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How many molecular subtypes? Implications of the unique tumor principle in personalized medicine

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Abstract

Cancers are complex multifactorial diseases. For centuries, conventional organ-based classification system (i.e., breast cancer, lung cancer, colon cancer, colorectal cancer, prostate cancer, lymphoma, leukemia, and so on) has been utilized. Recently, molecular diagnostics has become an essential component in clinical decision-making. However, tumor evolution and behavior cannot accurately be predicted, despite numerous research studies reporting promising tumor biomarkers. To advance molecular diagnostics, a better understanding of intratumor and intertumor heterogeneity is essential. Tumor cells interact with the extracellular matrix and host non-neoplastic cells in the tumor microenvironment, which is influenced by genomic variation, hormones, and dietary, lifestyle and environmental exposures, implicated by molecular pathological epidemiology. Essentially, each tumor possesses its own unique characteristics in terms of molecular make-up, tumor microenvironment and interactomes within and between neoplastic and host cells. Starting from the unique tumor concept and paradigm, we can better classify tumors by molecular methods, and move closer toward personalized cancer medicine and prevention.

Keywords

genomics; holistic; intratumor heterogeneity; molecular classification; molecular pathological epidemiology; MPE; neoplasia; phenome; systems biology; tumor–host interaction; unique tumor paradigm

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For centuries, a conventional organ-based classification system of cancer (i.e., breast cancer, lung cancer, colon cancer, prostate cancer and so on) has been successful in clinical practice and research, and used globally in cancer registries. Recently, molecular pathology and diagnostics have become an essential component in clinical decision-making to manage cancer patients [1–6]. To advance oncological and pathological sciences towards personalized medicine, we need informative biomarkers that can classify tumors or patients to aid specific therapeutic decision-making. Examples of well-established informative biomarkers are genetic changes in neoplastic cells, such as *KRAS* mutation in colon and lung cancers, *EGFR* mutation in lung cancer, *BRAF* mutation in colon cancer and melanoma and microsatellite instability in colon cancer [4–8]. In addition, epigenetic changes, in particular DNA methylation alterations, have emerged as promising tumor biomarkers [9–12]. DNA methylation analysis is readily applicable to formalin-fixed paraffin-embedded tissues, which exist for patients with long-term clinical follow-up, and can be utilized for large epidemiologic and translational investigations.

Cancer biomarkers are useful in not only cancer outcome research but also cancer epidemiology and prevention research. Accumulating evidence suggests that cancer risk factors influence carcinogenesis processes differentially by tumor molecular subtypes and neoplasia pathways to cancer [13–16]. To investigate complex relationships between etiologic factors and tumor molecular characteristics, molecular pathological epidemiology (MPE) has recently been established as the evolving interdisciplinary field of science [13–15,17].

As each individual human being is unique, each tumor is unique. This fact poses tremendous challenges in personalized cancer medicine and prevention. In this article, we provide insights into the uniqueness of each tumor and its interaction with its environment (both micro and macro), and the nature of molecular heterogeneity between tumors and within a single tumor. Although there have been numerous reviews on tumor molecular classification, the unique tumor paradigm has not been discussed. Only with a better understanding of the uniqueness of each tumor, can we move steps closer to true personalized medicine and molecular diagnostics.

Basic features of neoplasms: somatic mutation (alteration) theory

Characteristics of cancer include uncontrolled cellular growth, proliferation, invasion and metastasis. To achieve these capabilities, preneoplastic and neoplastic cells gain somatic molecular changes, which are considered to accumulate in a sequential fashion [18]. Now, genetic somatic mutations as well as somatic epigenetic changes can be incorporated into this multistep carcinogenesis scheme. We can observe a nonrandom accumulation of molecular events in tumors, perhaps owing to a predisposition to sequentially develop multiple aberrations and selection pressure for any given molecular change [19]. Thus, examining associations of molecular events (i.e., molecular correlates) in cancer can give us clues to the nonrandom processes involved in carcinogenesis pathways [19].

In addition, we need to consider that, if a somatic alteration is less influential, there is less selection pressure for or against the alteration, leading to more stochastic accumulation of somatic molecular changes. This type of stochastic appearance may be pronounced in genome-wide somatic DNA methylation changes observed in cancer [20,21]. However, since any given somatic change may not be totally neutral, it may still influence a certain step of the carcinogenesis process, depending on cellular genomic and epigenomic status and the tumor microenvironment at the particular tumor evolution step. Evidence has suggested that even a partial loss of function of a tumor suppressor can contribute to tumor

development [22]. Thus, stochastic-appearing somatic changes may still contribute to tumor characteristics and intratumor heterogeneity, and add uniqueness to each tumor.

Basic features of neoplasms: tumor microenvironment (tissue organization field theory)

Other important aspects of neoplasia are the tumor microenvironment and tumor–host cell interaction [14,23]. The concept that the tumor microenvironment and tumor–host interaction determine the evolution of neoplasm is termed 'tissue organization field theory' [24]. Neoplastic cells constantly interact with the extracellular matrix and host non-neoplastic cells, including inflammatory and immune cells, vascular endothelial cells, fibroblasts and other mesenchymal cells in the tumor microenvironment [23,25]. The tumor microenvironment provides neoplastic and host cells with various molecules, including oxygen, nutrients, growth factors and other chemical mediators that promote or suppress cellular survival, growth, invasion and metastasis. The tumor microenvironment is influenced by not only endogenous factors (e.g., genetic and genomic variation and hormonal milieu), but also exogenous factors, such as dietary, environmental and life-style exposures (Figure 1). Evidence suggests that inflammation and oxidative stress can induce somatic epigenetic aberrations [26,27]. Tumor–host interactions probably influence, and are influenced by, the genome, epigenome, transcriptome, proteome and metabolome of both the neoplastic and nontransformed host cells [14].

Importantly, this tissue organization field theory [24] and somatic mutation theory [18] are not mutually exclusive. It is likely that both mechanisms play important roles in the evolution of neoplasm and have essential implications in cancer prevention and treatment.

Unique tumor principle: each tumor is unique

Can any two individuals behave exactly the same way in response to every chemical, physiological or psychological stimulus? Essentially, each of us is a unique human being with a unique set of genomic, epigenomic, transcriptomic, proteomic and metabolomic variants in each of the numerous cell types that exist in the human body. Thus, a complex network of cellular and molecular interactions (i.e., interactome) in any given individual throughout the body is also unique. Given that tumors result from complex host–tumor interactions, a tumor in a specific individual must have unique characteristics from that in another individual. In addition to the uniqueness of a host individual, each tumor has its unique combination of genomic and epigenomic features [19]. Genomic and epigenomic analysis data on tumors support enormous intertumoral heterogeneity between tumors that evolved in different individuals [20,28–40]. Essentially, each tumor in each patient arises through a unique pathway that is unlikely to be exactly recapitulated by any other tumor [19]. In addition to the genetic and epigenetic basis of intra- and inter-tumor heterogeneity, exogenous exposures such as dietary and lifestyle factors [41–43] will add more complexity to the intra- and inter-individual heterogeneity of the tumor microenvironment, and hence further contribute to the uniqueness of each tumor [13–15]. Thus, the unique tumor principle takes into account both the somatic mutation theory and the tissue organization field theory [24].

Intra- & inter-tumor heterogeneity

Intertumor heterogeneity is closely related to intratumor heterogeneity. The variability among cancer cells within a single neoplastic lesion has been known as intratumor heterogeneity [44–47], which is increasingly evident in single-cell transcriptional profiling [48] or single-cell sequencing [49]. The fundamental basis underlying intratumor

heterogeneity is the molecular variability of the tissue microenvironment and neoplastic cells within a single tumor, in particular with epigenetic heterogeneity. Molecular heterogeneity within a single tumor poses a considerable challenge in cancer therapy, not only making prediction of tumor behavior very difficult, but also resulting in almost universal emergence of neoplastic clones resistant to a given therapy. Tumor progression has been regarded as an evolutionary process whereby molecular changes accumulate and clonal selection constantly takes place [50,51]. Any molecular feature can be an advantage or disadvantage (even with very small effect) during the selection process, and this selection pressure may change at different tumor progression steps, depending on the tumor microenvironment. Data suggest that epigenetic alterations provide such diversity in intratumor cell clonal populations perhaps due to a weaker selection pressure than driver mutations [21]. A molecular feature that can give an advantage for cellular survival in the bloodstream may not be an advantage and may not be manifested until tumor cells invade deeply in tissue and blood vessel walls, and into the bloodstream. Since the tumor microenvironment is unique in each individual, a unique set of selection pressures exist for numerous clones within each tumor. Thus, intratumoral heterogeneity in combination with the unique tumor microenvironment in each host probably contributes to the uniqueness of each tumor (as a totality of neoplastic cell clones).

Driver versus passenger: a tale of two seats, or a continuum?

Molecular alterations are typically classified into drivers versus passengers, particularly in genomics research [28–40]. However, this dichotomy is often too simplistic. Evidence supports a ‘continuum model’ for oncogene and tumor-suppressor gene function [22,52], which certainly appears to be more natural and plausible than ‘a black-and-white model’ or ‘all-or-none model’. In addition, accumulating evidence suggests that different mutations in the same gene can have different biological effects [53–56], which adds to the continuum model of mutation effects. Each of the so-called ‘passengers’ may have a very small effect on tumor properties, but a net effect of all of the passengers in a cell may be substantial, given the number of passengers and interactions between passenger alterations and the many different signaling pathways and biological processes. It may be the case that, in addition to several *bona fide* drivers, a net effect of small effects from all genomic, epigenomic, transcriptomic, proteomic and metabolomic alterations may constitute drivers for the development and progression of any given tumor.

How many tumor subtypes?

Now we come back to the question in the title – how many tumor subtypes are there? Certainly, tumor subclassification based on genetic features and epigenetic features is quite common and there are many classification systems [11,42,43]. To answer the question, we must consider the uniqueness of each tumor and each tumor pathway. Figure 2 illustrates an example of colon cancer. As already explained, each tumor undergoes its own unique tumorigenesis pathway (as indicated by each arrow in Figure 2). Although each tumorigenesis pathway is unique, there is a similarity in some pathways (e.g., ‘A’ in Figure 2), as opposed to other pathways (e.g., ‘B’ and ‘C’). It is reasonable to regard similar tumors (e.g., ‘A’) as one type, and other tumors (e.g., ‘B’ and ‘C’) as other types. When one looks into type A more closely, there are tumors that are even more closely related to each other (e.g., ‘A1’), as opposed to other type A tumors (e.g., ‘A2’ and ‘A3’). Thus, tumor type–subtype structure is hierarchical. Since each tumor pathway is ultimately unique, any number of tumor types or subtypes is possible, depending on how we classify tumors. An optimal tumor classification system needs to be determined.

In our experience, for example, colorectal cancers can be divided into subtypes and further into more subtypes, as we add more molecular classifiers. Colorectal cancers can be classified according to five tumor tissue biomarkers (which have been shown to be important in the carcinogenesis process) including *KRAS* (mutant vs wild-type) [57], microsatellite instability (high vs stable) [11,19,58,59], CpG island methylator phenotype (high vs low/negative) [19,42,43,60–63], LINE-1 methylation (high vs low; as a surrogate of global DNA methylation level) [64–68], and *TP53* (positive vs negative). From there, tumors can be classified into 32 (= 2^5) subtypes! Indeed, in our database of over 1000 tumors, there is a tumor subtype with any combination of these features. Similar data have been reported by Suehiro *et al.* [69]. Further adding to this complexity, there is evidence for the existence of three CpG island methylator phenotype subtypes [63,70–73], and LINE-1 methylation is a continuous variable that affects tumor behavior in a statistically linear fashion (i.e., there are multiple LINE-1 methylation subtypes) [74,75].

The goals of molecular classification are to identify shared characteristics within a group of tumors that may predict disease course and treatment response. Basically, we classify tumors by similarities between carcinogenesis pathways. There are numerous sub-subtypes, and we classify them into subtypes based on similarities between them. It is important to note that the tumor classification system is actually hierarchical as well as multidimensional, with different set of classifiers, including genomic, epigenomic, transcriptomic, proteomic, metabolomic and interactomic classifiers.

Organ-based classification of cancer has been effectively utilized for centuries. We routinely classify tumors by organ system, because organ-based classification can improve prediction of tumor behavior. However, it is evident that we cannot achieve personalized medicine, unless we go beyond organ-based classification into molecular classification [13–15]. There is the necessity of a paradigm shift from organ-based classification to molecular classification. Molecular classification should be routine data in any population-based cancer registry in the near future, in order to achieve our ultimate goals of personalized medicine, prevention and public health. It should be emphasized that molecular classification is not replacing traditional clinicopathologic classification, but rather adds new dimensions to it and refines our methods to better predict tumor evolution and behavior.

Molecular pathological epidemiology: integrative analysis of tumor cells, host & environment

As each tumor is unique, the rationale of tumor classification is that tumors that share certain characteristics may arise through similar pathways and manifest comparable etiology, evolution and behavior [19]. Investigations of the influence of genetic, dietary, lifestyle and environmental factors in relation to tumor molecular classification can provide insights into carcinogenic mechanisms, and represent the evolving interdisciplinary and transdisciplinary science of MPE [13–15]. The MPE paradigm is becoming widespread [16,42,43,76–89]. An integrative MPE analysis aims to decipher a tumor as a dynamic organism comprised of neoplastic and non-neoplastic cells and a micro- and macro-environment around the tumor. Thus, MPE has an affinity to systems biology in one sense [90–93]. In another sense, MPE as applied, translational, clinical and population science represents epidemiology on a large population of human subjects, while systems biology as fundamental and basic science typically relies on experimental model systems. Integrative MPE research not only provides epidemiology research with pathogenic insights, but also provides correlative pathology research with etiologic insights. By using a MPE research approach, a specific relationship between an etiologic factor and certain molecular characteristics may provide evidence to support a causal role for the etiologic factor [13–15]. The concept of etiological heterogeneity has been well supported by systematic analysis

of etiologies and subtypes of bilateral breast cancers [16]. Molecular classification plays a pivotal role in MPE, with the underlying unique tumor principle and the premise that tumors that share similar features may evolve and behave in similar manners.

With the advent of targeted therapeutic intervention, it has been shown that molecular classification can provide predictive biomarkers and contribute to patient management [8]. There are ample examples of such predictive tumor biomarkers, including *ERBB2* (*HER2*) alterations in breast cancer [8], *EGFR* and *ALK* alterations in lung cancers [94–96], *KRAS* mutations in colorectal cancer [97–99], *BRAF* mutations in melanomas [100–102], and *KIT* and *PDGFRA* mutations in gastrointestinal stromal tumors [103,104]. Notably, even the same mutation such as a *BRAF* c.1799T>A (p.V600E) mutation may not provide the same information on treatment response in different tumor types, probably due to differences in cell context as well as in the tumor microenvironment [102,105].

Implications for personalized medicine & molecular diagnostics

The unique tumor principle has substantial implications for oncology and molecular diagnostics. Medical sciences are based on the premise that we can predict disease evolution and outcome from systematic investigations on previous patients with the same disease; however, the unique tumor principle denies the presence of the exact same disease in different patients. In addition, the fact that each tumor is unique poses a significant challenge in the development of specific therapeutics to individual patients. It is necessary to test various drugs in model systems before they are tested by clinical trials. Modeling individual tumors *in vivo* or *in vitro* is never perfect, so long as one is not modeling actual tumors within the specific human body, organ or microenvironment to which those tumors belong. The uniqueness of individual tumors implies the existence of numerous tumor subtypes, and including every subtype in a given model system is almost an impossible task. Even making experimental models for several tumor subtypes is a very tedious task. For example, in making models for lung cancer classified by four binary markers, 16 models are needed to cover all permutations of molecular subtypes. To overcome this issue, it may be necessary to develop a model to generate multiple tumor types through multiple carcinogenesis pathways, which recapitulate an actual human population. A mutator phenotype model may be one such example [51,106].

Another substantial challenge is how we can achieve personalized medicine by a finite number of molecular classifiers, despite the fact that each tumor is truly unique. We believe that molecular classification based on shared tumor characteristics will help us to better predict tumor occurrence and behavior [15,19]. Refinement of molecular classification systems by future research can further refine the risk prediction. Since each tumor is unique, this refinement will continue to improve risk stratification of individual patients in the future.

Conclusion

In this era, in which we are heading towards personalized medicine [107,108], we need to be reminded of the principle that each tumor is unique in terms of molecular make-up, tumor microenvironment and interactomes within and between neoplastic and host cells. Despite the fact that each tumor is unique, tumor classification and molecular diagnostics can benefit individuals based on the premise that similar tumors arise, evolve and behave in similar manners. Starting from this basic unique tumor principle, we can better classify tumors by molecular diagnostics and move closer toward true personalized medicine.

Expert commentary

Despite recent findings on cancer genomic and epigenomic variations, many scientists are still confined to traditional disease classification. Molecular classification has been increasingly more common in many different cancer types. However, the principle of the uniqueness of each tumor has not been discussed in detail. Understanding of the fundamental heterogeneity of neoplastic disease is necessary to achieve personalized medicine.

Five-year view

In the next 5 years, there will be accumulating data on intertumor heterogeneity, especially by next-generation sequencing technologies. Data on intratumor heterogeneity will still be hard to accumulate, but investigators will be increasingly aware of its importance. Other important trends and directions in biomedical and public health sciences are holistic approaches, such as systems biology and MPE, respectively. There will be an increasing need to gather data on tumor heterogeneity in cancer registries around the world. It will be a substantial challenge in prioritization of the tumor biomarkers that will be recorded in the disease registries, among numerous biomarkers. There will be more collaborations between basic scientists, population scientists and translational scientists, to work together to achieve personalized medicine and prevention.

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Key issues

- To advance molecular diagnostics, a better understanding of intratumor and intertumor heterogeneity is essential.
- Tumor cells and the tumor microenvironment are influenced by genomic variation, hormones, and dietary, lifestyle and environmental exposures.
- Essentially, each tumor possesses its own unique characteristics in terms of molecular makeup, tumor microenvironment and interactomes within and between neoplastic and host cells.
- We need to be aware of the ‘unique tumor’ concept and paradigm, to better classify tumors by molecular methods, and move steps closer to personalized cancer medicine and prevention.

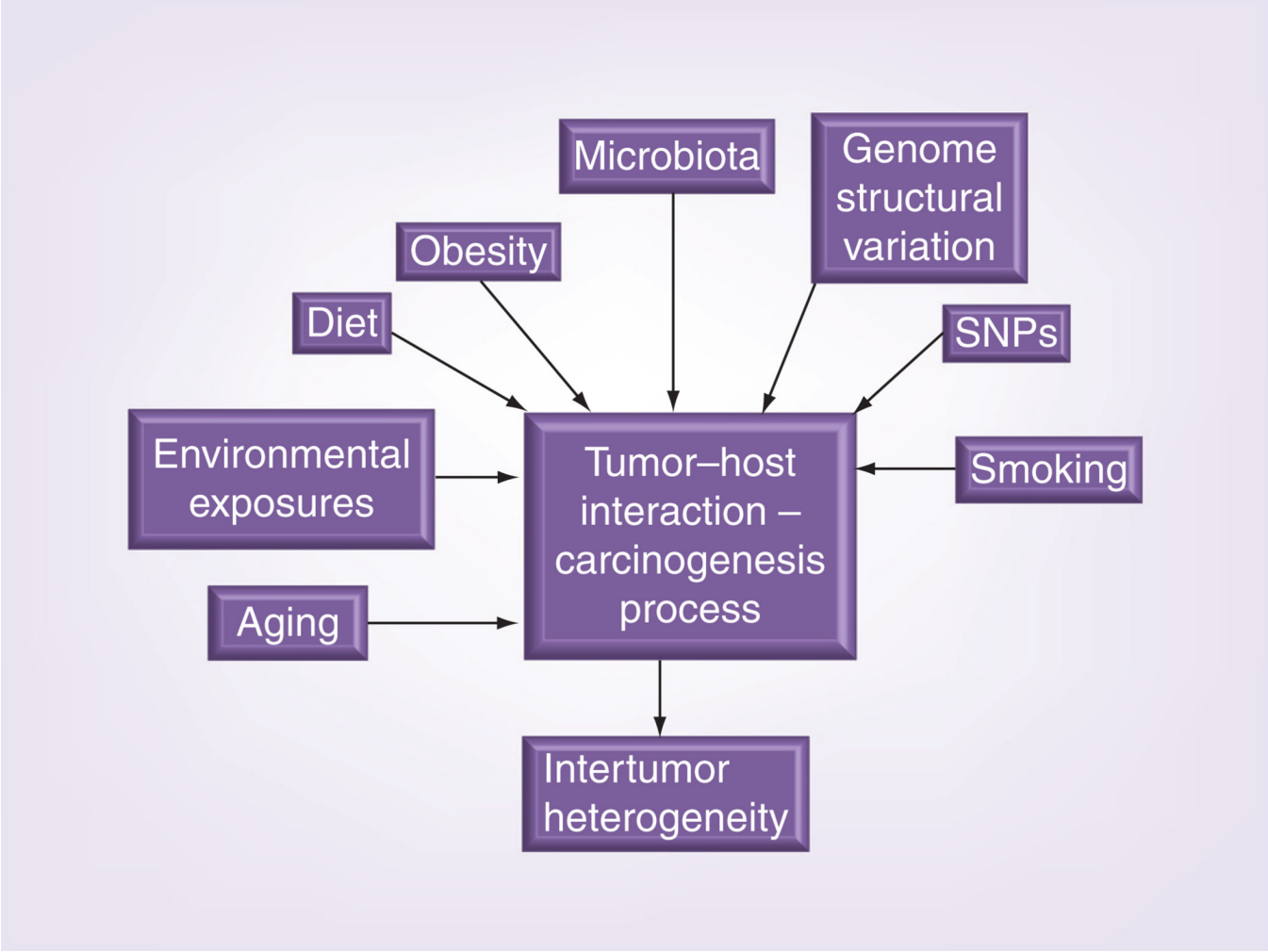

Figure 1. A variety of endogenous and exogenous factors contribute to a diversity of tumor–host interactions and carcinogenesis processes, leading to intertumor heterogeneity and the uniqueness of each tumor

To simplify, only several examples of such factors are shown, and no interaction between the factors is depicted. In addition, there is probably a chance factor in the process of carcinogenesis.

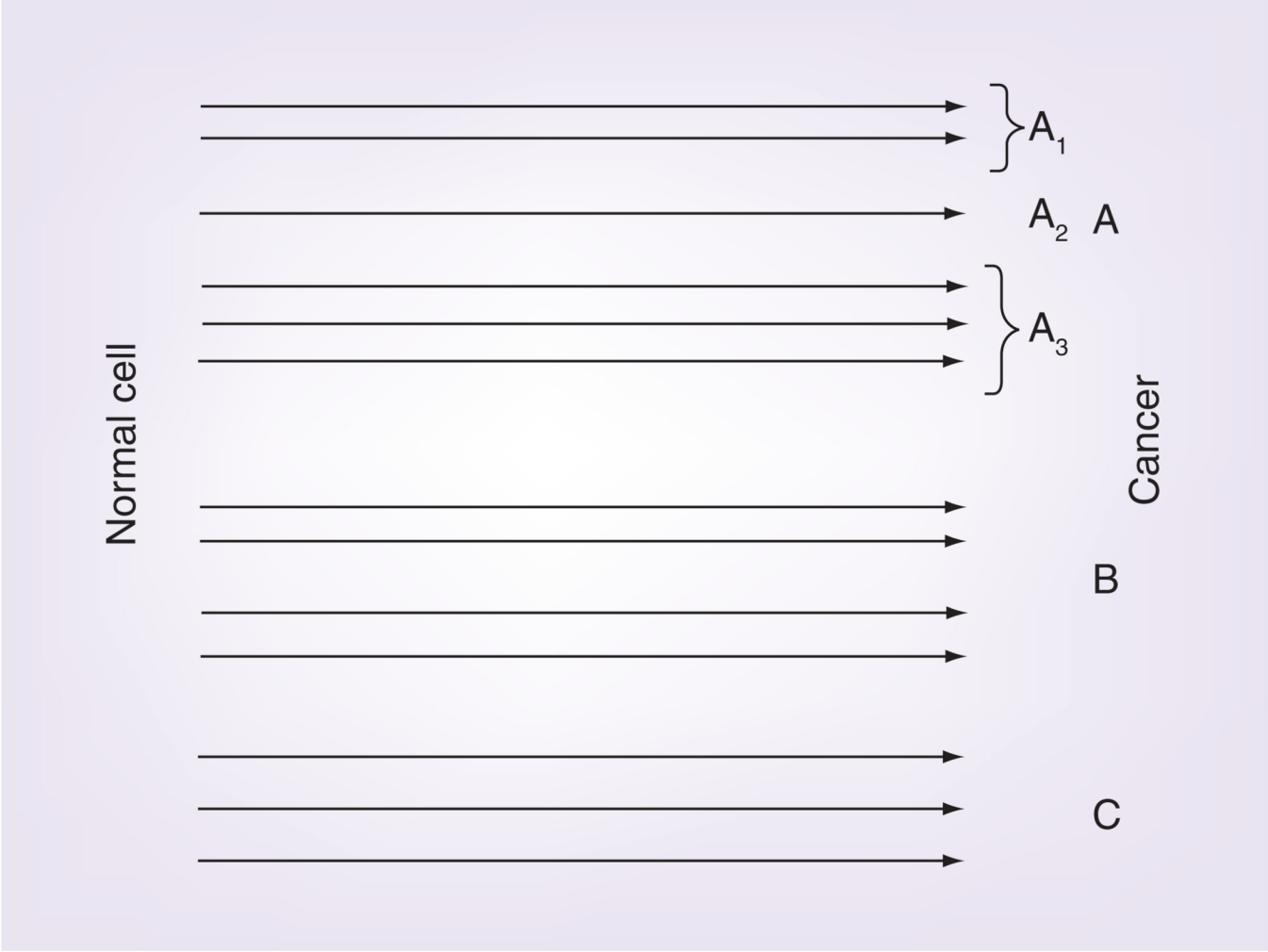

Figure 2. Tumorigenesis pathways

Each tumor pathway is unique, but we can classify similar tumor pathways into one type or one subtype. See explanation in the section titled ‘How many tumor subtypes?’