The genetic alterations in oncogenes generally lead to an increased function of the protein, whereas, in general, tumor suppressor genes are inactivated during carcinogenesis with apparent loss of function of the protein. However, the mechanisms of activation or inactivation are multiple, and the precises consequences on gain or loss of function are incompletely understood. K-ras and H-ras genes are examples of oncogenes preferentially altered by point mutation (codons 12, 13, and 61), generating a protein with constant GTPase activity. The c-myc gene can be activated by chromosomal translocation (in some leukemias) or by gene amplification (in some solid tumors). The p53 and Rb tumor suppressor genes are often knocked out by point mutation in one allele and by deletion (loss of heterozygosity) at the other. Others, like p16, have high rates of homozygous deletions or promoter hypermethylation. Some genetic defects are fairly characteristic for a given tissue type (most colorectal cancers have APC or β-catenin mutations). But the 'same players' are frequently involved in different tumors. Each human cancer can be regarded as a different molecular entity, with a different matrix of molecular targets, and it evolves with time (even as a result of systemic or local therapies) (26).

CARCINOGENESIS MODELS IN THE ERA OF GENOMICS AND PROTEOMICS

The recent progress in the physical mapping of the human genome, is already pointing towards a 'post-genome era'. Automated or semi-automated devices capable of reading thousands of genes are already available. Genomics and proteomics are here to stay (27), but their routine use in the clinic obviously requires proof of efficacy and judicious use. Apart from technical problems inherent to these techniques, which time will solve, there are some general obstacles to this promise:

1. The lack of genetic markers to cover 100% of all tumors.
2. The lack of knowledge on the functional implications of most genetic defects (the precise maps of these regulatory pathways are still under investigation, and may be to some extent tissue-specific).
3. The widespread location and heterogeneity of many of the gene mutations (particularly in large genes).
4. The lack of knowledge on the prevalence of these genetic defects in an apparently healthy population (people with no detectable cancer or histologically malignant lesions; for example, presence of molecular lesions in endometriosis or in apparently normal oral or bronchial mucosa).
5. The lack of knowledge on the significance (in terms of risk of developing cancer) of each of these genetic lesions individually or in different combinations. For example, which combinations of genetic lesions are more relevant to neoplastic transformation in a given tissue?

Key to the multistep genetic nature of cancer is that carcinogenesis is 'progressive'. In most epithelial tissues, progression means the sequential accumulation of somatic mutations. In some cases of familial predisposition to cancer some of these mutations are inherited. Gradually, a given target tissue experiences a transition from normal histology, to proliferative and/or dysplastic

changes, to so-called 'intraepithelial neoplasia' (IEN), which can be early or severe, to superficial cancers ('in situ'), and finally to invasive disease. In some instances, the process may be aggressive and relatively rapid (e.g., in the presence of a DNA repair-deficient genotype or an aggressive human papilloma virus), but in general these changes occur over a long period of time. In the breast, for example, it is estimated that progression from atypical hyperplasia through ductal carcinoma 'in situ' (DCIS) to adenocarcinoma may require 30 years or more (28,29). Similar observations have been made in other tissues, such as lung, head and neck, prostate, bladder, and colorectal tissue (30–34).

Patients with a head and neck squamous cell carcinoma (HNSCC) often develop multiple (pre)malignant lesions, ranging from leucoplakia to other cancers, which led Slaughter et al. (35) back in 1953 to postulate the concept of 'field cancerization'. The incidence rate of second primary tumors following a first diagnosis of HNSCC is 10–35%, depending on both the location of the first primary tumor and the age of the patient. The carcinogens associated with HNSCC (alcohol and tobacco smoking) are thought to induce mucosal changes in the entire upper aerodigestive tract (UADT), causing multiple genetic abnormalities in the whole tissue region. Similar arguments apply also to other tobacco-related cancers, like transitional cell carcinomas of the urogenital tract or bronchogenic carcinomas (36,37). An alternative theory for these observations is based on the premise that any transforming event is rare and that the multiple lesions arise due to the widespread migration of transformed cells through the whole UADT (38,39). However, most field changes appear to be induced by smoking, supporting the theory of carcinogen-induced field cancerization rather than field cancerization due to migrated transformed cells (40).

Other possible causes of 'field carcinogenic events' can involve hormonal factors (e.g., changes in the ovaries, breasts or prostate), hyperemia (increased proliferative and angiogenic activity in chronic cystitis, gastritis, esophagitis or colitis), chronic viral infections (e.g., Hepatitis B virus for hepatocarcinomas, Epstein–Barr virus for nasopharyngeal carcinomas or some lymphomas), aberrant methylation linked to ageing, free-radical-induced DNA damage (e.g., for cancers of the gastrointestinal tract), skin exposed to ultraviolet irradiation (e.g., actinic keratosis and squamous cell carcinomas), ionizing radiation-induced damage, or aberrant morphogenetic pathways. It is also possible that different carcinogenic pathways operate in different tissue fields belonging to the same organ. For example, adenocarcinomas of the right side of the colon are often associated with different clinical and molecular characteristics than adenocarcinomas of the colorectal region.

In this context, the old 'field cancerization' theory by Slaughter et al. (35) and the more recent 'multistep carcinogenesis' model by Fearon and Vogelstein (9) can now come together in a single model: if it does require some seven sequential carcinogenic 'genetic hits' in a single cellular clone for a malignant tumor to develop, it is mathematically more likely to occur in a tissue with a high background of genetic alterations in neighboring cellular clones, than in a tissue with a low background of such alterations, or with no detectable carcinogenic mutations at all (Figs. 1 and 2). The probability of a single clone accumulating seven independent but sequential genetic alterations leading to a malignant phenotype, without any similar events occurring in neighboring cells would seem to be rather low. This simple conclusion, and our ability to measure 'background carcinogenesis' in different parts of the body, might lead to several unexpected implications. Technology is just beginning to be sufficiently sensitive to start testing the hypothesis. One potential technical problem is that in premalignant tissue, the 'signal' (e.g., relevant oncogenetic lesions) might be diluted by the 'noise' (normal genome of most of the cells in the tissue), until the premalignant clones have expanded enough to become more numerous locally than normal cells.

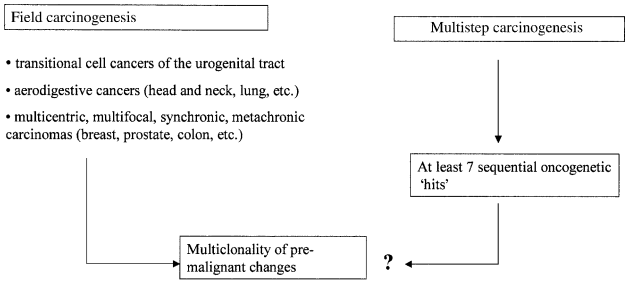
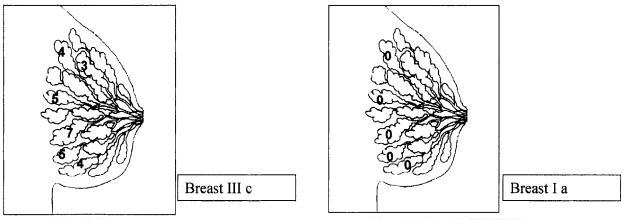


Fig. 1 Multiclonality of pre-malignant changes.



	Normal mucosa	Profilerative	Dysplastic
No Mutations	I a	I b	I c
<3 Mutations	II a	II b	II c
>3 Mutations	III a	III b	III c

Fig. 2 Combined histological and molecular staging system of tumors: numbers inside the drawings refer to numbers of relevant gene mutations/deletions/amplifications identified in various regions of the breasts.

However, it is only a matter of time before this goal is technically achievable.

IMPLICATIONS FOR SCREENING AND CHEMOPREVENTION

Cancer 'chemoprevention' can be defined as 'the treatment of carcinogenesis, its prevention, inhibition or reversal' (41). The term 'chemoprevention' is controversial. 'Chemo' may lead to a confusion with 'chemotherapy', and 'prevention' may not be the best word to define 'early detection' of cancer biomarkers. The subject is bound to grow very rapidly, both in terms of the identification, validation, and clinical relevance of cancer biomarkers (42), but also in terms of their impact on the quantitative estimation and prediction of individual human cancer risks (43).

A realistic aim is the development of a *'combined histological and molecular staging system'* (Fig. 2). For example, after a follow up of 5–10 years, one would expect more new cancers to develop in group IIIc of Fig. 2 (dysplastic changes and three or more than three significant mutations identified), than in group Ia (normal histology and no mutations identified). The clinical application of this concept and technology should then help to classify patients into various 'risk groups' very early on in the development of a malignant disease, allowing a tailor-made program for follow up and screening, as well as more appropriate therapeutic interventions. For example (44), a suitable combination of relevant biomarkers might help clinicians to identify smokers at high risk of developing lung cancer. Some smokers might be protected because of genetic polymorphisms of enzymes involved in the molecular activation of pre-carcinogens present in tobacco, whereas others may be more vulnerable to the carcinogenic effects because of genetic defects in DNA repair enzymes. Some molecular changes associated with ageing and carcinogenesis might be epigenetic (e.g., promoter methylation) rather than genetic (45). Even some pediatric malignancies might be secondary to abnormal morphogenetic events in utero (46).

It has been estimated that in the USA alone some 30% of people above the age of 60 can be found to have adenomas of the colon by colonoscopy, 70% of more of men above the age of 80 will have IEN of the prostate, 30% of people aged 60 or more have actinic keratosis on their skin, 20% of sexually active women above the age of 40 may have some degree of cervical IEN, at least 40% of heavy smokers can show metaplastic and/or dysplastic lesions in their bronchial mucosa, and some 20% of women with dense mammograms and aged more than 50 may show atypical cells on ductal washings (from the nipple) or ultrasound-guided biopsies. The use of a battery of genetic or protein biomarkers suitable for each of the main cancer types may soon help us to better define individual cancer risks. Perhaps, not too long from now, oncology units will be devoted to the 'treatment of carcinogenesis' just as much as to the 'treatment of cancer'.

The hypothesis presented here, that most epithelial cancers of adults may represent the end-result of carcinogenic 'field' tissue events, rather than localized isolated 'cellular clone' events will require the help of genomic (expression profiling) and proteomic (functional assays for individual and combined protein domains), as well as suitable mathematical epidemiological models of analysis. In summary, cancer is a ''state'', whereas carcinogenesis is a ''process''. By the time of clinical detection most cancers are probably ''monoclonal'', but carcinogenesis leading to these cancers is more likely to be ''polyclonial''.

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