A Cascade of Modules of a Network Defines Cancer Progression

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Abstract

Similar histologic subtypes of cancers often exhibit different spectrum of genetic and epigenetic alterations. The heterogeneity observed due to lack of consistent and defined alterations affecting a unique set of gene(s) or gene products in cancers derived from a specific tissue, or an organ, pose a challenge in unraveling the molecular basis of the disease. This dilemma also complicates diagnosis, prognosis, effective management, and treatment modalities. To streamline the available and emerging data into a coherent scheme of events, a multimodular molecular network (MMMN) cancer progression model is presented as a roadmap to dissect the complexity inherent to this disease. The fact that disruption/dysregulation of more than one alternate target gene could affect the functionality of each specific module of a cascade provides a molecular basis for genetic and epigenetic heterogeneity in any given cancer. Polymorphisms/mutations as well as the extracellular matrix and or the epigenetically/genetically conditioned surrounding stromal cells could also influence the rate of tumorigenesis and the properties of the tumor cells. The formulation of MMMN cancer progression models for specific cancers is likely to provide the blueprints for the markers and targets to aid diagnosis, prevention, and therapy of this deadly disease. (Cancer Res 2006; 66(15): 7379-85)

Introduction

Cancer is a complex disease that develops as a result of reversible or irreversible damage to critical genes in a multistep process involving the accumulation of genetic and epigenetic alterations (1–7). Such alterations lead to losses of or abnormal function of genes affecting processes that maintain or regulate orderly normal cell function resulting in the phenotypic manifestation of specific types of the cancer. The cells that have acquired the initial gatekeeper alterations undergo localized evolution at increments to convert the tumor cells to become aggressive in their ability to proliferate as well as to invade and spread to distant sites (7, 8). The aberrations in the status of the functionality of the normal gene that contribute to human cancer depending on the tissue type could be derived from overactive and/or deregulated oncogenes, which become activated due to alteration in one or both alleles, or from tumor suppressor genes whose functionality is eliminated when both alleles are damaged or lost (3). Due to incremental changes that accompany selection of cells with an advantage for survival during clonal evolution, the genetic and epigenetic outlook, as well as biochemical properties of tumor cells at the time of initiation, undergoes changes that match the inherent characteristics corresponding to each step of tumor progression as well as the advanced metastatic stage of cancer.

One of the major challenges in studying the genetics and epigenetics of cancer to identify markers or targets for diagnosis, prognosis, and therapy is the inconsistency in their identity and profiles observed in different samples for a similar type of tumor. In a recent study, from the analysis of genetic alterations in lung cancer during multistep progression, we proposed that different tumor phenotypes and heterogeneity in genetic alterations could be elucidated as a series of specific modules consisting of definable interconnected network of events that form a collection of aberrant functional units (Fig. 1; ref. 6). Independent studies attempting to integrate an enormous amount of expression profiling data using transcriptome analyses in the recent years also made predictions of modules of cellular machineries to build cancer genome concept maps (9–11). Although the latter approach attempted to provide clarity to common features of normal and cancer cellular functions (for e.g., cell division, transcription, apoptosis, proliferation, amino acid metabolism, angiogenesis, etc.), it lacked a scheme that will enable one to visualize and elucidate that progression to specific types of cancer as a process that occurs at different stages comprising of multiple interconnected modules consisting of unique and shared alterations. To streamline these concepts and integrate the plethora of molecular details that have emerged and continuing to emerge in the literature on genetic and epigenetic alterations, differential gene expression at the levels of transcripts and proteins and the posttranslational modifications that define the functional epigenome of cancer, a multimodular molecular network (MMMN) cancer progression model is outlined as a roadmap to dissect the complexity inherent to this disease (Fig. 2; Table 1).

The Multistep Cancer Progression Model and the Characteristics of Cancer Cells

For almost two decades, the multistep cancer progression model, popularly called the "Vogelgram" based on the progressive accumulation of genetic alterations involving critical tumor suppressor genes and oncogenes in a series of steps, has provided the framework to understand the initiation, progression, and spread of neoplasm (1). The Vogelgram is also instrumental in pointing out the importance of the cumulative accumulation of alterations and their order with respect to each other in the genesis of cancer (1, 7). More importantly, the concept of multistep cancer progression model established in colorectal cancer has served as a prototype for modeling cancer progression of many other cancers (7, 8, 12, 13). The underlying principles for the genesis of cancer has also been put forward and outlined as the self-sufficiency in growth signals, insensitivity to antigrowth signals, evasion of apoptosis, limitless replicative potential, sustained angiogenesis, and tissue invasion and metastasis (14). Furthermore, another important development during recent years is the increase in awareness of an

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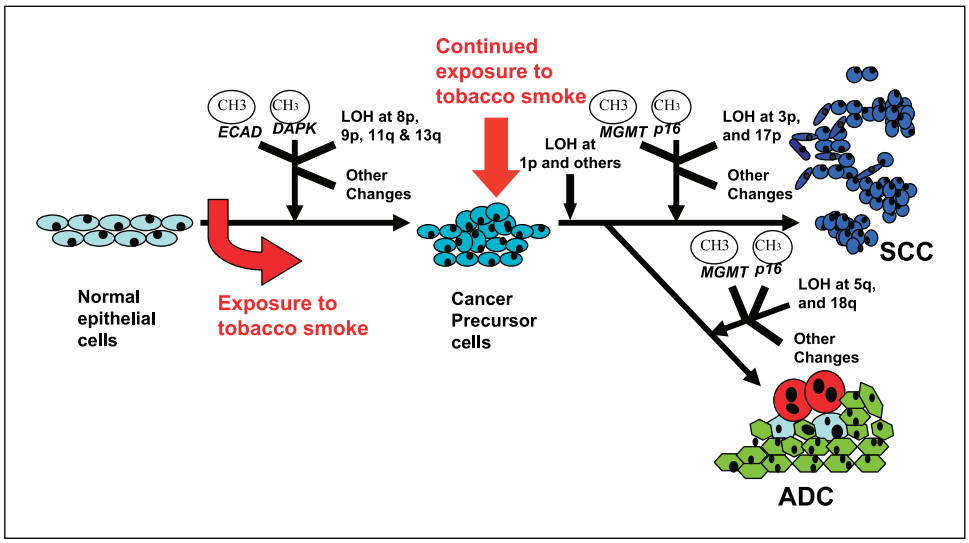

Figure 1. Progressive genetic and epigenetic alterations define histologic subtypes of multistage tobacco smoke–induced NSCLC. One of the major causes of lung cancer is the exposure to tobacco smoke, which is believed to mediate genetic alterations at multiple steps. The early genetic alterations at chromosomal sites 8p, 9p, 11q, and 13q, as well as DNA methylation in the promoters of *DAPK* and *ECAD*, are some of the early events that could inactivate the gatekeeper module in NSCLC. The cancer precursor cells harboring the initial critical alterations are receptive to or selected for additional genetic alterations induced by continued tobacco smoke exposure and provide a survival advantage at each of the steps leading to the genesis of lung cancer. The bronchial epithelial cells harboring early/gatekeeper alterations may acquire additional alterations in different histologic subtypes of NSCLC, such as LOH at the same chromosomal loci (e.g., 1p), specific unique LOH site(s) (e.g., 3p and 17p—squamous cell carcinoma and 5q and 18q—adenocarcinoma), leading to the development of a specific histologic subtype of NSCLC. Promoter DNA methylations of *ECAD* and *DAPK* are early events, whereas those of *p16* and *hMGMT* are late events in NSCLC. The genetic and epigenetic alterations indicated in groups may define in full or part of a module and the interconnecting multiple modules that are responsible for the genesis of NSCLC. This modular organization has to be confirmed and fine tuned in future studies before it could be successfully used in diagnosis, prognosis, and therapy of NSCLC.

active role played by the surrounding stromal cells and the extracellular matrix (ECM) of the target cancer or cancer precursor cells in modulating the altered gene function(s) in the target tumor cells (15, 16).

Alterations in Alternate Multiple Targets Affect Functional Modules

Non–small cell lung cancer progression. Recent observations on tobacco smoke–induced genetic and epigenetic alterations leading to the genesis of non–small cell lung cancer (NSCLC) in our laboratory and the review of accumulating literature suggest that cancer progression could occur in multiple stages of differentiation or dedifferentiation involving several functional modules where each module consists of axes of pathways that cross talk within as well as between different modules (Fig. 1; refs. 3, 6, 9, 17). Our studies suggest that tobacco smoke–induced NSCLC is mediated by genetic and epigenetic alterations at multiple steps. The early genetic alterations at chromosomal sites 8p, 9p, 11q, and 13q and promoter DNA methylation of the *ECAD* and *DAPK* genes are some of the prominent inactivations of the targeted genes that act alone or in combination in the first module (i.e., gatekeeper) of the network (Fig. 1). On the other hand, the prominent genetic alterations observed during progression to advanced stages of NSCLC include loss of heterozygosity (LOH) at chromosomal loci 1p, 3p, 5q, 17p, and 18q and epigenetic alterations due to promoter DNA methylation of the *p16* and *MGMT* genes (Fig. 1; refs. 6, 17). The fact that not all of the alterations are detected simultaneously in the majority of tumor samples of the same histologic subtype of cancer and the presence of at least a subset of alterations at all times in the majority of cancers suggest that there is targeting of alternate genes in the same functional network during cancer progression. When compared with morphologically normal-looking bronchial epithelial cells from smokers, similar frequencies of LOH were observed at some chromosomal loci (e.g., 1p) and increased frequencies of LOH at other sites in tumors (e.g., 3p and 17p—squamous cell carcinoma and 5q and 18q—adenocarcinoma), suggesting that albeit contributions by common alterations, there could be targeting of distinct genes during the development of specific histologic subtypes of NSCLC (Fig. 1). It is also noteworthy that although lung cancer is primarily caused by smoking of tobacco, ~10% of lung cancers could also arise in nonsmokers, likely due to other causes including genetic susceptibility, passive smoking, exposure to radon gas, asbestos or environmental pollutants, and dietary variables. One of the gene alterations often associated with nonsmoker NSCLC is mutations in the epidermal growth factor receptor (*EGFR*). Further studies will be necessary to determine whether mutations in *EGFR* or other target genes correspond to one of the modular subnetwork of the overall NSCLC network.

Targeting Alternate Genes in a Pathway/Subnetwork

There is evidence in the literature that supports inactivations of alternate target genes involved in the same pathway or axis as predisposing to or serving as early aberrations of cancer initiation, such as mutations in either *APC* or β-catenin in familial adenomatous polyposis, *TP53* or *CHEK2* in Li-Fraumeni syndrome, and in *hMSH2* or *hMLH1* in hereditary nonpolyposis colon cancer (7, 18–20). Additionally, there are several studies showing that loss of function due to inactivation of one factor could be compensated by activation of a second downstream factor or dysregulation of the functionality of a pathway due to changes in alternate targets in a linear axis of events. For example, a study examining a role for prohibitin (PHB) in epithelial cell migration

showed that in the axis, Ras-PHB-Raf–mitogen-activated protein (MAP)/extracellular signal-regulated kinase (ERK) kinase(MEK)–ERK, the loss of cell mobility due to inactivation of PHB could be compensated or substituted by activation of C-Raf, a downstream factor to regain function (21). Another study provided evidence for the mutually exclusive nature of rearrangements of the *RET* receptor (*RET/PTC*) and activating mutations in *BRAF* or *RAS* oncogenes in papillary thyroid carcinomas is potentially due to their function in a linear oncogenic signaling cascade, RET/PTC-RAS-BRAF (22). Furthermore, analyses of similar histologic subtypes of tumors showed the existence of axes, such as $p16^{INK4}$-cyclinD1-CDK4-RB, WNT-Frizzled/LRP5/6-DSH-APC/Axin/GSK3/β-catenin-TCF, Met-Gab1-Shp2-ERK/MAPK, and RTKs/IRS2-p85/PIK3CA/PTEN/PDK1/AKT2/PAK4 (23–26). Similar examination of several examples of this phenomenon resulted in an overall conclusion that with most pathways, inactivations or aberrant activations of genes or gene products in cancer could follow the "exclusivity principle" in targeting a single or few genes (5). In addition to examination of linear pathways, there is accumulating evidence that pathways do not exist in isolation but often interact with other pathways or lead to the activation of the same end product in a tissue-specific manner. For example, there is evidence that Smad and MAP kinase (MAPK) pathways interact and phosphorylation of Smad2 could be mediated by either transforming growth factor-β or Ras/MAPK pathway and induction of *cyclin D1*, a marker gene for cell proliferation, could occur downstream of either growth factor signaling involving the RAS/MAPK pathway or the receptor activator of nuclear factor-κB (NF-κB) ligand/NF-κB pathway (27, 28). Aberrant regulation of these genes as a constituent of a subnetwork has been implicated in a multitude of cancers. Consequently, integrated analyses suggest that, in general, targeting for inactivation or aberrant activation in a subnetwork/module of the defined functional unit is likely to occur in a tissue-specific manner and affect different targets (i.e., one or few of these genes) at a time in a specific tumor but yield the same outcome. Although modular aberrations are likely to occur as random events due to overall instability of the cancer genome, it is also equally likely to occur at times, in a sequence, due to selection and/or evolution that provides an advantage in survival that allows tumor progression in gradual shifts from earlier to the later stages of cancer.

MMMN Cancer Progression Model

Cascade of functional modules in a global network. Both solid tumors that are primarily of epithelial cell origin, and the hematologic malignancies (i.e., leukemia, lymphoma, and myeloma) accounting for >90% and 7% of all cancers, respectively, are believed to progress from an early to an advanced stage in a complicated series of events due to genetic and epigenetic alterations in target cells (1–8, 29, 30). The complexity in tumorigenesis influenced by various factors could be academically simplified to dissect the processes using a MMMN cancer progression model that is defined by a cascade of modular events encompassing multiple targets within each module (Fig. 2; Table 1). This framework explains the genetic and epigenetic heterogeneity that is observed during progression to tumors exhibiting similar pathologic characteristics. According to the MMMN cancer progression model, one can envision that inactivation, deregulation, or aberrant activation of individual modules could be mediated by different target gene or gene product alterations and thus the identity and combinations of these alterations in different tumors derived from similar tissues of origin could elicit a wide range of variations (i.e., profiles) as long as the disruption or dysregulation of the defined functional modules have been

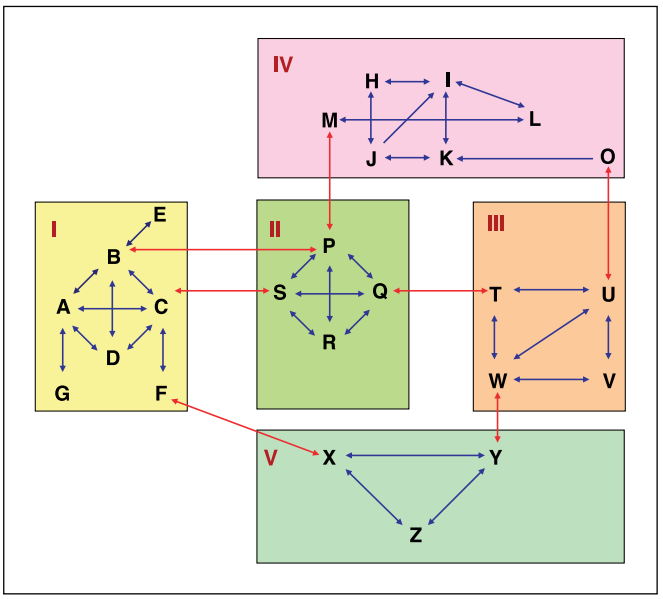

Figure 2. The MMMN cancer progression model. Cancer initiation and progression induced by environmental effects or other *in vivo* or *in vitro* conditions is mediated by aberrant activations or inactivations of functional modules of subnetworks. In this model, cancer initiation occurs due to inactivation of the gatekeeper network module (e.g., module I). The functionality of the gatekeeper subnetwork/module is mediated by an interconnecting subnetwork of pathways (axes) within a module. Thus, dysregulation or inactivation of the gatekeeper module predisposes the cells to become more receptive and susceptible to acquiring additional neoplastic alterations that occur in a series of events of modular inactivations or dysregulations (modules II, III, IV, V, etc.) leading to intermediate and late carcinoma and finally the advanced metastatic stage. Alternatively, alterations in any of the modules could occur independently and not in a sequence of events. Module II in this model represents the intermediate stage of the tumor progression. The terminal modules may represent a specific specialized histologic subtype (e.g., modules III, IV, and V) despite their origin from similar type of cells. Although the MMMN cancer progression model depicted here is with a branching structure to explain how different specialized histologic subtypes could arise from identical or group of cells from the same niche, a linear cascade of modules could explain the genesis of a specific histologic form/type of cancer that originated from cells with similar properties. The fact that there could be alternate target genes responsible for aberrant function in any one of the modules of the network could explain why there is often genetic or epigenetic heterogeneity in multistep cancer progression resulting in similar histologic type of cancer. In general, despite the direct relationship between the genetic and epigenetic alterations in the target cells and the phenotype, it is likely that the overall phenotypic effects elicited by the target tumor or tumor precursor cells could be influenced by the surrounding stromal cells and/or the ECM components. Nonetheless, the epigenetic and genetic alterations in the resident target cells are a prerequisite for the effects caused by the environment. The rates at which different functional modules become affected is likely to be governed by the preexisting alterations as well as the altered functionality of the cellular machineries responsible for the maintenance of genetic integrity, epigenetic code, and the tumor microenvironment. The imbalance in these tightly controlled processes could tilt the balance in favor of tumor evolution from an early stage toward an advanced stage. *Double-headed blue arrows,* intramodular connections; *double-headed red arrows,* extramodular connections, respectively. *Alphabetical letters,* specific genes, gene products, functional protein-protein, or protein-DNA interactions or axis/pathway that are nodal points in each of the module of the global network. *Letters connected by the double-headed red arrows,* similar or different interactors at the nodal points, which indicate the fact that they may or may not be always an integral part of only one of the resident module; if they are the same, then they may have similar or dissimilar module-specific activity and the different interactors may either enhance or suppress activities of each other. These various players and their activities should be related to each other using computer algorithms to depict the network interactions and downstream effects.

Table 1. Principles defining the MMMN cancer progression

1. Cancer progression is effected in a multistep process that involves aberrant activations or inactivations of target genes in a series of interconnected functional modules of a global network.
2. Altered functionality of a specific module may be achieved by targeting at least one but a required minimum number of gene(s) that could include preexisting mutations, genetic polymorphisms, and epigenetic and genetic changes occurring in a tissue-specific manner.
3. Genetic and epigenetic heterogeneity in altered genes in any given tumor despite similar tissue origin is derived from disruption/dysregulation of alternate target genes in a specific module.
4. The network module that becomes dysfunctional to initiate cancer is the gatekeeper functional unit, which predisposes the initial target cell(s) to acquire additional genetic and epigenetic alterations.
5. The network modules exhibiting aberrant functionalities in a series of events advance the tumor from the early to a late stage of cancer.
6. Although any alteration capable of inactivating a specific subnetwork module could occur at any time, the effect will be fully implemented/realized to elicit the properties corresponding to a particular module of the series only when the preceding module(s) have also become inactivated/dysregulated.
7. There could be overlaps in the functional roles or differential tissue/stage–specific activity of various players in different modules and thus more than one module could become affected at the same time due to targeting of an individual gene.
8. Alterations in a specific gene could mediate disruption of modules belonging to different stages in the same or different types of cancers.
9. Although the overall phenotypic effects/functional properties elicited by the target tumor cells could be influenced by the surrounding cells (e.g., stromal cells), autocrine/paracrine factors and/or the ECM components, the specific epigenetic and genetic alterations in the resident target cells is a prerequisite that defines the tumor characteristics.
10. The rates at which different functional modules become affected during tumor evolution determining the time required for transition from an early to a later stage of cancer are likely to be governed by the dysfunctional status of the cellular machineries responsible for the maintenance of genetic integrity, preexisting alterations, epigenetic code, and the tumor microenvironment.

achieved. Therefore, absence of consistent alterations in specific gene(s) or gene products in sporadic cancers and in cancers that are primarily induced by environmental effects to generate neoplastic precursor cells could be predicted to occur *via* inactivation or overactivation of multiple alternate early target gene(s) or gene products that act in one or more interconnected axes of events within a defined subnetwork known as a module in the global network (Fig. 2). The milieu of environmental effects responsible for the genesis of neoplastic precursor cells are often associated with autoimmune or chronic inflammatory reactions induced by biological agents, endogenous or exogenous chemicals, and physical agents such as heat, radiation, and foreign bodies (31). As a result of these complex processes, the network module that becomes inactivated leading to the initiation of cancer is defined as the gatekeeper functional unit (8). The cancer precursor cells harboring inactivated gatekeeper module could either take advantage of preexisting inherited or randomly acquired alterations or become increasingly receptive to additional genetic and epigenetic aberrations in making progressive transition to the advanced stages. The occurrence of a series of these events in interconnecting but defined modules of subnetworks in multiple stages could ultimately lead to development of advanced stages of cancer (Fig. 3). Altered functionality in any defined module may be achieved by targeting at least one gene or gene product but may require a minimum number of gene or gene product alterations that could include preexisting genetic polymorphisms, mutations, and epigenetic changes including imprinting at the level of nucleic acids or proteins. Thus, as a consequence of the functional network module inactivations or aberrant activations that occurs in multiple modules in a series of events, the tumor advances from an early to intermediate and later stages and finally to an acute or dormant advanced metastatic stage (Fig. 3). An acute advanced metastatic stage of cancer results in organ failures leading to the death of the patient.

The molecular basis of tumor cell behavior. There are at least four major preexisting or induced aberrant conditions that can affect the properties as well as rate of progression of tumor cells from an early to advanced stage often without directly being responsible for initiating the tumorigenic process. These conditions are as follows: (*a*) inherited genetic and epigenetic aberrations such as mutations, genetic polymorphisms, and imprinting; (*b*) aberrant functionality of the caretaker and chromosome segregator genes that are responsible for maintaining the genomic integrity; (*c*) the enforcers and targets that define the nature of the epigenome and the epigenetic code; and (*d*) the tumor microenvironment determined by the surrounding stromal cells, the constituents of the immune system, autocrine/paracrine factors, and/or ECM components.

If target cells already harbor inactivated or dysregulated module(s) due to inheritance, the rate of cancer progression will occur at an accelerated phase as soon as the preceding modules become sporadically inactivated or dysregulated. The series will become completed up to the last contiguous aberrant module in the sequence of the cascade of events required to complete the disease progression. Thus, individuals with inherited alterations corresponding to a later module could be cancer-free to a point until alterations in the earlier module(s) occur. However, once these individuals with predisposing alterations acquire the required alterations in the preceding module(s) in sporadic events, progression of the disease will occur at an accelerated phase. It should be noted that conditions (*b*) and (*c*) in some cancers could also be an integral part of the primary alterations that occur in a specific module. Despite the ability of any of these conditions to influence the modules at any time during cancer progression, the primary and critical nodal epigenetic and genetic alterations in resident target cells is a prerequisite and will ultimately dictate whether aberrant functionality of the module(s) are effected and/or transitions from an early to a later module will occur during tumor evolution/progression. This notion has been substantiated in recent reports on characterization of gene expression patterns and genetic alterations in breast and ovarian cancers. It was revealed that despite the observation of random as well as occasional targeted allelic imbalance in adjacent stromal cells,

significant number of targeted genetic alterations are selected and fixed during tumor evolution only in the target cancer epithelial cells (32, 33). In other words, the existence or creation of target cancer precursor cells harboring critical aberrant modules is a prerequisite for tumor formation despite the aberrant functionality/activity in the tumor microenvironment (i.e., condition; *d*). In certain situations, a group of cells of mesenchymal origin could also act as primary target cells if they harbor critical alterations, whereas the surrounding cells, even if they had a common origin, could play an accessory role by acting as the microenvironment during the tumor evolution of mesenchymal tumors.

Timing of alterations. Although genetic and epigenetic alterations within any of the modular subnetworks could occur at any time, effects leading to the differentiated stage corresponding to the module could only become fully realized when alterations in the preceding contiguous module(s) have already occurred. This notion is substantiated by the fact that several of the single gene defects or overactivities alone in the absence of an early alteration even in the presence of a late alteration or *vice versa* are insufficient to drive cells from initiation to an advanced tumor. For example, mutations in just one of the target genes, such as *APC* or *β-catenin*, *SMAD4* or *TP53*; or activation of *KRAS* alone during colon cancer progression; mutations in *BRACA1* or *BRACA2* alone, and overexpression of *HER2/NEU* during the development of breast cancer; mutations in *TP53, RB, p16*, or *PTEN*; or overactivity of *KRAS, MYC*, or cyclin D1 alone in lung cancer are insufficient to initiate and drive the neoplastic precursor cells to metastatic tumors (8, 12, 34, 35). Additionally, even the same altered gene could elicit different effects depending on the specific tissue type or in the context of the combination of other alterations or polymorphisms already present in the target cell. This phenomenon is further substantiated in animal models as the existence of defective *APC* allele in combination with a defect in *TP53* promotes mammary neoplasia, whereas in combination with defective *SMAD4* promotes development of intestinal malignancy (36, 37).

The number of gene alterations versus modular aberrations. The requirement for multiple alterations for the genesis of cancer has been established with the aid of mouse models and human cell lines in independent studies (38, 39). By comparing human cancer to mouse models, it has been proposed that disruption of a limited

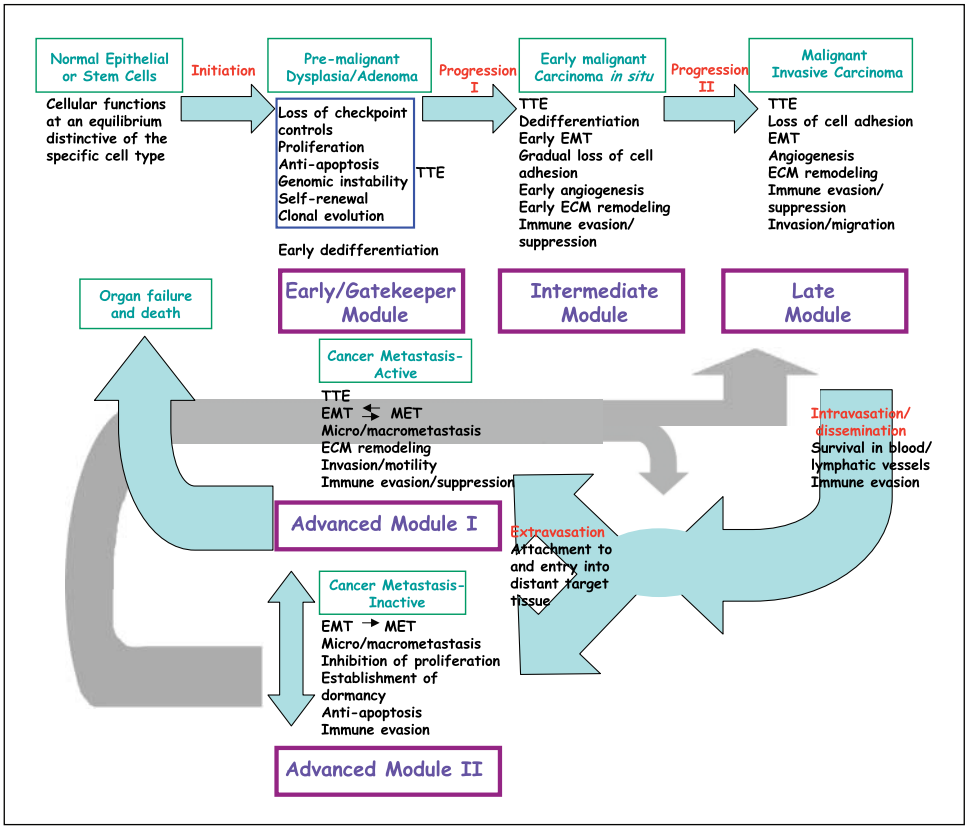

Figure 3. The modular organization of epithelial/stem cell–derived cancer progression. This example depicts that MMMN cancer progression from normal or stem cells to organ failures and death occurring in modular aberrations that represent early/gatekeeper, intermediate, late, and active/dormant advanced stages. It should be noted that although the localized cancer (i.e., benign) may consist of only early/gatekeeper or the early/gatekeeper and intermediate modules, the cancers that have acquired the properties to spread from the site of origin (i.e., malignant) may consist of other additional definable modules (e.g., invasive carcinoma, metastasis, etc.). Furthermore, in acute cases (advanced module I), the tumor cells continue to be highly proliferative and invasive as opposed to the dormant phase (advanced module II), where they remain nonactive. Whereas the cells in the advanced module I stage exhibit mesenchymal phenotype, the tumor cells in the dormant advanced module II stage are predominantly epithelial due to a switch from the mesenchymal to epithelial phenotype, a process that is not necessarily a simple reversal of the genetic and epigenetic alterations that originally led to the EMT from the target normal cells. A highly active advanced module I could cause damage to the host tissue and organ failures leading to the death of the affected individual. Although we have represented the modular organization in terms of aberrant functionalities of affected cells, the ultimate goal will be to decorate these with genes and gene products that are targeted for alterations and organized in interconnecting axes and networks. Thus, here the modules are represented by the functional status of cancer cells corresponding to a specific stage of cancer progression that is mediated by altered genes or gene products that may play roles in one or more module (s). *TTE,* tumor transition events; *EMT,* epithelial-mesenchymal transition; *MET,* mesenchymal-epithelial transition.

minimum number of pathways corresponding to a variable number of target genes contributes to the genesis of most if not all cancers (40). Despite the lack of dissection of the contributions of specific genes or gene products as alternate targets in the context of "aberrant functional modules" and the requirement for interdependency of interconnected altered modular functions in a cascade of events for cancer progression in these studies, they are highly consistent with the theme of the MMMN cancer progression model.

It is also becoming increasingly clear that there could be overlaps in the functional roles of various players in different modules during the genesis of cancer and thus more than one module could become affected at the same time due to targeting of an individual gene. For example, *KRAS* mutations are known to both initiate as well as participate in the genesis of malignant pancreatic cancer (12). Furthermore, with reference to Met activation, it has been shown to affect not only ETS/AP1 transcription factors and adhesion molecules *via* the Gab1-Shp2-ERK/MAPK cascade, but it could also influence the cytoskeleton and cell adhesion *via* the Ras-Rac-Pak cascade (24). Another intriguing phenomenon that is observed in cancer is that multiple apparently unrelated alterations can result in similar end effects affecting the status of a specific gene product. For example, mutations in *APC* or in the amino acid residues that undergo phosporylation of β-catenin could stabilize the protein causing constitutive signaling independent of WNT, an aberrant condition commonly observed in colon cancer (41). On the other hand, there is also data suggesting that aberrant accumulation of β-catenin in cancer cells is due to inactivation of p53, whereas the defect in the latter is generally associated with advanced stages of most cancers (42).

Conditioned stromal cells define the microenvironment for cancer progression. The stromal cells (e.g., endothelial cells, fibroblasts, myoepithelial cells, inflammatory cells etc.) surrounding the neoplastic epithelial cells are widely accepted to play critical roles in influencing the behavior of the tumor. The conditioning and activation of the microenvironment of stromal cells to support aggressive tumor cell behavior could result from coevolution of the surrounding cells that are likely to undergo transient/stable epigenetic changes, random or occasionally targeted/evolutionarily selectable genetic changes, and/or from the recruitment of appropriate cells (e.g., inflammatory cells) to serve as a reservoir of chemotactic, stroma-modulatory, and other factors (43). The distinctive gene expression patterns, nature of posttranslationally modified protein products, acquired ability to secrete various tumor-promoting factors, and remodeling of microenvironment consisting of a characteristic ECM by the tumor stromal cells are likely to enable them to determine the rate of acceleration of tumor progression.

MMMN Cancer Progression Model and the Cancer Stem Cells

We are currently in the midst of a debate over the clonal origin of the so-called "cancer stem cells." There are two schools of thoughts, one promoting the origin of tumors as exclusively from tissue stem cells that have acquired the necessary alterations to become cancer stem cells and the alternate hypothesis is based on the genesis of tumor cells due to a stochastic process in which multiple independent differentiated cells that have acquired the necessary alterations could elicit limitless proliferating potential and "stem cell–like" properties (44, 45).

The "holy grail" here is the identification of specific markers that will enable one to distinguish between cancer stem cells that originated from tissue stem cells and cancer precursor cells that are derived from reversion of differentiated cells to become "embryonic or stem cell like" by acquiring at least a subset of the primary properties of the latter type of cells. Although these are plausible hypotheses toward a common goal to elucidate the unique and identifying characteristics of cancer cells, one should be acutely aware of not letting the dogma overshadow the noble goal of pursuing smarter approaches in research that will allow us to eradicate all of the tumor cells in the affected individuals irrespective of whether they originated from cancer stem cells or through a stochastic process from any affected cell. The authors' own view is that it is unlikely that we will be able to trace all of the cancers to specific tissue stem cells with a likely exception of hematologic malignancies. Alternatively, cancer stem cells can potentially arise from any cell, including tissue stem cells. Irrespective of which side of this debate your leanings are, understanding of the heterogeneity in genetic and epigenetic alterations as well as distinctive gene expression patterns observed in cancer cells using modeling approaches, such as the one proposed here, the MMMN cancer progression model focusing on the properties of the cancer cells at different stages of disease manifestation is most likely to provide the "magic bullet" to help us conquer this epidemic.

Conclusions and Future Directions

Overall, the formulation of the MMMN cancer progression models for different cancers is an achievable task with the accelerated accumulation of enormous amount of high-throughput data and with development of new bioinformatics tools at the current age. Thus, construction of successful modular network models should enable us to understand the complexity in genetic and epigenetic alterations that occur in cancer and provide a futuristic view of the molecular understanding of cancer progression. Interestingly, it has already been established that similar genetic and epigenetic alterations of specific genes may play different roles in different cancers in a tissue-specific manner (3). For example, a familial cancer gene alteration for one cancer may serve as a sporadic cancer gene alteration in a different cancer occurring at a later stage of cancer progression (3). The MMMN models constructed for different cancers could shed light on similar and dissimilar end effects of specific alterations observed in a tissue-specific manner (e.g., *BRCA2* and *SMAD4* mutations initiate breast cancer and juvenile polyposis, respectively, but occurs at a later stage in pancreatic cancer) and could also help to define a specific stage of the tumor evolution based on common properties acquired by the cancer cells (e.g., dysplasia, carcinoma *in situ*, invasion, angiogenesis, intravasation, extravasation, micrometastasis, macrometastasis, bone metastasis, etc.). Despite the fact that determination of the identities of genes has become routine at this genomic age, the elucidation of gene function both in isolation and in the context of other gene products in the various intracellular niches remain a challenge. Thus, at this time, in general with few exceptions, we are only able to describe the various axes of functions organized in the modules of the MMMN cancer progression as end effects rather than with the aid of multidimensional interconnecting networks of genes and gene products (Fig. 3). Thus, a major challenge for future research will be to place the various genes and gene products in prospective with alternating

functions in the axes of modular groups of a global network with the aid of novel technologies and bioinformatics tools.

Because cancer is a disease that elicits the fundamental properties of dysfunctional cellular processes as a common theme, there will be plenty of overlap in observed specific genetic and epigenetic alterations arising from dissimilar tissue types. Thus, the detailed characterizations in terms of building MMMN cancer progression models for any one of the cancers is highly likely to advance the entire field forward in gaining an overall understanding of the molecular basis of cancer. Therefore, regardless of which cancer one wants to study as their favorite model system of choice, the discoveries made in any of the model systems will accelerate the ability to disentangle the intricacies of individual diseases. Once we have modular network models in place for each of the specific cancers, they will provide the blueprints for the markers and targets that can be taken advantage of to develop diagnostic, preventive, and therapeutic strategies for this deadly disease. In the global sense, the principles underlying the formulation of MMMN cancer progression models could also be expected to provide the necessary conceptual framework to develop similar models for other challenging complex diseases.

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